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1102 Bates Ave, Houston Tx 77030
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VIRTUAL GLOBAL CONGRESS ON SUSTAINABILITY FOR GROWTH & DEVELOPMENT - GCSGD 2020

GCSGD 2020 was organized by Einstein Research Academy (ERA) in association with Nilai University, Galgotias University and Fatima College (Autonomous) on July 18, 2020. The congress was aimed to bring together the academicians, scientists, and experts all around the world to share their expertise, knowledge and research findings towards global sustainability and growth, and also to motivate and strengthen interdisciplinary research among researchers and stakeholders worldwide to take the bold and transformative steps to steer the world towards sustainable and resilient path.

GCSGD2020 had attracted nearly 200 participants, with 4 plenaries and 8 invited talks by the experts from various disciplines, and around 120 oral and poster presentations on novel research findings by the researchers from numerous countries including Malaysia, India, Indonesia, Thailand, Bangladesh, SriLanka, Australia, France, and Libya. As the organizers of the GCSGD 2020, we are happy to publish the accepted conference articles related to life and health sciences in the Journal of Experimental Biology and Agricultural Sciences (JEBAS) as a special issue.

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THE EFFECT OF AEROBIC EXERCISES ON PAIN, QUALITY OF LIFE IN PRIMARY HEADACHE

Kiruthika Selvakumar*

Department of Physiotherapy, Universiti Tunku Abdul Rahman, Bandar Sungai Long, Kajang, Selangor, Malaysia

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ABSTRACT

Headache disorders are among the most common disorders of the nervous system. According to World Health Organisation reports that almost half of all adults worldwide experience a headache in any given year. Based on research, headaches are classified into primary and secondary headaches. Depending on global prevalence the most common primary headaches are migraine, tension-type, and cluster headaches. If left untreated it can result in increased pain, decreased quality of life. The objective of this literature article is to analyze the effect of aerobic exercise on pain and quality of life among subjects with primary headaches like migraine, tension-type, and cluster headache and to discuss the current updates in the literature. In this article, relevant data available in PubMed, Cochrane, and Medline databases were retrieved from 2010 to February 2020 using the search terms aerobic exercise and tension-type headaches, aerobic exercise and migraine, aerobic exercise and cluster headaches, pain, and quality of life. The search strategy identified five articles that considered the effect of aerobic exercise on primary headaches like a migraine; tension-type and cluster. Results have positive effects for aerobic exercise on tension-type headache, migraine headache mainly on pain intensity, whereas the quality of life is less studied. On the other hand, these studies did not provide a specific protocol or parameter on exercise intensities. The availability of data on the influence of aerobic exercise on primary headaches though is limited, aerobic exercises are the best option for reducing pain and improving quality of life in primary headaches, especially for tension-type and migraine-type headaches.

* Corresponding author

E-mail: kiruthika@utar.edu.my (Kiruthika Selvakumar)

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1 Introduction

According to World Health Organisation, headache is a disorder and most familiar problem of the nervous system. Headache is not only painful but also disabling. Headache disorders collectively ranked at third highest. It has been reported that the incidence of headache among adults is nearly half that is 47% (Jensen & Stovner, 2008). It is estimated that half to three-quarters of adults who are aged between 18–65 years have a headache in the last year. It is approximated that 1.7–4% of the world's adult population has pain due to headaches that last for more than 15 days (Yancey et al., 2014).

Headaches are classified into primary and secondary. Secondary headaches are uncommon, but their recognition is extremely important as early intervention may be lifesaving. Primary headaches can be further classified majorly into a migraine, tension type, clusters, etc. (Carbaat & Couturier, 2016). According to the International Classification of headache disorder, apart major subtypes include primary cough, primary exercise headache, a primary thunderclap headache, external pressure headache, primary stabbing headache, nummular headaches, hypnic headaches, etc. (Clinch, 2001).

Globally the incidence of headache disorder is still higher, with active headache around 47% for primary headache, 10% for migraine type headache, 38% tension type headache (Jensen & Stovner, 2008). The lifetime prevalence is still more higher with 66% for headache, 14% for migraine, 46% for tension type headache, and 3-4% for chronic headache (Stovner et al., 2007).

A cross-sectional study found 1-year prevalence and incidence of tension-type headache is 43.1%, active migraine is 18.1% and the lifetime prevalence of migraine is 32.8% (Hagen et al., 2018). In cluster type headaches the incidence up to 0.1% of the population only, but it's found to be higher in females than males in the ratio of 2.5:1 (Wei et al., 2018). Cough headaches are symptomatic in about 40% and associated with secondary conditions like Arnold-Chiari malformation, Vertebrobasilar disease. Primary exercise headaches are mostly of vascular origin.

Thunderclap is associated with vascular disorders and also intracranial vascular disorders. External pressure headache often results from compression or traction. Primary stabbing headache commonly associated with migraine headaches. Nummular headaches are less common. Hypnic headaches features are similar to tension-type headache, but recent studies found patient present with migraine like feature and occasionally present with nausea ("Primary Headache Disorders: Types," 2019). Hence from this prevalence it is found that migraine, tension-type and cluster headaches are the more common types.

The migraine type of pain is defined as pulsating or reverberate, and often unilateral lasting from 4 hours to a maximum of 72 hours may be associated with nausea, vomiting, sensitivity to surroundings like smell, vision, and light (Ahmed, 2012). It is found that subjects with this type of headache prefer to be in a dark, quiet room, and avoid physical activity. In tension type headaches the pain is usually aching or pressure or has a dense or rigid band around the head and this type of headache rarely affects activities of daily living.

In Cluster headaches, the pain is severe and described as a "suicidal headache". It is found to be more commonly prevalent in teenaged or adolescent men (3.5:1). The duration of pain range from 15 minutes to 3 hours and occurs from every other alternative day to up to eight days. The patient will be extremely restless and agitated and often sweats excessively (Ahmed, 2012).

The common feature of headache is pain. The causative factor for pain in primary headache is that an increase in chemical activity in the brain, blood vessels, or nerves surrounding the brain or muscles and in and around the head and neck region. DaSilva stated, "during a migraine attack, the patients' dopamine levels fell significantly". Hence, a drop in dopamine could cause the sensory structures to be more sensitive, so that normally painless or imperceptible sensory signals from the skin, muscle, blood vessels, and other structure would become more painful (Bailey, 2017). The hypothesis for migraine pain is that pain occurs due to waves or abnormal activity by groups of excitable cells in the brain, these, in turn, can release chemicals or neurotransmitters like serotonin mainly resulting in the narrowing of blood vessels. When serotonin or estrogen levels change in the blood, it results for some as migraine ("How a Migraine Happens - Theories about Migraine Pain," 2020).

The common theory for tension-type headache is that the increase in muscular pain sensation cause increases activation of the spinal cord and brainstem neurons involved in pain signaling as well as activation of neurons in areas of the brain that are involved with processing pain, like the thalamus and somatosensory cortex. This amplified activity causes the pain pathways to become highly sensitized, leading to exaggerated response events to smaller degrees of stimulation. Also, it is found that the mechanism involved with pain inhibition may also begin to fail the suppress pain signaling by compounding the effect (Bendtsen et al., 2010).Headache disorders if left untreated impose a recognizable burden on sufferers that includes impaired quality of life (Terwindt et al., 2003), sleep disorder especially insomnia (Kim et al., 2018), depression, and anxiety (Wei et al., 2016). A study found that subjects with migraine-type headaches were not able to carry out activities and the total loss of workdays per one year is 270 days per 1000 persons. For tension-type headaches, the estimated absenteeism of working days is 820 (Rasmussen et al., 1992).

Table 1 PICOS and eligibility criterion

PICO	Inclusion Criteria	Exclusion Criteria
Patients	Migraine headache, tension type headache, cluster headache	Non-human subjects, pregnant women, secondary headache
Intervention	Aerobic exercises	Manual therapy, no intervention including diagnostic or prevalence studies or secondary headache
Control	Relaxation exercise, Medications	-
Outcome	Pain, Quality of life	-
Study design	RCT, Case-Control, Case studies	Non-English, meta-analyses and systematic reviews

Table 2 Characteristics of Studies Included and the Outcome Measure

Study	No. of subjects	Age (Mean & SD)	Population	Design/ Sampling	Outcome measure
Sertel et al., 2017	60	39.26 ± 9.23	Tension- type headaches	Block Randomization	Visual analog scale and pain diary, pain disability index, headache impact tests and SF-36.
Krøll et al., 2018	52	42±10.9: Experimental 36±10.1: Control	Migraine and co-existing tension-type headache and neck pain	Randomized Controlled Clinical Trial	Pain frequency, intensity, and duration, neck pain, physicalfitness, level of physical activity, well-being.
Santiago et al., 2014	60	31±9: Experimental 35±8: Control	Chronic Migraine	Randomized Controlled Trial	Frequency, intensity and duration, days of the analgesic medication use, body mass index, beck depression inventory and beck anxiety inventory.
Varkey et al., 2011	91	44.3± 10.6	Migraine	Randomized Controlled Trial	Migraine status, quality of life, level of physical activity and oxygen uptake.
Andersen et al., 2011	198	44 ±11: 2 min group 42±11: 12 min group 43±10: control group	Headache (combined with migraine and Tension-type)	Randomized Controlled Trial	Frequency, intensity, and duration of headache.

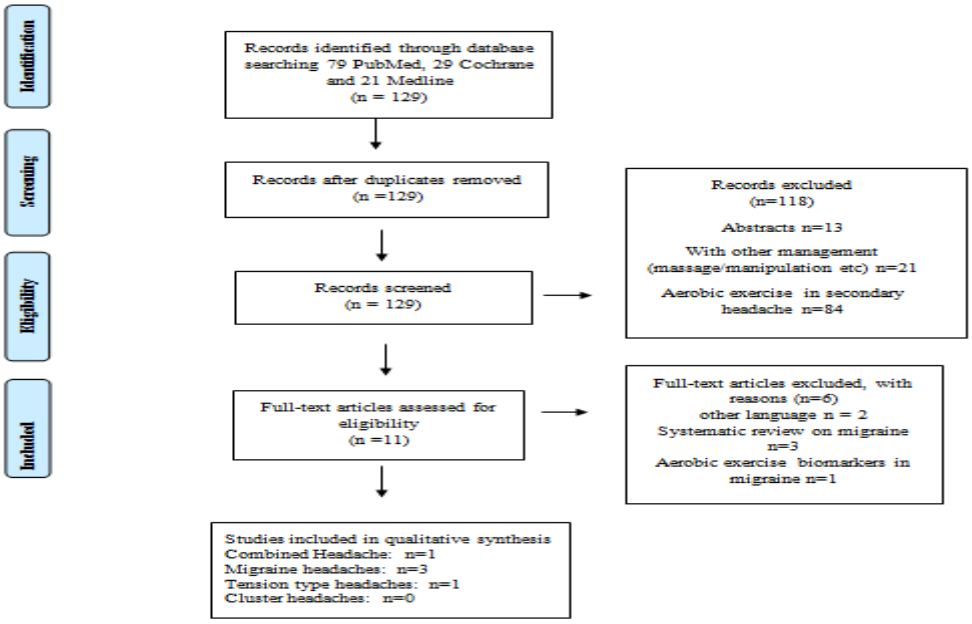

Figure 1 Flow Chart of Study Selection

Table 3 Interventions of Experimental and Control Groups with Results of Included Studies

Study	Experimental group	Intervention and Intensity for experimental group	Control group	Results
Sertel et al., 2017	Group 2: Aerobic exercise via step-dance accompanied by music	Step-dance training accompanied by music for 40 min, 3 sessions/ week for 6 weeks with RPE: 13-14 points.	Group1:BAT: relaxation, motion and massage Group 3: No exercises	BAT and Aerobic Exercise together can bring about reduction in pain intensity.
Krøll et al., 2018	Aerobic exercise	Bike/cross-trainer/brisk walking for 45 minutes, three times/week for 3 months with RPE: 14-16 points.	Controls continued usual daily activities	Exercise significantly reduced the burden of migraine and the ability to engage in physical activity because of reduced impact of tension-type headache and neck pain.
Santiago et al., 2014	Aerobic exercise and amitriptyline	Outdoor fast walking for 40 minutes with a frequency of 3 times/week for 12 consecutive weeks with Amitriptyline (25 mg/day)	Amitriptyline (25 mg/day)	amitriptyline was an effective treatment for chronic migraine, but its efficacy was increased when combined with aerobic exercise.
Varkey et al., 2011	Exercise	Indoor cycling 40 min 3 times/week, for 3 months with RPE 14-16 points.	Relaxation group: breathing, stress management 5-20 min. Topiramate group: 25mg every week till max dosage to 200mg/day.	Exercise may be an option for the prophylactic treatment of migraine in patients who do not benefit from ordo not want to take daily medication.
Andersen et al., 2011	Resistance type of aerobic training	Resistance training with elastic tubing at 2 min and 12 min/ day, 5 times a week for 10 weeks.	Health related information	Two minutes of daily resistance training for ten weeks reduces headache pain, frequency among office workers with neck/shoulder pain.

Among the conducted studies, 20% of the study population reported headaches one or more times a week, which was analyzed using the PedMIDAS scale, and the average score is over 12. Further, 10% of the school children aged between 12-15 years were analyzed for PedMIDAS and found to have a score of 16.8 whereas the PedsQL4 generic quality of life score is about 70.1.These results can indicate even children with headache have a poorer quality of life than compared to that of children with neurological or cardiovascular disorders like asthma, diabetes, or cancer. An average of 0.6 days of school was missed in 3 months across all school children (Kernick et al., 2009). These recognizable burdens are purely due to pain. Hence it is important to consider pain which in turn can affect the quality of life. The treatment for primary headaches is not common; it depends on severity, symptoms, and disability. Though pharmacological medications may be advised, it cannot be given for a longer duration due to its side effects. Thus, a non-pharmacological intervention is required to enhance the quality of life. Exercises can be of different types. It includes stretching, deep breathing and muscle relaxation, yoga, and cardiovascular exercises. Habitual aerobic exercise has a major advantage of preventing or reducing symptoms of several chronic diseases and medical conditions (Irby et al., 2016). Aerobic or cardiovascular exercise by definition is a form of bodily movement that is fuelled by aerobic energy-generating processes, where the energy demands of the exercise performed do not exceed the rate at which the cardiovascular system can supply oxygen to working muscles (McArdle et al., 1980). Aerobic exercises have already been proven to reduce the frequency, duration, severity, or associated disability in migraine (Irby et al., 2016).

When one exercise, the body releases endorphins, which are the body's natural painkillers and natural anti-depressants chemicals called enkephalins. This could mean providing a planned exercise can improve the overall function. According to the Centre of Disease Control and Prevention (CDC), an adult should exercise 150 minutes of moderate-intensity aerobic exercise and 2 or more days a week of muscle-strengthening each week for relief of migraine or primary headaches (Robbins, 2015). According to the American migraine foundation, the exercise program should include cardiorespiratory fitness, flexibility exercises, and muscular strengthening. As most of the studies found that aerobic exercises can reduce frequency, severity, and duration (Varkey et al., 2011; Santiago et al., 2014; Krøll et al., 2018). The purpose of this study is to investigate whether aerobic exercise influences pain and quality of life among subjects with primary headaches and are these studies are based on sufficient data.

Table 4 Interventions of Experimental and Control Groups with Results on Pain and Quality of Life

S. No	Experimental group	Control Group	Pain Intensity pre	Pain Intensity Post	QoL pre	QoL post
Sertel et al., 2017	Step-dance training accompanied by music for 40 min, 3 sessions/ week for 6 weeks with RPE: 13-14 points	Group 1:BAT: relaxation, motion and massage	6.10±1.02*	3.00±1.28*	36.51±6.24*	44.16±9.69*
			6.15±0.74	2.50±1.14	42.21±10.82	44.37±9.35
		Group 3: No exercises	5.90±0.71	5.65±0.74	37.87±10.03	40.85±11.54
Krøll et al., 2018	Bike/cross-trainer/brisk walking for 45 minutes, three times/week for 3 months with RPE: 14-16 points.	Controls continued usual daily activities	5.6(1.8) *	4.5(2.4) *	37.5(14.5) *	59.5(18.3) *
			5.7(1.8)	5.1(2.1)	40.0(14.8)	56.0(19.6)
Santiago et al., 2014	Outdoor fast walking for 40 minutes with a frequency of 3 times/week for 12 consecutive weeks with Amitriptyline (25 mg/day	Amitriptyline (25 mg/day)	8±8.38*	3±2.16*	--	--
			10±8.68	5±3.18		
Varkey et al., 2011	Indoor cycling 40 min 3 times/week, for 3 months with RPE 14-16 points.	Relaxation group: breathing, stress management 5-20 min. Topiramate group: 25mg every week till max dosage to 200mg/day.	50*	–8.8±3.2*	60*	5.7±1.9*
			39	-6.2±3.2	58	3.4±1.9
			40	-14.5±3.2	60	1.9±1.9
Andersen et al., 2011	Resistance training with elastic tubing at 2 min and 12 min/ day, 5 times a week for 10 weeks.	Health related information	3.8* (2 min)	-0.42*	--	--
			4.2* (12min)	–0.05*		
			3.6 (C)	-0.18		

*experimental group, (C)- Control group, QoL: Quality of life

2 Materials and Methods

2.1 Search strategy

To organize the search strategy, a PICO format was used (Table 1 - PICO - Evidence-Based Medicine, 2016). Three electronic databases were analyzed to identify eligible studies: PubMed, Cochrane, and Medline databases from the period of 2010 to February 2020. An additional search of grey literature for this study was not performed. The search terms used were aerobic exercise and tension-type headaches, migraine, cluster headaches, pain, and quality of life. The search strategy identified five articles that considered the effect of aerobic exercise on primary headaches.

3 Results

3.1 Study selection

The study yielded 79 studies in PubMed, 29 studies in Cochrane, and 21 in Medline concerning primary and secondary headaches. There were no duplications in the articles found. Of the 129 studies, 118 articles were excluded because 13 articles were abstracts though few were on primary headaches, 21 articles had inclusion as massage, manipulation in the intervention so was excluded and 84 articles were on secondary headache like stroke, brain injury, etc.; and only 11 studies were retrieved and screened on full text. After screening on full text, five studies were found eligible and were included in this study (Figure 1).

3.2 Study characteristics

The studies included were all RCTs, except for one. Studies included subjects with headaches like migraine, cluster, and tension-type. Of the 5 studies included, one was on combined headache (migraine and tension-type), three on migraine headache, and one on tension-type headaches. A total number of 479 subjects participated in these studies with 198 subjects with headache, 221 with migraine type, and 60 with tension-type headache. The mean age of all included subjects was 40 years. The average baseline headache duration of tension-type not specified (Sertel et al., 2017), for that of migraine type is 11.7 days (Varkey et al., 2011; Santiago et al., 2014; Krøll et al., 2018) and 5.6% for combined headache (Andersen et al., 2011).

3.3 Synthesis of the results

For each study, a summary of characteristics of participants, design, intervention, control, and outcome is presented in Table2 (Characteristics of studies included and outcome measure) and Table 3 (Interventions of experimental and control groups with results of included studies).

3.4 Intervention

Types of aerobic exercises used in the studies were screened and analyzed. One study used a step-dance board accompanied by music for 30 minutes with 5 minutes of warm-up and cool down. The intensity corresponds to 13-14 points of 6-20 point BORG scale (Sertel et al., 2017) one a combination of indoor or outdoor bike, cross trainer or brisk walking and exercise with the trained physiotherapist for 30 minutes with 10 minutes of warm-up and 5 minutes of cool down. The intensity corresponds to 14-16 points on the BORG scale (Krøll et al., 2018). Two, outdoor fast walking for 40 minutes (Santiago et al., 2014).

Third, indoor cycling for 20 minutes with 15 minutes of warm-up and 5 minutes of cool down. The intensitycorresponds to RPE scale 14-16 (Varkey et al., 2011). Fourth, training with elastic tubing either for 2 minutes or 12 minutes per day without any warm-up or cool down (Andersen et al., 2011). Table 3 summarizes the type and intensity of intervention of all included studies.

3.5 Control

All 5 studies had a control group. One study had 2 control groups one with a body awareness therapy (BAT) program that included relaxation, motion, and massage and one without intervention (Sertel et al., 2017). In other studies, patients with migraine included in the control group received no intervention (Krøll et al., 2018), treatment based on medication amitriptyline (Santiago et al., 2014). Further, one study had 2 control groups, among these, one with a relaxation exercise and another one is a treatment based on topiramate medication (Varkey et al., 2011). Other studies had treatment based on health information (Andersen et al., 2011). Table 3 summarizes the interventions of control.

3.6 Effect of aerobic exercise on Pain Intensity and Quality of Life

Among the selected five studies, four studies have reported a reduction of pain intensity following aerobic exercise.In all the analyzed studies, only pain intensity was considered whereas the duration and frequency are not studied.

In one of the study, pain intensity as measured by visual analogy scale was reduced from 5.90±0.71 to 5.65±0.74, and the pain disability index reduced from 27.10±9.97 to 26.10±10.39 compared to control groups (Sertel et al., 2017). In another study following the intervention, the pain intensity reduced from 4.5±1.4 to 3.9±1.8 (Krøll et al., 2018). With medication amitriptyline as control, the pain intensity in the aerobic exercise group reduced from 10±8.68 to 5±3.18 (Santiago et al., 2014), with topiramate as one of the control, the pain intensity in experimental as measured by visual analogy scale (point 1-100) reduced from 50% to 8.8±3.2 at last month of treatment (Varkey et al., 2011). In another study, the pain intensity remained unchanged following exercise (Andersen et al., 2011). Of the five studies, only 3 studies have reported about quality of life and its result following aerobic exercise. In a study, quality of life as assessed using SF 36 showed improvement from 36.32±6.24 to 44.16±9.69 (Sertel et al., 2017), in another study psychological wellbeing improved from 37.5±14.5 to 59.5±18.3 (Krøll et al., 2018).

In the second study, the quality of life as measured by the migraine-specific quality of life improved from 60% to 5.7±1.9 but the improvement was considerably less when compared to medication (Varkey et al., 2011).

Table 4 summarizes the effect of aerobic exercise on pain and quality of life with pre and post values of tension-type, migraine, and combined headaches (migraine and tension-type).

In another study, the pain intensity remained unchanged following exercise (Andersen et al., 2011).Of the five studies, only 3 studies have reported about quality of life and its result following aerobic exercise. In a study, quality of life as assessed using SF 36 showed improvement from 36.32±6.24 to 44.16±9.69 (Sertel et al., 2017), in another

In another study, the pain intensity remained unchanged following exercise (Andersen et al., 2011).Of the five studies,

4 Discussion

According to the Cleveland clinic, headache resulting in pain may be managed with pharmacological drugs. Medications can be classified into three types: symptomatic relief, which relieves symptoms associated with headache, or abortive therapy that stops the process that causes a headache, or preventive therapy that prevents headache itself. The common and major side effects of these medications are sleepiness, fatigue, nausea, difficulty thinking, dizziness, muscle weakness, and cramps (Boyles, 2003). Due to its greater side effects, a non-pharmacological intervention is required to treat

the symptoms to prevent the consequences and impairment. Hence physical activity and exercise are important to prevent headache disorder symptoms.

This study aimed to analyze the effect of aerobic exercise in subjects with a primary headache on pain intensity and quality of life. From the review, it is clear that only a few articles are available, that is only one study on combined headache (migraine and tension-type), one study on tension-type headache, and three studies on migraine-type headaches. Though the inclusion criteria were primary headaches and their subtypes, it ensured a homogenous group but on the other hand, the control groups were not homogenous because studies control had medication like amitriptyline (Santiago et al., 2014), topiramate (Varkey et al., 2011) and few had relaxation exercises like massage (Sertel et al., 2017), breathing exercise (Varkey et al., 2011) as control.

Based on the available literature review, there is moderate evidence that aerobic exercise can decrease pain intensity and low-level evidence that it can improve quality of life. This result is similar to the conclusion of Daenen et al.(2015). The moderate to low-level evidence could be due to a smaller number of studies. Aerobic exercise protocol as analyzed includes step-dance board (Sertel et al., 2017), a combination of indoor or outdoor bike, cross trainer or brisk walking (Krøll et al., 2018), outdoor fast walking (Santiago et al., 2014), indoor cycling (Varkey et al., 2011) and resistance training with elastic tubing (Andersen et al., 2011), indicates that there is no specific protocol being established.

The moderate to the low level of evidence may also be due to dropouts in the studies. The reasons for dropouts both in the experimental and control group being transportation difficulty (Sertel et al., 2017), family issues (Sertel et al., 2017), lack of time and energy, no explanation (Varkey et al., 2011; Sertel et al., 2017), pregnancy (Sertel et al., 2017), illness (Sertel et al., 2017), pain following test (Sertel et al., 2017), dissatisfied with treatment (Varkey et al., 2011; Sertel et al., 2017), started other treatment (Krøll et al., 2018), side effects due to drugs (Santiago et al., 2014; Varkey et al., 2011), noncompliance (Varkey et al., 2011), lost follow-up (Andersen et al., 2011). Most of these studies had 3 months (Varkey et al., 2011; Santiago et al., 2014; Sertel et al., 2017; Krøll et al., 2018), as well as 6 months (Varkey et al., 2011; Sertel et al., 2017; Krøll et al., 2018), follow up.

Cluster headaches on the other side were not studied for aerobic exercises. This could be because the most well-known treatment is 100% oxygen therapy. This technique is considered a level A recommendation and is unique to cluster headaches. The nil evidence for aerobic exercise can be because around 25% of patients who experiences cluster headache will never have another one. This condition as such can resolve in 15 years (Kandel & Mandiga, 2020). But these patients if left undiagnosed can experience detriments in their mental health, physical burdens, and loss of quality of life. It is also found many patients have suicidal ideation (Hoffman & May, 2018). Hence further studies are needed to check whether aerobic exercise can alter or resolve symptoms and reduce cognitive issues. Large population-based studies following experimental analyses concluded that if the physical activity is reduced or less, it is more often associated with higher or increased prevalence and frequency of migraine and other headaches (Queiro et al., 2009). Adolescent men with lower cardiovascular fitness are estimated to be at higher risk of developing prescription pharmacological drugs for migraine symptoms (Nyberg et al., 2019). Although participation in regular exercise is routinely recommended as a means of managing and preventing headache, the pieces of evidence for this recommendation or specific protocol to prescribe aerobic exercise is well less defined.

The regular practice of aerobic exercise seems to benefit patients with headache. These benefits could be majorly due to changes in nitric oxide levels (Narin et al., 2003), increased production of beta-endorphins especially in migraineurs headaches (Koseoglu et al., 2003), but these controlled studies do not define the optimal frequency and intensity of exercise. Despite the positive results for decreased pain, frequency, duration, frequency, and improvement in the quality of life, there are only a few studies about the real influence of aerobic type exercise on other clinical consequences of headache.Though it is well known that primary headache either combined or isolated subtypes are all neurovascular, neuroinflammatory disorders, beyond neurological and physiological pathways, improvements in aerobic fitness and symptoms are associated with overlapping changes in psychological, behavioral, and socio-cognitive factors which must be considered. It is important both for clinical and a public health perspective to identify brain activity, hormone levels over time for future considerations.

Conclusion

The availability of data on the importance and impact of aerobic exercise on pain and quality of life in primary headaches though is limited, further research is needed to estimate the definitive protocol. From these evidences, we can conclude that aerobic exercises are the best option for reducing pain and improving quality of life in primary headaches, especially for tension-type and migraine-type headaches.

Conflict of Interest: No conflicts exist.

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BRAIN COMPUTER INTERFACE (BCI) ON ATTENTION: A SCOPING REVIEW

Anita Prem*, K Mohanraj, A Rajan Samuel

Vinayaka Mission's College of Physiotherapy, Vinayaka Mission's Research Foundation (Deemed to be University), Salem-636308, Tamilnadu, India

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ABSTRACT

Technological innovations are now an integral part of healthcare. Brain-computer interface (BCI) is a novel technological intervention system that is useful in restoring function to people disabled by neurological disorders such as attention deficit hyperactivity disorder (ADHD), amyotrophic lateral sclerosis (ALS), cerebral palsy, stroke, or spinal cord injury. This paper surveys the literature concerning the effectiveness of BCI on attention in subjects under various conditions. The findings of this scoping review are that studies have been made on ADHD, ALS, ASD subjects, and subjects recovering from brain and spinal cord injuries. BCI based neurofeedback training is seen to be effective in improving attention in these subjects. Some studies have also been made on healthy subjects.BCI based neurofeedback training promises neurocognitive improvement and EEG changes in the elderly. Different cognitive assessments have been tried on healthy adults. From this review, it is evident that hardly any research has been done on using BCI for enhancing attention in post-stroke subjects. So there arises the necessity for making a study on the effects of BCI based attention training in post-stroke subjects, as attention is the key for learning motor skills that get impaired following a stroke. Currently, many researches are underway to determine the effects of a BCI based training program for the enhancement of attention in post-stroke subjects.

* Corresponding author

E-mail: anitapremphysio@gmail.com **(**Anita Prem**)**

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1 Introduction

In the world today, technology is inevitable in every aspect of life. Healthcare is an important industry in which technology plays a crucial role. Technological innovations are indispensable for treating disorders and sustaining health. This paper surveys studies on how brain-computer interface (BCI) has made inroads into cognitive rehabilitation, specifically in improving attention. Attention is a cognitive function that selects and acts on a particular stimulus among many other stimuli. It processes sensory stimuli and mental tasks like memory and thoughts (Esghaei & Daliri, 2014; Treder et al., 2014). William James, one of the most influential psychologists asserts that it is the taking possession of one out of several simultaneously possible objects or trains of thought, by the mind in clear and vivid form. He points out that attention is the focalization and concentration of consciousness (James,1891).

Sustained attention is the capacity to concentrate on a particular task for a long period without being distracted. Concentrating on reading or writing is an example. Improvement in attentional processing and reduction of reaction time to the target stimuli are the result of the training of sustained attention such as meditation (Lutz et al., 2009). Alternative attention indicates flexibility in mental activity. An example is reading instructions (learning task) and fixing an instrument according to the instructions (execution task). In other words, it is the alternating of concentration between two different cognitive tasks. An increase in mental fatigue is associated with impairment in alternative attention (Kawatani et al., 2011; Mizuno et al., 2011). The ability to select a particular stimulus among various stimuli is called selective attention. Recognizing a particular voice in a noisy surrounding is an example of selective attention. While selecting a particular signal among different signals, the reaction time and place of the target stimulus may be better predicted, and this improves the judgment of the task (Spence & Driver, 1997; Spence et al., 2001). Divided attention is the performance of two or more tasks simultaneously, so that attention is divided among these tasks. Attentional and processing resources are assigned differently between dual tasks in divided attention (Shapiro et al., 2006).

In the field of neurological rehabilitation, evaluating and managing cognitive function is an ingenious and hardly explored area. Attention Process Training (APT) method has been studied and found to be a valuable intervention method for patients with attention-deficit. However, this method has certain limitations like limited generalizability to wider samples, chance findings, and influenced outcomes.

The use of Brain-Computer Interface (BCI) to enhance motor and cognitive recovery is a novel approach. The use of BCI has certain advantages of brain activation monitoring, in particular, the monitoring of the global level of attention concerning a task, and the level of interhemispheric balance (Carelli et al., 2017). The need arises for the study on the use of BCIs for cognitive training to improve the patients' clinical conditions. Further, the treatment may be made more efficacious by providing real-time feedback, which enhances neural plasticity. The use of BCIs to improve attention has not been much explored. Some reviews discuss the current status of BCI interventions in varied uses. Studies in this area include BCIs using auditory, tactile, and motor imagery-based designs (Naci et al., 2012; Muller-Putz et al., 2013). BCI is a non-invasive biofeedback system that can be used in training for cognition, emotion function, and motor learning (Daly & Huggins, 2015). In BCI intervention, functional magnetic resonance imaging (fMRI), Functional near-infrared spectroscopy (fNIRS) and electroencephalography (EEG) can be used to restore some communicative ability in these patients (Luaute et al., 2015).

Among the non-invasive methods available for recording brain activity, EEG and fNIRS seem to be the best potential candidates for usage in post-brain injury rehabilitation (Dokkum et al., 2015). BCI in conjunction with a 3D virtual environment is found to be effective in the treatment of post-stroke patients (Denis et al., 2007). The combination of BCI-MI plus orthosis with physical training may help to improve upper limb motor control post-stroke (Ramos-Murguialday et al., 2013).

Improving attention in patients with disorders of attention using BCI is a relatively new approach. BCIs use mathematical algorithms to decipher nerve impulses. Patients can monitor the activity of their brain at the time of occurrence and regulate it by themselves. This is called "neuro bio-feedback therapy". BCIs commonly utilize EEG recordings, magnetoencephalography (MEG), near infra-red spectroscopy (NIRS), functional magnetic resonance imaging (fMRI), electrocorticography (ECoG), and multi-electrode intracranial implants (Mehdi et al., 2016). Meta-analyses and randomized controlled trials conducted on ADHD have shown that three standard neurofeedback training protocols are specific – theta/beta (TBR), sensorimotor rhythm (SMR), and slow cortical potential (SCP) (Enriquez-Geppert et al., 2019). A meta-analysis with 10 randomized controlled trials shows that the effects of neurofeedback in ADHD children increase in time, while the medication effects diminish with time, thus suggesting the long-term efficacy of neurofeedback (Van Doren et al., 2018). Some studies have been done on real time (RT) brainwave based neurofeedback for improving attention in older adults combating cognitive decline and brain aging (Jiang et al., 2017).

We here present a scoping review about BCI applications dealing with attention issues, in both healthy individuals and subjects with various cognitive impairments such as attention deficit hyperactivity disorder (ADHD), amyotrophic lateral sclerosis (ALS), autistic spectrum disorder (ASD), post stroke disabilities, cognitive deficit following brain and spinal cord injuries, and dementia.

Table 1 BCI on Attention Deficit Hyperactivity Disorder (ADHD)

Study	Paradigm/ Signals	Sample & Method	Tested Variables	Outcome Measure	Limitations
Lim et al. (2012)	Frontal EEG Fp1 & Fp2	20 ADHD unmedicated children (16M &74F) 6-12yrs. 3D graphic game (CogoLand)	Inattention symptoms (accuracy& execution time), hyperactive impulsive symptoms.	ADHD-Rating Scale based on the DSM-IV criteria	Uncontrolled open-label trial could have resulted in an exaggerated treatment effect.
Munoz et al. (2015)	Neuromodulation of Beta & Theta waves	Children with ADHD 8-13 yrs. without depression/ comorbidity. Mindwave neuro-headset. Videogames	Waiting ability, Planning ability, Ability to follow instructions & to achieve objectives.	E-sense attention meter (Mindwave), Theta/Beta ratio.	
Qian et al. (2018)	Frontal EEG Fp1 & Fp2	51 boys (ADHD) -- Intervention group (33) & Control group (18) 3 BCI based training sessions per week for 8 weeks, 3D graphic game (CogoLand)	Inattention & Internalizing symptoms	Resting-state functional magnetic resonance imaging method, DHD-RS clinician inattention scores, Child behaviour checklist	Physiological noise in the functional magnetic resonance imaging (fMRI) pre-processing techniques. Clinical hetero geneity of ADHD not taken into account.
Lim et al. (2019)	EEG waves Fp1 & Fp2	172 children with combined subtypes of ADHD aged 6 to12. BCI training -3 sessions / 8 weeks. 3 follow-up training sessions / month - 12 weeks	Clinician-rated inattention symptoms, Short term efficacy, maintenance effect and long-term effect.	ADHD rating scale (ADHD-RS), Child behaviour checklist (CBCL) &Paediatrics adverse events rating scale (PAERS).	The trial results cannot be generalized as 90% of the participants were males. Possibility of response bias

2 Materials and Methods

From February to April 2020, we searched the databases PubMed, Web of Science, and Scopus, using the search phrase "brain computer interface in attention". Since attention disorders come under a cognitive deficit, the terms "BCI or brain computer interface on cognition" were also included in the search. As BCI is a method of neurofeedback training, we later included the phrase "Neurofeedback training on attention using BCI". Most of the studies selected for this review are on training attention using BCI, but a few studies on evaluating attention while performing another motor task have also been included. A total number of 618 studies have been found with PubMed, 2282 studies with Web of Science, and 2313 studies with Scopus, using the above terms.

We filtered the search down to 23 researches relevant to this review, which are included in Tables 1-5. The study samples included in this review cover both subjects affected by cognitive impairments and healthy subjects without any such cognitive deficit. Only studies in English have been considered in this review.

3 Results and Discussion

3.1 BCI in Attention Deficit Hyperactivity Disorder (ADHD)

Brain-computer interface has been experimented with as a neurofeedback therapy for ADHD. A BCI-based 3D game for attention training and rehabilitation was devised specifically for

ADHD subjects (Jiang et al., 2011). In this approach, BCI technology is used to translate the user's mental state of attention into game control. When the user controls a virtual hand's movement in a 3D animation technique, the BCI engine measures his/her attention level. This system is found to be more economical, engaging, and easier to use than the robotic based system. It can also be used in the treatment of patients suffering from neurological disorders caused by trauma.

A group of researchers evaluated a BCI based attention training program for treating ADHD, which included dry sensors and blue tooth technology in place of EEG leads with a game CogoLand (Lim et al., 2012). The training was given for eight weeks in 24 sessions. A follow-up of booster training sessions was given for three months. After the intervention, the parents rated the significant improvement in inattentive and hyperactive-impulsive symptoms in the ADHD subjects. Children, who have inattentive and combined subtypes, are found to benefit from the treatment more generally than the hyperactive-impulsive subtypes. Studies on neuro-imaging have shown that the brain functions responsible for selective attention and response inhibition in children with ADHD may be functionally normalized by neurofeedback therapy (Beauregard & Levesque, 2006). The limitation of this study is an uncontrolled open-label trial, the parents who completed the behavioral rating scale were not blinded and the children's school teachers presented a high non-response rate.

A videogame was developed using a BCI to keep track of neurophysiologic signals (Munoz et al., 2015). The name of the game was "The Harvest Challenge" and the setting was a coffee plantation. The games using an avatar were meant to boister up the waiting and planning abilities and the abilities to follow instructions and to achieve objectives. When these abilities are improved, inattention impulsiveness can be brought under control. The modulation of Beta and Theta waves, with the help of an electrode placed in the central part of the frontal lobe of the brain, was used for training sustained attention in children with ADHD.

An experiment with a BCI based intervention was conducted to re-normalize brain functional network topology in children with ADHD (Qian et al., 2018). The researchers used the resting-state fMRI method to examine the changes in the topology of brain functional networks. After training, it was found that the inattention symptoms of the intervention group had greatly reduced, compared to the non-intervention group. The BCI-based intervention can help to bring back to normalcy the topology of brain functional network associated with improvement in behaviour, and also expedite brain maturation in children with ADHD (Qian et al., 2018). The limitation of this study is that the ADHD subtypes, which may have a unique response of brain networks, are which not taken into consideration, due to the limited sample size.

A randomized controlled trial (RCT) was carried out to find out whether inattentive symptoms in children with ADHD could be improved by administering a BCI based attention training programme for 8 weeks. A lower-intensity training was also given in the subsequent 12 weeks (Lim et al., 2019). The inattentive symptoms of ADHD showed significant improvement after the programme, when compared to the untreated wait-listed control group. Earlier studies across different populations have shown that ADHD subjects experience significant anxiety and mood symptoms which can increase their impairment (Jensen et al., 2001; Xia et al., 2015). This BCI-based attention training programme alleviated these internalizing symptoms in the subjects under study. This is important because, if ADHD children develop anxiety symptoms during treatment, there is likely to be a poorer outcome (Sciberras et al., 2014). As almost 90% of the participants in this study were males, the results of the trial cannot be generalized. Also, there is a possibility of response bias among the parents who participated.

3.2 BCI in Amyotrophic Lateral Sclerosis (ALS)

Studies on BCIs controlled by people with ALS have been conducted (Sciberras et al., 2014; Sellers et al., 2006; Hoffmann et al., 2008). A single-case report has demonstrated the possibility of long-term independent home use for severely disabled people with ALS (Sellers et al., 2010).

Home based BCI use has made a significant contribution to the quality of life and productivity of the user. Some researchers investigated how attention and memory processes support the control of a P300-based BCI in people with ALS (Riccio et al., 2013). The study was made on a sample of 8 subjects with ALS. They had to perform two behavioural tasks: a rapid serial visual presentation (RSVP) task, and a change detection task. A P300-based BCI spelling task was also given to the participants. They concluded that the potential to keep the attentional filter active while choosing a target controls the BCI performance.

In a further study, the capacity of a sample of ALS patients to control a P300-based BCI device for attention processing, concerning healthy subjects was investigated (Riccio et al., 2018). 13 ALS subjects and 13 healthy volunteers in the same age group and with the same education were given a P300-speller BCI task and a rapid serial visual presentation (RSVP) task. The RSVP task was meant to assess the temporal attentional filtering capacity and the ability to improve the attentive filter in the periodic regularity of selective attention (Riccio et al., 2018). ALS patients showed changes in both the ability and the timing in the execution of the P300-speller task. The finding was that the lower capacity for ALS to control a P300-speller was related to the ability to filter a target stimulus temporally from among a crowd of stimuli. A central issue of BCI research is to develop assistive technology (AT)

Table 2 BCI on Amyotrophic Lateral Sclerosis (ALS) & Autistic Spectrum Disorder (ASD)

Study	Paradigm/ Signals	Sample & Method	Tested Variables	Outcome Measure	Limitations
Riccio et al. (2013)	Scalp EEG signals Fz, Cz, Pz, Oz, P3, P4, PO7 & PO8	9 volunteers (6 M &3F) mean age 59.7 with ALS diagnosis. Task- controlling a 6 by 6 P300 speller.	Temporal attention capabilities, memory capacity & spatial filtering capacity.	P300 Speller Interface, Rapid Serial Visual Presentation (RSVP) task & Change Detection (CD) task.	
Amaral et al. (2018)	P 300 & alpha power	15 participants aged between 16 & 38 yrs. with ASD. VR interface- 7 sessions in 4 months. Follow up after 6 months – joint attention task	Identification of social attention items, sensory /cognitive awareness & adapted behaviour composite in DLS.	Eye-tracking, Autism Treatment Evaluation Checklist (ATEC) & Vineland Adaptive Behaviour Scale (VABS).	Clinical validation of the primary outcome measure (eye-tracking) is not done. Realistic nature of the VR environment may be improved.
Riccio et al. (2018)	P300 based electro des Fz, Cz, Pz, Oz, P3, P4, PO7 & PO8	13 ALS participants (8 M & 5 F) aged 62-75 & 13 participants (9 M & 4 F) with no disorders (control gp.). 2 sessions BCI session P300 speller BCI task Psychological session – RSVP task	Selective attention, working memory & executive functions.	The computerized test for attentional performance (TAP) & Wisconsin Card Sorting Test (WCST).	Can't be generalized as locked in state (LIS) not included.

devices to restore communication in people with severe motor disabilities (Millán et al., 2010). Attention is a complex territory of cognitive functions (Posner, 1975).There are many ways to measure the attention substrates. Different contexts and different approaches show different results and so a direct comparison is not possible.

3.3 BCI in Autistic Spectrum Disorder (ASD)

A clinical trial was made using a BCI to improve social attention in ASD (Amaral et al., 2018). The authors experimented with an EEG based BCI to train social cognition skills in ASD patients. Fifteen 22-23-year-old ASD subjects underwent the trial and they were made to participate in a BCI training schedule using a virtual reality (VR) interface. The training was spread over seven sessions in 4 months. The task consisted of identifying objects through the gaze direction of an avatar. The EEG P300 component recorded the attentional responses. A follow-up assessment was made after six months. The subjects showed improvement in adapted behaviour composite and their depression level also decreased. The limitation of the study is that the primary outcome measure has not been validated clinically. For training social attention skills more efficiently, the VR environment may be improved.

3.4 BCI on Attention in Stroke

One of the major causes of dysfunction in adults is said to be a stroke, and a large number of training-oriented rehabilitation techniques have been developed. But they have often failed to work successfully on patients with severe deficits.BCI holds promise for such patients by recording and decoding brain activity while trying to perform motor and cognitive tasks. It can instigate movement, provide feedback on motor imagery, and monitor the comprehensive level of attention in performing tasks and the level of inter-hemispheric balance. An important issue in motor learning is the amount of mental workload, or how hard the brain is working to meet task demands (Ayaz, 2012).

Near infra-red spectroscopy (NIRS) measured activity over the prefrontal cortex could discriminate between low and moderate levels of workload (Mandrick et al., 2013a; Mandrick et al., 2013b), with a plateau effect towards higher levels of workload. Further, whatever the task duration may be, NIRS is found to be highly responsive to a decrease in attention. From the results, it may be inferred that it is possible to monitor changes in attention during BCI training. Researchers investigated the use of BCI along with exoskeleton technology to bring about multimodal stimulation in the rehabilitation of stroke subjects (Kotov et al., 2019). The experimental group was given complex multimodal stimulation involving several techniques along with BCI training, while only BCI training was administered to the control group. Subjects in the main group showed better improvement in memory, attention, and other skills, than the subjects in the control group.

Table 3 BCI on Stroke, Brain & Spinal Cord Injuries

Study	Paradigm/ Signals	Sample & Method	Tested Variables	Outcome Measure	Limitations
Salisbury et al. (2016)	14 channel EEG recording (AF3, F7, F3, FC5, T7, P7, O1, O2, P8, T8, FC6, F4, F8, AF4)	Study 1 25 medically stable, spinal cord injury patients without severe cognitive/ psychiatric impairments Cube rotation task – three trials lasting 8 seconds each. Study 2 21subjects with brain injury. Virtual reality environment with BCI – 8 training sessions.	Cognitive flexibility, working memory, complex attentional processes, cognitive & motor inhibition & processing speed.	1.MATLAB scoring program 2. The Automated Neuropsychological Assessment Metrics Stroop & Go-No-Go computerized tests, Woodcock-Johnson 3rd Edition Pair Cancellation subtest, &Delis-Kaplan Executive Functions System Colour-Word Interference test.	Excessive reliance on technology & financial feasibility.
Kotov et al. (2019)	EEG Signals	44 patients of 2 groups-- The main gp. (22) given a program of complex multimodal stimulation, using BCI technology. The comparison group (22) had only training with the use of BCI.	Memory, attention, visual and constructive skills.		
Laiz et al. (2018)	16-channel biomedical signal amplifier with g.tec USB connection (Austria)	32 subjects 22 in the first group of 61-69 years, and 10 in the second group of 70-81 years. 5 neurofeedback training sessions alternated with 5 work memory training sessions for 5 weeks,	Visuo-spatial, spoken language, memory, intellectual processes & attention.	Luria DNA neuropsychological battery.	The sample size is small. Further recommendation of this study would be inclusion of a control group
Martin et al. (2018)	P300 control matrix	5 participants (4 M & 1 F) with traumatic brain injury(TBI) as end users & 5 participants without TBI as control group (1 M & 4 F)	Memory, semantic and reasoning skills, language, and learning, attention& concentration.	Evaluation questions	The small sample size. Since based on prototype development, the study lacks evidence on effectiveness.

3.5 BCI in Brain Injury & Spinal Cord Injury

Two original studies were made to show how virtual reality and BCI may be integrated into neurorehabilitation (Salisbury et al., 2016). The first study investigated the feasibility of BCI with inpatient spinal cord injury. For this, 25 medically stable patients with sustained cervical-level (48%) or thoracic-level (44%) SCIs and residual tetraplegia (52%), without severe cognitive or psychiatric impairment took part in the first study. The second study was conducted to explore the effect of two virtual environments on subjects with central nervous system insult. The participants were subjects with acquired traumatic brain injury, stroke, brain neoplasm, and anoxic injury. These studies gave a positive outcome regarding advanced technologies in the subacute stage of neurorehabilitation, concerning cognitive functions. The patients found the technology user-friendly and there was a remarkable improvement in their performance in the course of the sessions.

An attempt was made to design and validate a BCI for cognitive rehabilitation in brain-injured subjects based on a user-centered approach (Martin et al., 2018). The subjects with limited functional abilities were made to control computers through their brain waves with the help of the BCI. A research was conducted among community dwellers recovering from brain injury, with a group of therapists who worked together towards the rehabilitation of cognitive functions. The participants performed two tasks namely Find-a-Category and a Memory Card task. A home interface presented the therapy activities. This work was achieved by a group of academics, business partners, and service users.

Table 4 BCI on Healthy Elderly

Study	Paradigm/ Signals	Sample & Method	Tested Variables	Outcome Measure	Limitations
Lee et al. (2013)	Frontal EEG Fp1 & Fp2	31 healthy elderly 60-70yrs. Intervention gp.(15) Control gp. (16). Card-pairing memory game with BCI, 24 sessions each 30 mins.over 8 weeks; control gp. Week 9 to 16	Immediate & delayed memory, language, attention, visuospatial/ construction.	Usability & acceptability questionnaire Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) scores.	Covers only a small subset of English literate elderly. Concerns about RBANS. Failure to avoid placebo effect.
Lee et al. (2015)	Frontal EEG Fp1 & Fp2	39 Elderly of Chinese ethnicity 60-70yrs. 21 subjects in Intervention gp& 18 in control gp 24 half-hour sessions of BCI/ 8 weeks.	Immediate & delayed memory, language, attention, visuospatial/ construction.	Usability & acceptability questionnaire RBANS scores.	Translated RBANS forms not validated.
Gomez-Pilar et al. (2016)	Event-related desynchronization (ERD) and event related synchronization (ERS) of alpha and beta frequency bands.	63 subjects (older than 60 years) with 31 subjects (13M&18F) in neurofeedback (NFT) group & 32(9M&23F) in control group. NFT tasks during 5 sessions- imagine to lead the cursor to the correct target. With progression of difficulty level	Visuospatial skills, linguistic skills, memory, intellectual functions and attention.	Luria adult neuropsychological diagnosis	It is desirable to extend the population under study for statistical reasons. Failure to make a follow-up of the cognitive improvement
Yeo et al. (2018)	EEG Signals	Participants 60-80 yrs., with no neuro psychiatric diagnosis. 24 sessions in eight weeks and booster sessions for 3 months. BRAINMEM with game components	Attention, working memory &delayed recall.	Repeatable Battery for the Assessment of Neuro psychological Status (RBANS).	The sex moderation effect not studied

3.6 BCI in Dementia (mild)

Dementia is a disease that evolves in the elderly, with varying degrees, affecting the daily life of the people suffering from it. A group of researchers attempted to assess whether cognitive training through a BCI will be effective in such subjects (Laiz et al., 2018). Total 32 subjects took part in the study, among these 22 were in the first group of 61-69 years, and 10 were in the second group of 70-81 years. The Luria-DNA neuropsychological battery was used to evaluate the subjects before and after training. The cognitive areas evaluated were visuospatial and linguistic skills, memory, intellectual functions, and attention. After training, compared to the second group, the first group showed significant improvement in all these aspects. The conclusion is that a suitable cognitive programme with BCI may delay cognitive impairment.

Table 5 BCI on Healthy Adults

Study	Paradigm/Signals	Sample & Method	Tested Variables	Outcome Measure	Limitations
Rohani & Puthusserypady (2015)	Unipolar reference electrode at the left earlobe, a ground electrode at Fpz, an EOG electrode below left eye, & a measurement electrode at Pz.	6 healthy young subjects (5m & 1f) aged 24-32. 2 oddball attention experiments ANISPELL & T-SEARCH inside the VR classroom. 5 trials each.	Sustained visual attention and visual discrimination	Non-linear Support Vector Machine (SVM) Classifier to detect the P300 potential	.P300 is likely to change over time due to task adaptation.
Aliakbaryhosseinabadi et al. (2017)	Monopolar EEG signals from 18 channels AF3, AFz, AF4, F3, F1, Fz, F2, F4, 27 FC3, FC1, FCz, FC2, FC4, C3, C1, Cz, C2, C4	12 healthy participants (6M & 6F), mean age 24.25. Two tasks - The control with normal attention demand and the complex secondary task with diversion attention level	attention variations during movement execution	MATLAB to extract the time domain feature & EEGLAB to extract the time frequency features	.The combination of movement and attention made it impossible to ignore the influence of dual tasking.
Pei et al. (2018)	Alpha power density, theta & beta	20 healthy volunteer subjects with normal vision, 2 groups neurofeedback (NF) group(10) – alpha frequency neurofeedback Sham NF gp. (10) – random 4Hz neurofeedback 5 sessions on different days for 5-7 days. . Resting EEG recorded before & after each session	Episodic memory, working memory & attention network processes.	Word pair task, attention network test & backward digit span task.	Lack of auditory feedback is a limitation.
Firat et al. (2018)	EEG Signals	Seventy healthy participants. Six tasks using a Brain-Computer Interface system	Cognitive state cluster and task performance. Pupil dilation, blink rate, and Galvanic Skin Response (GSR) data.	Nasa-Task Load Index, Logistic Regression, Decision Tree, and Neural Networks	
Arvaneh et al. (2019)	P 300 based speller BCI	28 healthy young adult participants, (12 M& 16 F) aged 20 to 39 yrs. Randomly assigned to exp gp. (14) & control gp. (14). One session for 1hour & 45 mins. P300-based speller task. Questionnaire at the start& end of the session.	Changes in EEG during and pre-post training. Changes in cognitive performance	Response time of spatial attention task.	Long-term effects not investigated. Not applied to those at the risk of attention problems like the elderly.
Chuanqi et al. (2019)	EEG optical flow	10 subjects listening to and imagining 12 well-known short musical pieces	Classification accuracy on the Open Music Imagery Information Retrieval (MIIR) dataset	Open Music Imagery Information Retrieval Open MIIR dataset	
Karran et al. (2019)	32 electrode EEG montage	30 healthy female participants from Business School age 18-43. Participants split into 3 groups: no countermeasures (NCM), continuous countermeasures (CCM) & event-synchronized, level-dependent countermeasures (ECM). 15 min calibration task & 90 min business logistics task	Mental demand, physical demand, temporal demand, taskperformance, frustration level & effort.	Short version of the NASA-Task Load Index, the RAW-Task Load Index.	Functional near-infrared spectroscopy (fNIRS) did not prove a useful tool with high levels of discomfort—problematic for ecologically valid tasks in real-world scenarios.
Gaume et al. (2019)	Electro des on frontal, parietal and occipital regions – Fp1, Fp2, F7, F3, F4, F8, C3, C4, CP5, CP1, CP2, CP6, P3, P4, O1 & O2.	14 healthy subjects (11m & 3f) age 19-32. Motor control of a cursor using a joystick.	Mental, physical, temporal demand, performance, effort & frustration	NASA Task Load Index	Insufficient to prevent modelling confounders of attentional load.

3.7 BCI on Attention in Healthy Elderly

Cognitive decline in aging is a prevailing issue which calls for interventions for improvement in attention. Some studies have taken up this demand and applied BCI based interventions in the elderly. An experiment was carried out with an EEG based BCI training programme which combined the positive effects of traditional computerized training interventions with neurofeedback training (NFT) (Lee et al.,2013). An eight-week training made up of 24 sessions of half an hour each was given. A questionnaire on the users' experience and feedback was administered at the end. After every session, the subjects had to report adverse events, if any. The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) recorded a change of total score pre- and post training. This was evidence for the efficacy of the system. Positive results were found from the questionnaire. The participants did not complain of any adverse events. The visuospatial/constructional attention showed significant improvement. This BCI based intervention system has the potentiality to improve memory and attention in healthy elderly. Senior users find it to be safe, handy and easy to use. However, another trial with participants having a mild cognitive impairment and early dementia is warranted.

The researchers repeated the study on a Chinese, healthy, elderly population. The research was conducted to find out whether cultural and linguistic factors affected cognitive training (Lee et al., 2015). They examined the efficacy of their BCI cognitive training programme in healthy Chinese-speaking Singaporean elderly. The same protocol as in the previous experiment was followed, and the control group received the same intervention after an 8-week waiting period. A questionnaire on the users' experience and feedback was given at the end.

For safety, after each session, users were questioned whether they experienced any discomfort. The users reported a total of 16 adverse events, but they were graded "mild". Only one was considered "moderate". The researchers have shown that cultural and linguistic factors did not influence BCI cognitive training.

A study was conducted to find out whether neurocognitive improvements and EEG changes can be brought about in the elderly through neurofeedback training with a motor imagery-based BCI (Gomez-Pilar et al., 2016). The neuropsychological test scores of both the experimental group and control group were compared and after five NFT sessions, the results showed significant improvements in the NFT group, in the four cognitive functions, visuospatial, oral language, memory, and attention. This experiment proved that NFT performed by a Motor Imagery-BCI enhanced cognitive functions.

A randomized controlled trial was carried out to examine the effectiveness of a personalized BCI system for cognitive training in the elderly (Yeo et al., 2018). The subjects were 60–80 years old, without any neuropsychiatric issues. The subjects were divided into intervention and waitlist-control groups. The training system BRAINMEM was assigned to them. It has game components designed to improve attention, working memory, and delayed recall. 24 sessions were conducted over eight weeks followed by booster sessions once per month for three months. The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) was used to measure the outcome. The experimental group showed better performance than the waitlist group among men. Among the females, the between-group difference in improvement was not significant. The results of the study showed that a neurofeedback intervention is likely to be feasible in cognitive rehabilitation in the male elderly.

3.8 BCI on Attention in Healthy Adults

BCI can be a prospective instrument for training attention inside a VR classroom and this was investigated by some researchers (Rohani & Puthusserypady, 2015). They created a training environment using a low-cost infra-red camera making up the illusion of 3D. Six healthy young subjects aged 24-32 participated in the experiment. On the scalp region above the parietal lobe of each participant, a single electrode was placed to elicit the P300 potential. This was found to be a successful demonstration of a non-intrusive, portable low-cost system targeting attention in an engaging environment. The study was conducted on healthy subjects, but it suggests a positive effect on ADHD subjects also.

An attempt was made, using 12 healthy adults of mean age 24, to classify EEG signals to identify attentional variations while carrying out motor tasks (Aliakbaryhosseinabadi et al., 2017). The motor task was a simple ankle dorsiflexion movement during which an auditory oddball task was applied to divert the users' attention. The researchers analysed every participant's attention levels and their effects on motor tasks. Motor cortex channels showed higher accuracy than other channels. The conclusion was that synchronous BCI systems with time-frequency feature may be employed to assess attention variation. The limitation is that the combination of movement and attention made it impossible to ignore the influence of dual tasking.

Some researchers made cognition assessments using an integrated neurofeedback system with dry electrodes (Pei et al., 2018). This integrated system combines BCI technology with a multithreading design and uses wearable, multichannel, dry electrode EEG acquisition equipment. For this, 20 healthy volunteers were divided into an NF (neurofeedback) group & a sham NF group. They had five treatment sessions/week. Compared to the sham NF group, the NF group showed higher alpha frequency band power. Thus, the

NF group performed better in working memory. This integrated system is expected to be an effective assessment system for neurofeedback training and cognitive function and may be used personally and clinically. This system may be updated and improved. According to some studies, a combined visual and auditory feedback modality achieved better BCI performance, enabling participants to focus attention on the task (Gargiulo et al., 2012; Fabien et al., 2013).

A classification of BCI users based on cognition was made (Firat Ozkan & Kahya, 2018). The study was meant to examine cognitive state using BCI systems. Seventy healthy participants were selected to perform six tasks using a BCI system. After each task, the participants filled Nasa-TLX (task load index) forms so that their task performances could be measured. The K-means method was used to create cognitive state clusters from the data collected. From the obtained data, the participants were classified into low or high risk, based on their cognitive state. The processing of classified data was done to evaluate the consistency of this classification. A consistency between 87.1% and 100% with other techniques was observed.

The use of a BCI to modulate the level of sustained attention over a long duration business logistics task has been studied (Karran et al., 2019). Total, 30 healthy female participants from Business School aged between 18 and 43 were selected and allocated to groups of no countermeasures (NOCM), continuous countermeasures (CCM), and event synchronized, level-dependent countermeasures (ECM). The researchers worked under the hypothesis that self-regulating sustained attention through neurofeedback would have the following results: task engagement would become greater; error rate would be decreased, and task performance would be improved. The results proved that self-regulating sustained attention can keep the users engaged for a long time, and moderately enhance task performance while decreasing errors. A group of researchers introduced a cognitive BCI based continuous performance task to monitor variations in visual sustained attention (Gaume et al., 2019). For this, 14 healthy subjects, 11 males & 3 females aged 19-32 were selected for the study. The task involved the use of a joystick to control a cursor. The continuous task enables keeping track of variations of visual sustained attention. Furthermore, the researchers have designed a task in which cognitive functions other than sustained attention are minimally involved. To update the visual information from our sensory inputs continuously, sustained attention is necessary. This was their focus rather than processing of the information stored in working memory.

A P300-based BCI for improving attention was devised by some researchers (Arvaneh et al., 2019). They modified the P300-based speller BCI into an engaging neurofeedback game. They designed the game in such a way that it adapted its difficulty level according to the user's performance, requiring the production of a stronger P300. The neurofeedback training was for only 30 minutes. From the obtained results it was found that the training brought about a remarkable improvement in the Event-Related Potentials (ERP) components of the target trials. A weakening of the corresponding ERP components was observed in the non-target trials. A spatial attention task after the neurofeedback training proved that the response time was significantly improved in the experimental group. From the study, it can be inferred that this neurofeedback training tool is bound to improve attention, specifically for subjects with attention disorders. However, the long term effects of this training remain to be investigated.

A new approach to improve the accuracy of EEG classification in BCI was taken up by some researchers (Chuanqi et al., 2019). They pay attention to the fact that the human brain has different functional areas for different human activities. They propose an attention-based transfer learning framework that includes a cross domain encoder and an attention-based decoder with a recurrent neural network (RNN). They applied this approach to a dataset called Open Music Imagery Information Retrieval (Open MIIR), involving 10 subjects listening to and imagining 12 well-known short musical pieces. They show that brain functional areas associated with new activities may be discovered by using attention mechanisms.

Conclusion

Attention is a fundamental mechanism of the brain, enabling the selection of relevant information and suppression of irrelevant signals. This review has given an overview of the studies in BCI on attention in different neurological disorders as well as healthy adults and elderly. BCI based neuro-feedback training is found to be effective in enhancing attention in ADHD subjects. Researches support that BCI based attention training programme for ADHD is relatively simple to use, convenient and accessible over most other cognitive training programmes utilizing EEG information, and they also suggest that it could be developed into home-based treatment. In ALS subjects, behavioural tasks like visual presentation and change detection, as well as a P300 speller task are effective in improving attention.

In ASD subjects, social cognition is found improved due to BCI intervention. In spinal cord injury patients, BCI integrated with virtual reality was experimented with as neurorehabilitation with a positive outcome. An evaluation after cognitive training through BCI on subjects suffering from early dementia showed that such a cognitive programme may delay cognitive impairment.BCI based neurofeedback training promises neurocognitive improvement and EEG changes in the elderly. Different cognitive assessments have been tried on healthy adults, like classifying EEG signals to identify attentional variations during a motor task, BCI based

neurofeedback therapy with a multithreading design in cognitive assessment, BCI as a potential training tool for attention inside a virtual reality classroom, BCI based continuous performance task to monitor variations in visual sustained attention, and a P300-based BCI. In post-stroke subjects, BCI combined with exoskeleton technology of a multimodal stimulation offered better therapeutic results. More than half of stroke survivors suffer from cognitive defects, which determine broader outcomes than physical disability. Cognitive productivity can be reduced by impaired attention, even when other cognitive functions are intact. Attention is also associated with balance, functional independence, and daily living. Hence attention training using BCI is undoubtedly a useful tool in post-stroke rehabilitation. From this review, it is evident that hardly any research has been done on using BCI for enhancing attention in post-stroke subjects. So there arises the necessity for making a study on the effects of BCI based attention training in post-stroke subjects, as attention is the key for learning motor skills that get impaired following a stroke. Currently, various researches have been under investigation to determine the effects of a BCI based training programme for the enhancement of attention in post-stroke subjects.

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A SYSTEMATIC REVIEW ON THE PREVALENCE OF MENTALLY CHALLENGED ADOLESCENTS IN INDIA AND THE EFFICACY OF DIETARY SUPPLEMENTS WITH HERBS ON COGNITIVE FUNCTION

Manjula J, Karthiga K*

The Research Centre of Home Science, Fatima College (Autonomous), Madurai, India

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Herbs

ABSTRACT

Health is the state of well-being in physical, social, spiritual, and mental health. Apart from physical health, mental health is of prime importance. The brain is one of the most complex organs in the body. The brain has multiple tasking abilities like learn, play, concentrate, remember and it helps in sustaining a clear mind. Brain comprises around 60% fat with omega 3 fatty acid and Docosahexaenoic acid, these two supports the normal neuronal membranes. The modern diet lacking in omega 3 and DHA may harm cognitive development. In the world, India stands second place with a population of 135.26 crores. India comprises one fifth of the adolescent population that is around 27.05% of the total population. According to the American Association on Intellectual and Developmental Disabilities, "Mental retardation is a disability characterized by significant limitations both in intellectual functioning and in adaptive behaviour. Mental health in adolescents is neglected and it increased morbidity and mortality during recent years. Cognitive development in adolescence occurs in three main areas. The three areas are advanced reasoning skills, abstract thinking, and formal operational thinking. The psychiatric disorders in adolescents begin before the age of 14 and nearly 50% of adolescents are affected during this period. The remaining 20% of the adolescent's population are affected by predisposing or precipitating factor. Plants have been used as a medicine based on folk remedies and experience. Due to the immense potential of medicinal plants, the research work was planned to carry out on plant based medicine. Various nutraceuticals and pharmaceutical components have been derived from medicinal plants. Plants such as *Centella asiatica, Bacopa monnieri,* and various herbs are rich source of omega 3, Alpha-linolenic acid, Minerals and Vitamins which helps in cognitive development and work as memory enhancer in human being.

* Corresponding author

E-mail: arjun.maya1@gmail.com (Karthiga K)

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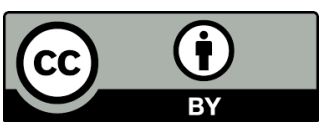

1 Introduction

India is the second-largest population in the world and comprises the largest population of adolescents with 27.05% (Plecher, 2020). Adolescents are individuals between 10 -19 years and they undergo an intervening stage with characteristic changes in social, emotional, cognitive, sexual, and physical development. Many problems in adulthood have their roots in adolescence. Adolescent health needs can be categorized into three types i.e. physical, psychological, and social. The major health issue faced by adolescents in recent years is mental illness. The integral part of health is mental wellness (Sivagurunathan et al., 2015).

The main challenge in mental illness is always a fight between one's change in mind and society. Adolescents will experience different and variety of emotional changes. The prevalence rate of mentally challenged adolescents in India ranges from 10.8% - 13.9% (Hossain & Purohit, 2019). In developing countries like India, there is less awareness about mental illness. In many areas, they are not even identified and managed. Our ancestors have been in close contact with the nature and environment. They have used the plants as the ingredients of food and also as a curative medicine too. Almost, in all cultures, plants have been used as medicinal resources. In the world, medicinal plants and their parts were used as drugs.

These plant and plant parts have many active components. These components act as a curative component for a particular disease (Kia et al., 2017). World Health Organization estimated that 80% of people worldwide depend on herbal medicines for their primary health issues (Tagboto & Townson, 2001). Cognitive development is the framework of remembrance, problem solving skills, decision making from childhood to adulthood (Sternberg, 2003). The present review is focusing on the prevalence of mentally challenged adolescents in India and also on the medicinal herbs that help in cognitive development and used as a memory enhancer.

2 Adolescence – A Dynamic Period

The most rapid phase of human development is adolescent. Biological maturity precedes psycho-social maturity. Adolescence is the period of transition between childhood and adulthood. The adolescent does not undergo only physical changes but they also experience cognitive, social/emotional, interpersonal changes as well. When they develop, adolescents are predisposed by environmental factors such as parents, peers, community, culture, religion, school, world events, and media. Every person differs in personality, interest, and other issues in an early, middle, late adolescence stage (AACAP, 2003).

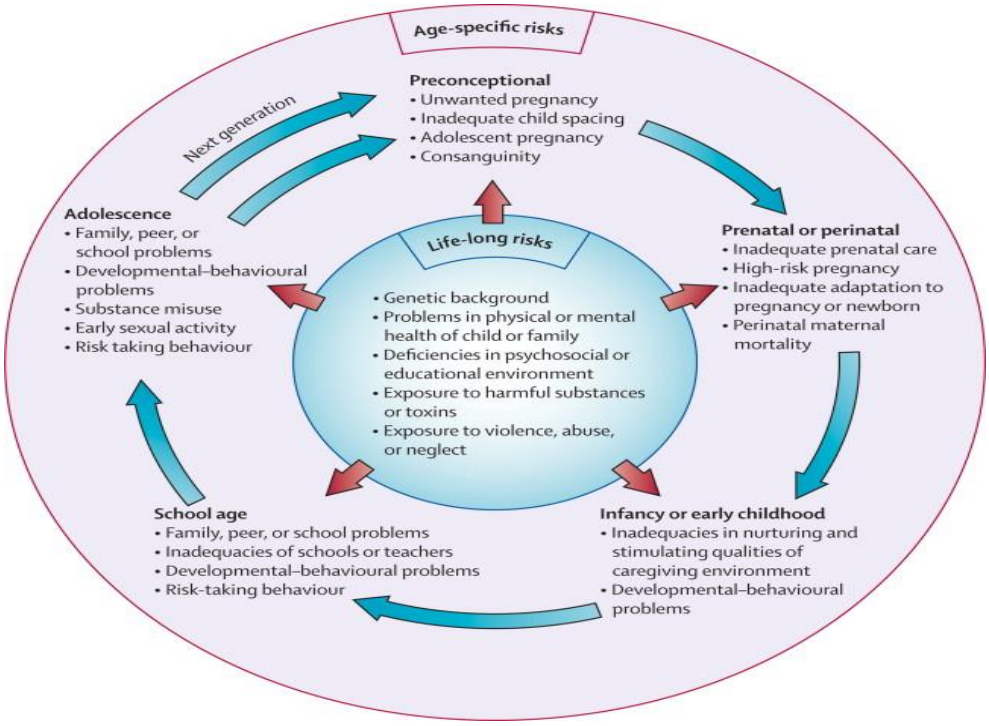

Figure 1 Child And Adolescent Mental Health Worldwide: Evidence For Action, 2011 (Christian et al., 2011)

Cognitive development is the attainments of a more fully conscious, self-directed, and self-regulating mind (Keating, 2004).

This is achieved principally through the assembly of an advanced 'executive suite' of capabilities (Donald, 2001), rather than through specific advancement in any one of the constituent elements. First, adolescents develop more advanced reasoning skills and deeper moral thinking. Second, adolescents move from thinking to reasoning. Third, the formal operational thinking characteristic of adolescence enables adolescents to think about thinking or meta-cognition (Renata, 2013).

3 Adolescent Population in India

Worldwide, Asia is the home for more than half of the adolescent population. In South Asia, the number of the adolescent is 350 million while this number was reported 300million in East Asia and the Pacific (United Nations, Department of Economic and Social Affairs, 2019).

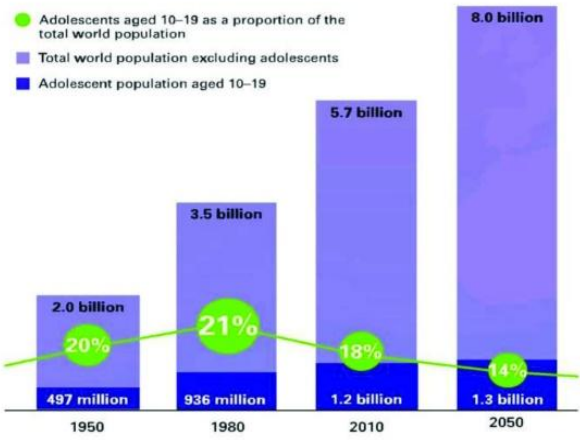

Figure 2 Proportions of Adolescents and World Population (Source: Progress for Children; A Report Card on Adolescents. UNICEF)

Table 1 Five Top Ranks of Adolescent Sex Ratio

Top five states/ UTs2011	Adolescent sex ratio (Females per 1000 males)
Lakshadweep	1053:1000
Arunachal Pradesh	983:1000
Odisha	981:1000
Meghalaya	979:1000
Chhattisgarh	972:1000

Source: A Profile of Adolescents and Youth in India, UNFPA, 2011

India, the second largest populous country has a total population of more than 1.21 billion and constitutes an adolescent population of 243 million (Youth in India 2017). The following list was top five states/Union Territories, the population of Indian adolescent collected in census 2011.

4 Prevalence of Mentally Challenged Adolescent in India

Worldwide the depressive disorder starts at an early age and with the prevalence rate of mental illness. Among children and adolescent the prevalence rate of mental disorders ranges between 1% and 51% (Sandal et al., 2017). The prevalence rate was from 10 % to 20% in adolescent between the age group 14 and 19 years (Kessler et al., 2007). Many mental health disorders like depression, anxiety and further more are present during adolescent. 20% of adolescent have an identifiable and curable mental health disorder. As per the National Mental Health Survey of India (2015–2016), the prevalence of psychiatric disorders among adolescents (13–17 years) is reported around 7.3%.

5 Natural Memory Boosters

From 16th century herbals were used to treat age related cognitive disorders. Many plants have been used to treat cognitive and other disorders. More than 150 plant species have been used in various preparations and mixtures (Desousa, 2014). The recent trend is using herbal medicines through which the overall condition of an individual, body, and mind equilibrium can be achieved. The herb which develops cognition is called medhya herbs (www.holistic herb.com, 2008). In Ayurveda, the mind power is categorized into three types called triads. The first one is acquisition power which is used to grasp a new thing or to understand or analyze it. The second is the power of retention which will retain the things what has been understood and the last one is recollecting power which helps in retrieving information that is stored and leaves in long term memory (Rathee et al., 2008). The nootropic herbs acts on the brain and the constituents or components isolated from this are named smart drugs. These drugs increase the blood circulation in the brain and enhance memory (Sahelian, 2000). Many plants have the capacity to recuperation from cognitive disorder and other memory related disorders. Bacopa moniera (Bramhi), *Ginkgo biloba*, Shankhpushpi, etc. and some other types of herbs can enhance cognition potential (Nabi,2014). The following is a list of effective herbs used in memory enhancement all over the world.

5.1. Ginkgo biloba

Ginkgo biloba belongs to the family Ginkgoaceae. Gingko leafs are used as a edible one. *Ginkgo biloba* eliminates the free radical and there by improve the behavioural adaptation for memoryenhancement. The active components present in it are

flavonoids such as quercetin, kaempferol and isorhamnetin. Minor flavonoids

proanthocyanidins, catechins, flavones and terpene ginkgolides (Birks et al., 2002).

5.2 Centella asiatica

Centella asiatica, belongs to the family Umbelliferae. It is commonly known as Centella asiatica, commonly known as Mandookaparni (Kokate., 2005). It is an Indian herb and it is also used as spice. The main purpose for its usage is, it helps in memory boosting, increase concentration, intelligence quotient in mentally retarded children (Sekar, 1996).

5.3 Bacopa monnieri

Bacopa monnieri belongs to the family plantaginaceae. It is used as memory and attention enhancement. It is given to infants for boosting the memory power. The active constituents present in the herbs are alkaloids and saponins. The major constituents are the steroidal saponins, Bacosides A and B. Thesaponins, baco side A and B are the active principles used for the enhancement ofcognitive function (Russo et al., 2005).

5.4 Acorus calamus

Acorus calamus belongs to the *family Acoraceae.* It is used as brain tonic. Stems, leaves and roots are used in ayurvedic medicine. It is helpful in treating brain tissue detoxification, concentration, thinking process (Chaturvedi et al.,2017). The constituents are alkaloids, flavonoids, gums, pectins, mucilage, phenols, quinone, saponins, sugars, tannins, and triterpenes. The active constituents includeflavonoids, lectins, phenols, and saponins.

5.5 Tinospora cordifolia

Tinospora cordifolia belongs to the family Menispermacaceae. It is used for treating Alzheimer's disease, improve cerebral ischaemia, regulation of cytokines, depression and attention deficit Hyperactivity disorder. They contain active constituents such as alkaloids, steroids, polysaccharides, glycosides (Joshi, 2013). The benefits of these herbs are enormous and it helps the human society. Hippocrates states "Nature is the best Physician" so it can treat each and every illness. The extract from leaves, roots and stem from these herbs have impending use in memory enhancement.

6 Efficacy of Herbal Dietary Supplements on Cognitive Function:

A dietary supplement is defined as "a manufactured or commercially available products which added purposely to food in an intention to achieve the targeted benefit" (Maughan et al., 2018). Mostly dietary supplements include specific components such as multivitamins, minerals, proteins, hormones, plant derived compounds, combination products, and non-categorical supplements like animal or synthetically derived substances. These dietary supplements are available in many forms like powders, capsules, tablets, energy drinks, and even cereals (Knapik et al., 2016). The global dietary supplements market estimated that in 2020 the market value will rise to 220.3 billion in USD (Zion Market Research, 2016).

Centellaasiatica is used as a memory enhancer. In a study conducted by AppaRao et al. (1977), thirty children were selected from the age of 7-18. The test was based on the general mental ability of mentally retarded children. Stanford Binet Test was performed for intelligence testing before the study and their urine samples were also tested for amino acid urea and other inborn errors.

Thirty children were separated into two groups. One group received 0.5g of *Centellaasiatica* tablet and the placebo received tablets made of starch. The final result was that the children who consumed *Centellaasiatica* tablet showed improvement in intelligence and general adaptive behaviour of the mentally retarded children.

Further, Dev et al. (2009) conducted a comparative study on the Cognitive effects of *C. Asiatica* in Healthy Middle Age Female and Male Volunteers with the range of 35-50 years. 22 females and 19 males were selected as participants and they were divided into two groups. Woodcock-Johnson Cognitive Abilities test III was performed before the trial.

The study concluded that *C. asiatica* has the potential to reduce the age related decline in cognitive function in healthy middle age and elderly adults. The placebo received colour coated pills and the other group received 3-4g of *C. asiatica* (according to body weight).

Similarly, Bacopamonnieri is used for cognition and memory enhancement. In a study conducted by Ramakrishnan et al. (2017) the efficacy of Brahmi was tested on Cardio Vascular Accident patients and cerebral palsy patients with behavioral problems. The groups were divided into two, in one group 15C.V.A patients werethere and inanother group 15cerebral palsychildren.They were given Brahmi tablets and were instructed to take the pills for 30 days.

Psychological well-being scale, bartheles index, FIM scale, the positive and negative schedule is used before and after the trial. The result revealed that there was an improvement in the cognitive function in CVA and Cerebral Palsy patients. On comparison,

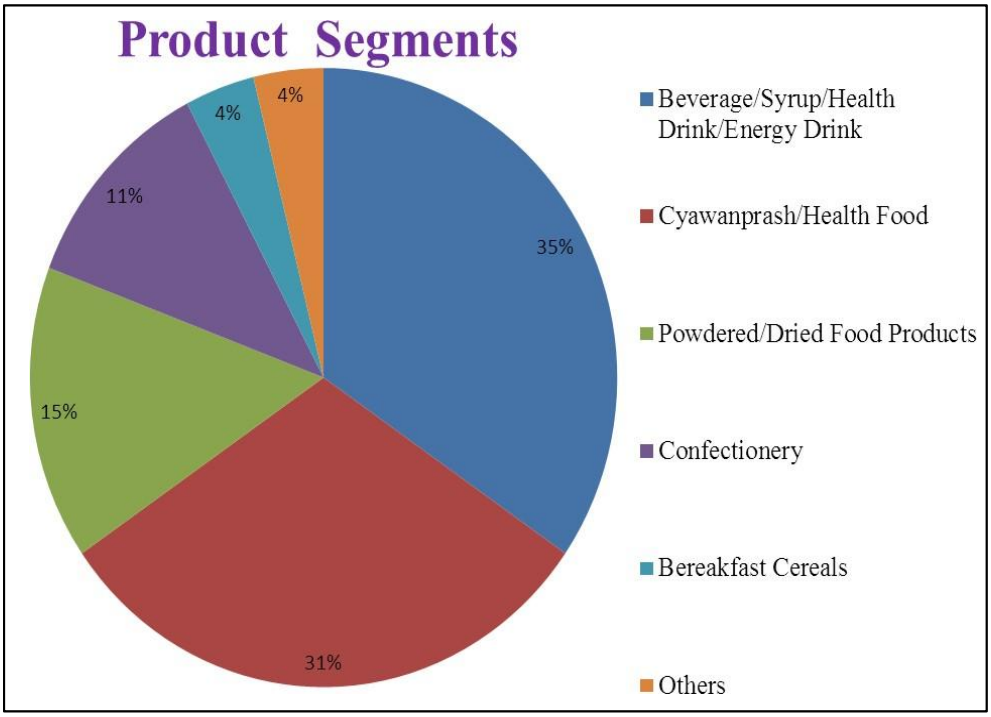

Figure 3 Sector wise Segregation of Products containing Brahmi as a functional Food ingredient (Source: Global New Products Database, 2017)

cerebral palsy patients benefited more.According to previous researches, the population below 18 years is 447million in India. Out of 447 million 29 million suffer from any one of the psychiatric problems. In India, the development of hildren and adolescent psychiatry is very slow and it is difficult to handle the huge problem (Aggarwal, 2003).

India with an abundant population of adolescent has a rising number of mentally challenged problems.

Conclusion

The lifestyle modification like dietary habits, lack of exercise, anxiety, stress, and emotional all leads to psychological problems. The main problem in adolescent is to develop both physical and psychological. These transitional changes lead to stress and in many cases, it leads to serious mental health conditions emerge during adolescence. The psychological tablet leads to increase in weight, hormonal changes, loneliness, and many other things. The plant sources are one which is beneficial to health, and it is non-hazardous when consumed. The plant materials contain a lot of primary, secondary, and many bioactive molecules. The main role of certain herbs helps in improving cognition, memory enhancement. Herbs are healing of the nation, so future research studies should be based on the molecular basis of herbs in humans.

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Conflict of interest

The authors declare that they have no conflict of interest.

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DISCOVERING THE TRUTH OF COVID 19 AND OTHER CONTAGIOUS DISEASES BY APPLICATION OF THE THEORY OF CHANGES AND THE THEORY OF YIN YANG

Cheok Wee Teck[1*], Cheok Yean Chin[2*]

[1]Inti International University of Malaysia, FHLS Department-TCM centre
[2]University of Putra Malaysia (UPM)-Nutritionist Department

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Keywords

Covid 19

Contagious Diseases

Microorganism

Decomposition

Mother Earth

Equilibrium

The Theory of Changes

ABSTRACT

The current pandemic COVID-19 continues to spread rapidly around the world. Many countries are still at the peak of infection. The prolonged lockdown has caused a serious impact on the socio-economy of the whole world. There is a growing concern about the link between animal's based food product with all the contagious diseases as most of the pandemic are initiated from farming animals. The micro study of the virus or bacteria could trace the infectious pathway of the contagious diseases and find substantial problem-solving solutions. Nevertheless, the root cause of the diseases remained unknown. Because of this, it is essential to evaluate the root cause of the disease in the macro-scope of study. By discovering the designated function of all the microorganisms from the insight perspective of the Theory of Changes and other Taoism theories, we could define the effective problem-solving solution. The result of the finding has discovered that the microorganisms which bring the so called "contagious diseases" are the cure for Mother Nature to resume its equilibrium. They are the agents of decomposition and transformation of Mother Nature. Coronavirus, other viruses, and bacteria are the essential microorganisms being created by nature to decompose the excessive animals' dead bodies that awash in the environment into smaller particles or subtracts, transforming them into useful nutrients to be absorbed and restored by Mother earth. After identifying the deadly microorganism as the cure for Mother Nature and recognizing the harm that we have done to Mother Nature, we could find out the root cause of the problem and solve the problem fundamentally.

* Corresponding authors
E-mail: weeteck.cheok@newinti.edu.my (Cheok Wee Teck);
setiatcm@gmail.com (Cheok Yean Chin)

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1 Introduction

The Novel Coronavirus "Covid 19" which occurred at the end of 2019 has initiated a pandemic. Although the exact origin of SARS-CoV-2, the etiologic agent of COVID-19, is currently unknown, there is substantial evidence to suggest the source of transmission of the virus occurred within the Wuhan wet market. In these markets, bats and wild animals are frequently sold and stored in close contact (Alonso Aguirre et al, 2020). Besides Covid 19, there are several diseases well known to both the industry and the general public that are directly related to all the domestic meat species of beef, pork, lamb, and poultry. These include BSE (bovine spongiform encephalitis) from beef cattle, Trichinosis and H1N1 from pork, Salmonella and Avian Influenza from poultry, Scrapie from lamb and mutton, Foot-and-mouth disease from livestock, and many others. These are known as zoonotic diseases or zoonoses (Centers for Disease Control and Prevention, 2017). There is a growing concern about the link between animal's based food products with all the contagious diseases.

This research is to identify the link between meat production and consumption with the Covid 19 and other contagious diseases, discover and evaluate their root cause from the perspective of The Theory of Changes and other Taoism theories, with the aims of defining the effective problem-solving solution. The discovery of 8 trigrams (八卦) by the Fu Xi (伏羲), the first writer of The Theory of Changes was based on the observation of all the phenomenon in the universe (Liang Yong Xuan & Lu Zhao Lin, 2005). In light of this, the observation of the phenomenon from the macro but insight perspective could help us to discover the truth of the contagious disease and provide us a better solution and prevention. This research will focus on the macro analysis of the natural setting of Heaven and Earth by using The Theory of Changes and other Taoism theories.

2 Discussion

Most of the recent researches are focusing on the micro study of the microorganism and identifying them as pathogenic factors. This study is based on a macro study on the microorganism to explore the true identity and function of the so-call life-threatening microorganism. Based on the great observation and justification by the Theory of Changes, findings of this study has proven that all the microorganism inclusive of Covid 19 are the important elements in the Mother Earth for decomposition and transformation settlements.

It provides a revolutionary perspective of microorganisms and rectifies the common wrong perspective on deadly viruses and bacteria. The human could only obtain their absolute solution in solving the threat of Covid 19 and other contagious diseases by identifying the true identity and function of the microorganism.

Excessive meat consumption and meat production not only harm innocent lives, affect the health of the public but also causing a detrimental effect on the earth. The existence of a huge amount of livestock, farming animals either land or aquatic and the leftover animals' dead bodies generated from the meat consumption have made our living, working and social environment disgustingly filthy. The methane created by livestock has proven to be the major cause of global warming in the world.

According to research, more than 200 million land animals are killed for food around the world every day. That comes out to 72 billion land animals killed for food around the world every year. Including wild caught and farmed fishes, we get a daily total of closer to 3 billion animals killed (Matthew Zampa, 2018). Compare to merely 7.8 billion world population (Kaneda et al, 2020), this amount of death is comparatively huge. It is generally believed that pests, insects, and microorganisms are the primary cause of the diseases. When the living and working environment of the human being are awash with the uneaten animals' dead bodies like the organs, skins, heads, and other inedible parts. It has provided a conducive breeding and living environment for bacteria, viruses, and other pathogenic factors.

《Plain Question. Liu Wei Zhi Da Lun》in Yellow Emperor Classic has stated: "Excessive is destructive, it should be neutralized to the moderate and right order." (《素问。六微旨大论》："亢则害，承乃治。") (Wang Qi Qing, 2003). The bigger destruction should be counteracted by the bigger neutralizer. The amount of livestock raised and animals killed is excessive. This should be withstood and neutralized to the right and moderate order before disastrous consequences happen. As a result, nature is carrying out the necessary settlements to purify the environment and restore the ecological balance.

Animals' dead bodies and excrement need to go through the decomposition process. They are broken into smaller parts for the earth and nature to absorb and dissolve. The earth has the function of transforming all the waste into usable elements. The earth has the characteristic of Kun (坤). According to Theory of Changes 《Zhou Yi 》："Earth is represented by Kun, thus the superior man should possess the great moral of tolerance and care for all living beings". (《周易》："地势坤，君子以厚德载物") (Yang Li, 2010).

The earth with the characteristic of loving kindness has the great contribution and devotion of accepting, carrying, and holding, preserving, sustaining, nourishing, and growing all things. It could accept the energy yang (solar energy) and at the same time tolerate the turbid and rubbish. Those who want to practice and cultivate the Dao or achieve superiority should learn from the earth. With her great tolerance, mother earth is willing to accept and hold all

objects lying upon her. Besides this, she is also willing to nourish the good and transforming the bad. The two major devotions and functions of Mother earth are "Nourishing and Transforming". It could have resembled the mother who is feeding her children with her breast milk and cleaning all excrements and urine discharged by her children. The Great Mother characteristic of the earth has been generally recognized and claimed by most of the people in the world. Thus, we usually call the earth as Mother Earth.

The functional characteristic of Kun could be explained better by the following two statements in the Theory of Changes 《Kun Hexagram. Explanation of Hexagram 》stated that: "Yuan, Heng, Li, Pin Ma, Zhen" (《坤卦卦辞》所言： "元,亨,利,牝马,贞) While the 《Qian Hexagram. Explanation of Hexagram 》stated: "Qian, Yuan, Heng, Li, Zhen." (《乾卦卦辞》原文所说："乾，元，亨，利，贞") (Yang Li, 2010). The first statement is explaining the characteristics of Earth "Kun"(坤), while the second statement is explaining the characteristic of Heaven "Qian" （乾）. Earth "Kun" (坤) is Yin, Heaven "Qian"（乾) is Yang. The function of the Kun could only be accomplished when there is an involvement of the Qian. The holy integration in between the Heaven "Qian"（乾） and Earth "Kun" (坤) (乾坤合德) or integration of Yin and Yang could generate a living environment which could sustain and nourish all the living beings.

They both accomplished the four processes together which are Yuan, Heng, Li, Zhen (元，亨，利，贞). Before they could accomplish these, the Heaven should resume his Qian（乾）status, while the Earth should restore to Pin Ma 牝马condition. Qian represents the Heaven, the movement of Heaven "Qian"（乾) is powerful and ever vigorous. Yuan (元) is the original qi or energy yang (solar energy) generated by the Heaven "Qian"（乾) to contribute to the Earth "Kun"(坤). Yuan (元) should be conceived by the Earth to nourish the plants and other creation lying upon her. Heng (亨) always be pronounced in the complete words call Heng Tong (亨通) which means proceed smoothly and prosperously. This is the result of the driving force of the energy Yang. Li (利) means benefiting. It means the Original Qi which is proceeding smoothly and prosperously could benefit the earth and all the living being. Li also could be explained as Yi (宜) which means a conducive living environment which could sustain all living being. The most conducive and sustainable living environment is the Consolidated Yang and Harmonized Yin (阴平阳秘) condition where the Yang is consolidated and restored firmly underneath the harmonized Yin. This condition is the most favorable condition which could be benefiting all the living being. Zhen (贞) means right and correct. To achieve a sustainable living environment or we call it Consolidated Yang and Harmonized Yin, everything should be in the correct and right pathway, or other more profound words, and the entire natural phenomenon should achieve equilibrium and strive for ecological balance.

As previously mentioned before the environment and ecological balance of this planet have been damaged by the farming industries and the wrong food consumption habit generally possesses by the majority of people of the world. The Earth "Kun" (坤) need to resume it by applying the Pin Ma (牝马) process. Pin (牝) means female, ma (马) means horse. Pin Ma (牝马) means female horse. The explanation of Hexagram is written by The King of Zhou Wen (周文王) of the Zhou Dynasty (周朝). Female possesses the Earth "Kun" (坤) characteristic. Kun (坤) major characteristics and functions are growing and transforming. Female animals and other creatures could procreate animals' offspring. To procreate they need the involvement of male animals and male creatures.

All the living beings on this planet are created by the nature with holy duties. They should accomplish their tasks to help the mother earth and father heaven to accomplish the process of sprouting (生), growing (长), transforming (化), harvesting (收) and restoring (藏). In other words, they should work hand in hand to ensure the process of Yuan, Heng, Li, Zhen (元，亨，利，贞) are running smoothly. These creatures are inclusive of all types of animals, insects, and microorganisms.

The King of Zhou Wen (周文王) of the Zhou Dynasty (周朝) used the horse to represent all animals and creatures probably because horses are the main transport for the peoples in ancient period, or in other words, horses are the most intimate friend for the human being. Besides that, all writing information in ancient times had to be carved on stone, bones, or bamboo. The record of the information could not be too lengthy. It should be short and precise. The readers should extend the meaning of one word by using logical and critical thinking. Thus, the usage of Pin Ma (牝马) was referred to all the living beings in nature.

As it is known that some creatures are good at spreading the seeds like the birds, bees, and certain insects. Most of the animals dropping and excrement could become the fertile fertilizers and nutrients for the mother earth and they are helping the growth of the trees and plants. The green coverage of plants has provided a Harmonized Yin on earth which could conceive the Consolidated Yang better. Certain animals are ensuring the health of the plants and controlling other animals.

Herbivores are eating grasses and leaves. They help to trim the plants to ensure the greenery of the plants. Some animals like lions, tigers, leopards, and other carnivores which are at the top of the food chain are controlling the numbers of other animals to avoid excessive conditions happen. They are "harvesting" the

excessive and unnecessary. After the animals were killed, nature would ensure all these animals' dead bodies with awful and disgusting smells are cleaned up properly. The leftover animals' dead bodies need to be decomposed into smaller particles for the earth to absorb and restore. To accomplish this, nature has created different types of scavengers or partial scavengers like raccoon, rat, coyote, opossum, Tasmania devil, hyenas, wild dogs, wolf, wolverine, lizards, Comodo dragon, vulture, condor, Hagfish, Black Hagfish, Feeder Shrimp and many others which are munching on the dead bodies.

In addition to these, nature has created different types of insects and worms like cockroaches, meat ants, fire ants, flies, burying beetles, tapeworms, earthworms, maggots, or even certain smaller microorganism like bacteria and viruses to fasten the decomposition process. They are helping the dead bodies to decompose and nutrients could be taken from dead bodies to the earth through the soil that is enriched with their excrement or castings. At last, all the dead bodies will be dissolved properly and being absorbed completely by the earth. They are performing the Pin Ma (牝马) duties. All in all, these animals and creatures are helping in the transforming, harvesting, and restoring process for the sole purpose of achieving Zhen (贞) where the Li (利)and Yi (宜) living environments are maintained properly. The Consolidated Yang and Harmonized Yin (**阴**平阳秘) condition of the mother earth could be sustained.

All the scavengers, certain insects, worms, and microorganisms are created by nature to decompose the animals' dead bodies into smaller particles or subtracts. It could be transformed into useful nutrients to be absorbed and restored by the earth. The data analysis by the article with the title: "Virus decomposition provides an important contribution to benthic deep-sea ecosystem functioning." indicate that the decomposition of viruses provides an important, previously ignored contribution to deep-sea ecosystem functioning and has an important role in nutrient cycling within the largest ecosystem of the biosphere (Dell'Anno et al., 2015). Research on Arbuscular mycorrhizal fungi has also proven that their existence is playing an important role in litter decomposition (Xiongshi Kong et al, 2018). Another research also revealed that the Thraustochytrids are cosmopolitan osmotrophic or heterotrophic microorganism that are considered as important decomposers in coastal ecosystems (Yoshitake Takao et al., 2015).

Good quality and effective subtractive system are vital to ensure the growth of the healthy and thriving plants. This is essential in creating a harmonized Yin and balance natural ecosystem. The plants could emancipate oxygen in to the air and absorb carbon dioxide making the environment habitable for living beings. This is essential in creating a harmonized Yin and balance the natural ecosystem. The plants could emancipate oxygen into the air and absorb carbon dioxide making the environment habitable for living beings. A good subtractive system is not only providing nutrients needed by the plants but also creating an environment that encourages and promotes the colonization of even more bacteria, viruses, and other microorganisms in the earth.

These additional microorganisms are necessary to break down the toxic waste substances remaining in the dead bodies' components, turn them into harmless nutrients that can be absorbed by the plants. Some bacteria and viruses could even improve filtration and purification of air, water, and soil making clearer air, water, and soil condition which could counteract the air, water, and soil pollution created by the animals' dead bodies. At last, a healthier and longer-lasting living environment which is fulfilling the Zhen condition was made perfectly.

By understanding the true meaning of the Theory of Changes, we realized that the existence of all the contagious diseases either the bacteria in the Black Death or viruses in the Covid 19, SARS, MERS, and Ebola are the essential microorganisms being created by nature to decompose the animals' dead bodies into the smaller particles or subtracts. By doing so, they could filter and purify the air, water, and soil. The more animals' dead bodies are created by the human being, the more powerful and lethal the microorganism will be generated by Mother nature to counteract the problem. As mentioned before that the Yellow Emperor Classic had stated: "Excessive is destructive, it should be neutralized to the moderate and right order" (《素问。六微旨大论》："亢则害，承乃治。") (Wang Qi Qing, 2003). The bigger destruction should be counteracted by the bigger neutralizer.

Human are not the natural carnivores and scavengers but pretend to eat like them, their internal body parts will be dissolved and decomposed by these microorganisms as well. All these microorganisms which supposed to be in the digestive system of the scavengers now exist in our digestive system. As human bodies are different from the structure of the carnivores and scavengers. Our digestive systems are not designed to counteract the adverse condition. Low acidic conditions in our stomach could not kill the microorganism and control their colonization. In order words, they are the suitable Pin Ma (牝马) agents to accomplish the decomposition and transformation process, but we are not.

The same microorganisms which are benefiting the scavengers and carnivores would become harmful to us. All these microorganisms are like flies which are very sensitive to the awful and disgusting smell of dead bodies. They will be attracted to those things which have an awful and disgusting smell or even transformed by themselves as colonization could be promoted in the right environment.

If we consumed plenty of meat (animals' dead bodies), definitely we would attract or generate all these deadly bacteria and viruses which could cause life threatening diseases. The capability of self-generation and self-transformation of the microorganisms could explain why some Covid 19 cases which have no contact with the pandemic area are infected out of no way. This could be the direction that the scientist should further their investigation for that unknown etiological pathway like the first 15 cases Covid 19in Italy which had no travel records to China (News Strait Times, 2020).

The Covid 19 cases happen in Esther Island which is located far from the Chili mainland where the community is in isolation. The first person who contracted Covid 19 in Esther Island is a Chilean woman who had not been overseas or to be in contact with any foreigner (RNZ News, 2020). Although the current studies have defined the pathophysiological characteristics of Covid 19, there is great uncertainty regarding its mechanism of spread. This review could provide some clues in finding the reason.

The existence of viruses, bacteria, and other microorganisms in the decomposition process of turbid, waste, dead bodies, and other organic materials could be excessive as well. As what have mentioned before: "Excessive is destructive." Nature needs to generate some elements to counteract them. As a result, some other microorganisms or natural substances will be generated by Mother Earth to neutralize the excessive to achieve equilibrium. Investigation of anti-HIV properties of oxihumate has shown that coal-derived humid acids and oxyfulvic acid, which are complex mixtures formed during the decomposition of organic matter including cellulose found naturally have been previously shown to have anti-HIV properties (Van Rensburg et al., 2000; Van Rensburg et al., 2002). The occurrence of the cure for HIV or anti-HIV properties in the decomposition process in nature has proven that the contagious HIV viruses are existing in the decomposition process as well in Mother earth.

The excessive HIV existence has generated anti-HIV properties. Besides that, these properties can be predicted to be effective against influenza but that remains to be proven (Kotwal, 2008). In short, all the relevant researches above have shown the complex counter-reaction process of the decomposition settlements of Mother Earth. The purpose of this reaction is to ensure the equilibrium of the ecosystem.

Scientists understand that the Black Death which struck Europe and Asia in the mid-1300s was spread by a bacillus called Yersina pestis, which lives in rodents. Rodents have developed immunity towards these bacteria, but not the fleas that feed on their blood. A toxin produced by Yersina Pestis is carried from rodents to the human when the fleas bite the humans (History.Com Editor, 2010). This organism settled on the humans' lungs, the lungs' immune system will resist this foreign particle and the goblet cells will generate mucus or phlegm. Prolonged inflammation in the lung for more than 4 to 7 days, the lungs will eventually liquefy and generate bloody sputum. Patients were suffered from difficulty in breathing and suffocation. This eventually causes death. Rodents are considered pests which brings the Yersina pestis and caused Black Death. In fact, rodents are not the main cause of the disease if we apply the Theory of Changes. As we know all the creatures created by nature have holy duties. No exceptional to rodents. Rodents that are living near human beings are partially omnivorous and scavengers. They are created by Mother Nature to clean up the remaining food and animals' dead bodies left by human beings. This theory explains the existence of dogs and cats in human society as well. When humans know how to use fire, they have climbed up to the top of the food chain and eat most of the animals inclusive of those carnivores. In nature, most of the scavengers are living near to the habitat of the carnivores. For example, the hyenas and wild dogs would choose to live near the lions' habitat as they know that they could find the dead bodies left over by the lions. The same theory applies to the human being. When humans start eating meat and creating plenty of animals' dead bodies, they will attract the scavengers like dogs, cats, and rodents to live in their living environment. Their existence is to clean up the dead bodies.

Thus we should not treat rodents as a pest as they have their living purpose in nature. One realistic fact that we should accept is "Filthy condition attract rodents and flies and not the rodents and flies created the filthy condition." If we always keep our living environment clean and avoid those smelly food, the chances of flies and rodents enter into your house is relatively low. Rodents are the agents which help in decomposition. Even the bacteria and viruses living in the rodents' bodies are also having the same purpose of decomposing the animals' dead bodies awash in the human living environment. They are the cure for the environment and not the pests or diseases. The ancient drawing of rodents has a Hua (化) word in the stomach Hua (化) means transforming. It shows that the rodents could transform the animals' dead bodies and unwanted food remaining into excrements that are easier for the earth to absorb. The colonization of more microorganisms in the excrements of the rodents could also break down the toxic waste substances and turn into the harmless nutrient to be absorbed by the earth. In other words, they are performing the Pin Ma (牝马) duties in decomposing, transforming, filtration and purification. As mentioned before, humans' bodies are not designed to perform the decomposition and transforming process, the incapability has finally led to death.

The Coronavirus which causes Covid 19 is like other viruses using protein to latch onto and invade human cells. This virus is part of

the RNA brigade of viruses which are much less stable than the DNA-based viruses. The instability in copying genetic code leads them to mutate easier and allows them to cross the huge division between different species. This makes the virus becomes more contagious and resistant to drugs. When encountering with the huge amount of the animals' dead bodies and pollutant, the Mother Earth need to create a more powerful and lethal virus to effectively settle the problems. Coronavirus attacks the human's lung and causes pneumonia where inflammation in the air sacs making the patient difficult to breathe. The deterioration of the condition without supporting care will probably lead to death. As we knew, the propagation of viruses either mitosis or meiosis depends on the specialized host cells to perform the complex metabolic and biosynthetic process. Metabolism is the biochemical processes that occur within a living organism which consists of anabolism (the buildup of substances) and catabolism (the breakdown of substances). The term metabolism is commonly used to refer specifically to the breakdown of food and its transformation into energy (Shiel Jr, 2018).

While biosynthesis in a living organism is a process in which substrates are converted to more complex products. Biosynthesis is usually synonymous with anabolism. The products which are produced as a result of biosynthesis are necessary for cellular and metabolic processes deemed essential for survival.

Catabolism is the breakdown of substances which is the decomposition process of Mother Earth, while anabolism is the buildup of substances which is the transformation process of Mother Earth to turn substances into useful energy or nutrients. Here we have to face the horrible facts that the Coronavirus is not a disease but a cure for Mother Earth and we are the virus for Mother Earth.

Human always blame that the animals or the insects (Entomopathogen) are the intermediate host for the contagious diseases. We kill them to prevent the outbreak but we never reflect upon ourselves that we are the root cause of the problem and not the innocent animals and insects. In fact, every living creature is the divine creation of Mother nature which has its holy duty and purpose. They are the members or agents of the Kun (坤) which assist in the Pin Ma (牝马) process. They are not the pest but the cure for ecological balance. The microorganisms that host on the animals' bodies are harmless to them. The pathogenic growths of the bacteria or viruses in the insect or animals are prevented by the host immune system, limitation of nutrients, or other competing microorganism parks inside their bodies. On contrary, certain microorganisms are found in the intestines of the animals could help the host to improve food digestion. The same as most of the E coli bacteria which are found in the human's and other warm blood animals' lower intestine could prevent the colonization of other pathogenic microorganisms and benefit the hosts by creating vitamin K2.

Thus the microorganism could be harmful to certain species and could be beneficial to another species. As we commonly acknowledge, a virus must use cell processes to replicate, and the viral replication cycle can produce dramatic biochemical and structural changes in the host cell, which may cause cell damage. These changes are called cytopathic (causing cell damage) effects, which can change cell functions or even destroy the cell (Lumen Learning, 2013).

A CT scan in COVID-19 extubated survivor: a study performed during recovery (26 days after onset of COVID-19 pneumonia). Image section at the level of the carina demonstrating widespread ground-glass opacification and considerable architectural distortion (George et al., 2020). In a longitudinal CT study of 90 patients with COVID-19, 94% of individuals had residual changes on CT at discharge (median duration of 24 days after symptom onset) with ground-glass opacity the most common pattern (Wang et al., 2020).

At discharge, in a study of 110 patients with COVID-19, 91 (83%) of whom had a mild-moderate disease and 19 (17%) of whom had severe disease, almost half had impairment of the transfer factor of the lung for carbon monoxide (TLco) (Mo et al, 2020). Ground-glass opacification/opacity (GGO) is a descriptive term referring to an area of increased attenuation in the lung. Architecture distortion is the result of the cell damage caused by the decomposition process being carried out by the Coronavirus.

The functional impairment of the lung has finally caused death. This damaging process is the decomposition process carried out by the virus. The microorganisms would be useful and beneficial if they were placed at the right time and in the right location. In short, the chances for us to acquire Covid 19 and other contagious diseases is relatively low if we don't allow the wrong microorganism to inhibit in our body and doing the unnecessary decomposition and transformation by adopting veganism and avoiding meat (animals' dead bodies) intake.

Conclusion

The destructive impact of animal agriculture and meat consumption culture on the environment and human health is undeniable. Meat industries are considered one of the prime causes contributing to the current six mass extinction. In the near decades, more than 10 thousands of animals will be on the brink of extinction. As we knew, all living beings inclusive humans, animals, plants, and other creations of nature should co-exist with

each other in the protection and nourishment of Heaven and Earth. The extinction and the destruction of any one of them will cause the ecological imbalance and lead to the breakdown of the process of Yuan, Heng, Li, Zhen (元亨利贞). The collapse of the Consolidated Yang and Harmonized Yin is unavoidable if the condition could not be neutralized and restored to the right order. At this verge of collapse, the Pin Ma (牝马) settlement has brought in by the Mother earth to prevent the deterioration of the equilibrium.

The lockdown of the whole world has brought a good rest to Mother Earth and her animal children. She is healing her wounds, taking a good rest, and breathing the sigh of relief, as well as her beloved children. During these socially isolated periods, many animals are enjoying temporary freedom and reclaiming their territories in the cities and villages. The endangered turtles in India and Brazil have been making the most of the deserted beaches to nest in peace. The pink jellyfishes which have been absent for years due to human activities are now flourishing beautifully along the coastline of Palawan, Philippines. Fishes and sea creatures are released back to the sea as demand dropped during the lockdown. The skies of most of the big cities in the world have turned blue as the carbon emission and pollution rate in the World were reduced at its utmost limit unprecedentedly.

Most importantly, the emerging of the Corona Viruses has substantially contributed to the decomposition and transformation of the overloaded dead bodies and animals' excrements into the useful elements which could be absorbed by Mother Earth. Given such a situation, we realized that the viruses are not the diseases but cure, and we are the virus to the planet which cause harm to the Mother Earth.

It is time for human to turn inward and reflect upon ourselves. What are the harms that we have done to our bodies, to the animals and to the mother earth? What are the sustainable lifestyle we should adopt for the good sake of our health, other earthlings and mother earth? The thorough evaluation by application of the Theory of Changes and the Yin and Yang Theory has proven that reduction in meat consumption and production could save us from being the victims of the process of decomposition and transformation of the viruses and other microorganism. After we have identified the deadly microorganism as the cure for Mother nature and recognize the harm that we have done to Mother Nature, we could find out the root cause of the problem and solve the problem fundamentally. The ecological balance of nature which has been damaged seriously by disruptive and excessive human activities needs to be settled with full dedication of the human being. Lockdown the whole world is not the appropriate long term approach. Humans should face the truth and solve the problem fundamentally. We could only gain peace and joy by giving peace and joy to Mother Nature and other living beings!

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THERAPEUTIC APPLICATIONS OF *Spirulina* AGAINST HUMAN PATHOGENIC VIRUSES

Sharolynne Xiao Tong Liang[1], Ling Shing Wong[2], Anto Cordelia Tanislaus Antony Dhanapal[3], Prakash Balu[4], Sinouvassane Djearamane[1*]

[1]Department of Biomedical Science, Faculty of Science, Universiti Tunku Abdul Rahman, Kampar, 31900 Malaysia
[2]Life Science Division, Faculty of Health and Life Sciences, INTI International University, Nilai, 71800 Malaysia
[3]Department of Chemical Science, Faculty of Science, Universiti Tunku Abdul Rahman, Kampar, 31900 Malaysia
[4]Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Chennai, Tamil Nadu, 600117, India

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ABSTRACT

Viruses can spread worldwide and the early detection of emerging infectious diseases and outbreaks in humans and animals is important for effective surveillance and prevention. Viruses such as human immunodeficiency virus (HIV), swine flu, and influenza virus are some of the viruses that spread diseases worldwide. However, the non-availability of effective antiviral drugs and the drug-resistance among the virus and host have become the major problems in controlling viral infections. The natural products from microalgae can be an alternative therapeutic agent to control viral infections in humans. *Spirulina* is a well-known cyanobacterium that has been consumed by humans as a food supplement for more than centuries without side-effects. *Spirulina* possesses high nutritional values and provides numerous health benefits to the consumers. *Spirulina* can be an alternative natural therapeutic agent for numerous virus infections as it contains several bioactive compounds with proven antiviral effect on enveloped viruses (Herpes simplex virus, measles virus, mumps virus) and non-enveloped viruses (astrovirus, rotavirus) by preventing the spread of the virus in the host cells. *Spirulina* also serves as a natural supplement that strengthens the immune system. This review focuses on the antiviral properties and immunostimulant effects of *Spirulina* as a potential therapeutic supplement on human health.

* Corresponding author
E-mail: sinouvassane@utar.edu.my/biochsinouvas07@gmail.com (Sinouvassane Djearamane)

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1 Introduction

Viruses are cellular and obligate intracellular parasites that can cause viral infection in host cells and are harmful to all living organisms including humans, animals, and plants (Koonin et al., 2006). Viruses can spread disease worldwide through many factors such as contaminated water and food, environmental factors such as air and soil, person to person, insects, and vectors. Early detection of emerging infectious diseases and outbreaks in humans and animals is important for effective surveillance and prevention (Ramakrishnan, 2013). There are around 219 human infecting virus species that are known, and the first human infecting virus discovered was yellow fever in 1901 (Woolhouse et al., 2012). Carcinogenic viruses and other disease causative viruses including Chikungunya virus, dengue virus, acute respiratory syndrome coronavirus (SARS-CoV), human immunodeficiency virus (HIV), influenza virus, Herpes simplex virus (HSV), severe swine flu, human papilloma virus (HPV), and human T-lymphotropic viruses (HTLV-I, HTLV-II) are the most important viruses spreading diseases worldwide (Ramakrishnan, 2013). Drug-resistance of the virus and the host have made the treatment of viral infection using chemical drugs difficult. One alternative way to overcome the drug-resistant issue is to substitute chemical drugs with natural biological compounds (Ramakrishnan, 2013). The natural products from microalgae can be an alternative therapeutic agent to effectively control the viral infections in humans.

Cyanobacteria are one of the oldest photosynthetic organisms that are found in different aquatic environments (Nuhu, 2013). *Spirulina* is known to be a "superfood", named by the World Health Organization (WHO), and is recommended to be a compact food during space travel for astronauts of National Aeronautics and Space Administration (NASA) (Koyande et al., 2019). *Spirulina* has been consumed for more than centuries as food and supplements for humans, traditionally by the native population at Lake Chad area and the Aztec population of Mexico (Djeramane et al., 2018; Koyande et al., 2019). The species of *Spirulina* includes *S. platensis*, *S. laxissima, S. subsalsa, S. fusiformis, S. maxima*, and *S. lonar*. However, between all the species, *S. platensis* and *S. maxima* are more popular and mostly used for nutritional and therapeutic purposes (Kameshwari et al., 2020).

Spirulina contains a high level of nutrients such as protein, lipid, carbohydrate, amino acids, vitamins, minerals, pigments, and others (Khan et al., 2005). Generally, *Spirulina* contains 55-70% protein, 15-25% carbohydrate, 6-13% nucleic acids, 5-6% lipid, and 2.2-4.8% minerals (Reboleira et al., 2019). Vitamins such as B_1, B_2, B_3 and B_{12}, photosynthetic pigments, and minerals like calcium, copper, iron, magnesium, phosphate, sodium, and zinc are rich in *Spirulina* species (Wan et al., 2016). *Spirulina* is a natural immunity booster, detoxifier, appetite suppressant, and antioxidant that provide a wide range of benefits upon consumption (Singh et al., 2020). The antibacterial, anticancer, antiviral, and anti-parasitic properties of *Spirulina* are being widely studied by researchers (Martínez-Galero et al., 2016; Fayyad et al., 2019; Abd El-Baky & El-Baroty, 2020; Joseph et al., 2020). Besides, *Spirulina* is also being used in clinical studies due to its high functional and nutritional properties in treating a range of pathogenic conditions such as allergies, inflammatory diseases, hypercholesterolemia, heavy-metal poisoning, cardiovascular diseases and radiation poisoning (Reboleira et al., 2019; Kameshwari et al., 2020).

2 Antiviral properties

The National Cancer Institute (NCI) research group screened the lipophilic and hydrophilic extracts obtained from 300 species of cyanobacteria to examine their antiviral properties and reported that about 10% of the cultures were shown to reduce the cytopathic effects induced by a viral infection (Patterson et al., 1993). The bioactive compounds of microalgae such as nostoflan from *Nostoc flagelliforme* and fucoidan from the sporophyll of *Undaria pinnatifida* exhibit an antiviral effect on several enveloped viruses (Hayashi, 2008) and, ichthyopeptins A and B, the two novel depsipeptides from *Microcystis ichthyoblabe* inhibit the viral protein of influenza A virus (Zainuddin et al., 2007). *Spirulina* naturally can inhibit the activity of viruses as it has all the biomolecules which can build a strong immune system that can scavenge free radicals (Ali & Saleh, 2012). The extracts isolated from *S. platensis* and *S. maxima* were reported to possess antiviral properties (Hernandez-corona et al., 2002; El-Baz et al., 2013; Kameshwari et al., 2020). The antiviral activity of *Spirulina* mostly depends on the richness of proteins (phycocyanin), sulphated polysaccharide fragments, fatty acids (sulpholipids), minerals, and some other constituents (Nuhu, 2013; Kameshwari et al., 2020).

The antiviral effect of *S. platensis* against enveloped viruses such as human cytomegalovirus (HCMV), HSV-1, measles virus, mumps virus, HIV-1, and influenza virus is mainly contributed by the acidic or sulphated polysaccharides, calcium spirulan (Ca-SP) isolated from hot water extract of *S. platensis* that acts through inhibiting the replication of the viruses (Nuhu, 2013; Ramakrishnan, 2013). The aqueous extract of *S. platensis* is found to significantly inhibit the *in vitro* replication of HIV-1in human T-cell lines, Langerhans cells, and peripheral blood mononuclear cells (PBMCs) (Hoseini et al., 2013; Ramakrishnan, 2013), with up to 50% viral reduction on PBMCs using extract concentration between 0.3 to 1.2 µg/mL (Ayehunie et al., 1998). Further, the water extract of *S. platensis* inhibited HSV-1 replication in HeLa cells by interfering with the entry of the virus into the host cells, although the extract is not virucidal (Hayashi et al., 1993). Besides the enveloped viruses, *S. platensis* also demonstrated the antiviral effects on non-enveloped RNA and DNA enteric viruses. The

ethanol extract of *S. platensis* caused a reduction of 76.7% on astrovirus type 1, 66.7% on Coxsackievirus, 53.3% on adenovirus type 7, 56.7% on rotavirus Wa strain, and 50% on adenovirus type 40. The virucidal effect of *S. platensis* extract using ethanol showed a higher reduction on RNA viruses than DNA viruses (El-Baz et al., 2013). Allophycocyanin isolated from *S. platensis* is proven to inhibit 50% of viral-induced cytopathic effect in African green monkey kidney cells and human rhabdomyosarcoma cells at concentrations of 0.056-0.101 µM on enterovirus 71 by interfering with viral RNA synthesis in infected cells. The treatment of allophycocyanin to the host cells before viral infection showed higher antiviral activity than treatment after infection (Shih et al., 2003). Other than *S. platensis*, Hernandez-corona et al., (2002) reported that methanol-water (3:1) extract of *S. maxima* exhibited the highest antiviral activity on HSV-2 among hexane, chloroform, methanol, and hot water extracts. *S. maxima* extract prepared using hot water showed less than 20% inhibition on adenovirus type 3 with IC_{50} 5.2 mg/mL and no inhibition was observed at concentrations below 2 mg/mL (Hernandez-corona et al., 2002; Rahman et al., 2006).

Abd El-Baky & El-Baroty (2020), reported that L-asparaginase (L-AsnA) purified from *S. maxima* showed a dose-dependent antiviral effect on Coxsackie B3 (CSB3) virus where 17.03 µg/mL of L-AsnA inhibited 50% of CSB3 virus and suggested that inhibition of viral replication cycle might be the mechanism behind the antiviral effect. Similarly, Sharaf et al. (2010) reported that the crude extracts of *S. fusiformis* caused inhibition of HSV replication in host cells on both pre and post-infection stages.

Further, *Spirulina* contains about 2-5% of sulpholipids, which are effective against the enzymatic activity of HIV-1 reverse transcriptase (RT). A minimum concentration of 24 nM of sulpholipids can exhibit 50% inhibition by selectively acting on DNA polymerase of HIV-1 RT (Hoseini et al., 2013). A study on carbohydrate-binding agents that demonstrated the inhibition of HIV-1 and other enveloped viral particles suggested that cyanovirin-N (CV-N) isolated from *Spirulina* has the potential as an anti-HIV therapeutic agent in the future (Balzarini, 2007). Table 1 and 2 shows the antiviral properties of *S. platensis* and *S. maxima*, respectively.

Table 1 Antiviral properties of *S. platensis*

Compound name	Virus	Antiviral property	Authors
Ca-SP from hot water extract	HSV-1, HCMV, Measles, Mumps, HIV-1, Influenza	Inhibit viral replication *in vitro*.	Nuhu, 2013; Ramakrishnan, 2013
Ethanol extract	Astrovirus type 1, Coxsackievirus, Rotavirus Wa strain, Adenovirus type 7, Adenovirus type 40	Higher virucidal effect on RNA viruses than DNA viruses (*in vitro)*.	El-Baz et al., 2013
Aqueous extract	HIV-1	Inhibit viral replication in PBMCs, human T-cell lines, and Langerhans cells (*in vitro)*.	Ayehunie et al., 1998
Water extract	HSV-1	Inhibit viral replication and prolong the survival time of virally infected hamster.	Hayashi et al., 1993
Allophycocyanin	Enterovirus 71	Delay viral RNA synthesis and activate apoptosis (*in vitro)*.	Shih et al., 2003

Table 2 Antiviral properties of *S. maxima*

Compound name	Virus	Antiviral property	Authors
Hot water extract	HSV-1, HSV-2	Inhibit viral infection in Vero cells by interfering viral infectious cycle, adsorption and penetration.	Hernandez-corona et al., 2002
Methanol-water (3:1) extract	HSV-2	Block viral infectious cycle at adsorption and penetration stages.	Hernandez-corona et al., 2002
L-AsnA	CSB3 virus	Inhibit viral replication cycle.	Abd El-Baky & El-Baroty, 2020

3 Immunostimulant effects

Numerous *Spirulina* supplements can be found in the market nowadays as they contain high nutritional values and are reported to exhibit immune-stimulating properties (Jung et al., 2019; Singh et al., 2020). *Spirulina* was found to activate macrophages, natural killer (NK) cells, T-cells, and B-cells (Nuhu, 2013; Singh et al., 2020), and further enhance immunity by increasing the production of antibodies, interferon-gamma (IFN-γ), and cytokines (Nuhu, 2013; Banakar et al., 2020).

Natural substances isolated from *Spirulina* are reported to be effective inhibitors against enveloped and non-enveloped viruses by interfering or blocking the adsorption and penetration of virus, and also inhibit viral replication in the host cells (Singh et al., 2020). Populations in Japan, Korea, and Africans at Chad area that consume *Spirulina* daily with an average of 3-13 g, reported to have lower cases of HIV and acquired immune deficiency syndrome (AIDS) as compared to the other populations that do not take *Spirulina* diet (Teas et al., 2004).

The HIV/AIDS prevalence of algae-consuming populations in Eastern Asia (Japan and Korea) is about 1/10,000 adults, as compared to Africa which has a high prevalence of 1/10 adults (Teas et al., 2004).

Conclusion and recommendation for future research

It is evident from the literature that *Spirulina* has high nutritional values and provides a wide range of nutritional and health benefits. Numerous research studies have proven that the extracts from *S. platensis* and *S. maxima* can inhibit the spreading of enveloped and non-enveloped viruses in host cells. Besides, the immune-stimulating effects of *Spirulina* products on human health offer *Spirulina* to be a potential therapeutic supplement. Further research is needed to determine its' usefulness against different viruses and unlock its potential. The multifunctional role of *Spirulina* makes it as an ideal natural drug with immense prophylactic and therapeutic properties.

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ANTIVIRAL PROPERTIES OF MICROALGAE AND CYANOBACTERIA

Manishaa Sri Mahendran[1], Sinouvassane Djearamane[2], Ling Shing Wong[3], Govindaraju Kasivelu[4], Anto Cordelia Tanislaus Antony Dhanapal[1*]

[1]Department of Chemical Science, Faculty of Science, Universiti Tunku Abdul Rahman, Kampar, 31900 Malaysia;
[2]Department of Biomedical Science, Faculty of Science, Universiti Tunku Abdul Rahman, Kampar, 31900 Malaysia
[3]Life Science Division, Faculty of Health and Life Sciences, INTI International University, Nilai, 71800 Malaysia;
[4]Centre for Ocean Research, MoES - Earth Science & Technology Cell (Marine Biotechnological Studies), Sathyabama Institute of Science and Technology (Deemed to be University), Chennai, 600119 India.

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ABSTRACT

The recent outbreak of Corona Virus Disease (COVID-19) and the surge in accelerating the development of a vaccine to fight against the SARS-CoV-2 virus has imposed greater challenges to humanity worldwide. There is lack of research into the production of effective vaccines and methods of treatment against viral infections. As of now, strategies encompassing antiviral drugs and corticosteroids alongside mechanical respiratory treatment are in practice as frontline treatments. Though studies have reported that microalgae possess antiviral properties, only a few cases have presented the existence of antiviral compounds such as algal polysaccharides, lectins, aggluttinins, scytovirin, algal lipids such as sulfoquinovosyldiacylglycerol (SQDG), monogalactosyldiacylglycerides (MGDG) and digalactosyldiacylglycerides (DGDG), and algal biopigments especially chlorophyll analogues, marennine, phycobiliproteins, phycocyanin, phycoerythrin and allophycocyanin that are derived from marine and freshwater microalgae. Given the chemodiversity of bioactive compounds from microalgae and the present scenario, algal biotechnology is seen as a prospective source of antiviral and anti-inflammatory compounds that can be used to develop antiviral agents. Microalgae with potential as antivirals and microalgae derived functional compounds to treat viral diseases are summarized and can be used as a reference in developing algae-derived antivirals to treat SARS-CoV-2 and other similar viruses.

* Corresponding author
E-mail: antoc@utar.edu.my (Anto Cordelia Tanislaus Antony Dhanapal)

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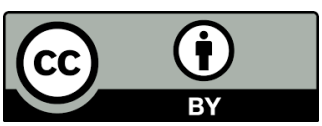

1 Introduction

Research activities to classify compounds used to prevent and treat severe and acute viral infections are now a priority and society has been hesitant and unable to seek scientific solutions (Liu et al., 2020). The complex life cycle and the distinctive nature of the viruses have made it highly difficult to discover definite remedies against viral infections (Murrell et al., 2011).

A variety of diseases, such as hepatitis C (HCV), human immunodeficiency virus (HIV) and dengue virus (DENV) still affects the majority of people of the world's population over decades, despite comprehensive studies of effective vaccines and viral infection treatment over the last century (Graci & Cameron, 2005).

To date, the development of vaccines against certain viruses such as HIV and HCV has proven to be an intractable strategy and specific vaccine are not found against many common viral infections, including herpesviruses (Ahmadi et al., 2015) and respiratory tract viruses such as Severe Acute Respiratory Syndrome and Middle East Respiratory Syndrome.

Besides, drug resistance of various viruses (HIV-1 and HSV), to available antiviral agents has often been a significant obstacle for antiviral medications (De Clercq, 2002).

The complex aquatic ecosystem has given the world of the marine environment a range of algae from micro-organisms to giant algae. Microalgae and cyanobacteria have been used for millennia as food in ancient Asian, African, and South American cultures. However, only by the middle of the past century biotechnology for microalgae begin to evolve (Mendes et al., 2003).

Microalgae and cyanobacteria provide immense prospects in industries such as pharmaceuticals, foodstuffs, or cosmetics for isolating natural substances of considerable commercial interest. Further, numerous research studies have evidenced the antiviral property of microalgal compounds (Mayer & Hamann, 2005) that proposes microalgae as a potent source for a natural antiviral agent. This review article summarizes the antiviral activities of the purified biomolecules from the microalgae.

2 Algae derived biomolecules as antiviral agents

2.1 Polysaccharides

Research by Gerber et al. (1958) found that an important source of antiviral agents with polysaccharides was the inhibition of influenza B viruses by marine algae polysaccharides. Anti-viral activity of other red algae-insulated polysaccharide fractions against HSV and HIV-1 have been recorded over the past two decades. Since then, several studies have shown that some algae-driven polysaccharides have antiviral potential and underlying action mechanisms as shown in Table 1.

The sulphated exopolysaccharides of marine microalgae such as *Porphyridium* sp. and *Cochlodidium polykrikoides* are found to interact with certain enveloped viruses like HSV and HIV, preventing them from reaching the host cells (Amaro et al., 2011) and also exhibited antiviral action against type 1 and 2 HSV in both *in vitro* and *in vivo* rabbits and rats (Huleihel et al., 2001). The antiviral property of the polysaccharides from *Porphyridium* sp. was due to the neighboring attachment of HSV-1 particles to polysaccharides (Batinic & Robey, 1992). Also, naviculan is an extracellular sulphated polysaccharide produced by *Navicula directta* diatom. The naviculan contains galactose, rhamnose, xylose, fucose, sulfate, and manna that reported to have an antiviral effect on HSV-1and HSV-2 (IC_{50} range 7–14µg / mL). Besides, naviculan hinders influenza viruses at the early stages of virus-related response, possibly preventing the virus from entering the host cells (Lee et al., 2010).

Cyanobacteria contain vital antiviral compounds. *Arthrospira platensis* is a blue-green alga that consists of exopolysaccharides. This exopolysaccharide has an antiviral activity for Koi Herpes Virus (KHV), which is accountable for significant economic losses in common carp and koi (Reichert et al., 2017). Polysaccharides enriched fraction at 18 to 36 µg/mL inhibits the replication of the virus (Haslin et al., 2001). *S. platensis* extract is known as calcium spirulan (Ca-SP) which is a form of sulphated polysaccharide. Ca-SP consists of ribose, mannose, galactose, fructose, and rhamnose which will intrude the replication of both enveloped (HIV, influenza A) and non-enveloped (HSV, polio, cytomegalovirus) viruses (Takebe et al., 2013).

2.2 Proteins

Proteins with antiviral activities are also produced by various species of cyanobacteria and microalgae. Glycoproteins or carbohydrate-binding proteins are known as lectins.

Lectins can bind with carbohydrates and carbohydrate moieties of glycoconjugates. Different lectins that exert anti-HIV activity by attaching strongly with carbohydrate moieties on the glycosylated HIV envelope have been identified over the last decade (Huskens & Schols, 2012).

HIV shows a mannose-rich glycoprotein gp120 on its surface envelope which is essential for the virus to bind to the target cells' cellular receptor CD4 (Tiwari et al., 2009). Besides this, there is a strong antiviral activity produced by *Scenedesmus obliquus* hydrolysates Sd, Sd1 and Sd2 against Coxsackie virus B (Afify et al., 2018).

Table 1 Polysaccharides from microalgae and cyanobacteria having antiviral activities

Compounds	Microalgae	Viruses	Authors
Sulfated polysaccharide	*Navicula directa*	Influenza-A	Ahmadi et al., 2015
Highly sulfated polysaccharide	*Porphyridium cruentum*	HSV-2, HSV-1, Vaccina	Huang et al., 2007
Sulfated exopolysaccharide	*P. purpureum*	Vaccina	Radonic et al., 2010
Sulfated polysaccharide	*Porphyridium cruentum*	Varicella zoster virus	Huleihel et al., 2001
Exopolysaccharides	*Rhodella reticulata*	Murine sarcoma and leukemia viruses	Talyshinsky et al., 2002
Extracellular sulfated polysaccharide	*Cochlodinium polykrikoides*	Influenza , parainfluenza-2	Hasui et al., 1995
Calcium spirulan	*Arthrospira platensis*	measles,influenza	Radonic et al., 2010
Nostaflan	*Nostoc flagelliforme*	Influenza A virus	Goss & Jakob, 2010
Carrageenan	*Gigartina skottsbergii*	Influenza virus, HSV-1,DENV, HSV-2, , HIV	Dye, 2014
Galactan	*Cryptonemia crenulata*	HSV-1, HSV-2, HIV-1, HIV-2, DENV	Delattre et al., 2011
Alginate	*Macrocystis pyrifera*	HIV	Zheng et al., 2020
Fucan	*Adenocytis utricularis*	HSV-1, HCMV, HSV-2, HIV-1	Salehi et al., 2020
Laminaran	*Ascophyllum nodosum*	HIV	Edwards et al., 2019

2.3 Lipids

Many algal lipids have exhibited antiviral activity to a lesser degree relative to polysaccharides and proteins. Compounds such as sulfoquinovosyldiacylglycerol (SQDG) and monogalactosyldiacylglycerides (MGDG) are primarily sulfolipids and glycolipids (Buck et al., 2006). The high anti-HIV activity was detected in *Lyngby alagerheimii* and *Phormidium tenue's* SQDG (Gustafson et al., 1989). *Spirulina*'s methanol extract with an IC_{50} value of 25.1µg/mL has antiviral activity against HIV-1 (Zalah et al., 2002; Yim et al., 2004; Li et al., 2008).

2.4 Pigments

Different pigments in microalgae and cyanobacteria have shown various biological activities. For example, chlorophyll analogs in *Dunaliella primolecta* (Ohta et al., 1998) have demonstrated anti-HSV activity. *Haslea ostrearia* is a marine diatom that produces blue pigment, a water-soluble fraction containing marennine (EC_{50} value of 14 µg/mL), was able to inhibit HSV-1 replication in vitro cells. Additionally, this element prolonged the development of syncytia caused by HIV-1 on cells of MT2 (Kamat et al., 1992; Shih et al., 2003). Phycobiliproteins, the natural coloring in food and pharmaceutical products, are the key photosynthetic additive in cyanobacteria and red algae. The two most commonly known phycobiliproteins are *Arthrospira* phycocyanin and *Porphyridium* phycoerythrin. They have reported showing antiviral properties that turned them into a promising material for health applications. Table 2 states the commercial application of the algae and cyanobacteria derived antivirus compounds.

Conclusion

Various biological activities, including the transcendent antiviral effect, have been documented for algal purified molecules. Numerous pharmacological studies have been only conducted in host cells as *in vitro* studies. Nevertheless, *in vivo* studies are essential to promote the usage of microalgal products in the pharmaceutical industry as effective anti-viral agents. Additionally, new experiments to explore the antiviral activities against infectious viruses, including clinical trials, are another area for further research.

Table 2 Commercial application of the algae and cyanobacteria derived antivirus compounds

Microalgae	Commercial Supplement	Brand	Benefits	Authors
Spirulina	Apogen Children Granules	Febico	Flu, influenza, enterovirus, virus respiratory syncytial and dengue virus	Febico, 2021
Gigartina papillata	Red Marine Algae Plus	Pure Planet	Oral herpes, shingles,genital herpes, mononucleosis, HIV, and influenza	Kelly Harrington, 2020
Aphanizomenon flosaquae	Blue-Green Algae	Source Naturals	HIV/AIDS	Zizzo et al., 2020
Spirulina	Apogen capsules	Febico	Flu, influenza, enterovirus, dengue virus, and respiratory syncytial virus	Febico, 2021

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NUTRACEUTICALS AND FUNCTIONAL FOODS, A STRATEGY TO EMPOWER WOMEN'S HEALTH

Neevitha Arunprabhu*

RASS Academy College of Nursing, Sivagangai, Tamilnadu,

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ABSTRACT

While healthy, balanced, and natural food meets the nutritional requirements of any healthy person, there are people, particularly women who need to be provided with supplements. Pregnant and lactating women, those with pre and post-menopause issues, and women with special dietary needs like sportswomen, models, etc., fall in this category. There is also a major issue of malnutrition endemic among a vast section of the society which, on account of the prevalent social mores and practices, impacts women more profoundly than the menfolk. On account of various sociological and economic factors, the special nutritional needs of women cannot be properly addressed through the normal consumption habits and nutraceuticals come as an effective solution to address this issue. Though not acknowledged to the extent it deserves, the nutraceuticals do play a decisive role in combating many life-threatening diseases like Cardio Vascular Diseases, Obesity, Hypertension, Arthritis, etc., both as an effective prophylactic as well as a curative aid to the mainline treatment. Known benefits of a carefully calibrated regimen of nutraceuticals are general performance enhancement, stress relief, increase in memory, immunity boosting, etc., Considering that women shoulder more than their share of family responsibility, women stand to be the main beneficiaries of such a health regimen, and in this sense, the nutraceuticals contribute to a considerable extent to the empowerment of women.

* Corresponding author

E-mail: nividasurendran@gmail.com (Neevitha Arunprabhu)

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1 Introduction

Women's health is a major concern in any society for several reasons. Women who constitute nearly half the population, during their life cycle, undergo many physiological changes that call for special attention to them not only in terms of medical care but also of nutritional requirements. But the fact of the matter is that a large section of the women population only has a secondary claim on the family resources mainly because their immense contribution to the economic activities goes unrecognized. Consequently, their deprivation level in terms of nutritional food is worse than that of men seriously impacting their health. Hence there is a strong case for various nutraceuticals or functional foods to fill the gap in nutrition and thus contribute to improving women's health and thereby empower them.

The other aspect of Women's Health focuses on the treatment and diagnosis of diseases and conditions that affect a woman's physical and emotional wellbeing and here also nutraceuticals have a major role to play. Nutraceutical as a food or part of a food that provides medical or health benefits which includes the prevention and treatment of disease (Bull et al., 2000). Functional foods have a potentially positive effect on health beyond basic nutrition (Zeratsky, 2018).

Apart from the adoption of preventive measures against diseases and malnutrition, a special drive to provide for instant nutrition by sustained awareness drive with special reference to functional foods and nutraceuticals is the need of the day. The potential health benefits of functional foods and nutraceuticals in fortifying women's health is too immense to be ignored. Goldberg (1994) specified that functional foods are any food with its positive impact on an individual's health, physical performance, or state of mind in addition to its nutritive values. The three basic conditions of functional foods to have functional health benefits such as follows:

- Presence of natural ingredients in the food
- Should be capable of adoption as part of the daily diet
- Should be able to regulate a particular body process, such as :
    a. Enhancement of the biological defense mechanism
    b. Prevention of a specific disease
    c. Recovery from a specific disease
    d. Control of physical and mental conditions
    e. Slowing the aging process

Nutraceuticals typically include food products like dietary fiber, prebiotics, probiotics, polyunsaturated fatty acids, antioxidants, and other different types of herbal /natural foods (Das et al., 2012). Women are identified as one of the most important consumer groups for functional foods as they need to face specific health issues and meet the nutritional needs during pregnancy, the postpartum period, and menopause (Carabin, 2006). This review study emphasizes the importance of women's health with a report on various review articles based on functional foods and Nutraceuticals. It is intended to create better awareness among women on the efficacy of various food products and the different options available for them to meet their specific requirements and thus help them become wise consumers.

This review paper summarizes the specific needs of women and the properties of functional foods and Nutraceuticals to reduce the risk of various diseases. The current research works and scientific evidence found available in recent journals and review articles provide data to analyze and understand the health benefits of functional foods for women. The main objective of the present paper is to impart nutrition education for women and encourage future studies on functional foods and Nutraceuticals.

2 Empathy: Health Promotion for Women

The process of health promotion enables people to improve their health and thus lead a more fulfilling life as part of the empowering process. The positive measures to strengthen women may suggest the following approaches:

2.1 Recommendation for healthy living

While addressing the specific health and fitness at different stages of women's life, we need to adopt healthy living by

- Planning a healthy diet(with fruits and vegetables, foods low in saturated and trans fats)
- Regular exercises
- Maintenance of healthy body weight
- Avoiding alcohol and smoking

People who are more physically active, with improved lifestyle changes can focus on the right energy balance and lead a healthy living (Nandakumar & Swarajyalakshmi, 2010).

2.2 Identification of vulnerable group

Nearly 85% of menstruating women (between the age group of 20-40 years) are experiencing one or more symptoms of Pre Menstrual Syndrome (PMS). The major symptoms of PMS which last for almost two weeks include mental and physical responses to hormonal imbalances (Alliance Integrative Medicine, 2020). Women at home are likely to suffer from overeating and hence they are subject to the risk of becoming overweight. Meal planning with less food wastage can avoid the consequences of "left-overs" (Anderson, 1985). The natural and preventive approach of women to manage the physiological changes and challenges identifies Nutraceuticals as a valuable tool in promoting better health (Olivo, 2016).

2.3 Concern on risk factors

Overweight women are particularly susceptible to cancer of the uterus, gallbladder, ovary, and breast (Antia & Abraham, 2005). Lack of motivation and enthusiasm prevents women from maintaining healthy body weight. But the combination of a sensible diet and regular exercise can help tackle the issue of overweight (Jindal, 2015). Women are unaware that micronutrient deficiencies can persist for an extended period in their life and need to be accordingly taken care of by proper nutrition supplements. The majority of the female population is susceptible to diseases such as anemia, osteoporosis, obesity, and depression during post menopause stage (Akkawi & Zmerly, 2018).

The secondary metabolites of functional foods and the cumulative effect of several components of Nutraceuticals may aid in the prevention and treatment of various diseases

Anemia among arthritic patients due to disease conditions as well as improper iron absorption results in women suffering more severe illness than men (Damle, 2005).

Women tend to have smaller and lighter bones than men. The low bone mass and menopause affect their bone health; this may lead to an increased risk of developing osteoporosis (Halvorsen, 2016). Table 1 lists the health benefits of various functional foods and Nutraceuticals for women indicating the area of their utility (Aoi et al., 2006; Rudkowska, 2008; Iron deficiency occurs in women due to inadequate intake of iron-rich foods. Moreover, menstruation increases the risk of having low body stores of iron (The Irish Nutrition & Dietetic Institute, 2015). Women are more prone to Urinary Tract Infections (UTI) during pregnancy and after menopause. About 50% of the women population develops at least one UTI during their lifetime (Naveen, 2011).

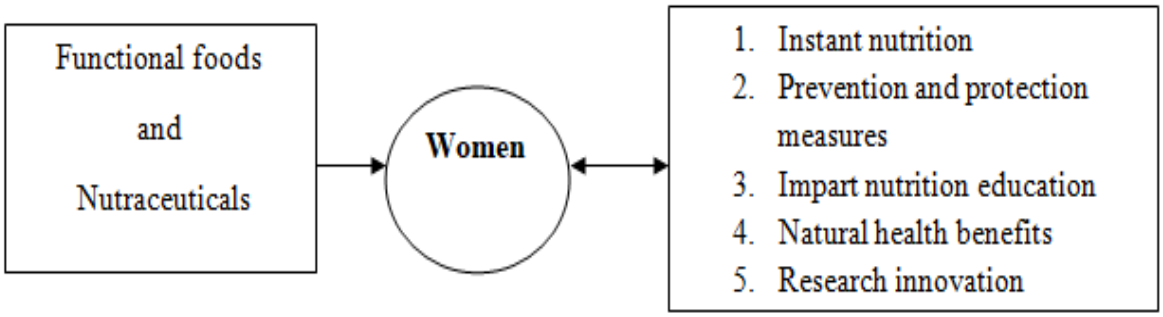

Figure 1 Schematic representation of functional foods and Nutraceuticals for women

3 Strategy: Functional foods and Nutraceuticals for Women

Any strategy to improve general health by necessity has to target women. Such an approach encourages them to consume highly fortified nutritional and functional snacks (Sloan, 2018).

It is advisable to gain nutritional knowledge about functional foods through gym instructors and nutritionists (Kapoor & Munjal, 2017). Figure 1 shows a schematic representation of Functional Foods and Nutraceuticals for women illustrating the strategy for improving women's health status.

Most of the degenerative types of arthritis like osteoarthritis, osteoporosis, and lumbar spondylitis intensify with weight gain. Sustainable health can be defined as "healthy and active aging avoiding the risk of diseases". Functional food markets with their products can contribute to sustainable health by protecting and improving public health (DelCastillo et al., 2018).

- Pomegranate Juice
- Citrus Fruits
- Avocado Milkshakes
- Berries And Cherries
- Tofu
- Sprouts
- Dates
- Almonds
- Milk And Its Products Like Yoghurt, Paneer
- Oats And Other Fortified Cereals
- Green Leafy Vegetables

4 Technology: Bioactive compounds and Biodiversity for Women

various illnesses. Chemoprevention uses natural or synthetic agents to reverse the process of carcinogenesis. Regular consumption of dietary phytochemicals for chemoprevention serves as an economical alternative to control cancer. Similarly, phenolic

compounds reduce the risk of cancer. Phytochemicals have a role in the regulation of body metabolism by

- Enhancing nutrient absorption
- Eliminating toxins
- Inhibiting the growth of harmful intestinal bacteria (Helal, 2019).

The polyphenols present in fruits, cereals, and vegetables are proven to be effective antimutagenic, anticarcinogenic, and antioxidants (Souyoul, 2018). Bioactive compounds are extra nutritional constituents present in small quantities in foods (Teodoro, 2019). The phenolics compounds of fruit residues like grape seed extract, anthocyanins of litchi pericarp, flavonoids in citrus peels, antioxidants of mango peels and stones provide a lot of scopes for exploring the therapeutic potential of Nutraceuticals by implementing low cost waste technology in agribusiness (Babbar et al., 2013).

The therapeutic capability, potential nutrients, and safety profile of nutraceuticals may be used to improve health, delay the aging process and prevent chronic diseases (Nasri et al., 2014). Any scheme aimed at making available essential nutrients including nutraceuticals and functional foods to all those who need them should have as part of its strategy the following components:

- Biodiversity/Conservation measures to ensure that there is no shortage in the supply of these vital health inputs.
- Sustained awareness drive focusing on the prevalence of poverty, malnutrition, and hunger and the measures required to be undertaken at all levels to overcome them.
- The 2030 agenda for sustainable development is achieved by dovetailing various local developmental activities to the former.
- Research and Development in the area of harnessing the biological significance of active chemical components of food by the rapid analytical methods.

Table 1 Health benefits of functional foods and Nutraceuticals for women

Functional Foods and Nutraceuticals	Properties	Reduces risk of
• Soy proteins and isoflavones • Omega 3 fatty acids • Plant sterols • Prebiotics and probiotics • Fiber-rich foods • Colored fruits • Pumpkin Seeds, Flax seeds, walnuts • Avocado • Sweet potatoes • Calcium, Iron, and Magnesium-rich foods	• Anti-inflammatory • Anti-oxidant • Immune modulation • enhances muscle strength • reduces lipid levels • improves bone health • increases memory • Anti-carcinogenic • Antimicrobial	• Cardio Vascular Diseases • Diabetes • Obesity • Hypertension • Arthritis • Cancer • PMS • UTI

5 Discussions

Functional Foods and Nutraceuticals with their significant health benefits enrich women's health in all the stages of life and significantly contribute to an enhanced quality of life. Though there is a growing case to support even more extensive use of functional foods and Nutraceuticals, there is also a need to carry out more research in understanding their mechanism on our body, especially of women. The health status of women can be improved with immune boosters, natural energizers, and antioxidants. However, the conflicting results on the effect of antiestrogenic soy isoflavones, additional techniques for vitamin D, and calcium absorption (fortification) are some of the areas which are crying for immediate resolution. Poor bioavailability of some essential nutrients and the low level of active ingredients in some of them poses a challenge of an altogether different kind.

Conclusion

Functional foods and Nutraceuticals play a central role in the fortification of women's health both as food supplements and therapeutic inputs. There is a need to devise a sustained action plan

that focuses on all essential areas like research, awareness, marketing, and accessibility. The important link is to make sure that women are integrated into the program of social welfare and community development that focuses on practical skills that make them aware of their own needs and responsibilities.

Making better women with good nutritional status and supportive health care at all stages of life is an essential but not necessarily an easy task. Recent studies on functional foods and Nutraceuticals have concentrated on encouraging women to improve nutritional knowledge. This helps in the maintenance of normal wellbeing by reducing the risks of various disease conditions.

The study on biological properties of fruit residues such as anticarcinogenic, antimutagenicity, and antiaging activity has opened new vistas in the area of the development of high-quality food products. Simple dietary advice by a qualified nutritionist for the consumption of functional foods and Nutraceuticals can be a gamechanger in transforming women's health. This paper can serve as a survey article for students to pursue their research.

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IMPORTANCE OF NUTRITION ASSESSMENT IN CRITICALLY ILL PATIENTS

V. Suganya[1]*, Vasantha Esther Rani[2]

[1]Madurai KamarajUniversity, Madurai, India
[2]Head of the Home Science Department, Fatima College, Madurai, India

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Subjective Global Assessment

Acute Physiology and Chronic Health Evaluation

Body Mass Index

ABSTRACT

A timely assessment of nutritional status in critically ill patients is crucial. This was an observational prospective hospital study carried out for 3 months from December 2019 to February 2020 in an ICU in a multispecialty hospital in Madurai, Tamilnadu, India. All adult patients with ≥ 72 hours of ICU-LOS were included and their nutritional assessment was completed by SGA (Subjective Global Assessment) forms within 24 hours of ICU admission. Data collected regarding variables such as Age, Nutritional status, APACHE IV, SOFA score, presence of Comorbidities. All patients were studied about their duration of stay in ICU and hospital, Predicted Mortality rate, & discharge outcome.120 patients were involved in the study in the age 62. 2 ± 14.years, among this 55.3% were males and rest 44.7% were females. Their complete nutritional assessment revealed that 50%, 43%, and 17% were well nourished, moderately malnourished, severely malnourished respectively. A strong correlation was observed between nutritional status, morbidity, and their prolonged stay in ICU. Mean ICU & hospital mortality percentage was 37%, 45% respectively. The average duration of stay in the ICU and hospital was 13, 20 days respectively. Participants anthropometric data decreased considerably (mean weight, MUAC, calf circumference) during ICU discharge day. BMI comparison of admission and discharge didn't show significant change. Timely nutrition intervention and medical nutrition therapy reduce the duration of patient stay in ICU and hospital and improved patient outcomes. The disease severity scoring system can be used as guidance for objective assessment of disease outcomes and estimation of the chance of recovery.

* Corresponding author

E-mail: suganya101973@gmail.com, suganya_v@apollohospitals.com (V. Suganya)

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1 Introduction

Malnutrition is a global health problem in hospitalized patients, especially within the critical care setting. Especially for critically ill patients, malnutrition is noted as a major consequence of hospitalisation. Hyper metabolism in patients increases nutrient requirements but the patient's energy and protein intake were considerably less leading to malnutrition. Many research with critically ill patients showed that malnutrition before admission to the ICU leads to the diminished status of macro and micronutrients in their bodies. Nutritional Assessment on admission is the first and foremost step in a nutritional intervention which will help in reducing the duration of ICU and hospital stay and appropriate nutrition therapy prevent complications and infections thereby mortality risk will be reduced.

In critically ill patients, SIRS (systemic inflammation response syndrome) impacts a catabolic state that increases metabolic demand and accelerates the development of malnutrition (Heyland et al., 2011; Blaser et al., 2017). This study was conducted to assess the nutritional status of critically ill patients on ICU admission and discharge day through various clinical indicators and to highlight the importance of nutritional assessment for critically ill patients. Further, this study also correlated the mortality prediction score APACHE IV, SOFA scores on admission with patient's duration of stay in ICU and hospital along with patient outcome (Hopkins et al., 2017). The SOFA score is considered as an important tool in defining both the clinical condition of the individual patient and the response to therapies in the context of clinical trials (Simon Lambden et al., 2019). ICU patients were assessed with various clinical indicators to categorise their nutritional status. The anthropometric indices include BMI (body mass index), MUAC (mid upper arm circumference), and calf circumference. The body composition markers and biochemical investigations comprise visceral protein, total protein, albumin, and lymphocyte. A clinical questionnaire called SGA (Subjective global assessment) was applied in the study to analyse the participant's level of nutritional status. The SGA form widely accepted tool for identifying hospitalised patient's nutritional status including critically ill patients (Hopkins et al., 2017). Subjective global assessment (SGA) is an inexpensive, and quick method that can be conducted at the bedside. It is a reliable tool for inferring outcomes in critically ill patients (Herridge et al., 2016; McClave et al., 2016; Mehta et al.,2018).

2 Materials and Methods

2.1 Study Design

This was a hospital based prospective study duration, which was conducted for 3 months from December 2019 to February 2020, in a multidisciplinary ICU with a bed capacity of 21 beds in India, Tamilnadu, Madurai. All adult patients admitted in ICU with ≥ 72 hours of ICU-LOS were included and their nutritional assessment was completed by SGA (Subjective Global Assessment) forms. This process was completed soon after their ICU admission (within 24 hours). Reassessment was done once in seven days and during discharge and this was utilized for monitoring and evaluation of patient nutritional status.

2.2 Study Population

The sample size for the study was derived as 120 critically ill patients. The formula used for the study was n = Z2 (SD2/d2), taking a value of Z = 1.96 with 95% confidence intervals, and d = 0.7 as the margin of error in estimating the mean or effect size. A total of 325 patients admitted to the multidisciplinary ICU were screened but only 120 patients were in the eligibility criteria. The inclusion criteria considered as 18 years of age, adult patients with ≥ 72 hours of ICU-LOS were included and other patients received from other hospitals ICU'S were excluded from the study. Approval obtained from Clinical Research and Institutional Ethics Committee of multispecialty hospital IEC application number - ASH/ACAD-004/12-19, as per the requirement of the ethics committee informed consent was obtained from the patient's caretaker who admitted the patient.

2.3 Data Collection

Participant's unique ID number noted and demographic and clinical details collected along with, admission diagnosis, age, sex, category of admission - medical/surgical were noted within 24 hours of ICU admission. In this study mortality risk assessment score namely APACHE IV Score (Acute Physiology and Chronic Health Evaluation) (Chakravarty et al., 2013; Lew et al., 2017) was employed, SOFA (Sequential Organ Failure Assessment) score was used to assess the disease severity of the patient. GCS (Glasgow Coma Scale) marked to analyze the conscious level of the patient, all these assessments were computed for every patient in the hospital during ICU admission and discharge days. On the day of ICU admission and discharge anthropometric indices were collected, for every patient in which height measuring ulna length and weight calculated from the patient's height. BMI was computed by using the formula weight in kg divided by height in meter square. MUAC (Mid-upper-arm Circumference) and calf circumference were measured as shown in Figure: 1.Public Health sponsored study acknowledged that MUAC has been used for evaluation of adult nutritional status as well, especially in resource-limited settings, including India. MUAC can be an efficient indicator of adult undernutrition—comparable or even better than BMI (Das et al., 2020).

SGA questionnaire was used for every patient on the date of ICU admission and discharge. SGA is a widely accepted nutritional

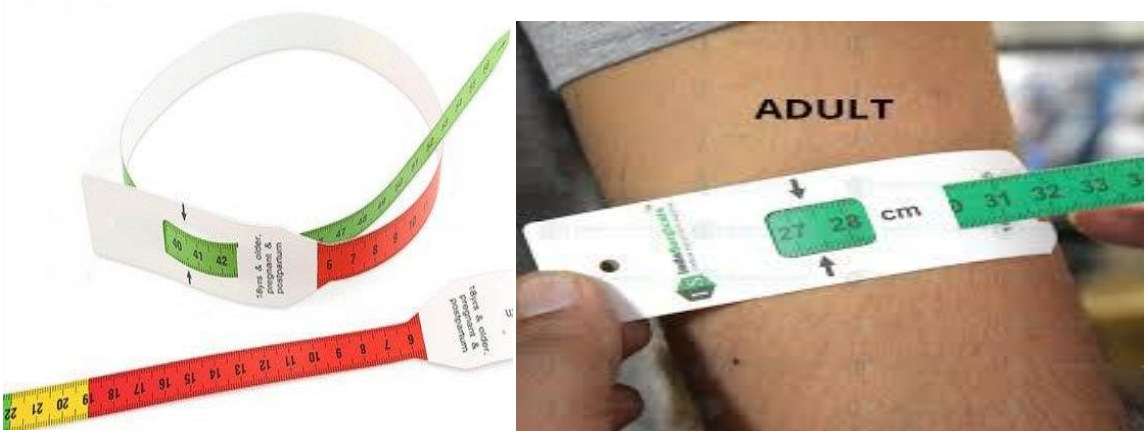

Figure 1 MUAC TAPE Colour Indicator tape

Table 1 BMI and MUAC Comparison

	Corresponding BMI category			
	$<17\,kg\,m^{-2}$ (moderately underweight)	$<18.5\,kg\,m^{-2}$ (underweight)	$\geq 25\,kg\,m^{-2}$ (overweight)	$\geq 30\,kg\,m^{-2}$ (obese)
MUAC (cm)				
Men	22.9	<23.9	≥28.1	≥31.4
Women	22.9	<23.9	≥28.4	≥31.8

Source: researchgate.net

assessment tool. It includes details such as weight-loss history, diet intake pattern, GI (gastrointestinal) symptoms, functional capacity, and comorbidity details. This questionnaire also indicates details related to muscle wasting and wastage of subcutaneous fat. All mentioned data were entered to finalize the score as A, B, or C an indicator of the degree of malnutrition. Score A indicates well nourished, B denotes mild to moderate malnutrition, and C indicates severe malnutrition On ICU admission and discharge date biochemical indices such as total protein, albumin, phosphorous, magnesium, calcium, and TLC -total lymphocyte count were collected (Auto analyzer was used).

Important details like ventilator-free days and duration of ICU / hospital stay (in days) was recorded and compared with APACHE IV SCORE (predictors for mortality rate and length of ICU stay).

2.4 Statistical Analysis

Statistical package for social sciences was used (SPSS version 19) for statistical analysis. The data were represented as mean and standard deviation. The comparison of data between admission and discharge were done by a paired t-test. The SGA distribution was compared by using the sign test. The duration of ICU/hospital, Ventilator free days, and malnutrition status analysis related to ICU admission and discharge day was compared by using Pearson's Coefficient correlation $P \leq 0.05$.

3 Results

Complete data collection was carried out for the 325 ICU admitted patients for 3 months, among this finally 120 eligible patients' results were presented in this observational study (Table 2).

In table 2, the Patient's age, sex, and admission category were given, it revealed that the average patient's age was 62.2±14 years, among these 55.3% were males, and the rest 44.7% were females. Further, among the selected respondents, 52.8% were admitted for medical management and 47.2 % for surgical intervention.

Results given in table 3 provides patient admission diagnosis among the study population. Among the diagnosis, the highest number of patients was reported for Trauma cases (%), this was followed by Neurological disorders. While the lowest patients number was reported for Sepsis (5%), Renal (5%), and Orthopaedic. Mortality and disease severity score was provided in table 4. The patient's clinical outcomes were shown in this table 5.

Table 2 Age, Sex & Admission category of the patients

Variable	N, 120Mean±SD or n (%)
Average Age (y)	62.2±14.1
Male	55.3%
Female	44.7%
Admission Category:	
Medical	66 (52.8%)
Surgical	59 (47.2%)

Table 3 Diagnosis of the selected Patient during ICU admission

Diagnosis	Patients (%)	Diagnosis	Patients (%)	Diagnosis	Patients (%)
Cardiovascular	12(6.6%)	Gastrointestinal	11(9.2%)	Renal	6(5%)
Neurological	22(26.6%)	Orthopaedic	2(1.6%)	Respiratory	14 (11.7%)
Trauma	40(33%)	Sepsis	6(5%)	Cancer	7 (5.8%)

Table 4 Mortality and Disease severity score

Variable	N, 120Mean±SD or n (%)
APACHE[†] IV score	70 ±21
Admission SOFA score	8.4 ±2.8
Admission GCS	7±3.2

Here, APACHE (Acute physiology and chronic health evaluation); SOFA (Sequential organ failure assessment); GCS (Glasgow coma scale)

Table 5 Clinical and Mortality related patient outcome details

	Mean±SD, Median (IQR)
Duration of ICU stay (d)	13 (7-22)
Duration of hospital stay (d)	20 (12-39.5)
Mortality in ICU (n, %)	37 (29.6%)
Mortality in hospital (n, %)	45 (36%)
Duration of ventilator-free days (d)	4 (0.5-10)

IQR: Interquartile range; SD: Standard deviation; ICU: Intensive care unit

Table 6 Anthropometric Measurements

Measurements	Admission day	Discharge day	P value
Weight (kg)	63.4±12.7	56.6±11.6	<0.001[‡]
BMI (kg/m^2)	25.7 (23.3-28.8)	22.8 (21.3-26.5)	0.088
MUAC (cm)	30.1±4.5	27.6±4	<0.001[‡]
Calf circumference (cm)	32.2±3.8	29.1±3.7	<0.001[‡]

BMI: Body mass index; MUAC: Mid-upper-arm circumference

Table 7 Subjective Global Assessment Percentage (SGA %)

SGA (n, %)[†]	Admission day	Discharge day
A (Well nourished)	60 (50%)	45 (37.5%)
B(Moderately malnourished)	43 (35.8%)	52 (43.3%)
C (Severely malnourished)	17 (14.2 %%)	23 (19.2%)

SGA: Subjective Global Assessment,

Table 8 Biochemical parameters on Admission and Discharge Day

	Admission day	Discharge day	P value
Total protein (g/dl)	6±1.05	6.2±1	0.968
Albumin (g/dl)	3.4±0.6	3.3±0.6	0.16
TLC	1242.5 (787.6-2099)	1279.2 (1042-1699)	0.146
Mg (mg/dl)	2.2±1.2	2±0.4	0.013‡
P (mg/dl)	3.5±1.2	3.3±0.7	0.72
Ca (mg/dl)	8.4±0.67	8.5±0.6	0.43

TLC: Total lymphocyte count; Mg: Magnesium; P: Phosphorous; Ca: Calcium

Table 9 SGA status correlation on ICU discharge day, admission day, and outcomes

Measurements on admission day	Correlation coefficient (Rho Spearman)	P value
Weight (kg)	−0.412	<0.001†
BMI (kg/m^2)	−0.426	<0.001†
MUAC (cm)	−0.459	<0.001†
Calf circumference (cm)	−0.481	<0.001†
Total protein (g/dl)	0.106	0.334
Albumin (g/dl)	0.132	0.221
TLC	−0.127	0.244
Mg (meq/l)	−0.059	0.622
P (mg/dl)	−0.021	0.852
Ca (mg/dl)	−0.179	0.062
Length of ICU stay (d)	0.328	0.002†
Length of ventilator-free days	0.288	0.11

†P≤0.05 was considered statistically significant

The mean duration of stay in ICU and hospital was 13 and 20 days respectively. On an average the patient's stay in ICU were 7.9 days and 27.4 days in the hospital. Ventilator free days were noted as 4 days (mean value taken). In addition to this ICU & hospital mortality percentage was indicated as Mean 29.6% and 36% respectively. Anthropometric details collected during admission and discharge days were provided in table 6. SGA on Admission and discharge were shown in table 7. A significant decrease was noted in the Nutritional status of the ICU patients. Nutrition status was inversely proportional with the duration of ICU stay. On ICU discharge day malnutrition scores increased significantly in comparison with admission-day scores. Biochemical parameters recorded on the admission day and discharge day were tabulated in table 8. Except for magnesium, the rest of the data did not change significantly during ICU stay but magnesium level decreased during ICU discharge day. Discharge day malnutrition score and duration of ICU stay showed a positive significant correlation (Table 9). No significant correlation was noted in the discharge day malnutrition details and ICU ventilator independent days.

4 Discussion

Malnutrition in critically ill patients during ICU stay had a strong association with increased risk of mortality and morbidity rate. As per the study, nutrition assessment revealed that the malnutrition percentage of ICU patients on admission day (50%) had increased significantly on discharge day (62.5%). As per the SGA study, ICU patient's malnutrition prevalence on admission day was 50.0% (35.8% & 14.2% were mildly-moderately and severely malnourished respectively) which significantly increased on discharge day and it reached 62.5% (43.3% & 19.2%). In the current research, the variation in the nutritional status of the participants is related to age, the previous health status of the patients, and presence of comorbidities, 41.2% were with a history

of hypertension and 48.5% were diabetic and 60.3% of participants were in the geriatric age group. All these factors like age, comorbidities, and health status hurt their nutritional status. In the present study, malnutrition is strongly associated with a longer duration of ICU/ hospital stay, and it is evident when data were compared between malnourished and well-nourished patients. Medical Nutrition therapy always plays an important role in patient outcomes and recovery. In critical care units the anthropometric measures, MUAC measurements were utilised for assessing adult malnutrition.

This study showed a significantly lower level of values such as mean MUAC, Calf circumference among mild/moderate and severe malnourished patients versus well nourished patients. Accordingly, Haung et al. (2000) a reduction in anthropometric indices during a patient's ICU stay (Mehta et al., 2018).

The patient's detailed assessment depicted that mean weight, calf circumference, and MUAC, had significantly reduced. Similarly, Nematy et al. (2012) findings showed a significant decrease in weight and MUAC readings among critically ill patients. This indicated a higher incidence of malnutrition among ICU patients (Mendes et al., 2017; Vallejo et al., 2017; Wischmeyer, 2017). Besides this study also highlighted a decrease in nutritional status on discharge day (table 7).

Further, the present study also revealed that the serum albumin levels were significantly lower in malnourished patients (mild or severe) when compared with well-nourished groups and this low albumin levels were associated with an extended period of hospital stay. In the present study, the overall mortality rate was 41.2% whereas mortality rate of 55.9% and 26.5% was observed among malnourished and well nourished group respectively which was significantly lower in well nourished participants. So malnutrition is strongly associated with negative outcomes of critically ill patients in the term of increased morbidity and mortality (Nicolo et al., 2016).

Binary logistic regression was performed with lower nutritional status detected by SGA data and it was an independent predictor noted with a negative correlation with mortality. It also clearly indicates the mortality risk was two times higher for a malnourished patient compared to well-nourished participant. The predicted mortality risk scoring - APACHE IV and SOFA scores correlated positively with the observed mortality rate and length of stay in ICU. Bendavid et al. (2017) study result well correlated with the present study that malnourished patient's mortality rate was higher compared with the well-nourished patients (Schiesser et al., 2009; Vallejo et al., 2017). During ICU stay the Biochemical data evaluation revealed a significant decrease in serum magnesium concentration. Hypomagnesaemia was considered as a single indicator of disease severity, morbidity, and mortality in ICU patients (Singer et al., 2019). The low magnesium level was prevalent due to poor nutritional status and low magnesium intake during hospitalisation.

In this study, critically ill patient's anthropometric indices (table 6) were considered as good predictors of nutritional outcomes (malnutrition severity scoring system) compared to patient's biochemical data (Table 8 & 9). Moreover, albumin level and SGA showed a significant correlation (Table 8). Thus, SGA analysis along with anthropometric parameters was a valuable and effective method in the evaluation of malnutrition status of hospitalised patients, especially for critically ill patients. The study limitation was that heterogeneous ICU patients with different medical or surgical problems were included in the study. However, these different types of ICU patients were intentionally involved in the study because the main aim of the study was to identify the malnutrition prevalence among critically ill hospitalised patients on admission and discharge days without considering the causes of their diseases.

Conclusion

To conclude, Subjective Global Assessment revealed that malnutrition on ICU discharge day increased to 57.32%. It was highlighted that nutritional assessment with detailed anthropometric details should be collected and SGA to be done for every patient entering into ICU. This initial approach paved the way to implement the timely nutritional intervention, and it reduced nutrition-related complications and resulted in an appropriate Nutrition care process.

The study clearly stated that prioritizing nutritional assessment early at ICU admission paved the way to the appropriate nutritional plans which minimize the impact of malnutrition on the patient's outcome and it helped to reduce the duration of ICU and hospital stay. SGA, timely nutrition intervention and medical nutrition therapy prevent hospital-acquired infection and thereby mortality rate reduced. The nutrition support team and Nutrition protocol implementation in all ICU is crucial and it is very important to achieve a positive outcome for a critically ill patient.

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Conflict of Interest: None declared.

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A NARRATIVE REVIEW ON MINDFULNESS PRACTICES IN OPTIMIZING PERFORMANCE AMONG SPORTS INDIVIDUALS

Vinodhkumar Ramalingam[1], Cheong Soon Keng[2], Poh Foong Lee[3*]

[1]Faculty of Health and Life sciences, INTI International University, 71800 Nilai, Malaysia.
[2]Faculty of Medicine and Health Sciences, University Tunku Abdul Rahman, 43000 Kajang, Malaysia.
[3]Lee Kong Chian Faculty of Engineering and Science, University Tunku Abdul Rahman, 43000 Kajang, Malaysia.

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KEYWORDS

Athletes

Depression

Mindfulness

Sports anxiety

Sports performance

ABSTRACT

Mindfulness practice has become an increasingly popular intervention in optimizing athletic performance in sports. Numerous studies have reported on applying mindfulness for improving the performance of various sports such as tennis, table tennis, shooting, cricket, archery, golf, running, hockey, swimming, and cycling. This narrative review addresses different existing mindfulness programs that enhance sports performance, the outcome measures of mindfulness therapy, and identifies the anxiety and depression that affect the performance of sports individuals. To cope with the issues, the efficacy of mindfulness in performance enhancement and future research directions on mindfulness needs attention.

* Corresponding author
E-mail: leepf@utar.edu.my (Poh Foong Lee)

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1 Introduction

The term mindfulness refers to the way that individuals pay attention to their own experiences from moment to moment with non-judgmental attitudes (Kabat-Zinn, 2009; Kabat-Zinn, 2012). Quite recently, considerable attention has been paid to mindfulness practice, it originated from Buddhist tradition where it developed through meditation and involves cultivating an ability to be non-reactive to thoughts, feelings, and body sensations (Mize, 2015). The mindfulness practice is an act of focusing or attention on being in the present moment, for instance by focusing completely on taking in its scent, warmth, taste, drinking a hot cup of tea, and removing overpowering emotions from the mind. The mindfulness model by Hölzel et al. (2011) specifies this underlying mechanism act as an active component in mindfulness practice where emotion regulation, perspective on the self, and attention regulation creates body awareness. According to Ludwig & Kabat-Zinn (2008), the mindfulness concept comprises five major characteristics which include the experience of being present, awareness, acceptance, attention, and the transformative process of being mindful and moving towards a more integrated way of living. By considering these characteristics, towards individual sports achievements attention is one of the important factors, that stimulate the inner thoughts and emotions of an athlete through mindfulness (Lutkenhouse, 2007).

Therefore, the purpose of this review is to identify the different types of anxiety and depression, which affects individual sports performance and the effect of various mindfulness approaches that enhancing sports performance. And to find the outcomes measurement tools applied in mindfulness therapy. The sports enhancement mindfulness programs were originated from various mindfulness methods like Mindfulness-based stress reduction (MBSR), Mindfulness-based Cognitive therapy (MBCT), Mindfulness – acceptance commitment (MAC), Mindful sports performance enhancement (MSPE), and Mindfulness Meditation Training for sports (MMTS) as shown in Table 1.

Table 1Diverse approaches in Mindfulness

Source	Mindfulness techniques	Duration of the intervention
Kabat-Zinn, 1990	Mindfulness-based stress reduction (MBSR)	Breathing and mindfulness techniques for 90 minutes in a week for 8 weeks
Segal et al., 2002	Mindfulness-based Cognitive therapy (MBCT)	Mindful attention, nonjudgmental acceptance of internal states, and commitment to achieving valued goals for 2 hours per week for 8 weeks
Gardner & Moore, 2004	Mindfulness – acceptance commitment (MAC)	Seven weekly meeting or modules includes in-session exercises and discussion on the skills being learned
Kaufman et al., 2009	Mindful sports performance enhancement (MSPE)	Mindfulness exercises, including walking meditation, adapted to participants' sport of focus weekly 90-minute for 4 weeks
Baltzell & Akhtar, 2014	Mindfulness Meditation Training for sports (MMTS)	6 weeks program consisting of two sessions (30 minutes) per week, and integrating mindfulness training and traditional psychology skills training (e.g., imagery and self-talk)
Pineau et al., 2014	Expand version of MSPE	Raisin exercise, the body scan, mindful breathing, the sitting meditation, mindful yoga, and the walking meditation 6 weeks program consisting of weekly 90-minutes group sessions and daily home practice

Mindfulness practice prepares an individual's brain prefrontal cortex to be calm and prepare the mind to stay focused by avoiding distractive thoughts and perform at the best to reduce the stress response in the brain (Kabat-Zinn, 1990). On the other hand, a higher level of mindfulness practice activates the insular cortex of the brain and brings an individual's attention inward (Huang, 2016), which calms the participant's mind by lowering the stress hormone (resting cortisol levels) (Rosenkranz et al., 2013). Further, Dr. Kristen Race, founder of "Mindful Life" reported that the awareness and recognizing of the body movements enhanced the association between the body and mind (Yeh, 2016). This would in turn help in sensing the physiological responses, like muscle tension or short breathing patterns that make an individual respond immediately with a conscious mind by aware of what is going on both physically and mentally (Race, 2014). From Denny & Steiner, (2009) findings among Stanford student-athletes (N=140) toward coping with performance hardship showed that internal factors like mindfulness, self-restraint, locus of control, and self-esteem were more potent, better account for happiness, and maintained throughout than the external factors (playing time, scholarship) that referring to the mindfulness model (Hölzel et al., 2011). These reports have shown that the mindfulness practice had brought more inner peace to calm the sports individuals.

2 Psychological status of an athlete

2.1 Common psychological challenges and Mindfulness effects

In sports, athletes generally engage with positive emotions like joy and happiness that brings in by their satisfying performance, whereas concurrently, they do experience negative emotions such as anger, distress, anxiety, over-arousal that may hurdle their way in performance. The right amount of good stress helps sports individuals to be prepare, focus, and perform their sports at an optimal level. Equally bad stress, distress may hinder athletes to accomplish a task. Besides, anxiety among sports individuals can be recognized in three levels, cognitive as a thought process, somatic as a physical response, and behavioral as patterns of behavior (Karageorghis & Terry, 2010; Weinberg & Gould, 2011). Additionally, a study by Elmagd (2016) indicates that the optimal positive emotions capable to initiate and maintain the required amount of effort to perform a task towards their success. As evidenced, Pre-competition stress (PCS) was considered common stress among athletes which usually distracts athletes from their specific sports task, fails to cope with the performance during the competition (Miles et al., 2016).

A study by Freeman & Rees (2009) suggested that among 118 high-performance male golfers proved that positive emotions such as excitement and happiness were associated with better sport performances, while negative emotions such as anger, anxiety, or shame were associated with poor sports performance. Another study by Lane et al. (2010) studied the association of emotions with optimal and dysfunctional performance among 284 volunteer students participants from various sports which includes track and field, soccer, rugby union, hockey, basketball, volleyball, cricket, netball, lacrosse, tennis, badminton, martial arts, golf, water polo, boxing, and distance running. Similar to the previous study, Lane et al. (2010) reported that positive emotions like happiness, calmness, and vigor were associated with optimal sports performance whereas negative emotions such as anger and confusion were associated with poor performance. Though these studies applied different emotional tools such as Stress Appraisal Measure (SAM), a 20-item self-reported questionnaire to report perception of support availability, emotional intelligence scale, and the 24-item Brunel Mood Scale (BRUMS) to test the participants' emotional state. However, both these studies reported positive emotions are the key factor that optimizes sports performance (Denny & Steiner, 2009; Lane et al., 2010).

The response of 4-weeks Mindfulness Meditation Therapy (MMT) on elite shooters showed an effect on HPA-Axis by decreasing the level of salivary cortisol among experimental participants (n=48) as a reliable physiological marker in PCS compared with the control group (n=48) (John et al., 2011). Another study on junior elite soccer players (n=41) showed that 67% of the players in the mindfulness-based intervention remained injury-free and their attention level possibly decrease their injury risks (Ivarsson et al., 2015). The effectiveness of mindfulness was measured with the psychological instrument by using the Mindful Attention Awareness Scale (MAAS) on anxiety and sports performance training techniques among badminton players (n= 40). The results showed a reduction in post-test anxiety as compared to the pre-test results. Besides, the mindfulness scores among players showed improvement in their awareness and enhanced athletic performance (Moghadam et al., 2013). The authors found the impact of mindfulness influence burnout of stress level among 382 athletes towards perceived performance in their academic and sports activities in 79% of respondents. However, the participants perceived performances in sports were negatively related to their burnout level. The study suggested that mindfulness can be an important stress buffer and may help elite youth athletes to avoid burnout and perform better (Moen et al., 2015). The studies applied MAAS as the measuring tool following their mindfulness intervention (Moghadam et al., 2013; Jekauc et al., 2016).

2.2 Sports Individuals anxiety and depression

Mental illness greatly affects the day-to-day healthy living, which might cause serious functional impairments such as anxiety and depression that are found to be predictors of poor athletic performance. According to the American College Health

Association (ACHA) survey, 30 % of the 195,000 student-athletes have been identified as having depression over the past 12 months, and 50 % reported having anxiety over the same period which might be due to pre-competition anxiety (PCA) (Davoren & Hwang, 2014). Ferreira et al. (2007) reported that the incidence of PCA among (n= 42) Paralympic athletes. They studied the temporal patterning components analysis for 20 minutes, 2 hours, and 1-week among these athletes found that anxiety is a common problem, and suggested the importance of clinical intervention to deal the emotional distress, to benefit the individual against the emotional problem, and support them to cope with their stress.

Sports injuries are defined as tissue damage or physical trauma along with functional impairment (Scott-Hamilton et al., 2016). The athlete who has suffered injury is potentially at the risk of developing posttraumatic stress disorders and depression 6- times more in chances as compared to the non-injured athletes. Besides, the injured athletes also exhibit greater anxiety and lower self-esteem (O´Connell & Manschreck, 2012). In chronic sports injuries, athletes are more likely to become depressed than their teammates. Practicing in elite-level sports offers a completely different set of circumstances for athletes that place them in a high-pressure career with stressors and limitations that tend to cause injury (Schaal et al., 2011). A study among rugby players (n=470), examined the role of psychological factors, their prediction, and sport-related injury prevention to assess somatic anxiety, worry, and concentration disruption. Their results showed that social support, coping skills, and previous injury closely interacted with life stress and injury (Maddison & Prapavessis, 2005). Another study by Li et al. (2017) measured preseason anxiety and depression and reported a total of 597 injuries with 40.6% preseason injuries, 28.8%, and 21.7% of athletes suffered from anxiety and depressive symptoms. Furthermore, athletes with pre-season anxiety symptoms showed a higher injury incidence rate compared to athletes without anxiety symptoms. In general, anxiety increases fear (Scott-Hamilton et al., 2016), when this persists over a period that affects the personality of athletes as trait anxiety, whereas that affects temporary as state anxiety (Ensari et al., 2015). On the other hand, the state anxiety classified depends on the mental state and physical state as cognitive anxiety and somatic anxiety respectively (Waechter & Stolz, 2015).

2.3 Mindfulness in optimizing sports performance

Elite athletes face a variety of challenges in their careers, leading to important steps to ensure that sports psychologists have an appropriate response to athletes' challenges in the field of sport (Birrer et al., 2012). Over the last 30 years, the techniques used were primarily from psychological skills training (PST) with a set of systems for self-talk, imagery, goal setting, and arousal regulation based on cognitive-behavioral theories. Recently, the rise of interest in mindfulness-based interventions has increased in sport psychology which ultimately creates an impact on optimizing sports performance (Elmagd, 2016). The 8-week mindfulness intervention among the competitive cyclist (n=27) showed increased attention of the athletes, decreased sport-specific anxiety, and sport-specific pessimism compared to the control group (n=20) cyclist (Scott-Hamilton et al., 2016).

Mindfulness mediation practice can reduce anxiety (Keng et al., 2011) and this practice has been reported to have associated with the ability to let go of and decreased the occurrence of negative thoughts (Evans et al., 2008). The trait mindfulness among 133 elite athletes and their levels of engagement from various sports showed that trait mindfulness encouraged a positive functioning in high demand situations, which improves athletes' performance. Besides, mindfulness was found to be able to reduce the state anxiety before a competition of the athlete (Röthlin et al., 2016). A study by Walker (2016) explored the relationship between mental toughness and mindfulness among provincial adolescent female hockey players (N=484), found that mindfulness exhibited positive correlations with positive emotions (Walker, 2016).

The mindfulness acceptance commitment (MAC) approach among 118 collegiate sports participants from various sports like soccer, field hockey, crew, and wrestling, exhibited a significantly greater increase in coach ratings and increase in flow experience comparing with PST participants (Gardner & Moore, 2004; Lutkenhouse, 2007). Another study by Vøllestad et al. (2011) investigated the effect of MBSR on self-referred participants (n=76) under heterogeneous anxiety disorders reported to have higher relief from depression, anxiety, and trait anxiety. Further, the study evidence that mindfulness has fully mediated a change in acute anxiety level and partial changes in trait anxiety level.

Kaufman et al. (2009) developed MSPE to track the sports performance, affected flow states, and psychological characteristics of the archers (n=11) and the golfers (n=21). Their results indicate that MSPE has been a positive intervention to improve sport-confidence flow, knowledge, and aspects. A further study on the long-term effects of MSPE psychological aspects of the individual athlete and their athletic performance was conducted among golfers, archers, and long-distance runners (N = 25) in the set duration of one-year. Their results showed the athletes manage to cope up with the depression level. Specifically, the long-distance runners showed improvement in their time duration, performance, and trait variables. The authors recommended MSPE as a promising approach for sports individuals that enhance long-term changes in trait variables towards athletic performance (Thompson et al., 2011; Kaufman et al., 2018). Another study

among (n=45) collegiate athletes who attend 75-min, 6-week MSPE reported positive psychological assistance such as mental strength and relaxation (Mistretta et al., 2017).

Mindfulness Meditation Training for Sport (MMTS) is a mindful based intervention that was introduced by Baltzell & Akhtar (2014) with traditional psychological skills training among female collegiate athletes (n=42)**,** showed that athletes who received MMTS had increased in mindfulness score. Another study explored mindfulness intervention on women soccer players, coaching staff, and their entire team. Their result showed in the beginning participants mostly had difficulty in understanding the process of meditation, however after undergoing MMTS, they reported an increased ability to accept and experience positive emotions, both on and off their field. At the session end, the participants felt sport-focused mindfulness programs help in enhancing sports performance (Baltzell et al., 2014). Similarly, a study by Cote et al. (2019) on MMTS 2.0 among collegiate tennis players (n=9) improved their sports concentration, adaptability, and tolerance level.

Table 2 Mindfulness study design, population, interventions, tools, and results

Author reference	Study design	Study population	Intervention	Tools used	Results
Röthlin et al., 2016	Cross-sectional study	(N=133) Elite athletes	Perception of Trait mindfulness to promote positive sports performance in high demanding situations in sports	37-item Comprehensive Inventory of Mindfulness Experiences	Trait mindfulness reduced performance worries and influencing athletes' performance in their specific sports
Walker, 2016	Experimental study	(N=484) adolescent female hockey players	Relationship between mental toughness and mindfulness	14-item Sports Mental Toughness Questionnaire 10-item Child and Adolescent Mindfulness Measure	Athletes in the high mindfulness group reported significantly higher levels of control and general mental toughness Mindfulness exhibited significant positive correlations with confidence, constancy, and control, as well as with total mental toughness
De Petrillo et al., , 2009	Experimental study	N=25 Runners	Determined the effects of 4-weeks Mindful Sport Performance Enhancement (MSPE)	39-item Kentucky Inventory of Mindfulness Skills (KIMS) 13-item Toronto Mindfulness Scale (TMS). Mindfulness Practice Log	MSPE group showed significantly more improvement in perfectionism compared with controls groups
Kaufman et al., 2009	Experimental study	N=11 archers and N= 21 golfers	4-weeks MSPE to check the performance and psychological characteristics of participants	39-item Kentucky Inventory of Mindfulness Skills (KIMS) 13-item Toronto Mindfulness Scale (TMS). Daily Mindfulness Practice Log	MSPE is a promising intervention to enhance flow, mindfulness, and aspects of sport-confidence

Thompson et al., 2011	Longitudinal study	archers, golfers, and long-distance runners (*N* = 25)	One-year follow-up (MSPE), a program designed to improve athletic performance and psychological aspects of the sport.	39-item Kentucky Inventory of Mindfulness Skills (KIMS)	MSPE techniques as a promising intervention for long-term changes in trait variables that may contribute to optimal athletic performance
Baltzell & Akhtar, 2014	Experimental study	Female athletes (N = 42) soccer players (N=19), rowers (N=23)	Examined the impact of a twelve-session, 30-minute mindfulness meditation training session for sport (MMTS) intervention	Mindfulness Attention Awareness Scale (MAAS), the Positive Affect Negative Affect Scale (PANAS), the Psychological Well-Being Scale and the Life Satisfaction Scale	Paired sample t-tests highlight significant increases in mindfulness scores for the intervention group ($p < .01$)
Baltzell et al., 2014	Longitudinal study	N=7 soccer players	6-week, 12 session mindfulness meditation training for sport (MMTS) program	Interview	Participants reported an enhanced ability to accept and experience a different relationship with their emotions, both on and off their field, during post-intervention
Cote et al., 2019	Longitudinal study	N=9 Division I varsity tennis collegiate athletes	one-hour program or in two 30-minute segments for 6 days, 6 weeks MMTS 2.0 program addressing the psycho-education, guided practice, and group discussion	Participants completed pre-post interview on the MBI	Interviews highlights MMTS 2.0 is a practical and valuable intervention for collegiate athletes
Scott-Hamilton et al., 2016	Experimental study	N=27 cyclists in the mindfulness intervention condition, N= 20 cyclists in the control condition	8-weeks mindfulness training increases athletes' mindfulness and flow experience and decreases sport-specific anxiety and sport-specific pessimism	Participants completed baseline and post-test measures of mindfulness, flow, sport-anxiety, and sport-related pessimistic attributions	Results suggest that mindfulness-based interventions tailored to specific athletic pursuits can be effective in facilitating flow experiences
John et al., 2011	Experimental study	96 male elite Shooters, experimental and control (48 in each)	4- weeks of MMT and one week study to determine the follow-up effect	Salivary Cortisol (SC), a reliable physiological marker of HPA- axis response in reducing Pre-competitive stress (PCS)	Reduction of PCS level and increase in shooting performance

Conclusion

This review on various mindfulness approaches has been proven to be effective in managing the sports individuals' state anxiety and depression, hence be able to enhance performance in individual sports. Further, it conveys for the sports rehabilitation specialists that mindfulness practice has diverse approaches that are transfigured from cognitive-based therapy to a mindful approach, namely from MBSR to MMTS.

However, the majority of the study reported the effectiveness of mindfulness practice was assessed by using the very subjective questionnaire MAAS for testing the participants' mindfulness attention expect one study applied salivary cortisol testing. Hence, this review suggests the need for an objective assessment method to assess the impact of mindfulness intervention in sports rehabilitation.

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Disclosure of interest

The authors declare that they have no competing interests.

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CHEMICAL INVESTIGATION AND ANTIPROLIFERATIVE STUDIES OF ISOLATED POLYISOPRENYLATED BENZOPHENONES FROM STEM-BARK OF *Garcinia maingayi*

Sangeetha Arullappan[1*], Wong Fai Chu[2], Lim Chan Kiang[3], Vivien Jong Yi Mian[4], Sim Kooi Mow[5]

[1]Department of Allied Health Sciences, Faculty of Science, Universiti Tunku Abdul Rahman, Perak Campus, Bandar Barat, Jalan Universiti, 31900 Kampar, Perak, Malaysia.
[2, 3, 5]Department of Chemical Science, Faculty of Science, Universiti Tunku Abdul Rahman, Perak Campus, Bandar Barat, Jalan Universiti, 31900 Kampar, Perak, Malaysia.
[4]Centre for Applied Sciences, Faculty of Applied Sciences, Universiti Teknologi MARA, Samarahan Campus 2, Jalan Meranek, 94300 Kota Samarahan, Sarawak, Malaysia.

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ABSTRACT

In the current study, sequential solvents extraction from the stem bark of *Garcinia maingayi*, a native plant to Malaysia has led to the isolation of four polyisoprenylated benzophenones: 30-*epi*-cambogin **(GB 1)**, 14-deoxy-30-*epi*-cambogin **(GB 2)**, guttiferone F **(GB 3)**, and 14-deoxy-guttiferone F **(GB 4)**. The structures were elucidated using IR, optical rotation, and NMR spectral data. The compounds were evaluated for antiproliferative effect using MTT assay, apoptosis using Annexin V/7-AAD flow cytometry, cell cycle progression, and activation of caspases 3/7, 8 and 9 and BCL2 mRNA expression in MCF-7, HeLa, and HepG2 cancer cell lines. Compounds **GB 1** to **GB 4** exhibited a remarkable antiproliferative effect on HeLa, MCF-7, and HepG2 cells with IC_{50} values ranging from 5 to 45 µM. Compounds **GB 1** to **GB 4** induced significant cell cycle arrest in the G1 phase corroborated with the decrease in the number of MCF-7 and HepG2 cells in S and G2/M phases ($P<0.05$). Compounds **GB 1** to **GB 4** induced apoptosis at 48 h. Further, among these, compounds **GB 1** and **GB 2** induced significant levels of caspases 3 and 9 in HeLa cells, while **GB 3** induced caspase 9 activities in both MCF-7 and HepG2 cells. No significant induction of caspase 8 was observed suggesting that the apoptotic effects are mainly mediated through the intrinsic pathway. Only compound **GB 1** inhibited the BCL2 mRNA expression significantly in all treated cancer cells. In conclusion, these compounds possess anticancer properties and thus further investigation is crucial on the mechanistic study, structure-activity relationship, and identification of putative molecular targets.

* Corresponding author
E-mail: sangeetha@utar.edu.my (Sangeetha Arullappan)

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1 Introduction

Therapeutic uses of medicinal plants in the pharmaceutical drug discovery against cancer have been tested over the past century. World Health Organization (WHO) estimated 21.7 million people will be diagnosed with cancer and 13 million people will die because of cancer by the year 2030 (Ferlay et al., 2015). Cancer incidence rates are increasing rapidly and have become the second leading cause of death globally. In line with the drug development, the *Garcinia* species is one of the important plants studied greatly for medicinal values.

Garcinia is a polygamous dioecious evergreen fruit tree or shrub that belongs to Guttiferae (Clusiaceae) family (Chai, 2000; Joseph et al., 2005; Jabit et al., 2009; Hemshekhar et al., 2011). Approximately 450 species of *Garcinia* are widely distributed in tropical Asia, South Africa, and Polynesia as well as in Australia (Kumar et al., 2013; Patil & Appaiah, 2015). *Garcinia* is widely found in Malaysia specifically in Kedah, Perak, Pahang, Terengganu, Johor (Kochummen, 1998; Jabit et al., 2009) and primarily present in Sabah and Sarawak (Repin et al., 2012; Ling & Julia, 2012). Detailed chemical studies on *Garcinia* species using nuclear magnetic resonance (NMR) spectroscopy revealed the presence of triterpenoids (stigmasterol, sitosterol), benzophenone (isoxanthochymol), xanthone (1,3,7-trihydroxy-2-3-(3-methylbut-2-enyl)xanthone), and benzoic acid derivative (3,4-dihydroxy-methylbenzoate) (Cheng & Cheow, 2008). The presence of phenolic compounds contributed to antioxidant properties (Krishnamoorthy et al., 2014), while xanthone and benzophenone have cytotoxic properties toward various human cancer cell lines (Kumar et al., 2013).

Two newly isolated caged xanthones from *G. wightii* viz., wightiic acid and 16-O-methyl wightiic acid also showed antiproliferative activities. However, the wightiic acid showed exceptional antiproliferative activity with IC_{50} of 4.7 and 5.2 µMin A-375 and MCF-7 cells, respectively (Lekshmi et al., 2020). *Garcinia* xanthone I, a newly isolated xanthone from *G. xanthochymus* induce HepG2 apoptosis through the mitochondrial pathway and proved as a potential drug in the treatment of liver cancer (Jin et al., 2019). There is a lack of study on the Malaysian species of *G. maingayi*. The current study was carried out to reveal the effect of the pure bioactive compounds on various biological activities and their possible mechanism of action. The ability to induce apoptosis is the primary characteristic in considering the efficacy of the compound as a chemopreventive agent is shown in this research.

2 Materials and Methods

2.1 Plant material

Stem-bark of *G.maingayi* was collected from the forest of Semengok, Sarawak, Malaysia in June 2014. The plant was authenticated by Mr. Tinjan Anak Kuda, a botanist from the Forest Department, Sarawak. A voucher specimen of UITM 3017 was deposited at the herbarium of Universiti Teknologi MARA, Sarawak.

2.2 Isolation and purification

The 2.7 kg dried stem-bark of *G.maingayi* was sequentially extracted using hexane, ethyl acetate, dichlomethane, ethanol, and methanol for three days at room temperature. Each filtrate was concentrated using a rotary evaporator at 40 to 60°C. The crude extracts were chromatographed on a silica gel (Merck) column with increasing polarity of organic solvents at various ratios. These steps were repeated successfully using gravity column chromatography and preparative thin-layer chromatography to obtain pure compounds.

2.3 Chemical investigation

The pure compounds were elucidated using Jeol JNM-ECX (Japan), 400 MHz NMR spectrometer with the use of trimethylsilane (TMS) as internal and reference standards for ^{1}H NMR, ^{13}C NMR, HMQC, and HMBC spectra. IR spectra were analyzed using Fourier Transform Infrared (FTIR) (Perkin Elmer). Optical rotations were measured on Jasco Europe P-2000 digital polarimeter.

2.4 Antiproliferative analysis

2.4.1 Cancer cell lines

Human cervical epithelial carcinoma cell line (HeLa)(ATCC®CCL-2™), human breast adenocarcinoma cell line (MCF-7)(ATCC®HTB-22™), and human hepatocellular carcinoma cell line (HepG2)(ATCC®HB-8065™) were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 100 UI/ml penicillin and 100 UI/ml streptomycin (Sigma-Aldrich, St. Louis, MO, USA). All the cell lines were maintained at 37ºC in a 5% CO_2 incubator.

2.4.2 MTT assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described by Leong et al. (2007) and Bradshaw et al. (2005) with some modifications was used. Cancer cell lines were seeded into 96-well plates (Nunc, Denmark) at concentrations ranging from $1x10^4$ to 10^5 cells. Compounds, DMSO, and doxorubicin hydrochloride (positive control) (Nacalai Tesque, Japan) were added into respective wells and incubated for 48 h at 37ºC in 5% CO_2 incubator. MTT reagent (5 mg/ml) (Sigma, UK) was added and incubated further for 4 h, followed by the addition

of 100 µl of DMSO. The absorbance was measured using a microplate reader (Omega, Germany) at 570 nm. The dose-response curves and 50% growth inhibition concentrations (IC_{50}) were determined from GraphPad Prism (version 8.0.2).

2.4.3 Flow cytometry

Propidium iodide staining coupled with flow cytometry was used in the evaluation of cell cycle progression. The population of apoptotic cells was measured using the PE Annexin V Apoptosis Detection Kit (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions. Both floating and attached cells were collected, stained, and analyzed using a FACS Calibur flow cytometer and the CellQuest Pro software (version 5.1.1; BD Biosciences, USA) (BD Biosciences, 2008).

2.4.4 Caspases activation

The catalytic activity of caspases 3/7, 8, and 9 was measured at 48 h after treatment using Caspase-Glo 3/7, Caspase-Glo 8, and Caspase-Glo 9 assay kits (Promega, Madison, WI, USA) based on manufacturer's guidelines (Promega, 2018).

2.4.5 BCL2 mRNA expression

Treated cancer cell lines were further preceded to RNA extraction and the cDNA was synthesized using high capacity RNA-to-cDNA master mix based on manufacturers' protocol(Qiagen, Valencia, CA, USA; Applied Biosystems, Carlsbad, California, USA) (Qiagen, 2013). FastStart Universal SYBR green master reagent (Roche, Indianapolis, USA) and Bio-Rad iQ5 real-time PCR detector system (Bio-Rad, Richmond, CA, USA) (Pabla & Pabla, 2008) were used. Bio-Rad iQ5 Optical System Software V1.0 was used for data analysis. Table 1 shows primer sequences used in the expression. The 40 cycle's conditions were 3 min at 94°C followed by 40 s at 94°C, 40 s at 60°C, and 25 s at 72°C. The housekeeping gene, GAPDH was used in the normalization (Reboucas et al., 2013).

2.5 Data analysis

Data were reported as mean±standard deviation from a minimum of three independent experiments. Levels of significance were determined using student t-test, whereas treatments were compared to the control. $P < 0.05$ was considered statistically significant unless otherwise specified.

3 Results and Discussion

3.1 Structural elucidation

The 30-*epi*-cambogin (GB 1), 14-deoxy-30-*epi*-cambogin (GB 2), guttiferone F (GB 3) and 14-deoxy-guttiferone F (GB 4) were isolated from *G. maingayi* (Figure 1).Table 2 summarizes the detail of compounds, while Table 3 shows the ^{1}HNMR and ^{13}CNMR spectra of the compounds GB 1 to GB 4. The molecular formula of GB 1 was $C_{38}H_{50}O_6$ with the mass of 602 and optical rotation ([α]D) of -129º. The molecular formula of GB 2 was $C_{38}H_{50}O_6$ differing from GB 1 by an oxygen atom. Compounds GB 1 and GB2 were different on the aromatic ring in which GB 1 has two OH groups at C13 and C14, while GB 2 has only one OH group at C13. The molecular formula of GB 3 is similar to that of GB 1 with $C_{38}H_{50}O_6$, while the molecular formula of GB 4 is similar to that of GB 2.

The NMR spectra of GB 4 were generally similar to that of GB 3 except for the absence of an OH group at C14 which was replaced by an aromatic proton. Compounds GB 1 and GB 2 were obtained as a yellow powder, while compounds GB 3 and GB 4 as yellow and honey consistency.

Compounds GB 1 and GB 3 were also reported obtained from the root wood of *Allanblackia stuhlmannii* (Engl.) in the early phase of research (Fuller et al., 1999) with the same structural properties. However, compounds GB 2 and GB 4 obtained in this study are new compounds and reported isolated for the first time from Malaysian species of *G. maingayi*.

3.2 Antiproliferative effect

Figure 2 shows the dose-response curves of cancer cell lines treated with various concentrations of compounds **GB 1** to **GB 4** for 48 h. These compounds exhibited cytotoxicity with IC_{50} values in the range of 5 to 45 µM as shown in Table 4. The number of cells reduced drastically upon treatment as compared to vehicle control (0.1% DMSO) indicating cytotoxic effect (Figure 3).

The monolayer cells detached and form spherical shapes revealing death mode upon treatment. The original anchorage-dependent cancer cells losses their adhesiveness or lose their substrate attachment and becomes rounded, further shrinks due to cell death (Freshney, 2005; Kleinsmith, 2006). These morphological changes confirm the presence of anticancer properties in the isolated compounds that act as mitogen factors.

Visible cell death suspensions upon treatment were seen in HeLa cells as compared to other cells. Kenji et al. (2003) reported garcinol, isogarcinol and xanthochymol from *G. purpurea* displayed a strong apoptosis-inducing effect against human leukemia cell lines namely NB4, HL60, U937, and K-562 via nuclear fragmentation and DNA ladder formation. Kai-Wei et al. (2012) reported garcinielliptones S and phloroglucinol from *G. subelliptica* remarkably increased the cell death of human bladder carcinoma cells (NTUB1) at 72 h with $\mathbf{IC_{50}}$ values of 45.1 ± 7.8, 13.5 ± 2.3 and 3.3 ± 0.1 µM, respectively. These studies were in agreement with the current study supporting that the compounds isolated from *G. Maingayi* are potential antiproliferative agents.

3.3 Cell cycle progression

The effects of compounds **GB 1** to **GB 4** in MCF-7 and Hep G2 on cell cycle progression is shown in Figure 4. These compounds induced significant cell cycle arrest in **G1** phase corroborated with the decrease in the number of cells in S and G2/M phases ($P<0.05$). Due to excessive cell death, cell cycle analysis was not able to perform on HeLa cells. The link between apoptosis and cell cycle progression in cancer cells is considered a possibly effective approach to control tumour growth (Roger & Mike, 2006). A classic cell cycle progression is represented by early-G1, late-G1, S, and G2/M phases. Higher G1 populations were seen in both MCF-7 and HepG2 treated cells, and this causes a concomitant decrease in the population of cells in the G2/M phase as compared to control. The results showed the G1 cell cycle arrest induces cell death in the cancer cell lines. The current study agrees with Zhang et al. (2016) that a novel compound nujiangexathone A from *G. nujiangensis* suppressed HeLa by arresting the cells at G0/G1 phases from 61.65 to 73.65% and decreased the cells in the G2/M phases from 23.55 to 12.60% after 48 hours at 20 µM.

3.4 Induction of apoptosis

Compounds **GB 1** to **GB 4** induced apoptosis in all the cancer cell lines by a significant percentage of apoptosis following treatment for 48 h using Annexin V/7-AAD staining. Compounds **GB 1** and **GB 2** exhibited the highest percentage of apoptosis in HeLa cells, while the lowest was shown by compound **GB 2** in HepG2 cells. However, 0.1% of DMSO as vehicle control showed a lower apoptotic rate indication the cells are viable. Figure 5 shows the scatter plots of the treated cancer cells entering to first early apoptotic stage and finally to the late apoptotic stage. It is seen that lesser treated cells enter the necrotic stage (upper left).

3.5 Activation of Caspases

Caspases 3/7, 8, and 9 activities in HeLa, MCF-7 and HepG2 activation following treatment exhibited a remarkable apoptotic effect (Figure 6).

Table 1 Primer sequences for qPCR

Gene	Forward primer	Reverse primer
BCL2	5'-ATCGCCCTGTGGATGACTGAGT-3'	5'-GCCAGGAGAAATCAAACAGAGGC-'3
GAPDH	5'-GTCTCCTCTGACTTCAACAGCG-3'	5'-ACCACCCTGTTGCTGTAGCCAA-3'

Table 2 Summarised details of the isolated polyisoprenylated benzophenones from the stem-bark of *G. maingayi*

Features	GB 1	GB 2	GB 3	GB 4
Findings	Known compound	New compound	Known compound	New compound
Isolated from Crude extracts	Methanol	Dichloromethane	The mixture of ethanolic and hexane	Ethyl acetate
Name	30-*epi*-cambogin	14-deoxy-30-*epi*-cambogin	Guttiferone F	14-deoxy-guttiferone F
Form	Yellow powder	Yellow powder	Yellow and honey consistency	Yellow, honey consistency
Molecular formula	$C_{38}H_{50}O_6$	$C_{38}H_{50}O_5$	$C_{38}H_{50}O_6$	$C_{38}H_{50}O_5$
Molecular mass	602	586	602	586
Optical rotation	-129º	-143º	-298º	-255º

Table 3 ^{1}H NMR and ^{13}C NMR spectra of compounds GB 1 to GB 4

Position	GB 1	GB 2	GB 3	GB 4	GB 1	GB 2	**GB 3**	**GB 4**
		δC,ppm				δH, ppm (type)		
1	171.7	171.9	199.0	198.5	-	-	-	-
2	125.2	125.2	116.0	116.5	-	-	-	-
3	194.6	193.9	193.9	193.6	-	-	-	-
4	68.2	68.3	69.8	69.9	-	-	-	-
5	46.1	46.4	49.7	49.8	-	-	-	-
6	46.2	46.4	46.9	46.8	1.33, m	1.45, m	1.45, m	1.41, m
7	39.3	39.7	42.7	42.7	1.86, dd (14.6, 7.3) 2.14, d (14.6)	1.98, dd (14.2, 7.3) 2.28, d (14.2)	2.03, m 2.35, d (14.2)	2.04, m 2.35, d (13.8)
8	51.2	51.3	58.0	57.9	-	-	-	-
9	207.3	207.4	209.3	209.3	-	-	-	-
10	193.2	193.7	195.0	195.4	-	-	-	-
11	129.9	133.2	127.8	132.1	-	-	-	-
12	114.3	114.8	116.5	115.8	7.21, d (1.8)	7.29, t (1.4)	6.91, m	6.87, m
13	144.7	156.2	143.8	156.1	-	-	-	-
14	150.6	121.7	149.9	121.2	-	7.14, dt (7.8, 1.4)	-	6.90, m
15	114.4	129.7	114.4	128.9	6.61, d (7.9)	7.18, t (7.8)	6.58, d (8.0)	7.10, t (7.8)
16	123.8	120.6	124.2	120.1	6.87, dd (7.9, 1.8)	6.96, dt (7.8, 1.4)	6.93, m	6.85, m
17	25.5	25.7	27.1	26.3	2.30, dd (13.7, 5.2) 2.54, dd (13.7, 5.5)	2.43, dd (13.7, 5.5 2.64, dd (13.7, 8.2	2.72, m 2.58, d (12.4)	2.71, m 2.52, d (13.7)
18	119.6	119.9	120.2	120.0	4.77, m	4.90, t (5.5)	5.08, m	-
19	134.6	139.0	135.4	137.7	-	-	-	5.08, m
20	25.9	26.2	26.2	26.2	1.47, s	1.58, s	1.79, s	1.78, s
21	17.9	18.1	18.3	18.1	1.45, s	1.57, s	1.73, s	1.68, s
22	22.3	22.6	22.8	22.7	1.03, s	1.15, s	1.15, s	1.11, s
23	26.6	26.9	26.5	27.1	0.85, s	0.97, s	1.00, s	0.99, s
24	29.2	29.4	29.0	29.1	2.03, m 2.49, m	2.15, m 2.59, m	2.05, m 2.10, m	2.02, m 2.09, m
25	124.8	125.0	123.9	123.9	4.77, m	4.90, t (5.5)	4.92, t 7.3)	4.90, m
26	133.0	133.8	133.1	133.0	-	-	-	-
27	25.8	26.0	25.8	25.9	1.54, s	1.66, s	1.65, s	1.67, s
28	17.9	18.0	18.1	18.0	1.56, s	1.65, s	1.52, s	1.51, s
29	28.3	28.4	36.3	36.2	0.84, m 2.91, dd (14.0, 3.7)	0.93, m 3.03, dd (14.2, 3.7)	1.98, m 1.88, dd (14.0, 4.2)	1.90, m 1.93, m
30	42.8	42.9	43.7	43.6	1.28, m	1.39, m	2.67, m	2.74, m
31	86.7	86.7	148.1	148.2	-	-	-	-
32	28.5	28.6	112.8	17.8	0.78, s	0.88, s	4.36, s 4.40, s	1.60, s
33	21.1	21.3	18.0	112.7	1.12, s	1.23, s	1.59, s	4.40, s 4.43, s
34	29.6	29.7	32.7	32.7	1.67, m 1.91, m	1.79, m 2.02, m	2.03, m	2.00, m 2.12, m
35	121.3	121.5	122.7	122.7	5.07, t (7.3)	5.15, td 6.6,1.4)	5.03, t 6.8)	5.04, m
36	133.7	134.7	132.1	135.1	-	-	-	-
37	25.6	25.9	25.9	25.8	1.65, s	1.74, s	1.69, s	1.67, s
38	18.0	18.2	18.1	18.2	1.48, s	1.60, s	1.54, s	1.54, s

*Recorded at 400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR in δ ppm: assignments based on DEPT, HMCQ and HMBC [s singlet, m multiplet, d doublet, dd doublet of doublet, t triplet]

Table 4 *In vitro* cytotoxic effect of compounds GB 1 to GB 4 as expressed in IC_{50} values at 48 h

Sample	IC_{50} values (µM)		
	HeLa	**MCF-7**	**HepG2**
GB 1	13.24 ± 1.53	5.48 ± 0.20	8.02 ± 1.53
GB 2	15.49 ± 1.39	5.30 ± 0.32	9.25 ± 1.57
GB 3	33.71 ± 2.33	12.46 ± 0.57	18.03 ± 1.99
GB 4	45.10 ± 1.27	22.97 ± 0.97	24.97 ± 1.04
Doxorubicin	0.29 ± 0.01	1.45 ± 0.69	0.45 ± 0.01

Compounds **GB 1** and **GB 2** induced significant (>two-fold induction and P<0.01 by student t-test) levels of caspases 3/7 and 9 activities in HeLa cells, while compound **GB 3** induces caspase 9 activities in both MCF-7 and HepG2 cells. Compound **GB 4** is the least potent among all the compounds tested, as apoptosis was only detected in MCF-7 and HepG2 cells. Compound **GB 4** induces significant caspase 9 activation suggesting activation of the intrinsic pathway.

Interestingly, compound **GB 4** did not induce caspases 3/7, 8, or 9 activities in HepG2 cells despite a significant induction of apoptosis suggesting that the compound might induce cell death through a caspase-independent mechanism. No induction of caspase 8 was observed suggesting that the apoptotic effects induced by compounds **GB 1** to **GB 4** are mainly mediated through the intrinsic apoptotic pathway. Activation of the Bcl2 protein family usually occurs in response to DNA damage via the mitochondrial pathway (Damagoj & Wayne, 2007).

Similarly, Ahmed et al. (2012) and Sethi et al. (2014) reported garcinol from the fruit rind of *G. indica* killed prostate (LNCaP, C4-2B and PC3) and pancreatic ca(BxPC-3) cancer cells by downregulating NF-kB and phosphoinositide 3- kinase (PI3K)/serine/threonine-specific protein kinase (Akt) signaling pathways. Besides, Xiong et al. (2014) cytochrome c (Crompton, 2000). This process will further induce cell death selectively and eliminates tumour cells.

3.6 BCL2 mRNA expression

Results given in figure 7 shows compound **GB 1** inhibited BCL2 mRNA expression significantly (P< 0.01) in HeLa, MCF-7, and HepG2 cells, while no such inhibitory effect was observed in other compounds treated cells.

The levels of BCL expression was reduced by 3.5, 3.2, and 1.8-fold in HeLa, MCF-7, and HepG2 cells, respectively. Bcl2 family of proteins is an anti-apoptotic marker and central regulators of mitochondrial cell-intrinsic apoptotic which is directly related to apoptosis induction. The results clearly shown that the BCL2 are down-regulated in all the cancer cells treated with compound **GB 1**. The BCL2 itself can bind to pro-apoptotic members such as Bax and thus releases cytochrome c (Crompton, 2000). This process will further induce cell death selectively and eliminates tumour cells.

Conclusion

In a nutshell, compounds **GB 1** to **GB 4** induced not only cell cycle arrest but also apoptosis in cancer cell lines in a dosage-dependent manner. However, only compound **GB 1** inhibited the BCL2 mRNA expression. Further investigation on the mechanistic study, chemical modification, structure-activity relationship, and identification putative molecular target of compounds **GB 1** to **GB 4** through *in silico* molecular docking can be considered.

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Conflicts of Interest

The authors declare no conflict of interest.

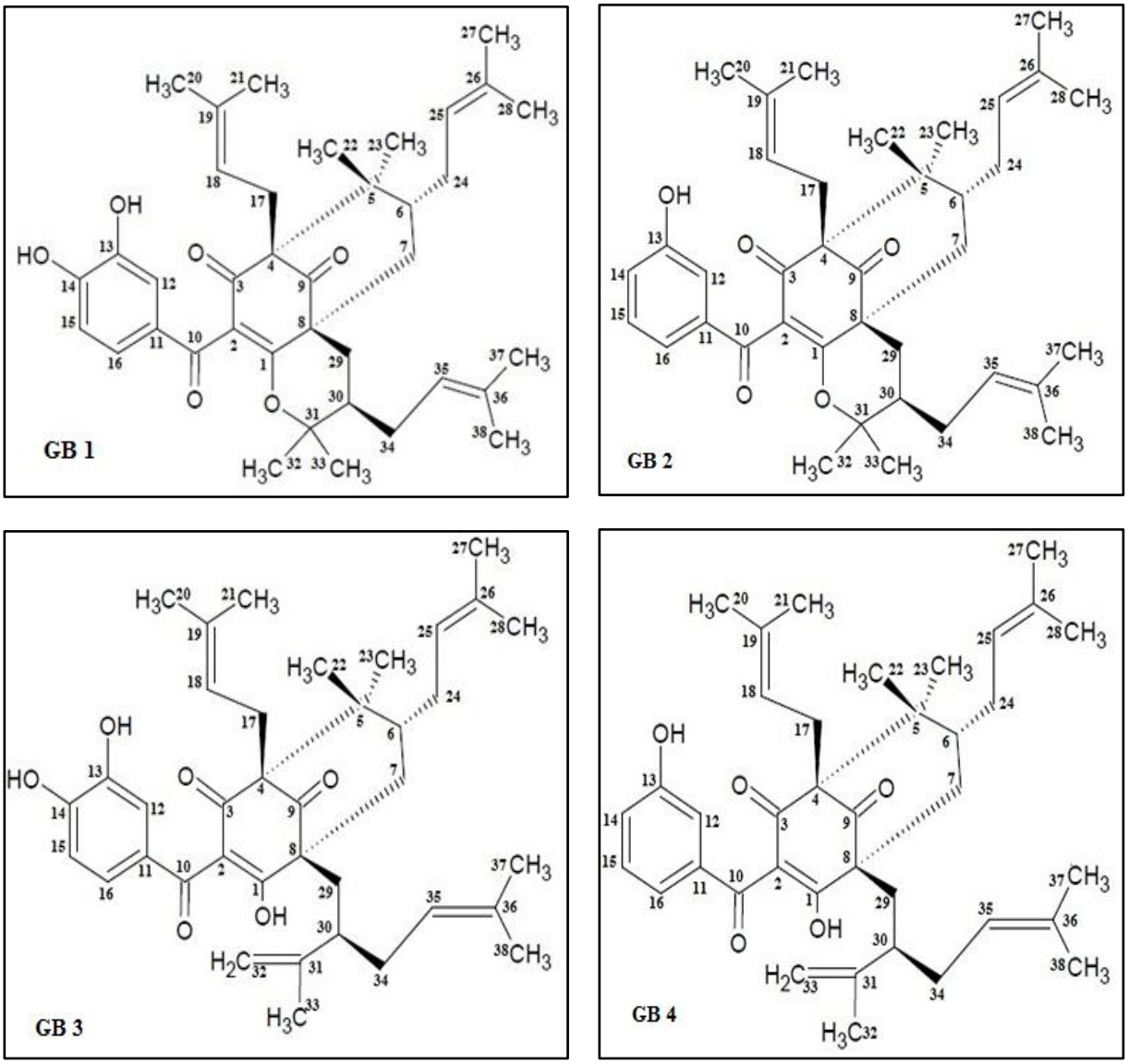


Figure 1 Chemical structures of 30-*epi*-cambogin (**GB 1**), 14-deoxy-30-*epi*-cambogin (**GB 2**), guttiferone F (**GB 3**) and 14-deoxy-guttiferone F (**GB 4**)

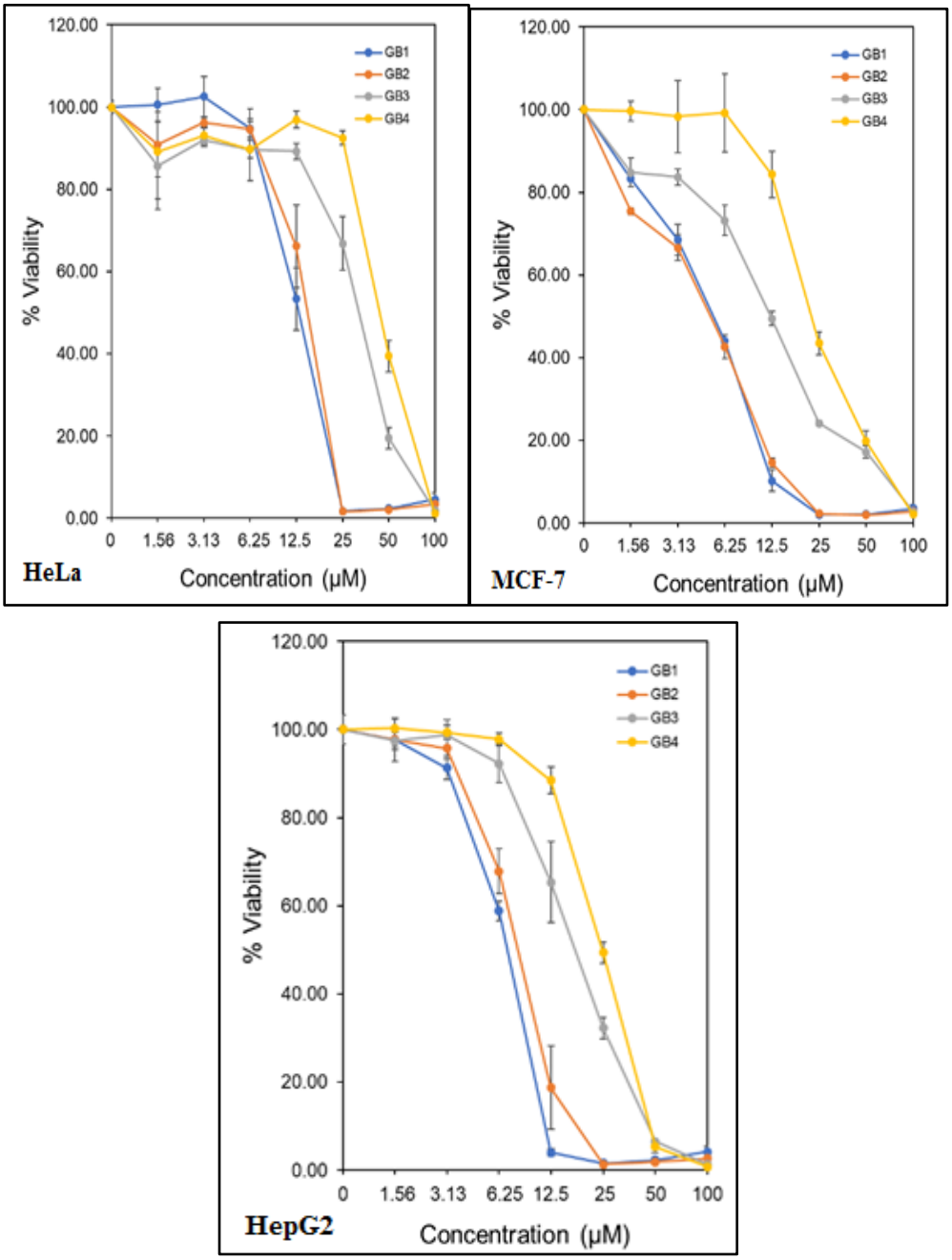


Figure 2 The antiproliferative effect in HeLa, MCF-7 and HepG2 cancer cell lines treated with compounds **GB 1** to **GB 4** at 48 h. The points represent means ± standard deviation (n=3)

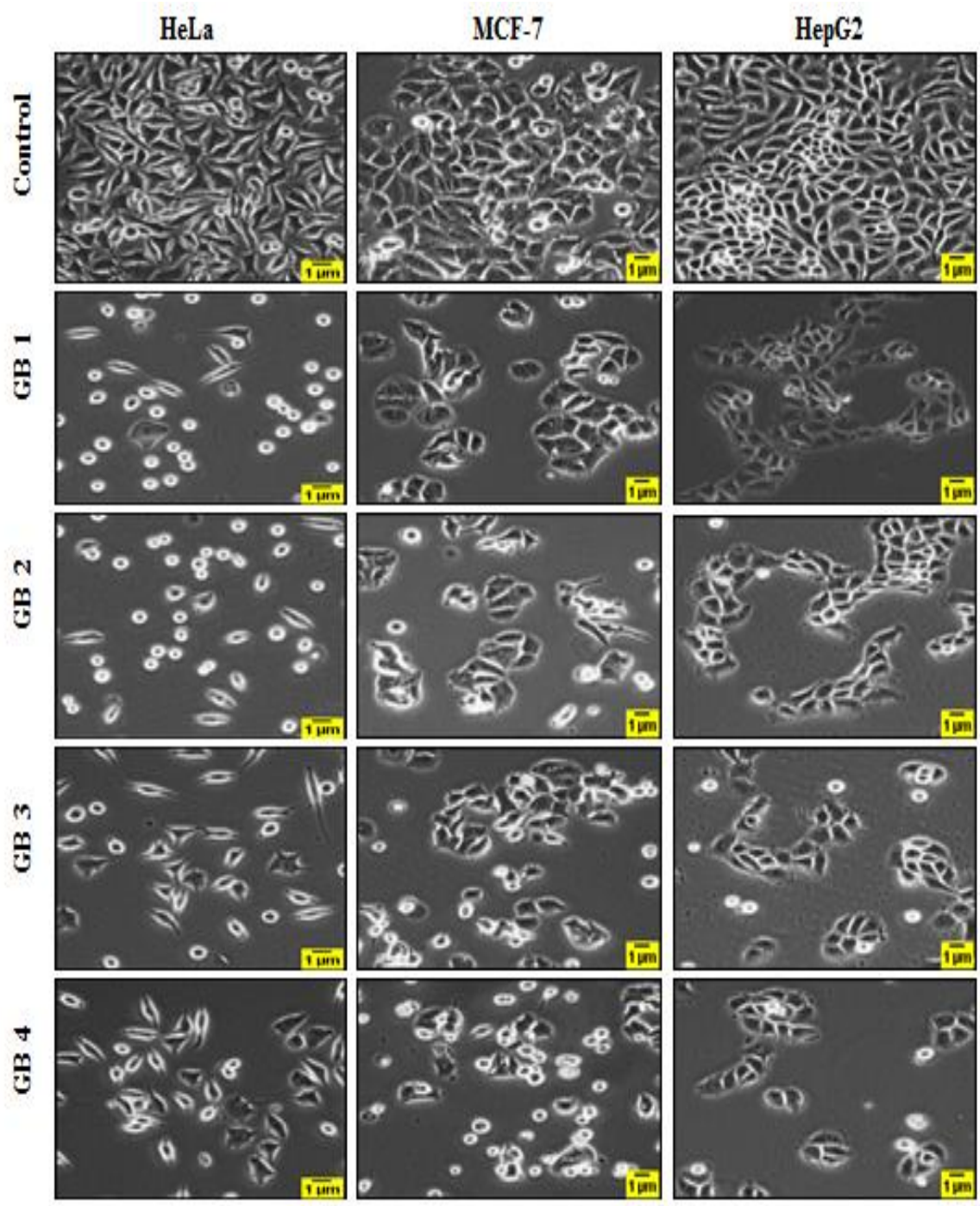
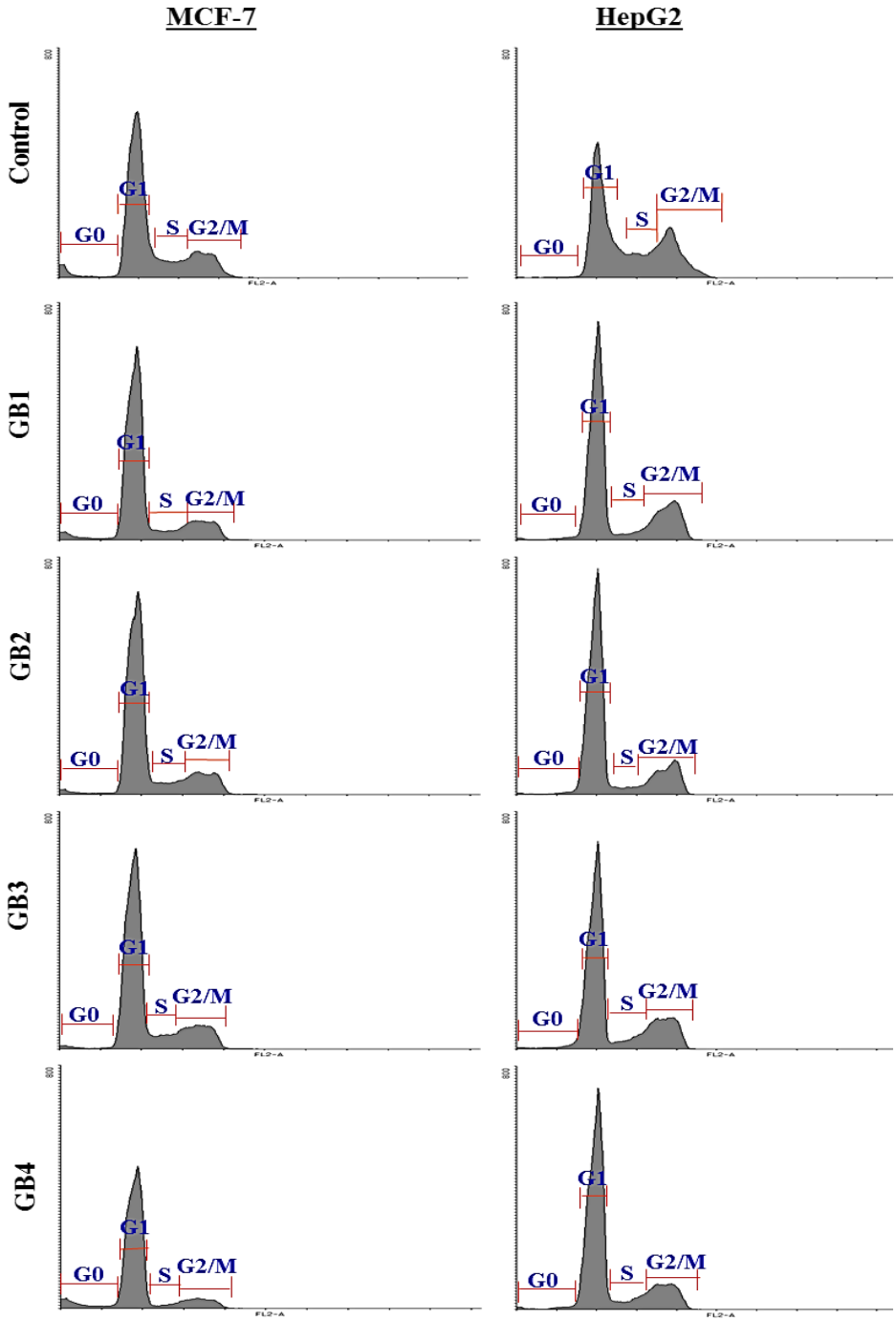


Figure 3 Morphological changes in MCF-7, HeLa and HepG2 cells induced by compounds **GB 1** to **GB 4** and 0.1% DMSO (vehicle control) using microscopy analysis (100x)



|Figure 4 Cell cycle arrests induced by compounds **GB 1** to **GB 4** in MCF-7 and HepG2 cellsat 48 h, followed by propidium staining and flow cytometry

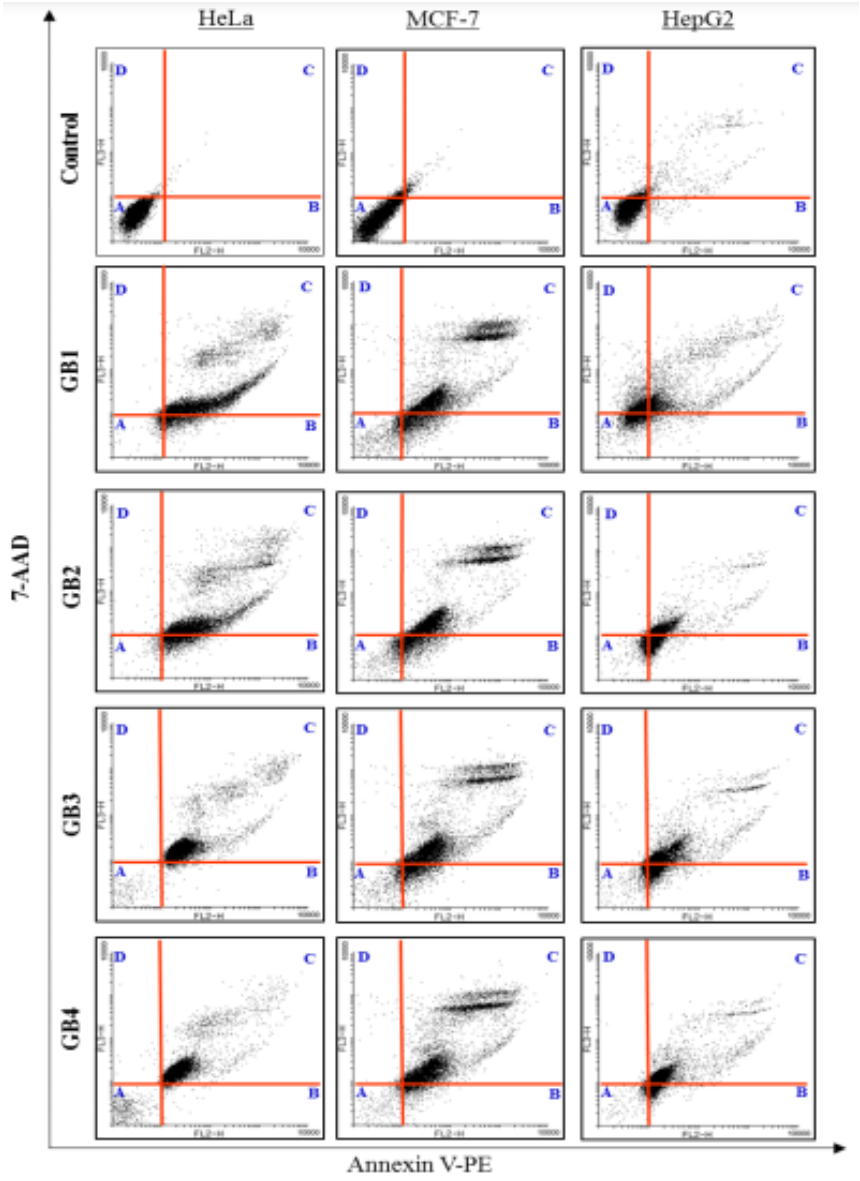


Figure 5 Flow cytometric PE Annexin-V/7-ADD binding profiles of MCF-7, HeLa and HepG2 cancer cell lines treated with 0.1% DMSO (vehicle control) and compounds **GB 1** to **GB 4** at 48 h. Regions labelled A indicating viable cells, B is early apoptotic cells, C is late apoptotic cells and D is necrotic cells

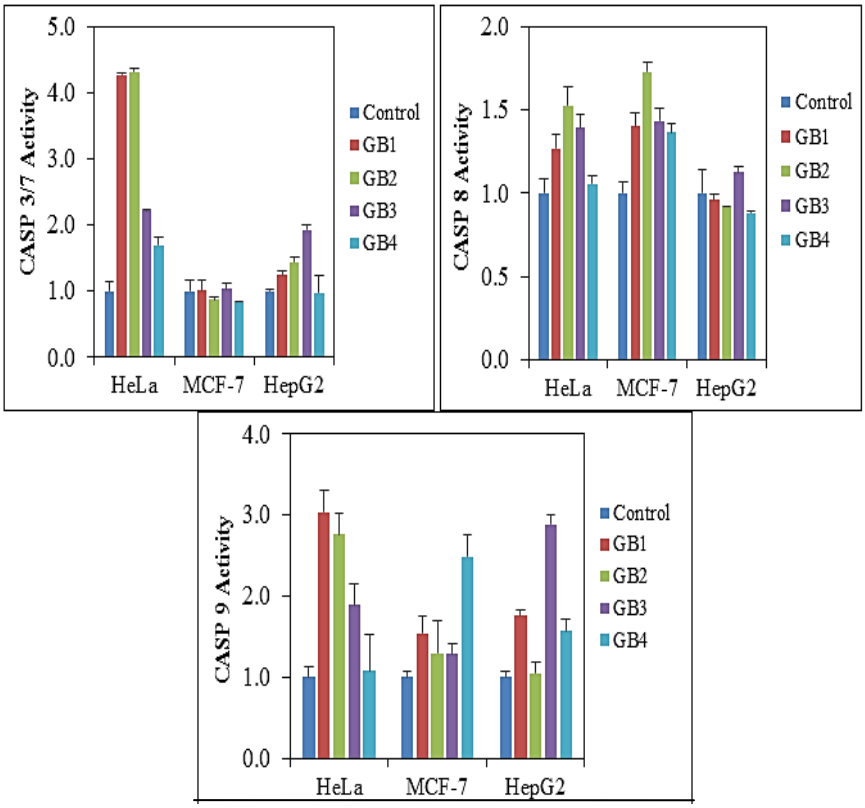


Figure 6 Effects of compounds **GB 1** to **GB 4** on caspases 3/7, 8 and 9 activities in HeLa, MCF-7 and HepG2 cells. The bars represent the mean ±standard deviation (n=3) and * indicates fold change > 2 and P < 0.05 (Student t-test)

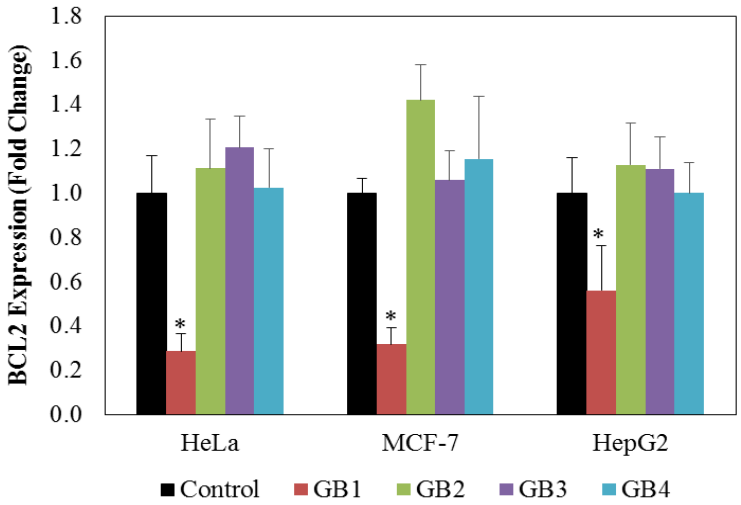


Figure 7 BCL2 mRNA expression profile in MCF-7, HeLa and HepG2 cancer cell lines treated with compounds **GB 1** to **GB 4** at 48 h. Bars indicate mean ± standard deviation (n = 3). * indicates P < 0.01, Student t-test

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CHALLENGES OF PHYSIOTHERAPIST DURING PROSTHESIS REHABILITATION IN MALAYSIA

Chong Li Chi, Vinodhkumar Ramalingam*

Faculty of Health and Life Science, INTI International University, Nilai, Malaysia

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KEYWORDS

Prosthesis Rehabilitation

Quality of life (QOL)

Mortality Rate

Challenges

ABSTRACT

The present study reported the challenges faced by the physiotherapist during prosthesis rehabilitation in Malaysia. Mortality due to lower limb amputation is a concern in recent days that can be improved by providing proper rehabilitation. However, challenges faced by the physiotherapist during rehabilitation are expected to affect the effectiveness of the rehabilitation. Hence, it is important to identify the possible challenges faced by the physiotherapist during prosthesis rehabilitation to take the necessary precautions for better outcomes. A total of 200 Malaysian physiotherapists who have experience in rehabilitating amputees were selected to participate in this online survey. The participants were requested to complete a self-administered electronic questionnaire containing 20 questions. The present study reported a few challenges faced by the physiotherapists in Malaysia during prosthesis rehabilitation such as "encountering patient that refuse to perform exercises is common" (77%), encountered high physical demand (81.5%), encountering language barriers (48.5%), patients often encounter difficulty in assessing health service (63.5%) and encountering prosthesis problems (67%). The barriers were inconsistent and complex, including patient factors, healthcare provider's factors, environmental factors, other factors. The study has highlighted challenges faced by the physiotherapists during prosthesis rehabilitation to raise awareness among physiotherapists and at the same time create ideas for specific health care practitioners to overcome the barriers, therefore shortening the length of rehabilitation and enhancing the effectiveness of the rehabilitation to lower the mortality.

* Corresponding author

E-mail: vinodh.ramalingam@newinti.edu.my (Vinodhkumar Ramalingam)

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1 Introduction

WHO estimated that the number of disabled people in a developing country who need a prosthetic and orthotic device is 0.5% of the total population. The prediction also suggested that around 160,000 Malaysian need prosthetics and orthotic devices (Arifin et al., 2017; Karim & Ming, 2020). Based on the study of Craig D. Murray, prosthesis usage is shown to have much deep personal meaning for amputees.

Therefore, it is suggested that health professionals play a very important role in preserving amputees' valued identities and allow them to manage their disability status (Murray, 2009). Mortality rates after lower limb amputation are respectively high according to Fortington et al. (2013) however; the mortality rate could be lowered by providing proper rehabilitation services. Studies indicated patients with amputation showed better survival rate, greater success with prosthesis fitting, more often return home, improvement in physical function, increased vitality, and reduction in body pain after numbers of inpatient rehabilitation (Pezzin et al., 2000; Christiansen et al., 2015; Ruth & Bphty, 2019). Besides, the quality of life of persons with lower-limb amputation is reported to be maintained or improved through inpatient rehabilitation (Zidarov et al., 2009). Exercises to improve balance and coordination, range of motion, gait, cardiovascular endurance, strength, and prosthetic fit education are important for prosthetic rehabilitation (Imamphd et al., 2019).

However, the challenges faced by the physiotherapist during rehabilitation might affect the effectiveness of the rehabilitation (Afzal, 2017). Lower limb amputees fall incidence is the common challenge faced by the physiotherapist during prosthetic rehabilitation (Steinberg et al., 2019).

Whereby, effective prosthetic rehabilitation is vital in maintaining or improving the quality of life and the physical function of an amputated patient. Hence, it is important to find out the challenges faced by the physiotherapist during prosthesis rehabilitation to enhance the effectiveness of the rehabilitation. Numerous studies reported challenges faced by different health care providers in different areas (Litchfield & MacDougall, 2002; Barber et al., 2015; Ennion & Rhoda, 2016; Afzal, 2017).

However, there is very limited data shows the challenges faced by the physiotherapist during prosthesis rehabilitation. Steward et al. (1999) as well as Parnell & Urton (2020) proposed the importance of identifying the possible challenges faced by therapists during rehabilitation to overcome future challenges. Hence, this study is to investigate the challenges faced by physiotherapists during prosthesis rehabilitation.

Also, the results may be used to raise awareness and create ideas among physiotherapists to overcome the barriers, thus enhancing the effectiveness of rehabilitation and lower the mortality rate (Fortington et al., 2013).

2 Materials & Methods

This observational study use data of self-administered validated questionnaire to investigate the challenges faced by the physiotherapist during prosthesis rehabilitation in Malaysia. The online questionnaire consists of two sections. In the first section, demographic data included physiotherapists' gender; the level of certification; and working experiences with the year of service were displayed and the respondents were asked to select the responses from the list. In the second section, 20 questions regarding the challenges physiotherapists faced during prosthesis rehabilitation were listed and the respondents were instructed to rate the Likert scale based on their own direct experience. Score 1 and 2 on the Likert scale was considered as disagreeable and was categorized as 1, score 3 on the Likert scale was considered as neutral and was categorized as 2, score more than 3 on the Likert scale was considered as agreeable and was categorized as 3.

These 20 questions were categorized into 5 factors: patient factors, healthcare provider factors, cultural factors, environmental factors, and other factors. There are seven questions for patient factors, eight questions for healthcare provider factors, one question for cultural factors, and two questions for environmental factors and other factors respectively.

The physiotherapists working in Malaysia with at least a diploma level of certification and registered under the Malaysian Physiotherapy Association was considered as inclusion criteria. However, physiotherapists with working experience of fewer than 6 months were excluded. The minimum sample size of 377 was fixed based on the estimated population of therapists in Malaysia, approximately 19000, by using the Raosoft sample size calculator. The online questionnaire was posted on the Malaysian physiotherapy Facebook page for a month from February until March 2020 after approval by the admin of the group. The research purpose was explained to every participant and informed consent was taken from respective participants before participation as shown in Figure 1. The study was approved by the research committee. IBM SPSS Statistics Version 21 was used to analyze the collected data. All descriptive information such as demographic data (name, age, gender, and qualification), working experience, and challenges that the physiotherapist faced during prosthesis rehabilitation were analyzed using central measures of tendency such as frequency, distribution, mean, and standard deviation.

Table 1 Respondent characteristics

Characteristics	Demographic
Gender	n (%)
Male	61 (30.5%)
Female	139 (69.5%)
Educational level	
Diploma	78 (39.0%)
Degree	110 (55.0%)
Master	12 (6.0%)

Table 2 Challenges of physiotherapists presented in mean and standard deviation

Mean	SD	Challenges
		Patient's factors
2.22	.814	Encountering patient with strong beliefs
2.05	.813	Encounter patient with racial preference/ stereotype
2.40	.777	Encounter patient with mental illness
2.36	.808	Patients view physiotherapist without a specific professional identity
2.69	.613	Encountering patient that refuse to perform exercises
2.55	.700	Patient with obesity is more difficult to train when compared to the patient that is not obese
2.66	.587	Patient's adherence to exercise is poor
		Healthcare providers factors
2.38	.799	No coordination/ communication of efforts between multidisciplinary team
2.41	.745	Lack of job structure
2.51	.695	Lack of practice for prosthesis rehabilitation
2.38	.805	Workload during prosthetic rehabilitation is high
2.10	.868	Prevent fall incidence during prosthesis rehabilitation is difficult
2.19	.811	Feeling the burden of transdisciplinary
2.31	.822	Emotional stress is commonly faced by therapists
2.77	.530	Encountered high physical demand
		Environmental factors
2.67	.584	Encounter with bigger gaps between follow-ups
2.57	.623	Patients often encounter difficulty in assessing health service
		Cultural factor
2.25	.808	Encountering language barriers
		Other factors
2.63	.612	Limited fund and resources
2.64	.551	Encountering prosthesis problems

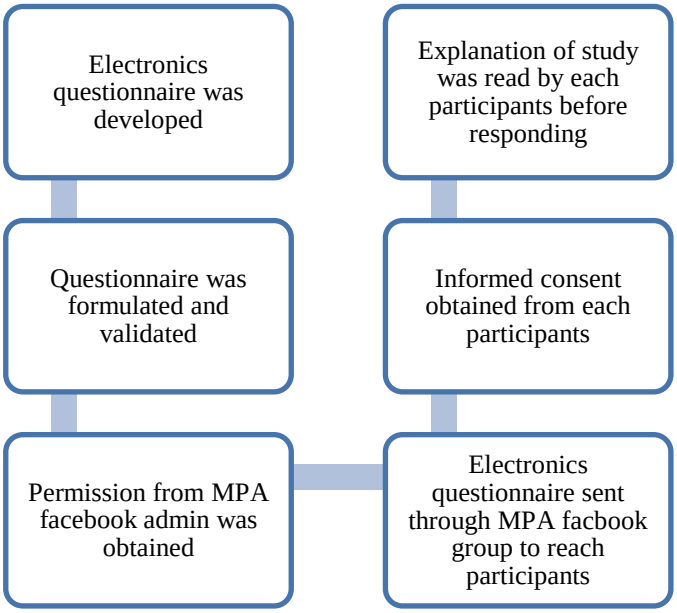

Figure 1 Flow chart for the process of data collection

Table 3 Patient factors presented in frequency, mean and standard deviation

Patient factors	Frequency (n)	Disagreeable	Neutral	Agreeable
Challenges	Mean (SD)		n (%)	
Strong Beliefs	2.22 (.814)	49 (24.5%)	59 (29.5%)	92 (46.0%)
Stereotype	2.05 (.813)	61 (30.5%)	68 (34.0%)	71 (35.5%)
Mental illness	2.40 (.777)	36 (18.0%)	48 (24.0%)	116 (58.0%)
No professional identity	2.36 (.808)	42 (21.0%)	45 (22.5%)	113 (56.5%)
Refuse to exercise	2.69 (.613)	16 (8.0%)	30 (15.0%)	154 (77.0%)
Obese patient	2.55 (.700)	24 (12.0%)	42 (21.0%)	134 (67.0%)
Poor exercise adherence	2.66 (587)	12 (6.0%)	43 (21.5%)	145 (72.5%)

3 Results

3.1 Respondent Characteristics

In total, only 225 physiotherapists responded to the electronic questionnaire and 25 responses were excluded based on exclusion criteria. Finally, 200 (n=200) responses were included for data analysis; the response rate was 88.89% among the respondents. Among 200 respondents, 30.5% were male and 69.5% were female, 39% were diploma graduates, 55% were degree graduates and only 6% were master graduates as shown in Table 1.

3.2 Challenges faced by physiotherapists during prosthesis rehabilitation

The mean and standard deviation were calculated individually for 20 challenges as shown in Table 2. Mean score out of 3.0 was calculated based on the scoring from the Likert scale (Beglar & Nemoto, 2014). The challenges are categorized into patient factors, healthcare provider's factors, cultural factors, environmental factors, and other factors. Based on the data, it is evident that the majority of the physiotherapists in Malaysia have faced the challenges stated in the questionnaire.

3.2.1 Patient factors

Table 3 shows the frequency, mean and standard deviation for patient factors. All challenges under this category scored mean more than 2.0 hence, all of the challenges were considered as agreeable. The second challenge, encounter patients with racial preference/ stereotype is common, (eg: either prefer same race or vice versa) reported the lowest mean score. Among the attended respondents, 30.5% (n=61) voted for disagreeable, 34.0% (n=68) for neutral, and 35.5% (n=71) for agreeable.

Further, the fifth challenge, encountering patient that refuse to perform exercises is common, reported the next higher mean score. In this challenge, 8.0% (n=16) of the respondents voted for disagreeable, 15.0% (n=30) for neutral, and 77.0% (n=154) for agreeable.

Table 4 Healthcare providers factors presented in frequency, mean and standard deviation

Healthcare providers factors	Frequency (n)	Disagreeable	Neutral	Agreeable
Challenges	Mean (SD)		n (%)	
No coordination between MDT	2.38 (.799)	40 (20.0%)	44 (22.0%)	116 (58.0%)
Lack of job structure	2.41 (.745)	31 (15.5%)	55 (27.5%)	114 (57.0%)
Lack of practice	2.51 (.695)	23 (11.5%)	54 (27.0%)	123 (61.5%)
High workload	2.38 (.805)	38 (19.0%)	51 (25.5%)	111 (55.5%)
Fall prevention	2.10 (.868)	66 (33.0%)	48 (24.0%)	86 (43.0%)
Burden of MDT	2.19 (.811)	50 (25.0%)	62 (31.0%)	88 (44.0%)
Emotional stress	2.31 (822)	46 (23.0%)	47 (23.5%)	107 (53.5%)
High physical demand	2.77 (.530)	10 (5.0%)	27 (13.5%)	163 (81.5%)

Table 5 Cultural factors presented in frequency, mean and standard deviation

Cultural factor	Frequency (n)	Disagreeable	Neutral	Agreeable
Challenges	Mean (SD)		n (%)	
Language barrier	2.25 (.808)	46 (23.0%)	57 (28.5%)	97 (48.5%)

Table 6 Environmental factors presented in frequency, mean and standard deviation

Environmental factors	Frequency (n)	Disagreeable	Neutral	Agreeable
Challenges	Mean (SD)		n (%)	
Larger follow-up gaps	2.67 (.584)	12 (6.0%)	41 (20.5%)	147 (73.5%)
Difficulty in assessing	2.57 (.623)	14 (7.0%)	59 (29.5%)	127 (63.5%)

Table 7 Other factors presented in frequency, mean and standard deviation

Other factors	Frequency (n)	Disagreeable	Neutral	Agreeable
Challenges	Mean (SD)		n (%)	
Limited funds and resources	2.63 (.612)	14 (7.0%)	46 (23.0%)	140 (70.0%)
Prosthesis problems	2.64 (.551)	7 (3.5%)	59 (29.5%)	134 (67.0%)

3.2.2 Healthcare provider factors

Table 4 shows the frequency, mean and standard deviation for healthcare providers factors. All challenges under this category scored mean more than 2.0 hence, all of the challenges were considered as agreeable. The twelfth challenge, prevent fall incidence during prosthesis rehabilitation is difficult, gained the lowest mean score. 33.0% (n=66) of the respondents voted for disagreeable, 24.0% (n=48) for neutral, and 43.0% (n=86) for agreeable. Whereas, the fifteenth challenge encountered high physical demand, scored the highest mean score as 5.0% of the respondents (n=10) voted for disagreeable, 13.5% (n=27) for neutral, and 81.5% (n=163) for agreeable.

3.2.3 Cultural factor

Table 5 shows the frequency, mean and standard deviation for the cultural factor. Encountering language barriers is common; this challenge was considered agreeable as the mean reported score was more than 2.0. On this, 23.0% (n=46) of respondents voted for disagreeable, 28.5% (n=57) for neutral, and the rest 48.5% (n=97) for agreeable.

3.2.4 Environmental factors

Table 6 shows the frequency, mean and standard deviation for environmental factors. All challenges under this category scored mean more than 2 hence, all of the challenges were considered as agreeable. Encounter with bigger gaps between follow-ups (>2 weeks) due to unforeseen circumstances is common; this challenge reported the third-highest mean score among all of the challenges. Among 200 respondents, 73.5% (n=147) voted for agreeable.

The eighteenth challenge, patients often encounter difficulty in assessing health service, 7.0% (n=14) of the respondents voted for disagreeable, 29.5% (n=59) for neutral, and 63.5% (n=127) for agreeable.

3.2.5 Other factors

Table 7 shows the frequency, mean and standard deviation for other factors. Limited funds and resources, this challenge is considered as agreeable as the mean score was more than 2.0. Among 200 respondents, 7.0% (n=14) of the respondents voted for disagreeable, 23.0% (n=46) for neutral, and 70.0% (n=140) for agreeable. The twentieth challenge, encountering prosthesis problems are common, this challenge was considered as agreeable as well and among the replied respondents, 3.5% (n=7) of the respondents voted for disagreeable, 29.5% (n=59) for neutral, and 67.0% (n=134) for agreeable.

4 Discussion

The purpose of this study was to investigate the challenges faced during prosthesis rehabilitation from the perspective of physiotherapists. The challenges identified can be categorized into five categories: patient factors, healthcare provider's factors, cultural factors, environmental factors, and other factors.

This study was unique in its use of quantitative research principles to investigate the barrier during prosthesis rehabilitation in Malaysia. There were several qualitative studies have explored the barriers faced by different groups of healthcare providers from different areas, however, few studies have explored physiotherapists' perceptions related to prosthesis rehabilitation in Malaysia. Therefore, the present study results provide an exceptional insight into prosthesis rehabilitation to complement the current literature.

4.1 Patient factors

In this study, 46.0% of the respondents experienced treating a patient with strong belief during prosthesis rehabilitation (Table 3). External evidence in support of this finding is available from a recent study in South Africa (Ennion & Rhoda, 2016). According to this, traditional healers were recognized as part of the multidisciplinary team (MDT) and to work closely with the hospitals. The amputation and mortality rate could be lowered if traditional healers are to be recognized as part of the MDT and trained to refer the patients to hospitals. However, many patients chose to believe that illness was related to bewitchment and sought help from traditional healers to appease their ancestors and remove the "curse". This greatly reduces their trust and adherence to western medicine and rehabilitation (Ennion & Rhoda, 2016). Among the replied respondents, 35.5% of the respondents voted agreeable for the statement "racial preference/ stereotype" as shown in Table 3, this indicated that physiotherapists involved in prosthesis rehabilitation in Malaysia did experience treating patients with racial preference or stereotype. This finding is also supported by the study done in South Africa regarding prosthesis rehabilitation (Ennion & Rhoda, 2016), however, most of the physiotherapists in Malaysia is from local instead of the foreign country. Furthermore, the Malaysian concept is to strengthen relationships and cooperation among the multi-ethnic people in this country. According to Prime Minister, One Malaysia concept "we stand, we think, and we act as Malaysians and we take actions based on the needs of all ethnic groups in our country" (Zainal & Salleh, 2010). Hence, this might not be a big challenge for prosthesis rehabilitation.

Table 3 shows that 58.0% and 67.0% of the respondents agreed with treating patients with mental illness and obesity is difficult. Similarly, Potter et al. (2003) interviewed physiotherapists working in the Perth metropolitan area, reported that patients with physical and psychological problems are hard to manage.

The reason behind the difficulty in treating an obese patient is owing to low levels of physical activity with poor adherence to exercise interventions as well as lack of motivation (You et al., 2012; Firth et al., 2016). Furthermore, Potter et al. (2003) highlighted that, physiotherapist skill and psychological skill training need to be trained and suggested that communication as well as behaviour modification strategies for improving and assisting the work for patients with difficultly. However, a study by Vivas et al. (2017) reported that obesity does not show a disadvantage with regards to validated outcomes, such as the 2-min walk test, L-test or SIGAM score at discharge after inpatient amputation rehabilitation, hence obesity should not be a barrier to offering inpatient rehabilitation for amputation patients.

In this study, 56.5% of the respondents agreed with patients' view of therapists without specific professional identity (Table 3). This finding is corroborated by a study done in the UK by Timmons and East regarding uniforms, status, and professional boundaries in hospitals (Timmons & East, 2011). This might result in a sense of loss and disempowerment by the therapist, hence reducing their commitment during rehabilitation. As shown in Table 3, 77.0% of the respondents and 72.5% of the respondents agreed for patients refuse to perform the exercise, and the patient's adherence to exercise is poor.

This finding is supported by a study done by Parry et al. (2017) regarding factors that affect the implementation of early rehabilitation into intensive care unit practice. The reason behind this might be owing to the patient's motivation and intake of sedative medications. To overcome this barrier, communication and education with patients and their families are very important; hence it is encouraged to involve patients and family in setting individualized patient goals.

4.2 Healthcare provider's factors

In this study, the majority of the respondents (44.0%) agree that there is a lack of coordinated effort among MDT (Table 4). Ennion & Rhoda, (2016) found that the members of the MDT might not know their roles. For instance, most health professionals do not include and recognize amputees as members of MDT; surgeons spend time only in performing surgery instead of discussing patient goals and rehabilitation outcomes; as well as barriers between prosthetists and physiotherapists. Team working is considered essential, as a wide range of knowledge and skills is required to diagnose and assess impairments; activity limitations, and participation restrictions; as well as select treatment options (Neumann et al., 2010). According to Neumann et al. (2010) and Clarke (2013), the lack of MDT approach might negatively affect

the outcome of rehabilitation and cause prosthesis problems due to inadequate rehabilitation for prosthetic fitting.

Among the respondents, 57.0% and 61.5% of the respondents agreed that there is a lack of job structure and lack of practice for prosthesis rehabilitation respectively as shown in Table 4. Afzal (2017) looked at the major challenges faced by the physiotherapy profession is expanding its role in health, prevention, and wellness service, and found a lack of job structure and lack of practice for prosthesis rehabilitation. The reason behind the lack of practice for prosthesis rehabilitation might be owing to different factors, a variety of levels of amputation as well as personal and environmental factors. A recent study regarding physiotherapy and rehabilitation approaches to lower-limb amputation by Ülger et al. (2007) suggested that the applications of various physiotherapy methods for prosthesis rehabilitation. Moreover, published evidence-based clinical guidelines for the physiotherapy management of adults with lower limb prosthesis which are readily accessed online can be used as a source of reference (Broomhead et al., 2012).

In this study, 43.0% of the respondents voted agreeable for prevention of fall are difficult (Table 4). This finding has not been reported widely in the literature. However, the study by Pauley et al. (2006) highlighted fall incidents among patients with lower limb amputation during inpatient rehabilitation is as high as 20% among all the amputees involved in the inpatient rehabilitation. Also, the same authors predict that older age, the greater number of comorbidities, cognitive impairment, and the use of a greater number of medications can increase the incidence of falls (Steinberg et al., 2019). Among the tested respondents, 44% of the respondents claimed that there is the burden of transdisciplinarity as shown in Table 4. This finding is also similar to the study carried out in South Africa on prosthesis rehabilitation.

According to Ennion & Rhoda, younger inexperienced therapists might be confused about transdisciplinarity and feel the burden. For instance, crossing the boundaries of discipline-specific scopes of practice considered as illegal implications especially when an injury to the patient occurs (Ennion & Rhoda, 2016).

As shown in Table 4, more than half of the respondents voted agreeable for high workload and emotional stress. Furthermore, 81.5% of the respondents voted agreeable for high physical demand. According to Pavlakis et al. (2010), physiotherapist plays an important role during the rehabilitation process, therefore emotional stress is susceptible for a physiotherapist. This study also suggested that low salary, employment sector, age group, and job-related stress will lead to burnout syndrome and hence affect the effectiveness of the rehabilitation.

4.3 Cultural factor

Among the respondents, 48.5% experienced a language barrier during prosthesis rehabilitation in this study (Table 5). These results are in agreement with the findings of Ennion & Rhoda (2016), language barrier is also reported during prosthesis rehabilitation in South Africa. The reason behind this was owing to the majority of the healthcare professionals were English speaking, however, only patients from several areas can speak and understand English.

Patients who are older, less educated, low income, and underinsured are believed to be low English proficient (LEP) (Wilson et al., 2005). English proficient status patients negatively affected the rehabilitation team's ability to diagnose, counsel, educate, and provide quality rehabilitation service (Neumann et al., 2010; Clarke, 2013).

A study by Wilson et al, suggested that an increasing number of language-concordant physicians might reduce language barriers and improve the quality of care for LEP patients (Wilson et al., 2005).

4.4 Environmental factors

In this study, the majority of the respondents (73.5%, 63.5%) voted for agreeable to both bigger follow-up gaps and difficulties to assess healthcare service (Table 6). This finding is supported by a study conducted in South Africa as well. Patients often struggled to follow up for prosthesis rehabilitation after discharge from inpatient rehabilitation due to some unforeseen circumstances such as lack of transportation and bad weather. These circumstances require patients to make use of public transport or request somebody to send them to attend clinics or follow-up at the hospital, which the majority of the patients refused to do so to avoid troubling others (Ennion & Rhoda, 2016; Stuckey et al., 2020).

4.5 Other factors

Respondents (70.0%) in this study highlighted that there are limited funds and resources when providing prosthesis rehabilitation (Table 7). Previous studies have noted that there is a lack of sufficient prosthetists being trained to meet the growing demands of persons with lower limb amputation in developing countries (Pearlman et al., 2008; Sanders, 2020). Other than that, the study carried out in South Africa on prosthesis rehabilitation and the recent guidelines on "rural-proofing policy and resourcing for health in rural areas" also reported similar results (Rural-Proofing for Health: Guidelines, 2015; Ennion & Rhoda, 2016). Considering the increased demand for rehabilitation services in rural settings owing to the increased incidence of disabilities, however, few rehabilitation therapists were employed hence the

shortage of therapy staff is to be a concerned (Wilson et al., 2009; Sanders, 2020). This problem is then leading to high workload (55.5%), emotional stress (53.5%), and high physical demand (81.5%) to the therapists in charge, where the majority of the respondents in this study were also agreed with the statement (Table 4). As a result of these stresses, the therapist in charge might be discouraged to continue to work in that setting or suffer burnout (Ennion & Rhoda, 2016). Among the respondents, 67.0% have experienced treating patients with prosthesis problems as shown in Table 7. External evidence in support of the present study finding is the study done in the Netherlands regarding the functional performance, participation, and autonomy after discharge from prosthetic rehabilitation are their autonomy outdoors and family role (Van Twillert et al., 2014). It is proved that the patients' satisfaction level towards their prosthetic limbs can affect rehabilitation interventions (Webster et al., 2012).To overcome this barrier, a study done in Australia proved that introduction of the interim prosthetic program (IPP) successfully reduced time to all primary rehabilitation (Hordacre et al., 2013).

Conclusion

Prosthesis rehabilitation was a vital process for amputees to lower the mortality rate and improve or maintain the general quality of life of the patient. There were several barriers faced by physiotherapists during prosthesis rehabilitation, which can be categorized into patient factors, healthcare provider's factors, cultural factors, environmental factors, as well as other factors. This study also created some ideas on modifications that could be done to facilitate prosthesis rehabilitation, including enhanced planning of a comprehensive amputation rehabilitation program, increased investment, improved planning and communication between medical units involved. Furthermore, recruiting different races and incorporating different languages in rehabilitation could be a viable option to address the identified challenges during prosthesis rehabilitation.

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TOXICITY OF ZINC OXIDE NANOPARTICLES ON HUMAN SKIN DERMAL CELLS

Harshyini Maheswaran[1], Ling Shing Wong[2], Anto Cordelia Tanislaus Antony Dhanapal[3], Ramasamy Thangavelu Narendhirakannan[4], Ashok Kumar Janakiraman[5], Sinouvassane Djearamane[1*]

[1]Department of Biomedical Science, Faculty of Science, Universiti Tunku Abdul Rahman, Kampar, 31900 Malaysia
[2]Life Science Division, Faculty of Health and Life Sciences, INTI International University, Nilai, 71800 Malaysia
[3]Department of Chemical Science, Faculty of Science, Universiti Tunku Abdul Rahman, Kampar, 31900 Malaysia
[4]Department of Biochemistry, Kongunadu Arts and Science College, Affiliated to Bharathiar University, Coimbatore, Tamil Nadu, India
[5]Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, UCSI University, 56000 Cheras, Kuala Lumpur, Malaysia

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ABSTRACT

Zinc oxide (ZnO) has special physical and chemical characteristics which enable it to be utilized in numerous applications including electronics, sunscreens, pigments, and most notably in biomedical applications. Nanoemulsions containing zinc oxide nanoparticles (ZnO NPs) are progressively sought-after as an active component in cosmetic formulations and are used in sunscreens, moisturizers, and antiaging products. Zinc paste bandages including Unna boot consist of open wove cotton gauze treated with ZnO paste are now common medicaments for leg ulcers. The damaged and broken skins are vulnerable to ZnO NPs uptake. This being the case, ZnO NPs on the skin surface can affect the functions of surrounding cells in numerous ways by penetrating into the skin cells. This could exert toxicity effects on the skin cells over time depending on the concentration and site of ZnO NPs exposure. This review brings together some findings regarding the toxicity of ZnO NPs on human skin dermal cells and thus in turn enlightens the safer usage of ZnO NPs in skin care applications.

* Corresponding author

E-mail: sinouvassane@utar.edu.my; biochsinouvas07@gmail.com (Sinouvassane Djearamane)

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1 Introduction

Richard Feynman, the Nobel Prize winner in 1959, was the foremost to foresee the advent of modern technology that might work with materials on just a range of 1–100 nm. Nanotechnology's true promise lies in the potential for manipulating materials at the same unimaginably small scale (Tocco et al., 2012). The reverberations that nanotechnology has in our lives at this moment in time are mountainous. Current implementations contribute to the instigation of devices, systems, and structures that are capable of revolutionizing medical therapeutics and diagnostics which have yet to be seen (Rajeshkumar et al., 2019). According to a study from the Consumer Product Inventory of Nanotechnology, silver nanoparticles (Ag NPs) are the most commonly used nanomaterials as they are present in 435 (24%) consumer products on the market (Therapeutic Goods Administration, 2006; Vance et al., 2015; Zhang et al., 2016).

Most of the metal nanoparticles (MNPs) such as copper oxide, magnesium oxide, silver, and zinc oxide nanoparticles exhibit antimicrobial properties that have been imparted into the packaging to kill harmful microorganisms (Makhlouf &Tiginyanu, 2011). In this regard, antimicrobial MNPs have positive results in hindering food deterioration as well as prolonging food's shelf life because of the intrinsic physicochemical properties of inorganic MNPs which enable the unrestrained development of reactive oxygen species (ROS) that primes to oxidative stress and ensuing cell damage (Fu et al., 2014). Given their low toxicity, price, and ultraviolet barrier properties, zinc oxide nanoparticles (ZnO NPs) are favored over Ag NPs (Chaudhry et al., 2008; Llorens et al., 2012). Drug delivery has drawn awareness of researchers and pharmaceutical companies that nano-mediated systems could constitute an acceptable exchange therapy for conventional drugs since they can provide a more impactful drug build-up in the site of infection with reduced side effects (Grumezescu, 2016).

2 Zinc Oxide Nanoparticles

Since the early 1960s, ZnO has been synthesized in thin films to be used as catalysts, sensors, and transducers. Due to its distinctive and precise properties, ZnO has attracted attention for a broad array of applications like those of electrical conductors, optical (pigment applications), and thermal since it is stable at temperatures more than 1800°C (Moezzi et al., 2012). Zinc oxide nanoparticles (ZnO NPs) are also known as oxydatum, ketozinc, permanent white, zincioxicum, and oxozinc (AZoNano, 2013). ZnO NPs have inherent qualities determined primarily by size, crystallinity, composition, and morphology. Their characteristics, such as morphological, chemical, and mechanical features, alter concerning the nanometer level. In addition to the stated properties, ZnO NPs also have a high electrochemical coupling coefficient, high chemical stability, and high photostability (Kołodziejczak-Radzimska & Jesionowski, 2014). Not only ZnO NPs work as credible physical ultraviolet A (320-400 nm) as well as ultraviolet B (290-320 nm) filters (Nohynek et al., 2007; Zvyagin et al., 2008) that are present in sunscreens to shield users from the damaging outcomes of UV rays (Maverakis et al., 2010; Djearamane et al., 2019), it also reduces the reliance on systemically absorbable chemical sunscreen agents (Gonzalez et al., 2006). These formulations aimed to shield the skin from sun-induced erythema, decrease photoaging as well as possibly decrease the likelihood of skin cancer (Holmes et al., 2016) by lingering at or close by the surface of the skin because there is no reasoning for their entry into the skin (Gamer et al., 2006; Cross et al., 2007). However, contact with ZnO NPs through the usage of consumer products and also through occupational and environmental exposure might bring health hazards to humans.

3 Toxicity of ZnO NPs on Skin Cells

The skin as the body's key defence organ is well equipped to halt the penetration of materials through its surface (Lin et al., 2015). Mammalian skin is divided into many layers: stratum corneum (SC), epidermis, dermis, and subcutaneous layer. SC is the barricade that limits the penetration rate of many topically applied materials (Schaefer et al., 2003). Nanomaterials including nano-emulsions made up of oils, emulsifiers and aqueous vehicles are being processed to create a stable network of nano sized globules that are increasingly popular in cosmetics and medicine (Nohynek et al., 2007). For instance, the Nano Gel platform developed by Tri-K Industries presents a simplistic production process for refined nanoemulsion products that prevents transepidermal water loss (TEWL) and enables it suitable for many applications including anti-aging products, moisturizers, and sunscreens (Müller et al., 2002).

Zinc is an essential micronutrient for human health (Rostan et al., 2002). Zinc has a prominent part in influencing each stage of the wound alleviating phase; from membrane restoration, oxidative stress, coagulation, inflammation, and immune response, tissue re-epithelialization, angiogenesis, to scarring (Sekhon & Sen Gupta, 2017). Zinc is particularly essential to the skin (Rostan et al., 2002) because the skin contains high zinc in the epidermis (Gupta et al., 2014). Because of its multitude in the epidermis, a slight zinc inadequacy can lead to coarsened skin and hinder the curing of wounds (Lansdown et al., 2007). Unna boot bandages of zinc paste comprised of open wove cotton gauze infused with ZnO paste persist as the traditional procedure for leg ulcers (Williams, 1999).

Numerous factors possibly influence the absorption of ZnO NPs into the skin following its superficial application. Firstly, the surface area to volume ratio increases with smaller NP's that enhances the reactivity of NPs with penetration capacity into the

skin. The amount of cellular uptake of NPs increases with the decrease of size as the submicron ZnO or ZnO microparticles penetrate slower than ZnO NPs (He et al., 2010; Reed et al., 2012). Secondly, the type of zinc ion formed following ZnO dissolution and then readiness to penetrate into the skin cells is pH-dependent. The rate of dissolution of ZnO into Zn^{2+} is faster at lower pH (Han et al., 2010; Bian et al., 2011). Thirdly, high pH (>9) or low pH (<3) damages human skin integrity which brings the risk of NPs entry into the skin cells (Holmes et al., 2016). Fourthly, the capability of zinc ions and ZnO to traverse the SC is reliant on the essence of their engagement with the SC (Hostynek, 2003). The noticeably elevated uptake of Zn in human skin at lower pH occurred because human SC is semipermeable for cations as Zn is in the cationic form at lower pH (LareseFilon et al., 2011). Lastly, in the topical formulations, zinc absorption into the skin cells is determined by the concentration of zinc present in the topically applied substance (Zhong, 2004).

The controversy regarding the safety of ZnO to humans began in the late 1990s when ZnO had been utilized for obstructing UV radiation in sunscreens. Studies indicated that ZnO NPs cannot cross the skin barrier, so it stays on the skin's external layer and therefore does not cause toxicity (Klingshirn, 2007; Moezzi et al., 2012). Furthermore, ZnO NPs pooled on the exterior of skin and inside the skin furrows through the intact epidermis have not been reported to penetrate or cause cellular toxicity. Mohammed et al. (2019) also concluded the safe use of sunscreen products. However, the dermal absorption of ZnO has been documented with a ZnO cream (Triple Treatment, Smith & Nephew, Hull, UK) administered on the forearm for a duration of 3 hours (Gorodetsky et al., 1999). Another report showed low zinc ion uptake by skin in normozincemic humans through topical ZnO (Hostýnek et al., 1993). When the superficial keratinocytes are shed off naturally, the zinc ion in the epidermal keratin will be gone while a portion of zinc entering deeper skin layers will then be taken up into the circulatory system (Lansdown et al., 2007). Although topically spread ZnO does not enter the viable epidermis, ZnO hydrolyzes on the skin superficial, which raises the zinc ion concentration in SC and the epidermis, thus in the systemic circulation as well as in urine (Holmes et al., 2016). It has been mentioned that little quantities of zinc from ZnO do get absorbed through the human skin (Hayden et al., 1997). Zyyagin et al. (2008) demonstrated that the caprylic capric triglycerides formulation of ZnO NPs encouraged the passive diffusion of NPs through the lipophilic intercellular passage. This passage depicts the fundamental transdermal penetration pathway. ZnO NPs mineral constituents are found on the skin barrier as well as in the region of desquaming corneocytes as shown by electron micrographs of human skin (Zvyagin et al., 2008; Espitia et al., 2012). At lower pH, more ZnO undergoes acid-catalyzed hydrolysis to liberate zinc ions. Zn^{2+}will be the main ion in the solution and that zinc ion penetrates the SC and thence into the viable epidermis (Bian et al., 2011). An insoluble precipitate forms at pH 9 as the zinc is in the $Zn(OH)_{2(s)}$ form because of the existence of a zero charge point at around pH 9.4. Hence, the amount of zinc ions accessible to pass through the skin is lesser at higher pH due to a decrease in $Zn(OH)^{+}$ ions dissolution (Holmes et al., 2016). The uncertainty now is if the rise in free zinc may affect the intracellular zinc homeostasis. Studies on the toxicity of skin cells by differing labile zinc concentrations are doubtful. It turned out to be that the viability of human skin fibroblasts with the company from oxidative stress from UVB as well as UVA radiation has been assisted with the rise in intracellular zinc (Richard et al., 1993).

The clinical relevance of the transdermal route in alleviating the symptoms of zinc deficiency is uncertain. After covering the extensive skin areas using topical ZnO in petrolatum, neither Morgan et al. (1980) nor Derry et al. (1983) managed to track an elevated serum zinc level. Systemic zinc uptake by topical zinc is significantly enhanced in the absence of a skin barrier. Following the usage of ZnO-medicated adhesive dressing, serum zinc concentration elevated was reported among the victims of substantial partial-thickness as well as a full-thickness burn injury. There were no substantial variations in serum zinc among ZnO-treated and control-treated patients with small wounds about $10cm^2$ (Lansdown et al., 2007). Skin diseases like psoriasis vulgaris produce hyperkeratosis that might end in reduced topically applied substance uptake (Korting et al., 1990). This is reasoned by the thickening of the epidermis as a result of inflamed skin which ultimately enhances the skin as a barricade (Walker et al., 2003). Other skin diseases such as eczema and podoconiosis (elephantiasis) with rupture in the SC permit the penetration of topically applied substances as the reduction in the barrier capacity of the skin (Korting et al., 1990). Hence, the studies have confirmed that the damaged skin is susceptible to ZnO uptake (Williams, 1999, Walker et al., 2003, Lansdown et al., 2007). Earlier studies reported that exogenous zinc ions are useful as they can avoid herpes infections (Read et al., 2019) and serve to shield the skin as an antioxidant (Prasad, 2014). Two modes of action involving zinc ions applied topically as an antioxidant was suggested. Firstly, Zn^{2+} substitutes Fe^{2+} and Cu^{2+} on cell membranes along with proteins. Secondly, Zn^{2+} might stimulate metallothione in forming a zinc-thiolate moiety that operates as a chelating agent for damaging free radicals (Rostan et al., 2002). In a hamster ear experiment, the topical formulation of zinc ions was proven to simulate metallothionein. The skin contains metallothionein, which will cling to additional labile zinc to ensure an ideal concentration of zinc ions. Small concentrations of free zinc originating from ZnO that enter intact skin may be advantageous to serve as an antioxidant (Morgan et al., 1993).

Besides, when ZnO NPs undergoes hydrolysis, it produces zinc ions and reactive oxygen species (ROS). This further activates different cytotoxic pathways such as intracellular calcium flux, plasma membrane leakage, and mitochondrial depolarization (George et al., 2009).

Conclusion and Recommendation for Future Research

From the earlier study findings, it is evident that ZnO NPs do not penetrate the intact healthy skin and therefore do not cause toxicity effect. On the other hand, some studies have reported the penetration of zinc ions through the stratum corneum of damaged and compromised skin. The information regarding the toxic effects of ZnO NPs in skin cells is scarce. Therefore, future research studies are needed in both *in vitro* and *in vivo* to address the dose and duration of exposure required causing the potential toxic effects of ZnO NPs upon entry into the skin cells.

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CHANGES IN EATING BEHAVIOURS FOLLOWING BARIATRIC SURGERY: A PROSPECTIVE STUDY

Kavitha Subramaniam[*1,2], Wah-Yun Low[3], Peng-Choong Lau[4], Kin-Fah Chin[4], Karuthan Chinna[5], Nik Ritza Kosai[6], Mustafa Taher[6], Reynu Rajan[6]

[1]Medical Education, Research and Development Unit, Faculty of Medicine, University of Malaya, Malaysia
[2]Department of Physical and Mathematical Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900, Kampar, Malaysia
[3]Dean's Office, Faculty of Medicine, University of Malaya, Malaysia
[4]Department of Surgery, Faculty of Medicine, University of Malaya, Malaysia
[5]School of medicine, Faculty of Medical and Health Science, Taylor's, University Malaysia
[6]Minimally Invasive, Upper gastrointestinal and bariatric surgery, Department of Surgery, Faculty of Medicine, University Kebangsaan Malaysia.

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ABSTRACT

Adopting healthy eating behavior is important in achieving successful weight loss after bariatric surgery. This study aims to determine the changes in eating behaviors 6 months after surgery. Fifty-seven patients who went through bariatric surgery in two tertiary referral hospitals were recruited and interviewed before surgery (T0), three (T1), and six (T2) months after surgery. Eating behaviors were assessed using the Dutch Eating Behavior Questionnaire (DEBQ), which measured emotional, external, and restrained eating. Higher subscale scores indicate strong behavioral traits. Other information including psychological distress, quality of life, socio-demography, and morbidity were collected. Generalized Estimating Equation (GEE) model was developed to study the change in eating behaviors and its' predictors over time. Participants of the study were mostly women, from the Malay ethnic group and the average age at the time of surgery was 39.4 years. Emotional and external eating scores changed significantly over time with the values recorded at various time intervals as follows: 2.06 and 2.86 before surgery; 1.64 and 2.25 three months after surgery; and 1.81 and 2.40, 6 months after surgery. Reduction in the third month followed by a slight increase at the sixth was noted. Restrained eating did not show a significant change. The presence of diabetes was also associated with higher emotional and external eating scores. Further, higher anxiety scores were associated with higher external eating. Favorable changes in eating behaviors were noted after bariatric surgery. However, a risk of a reversal in the improvement was present. The emotional and external eating behaviors in surgical candidates should be identified and addressed accordingly with special attention to diabetic patients.

* Corresponding author

E-mail:eskei13@yahoo.co.uk, kavithas@utar.edu.my (Kavitha Subramaniam)

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1 Introduction

To date, bariatric surgery is the most durable weight-loss solution for patients with morbid obesity (Carter, 2015). Post-surgically, patients lose up to 60% of excess weight (Coleman et al., 2014), and 85% of the patients were able to maintain 50% of the excess weight loss (Madura & Dibaise, 2012). Despite the promising overall outcome, individual variations in the post-surgical weight loss achievement have been reported, where some patients experienced sub-optimal weight loss (Campos et al., 2009; Conceição & Goldschmidt, 2019; Geerts et al., 2020) and some regained weight (Odom et al., 2010; Baig et al., 2019; Conceição & Goldschmidt, 2019). Surgical patients who practiced healthy lifestyles, which include controlled eating and regular physical exercise had better post-surgical weight loss (Madura & Dibaise, 2012; Monpellier et al., 2019). Conducive eating behaviour for better weight loss outcomes included adherence to dietary recommendations given by health professionals (Robinson et al., 2014) and having controlled food urges (Odom et al., 2010). Post-operative disordered eating behaviours such as binge eating (Luiz et al., 2016; Conceição & Goldschmidt, 2019), grazing (Wimmelmann et al., 2014; Pinto-Bastos et al., 2019) night eating (Wimmelmann et al., 2014), emotional eating (Chesler, 2012; Novelli et al., 2017; Conceição & Goldschmidt, 2019) and loss of control eating (White et al., 2010; Geerts et al., 2020) were associated with poor weight loss outcomes.

The surgical option for weight loss is often provided to patients with morbid obesity, who had futile efforts to lose weight via the conventional treatment. Previous failed attempts to lose weight lead patients to think that obesity and the eating behaviours are beyond their ability to control (da Silva & Maia, 2012; Opolski et al., 2015) and weight-loss surgery is seen as a "miracle moment" that is to happen to them, which is as an external solution that will help them to change their lifestyle without any effort from themselves (da Silva & Maia, 2012). Such understanding is of concern, as previous findings have reported high rates of disordered eating behaviour traits, such as emotional eating, binge eating, binge eating disorder, and food craving among the surgical candidates (Opolski et al., 2015), that continued to exist post-surgically in some patients (White et al., 2010; Conceição et al., 2014; Nasirzadeh et al., 2018; Conceição & Goldschmidt, 2019;), resulting in poor weight loss outcome (White et al., 2010; Chesler, 2012; Wimmelmann et al., 2014; Luiz et al., 2016; Novelli et al., 2017; Nasirzadeh et al., 2018). Controlled eating after bariatric surgery is pertinent for weight loss, weight maintenance, and the overall well-being of the patients. Knowledge of predictors of changes in eating behaviour among bariatric surgery candidates is very limited. Such knowledge will be helpful for patient-care to achieve adequate weight loss and health. This study was thus designed to identify the changes in disordered eating behaviors from pre to six months post-surgery and identify demographic and psychological predictors of changes in eating behaviors.

2 Materials and Methods

2.1 Selection of participants and Data Collection

Fifty-seven bariatric surgery candidates from two referral hospitals in Kuala Lumpur were recruited upon written consent. The participants were assessed at three timelines, before surgery (T0), three (T1), and six (T2) months after surgery. Forty-five participants (79%) turned up for the second interview (T1), and 43 (75%) turned up for the third interview (T2). Thirty-six participants (63%) completed all three follow-ups. The surgeries performed on the patients were Laparoscopic Roux-en-Y gastric bypass (LRYGB) (n=30, 53%), Laparoscopic sleeve gastrectomy (LSG) (n= 23, 40%), and one anastomosis mini gastric bypass (OAMGB) (n=4, 7%).

2.2 Assessments

This study is part of a larger research that aimed to determine the changes in health-related quality of life, psycho-behavioural variables, sexual function, and weight loss following bariatric surgery. Changes in eating behaviour are discussed in this study. Eating behavioural traits were assessed with the aid of the Dutch Eating Behaviour Questionnaire (DEBQ)(Van Strien et al., 1986). The DEBQ instrument consists of 33 items that assess three eating behaviours viz., (i) emotional eating, that is, eating in response to emotional cues or to regulate emotion; (ii) external eating, that is, eating in response to external food-related cues such as sight and smell of food; and (iii) dietary restraint (restrained eating), that is, dietary control via cognitive cues to influence one's body weight (Van Strien et al., 1986). A higher mean score for each subscale denotes a stronger behavioural trait. The DEBQ instrument has satisfactory construct validity and internal consistency when tested on the Malaysian adult sample (Subramaniam et al., 2017). Emotional, external, and restrained eating behaviours were included in this study as the behaviours were associated with eating disorders that are common among adults with obesity such as disinhibition, binge eating, night eating, and food addiction (Chesler, 2012; Nolan & Geliebter, 2012; Dochat et al., 2019).

Participants were screened for anxiety and depression using the Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983). The anxiety subscale score of 7-9 indicates possible anxiety, 10-13 indicate probable anxiety, and 14 and above for severe anxiety. The depression subscale score of 8 -10 indicates possible depression, 11-14, indicates probable depression, and 15 and above, severe depression (Zigmond & Snaith, 1983). The HADS instrument was reported to be suitable to be used in the Malaysian population (Quek et al., 2007). The possible, probable,

and severe anxiety and depression were combined to form the clinically significant anxiety and clinically meaningful depression categories due to small sample sizes.

The health related quality of life (HRQoL) was measured using the Short-Form 36 (SF 36) instrument (Ware & Sherbourne, 1992). The SF-36 contains 36 items that assess 8 dimensions of health, namely physical functioning, role limitations due to physical problems (termed 'role physical'), bodily pain, general health, vitality, social functioning, role limitations due to emotional problems (termed 'role emotional') and mental health. Each subscale score ranges from 0-100 with higher scores indicating better health status. The subscales are further grouped to obtain a composite score for physical health (Physical Component Score, PCS) and mental Health (Mental Component Score, MCS). The instrument showed good structural validity and reliability (Chronbach's alpha value above 0.7 for all subscales with exception to SF, which had a value of 0.62) when tested on the general Malaysian population (Sararaks et al., 2005) and thus it is a suitable measure of HRQOL for the Malaysian population (Sararaks et al., 2005).

Basic socio-demographic information such as gender, age, ethnicity, marital status, and occupation were collected. Several personal and family health indicators were assessed during the interview, which includes the following: duration of obesity (childhood or later), presence of overweight or obese family members, and incidence of comorbidities among first-degree family members. Information about the presence of obesity-related comorbidities such as diabetes, hypertension, dyslipidemia, fatty liver disease, sleep apnoea, and conditions that cause difficulty in walking was recorded from the patients' medical records.

The height and weight of the participants were measured during all three follow-up sessions using the standard hospital scales and rounded to the nearest 0.1cm, and 0.1kg. Body Mass Index (BMI) was estimated in the mathematical format of (weight, kg)/(height, m)2. Total weight loss (TWL) % was estimated in the mathematical format of [(preoperative weight - postoperative weight/ preoperative weight)] X 100%. Excess BMI loss (EBMIL)was estimated in the mathematical format of [(Pre-treatment BMI –current BMI)/ ((pre-treatment BMI- 25)] X 100%.

2.3 Compliance with Ethical Standard

Ethics approval was obtained from both hospitals' ethics committees (First hospital: MEC Ref No 732.19; Second Hospital: JEP-2016-276). This study is research involving human participants. All participants were recruited in the study upon written informed consent.

2.4 Statistical Analysis

Characteristics of the groups studied at each timeline was compared to determine if the groups that were followed-up were the same as the original recruited group at T0. Chi-square test was conducted to compare the categorical variables whereas the Kruskal-Wallis test was conducted to compare the distribution of continuous variables. Friedman's test was conducted to study the change in the distribution of variables over time. Wilcoxon sign-rank test with Bonferroni's adjustment was the post-hoc test conducted. Kendall's W test was used as the effect size where a value 0.1 indicates a small effect size, 0.3 moderate and 0.5 and above a indicates large effect. Spearman's rank correlation coefficient was used to study the relationship between variables. The Generalised Estimating Equation (GEE), was used to study the changes in eating behaviour over time, and predictors of change, if any. The GEE technique does not exclude incomplete cases from the analysis, and it produces findings based on all available data. It also does not require the normality assumption (Naseri et al., 2016). Pre-surgical and time-varying factors were included as predictors of changes in eating behaviour. The pre-surgical factors tested in the model were age, gender, ethnicity, marital status, initial BMI, and baseline comorbidities. Time-varying factors studied were psychological distress, HRQoL, surgery-related complications at follow-ups, and longitudinal BMI. Statistically significant variables ($p<0.05$) that improved the overall fit of the model were retained in the final regression equation.

3 Results

3.1 Socio-demographic characteristics and weight loss

A total of 57 bariatric surgery patients participated in the study, of whom 45 (79%) turned for the third-month assessment and 43 (75%) turned up for the sixth-month assessment. As the follow-up rates below 80% are a concern, the characteristics of the participant group included in each timeline were compared to determine if they were similar to the initial group recruited. The socio-demographic and initial weight (baseline weight) characteristics of the participants did not differ across time. The sub-group studied is not different from the original group recruited and the findings from follow-up data could be used to generalise for the included participants (Table 1). Little's Missing completely at random (MCAR) test was conducted to determine if the missing pattern was MCAR. The MCAR test was conducted across all timeline together (χ^2=12.78, p=0.17), only T1 data (χ^2=3.16, p=0.37) and only T2 data (χ^2=9.55, p=0.48). It was found that the missing pattern was MCAR in all the comparisons. The missing pattern analysis shows that the assumption needed for the regression analysis is fulfilled. The MCAR missing pattern confirms that the missingness is not dependent on any variables or outcome.

Table 1 Socio-demographic characteristics and baseline anthropometric measures of the participants included at T_0, T_1 and T_2

Characteristics	Participants at T_0 (n=57) n (%)	Participants at T_1 (n=45); n (%)	Participants at T_2 (n=43) n (%)
Gender			
Female	37 (65.0)	29 (64.4)	27 (62.8)
Male	20 (35.0)	16 (35.6)	16 (37.2)
Age (Years) †			
Mean, (sd)	39.40, (10.01)	38.18 (9.79)	40.19 (10.13)
Median, (IQR)	39.00, (12.00)	36.00 (12.5)	40.0 (14.0)
Ethnicity			
Malay	43 (75.4)	36 (80.0)	33 (76.7)
Indians	10 (17.5)	8(17.8)	(14.0)
Chinese and Others	4 (7.0)	1 (2.2)	4 (9.4)
Marital Status			
Married	47 (82.0)	36 (80.0)	37 (86.0)
Single / widower/ widowed	10 (18.0)	9 (20.0)	6 (14.0)
Educational Qualification			
Primary/Secondary	18 (31.6)	13 (28.9)	14 (32.5)
College	13 (22.8)	11 (24.4)	10 (23.3)
University	26 (45.6)	21 (46.7)	19 (44.2)
Housing situation			
Own House	47 (82.5)	35 (77.8)	36 (83.7)
Rented House/ Quarters / others	10 (17.5)	10 (22.2)	7 (16.3)
Mode of transport			
Own transport	52 (91)	41 (91.1)	39 (90.7)
Public transport/ Others	5 (9)	4 (8.9)	4 (9.3)
Weight (mean ± sd)	123.07± 33.0	120.36 ± 34.88	125.36 ± 34.30
BMI (mean ± sd)	45.46 ± 9.93	44.72 ± 10.47	46.24 ±10.21

sd - standard deviation, Ethnicity comparison was made by combining Indians and Chinese and others in one category, the Non-Malay group. The Malay and Non-Malay group were compared. Chisquare-test was conducted to compare categories; Kruskal-Wallis test was conducted to compare the weight and BM across the three groupsI.*p<0.05, ** p<0.01.

The majority of the participants were Malays and women. The mean age of the participants was 39.40 (SD=10.01) years and an average initial BMI of 45.52 (SD=9.94) (Table 1). The mean TWL achieved at the third and sixth months were 22.28% (SD=7.82) and 23.83% (SD=7.40) respectively. The mean EBMIL achieved at the third and sixth months were 40.55% (SD=18.33) and 63.33% (SD=33.18) respectively.

The most pronounced weight loss occurred in the first three months. The baseline prevalence of co-morbidities was as follow: diabetes (47%); hypertension (37%); dyslipidemia (35%); obstructive sleep apnoea (21%); fatty liver (7%), and problems affecting mobility (such as knee pain) was 21%. Family support during the time of illness was assessed with one question, “Who usually takes care of you if you become ill?” The majority of the participants (n=34, 60%) stated that their spouses took care of them when they were ill, followed by parents (n=8, 14%), children (n=3, 5%), and siblings (n=2, 3.5%). The remaining10 participants (17.5%) stated that they took care of themselves. No one stated that they were taken care of by a non-family member.

3.2 Psychological distress

The mean and median anxiety and depression scores reduced over time with a moderate effect size. This was accompanied by a reduction in the prevalence of clinically meaningful anxiety and depression across the study. A significant change in anxiety score was noted between the T0 and T2 timelines whereas a significant reduction in depression was noted from T0 to T1 and T0 to T2 (Table 2).

3.3 Health Related Quality of Life

The mean and median physical component summary scores increased over time. Post-hoc comparison showed that there was a significant improvement across all timelines with a large effect size (Table 2). The Mental component summary scores improved across time with a small effect size. Significant improvements were noted between T0 to T1 and T0 to T2 timelines (Table 2).

Table 2 Distribution of eating behaviour and psychological distress across study duration

Factor	Timeline T_0	T_1	T_2	χ^2,p *	Effect Size
Emotional Eating					
Mean, (sd)	2.06, (0.94)	1.64, (0.80)	1.81,(0.81)	3.928,	0.082
Median,(IQR)	1.73, (1.44)	1.23, (1.13)	1.58, (1.00)	p >0.05	
External Eating					
Mean, (sd)	2.86, (0.68)	2.25, (0.79)	2.40,(0.68)	17.156[ac]	0.378
Median, (IQR)	2.80, (0.80)	2.10, (1.53)	2.30, (0.93)	p <0.01	
Restrained Eating					
Mean, (sd)	2.65, (0.73)	2.93, (0.86)	2.74, (0.77)	3.489	
Median, (IQR)	2.70, (0.90)	2.90, (1.48)	2.80, (1.13)	p >0.05	0.027
Anxiety					
Mean, (sd)	4.88, (3.23)	3.43, (2.94)	2.40, (2.47)		
Median, (IQR)	4.00 , (5.0)	3.00, (4.00)	2.00, (4.00)	32.53[c]	0.465
Rate n (%)	12 (21.0)	4 (7.0)	2 (3.5)	p<0.01	
Depression					
Mean, (sd)	3.72,(2.89)	2.02,(2.59)	1.60, (2.13)		
Median, (IQR)	3.00, (5.00)	1.00, (2.00)	1.00, (3.00)	28.82[ac]	0.412
Rate n (%)	4 (7)	3 (5.3)	1 (1.8)	p<0.01	
HRQoL-PCS					
Mean,(sd)	42.77, (8.96)	49.85,(6.87)	51.59, (7.88)	39.31[abc]	0.546
Median, (IQR)	45.11, (14.25)	51.23, (10.44)	53.11, (7.96)	p<0.01	
HRQoL-MCS					
Mean, (sd)	50.19, (8.98)	53.90, (8.57)	54.79, (7.82)	7.26 [ac]	0.101
Median, (IQR)	50.53, (10.67)	57.37, (10.51)	56.73, (9.95)	p<0.05	

HRQoL-Health Related Quality of Life, PCS-Physical Component Summary, MCS- Mental Component Summary, *Friedman's test, sd-standard deviation, IQR- Inter Quartile Range. Prevalence of clinically significant anxiety and depression are reported below mean scores.T_0- before surgery T_1-Three months after surgery T_2-Six months after surgery, a-significant difference between T^0 and T^1, b-significant difference between T^1 and T^2, c-significant difference between T^0 and T^2.

3.4 Eating Behaviours

The scores of emotional and external eating behaviours decreased from the baseline to T1 but increased at T2.

On the other hand, the score of restrained eating increased at T1 and decreased at T2. Friedman's test outcomes showed that only external eating changed over time. Post-hoc comparison showed that the scores reduced significantly from T0 and T1 and T0 and T2. In the GEE analysis, emotional and external eating scores changed in the six months, whereas the restraint eating did not (Table 3).

The GEE analysis does not exclude incomplete cases and is thus deemed to be a more robust analysis. The emotional eating score changed at the rate of $-0.32t + 0.79t^2$ per month. The positive term for the squared variables shows a minimum point. The presence of diabetes is associated with a higher emotional eating score.

The external eating score changes at the rate of $-0.38t + 0.06\ t^2$ per month. Higher anxiety scores and the presence of diabetes were associated with increased external eating scores. Demographic, socio-economic, comorbidity, and HRQoL variables were not associated with a change in emotional and external eating scores across time.

4 Discussion

This study was aimed to identify the pattern of changes in eating behaviours post-surgery and predictors of changes in eating behaviours. Emotional and external eating changed significantly over time. The reduction in emotional and external eating scores at the 3rdand 6th post-surgical months echoed previous findings which reported score reduction in uncontrolled eating (Laurenius et al., 2012) and emotional eating (van Hout et al., 2007) at the 6th week and 6th month after surgery. Both the authors found the improvements to have waned off during one and two year follow-ups (van Hout et al., 2007; Laurenius et al., 2012).

Recent finding with longer follow-up intervals showed that mean emotional, external, and restrained eating scores showed maximum

Table 3 Predictors of changes in emotional and external eating across study duration

Factor	Regression coefficient β (95% Confidence Interval of β)
Emotional Eating	
Intercept	1.71 (1.39, 2.04)
Time (month)	-0.32 (-0.52, -0.12)
Time^2 (month)	0.79 (0.05, 1.52)
Diabetes Yes No †	 0.79 (0.05, 1.52) 0.00
External Eating	
Intercept	2.30 (2.00, 2.61)
Time (month)	-0.38 (-0.54, -0.22)
Time^2 (month)	0.06 (0.02, 0.10)
Anxiety	0.06(0.01, 0.10)
Diabetes Yes No †	 0.70 (0.15, 1.25)

†-reference category

reduction at the 15th month after surgery, followed by an increase at the 24th and 36th, and 48th months (Monpellier et al., 2019).Another study showed that binge eating, emotional eating, and loss of control eating behaviours reduced one year after surgery, and increased thereafter at the second and third post-surgical years (Nasirzadeh et al., 2018).The increasing trend at a shorter follow-up duration of sixth-month post-surgery noted in the current study is in coherence with the previous finding which showed that disordered eating behaviorsre-emerged as early as 4-9 months after bariatric surgery (Conceição & Goldschmidt, 2019).

Further research with longer study duration and shorter follow-up intervals is needed to obtain a more precise picture of the improvement in emotional and external eating disorders over time. An important fact highlighted by all these studies is that there is a clear possibility of the improvement in eating behaviour achieved after the surgery waning off with time. Post-surgical disordered eating behaviour traits have a negative impact on weight loss (White et al., 2010; Konttinen et al., 2015; Nasirzadeh et al., 2018; Conceição & Goldschmidt, 2019; Monpellier et al., 2019). Thus, in addition to the surgery induced anatomical change that reduced the eating capacity, behavioural change in the patients is also needed to optimize the benefit of bariatric surgery. Monitoring and addressing the negative eating behavioural traits at the early phase are necessary to achieve the desired weight loss outcome in the long term. Further research on effective interventions for disordered eating behaviour among bariatric surgery candidates is warranted.

Diagnosis with diabetes was found to be associated with higher emotional and external eating scores. A review reported that the prevalence of disordered eating was as high as 40% among patients with diabetes, with binge eating and night eating syndrome being the most common (García-mayor & García-soidán, 2016). Another study that recruited diabetes patients and healthy controls showed that diabetes patients had a much higher rate of binge eating and disordered eating than healthy controls (Nicolau et al., 2015). As almost half of the surgical patients in the current study (47%) have been diagnosed with diabetes, the association between emotional and external eating with baseline diabetes could be reflecting the previous findings on the association between diabetes and disordered eating. This is something that warrants attention as among the diabetic patients, disordered eating were associated with poor triglyceride levels (Nicolau et al., 2015), poor metabolic controls (García-mayor & García-soidán, 2016), and diabetic complications (García-mayor & García-soidán, 2016). Bariatric surgery patients who were diagnosed with diabetes were reported to achieve lower weight loss outcomes (Barr et al., 2019).

Previous studies reported remission and improvement of diabetes following bariatric surgery (Sjostrom, 2013; Sillén et al., 2017). The current finding shows that at the early stage after surgery, the diabetic group had unhealthy eating habits which might directly affect weight loss and indirectly the benefits of weight loss including remission of diabetes. Future studies should investigate if eating disorders cause poor metabolic controls among the surgical candidates with diabetes, like that reported for the clinical diabetic population, and also if the disordered eating behaviors affect the chances of diabetes remission.

Anxiety was found to be associated with the increased external eating score. The association between psychopathology and disordered eating behaviors in bariatric patients have been shown by previous researchers. Higher emotional and external eating

scores were associated with higher depression scores (Sevinc et al., 2016). Emotional eating was found to partially mediate the relationship between body image dissatisfaction and depression and fully mediate the association between body image dissatisfaction and suicidality (Geller et al., 2019).

Bariatric surgery patients with food addiction, a type of disordered eating behaviour, was found to have a higher rate of current anxiety disorders and lifetime major depressive disorders (Benzerouk et al., 2018). The presence of disordered eating behaviours could have affected the patient's mental well-being and similarly, the patients could have succumbed to unhealthy eating behaviours due to underlying psychopathology. The mental well-being of the surgical candidates should be monitored regularly and new conditions or re-emerging conditions should be treated.

This study has some limitations. Firstly, the low follow-up rate is a concern, despite the follow-up samples having the same demographic characteristics as the baseline group. Secondly, the study was conducted in only two centers located in the capital of the country, and thus may not be representative of the bariatric surgery patients in suburban and rural settings. The post-surgical biomedical assessments were not standardised across institutions and thus the blood glucose level could not be assessed across time. Consequently, it could not be concluded whether the improvement in diabetes at the post-surgical timeline was influenced by disordered eating. The self-rated instruments, despite the established validity, impose the possibility of over or under-reporting symptoms. Despite these limitations, this study provides information on factors that influence the change in eating behaviours post-surgery, an area that had not been well explored.

Conclusion

Emotional and external eating reduced from pre to three months post-surgery but increased thereafter to a level that was lower than baseline. This shows the possibility of reversal of the improvements after six-months. Baseline diabetes was associated with higher emotional and external eating at follow-ups. The disordered eating behaviors among surgical candidates should be monitored and addressed accordingly with special attention to the ones with comorbid diabetes. Future researches that have a longer study duration and include semi-urban and rural patients are warranted.

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ANTI-BACTERIAL ACTIVITY OF *Trigonella foenum-graecum* AGAINST SKIN PATHOGENS

Geetha Subramaniam*, Rayshen Renganaden Poolee Cootee, Cheah Cheng Han, Lalita Ambigai Sivasamugham

INTI International University, Persiaran Perdana BBN, Putra Nilai, 71800 Nilai, Negeri Sembilan, Malaysia

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Keywords

Trigonella foenum graecum

MRSA

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Anti-bacterial

ABSTRACT

The increase in antibiotic resistance globally necessitates the search for alternative therapeutic agents. Among the common antibiotic-resistant bacteria, MRSA and MRSE cause a wide range of infections with limited treatment options. *Trigonella foenum-graecum* has been widely used particularly in Asian countries in food and as a supplement. In this study, the anti-bacterial activity of *T. foenum-graecum* essential oil, boiled aqueous and ethanolic extracts was determined against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes*, MRSA, and MRSE by using the agar well diffusion assay. For determination of the zone of inhibition, Clindamycin (2 µg/disc) was used as a comparison for the anti-bacterial susceptibility results. The maximum zone of inhibition was observed with the boiled aqueous extracts of *T. foenum-graecum* against MRSA (20 mm), while the essential oil and ethanolic extracts of *T. foenum-graecum* did not show any zones of inhibition against all the tested staphylococcal and *P. acnes* isolates. Qualitative phytochemical analysis revealed the presence of flavonoids and terpenoids in all three extracts tested, among these boiled aqueous extract contained the highest number of phytochemicals which could account for the higher anti-bacterial activity exhibited by the boiled aqueous extracts. Hence, the *T. foenum-graecum* seeds show a potential application as an anti-bacterial agent against skin pathogens, particularly in the formulation of a skincare product.

* Corresponding author

E-mail: geetha.subramaniam@newinti.edu.my (Geetha Subramaniam)

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1 Introduction

Plant extracts have been used throughout generations to remediate human diseases (Brown & Wright, 2016). It is believed that up to 70% to 90% of the populations in developing countries use plant extracts as remedies (Chin et al., 2006). Indiscriminate uses of antibiotics has led to the emergence of antibiotic-resistance in pathogenic bacterial strains and this increases the demand for alternative anti-bacterial substances obtained from natural sources, mainly from medicinal plants (Khan et al., 2017).

Infections caused by Methicillin-resistant *Staphylococcus aureus* (MRSA) and Methicillin-resistant *Staphylococcus epidermidis* (MRSE) are a major health problem, globally (Livermore, 2000). According to the CDC (Centers for Disease Control and Prevention, 2019), MRSA is responsible for skin, wounds, bloodstream infections, and pneumonia. In the US (United States) 120,000 cases of severe MRSA infections followed by 20,000 deaths per annum were recorded in 2017, and it was found that these isolates also had resistance against various commonly used antibiotics such as nafcillin, oxacillin, and cephalosporins (CDC, 2019). MRSE, on the other hand, is becoming increasingly prevalent as a cause of bacteremia (Kleinschmidt et al., 2015). Furthermore, the prevalence of multidrug resistance in MRSA isolates, in particular, is resulting in an increase in mortality rates as well as healthcare costs (Lakhundi & Zhang, 2018). As there are decreasing antibiotic treatment options for MRSA and MRSE infections, there is an urgent need for alternative remedies to help curb the spread of this alarming threat.

Trigonella foenum-graecum (commonly known as fenugreek), is a member of the family Leguminosae. The seeds and leaves of *T. foenum-graecum*, are frequently used as spices to enhance food flavor and are a rich source of nutrients and natural fiber (Srinivasan, 2006). The anti-oxidative and anti-diabetic properties exhibited by *T. foenum-graecum* have resulted in the common use of this herb in Ayurvedic medicine (Dixit et al., 2005; Kannappan & Anuradha, 2009). Antimicrobial screening using fenugreek seed extracts has shown positive inhibitory effects against *S. aureus*, *S. epidermidis*, *Escherichia coli*, and *Proteus vulgaris* which shows potential antimicrobial effects against these pathogens (Walli et al., 2015).

The chemical constituents in *T. foenum-graecum* that contribute to the therapeutic benefits of this plant include flavonoids, saponins, and other rare constituents such as coumarin, trigonelline, fenugreekine, and many more (Michael & Kumawat, 2003; Snehlata & Payal, 2012). These phytochemicals have been linked to antibacterial activity against various bacterial species (Gill & Holley, 2006; Tiwari et al., 2009). Another factor that could influence the intensity of the antimicrobial activity of plant extracts, in general, could be the solvent used to extract the phytochemical constituents (Dhanani et al., 2017; Fehli et al., 2017).

This research was carried out to analyze the phytochemical content of the essential oils, ethanolic extracts, and aqueous extracts of *T. foenum-graecum* and the potency of its anti-bacterial effect against MRSA and MRSE and other skin pathogens.

2 Materials and Methods

2.1 Bacterial isolates

Bacterial isolates that were used included MRSA, MRSE, *S. aureus*, *S. epidermidis*, and *Propionibacterium acnes*. All these isolates were provided by INTI International University, Malaysia, and were cultured on nutrient agar before further testing.

2.2 Confirmatory tests

Basic biochemical tests were performed on each bacterial isolate to confirm their identity. The Gram stain was done using an overnight bacterial culture smear as previously described (Beveridge & Davies, 1983). The presence of catalase in each bacteria was determined using 3% (v/v) H_2O_2 mixed into a single colony of the individual cultures and observing for the formation of bubbles (Taylor & Archanzar, 1972). All isolates were streaked onto mannitol salt agar (MSA) and incubated at 37^0 C overnight to observed bacterial growth and change in the color of media. MRSA and MRSE isolates were confirmed as being resistant using the cefoxitin disk diffusion assay (Bonjean et al., 2016). All bacterial culture work was carried out under aseptic conditions in a lamina air-flow cabinet.

2.3 *T. foenum-graecum* seed extract preparation

T. foenum-graecum seeds were obtained from two different states of Malaysia viz., Penang (S1) and Negeri Sembilan (S2) for a more unbiased testing to determine if the results obtained were similar despite the origin of the plant material. The seeds were rinsed briefly with distilled water and 70% (V/V) ethanol before drying under the sun for 1 day. From the collected seeds, 50% of the healthy seeds were ground into a fine powder and extracted with 80% ethanol using a Soxhlet extractor, and concentrated using a rotary evaporator (Chua et al., 2019). The remaining seeds were boiled with distilled water in a ratio of 1:2 for 15 minutes, filtered, and cooled at room temperature. The pure essential oil of *T. foenum-graecum* (Naturelife and BioFinest) was purchased online.

2.4 Determination of anti-bacterial activity

A fresh bacterial suspension was prepared in sterile distilled water and compared against the 0.5 McFarland standard to obtain a concentration of approximately 5X105 CFU/mL. The suspension

(50µL), was lawned onto Mueller-Hinton agar (MHA) plates and left to dry for 15 minutes in a biohazard cabinet (CLSI, 2018). A well was bored into the middle of the quadrant of the agar plates. A total volume of 400 µL of plant extract was applied into the appropriate well of the plate. Concentrated essential oil (50µL) was applied to sterile disks and placed on the labeled quadrant. Clindamycin (2µg/disk), was used as a comparison of anti-bacterial activity. The zone of inhibition diameter was measured after a 24-hour incubation at 37○C. The diffusion assays were performed in triplicates.

2.5 Phytochemical screening

Qualitative analysis of phytochemical compounds present in *T. foenum-graecum* was performed on the essential oil, ethanolic extracts, and boiled aqueous extracts. The phytochemicals screened for included secondary metabolites such as flavonoids, saponins, terpenoids, alkaloids, phenols, and tannins, using different methods as previously described (Kumari et al., 2016).

2.6 Statistical analysis

Statistical comparisons were performed using ANOVA Post Hoc Tests in IBM SPSS for one-way ANOVA. P-value is lesser than 0.05 were used to confirm significant differences between the means of different sets of data as this experiment was repeated in triplicates to obtain more reliable results.

3 Results and Discussion

Essential oil and two different solvent extracts of *T. foenum-graecum* seeds (obtained from two different locations of Malaysia) were used to determine the antibacterial effects of these preparations against common skin pathogens. Two sources of seeds were used to ensure the antibacterial activity was due to the active ingredients of *T. foenum-graecum* seeds and not affected by the particular source from which it was obtained. In this study, both seed sources yielded similar results in the assays conducted, therefore the combined mean zones of inhibition were analyzed.

Previous studies have shown that essential oils generally have significantly higher activity against bacterial isolates, particularly against Gram-positive bacteria, which could be because essential oils are generally in concentrated form and contain a higher quantity of terpenoids (Subramaniam et al., 2020).

Hence, the essential oils are usually used in smaller quantities of testing in disk diffusion assays. The concentration of the ethanolic extract of *T. foenum-graecum* was almost half of that of the boiled aqueous extract (Table 1).

Significant differences in anti-bacterial activity were observed among the three different extracts of *T. foenum-graecum* ($p < 0.05$), with the boiled aqueous extract exhibiting the highest activity at a concentration of 500 mg/ml (Table 1).

Overall, the anti-bacterial activity of boiled aqueous extracts of *T. foenum-graecum* was more effective against the staphylococci isolates but not against the *P. acnes* isolate. While the ethanolic extracts are more effective than aqueous extracts as has been previously reported for the other plant's ethanolic extracts (Fehli et al., 2017). However, despite the lower concentration of the ethanolic extract in this study, a zone of inhibition would have been expected to form, whereas none was observed.

The antibacterial activity of *T. foenum-graecum* was evaluated against clindamycin (02.ug/ml) to assess the significance of this herb against the currently used antibiotic in the treatment of staphylococcal infections (Figure 1). Although, clindamycin was generally more effective against the tested bacterial isolates it should be noted that zones of inhibition more than or equal to 15 mm are considered significant and the isolate tested is said to be susceptible to the agent tested (BSAC, 2015).The MRSA isolate was susceptible to the boiled aqueous extract of *T. foenum-graecum* but resistant to clindamycin which is a promising result of the anti-bacterial effects of this seed extract against resistant pathogens.

Phytochemical screening of the three extracts of *T. foenum-graecum* showed a higher number of phytochemicals present in the boiled aqueous extract compared to the essential oil and the ethanolic extract (Table 2). This could be the reason for the more significant anti-bacterial effects of the boiled aqueous extract on the bacteria tested compared to the other two extracts.

The absence of alkaloids could have an impact on the overall anti-bacterial activity of the essential oil and ethanolic extracts. Similar findings were observed by Chua et al. (2019), whereby the ethanolic extracts of *T. foenum-graecum* did not yield any zones of inhibition when tested against *S. aureus*.

Aqueous extracts have generally been known to have less activity against bacteria tested (Ngo et al., 2017; Subramaniam et al., 2020). This could be due to the lower content of secondary metabolites extracted as found by Ngo et al. (2017). However, in this study, it was reported that boiled extracts of *T. foenum-graecum*, had significant antibacterial activity against the staphylococci isolates tested. This is concurrent with the findings of Walli et al. (2015) who showed that boiled aqueous extracts of *T. foenum-graecum* had significant antibacterial activity against both Gram-positive and Gram-negative bacteria, compared to hot and cold aqueous extracts which yielded little or no activity. Hence the boiling of the seeds of this plant could have contributed to the increase in secondary metabolites present in this preparation. A study by Truong et al. (2019) also showed comparably high phytochemical extraction yield compared to ethanol, in their study with *Severinia buxifolia*.

Table 1 Anti-bacterial activity of fenugreek essential oil and seed extracts against skin pathogens

Extracts	Concentration of extracts	Mean zone of inhibition (in mm)				
		S. aureus	*S. epidermidis*	*P. acnes*	MRSA	MRSE
Essential oil	N.A.	0	0	0	0	0
Ethanolic	330mg/ml	0	0	0	0	0
Boiled aqueous	500 mg/ml	16.83	17.0	12.0	20.16	17.12

Table 2 Phytochemical screening of fenugreek essential oil and seeds crude extracts

Phytochemicals	Extracts		
	Essential oil	Ethanolic	Aqueous
Flavonoids	P	P	P
Saponins	P	N	P
Terpenoids	P	P	P
Alkaloids	N	N	P
Phenols & Tannins	N	N	P

P indicates presence while N indicates the absence of phytochemicals in the plant extracts

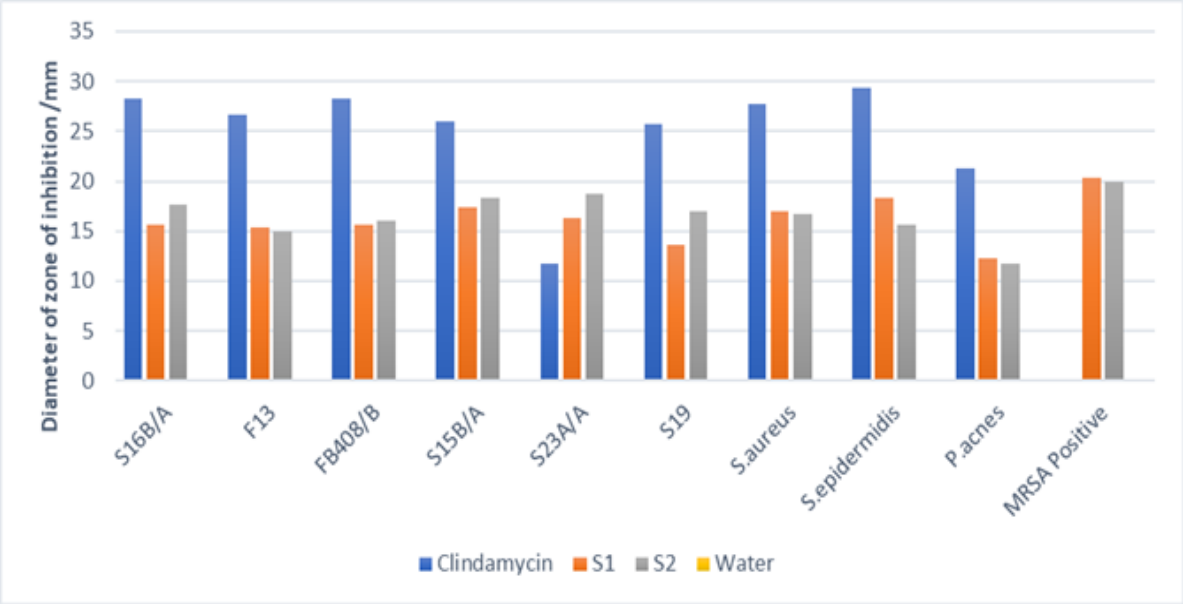

Figure 1 Mean zones of inhibition of *T. foenum* graecum boiled aqueous extracts (500 mg/ml) against staphylococcal and *P. acnes* isolates
*Note: Staphylococcal isolates used in this study were MRSA (S16B/A, F13, FB408/B) and MRSE (S15B/A, S23A/A, S19), *S. aureus* and *S. epidermidis*

HPLC analysis of the secondary metabolites contained in various solvents revealed that the best concentration of ethanol for the extraction of these bioactive compounds was 50% and much lower for 100% ethanol (ElNour et al., 2015; Ngo et al., 2017). This could explain why the ethanolic extracts in this study (85%) did not yield any zones of inhibition when tested against the bacterial isolates.

A study by Hozzein et al. (2020) showed that treatment of plant material with CO_2 induced accumulation of secondary metabolites including phenolics, flavonoids, saponins, and alkaloids in the fenugreek seed extracts. This pre-treatment of the fenugreek seeds could be used to further enhance the quantity and phytochemical activity of the boiled extracts.

MRSA and MRSE are becoming more prevalent as community-acquired pathogens. Many individuals harbor these resistant pathogens in a carrier status. This poses a challenge as they could be spreading these multi-drug resistant bacteria unconsciously making the threat of antibiotic resistance even more difficult to overcome. The development of skincare products using plant sources is common in some Asian countries but to date, *T. foenum-graecum* has not been utilized in this manner. The fact that there is significant anti-bacterial activity exhibited by the boiled aqueous extracts of *T. foenum-graecum* against the staphylococci including the resistant members of this group, is promising.

Conclusion

This study shows the potential benefits of *T. foenum-graecum* as an anti-bacterial agent against common skin pathogens including MRSA and MRSE. Further studies should be conducted on more bacterial isolates from skin samples to conclusively prove the potential benefit of *T. foenum-graecum* is being utilized as a skincare product.

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Conflict of interest

The authors declare that they have no conflict of interest.

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EFFICACY OF FLAXSEED CRACKERS IN AMELIORATING CLINICAL AND BIOCHEMICAL HYPERANDROGENISM IN YOUNG ADULT SOUTH INDIAN WOMEN DIAGNOSED WITH PCOS

Jeyamani Divya Christodoss[1*], Priya D John MD[2], Vasantha Esther Rani[3]

[1]Assistant Professor, Loyola College, Chennai
[2]Assistant Professor and Senior Clinician, Emory University, GA, USA
[3]Head and Professor, Fatima College, Madurai

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KEYWORDS

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Insulin

FG score

Menstrual cycle

ABSTRACT

Young women of reproductive age are more prone to hormonal/endocrine disorders, the most common being Polycystic Ovarian Syndrome (PCOS). Research in different cultural contexts to enhance the physical, emotional, social, and reproductive health of women has been the focus of several scientific studies. High lignan foods reduce the bioavailability of free testosterone through increasing Sex hormone Binding globulin (SHBG) levels. A randomized single-blind placebo-controlled trial was carried out among women of 20 - 25 years from a single on-campus hostel facility in Chennai, India based on the Rotterdam criteria of PCOS diagnosis. The results showed a statistically significant lower serum free testosterone concentrations (1.02 ± 0.428 ng/dl) in the flax group compared to the placebo (1.64 ± 0.504 ng/dl), after the study period. Similarly, it was noted that the lowered levels of Sex hormone Binding Globulin (SHBG) were elevated to 15.22 ± 14.593 nmol/L as compared to only 9.64 ± 5.124 nmol/L in the placebo though not statistically significant, thus creating a promising platform for improving the imbalances in the hormone levels. Also, elevated serum insulin concentration was lowered in the flax group (15.67 ± 6.928 µU/mL) than the control group (19.09 ± 8.526 µU/mL). The FG score significantly reduced in the flax group thus alleviating the clinical manifestation of hirsutism. Lowering of free testosterone, serum insulin, and elevation of serum SHBG levels is attributed to the phytoestrogen (SDG) present in flaxseeds and can be an ideal alternate replacing the conventional drugs for PCOS.

* Corresponding author

E-mail: divyajc@yahoo.com (Jeyamani Divya Christodoss)

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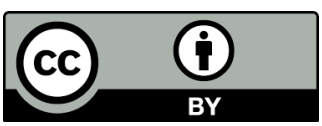

1 Introduction

Polycystic Ovarian Syndrome (PCOS) was first characterised with significant clinical implications like menstrual irregularities, hirsutism, infertility with the suboptimal obstetrical outcome along with metabolic imbalances such as impaired glucose tolerance, type 2 diabetes mellitus, dyslipidemia, coronary artery disease, and endometrial hyperplasia on a long term basis (Stein & Leventhal, 1935).

PCOS is a commonly prevalent endocrine disorder diagnosed in women with features related to ovulatory and menstrual irregularities, hyperandrogenaemia with manifested clinical features such as hirsutism, acne, and androgenic alopecia, along with polycystic ovarian morphology (Azziz et al., 2006). It is often associated with metabolic aberrations leading to insulin resistance and compensatory hyperinsulinemia, which is the main factor responsible for altered androgen production and metabolic dysregulation (Escobar et al., 2005).

PCOS affected 116 million women (3.4%) all over the world as estimated by World Health Organization (WHO) in 2012 (Vos et al., 2012) with a high global variability ranging from 2.2% to 26% (Vidya Bharathia et al., 2017). This wide range of prevalence rates across the globe is a clear indication of the fact incidence of PCOS may vary between different races and ethnicities (Li et al., 2013, Chang et al., 2016, Huang & Yong, 2016). Based on the ethnicity, it is reported that among the Asian population, there is an increased manifestation of hirsutism with early onset of symptoms, and increased risk of insulin resistance and metabolic risk factors associated with Caucasians (Kim & Choi, 2019). In India, the prevalence of PCOS ranges from 3.7 to 22.5 percent based on the population studied and the criteria adopted for diagnosis (Ganie et al., 2019) and middle and high-income groups of the urban sector show higher prevalence than the rural population (Balaji et al., 2015; Vidya Bharathia et al., 2017). Also, it was observed that among women from the South Indian union territory of Pondicherry, about 26% of them had hirsutism, 94% had oligomenorrhea, 80% had ovarian morphological changes, and 6% had amenorrhea, which demonstrates a high correlation between menstrual irregularity, PCOM and clinical features of PCOS (Sheelaa & Radha, 2014).

PCOS is characterized by metabolic and ovarian dysfunction. Metabolic derangements are known to respond to lifestyle modifications, which are advocated as the first line of treatment for PCOS, which includes changes in dietary pattern, exercise level, and behavioral therapies (Ebrahimi et al., 2017). Obesity is common among women with PCOS. Predominantly 50% of women with PCOS are obese, and lowering body weight is proven to be supportive in normalizing hormonal levels and clinical symptoms (Gambineri et al., 2002). High fiber foods have been known to reduce weight by several mechanisms, including promoting satiety, decreasing absorption of macronutrients, and alteration of gut hormones (Slavin, 2005).

While lifestyle modification is a pertinent recommendation for PCOS, decreasing insulin resistance by weight loss alone is difficult. Metformin, commonly used as an antidiabetic drug, works on insulin resistance and has been used in PCOS extensively. While metformin was shown to reduce serum insulin levels, it has not been shown to reliably restore hirsutism, androgen levels, or menstrual cycles, which are common clinical symptoms in PCOS (Johnson, 2014). Metformin has also been proven to show a higher incidence of gastrointestinal side effects, thus leading to drug discontinuation (Costello et al., 2007).

Interestingly extensive medicinal herbs have gained popularity as an ideal alternative remedy for disease prevention in the past few decades (Legro et al., 2013). Earlier scientific studies have indicated that high fiber foods, specially lignan rich, have decreased serum testosterone concentration by increasing their exertion in the enterohepatic circulation (Adlercreutz et al.,1987) and they also lower the circulation of bioavailable free testosterone by enhancing the levels of SHBG (Low et al., 2005).

Functional foods or Nutraceuticals are classified as foods that have been proven scientifically to have health benefits or disease-preventing properties along with the fundamental nutritional properties present in them. Whole flaxseed, flax meal, and milled flax have been found to have many health-promoting and disease-preventing properties (Bloedon & Szapary, 2004). The flaxseed (*Linum usitatissiumum*) is proven to be a rich source of the biologically active constituents made up of omega 3 fatty acid namely Alpha Linolenic Acid (ALA), phytoesterogenic lignans, namely Secoisolariciresinol Diglycosidic (SDG), and dietary fibers (Yari et al., 2016). It has been proven that flaxseed is also a functional ingredient used in baking applications contributing to improved tenderness and crunchiness of the product. The lignan present in flaxseed is found to be not affected by the high temperatures used in baking while incorporated into baked products and has gained popularity as a bakery ingredient (Lipilina & Ganji, 2009).Given this background of an increased clinical manifestation of PCOS in a South Indian population, this present study is focussed on assessing the efficacy of functional foods in ameliorating the clinical and biochemical manifestation of PCOS among young adult women from an urban population in South India. The study aims to assess the impact of flaxseed crackers, a baked product, on the metabolic and hormonal status among young women with COS and create awareness on the importance of healthy dietary and lifestyle habits in improving the prognosis of the disorder.

2 Materials and Methods

2.1 Study design

The design of this pilot study was a single-blind with two arms, placebo controlled randomized trial consisting of an intervention arm supplemented with flaxseed cracker (30g/day) and plain wheat cracker (30g/day) in the control arm. The present study protocol evaluated the efficacy and the safety of milled flaxseed crackers supplementation on the clinical and biochemical key features among young adult women of South Indian origin in the age group of 20-25 years. Milled flaxseed was purchased from the local market and baked into crackers weighing 15 g each and was stored in an airtight, cool, and dark container before the study's onset. Similarly, wheat crackers weighing 15 g each were prepared and stored for the control arm. The dosage of 30g/day of flaxseed was decided based on the findings of previous systematic reviews and meta-analyses (Sartang et al., 2017, Rahimlou et al., 2019). And it was taken care of to see that greater than 50g/day was avoided to prevent any side effects (Carraro et al., 2012). The study design and the methods were approved by the Independent Ethics Committee (IEC), Ethica Norma for Human Clinical Trial and all participants provided signed informed consents.

2.1 Participants

Participants for the study were selected based on Rotterdam criteria of diagnosis for PCOS (Wang & Mol, 2017) from a single on-campus hostel facility in the metro of Chennai, Tamil Nadu, South India. Willing participants for the study were assessed by a gynecologist for identifying clinical symptoms of PCOS (mFG score) and PCOM using the ultrasound technique and evaluated if they met the Rotterdam criteria. According to the Rotterdam criteria, any two of the following symptoms were used for inclusion of the subjects, that is, irregular periods 8-9 cycles/year, signs of hyperandrogenism with a FG score greater than 8 (Aswini & Jayapalan, 2017), serum testosterone levels > 180 ng/dl and polycystic ovaries in the ultrasound. Totally 24 participants within the age range of 20-25 were assigned into two arms of the study. The experimental arm (12 participants) received milled flaxseed in the form of crackers (30 g), and the placebo arm (12 participants) received wheat crackers (30 g). The intervention was administered for 5 months. Recruitment of subjects was done based on a randomization schedule using an online randomization generator. Biochemical levels for serum SHBG, Total testosterone, and Insulin concentrations were analyzed in a NABL accredited laboratory before and after the supplementation period. Free testosterone and bioavailable testosterone levels are deduced from estimated total testosterone and albumin levels in the blood (Figure 1).

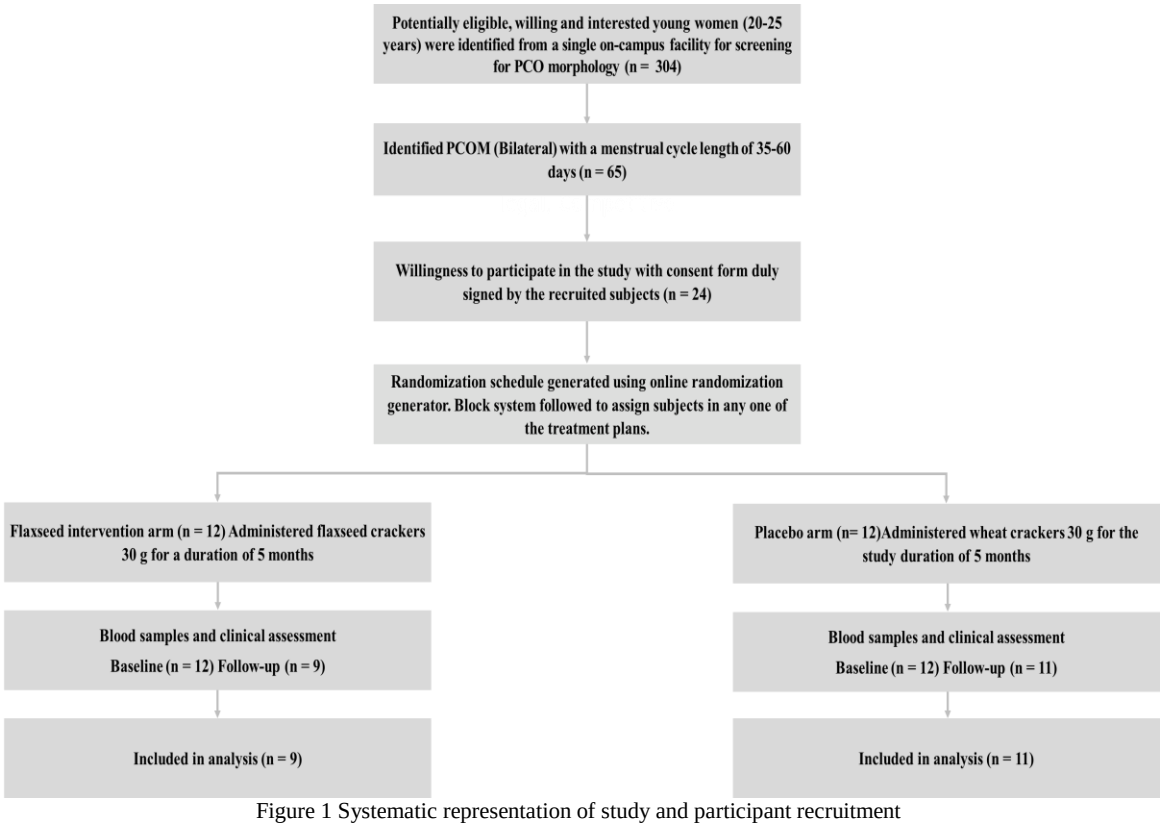

Figure 1 Systematic representation of study and participant recruitment

2.2 Inclusion criteria

Female participants aged between 20 and 25 years (both inclusive) residing in an on-campus hostel facility in the metro of Chennai. BMI between 18.5 and 40 (both inclusive). Diagnosed for any two of the following symptoms as per Rotterdam criteria, that is, Oligomenorrhea/Amenorrhea (two years after menarche) i.e., irregular periods 8-9 cycles/year, signs of hyperandrogenism (Clinical or Biochemical), Hirsutism (FG score greater than 8), Testosterone levels > 6.2 nmol/L, Polycystic ovaries in ultrasound, Signs of hyperinsulinemia: Obesity, Acanthosis Nigricans. Ability to understand and willingness to sign statements of informed consent.

2.3 Exclusion criteria

Women on other medications for PCOS such as oral contraceptives, metformin, spironolactone, cimetidine, cyproterone acetate, cyproheptadine, and glucocorticoids. Any known history of hypersensitivity or idiosyncratic reaction was observed to any part of the flaxseed and other members of the Linaceae plant family. Women are known to have past episodes of allergies with a clinical significance which includes drug allergies or bronchial asthma due to allergic reaction. Women with known occurrences of heart and pulmonary diseases, liver and renal disorders, gastrointestinal disturbances and gastric surgery, hormonal imbalances, skin allergies, neurological and psychiatric disorders will be excluded from the study. Known history of any major illness and presence of any significant organ abnormalities. Women administered with over the counter (OTC) and prescribed medications, enzyme modifying medication, and systemic medication taken within the last 30 days before the start of the study were not included.

2.4 Outcome measures for the study

Primary biochemical outcome measures were serum insulin level, Total testosterone, SHBG, free testosterone, and bioavailable testosterone, along with hyperandrogenic clinical variables such as hirsutism and menstrual cycle status. Clinical evaluations were determined using a modified Ferriman–Gallwey score (Aswini & Jayapalan 2017). Menstrual cycle regularity was evaluated using a questionnaire and personal interviews before and after the study period.

2.5 Laboratory methods

Blood samples from the participants were collected in the morning after an overnight fast before and end of the study period. Quantitative determination of serum total testosterone concentration and serum insulin concentration were done using Electro-Chemiluminescence immunoassay and serum SHBG concentration was analyzed using Chemiluminescence immunoassay in a NABL accredited laboratory in the metro of Chennai.

2.6 Statistical analysis

The data collected were expressed as Mean ± Standard Deviation (SD). Statistical analyses were done with the software IBM SPSS version 20 with a p-value of < 0.05 being statistically significant. The statistical tools, independent sample t-test, and t-test for paired samples compared the parameters between the two groups before and after supplementation of the flax crackers and placebo. Descriptive statistics like frequency and percentage were used to identify the sample characteristics.

3 Results

In the present pilot study, 24 participants were recruited, and among these, 20 (83%) of them completed the study trial. Three participants from the flaxseed group and one participant from the placebo group discontinued the study citing personal reasons of shifting to their hometown, and non-compliance of the participants throughout the study protocol. More than 90% of the flax crackers were consumed by the participants in the intervention group. Compliance with the study intervention was ascertained by scheduled periodic phone calls and in-person visits to the study participants. None of the participants complained of any side effects related to flax consumption such as bloating and abdominal pain. The baseline characteristics of participants in both groups are presented in Table 1. It was noted that there was no significant difference for BMI (Table 1) in both baseline and final values for both the intervention and control groups.

Table 1 Subject Characteristics and key PCOS features

Characteristics	Flax arm		Control arm	
	Baseline	Final	Baseline	Final
Age (years)	21.44 ± 1.065	21.44 ± 1.065	21.72 ± 0.962	21.72 ± 0.962
BMI (kg/m^2)	27.42 ± 6.856	27.24 ± 7.021	25.55 ± 2.989	25.73 ± 3.19
FG score	15.22 ± 4.871	11.11 ± 2.766	11.45 ± 2.74	11.36 ± 2.99
Total Testosterone (nmol/L)	1.26 ± 0.333	1.29 ± 0.314	1.60 ± 0.588	1.81 ± 0.560
Free Testosterone (ng/dl)	1.02 ± 0.017	1.02 ± 0.18	1.18 ± 0.481	1.52 ± 0.426
SHBG (nmol/L)	13.66 ± 7.246	15.27 ± 3.620	16.70 ± 8.991	9.73 ± 4.848
Insulin (µU/mL)	16.16 ± 5.406	15.57 ± 6.603	15.44 ± 4.704	19.01 ± 8.107

Values are actual representing Mean ± SD;

It was observed that participants supplemented with flax crackers for 5 months had a statistically significant lower serum free testosterone concentration (1.02 ± 0.428 ng/dl) compared to the placebo (1.64 ± 0.504 ng/dl), with t = -2.897, p = 0.010 (< 0.05) after the study period (Figure 2). Serum total testosterone concentrations in the flax group (1.33 ± 0.500 nmol/L) was lowered as compared to the control (1.73 ± 0.467 nmol/L) though not statistically significant, t = -1.818, p = 0.086 (> 0.05) (Figure 3).

SHBG, which was lowered at the beginning of the study, was elevated to 15.22 ± 14.593 nmol/l as compared to the placebo being only at 9.64 ± 5.124 nmol/l after the period of supplementation (Figure 4). Though this is not statistically significant, t = 1.189, p = 0.250 (> 0.05), it does create a promising platform for improving the imbalances in the hormone levels by increasing the serum levels of SHBG, which in turn binds with the free circulating testosterone rendering it biologically inactive.

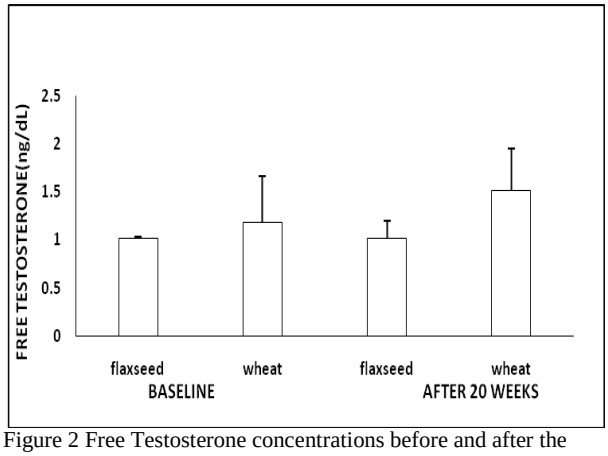

Figure 2 Free Testosterone concentrations before and after the supplementation (Data expressed as mean ± SD; statistically significant at 0.05)

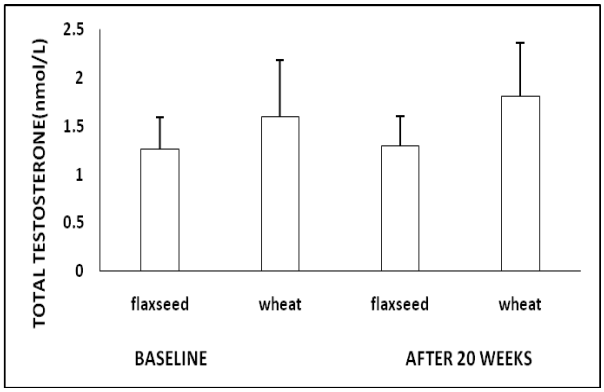

Figure 3 Serum Total Testosterone concentrations before and after the supplementation (Data expressed as mean ± SD; statistically significant at 0.05)

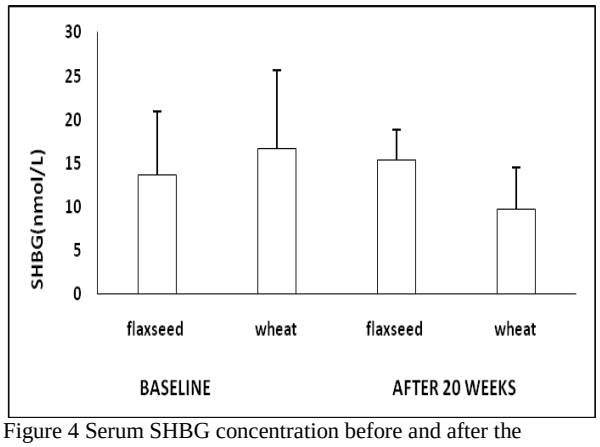

Figure 4 Serum SHBG concentration before and after the supplementation (Data expressed as mean ± SD; statistically significant at 0.05)

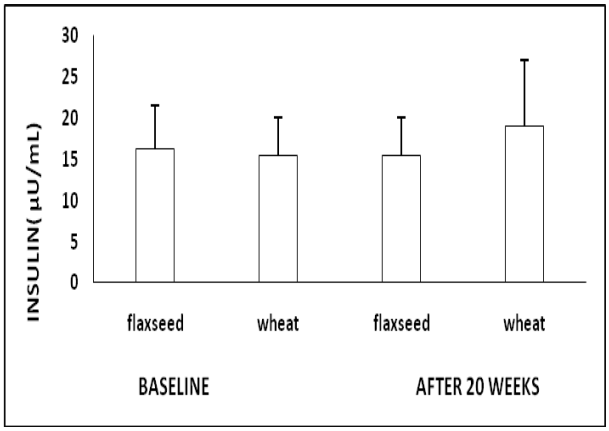

Figure 5 Serum Insulin concentrations before and after supplementation (Data are expressed as mean ± SD; statistically significant at 0.05)

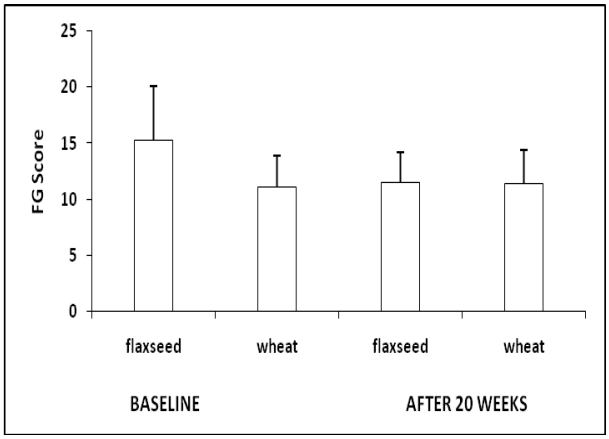

Figure 6 FG score before and after the supplementation (Data expressed as mean ± SD; statistically significant at 0.05.)

Table 2 Menstrual cycle status of participants before and after the study period

Menstrual regularity status	Flax (n = 9)		Placebo (n = 11)	
	Beginning	End	Beginning	End
Regular n (%)	4 (44.4%)	7 (77.8%)	5 (45.5%)	7 (63.6%)
Irregular n (%)	5 (55.6%)	2 (22.2%)	6 (54.5%	4 (36.4%)

Various scientific studies have established the inverse relationship between SHBG and insulin concentration. It was noted that the elevated serum insulin concentration was lowered in the flax group (15.67 ± 6.928 µU/mL) as against the control group (19.09 ± 8.526 µU/mL) (Figure 5). At baseline, 55.6% of the participants in the flax group had menstrual irregularity. At the end of the study period, menstrual regularity increased among the participants in the flax group (77.8%) in contrast to the control group (63%) (Table 2).

4 Discussion

Young adulthood is the prime period in one's lifetime, a time of peak strength, physical performance, and in women, it is also the period of peak fertility, thus necessitating the need for healthy food habits, physical activity, and sleep pattern to augment a sound reproduction during this phase of life.

As shown in Figure 6, the FG score reduced in the flax group before (15.22 ± 5.166) and after (11.11 ± 2.934) supplementation and was found to be statistically significant in comparison to the placebo at a 5% level of significance ($p = 0.043$). Thus, the intervention with flax crackers proves to be a strong contributor in alleviating the clinical manifestation of hirsutism among young women with PCOS. Increased globalization and spearheading various workforces in different sectors of occupations have made women play a pivotal role in society. Predominantly young adult women of reproductive age are more prone to hormonal and metabolic disorders and the most common among them is Polycystic Ovarian Syndrome.

Flaxseed is a promising functional food ingredient that is used extensively in baked foods, fruit juices, dairy and dairy products, extruded pasta products, and meat products. The very fact of consuming flaxseed in the form of whole seed, milled flour, and roasted seed, provide basic nutrition coupled with various health benefits qualifying it to be considered as a functional food (Goyal et al., 2014). Several other scientific studies have proven its efficacy in decreasing androgen levels and normalizing lipid levels, though most of the studies are in males (Pan et al., 2009). In the past decade, there have been several research findings adopting animal and human trials in treating PCOS, which is also an androgen-related disorder. It has been established that milled flaxseed consumption brings about a significant improvement in the regularity of the menstrual cycle (Oner, 2013).

In this present preliminary data, it was shown that there was a significant increase in the SHBG in the group supplemented with flax crackers. It was also observed that there was a significant reduction in the serum-free testosterone level with flax crackers. Total testosterone and serum insulin showed a trend towards improvement though statistically not significant. Also, an improvement in menstrual cycle regularity in the group supplemented with flax crackers was seen. The current study observed that the participants on flaxseed crackers showed a significant reduction in the FG score at the end of the study period, thus providing an ideal alternative to the drugs administered for the same.

The Total testosterone, Free testosterone, and Sex Hormone-Binding Globulin (SHBG) levels were included as part of the clinical assessment of the female androgen levels in PCOS (Haidari et al., 2019).

Excessive androgen secretion in PCOS conditions causes an increase in the number of immature follicles and it is found that flaxseed significantly reduces serum androgen levels. It has been reported in previous research findings by Sturgeon SR et al. and Debra Novak et al. that a decline in testosterone, oestradiol, and estrone levels was observed following dietary flaxseed supplementation, especially in overweight/obese women (Debra et al., 2007, Sturgeon et al., 2008).

SHBG is a significant protein found to bind to some of the total testosterone, thereby reducing the free testosterone availability in blood circulation (Farzana et al., 2015) thus being an effective indicator for the determination of androgen levels. Lowered levels of SHBG indicate elevated active testosterone levels in the blood predisposing women to greater intensity of manifestation of clinical symptoms of acne, hirsutism, and male pattern alopecia. PCOS patients are therefore advised to take a diet that is rich in lignans and fiber to maintain optimum levels of SHBG in the blood and flaxseeds are a very good source of fiber and lignans. These seeds also contribute to high amounts of omega-3 and omega-6

fatty acids proven to have very high anti-inflammatory properties, which regulate glucose metabolism and increased insulin-sensitivity.

Previous studies have reported that there is an inverse relationship between SHBG and insulin levels, wherein insulin acts indirectly by decreasing the hepatic biosynthesis of SHBG (Khani et al., 2017). Furthermore, research findings have reported flaxseed or its isolated lignan (SDG) counterpart to reduce the insulin-like growth factor I (IGF-I) levels in the blood. Furthermore, it is reported by Sharon E et al. that consumption of flaxseed or its isolated lignan (SDG) reduces the insulin-like growth factor I (IGF-1) levels in the plasma found in rats with breast cancer (Rickarda et al., 2000). It has been found that IGF-1 increases LH secretion, which leads to hyperinsulinemia and lowers SHBG levels (Hara et al., 2012). Thus, a reduction in plasma IGF1 and subsequently insulin levels can lead to a decrease in LH secretion, causing an elevation in SHBG and lowering of testosterone. It has been established that the pharmaceutical drug metformin functions in lowering insulin by its impact on IGF-1 levels. So, there could be a possibility that flaxseed could bring about a similar change in the insulin levels in PCOS (Evanthia & Farid, 2009). It has also been reported that metformin therapy increases circulating IGFBP-1 levels and thereby lowers the free-bioactive IGF-I in the blood circulation (Kolodziejczyk et al., 2000).

Elevation of testosterone leads to a male-pattern terminal hair growth pattern in females termed as hirsutism and is generally assessed using the mFG score, the most common diagnostic criterion of hyperandrogenism in clinical practice (Aswini & Jayapalan, 2017). Anti-androgenic medications like flutamide blocks androgen receptors and finasteride prevent the conversion of testosterone to more active androgens, thereby facilitating a reduction in excess hair growth by lowering circulating androgens, however, though side effects are rare they have harmful effects on the liver (A Guide for Patients Revised American Society for reproductive medicine Hirsutism and Polycystic Ovary Syndrome (PCOS), 2016). On the contrary, flaxseeds have been proven; however, to reduce liver enzymes and improve the status of non-alcoholic liver disease, thus creating a protective role in maintaining the health of the liver (Yari et al., 2016).

Conclusion

Increased globalization has made women play a pivotal role in society. Their health status determines their productivity at home and work and the nation at large. With the increased economic independence of women, especially in the urban sector owing to their sedentary lifestyle coupled with affordable calorie-dense food, research in different cultural contexts for enhancing the productivity of women by improving their physical, emotional, and social well-being is imperative. It has also been proven that women belonging to the reproductive age are more susceptible to hormonal /endocrine imbalances and the most common being PCOS. In this randomized controlled clinical trial among young adult women, it has been found that milled flaxseeds baked as crackers for convenience of consumption and palatability have significant androgen and metabolic lowering potential ameliorating the clinical and biochemical symptoms in PCOS. Thus, in this present study, flaxseed crackers containing 30 g of milled flax powder taken for 5 months had beneficial effects on lowering the insulin levels, androgen, and sex hormone transporters, regularising menstrual cycle, and reducing mFG score, thereby decreasing hirsutism among young women. Considering this improvement in the metabolic and hormonal levels in PCOS diagnosed women and also lowering the clinical manifestation of hirsutism, flaxseeds can be incorporated for developing functional food traditional Indian products and can serve as an ideal alternative source of plant origin as a substitute for pharmaceutical formulations for the treatment of PCOS. The limitation of the present study was the small sample size; while the results were promising, the study necessitates validation with larger sample size.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

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EFFECTS OF INDIVIDUALIZED TRAINING AND RESPIRATORY MUSCLE TRAINING IN IMPROVING SWIMMING PERFORMANCE AMONG COLLEGIATE SWIMMERS - AN EXPERIMENTAL STUDY

Sivaguru Muthusamy[1,2], Ambusam Subramaniam[1*], K. Balasubramanian[2]

[1]Physiotherapy programme, INTI International University, Nilai, Negeri Sembilan, Malaysia
[2]Department of Physical Education & Health Sciences, Alagappa University, Tamil Nadu, India

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ABSTRACT

Previous researches have been conducted to determine the types of training to improve swimming performance. Nevertheless, no study has been done on the individualized training approach among swimmers. Hence, this study aimed (i) to examine the effects of respiratory muscle training on swimming performance (ii) to examine the effects of combined respiratory muscle training with individualized training on swimming performance, and (iii) to compare the differences between the isolated respiratory muscle training, combined intervention of respiratory muscle training with individualized training and usual training on swimming performance. For this, 45 collegiate swimmers with no previous injuries and swims regularly for at least 1 hour per week participated in the study. Participants were randomly assigned into three groups; Group A: Respiratory muscle training and Individualized Training, Group B: Respiratory muscle training alone, and Group C: Usual training session. The difference within the groups after four weeks of the intervention was analyzed using Paired T-test, while the differences between intervention groups were analyzed using repeated measure two-way ANOVA. Both the intervention groups (Group A and B) showed significant improvement after four weeks of intervention, whereas in group comparison, Group A showed tremendous improvement in swimming performance ($F(17,238) = 8.385$, $p<0.05$, $n_p^2 = 0.375$). Thus, the current study has proven that the combination of respiratory muscle training with individualized training could further enhance the swimming performance in terms of heart rate, Vo2 max, stroke volume, perceived exertion, and SWOLF score. Future studies on athletic swimmers with a larger sample size are recommended to further examine the individualized training approach.

* Corresponding author

E-mail:ambusam_7@hotmail.com (Ambusam Subramaniam)

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1 Introduction

Regular physical activity is the key to maintain health and well-being regardless of age. In recent years, people have gained interest in swimming and consider it as a regular form of physical activity. Swimming has been stated to be able to sustain good anthropometric indicators, influence the effect of blood pressure, reduce morbidity risks, and even in general well-being (Cox et al., 2006; Amaro et al., 2017; Cavaggioni et al., 2019). Freestyle swimming is the most common technique which requires proper breathing at various stroke cycle (Tovin, 2006). Swimming, either competitive or amateur, requires good skeletal muscle strength and endurance in addition to strong respiratory muscle function (Lavin et al., 2015). Good respiratory muscle strength and good breathing techniques are needed to complete the whole cycle at a given time. Thus, repetitive activity such as swimming strokes with poor biomechanics and breathing pace may lead to injury and early fatigue, consequently affecting the swimming performance.

Few studies have shown that respiratory muscle training (RMT) able to progress the performance quality among competitive swimmers (Lemaitre et al., 2013; Amaro et al., 2017; Szczepan et al., 2020). Moreover, RMT has the potential benefits in reducing breathlessness, perceived exertion, improving respiratory strength, increase endurance, and enhancing pulmonary function comparing the usual training (Illi et al. 2012; Lemaitre et al. 2013; Yasemin et al., 2019). Similar results were reported in another systematic review with meta-analyses showing that respiratory muscle training can improve sports performance which is highly dependent on training methods employed and the intensity of training (HajGhanbari et al., 2013).

Strength and power are major determinants of swimming performance (Garrido et al., 2010). Individualized training (IT) has the potential to develop respiratory muscle strength, especially under dynamic conditions. Besides, IT can also improve power which represents the ability to perform movements at high speed and/or exert high strength in a short period (Sadowski et al., 2012). The benefits of the IT program were seen via the improvement of the sports performance among various sports fields (Harms, 2007; Illi et al., 2012; HajGhanbari et al., 2013; Jiménez-reyes et al., 2017). Although the effect of the individualized training program is not yet proven among swimmers, limited researches have been carried out on the effects of respiratory muscle training in improving swimming performance (HajGhanbari et al., 2013; Lemaitre et al., 2013; Lavin et al., 2015). Nevertheless, the individualized training performance should also be taken into consideration in improving the swimming performance. However, there is no evidence yet to prove whether individualized training with respiratory muscle training able to further improve swimming performance. Hence, this study aims: (i) to examine the effects of respiratory muscle training on swimming performance among collegiate swimmers, (ii) to examine the effects of combined respiratory muscle training with individualized training on swimming performance among collegiate swimmers, and (iii) to compare the differences between the isolated respiratory muscle training, combined intervention of respiratory muscle training with individualized training, and usual training on swimming performance among collegiate swimmers.

2 Materials and Methods

2.1 Subjects

Forty-five (23 males and 22 females) collegiate swimmers (Age: 19.58±1.118) were recruited for the current study. The participants were categorized into three groups i.e. two experimental and one control group using computerized randomization (n= 15 each group). The participants met all the inclusion and exclusion criteria.

The swimmers were freestyle swimmers, aged between 18-21, and swim regularly for at least 1 hour in total per week. Participants will be excluded from the study if they have any central nervous systems (CNS) disorders such as seizure, epilepsy, multiple sclerosis; respiratory disorders; previous injuries involving upper and lower limbs; BMI greater than 24kg/m^2; and those who are undergoing other training regimens aside from swimming. All the participants were provided with written informed consent in this study. The research was conducted following the ethics committee of INTI International University.

2.2 Experimental design

All the participants were assigned through randomized allocation into three groups; two experimental groups and one control group. The experimental groups consist of Group A: Respiratory muscle training (RMT) and Individualized training (IT), Group B: Respiratory muscle training (RMT), and Group C: Usual training session (TS). The measurements of the swimming performance were taken at two periods viz. (i) Before the intervention period and (ii) After 4 weeks of the intervention of RMT, IT, and TS respective to the groups.

During the first period, a baseline measurement is taken, all the participants were required to swim 100 m distance. The baseline measurement consists of the time taken to complete 100m distance, max HR, VO2 max, strokes, and Swim Golf (SWOLF). The BORG Scale of perceived exertion was recorded at the starting and upon completion of a 100m distance of swimming. In the second phase, the participants were randomly allocated into three groups (Group A, B, and C). The participants were given 4 weeks of training based on the group respectively and the measurements were taken again at the end of the 4th week. All three groups were assessed at the same time.

Table 1 Baseline characteristics based on the group allocation (Week 1)

	Group A (n=15)	Group B (n=15)	Group C (n=15)
Gender (n/%) Male Female	 7 (46.7) 8 (55.3)	 8 (53.3) 7 (46.7)	 8 (53.3) 7 (46.7)
Age	19.6 ± 1.12	19.53 ± 1.19	19.6 ± 1.12
Time taken for 100m distance	3.66 ± 0.30	3.56 ± 0.34	3.49 ± 0.48
Max HR	161 ± 12.49	165.67 ± 13.8	168.13 ± 10.00
Vo2 max	41.93 ± 3.47	42.53 ± 2.67	41.13 ± 2.00
Borg scale	5.27 ± 0.70	5.20 ± 0.68	4.80 ± 0.94
Strokes	79.4 ± 5.26	79.33 ± 6.14	76.67 ± 5.82
Swim golf (SWOLF)	298.72 ± 20.50	292.93 ± 21.30	286.11 ± 32.15

All the data presented in mean ± standard deviation except gender presented in frequencies (percentage); HR = Heart rate; VO_2 max = maximal oxygen consumption

Table 2 Measures of swimming performance before and after respiratory muscle training (RMT)

	Pre-training (Mean ± SD)	Post-training (Mean ± SD)	Mean differences	95% CI for the Mean (Lower to Upper)
Time taken for 100m distance	3.56 ± 0.34	3.48 ± 0.35	0.075	-0.06 to 0.208
Max HR	166.67 ± 13.80	156.0 ± 15.63	9.667*	6.247 to 13.06
VO_2 max	42.53 ± 2.67	46.73 ± 2.66	-4.200*	-5.527 to -2.873
Borg scale	5.20 ± 0.67	4.20 ± 0.78	1.000*	0.581 to 1.419
Strokes	79.33 ± 6.13	70.07 ± 4.86	9.267*	7.117 to 11.416
Swim golf (SWOLF)	292.93 ± 21.30	279.15 ± 21.45	13.787*	5.94 to 21.63

*$p < 0.05$; HR = Heart rate; VO_2 max = maximal oxygen consumption

Table 3 Measures of swimming performance before and after combined respiratory training and individualized training (RMT+IT)

	Pre-training (Mean ± SD)	Post-training (Mean ± SD)	Mean differences	95% CI for the Mean (Lower to Upper)
Time taken for 100m distance	3.66 ± 0.30	3.47 ± 0.36	0.185	-0.070 to 0.441
Max HR	161.00 ± 12.49	153.73 ± 12.71	7.267*	3.354 to 11.180
VO_2 max	41.93 ± 3.47	48.93 ± 3.86	-7.00*	-8.538 to -5.462
Borg scale	5.27 ± 0.70	3.67 ± 0.62	1.600*	1.192 to 2.008
Strokes	79.4 ± 5.26	68.67 ± 4.61	10.733*	8.523 to 12.943
Swim golf (SWOLF)	298.72 ± 20.50	276.87 ± 23.14	21.853*	6.436 to 37.27

*$p < 0.05$; HR = Heart rate; VO_2 max = maximal oxygen consumption

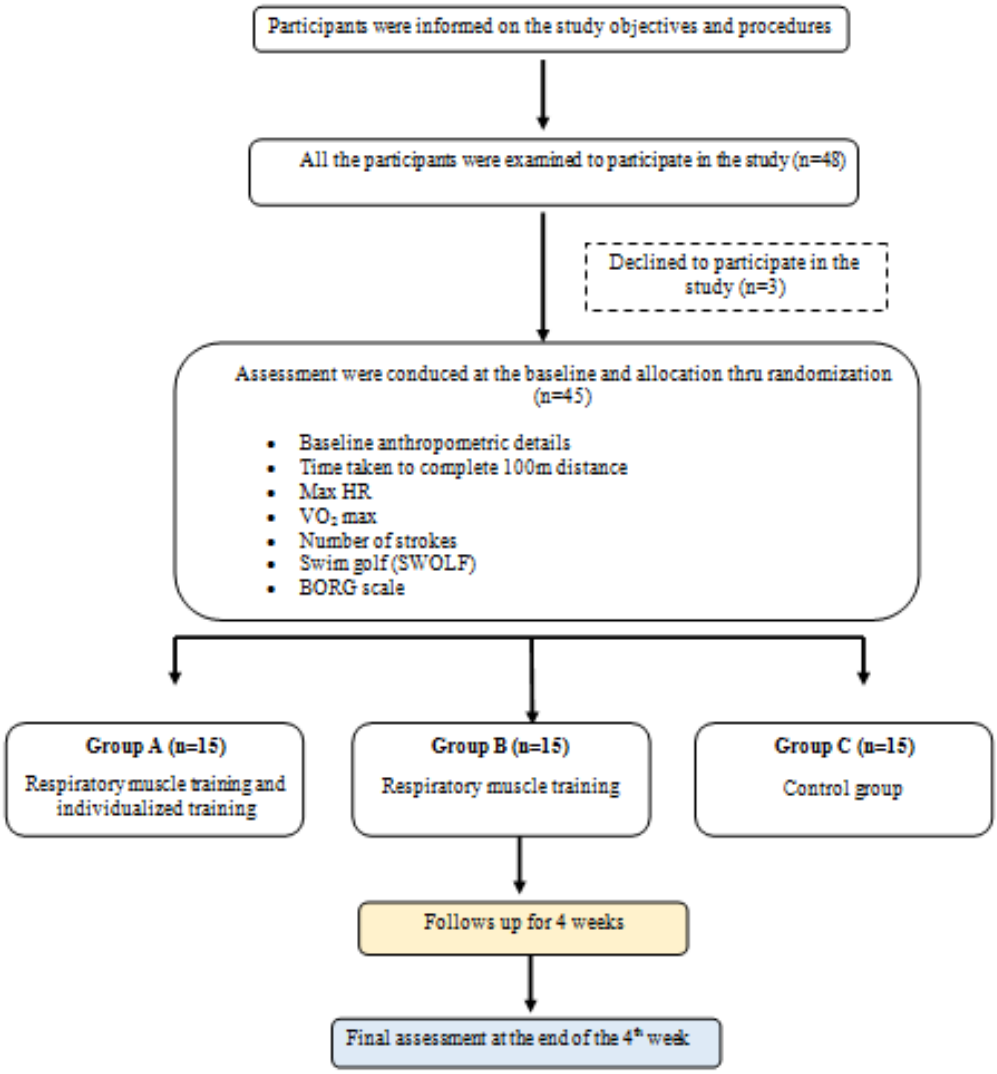

Figure 1 Study Flow chart

2.3 Training protocol

Once the participants allocated to the groups, information and procedure regarding the training sessions were informed. For Group A which is RMT+IT, the individualized training intensity based on each participants resting heart rate, maximal heart rate (220-Age), and 10 point Borg's scale was used to prescribe the training intensity (Sadowski et al., 2012; Grant & Kavaliauskas, 2017). Training intensity was fixed at 70%-85% of maximal heart rate (aerobic zone) (Grant & Kavaliauskas, 2017). During the first week of training, exercise intensity started with 75% and gradually progressed based on the individual's capacity. These individualized training also incorporated with RMT throughout the exercise sessions. The intensity for the RMT was prescribed from low level to moderate level (Based on the medium resistance power breathe trainer) (Cunha et al., 2019).

For Group B (RMT) throughout the training session starting from warm-up to cool down, respiratory muscle training was given and participants were instructed to continue their routine day-to-day activities (Lemaitre et al., 2013; Cunha et al., 2019). While for group C participants with routine training sessions were provided with a common exercise load and general land based training protocol prescribed by a swimming coach (Garrido et al., 2010). The core training session includes an aerobic training period such as jogging, cycling (static and non-static), and moderate resistance training focusing on the major muscles of freestyle swimming (Garrido et al., 2010).

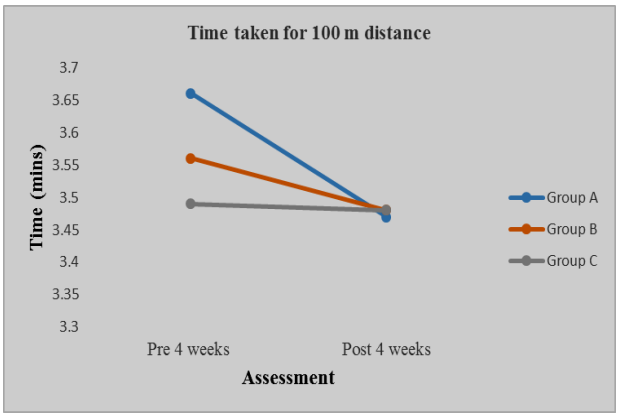

Figure 2 The time taken for subjects to complete 100m distance before and after four weeks of intervention, *Pre–post comparison, P ≤ 0.05

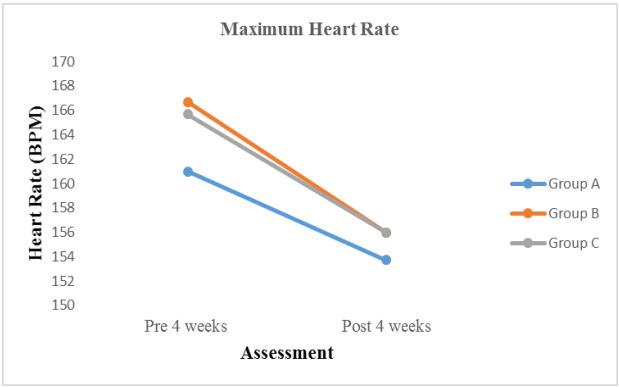

Figure 3 Maximum heart rate before and after four weeks of intervention (BPM = beat per minute), *Pre–post comparison, P ≤ 0.05

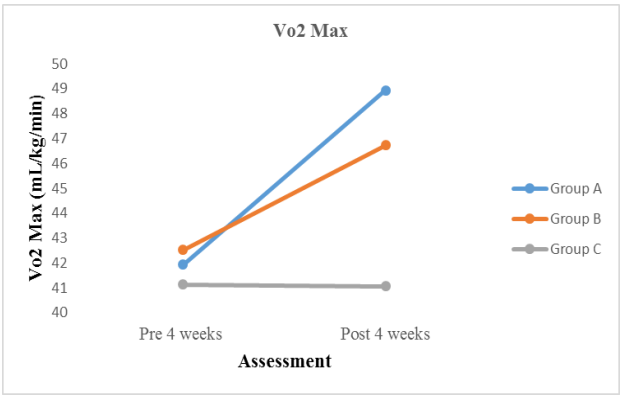

Figure 4 VO_2 max before and after four weeks of intervention, *Pre–post comparison, P ≤ 0.05

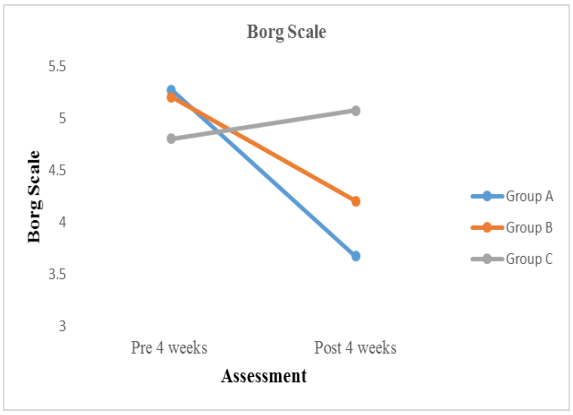

Figure 5 Rating of perceived exertion before and after four weeks of intervention, *Pre–post comparison, P ≤ 0.05

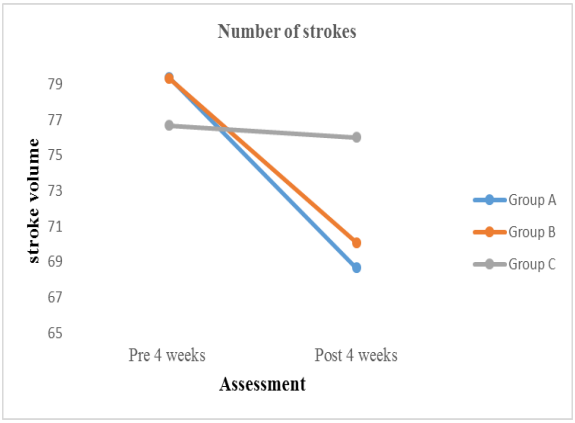

Figure 6 Number of strokes before and after four weeks of intervention, *Pre–post comparison, P ≤ 0.05

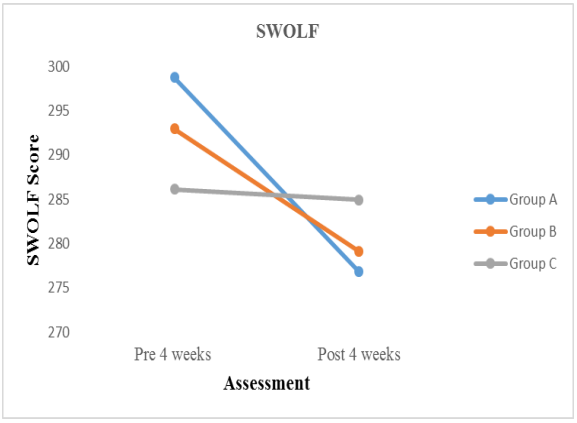

Figure 7 SWOLF Score before and after four weeks of intervention, *Pre–post comparison, P ≤ 0.05

For all the three groups, participants were scheduled with training sessions of five days weekly and an adequate amount of rest was given between various parts of training sessions. The training sessions and progression was monitored by an expert swimming coach whereas the RMT training and levels were prescribed by an expert cardiorespiratory physiotherapist. The study procedure is explained in Figure 1.

2.4 Instruments

The measurement of SWOLF, swim distance, heart rate, and Vo2max were recorded using the Garmin Forerunner 935 watch. The Garmin was connected to the smartphone through the Garmin app to record the data. To measure the heart rate accurately, the Garmin watch was strapped on the chest with a sensor connecting to the watch and it was worn throughout the 100m swimming distance. Precautions were taken to prevent the strap from restricting the breathing of the participant or creating any discomfort during the swimming.

Respiratory muscle training was given with the 'Power breathe®' medium resistance trainer (Nepomuceno et al., 2016). This medium resistance device was chosen for intermediate users. All the participants using the power breath were given clear guidance with a practical demonstration before given to them. This trainer has different levels of resistance setting starting from level 0 to level 9 (Amaro et al., 2017). An adjustable knob at the bottom of the device helps to increase or decrease the resistance as the higher level, the more is resistance. For the current study, level 2 to 8 has been prescribed for different groups with different exercise sessions.

2.5 Statistical Analysis

Shapiro-Wilk test was used to check the distribution of the data & all the data were found to be normally distributed. Mean ± standard deviation was used to present the values of each variable. For statistical analysis, the differences within the group were analyzed using paired T-test while the difference between the groups was assessed using repeated measure two-way ANOVA. The significant level was set at 0.05 with 95% confidence level analyse.

3 Results

3.1 Demographics details of the subjects

In the current study, forty-five subjects were recruited. The baseline demographics details of the subjects were presented according to the group allocation in Table 1.

3.2 Effects of respiratory muscle training (RMT) on swimming performance

The four weeks of respiratory muscle training among fifteen collegiate swimmers have shown significant improvement in the swimming performance ($p < 0.05$) except the time taken for the swimmers to complete the 100m. The results are presented in Table 2.

3.3 Effects of combined respiratory muscle training and individualized training (RMT+IT) on swimming performance

The differences of combined respiratory muscle and individualized training were evaluated pre and post 4 weeks intervention among fifteen swimmers. All the variables showed significant improvement except the time taken for the swimmers to complete the 100m distance similar to the respiratory training muscle group (Table 3).

3.4 Comparison between intervention (RMT+IT, RMT, and TS) on swimming performance

A repeated measure two-way ANOVA was used to compare the three groups (Two interventions and one control). A significant differences were found between the groups {$F(17,238) = 1483.02$, $p<0.05$, $np2 = 0.991$} and the improvement within the group after four weeks of intervention ($F(1,14) = 35.88$, $p<0.05$, $np2 = 0.719$).

Besides, the interaction of the group × time also showed significant effect ($F(17,238) = 8.385$, $p<0.05$, $np2 = 0.375$). All the results showed a large effect size as there is a clear significant relationship between the group and the improvement over 4 weeks. Figure 2 - 7 present the swimming performance in comparison of the three groups.

4 Discussion

The present study showed that both intervention groups; RMT+IT and RMT showed significant improvement in swimming performance after four weeks of intervention except for the time taken to complete the 100m distance. The comparison between the three groups reported significant differences as the combined intervention of RMT+IT showed better improvement in the time taken to complete the 100m distance, max HR, Vo2 max, perceived exertion, number of strokes, and the SWOLF score compared to isolated RMT and TS.

In this study, isolated RMT has shown improvement in the swimming performance among 15 collegiate swimmers after four weeks as there is a significant difference in max HR, Vo2 max, Borg scale, number of strokes as well as the SWOLF score. This is similar to previous studies as meta-analysis on the impact of respiratory muscle training on the performance among various athletes has reported excellent improvement in terms of athletic performance, respiratory muscle strength, and endurance in

comparison to usual training (HajGhanbari et al., 2013). The same meta-analysis has also stated that there is an improvement in aerobic metabolism and oxygen delivery due to changes in aerobic capacity of primary and accessory respiration muscle after RMT intervention (HajGhanbari et al., 2013). Indirectly, these changes have contributed to the delayed onset of fatigue and decrease competitive blood flow (Harms, 2007; Witt et al., 2007). Although the previous study on acute and chronic pulmonary responses has reported that with an increase of respiratory muscle strength, the pulmonary functions also improved concurrently (Yilmaz & Özdal 2019). Interestingly, this study also has reported the correlation between the detriments of respiratory muscle fatigue with an increase in respiratory muscle strength (Yilmaz & Özdal 2019). Thus, it can be concluded that with regular RMT, the swimming performance can be enhanced further.

Despite the improvement shown in the RMT group, the current study intended to observe the changes in swimming performance by incorporating both respiratory muscle training and individualized training among the collegiate swimmers. The results have shown a vast difference in the swimming performance comparing to isolated respiratory muscle training or the usual training performed by the collegiate swimmers. The combined intervention of both RMT and IT has shown tremendous improvement in the time taken to complete the 100m distance, max HR, VO_2 max, perceived exertion, number of strokes, and the SWOLF score. Individualized training has been used to improve the performance among various sports including marathon and distance runners; football, soccer, tennis, rugby, and others (Manzi et al., 2009; Manzi et al., 2012; Ulbricht et al., 2013; Jiménez-reyes et al., 2017; Cavaggioni et al., 2019).

In a study conducted among youth elite football players, IT has been regarded as an effective approach in enhancing the player's performance (Mujika et al., 2007). Similarly, in another study among distance runners, IT has shown an increased endurance level after eight weeks of training (Manzi et al., 2009).

The ballistic (jumping) performance has also shown significant improvement with the IT approach compared to the normal resistance training among forty-eight trained soccer and rugby players (Jiménez-reyes et al., 2017).

Although IT has been used to increase performance among various sports, to the best of our knowledge, there is no study has been conducted among swimmers using the IT approach.

The novelty of the current study to combine both the intervention of RMT and IT among the swimmers to improve various parameters (HR, Stroke Volume, VO_2 max, perceived exertion, and SWOLF score), which may further increase the swimmer's performance especially among the athletic swimmers.

The main limitation of the current study is the small sample size of swimmers in each intervention group. Despite this, the researchers in the current study have followed stringent & consistent observations leading to significant results statistically.

Further, the study has been conducted only for a short duration of time. Future studies were encouraged to be conducted in a longer duration of time to assess the chronic adaptations of RMT and IT on swimming performance with larger sample size, especially among the athletic swimmers.

Conclusion

The results of the current study indicate that the combined intervention of RMT and IT has shown better improvement compared to the isolated RMT and TS among the swimmers. The result suggests that training coaches can adapt the RMT and IT approach in their training to cater to the specific needs of the swimmers to enhance the development and performance, especially among athletic swimmers.

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THE COMBINATORIAL EFFECTS OF *Azadirachta indica* LEAF EXTRACTS WITH AMIKACIN AND TETRACYCLINE AGAINST CLINICALLY IMPORTANT BACTERIA

L.A. Sivasamugham*, K. Tze Sin, K. Thrumaran, G. Subramaniam

Faculty of Health and Life Sciences, INTI International University, Persiaran Perdana BBN, Putra Nilai, 71800 Nilai, Negeri Sembilan, Malaysia

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Keywords

Neem leaf extract

Amikacin

Tetracycline

Antimicrobial

Synergistic

Antagonistic

ABSTRACT

Antibiotic-resistance is a major threat in the treatment of diseases caused by resistant bacteria. Combination of plant extracts with antibiotics can serve as an alternative to antibiotics. *Azadirachta indica* (neem plant) has many antimicrobial properties due to the presence of secondary metabolites such as alkaloids and flavonoids. In this study, the combinatorial effects of neem leaf extracts with amikacin and tetracycline against eight clinically important gram-positive and gram-negative pathogens were investigated using the agar well diffusion assay. Synergistic effect of neem leaf extract and tetracycline was observed against *Propionibacterium acnes*, *Bacillus subtilis*, and *S. pneumoniae* with a significant enlargement ($p<0.05$) in the diameter of the zone of inhibition. However, the same combination showed insignificant inhibition against *S. faecalis*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Staphylococcus aureus*. The neem leaf extract-amikacin combination showed insignificant antibacterial activity against *Staphylococcus aureus*, *S. epidermidis*, *S. pneumoniae*, and *Pseudomonas aeruginosa*. An antagonistic effect was observed when *Bacillus subtilis* was exposed to the same combination as there was a significant reduction ($p<0.05$) in the zone of inhibition. This study suggests the potential development of the neem leaf extract-tetracycline combination as an antibacterial agent against *P. acnes*, *B. subtilis*, and *S. pneumoniae*. However, this preliminary data requires further investigation and test on a wider range of clinical isolates to make a more decisive conclusion. The antagonistic effect of the neem leaf extract and amikacin suggests that the individual agents are potent as antibacterial agents than the combination.

* Corresponding author

E-mail: lalitaa.sivasamugham@newinti.edu.my (Lalita Ambigai Sivasamugham)

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1 Introduction

Antibiotic resistance is an emerging global health threat (WHO, 2020). The increase of antibiotic resistance urges the need to discover and develop alternative antimicrobial agents from other potential sources including plants (Othman et al., 2019). The use of medicinal plants in treatment has been recorded since ancient history (Petrovska, 2012). Studies show that about 80% of the global population use plants for their primary health care (Ekor, 2014). The medicinal plants such as *Allium sativum (garlic) and Prosopis juliflora* (mesquite) contain phytoconstituents such as alkenyl phenols, alkaloids, and flavonoids that have antimicrobial properties (Emad, 2011). Plant extracts also generally show no or very low toxic effects (Cowan, 1999). Also, the cost of production is low (Teka et al., 2015; Cheesman et al., 2017).

Azadirachta indica is widely used as a traditional medicine due to its antimicrobial and other medicinal properties. Studies show antimicrobial activities of the different parts of the neem plant (Raja Ratna Reddy et al., 2013). A combination of neem leaves extracts with other medicinal plants exhibited synergistic antibacterial effects against *Pseudomonas aeruginosa, S. mutans, S. gordonii*, and *Candida albicans* (Bhuva & Dixit, 2015; Saini et al., 2020). Thus, this study was aimed to investigate the combinatorial effects of *A. indica* leaf extract with amikacin and tetracycline against eight clinically important bacteria using the agar well diffusion assay apart from the qualitative screening of the phytochemicals in the leaf extract.

2 Materials and Methods

2.1 Pure Culture Isolation

The bacterial cultures used in this study were *Streptococcus faecalis, Enterococcus faecalis, S. aureus, S. epidermidis, Propionibacterium acnes, Bacillus subtilis, Serratia marcescens,* and *Pseudomonas aeruginosa.* These cultures were in glycerol stock and were obtained from the culture collections of INTI International University. All bacterial cultures were inoculated onto nutrient agar and incubated at 37° for 24 h to obtain homogenous colonies.

2.2 Confirmatory Tests

The pure cultures were Gram-stained using the method described by Moyes et al. (2009). The Gram-positive bacteria were subjected to catalase test as described by Iwase et al. (2013) and grown on mannitol salt agar. The IMVIC (Indole, methyl red, Voges-Proskauer, and Citrate) test, oxidase test, and the triple sugar iron test was used to confirm the identity of the Gram-negative bacteria (Holt, 1994; Cappuccino & Sherman, 2005).

2.3 Ethanolic *A. indica* Extract Preparation

Fresh neem leaves were obtained from a residential garden in Seremban, Malaysia. The leaves were rinsed in the following order; water, 80% (v/v) ethanol, and sterile deionized water (Noor et al., 2011). The dust-free neem leaves were sun-dried for 24-48 h, ground using a micro-fine multipurpose powder dry medicine blender (Himitzu), and sieved with a 40-mesh sieve. The neem leaf powder (35 g) was suspended into 70 mL of 80% (v/v) ethanol to a ratio of 1:2. The mixture was incubated in an orbital shaker at room temperature for 24 h and centrifuged at 10,000 rpm for 15 min at room temperature (Itelima et al., 2016). The supernatant was filter-sterilized (0.2 µm) and analyzed for its phytoconstituents before subjecting to the agar well diffusion assay (Echegoyen et al., 2015).

2.4 Agar Well Diffusion Assay

The antibacterial activities of the leaf extracts were detected using the agar well diffusion assay (Perez et al., 1990). The overnight bacterial cultures which were adjusted to 0.5 McFarland standard was lawned onto MH agar using a sterile wooden applicator. Four wells were made onto the agar by puncturing the agar using a sterile micropipette tip. Wells in quadrants 1 and 2 were filled with 200 µL of neem extracts (0.5 g/mL) and 200 µL of antibiotics respectively (Clinical Laboratory Standard Institute, 2017). Quadrant III was filled with 200 µL of 80% (v/v) ethanol whereas; Quadrant IV had 200 µL of neem extract (0.5 g/mL) and antibiotic combination. The agar was incubated at 37°C for 24 hours. This assay was done in triplicates. The effects of the neem leaf extract-antibiotic combinations were analyzed by measuring the diameter of the zone of inhibition (in mm) using a metric ruler.

2.5 Qualitative Phytochemical Screening

The ethanolic neem leaf extracts were screened for phytochemicals using previously described methods to Shu'aibu et al. (2015).

2.6 Statistical Analysis

ANOVA in IBM SPSS Statistics Version 22 was used to determine the significant differences between the neem leaf extracts, antibiotics, and the neem leaf-antibiotic combination when tested against different bacterial strains with a significant value of $p<0.05$.

3 Results and Discussion

Amikacin is a semi-synthetic aminoglycoside used against a wide spectrum of Gram-negative bacteria, such as *Serratia* spp. and *P. aeruginosa* as well as Gram-positive bacteria, such as *Staphylococcus* (eMC, 2020). Tetracycline is a polyketide

antibiotic that is effective against both Gram-positive and Gram-negative bacteria (Eliopoulos & Roberts, 2003).

The combinatorial effect of neem leaf extract with tetracycline showed a significantly larger zone of inhibition ($p<0.05$) compared to the individual applications of neem leaf extract and tetracycline against *P. acnes, B. subtilis, and S. pneumoniae* (Figure 1). This indicates the synergistic effect of the neem leaf extract-tetracycline combination against these bacteria. This finding agrees with the study by Rasha et al. (2015) that showed the synergistic effect of *V. nilotica and S. alexandrina* leaf extract-tetracycline combination against *B. subtilis* with a significant increase in the diameter of the zone of inhibition. Kusum & Sharmita (2015) also reported the synergistic effect of neem leaf extract against *E. coli* when combined with tetracycline. The synergistic activity of the neem leaves extract-tetracycline observed in this study could have been attributed to the bioactive phytoconstituents of the neem leaf extract that enhanced the antibacterial activity of tetracycline (Aslam et al., 2009). Sibanda & Okoh (2007) suggested that bioactive phytoconstituents could inhibit the activity of the efflux pumps in bacteria. Efflux pump is a transport protein found in Gram-positive and Gram-negative bacteria that extrudes toxic compounds such as antibiotics out of the bacterial cells, making the cells to resist the action of antibiotics (Webber & Piddock, 2003). The inhibition of the efflux pump activity increases the entry of antibiotics into bacterial cells enabling them to reach the target site and destroy the cells (Lomovskaya & Bostian, 2006). Efflux pump inhibitors such as ferruginol isolated from *Chamaecyparis lawsoniana* inhibited the tetracycline-resistant efflux pump, quinolone-resistant efflux pump, and erythromycin-resistant efflux pump in *S. aureus* which improved the antimicrobial effect of tetracycline, quinolone, and erythromycin respectively (Aiyegoro et al., 2011).

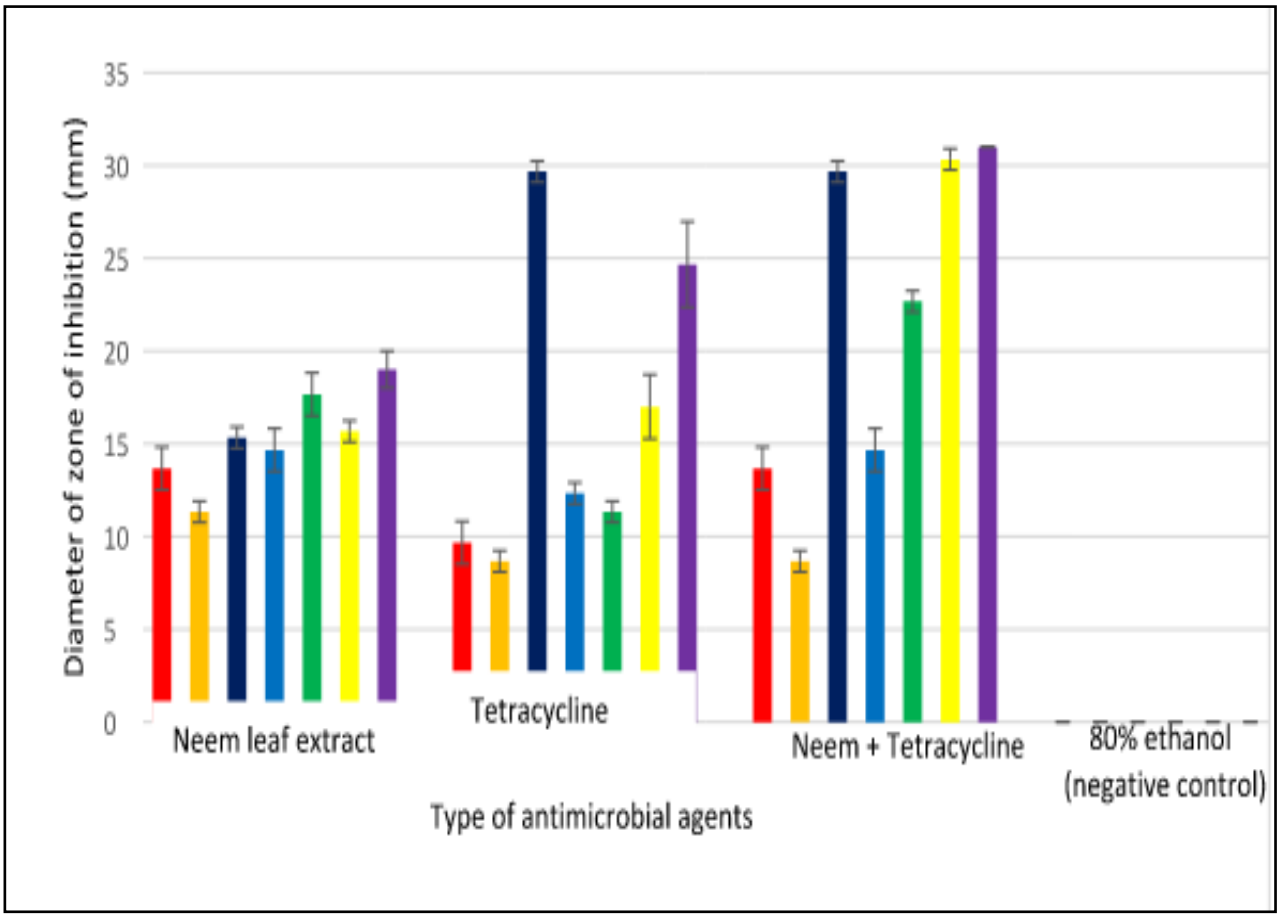

Figure 1 The mean diameter of zone of inhibition (mm) produced by neem leaf extract, tetracycline and combination of neem leaf extract with tetracycline against the tested bacteria

Qualitative phytochemical screening of the neem leaf extract showed the presence of glycosides, alkaloids, phytosterols, and flavonoids (Table 1) and this was consensus with the findings by Pandey et al. (2014) and Al-Hashemi & Hossain (2016). These phytochemicals could have exhibited a concerted effect eventually causing *P. acnes, B. subtilis and S. pneumoniae* to be more permeable to the action of tetracycline.

Table 1.Qualitative Phytochemical screening of neem leaf extract

Phytochemicals	Ethanolic Extract
Alkaloids	X
Flavonoids	X
Glycosides	X
Phytosterols	X
Saponins	O

Here X indicates presence and O indicates the absence of the specified phytochemicals in the leaf extract

Flavonoids are suggested to inhibit bacterial growth by inhibiting nucleic acid synthesis, plasma membrane function apart from inhibiting the activity of porin on the bacterial cell membrane. Flavonoids could have also altered the bacterial membrane permeability causing the cells to be more susceptible to the action of tetracycline (Xie et al., 2015). Alkaloids present in the neem leaf extract could have inhibited the efflux pump actions in the tested bacteria as reported by Mabhiza et al. (2016).

Another possible reason for the synergistic effect is the bacterial protein synthesis inhibition that resulted in a potent tetracycline activity (Aiyegoro et al., 2011). In this study, the qualitative preliminary phytochemical screening showed the presence of glycosides, alkaloids, phytosterols, and flavonoids but a quantitative analysis will identify the exact components that exhibited the synergistic activity with tetracycline. The findings in this study strongly suggest the potential use of the neem leaf extract-tetracycline combination as an effective antibacterial agent against *P. acnes, B. subtilis, and S. pneumoniae*. The combination might reduce the development of tetracycline-resistant bacteria and reduce the dose of tetracycline required to treat infections caused by these bacteria. However, further investigation and tests on different strains of *P. acnes*, B. *subtilis*, and S. *pneumoniae* are required before a more decisive conclusion can be made.

The combination of neem leaf extract and tetracycline showed no difference in the inhibition against *S. aureus, S. epidermidis, S. faecalis, and E. faecalis* (Figure 1). This might be due to the MIC of the combination of neem leaf extract and tetracycline that was not potent enough to inhibit the bacterial activity (Aiyegoro et al., 2011). Apart from that, the genetic variation in neem plant and bacteria might reduce the effectiveness of antimicrobial activity of neem leaf due to the expression of a certain gene in bacteria that could alter or destroy the antimicrobial molecules found in the extract (Muniata & Arias, 2016). However, the mode of action and mechanisms of active phytoconstituents of the neem leaf extract has to be studied to have a greater insight on this.

The neem leaf extract-amikacin combination showed a significant reduction ($p<0.05$) in the diameter of the zone of inhibition against *B. subtilis* indicating an antagonistic effect (Figure 2).

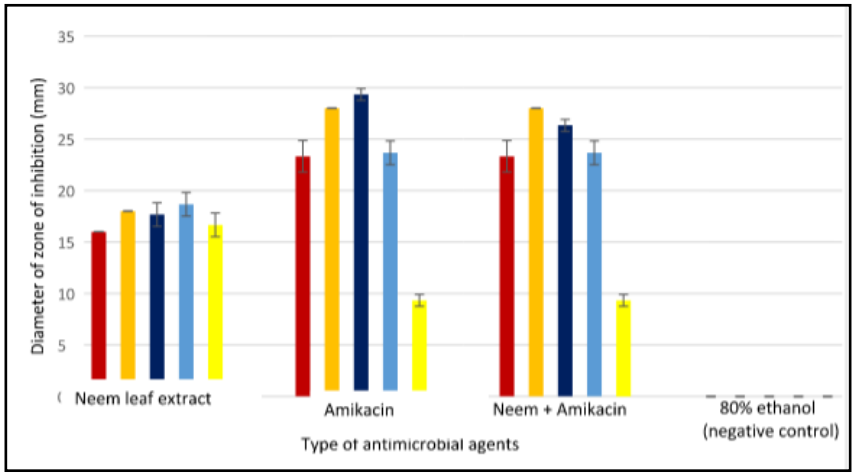

Figure 2 The mean diameter of zone of inhibition (mm) produced by neem leaf extract, amikacin, and combination of neem leaf extract with amikacin against the tested bacteria

This antagonistic effect might be due to the presence of certain phytoconstituents in the extract that had competitive binding with amikacin to the bacterial membrane hence reducing its antibacterial activity (Passat, 2012; Eze et al., 2013). The antagonistic effect between neem leaf extract and amikacin indicates that amikacin should be used individually as the combination showed reduced activity and possibly an adverse effect.

The combinatorial effect of neem leaf extract and amikacin against *S. aureus, S. epidermidis, S. pneumoniae, and P. aeruginosa* showed no significant difference in the diameter of the zone of inhibition indicating that the combination had no synergistic or antagonistic effect against these bacteria. Similar findings were reported by El-Zawahry et al. (2013) where the combinatorial effect of tamarind extract with amikacin showed a similar diameter of zone of inhibition with amikacin. This might be due to the absence of interaction between phytoconstituents of the neem leaf extract and amikacin (Bhuva & Dixit, 2015). This indicates that it is not crucial to combine neem leaf extract and amikacin for therapeutic purposes. However, to date, no study had been done on the combinatorial effect of neem leaf extract and amikacin against the bacteria used in this study. Hence, further investigations must be done to make a more decisive conclusion.

Conclusion

The combination of neem leaf extract with tetracycline showed synergism against *P. acnes, B. subtilis, and S. pneumoniae* based on the zone of inhibition suggesting the potential combination to treat related infections. Antagonistic effect exerted by amikacin and neem leaves extracts combination against *B. subtilis* suggests that they should not be combined. However, these are preliminary findings and thus require further investigation and test on a wider range of clinical isolates to make a more decisive conclusion on the potential use of neem leaf extract with tetracycline and amikacin.

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Conflict of interest

The authors declare that they have no conflict of interest.

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INFLUENCE OF GENDER ON PAIN, QUALITY OF LIFE, AND PHYSICAL ACTIVITY IN PATIENTS WITH KNEE OSTEOARTHRITIS

Tan Xue Min, Vinosh Kumar Purushothaman, Yughdtheswari Muniandy*

INTI International University, Faculty of Health Sciences, Physiotherapy program, Persiaran Perdana BBN, Putra Nilai, 71800 Nilai, Negeri Sembilan, Malaysia

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Pain

Knee osteoarthritis

Physical activity

Quality of life

ABSTRACT

Osteoarthritis is the most common cause of knee pain which contributes to adults' disability. Females have an increased risk of knee osteoarthritis. However, little is known about the gender influence in pain perception, quality of life (QOL), and physical activity. Hence, this study aimed to investigate the influence of gender on pain perception, QOL, and physical activity in patients with knee osteoarthritis. A cross-sectional study design with a total of 186 patients(mean age of 56.64 ± 6.49) with knee osteoarthritis were recruited. Pain intensity, level of physical activity, and QOL of patients were assessed using a visual analog scale (VAS), global physical activity questionnaire (GPAQ), and Short Form-36 (SF-36) questionnaire. Among the studied patients, 52% of patients with knee osteoarthritis had a relatively low level of physical activity with females representing the majority of them. Lower mean was observed in QOL and physical activity whereas pain intensity was higher in females compared to males ($p < 0.05$). Pearson correlation demonstrated a strong negative correlation between physical activity and pain ($r = -0.77$, $n = 186$, $p < 0.01$), and a weak correlation exist between physical activity and all domains of QOL except for the functional capacity and body pain. Majority of female participants with knee osteoarthritis exhibit poor QOL, physical activity, and increased pain intensity as compared to males. Clinicians need to be aware of the influence of gender in treating patients with knee osteoarthritis.

* Corresponding author

E-mail: eshwari_physiorehab@yahoo.com (Yughdtheswari Muniandy)

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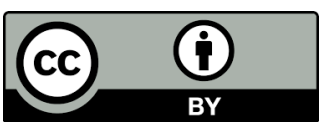

1 Introduction

Osteoarthritis is the most widespread chronic degenerative musculoskeletal disease, leads to pain and physical disability in adult individuals (Alenazi et al., 2020). It is a disease that progresses over time in joints causing significant changes in the tissue architecture, its metabolism, and function (Primorac et al., 2020). It commonly affects the joints in the hands and weight-bearing joints including knees, hips, feet, and spine (Chinese Orthopaedic Association, 2010). There were approximately 8.5 million adults in the UK and 27 million US adults have clinical osteoarthritis (Dillon et al., 2006).

The primary burden of osteoarthritis is pain and physical impairments which significantly influence the quality of life (QOL) of individuals (Elliott et al., 2007; Boutron et al., 2008; Yildiz et al., 2010). Several risk factors such as pain, inaccuracy proprioception, and muscle weakness have been identified in the declination of physical function in osteoarthritis patients (Dunlop et al., 2005; Dekker et al., 2009). Studies have reported that lack of regular physical activity is one of the most predominant risk factors in deteriorating the functions of activities of daily living (ADL) in patients with osteoarthritis (Dunlop et al., 2010). It can be interpreted as a pattern of avoidance for certain patients with osteoarthritis who are likely to delay physical activity in fear of escalating pain in the injured joint (Steultjens et al., 2002; Somers et al., 2009).

Apart from the pain and functional limitation, osteoarthritis also affects an individual's psychological well-being which negatively affects the quality of life (Elizabeth & Rena, 2011; Sharma et al., 2016). Female gender is a major predisposing factor of knee osteoarthritis (Ferre et al., 2019). There is a dearth of research in this field, in which the effects of gender on pain severity, quality of life, and physical activity is uncertain. Therefore, the present studies aim to identify the influence of gender on pain, quality of life, and physical activity in patients with knee osteoarthritis and determining the relationship of physical activity with pain and quality of life.

2 Materials and Methods

A cross sectional study using the convenience sampling method was adopted. For this analysis 186 participants were recruited. The inclusion criteria were participants between the age of 40 to 60 years, who have been diagnosed with knee osteoarthritis and presented with complaints of knee pain for the past 6 months or more. Whereas, the exclusion criteria included participants with a known history of a traumatic knee injury, undergone knee replacement surgery, rheumatoid arthritis, central or peripheral nervous system disorder, or a condition that limits pre-mobility status. Before collecting data, all participants were provided details about the intent and procedure of research, and written informed consent was taken. This study was approved by the Research and Ethics Committee of INTI International University.

All the eligible participants were given a form to fill that comprised of demographic details such as age, gender, occupation, level of physical activity, and pain assessment chart. The participants were screened for pain using the Visual Analog Scale (VAS). It is a 10cm continuous scale with one end representing the maximum intensity and the other end of no pain (Crichton 2001; Kahl & Cleland 2005). Participants were asked to measure their pain level at rest and during activity. The greatest benefits of VAS are the simplicity of its design and usage. Besides that, VAS is highly reliable with an intraclass correlation coefficient (ICC) between 0.71 to 0.99 (Kahl & Cleland 2005).

Meanwhile, a global physical activity questionnaire (GPAQ) was used to assess the level of physical activity. It includes 16 questionnaires to identify physical activity at work, travel to and from places, and recreational activities. The physical activity level of each participant was measured using the metabolic equivalent of task-minute per week by combining the score of work, travel, and recreational MET-minutes/week (Herrmann et al., 2013). Participants were categorized into 3 levels, high > 6.0 MET-min/ week; moderate between 3.0 to 6.0 MET-min/ week; and low below < 3.0 MET-min/ week (Bull et al., 2009). The QPAQ is found to be valid and reliable which demonstrated moderate to strong positive relationships ranging between 0.45 to 0.65 (Bull et al., 2009).

Short Form-36 (SF-36) was used for the assessment of the quality of life (Ware, 2000). It is a 36 item questionnaire that has been divided into 8 domains including physical functioning (10 elements), social functioning (2 elements), role limitations due to physical health (4 elements), role limitation due to emotional problems (3 elements), mental health (5 elements), energy and vitality (4 elements), bodily pain (2 elements) and general health (5 elements). Each item is recorded on a scale with values from 0 to 100 which corresponds to the worst and best health status respectively. Aggregate scores were compiled into a percentage. A greater score indicates better QOL (Ware Jr & John, 2000; Salaffi et al., 2005; Alkan et al., 2014). Each item has to carry equal weight. The SF-36 demonstrated high internal consistency (Cronbach's alpha ranged from 0.65 to 0.94) and excellent reliability (ICC between 0.75 t0 0.97) (Heyland et al., 2000). Statistical software package SPSS (Version 25.0) was used to interpret data. The Kolmogorov-Smirnov test, Q-Q plot, and skewness ranging between -1 to 1 was used to check the assumption of normality. The T-test was used for the assessment of the mean of the two groups. The correlation between physical

activity and other factors were tested using Spearman's correlation analysis. The findings were defined at a confidence level of 95% with a significant level at p <0.05.

3 RESULTS

Total 186 participants (91 males 95 females) were recruited with a mean age of 56.64 ± 6.49. The sociodemographic data of participants with knee osteoarthritis are as depicted in Table 1. The highest percentage of participants were between the ages of 50 to 59 years, with most of them were Malay. Most of the participants complain of moderate-intensity (between 5 to 7) pain 65.6%, followed by mild intensity (between 1 to 4) of pain 28% and 6.5% with severe pain intensity (between 8 to 10).

3.1 Level of Physical Activity in Knee Osteoarthritis

This study demonstrated that almost more than half of participants (52%), presented with a low level of physical activity (< 3.0 MET –min/week), followed by 44% of participants with moderate physical activity (3.0 to 6.0 MET-min/ week) and 8% of participants with a high level of physical activity (> 6.0 MET-min/week). The mean score of the total GPAQ in MET-min per week was 936 ±593 for males and 598 ±265 for females with the highest mean in the work domain followed by recreation and transport as depicted in table 2. There is significant difference among genders for all the physical activity domain (p value < 0.001).

Table 1 Demographic characteristics of the participant with knee osteoarthritis

Variables	Participants (n= 186) n (%)
Age (mean ± SD)	56.64 ± 6.49
Age Range	
40-49	33 (17.7)
50-59	79 (42.5)
≥60	74 (39.8)
Gender	
Male	91(48.9)
Female	95(51.1)
Ethnicity	
Malay	131 (70.4)
Chinese	41 (22.0)
Indian	13 (7.0)
Others	1 (0.5)
Pain intensity (mean ± SD)	52.20 ± 15.05
Pain intensity range	
Mild (1 to 4)	52 (28.0)
Moderate (5 to 7)	122 (65.6)
Severe (8 to 10)	12 (6.5)

According to the SF-36 in measuring the QOL, the mental health domain achieves the maximal value for both males and females with mean + SD of 72.7± 7.6 and 70.3± 7.4 respectively, while the lowest domain in males is for general health status with a mean + SD of 42.9± 8.6 and functional limitation domain in females with mean + SD of 37.7± 16.7.

Table 4 demonstrate statistical significant difference observed for functional capacity, p = 0.019, functional limitation, p = 0.004, bodily pain, p < 0.001, general health status 0.048 and mental health, p = 0.029. Examination of the mean scores indicates that there was remarkable QOL achieved for all the variables in males as compared to the females.

Table 2 Physical activity level of participants with knee osteoarthritis

Physical activity domain (MET-Min per week)	Male (n=91) Mean ± SD	Female (n=95) Mean ± SD	Significant value p
Work	685 ± 685	478 ± 315	<0.001
Transport	24.1 ± 102	16 ± 66	<0.001
Recreation	226 ± 222	104 ± 150	<0.001
Total GPAQ	936 ± 593	598 ± 265	<0.001

Table 3 Participants who did not met recommended physical activity level according to age

Age (Year)	Male (n= 38) n (%)	Female (n = 58) n (%)	Both Sex (n = 96) n (%)
40-49	1(5.3)	3 (21.4)	4 (2.2)
50-59	8 (22.9)	22 (50.0)	30 (16.1)
≥60	29 (78.4)	33 (89.2)	62 (33.0)
Total	38 (41.8)	58 (61.1)	96 (51.6)

Table 4 Comparison of SF-36 score between gender among participants with knee osteoarthritis

Quality of life domains	Male (n=91) Mean ± SD	Female (n=95) Mean ± SD	Significant value p
Functional Capacity	52.2±17.6	46.7± 14.0	0.019*
Functional Limitation	45.1±17.9	37.7± 16.7	0.004*
Bodily Pain	55.8± 18.9	38.8± 13.7	0.001**
General Health Status	42.9± 8.6	40.7± 6.5	0.048*
Vitality	61.6± 11.3	58.7±8.7	0.057
Social Aspects	71.3 ± 18.3	67.5±15.3	0.123
Emotional Aspects	58.3± 21.4	55.7±18.4	0.39
Mental Health	72.7± 7.6	70.3± 7.4	0.029*

$p < 0.001$**, $p < 0.01$*

Table 5 Comparison of pain intensity between gender

Pain Intensity	Male (n=91) Mean ± SD	Female (n=95) Mean ± SD	Significant value p
VAS	48.6 ±16.2	55.6 ± 12.9	0.001**

$p < 0.001$**

Table 6 Relationship between physical activity with pain intensity and quality of life

Variables	Correlation coefficient *r*	Significant value p
Physical activity - Pain intensity		
VAS	- 0.777	**<0.001****
Physical activity - QOL		
Functional Capacity	0.639	**<0.001****
Functional Limitation	0.401	**<0.001****
Bodily Pain	0.652	**<0.001****
General Health Status	0.279	**<0.001****
Vitality	0.257	**<0.001****
Social Aspects	0.312	**<0.001****
Emotional Aspects	0.241	**<0.001****
Mental Health	0.234	**<0.001****

$p < 0.001$**

3.3 Severity of pain in Knee Osteoarthritis

In terms of severity of pain, 65.6% of participants presented with moderate pain (between 5 to 7), followed by 28.0% of them with mild pain (between 1 to 4), and 6.5% complaints of severe pain (between 8 to 10). There was a statistically significant difference in pain intensity observed, $p < 0.001$ between genders as showed in table 5. Analysis of mean revealed a greater intensity of pain reported by females compared to males.

3.4 Relationship between physical activity and other parameters

The relationships between the level of physical activity with pain intensity and QOL was analyzed using Pearson's correlation as depicted in table 6.

between gender in participants who did not accomplish WHO recommendations for health (p=0.150). A strong negative correlation exists between level of physical activity and pain, which was statistically significant ($r = -0.777$, $n = 186$, $p < 0.001$). This indicates that with increase physical activity level, there is reduce in pain intensity. In terms of the relationship between physical activity and QOL, a weak correlation exists for all the domains except functional capacity and body pain which demonstrate a moderate positive correlation.

4 Discussion

The current study aimed to determine the influence of gender on pain, quality of life, and physical activity among adults with knee osteoarthritis. The research findings indicate that the prevalence of

knee osteoarthritis was greater in females as compared to males. osteoarthritis.

Similarly, previous studies have supported that females are at greater risk of osteoarthritis (Sowers et al., 2000; Srikanth et al., 2005; Hame & Alexander, 2013; Prieto-Alhambra et al., 2014; Ferre et al., 2019). This can be due to lower pain threshold and less tolerance to painful stimuli by females as compared to males (Chesterton et al. 2003; Riley et al. 1998). In addition to this, reduce in estrogen production due to menopausal changes can further accelerate the process of degeneration (Hame & Alexander, 2013). An earlier study has also documented an increased progression of cartilage degeneration and significant loss of tibial and patella cartilage in females than males (Eyre, 2004; Hanna et al., 2009). It has been reported that the rate of loss of cartilage at the tibia and patella in females is three to four times higher than in males (Hanna et al., 2009). Such findings show that females are more predisposed to osteoarthritis in the knee.

Results of the current study suggested that the highest percentage of participants were between the age of 50 to 59 years and with age beyond 60 years there is decreased in the incidence of osteoarthritis. A similar trend was also observed in the previous study (Prieto-Alhambra et al., 2014). Osteoarthritis progress rapidly in females during menopause aged between 55 to 60 years and gradually decline in the elderly. While for males, the incidence of osteoarthritis has increased gradually until the last stage of life (Prieto-Alhambra et al., 2014).

In terms of physical activity, the present study demonstrated that most of our participant did not achieve the guidelines for physical activity suggested by the World Health Organization (WHO) which is a minimum of 150 minutes of moderate-intensity of physical activity per week or 75 minutes of vigorous intensity of physical activity per week achieving 600 MET-min per week. It is notable, that the prescribed amount of physical exercise was not reached by a large percentage of participants and the incidence of knee osteoarthritis increases with age.

The outcome of the present study was further strengthened by previous studies which found females are insufficiently active in comparison with males (Troiano et al. 2008; Hamrik et al. 2014; Sparling et al. 2015). Factors that hinder the patient with knee osteoarthritis involve physical activity is not due to the onset of disease or pain but due to external factors such as time constraints and non-prioritization of physical activity (Mesci et al., 2015). In other cases, pain related fear has also contributed to immobility in a later stage, leading to inactivity (Steultjens et al., 2002). It is important to maintain regular physical activity to minimize and prevent functional and mobility-related disability and reducing the like hood of developing severe cardiovascular disease, metabolic disorder, perception deficit, osteoporosis, and muscle weakness (Rosenberg et al., 2011; McPhee et al. 2016; Chen et al., 2017).

The present study revealed that patients with knee osteoarthritis have overall impaired QOL primarily in the functional limitation domain. Similar results were obtained in a previous study which demonstrated patients with knee osteoarthritis had a significantly poorer quality of life (Salaffi et al., 2005). Besides that, physical health components showed significantly lower scores than the mental health component in the present study. This was consistent with other studies that reported the subscales related to physical health had relatively lower scores compared with the components of mental health (Lam & Lauder, 2000; Zakaria et al. 2009). Our study also demonstrated statistically significant difference in functional capacity, $p = 0.019$, functional limitation, $p = 0.004$, bodily pain, $p < 0.001$, general health status 0.048 and mental health, $p = 0.029$. Examination of the mean scores indicates that there was a greater quality of life for all the variables in males as compared to the females. The gender disparity in influencing the quality of life could be due to mechanical, environmental, and psychosocial factors such as employment, education, physical activity, muscle power, and pain coping skills (Rapp et al. 2000; Adamson et al. 2003; Dunlop et al. 2005).

In the present study, we noted that almost 65.6% of participants with knee osteoarthritis complain of pain. Pain is the hallmark symptom of osteoarthritis which limits the function and reduces the QOL (Dominick et al. 2004; Ayis & Dieppe, 2009; Neogi 2013). It is the important precursor of physical disability and key symptom that influences the decision making of the patient with knee osteoarthritis in seeking medical treatment (Neogi, 2013). The findings of this study also suggest a remarkable difference between gender in pain perception ($p <0.001$). Such results were comparable to prior research with women experiencing greater pain than men owning to lower pain threshold and tolerance to painful stimuli (Riley et al.,1998; Keefe et al., 2000; Chesterton et al., 2003; Tonelli et al., 2011). Present study findings can have significant implications for clinicians in treating pain. Clinicians might need to be aware that treatments may have different effects on pain perception when treating women versus men with knee osteoarthritis (Keefe et al., 2000).

In regards to the relationship between the QOL, pain, and level of physical activity the present study demonstrates a low positive correlation between QOL and physical activity in knee osteoarthritis patients. Current results were found to be consistent with the systemic reviews which have shown the positive correlation between regular physical activity and health-related QOL (Bize et al., 2007; Klavestrand & Vingard, 2009). In terms of the relationship between pain and physical activity, the present study reported a strong, negative correlation between pain and

physical activity, which indicates that with increase physical activity level, there is reduce in pain intensity. These results were similar to a study done by Wilkie et al. (2007) which reveals a strong association of knee pain with restricted mobility outside the home. Inactive patients were avoiding some movements or activities due to fear of pain although there was no real physical cause of pain over their knees (Steultjens et al., 2002). Hence, patients with knee osteoarthritis may develop progressive loss of physical function, the decline in QOL, and became dependent on their ADL (Loza et al., 2009).

Conclusion

In summary, present study reports greater numbers of female participants with knee osteoarthritis have relatively poor physical activity and quality of life and increase pain intensity as compared to males. This highlights the needs of clinicians to be aware of the gender influences in managing patients with knee osteoarthritis.

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Conflict Of Interest

The author(s) declare(s) that there is no conflict of interest.

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PRE-TREATMENT TEMPERATURE AND MULTI-RESPONSE SURFACE OPTIMISATION OF ULTRASOUND-ASSISTED EXTRACTION OF ANTIOXIDANTS FROM RED DATES

Haripriya Ravikumar, Chua Bee Lin*, Chen Yen Leng, Ameena Ali, Choo Choong Oon

School of Computer Science and Engineering, Faculty of Innovation and Technology, Taylor's University, 1, Jalan Taylor's, 47500, Malaysia

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ABSTRACT

Ziziphus jujube known as red dates are natural flora, are a rich source of antioxidant bioactive compounds and are widely used in making Chinese traditional medicine. However, the optimization of extraction conditions and demonstration of extraction kinetics of red dates remains a gap. Therefore, the main objective of this research was to enhance the antioxidant activity via DPPH, crude extract yield, and TPC by response surface methodology (RSM). Also, mathematical modeling of the TPC extraction kinetics was performed. Single-factor experiments were adopted to identify the preliminary RSM ranges of four extraction parameters such as liquid-solid ratio (10, 20, and 30 ml/g), extraction temperature (50, 60 and 70°C), time (40, 50 and 60min), and ultrasonic power (70, 80 and 90%). The extraction kinetics based on RSM optimized conditions were modeled into six extraction kinetic models. As result, the highest crude extract yield (4.56 g), highest TPC (0.023 g GAE/g extract), and highest antioxidant activity (85.88%) were obtained at 60ºC. The optimum values were liquid-solid ratio 30ml/g, extraction temperature 60°C, time 60 min, and ultrasonic power 70%. The antioxidant activity of red dates after optimization (90.59%) was higher than that of synthetic antioxidants, Butylated Hydroxytoluene (84.71%), and Butylated Hydroxyanisole (77.73%). Furthermore, the best-fitted kinetic model was the second-order kinetic model due to its coefficient of determination (R^2) at 0.9849, being the closest to 1 and its root mean square error (*RMSE*) was the lowest, 0.001028 among other models.

* Corresponding author
E-mail: beelin.chua@taylors.edu.my (Chua Bee Lin)

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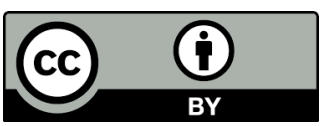

1 Introduction

Ziziphus jujuba Mil., referred as a red dates or Chinese dates belonging to the family Rhamnaceae, is a native fruit of Chinese culture (Ali et al., 2017; Bee Lin & Yen Leng, 2018). It is an outstanding source of bioactive compounds whose properties range from nutritional to nutraceutical. Red dates contain a high amount of macronutrients, vitamins, and minerals that are highly beneficial for those suffering from health issues such as insomnia, blood pressure, and high cholesterol (Morales et al., 2014). Research had proven that the most pronounced attribute of red dates was its antioxidant potential. Recently, research on preservation and extraction of antioxidants from natural sources are more driven by the fact that synthetic antioxidants like BHT and BHA may possess health hazards and they are restricted to use in food materials since they are a potent source to cause liver damage (Balyan & Sarkar, 2017). Thus, investigation of the antioxidant potential of red dates to prevent chronic disease is worthwhile. In a recent study on southern Morocco dates variety, the results revealed that total phenolic content and antioxidant activity are a promising source for antioxidants (Alahyane et al., 2019). Due to high nutritional and nutraceutical properties, there are limited study reported the optimization of extraction conditions which could result in increasing the yield of the crude extract along with higher TPC and antioxidant activity in red dates. Response surface methodology is also known as RSM optimization along with Box-Behnken Design (BBD) experimental design was commonly used due to its high coherence, lesser runs, and low cost (Anuar et al., 2013; Ali et al., 2018). However, there is limited study on the optimization of extraction conditions of red dates based on multiple responses simultaneously (crude extract yield, TPC yield, and antioxidant activity) (Ngo et al., 2017).

In real-life scenarios, extraction is not preceded immediately after the plant or plant parts are harvested. In such situations, high moisture content can promote microbial contamination, degrade the bioactive compounds, and ultimately results in loss of its antioxidant potential (Toğrul & Pehlivan, 2004; Chua et al., 2019a; Chua et al., 2019b; Choo et al., 2020). Thus, drying is implemented to enhance the quantity and quality of the plant and plant parts for further processing. High temperature may induce degradation of the compounds and low temperature may not have enough energy to remove the moisture to the desired level. Therefore, the oven drying method is more convenient and its drying process is uniform. Besides that, rapid evaporation of water takes place in the oven due to high vaporization. Thus, the present study aimed to identify the appropriate drying temperature that yields high TPC and antioxidants in red dates for future reference. On large scale, solid-liquid extractions are enforced to acquire the crude extract from any medicinal herb. Extraction techniques have evolved from the conventional (maceration, soxhlet, and distillation) to non-conventional (microwave-assisted (MAE), ultrasound-assisted (UAE), supercritical fluid (SFE), pulse electric, enzyme assisted, pressurized liquid, pressurized hot water) extraction techniques. Among all extraction methods, the ultrasound-assisted extraction method (UAE) is the method opted by researchers in terms of simplicity and effectiveness with reduced solvent and energy usage (Ali et al., 2018). Its fundamental benefit lies in its potential to extract and retain the bioactive compounds at a relatively low temperature without compromising the yield with equal effectiveness (Dias et al., 2017).

Mathematical modelling provides a swift and inexpensive method to determine, predict and control the extraction process. Both statistical and empirical approaches are adopted by researchers for the complete conception of the entire process and the significant factors that influence them. From a statistical point of view, response surface methodology (RSM) is implemented to examine and optimize the whole process. When the empirical approach is concerned, kinetic models are utilized to investigate the kinetics of the process (Dias et al., 2017). Response surface methodology (RSM) has been successfully implemented in the previous works to optimize the extraction of antioxidants. The most widely used kinetic models for describing the extraction of antioxidants are the logarithmic model, Peleg's model, second-order rate law, Page's model, Ponomaryov's model, two-site kinetic model, Power-law, Fick's law of diffusion (Ali et al., 2018). As far as our understanding, there remains a major gap involving the preservation and extraction of antioxidants from red dates efficiently and effectively. Thus, it is important to study the drying and extraction processes of red dates which affect the phenolic content and antioxidant activity. Firstly, the current study aimed to identify the best drying temperature of the antioxidants in red dates. Secondly, study was conducted to adopting both statistical and empirical approaches. The goal of prevailing research is also to determine the effectiveness of ultrasound on the extraction of antioxidants from red dates and optimise its extraction conditions via RSM and kinetics modelling. Furthermore, the comparison will be drawn between the optimised red dates extract and synthetic commercial antioxidants (BHT and BHA) to ascertain the antioxidant potential of red dates.

2 Materials and Methods

2.1 Chemical Reagent

The reagents and solvents used for this study were gallic acid, 99.9% ethanol (HPLC grade 99.9%), FC reagent, sodium carbonate (Na_2CO_3), and DPPH (2,2-diphenyl-1-picrylhydrazyl). Chemicals mentioned above are purchased from Evergreen

Engineering & Resources (Evergreen Engineering & Resources Sdn. Bhd., Selangor, Malaysia) and Chemolab (Chemolab Supplies Sdn. Bhd., Selangor, Malaysia).

2.2 Preparation and Pre-Treatment of Red Dates

Red dates (900g) were purchased and thoroughly washed with distilled water before the seeds of the fruits were removed. The initial weight was measured by a digital balance (TX423L, Shimazdu Corporation, Japan). About 50g of red dates were dried using an air-forced convective oven (Model UN75, Memmert, Germany) at different temperatures of 50ºC, 60ºC, 70ºC, and 80ºC until a persist weight was noted. The dried dates were ground to powder with help of a household blender and stored for future use. Subsequent analysis of crude extract yield, total phenolic content, and antioxidant activity (described in subsequent sections) were implemented by the extraction method reported in section 2.3 to identify the optimum pre-treatment temperature before extraction (Bee Lin & Yen Leng, 2018).

2.3 Ultrasound-Assisted Extraction (UAE) and Total Phenolic Content (TPC) Analysis of Red Dates

Ultrasound-assisted extraction of red dates was conducted according to the procedures reported in previous research with slight modifications (Al Harthi et al., 2015; Ali et al., 2017). Powdered red dates were extracted with ethanol (1:15 g/ml) for an hour in an ultrasonic bath (P120H, Elmasonic, Germany). The sonicated water bath functioned at 60°C, 70% input power, and 37 kW of operating frequency. Following the extraction, the mixture was filtered using Whatman filter paper no. 1. The supernatant was evaporated using a rotary vacuum evaporator (Hei-VAP Platinum 3, Heidolph, Germany) at 40°C and 1 bar. The dried crude extract was stored under 4°C for further analysis.

2.4 Quantification of Crude Extract Yield and TPC from Red Dates

The crude extract yield was measured using an analytical balance (HR-250AZ, A&D, Japan) and equation (1). To determine the TPC in the respective extract, 1 g of Folin-Ciocalteu (FC) phenol reagent was mixed with 99.9 ml of deionized water to prepare 10% (v/v) of Folin-Ciocalteu phenol reagent solution (Qu et al., 2010). Further, 7% of sodium carbonate (Na_2CO_3) solution was prepared in the same manner. Samples were developed by dissolving 1 g of crude extract in 15 ml of distilled water. To the above extract, 5 ml of 10% FC reagent, 4 ml of 7% Na_2CO_3 solution, and sample extracts were all mixed and kept at room temperature (26ºC) for an hour. About 0.2 ml of the resulting solution was poured into 96-well plate and the absorbance was noted via microplate spectrophotometer at 765 (Epoch 2, BioTek, USA). Pure ethanol was used as a blank solution. TPC in red dates extract was determined regarding the gallic acid calibration curve ($r^2 = 0.9792$) that was procured similarly. The experiments were performed in triplicates and denoted as g gallic acid equivalent/g of dried weight extract ± standard deviation.

$$\text{Crude extract yield} = \frac{W_c}{W_d} \times 100\% \quad (1)$$

Where W_c and W_d are the weight of the crude extract and red date powder sample in grams, respectively.

2.5 Determination of the Antioxidant Activity of Red Dates

To determine the antioxidant potential of red dates, DPPH analysis was used with slight modifications (Qu et al., 2010). About 270 µl of 0.1 mM DPPH solution in ethanol was added to 30 µl of sample extract and the resulting mixture was kept in dark for about 30 min. Later, 0.2 ml of the same mixture was transferred into a 96-well plate and the absorbance of the samples was read by microplate spectrophotometer at 515 nm. The antioxidant activity was calculated using Equation. (2). Each procedure was repeated thrice and values were expressed as % activity ± standard deviation.

$$\text{Scavenging activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100\% \quad (2)$$

Where $Abs_{control}$ is the absorbance of the control sample and Abs_{sample} is the absorbance of the extracted sample (Chua et al., 2019a; Choo et al., 2020).

2.6 Identification of Best Drying Temperature and Response Surface Methodology (RSM) Optimisation

The drying of red dates and extraction procedures were described in sections 2.2 and 2.3. Four different drying temperatures with a 10°C interval (50 to 80°C) were studied. Single-factor experiments were performed based on the selected drying temperature to investigate their impact on three targeted responses (crude extract yield, TPC, and antioxidant activity). The preliminary response surface methodology (RSM) operating ranges of the extraction parameters were identified and validated from single-factor experiments (Kang et al., 2019; Choo et al., 2020).

$$Y = \beta_o + \sum^{k} \beta_i x_i + \sum^{k} \beta_{ii} x_i^2 + \sum_i \sum^{k} \beta_{ij} x_i x_j \quad (3)$$

2.7 Optimisation of Extraction Conditions and Validations of Optimum Extraction Conditions

A four-factor, three-level Box-Behnken experimental Design (BBD) with a total of 28 runs was adopted to optimize the

ultrasound-assisted extraction of antioxidants from red dates (Qu et al., 2010) using Design Expert Software Ver. 7.0 (Stat-Ease Inc, Minneapolis, USA). The three responses for optimization, namely crude extract yield (y_1), TPC (y_2), and DPPH scavenging activity (y_3) were fitted to a second-order polynomial regression equation Eq. (3).

Where Y is the response variable, and X_i and X_j are the independent variables (i and j range from 1 to k), β_o is a constant, β_i, β_{ii}, and β_{ij} are the regression coefficients of linear, quadratic, and interactive terms respectively, k is the number of parameters (4 for present research).

Table 2 shows the BBD runs, experimental and predicted responses which are average of triplicate experiments ± standard deviation. Analysis of variance (ANOVA) was used to identify the significance ($\rho < 0.05$) of the developed models as well as the extraction parameters. The optimum conditions were validated with additional experiments and the differences between the experimental and predicted results of the responses (y_1, y_2, and y_3) were calculated using Eq. (4).

$$\text{Difference (\%)} = \frac{|y_{\text{experimental}} - y_{\text{predicted}}|}{y_{\text{experimental}}} \times 100\% \qquad (4)$$

2.8 Extraction Kinetic Modelling of Red Dates and Validation

The kinetic models of interest for the present study were Page's model, first-order, second-order, Peleg's model, Logarithmic model, and Ponomaryov's model kinetic model. All six models were used to elucidate the extraction parameters of TPC (Table 1). The kinetic models were solved and the statistical coefficient of R^2 and RMSE were determined using Microsoft excel solver (Microsoft Excel 2010, USA) and Matlab curve fitting tool (The MathWorks Inc., USA). The final results of the best-fitted kinetic model was obtained by comparing the experimental and predicted values of TPC under controlled conditions for three different extraction periods of 45, 65, and 85 min.

Table 1 Popular Extraction Kinetic Models for Plants Model

Name	Equation	Range of Constants	Applications	Reference
Peleg's	$C(t) = \mathrm{C}_0 + \frac{t}{\mathrm{K}_1 + \mathrm{K}_2 t}$	K_1: 0.063-4.414 K_2: 0.025-0.039	*Asteraceae* plants, *Vitis vinifera*, pomegranate marc, Soybeans	Jokic et al., 2010; Qu et al., 2010
Page's	$C(t) = -e^{-\mathrm{k}t^{\mathrm{n}}}$	k: -2.737-(-0.297) n: 0.055-0.481	*Asteraceae* plants, *Theobroma cacao*, soybeans	Jokic et al., 2010; Hii et al., 2009
Ponomaryov's	$1 - \frac{q_i}{q_e} = \mathrm{b} + \mathrm{k}_1 t$	b: 0.754-0.773 k_3: 0.00076-0.00103	Pomegranate marc, barley	Qu et al., 2010; Paunović et al., 2014
First Order	$\mathrm{C}_\mathrm{t} = \mathrm{C}_\mathrm{e}(1 - \mathrm{e}^{-\mathrm{k}_1 \mathrm{t}})$	k_1: 0.00794-0.187	Jamun, *Tilia* sapwood	Balyan & Sarkar, 2017; Harouna-Oumarou et al., 2007
Second Order	$C_t = \frac{C_e^2 \mathrm{k} t}{1 + C_e \mathrm{k} t}$	k_2: 0.115-6.314	Pomegranate marc, *Asteraceae* plants	Qu et al., 2010
Logarithmic	$C(t) = \mathrm{a} \cdot \log t + \mathrm{b}$	a: 2.466-6.3379 b:-3.277-14.447	*Asteraceae* plants, soybeans	Guerrero et al., 2008

Here $C(t)$: Concentration of TPC at time t, C_0: Initial concentration of TPC, C_e: Equilibrium concentration of TPC in extract, K_1 : Peleg's rate constant (g_{DM} mg_{GAE}^{-1}), K_2: Peleg's capacity constant (g_{DM} mg_{GAE}^{-1}), k,n: Page's constants, q_i: Concentration of TPC in the plants during extraction, q_e: Equilibrium concentration of TPC in the plants, b: Washing coefficient, k_3: Slow extraction coefficient (min^{-1}), k_1: first order rate constant (min^{-1}); k_2: second order rate constant (g_{powder}/mg min), a, b: Logarithmic constants

3 Results and Discussion

3.1 Impact of Drying Temperature on Antioxidant Activity of Red Dates

The impact of four different drying temperatures on red dates was investigated based on crude extract yield, TPC, and antioxidant activity (Table 2). The results portrayed at 60°C had the highest yield (4.56 g), TPC (0.023 g GAE/g extract), and DPPH antioxidant activity (85.88% activity) among the four temperatures investigated. The heat energy produced at this temperature was sufficient to disintegrate the bonds linking phenolic compounds and the insoluble fibres of red dates (Qu et al., 2010).

Thus, the resulting antioxidant activity was improved with increased phenolic content extraction at 60°C. An incremental decrease in the results was observed beyond 60°C drying temperature. This might due to the thermal degradation of TPC that occurred at temperatures above 70°C. The loss of TPC decreased the antioxidant activity of red dates. The current findings are consistent with previous reports (López-Vidaña et al., 2016). Thus, a drying temperature of 60°C was used to dry the red dates for further extraction stages.

Table 2 Effects of Drying Temperature on Antioxidant Activity, TPC and Crude Extract Yield

Temperature (°C)	Crude extract yield (g)	TPC yield (g GAE/g extract)	Antioxidant activity (%)
50	4.44±0.067	0.022±0.00006	85.18±0.98
60	4.56±0.089	0.023±0.00009	85.88±1.24
70	4.41±0.022	0.022±0.00004	84.71±0.75
80	4.37±0.038	0.021±0.00001	84.47±0.42

3.2 Single-Factor Experimental Design

Preliminary single factor experimental design was conducted to identify the response surface methodology (RSM) ranges for four extraction factors: liquid-solid ratio, extraction temperature, time, and ultrasonic power. The enactment of each factor on crude extract yield, TPC, and antioxidant activity are detailed in section 3.2.1 to 3.2.4 and the respective graphs are provided in online resource (Bee Lin & Yen Leng, 2018).

3.2.1 Impact of Solid-Liquid Ratio on Crude Extract Yield, TPC and Antioxidant Activity

The solid-liquid ratio is a significant factor that affects the crude extract yield, TPC, and antioxidant activity. Based on the results, the extract yield increased with a gradual rise in the ratio. The maximum extract yield of 1.905 g was achieved at the highest amount of 30 ml/g. At high ratios, more solvent was present in the extracting solution creating a higher concentration gradient that allowed the larger mass transfer of the system (Ali et al., 2018).

Moreover, the rate of diffusion was high because of the increase in the surface contact area between solvent and sample. TPC and DPPH antioxidant activity followed the trend of extract yield where the highest values of 0.0277 g GAE/ g extract and 91.69% antioxidant activity were also obtained at 30 ml/g. Therefore, the optimum range of the liquid-solid ratio used for optimization was 10, 20, and 30 ml/g. Similar findings were reported in a recent study (Alahyane et al., 2019). Hence, from the recent and current study, it is more evident that dates are loaded with high antioxidants.

3.2.2 Impact of Ultrasonic Power on Crude Extract Yield, TPC and Antioxidant Activity

Based on the results, all three outcomes increased with an increase in ultrasonic power. These outcomes might be caused by the formation and collapse of cavitation bubbles which was directly proportional to the amplitude of ultrasonic waves that passed through the medium (Kong et al., 2015). The larger frequency at high ultrasonication power led to intensified cavitation and the resulting shear stress caused by microstreaming from cavitation disrupted the cell wall. This mechanism accelerated the penetration of the solvent and released the antioxidants into and from red dates (Chemat et al., 2017). The highest yield (1.785%), TPC (0.0198 g GAE/ g extract), and DPPH antioxidant activity (86.1176% activity) of red dates were noted at 90% ultrasound power. At the same time, it was also noted that the results showed a drastic

decrease after 90% ultrasound power in all three aspects. The undesired outcomes might be due to the disintegration of the bioactive compounds and subsequently degraded its antioxidant potential. Previous studies had shown that high ultrasonication power increased the temperature of the extracting solvent which in turn degraded the thermos sensitive compounds and ultimately caused it to lose its properties (Zhou et al., 2017). Thus, the optimized ultrasonic power range was 70, 80, and 90%.

3.2.3 Impact of Extraction Temperature on Crude Extract Yield, TPC and Antioxidant Activity

Temperature rise rapidly favoured the extraction process, particularly TPC and DPPH antioxidant activity. The increasing temperature reduced the solvent viscosity and surface tension. This positively enhanced the cavitation mechanism (Qu et al., 2010). As a result, the collapse of the cavitational bubbles occurred and augmented the release of antioxidants from within. Furthermore, the swelling and loosening effect resulted from the thermal effect at high temperature might increase the diffusivity of the solvent into the red dates' matrix and improved the mass transfer of the system (Moorthy et al., 2017). The best results of extraction yield (1.805 g), TPC (0.0232 g GAE/g extract), and DPPH antioxidant activity (85.333% activity) were achieved at 60°C.

It is also known that high temperatures may also result in degradation of the phenolic content as observed during the experiments of ultrasound power. Similar results were also noted as temperature beyond 60°C reduced all three outcomes. Thus, it can be said with certainty that the phenolic content in red dates is thermos-sensitive and attention must be paid to this variable during the extraction process. Considering all factors, the appropriate temperature range of extraction for optimization was 50, 60, and 70°C.

3.2.4 Impact of Extraction Time on Crude Extract Yield, TPC and Antioxidant Activity

A positive effect on extraction time was observed on all three results. The best outputs of extraction yield (2.007 g), TPC (0.0225 g GAE/g extract), and DPPH antioxidant activity (80.7843% activity) were obtained at 50 min extraction duration. The fibrous structure of red dates would require a certain amount of time for effective extraction of the antioxidants. The initial increase in the outcomes could be attributed to the straightforward mechanism of ultrasound that rendered the plant cells during 50 min for effective extraction to take place. The results began to decline past this point could be due to several reasons. Firstly, the loss of the phenolic compounds that led to the degradation of its antioxidant potential might be caused by the overexposure of ultrasound waves and excess heating over time. The degradation of antioxidant activity is due to the structural disintegration of phenolic compounds (Alzorqi et al., 2017). Secondly, the extraction of insoluble impurities due to the rupture of the red date's cell wall might lower the solvent's permeability into the fruit's matrix (Qu et al., 2010). From the results obtained, the selected optimum time range of extraction for RSM optimization was 40 to 60 min.

3.3 RSM Optimisation

Adopting the results of the single-factor experiments, antioxidant extraction from red dates were optimized for the principal parameters of solid-liquid ratio (A), ultrasonic power (B), extraction temperature (C), and extraction time (D) following the BBD experimental design. BBD design with the responses of crude extract yield, TPC, and DPPH antioxidant activity obtained for each run is presented in Table 3.

The data of yield, TPC, and antioxidant activity ranged in between 0.845 and 2.175 g, 0.0155 and 0.032 g GAE/g extract, 67.97 and 96.36% activity, respectively. Since the primary goal of this present research is to escalate the antioxidant capacity of the red dates, optimization was held only by focusing on the results of DPPH antioxidant with the highest desirability throughout numerical optimization.

3.3.1 Second-Order Polynomial Regression Model Fitting

Three different quadratic models were developed for three responses using multiple regression analysis. ANOVA was adopted to analyze the variables and the RSM models where significance was considered when $\rho < 0.05$.

Additionally, F-test was also conducted to further verify the importance of the variables statistically. The final developed models excluding the non-significant terms of each response were given in Eqs. (4), (5), and (6). Overall, the developed models for crude extract yield, TPC and DPPH antioxidant activity were found to be significant with F-values of 5.08 ($\rho = 0.0029$), 5.78 ($\rho = 0.0016$) and 8.83 ($\rho = 0.0002$), respectively. Additionally, the correlation coefficient (R^2) of the three models were found to be 0.8455, 0.8042, and 0.8840 showing a strong correlation between the experimental and predicted responses (Moorthy et al., 2017).

The adjusted correlation coefficients (R^2) for all three models were determined to be 0.7412, 0.7670, and 0.8352, respectively. Besides, the absence of fit was not statistically significant in all instances (Xu et al., 2017). Coefficient of variance (CV) less than 10% and adequate precision greater than 4 was highly desirable (Ali et al., 2018). CV for crude extract yield (7.86), TPC (8.56), and antioxidant activity (4.05) together with adequate precision for crude extract yield (16.4715), TPC (14.4323), and antioxidant activity (14.9205) solidified the validity of the developed models.

Crude extract yield= -19.0265+0.0323A+0.3460C+0.3526 D-0.00275CD-0.001555C^2-0.001743D^2 (4)

$TPC = 0.028 - 0.002498C + 0.001725D - 0.0026BC - 0.0066C^2$ (5)

DPPH antioxidant activity= -472.8267+1.02092A+1.78563I +13.76460C+2.28608D+0.032353BC-0.031961CD- 0.024037B^2 − 0.125086C^2 (6)

The results of ANOVA revealed some quite interesting outputs as it would seem ultrasound power did not have any statistically significant influence on the responses except for antioxidant activity. Furthermore, its negative quadratic effect can be observed for the antioxidant activity only. On the other hand, extraction temperature and time had a high significantly positive linear as well as negative quadratic influence on all three responses. Total phenolic content was not influenced by the liquid-solid ratio, however, this variable had a significant positive linear influence on both crude extract yield and DPPH antioxidant activity. The interactivity between ultrasound power and temperature had both positive and negative effects on TPC and antioxidant activity, respectively. Lastly, the interaction between time and temperature have a significant negative influence on crude extract yield.

In other words, it can be said with certainty that the two factors that majorly influenced the extraction of antioxidants from red dates are none other than temperature and time. The 3D response plots graphically showing the effects of the independent variables on all three responses are provided in Figure 1.

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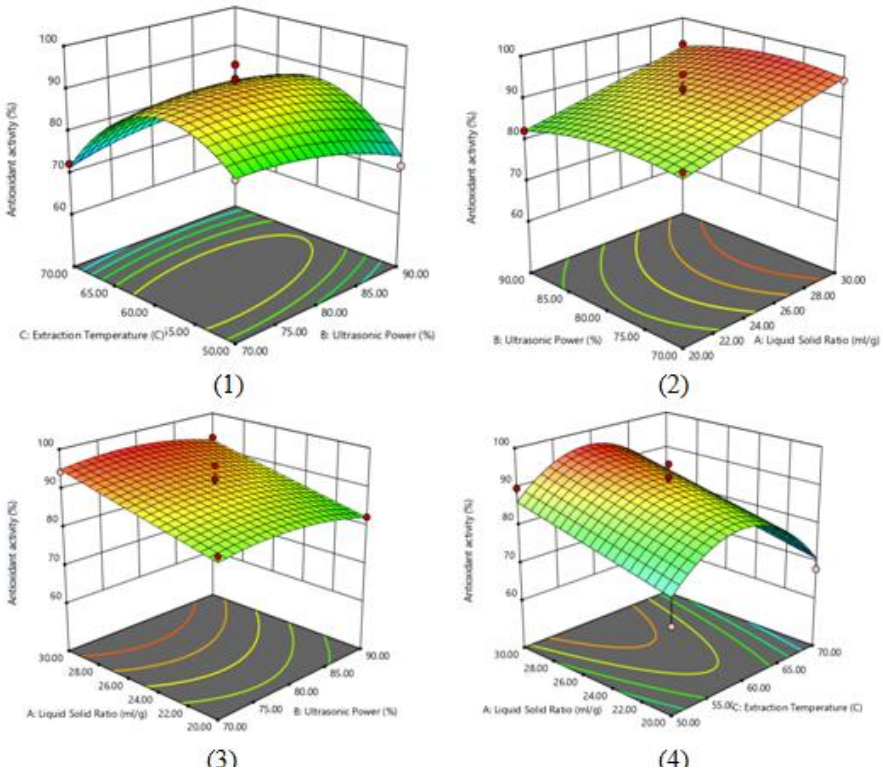
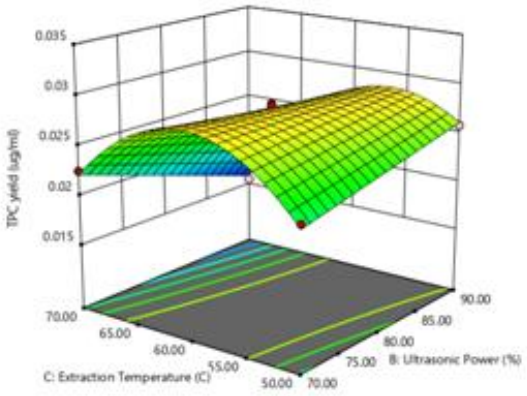
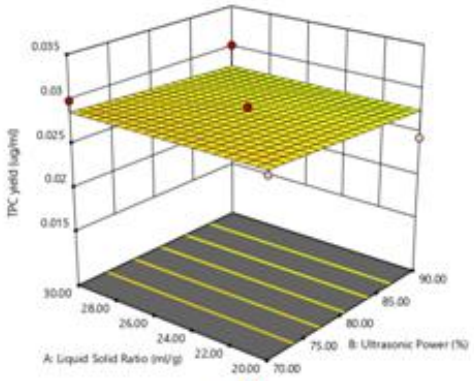
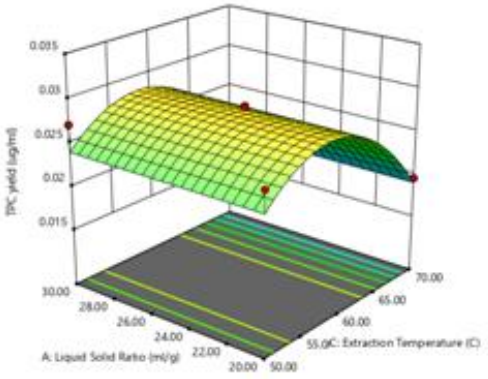



TPC yield (ug/ml)
0.035
0.03
0.025
0.02
0.015
70.00
65.00
60.00
55.00
50.00
C: Extraction Temperature (C)
90.00
85.00
80.00
75.00
70.00
B: Ultrasonic Power (%)

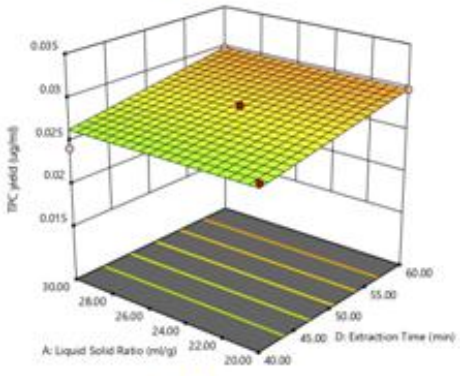
(5)


TPC yield (ug/ml)
0.035
0.03
0.025
0.02
0.015
30.00
28.00
26.00
24.00
22.00
20.00
A: Liquid Solid Ratio (ml/g)
90.00
85.00
80.00
75.00
70.00
B: Ultrasonic Power (%)

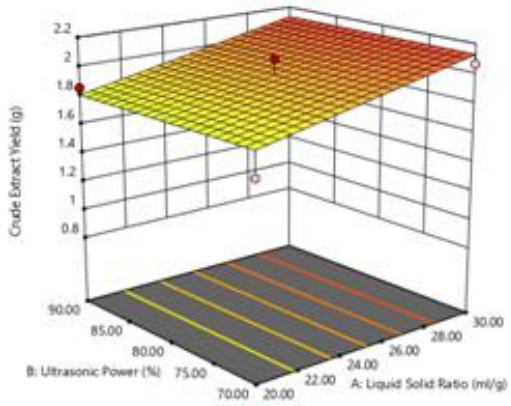
(6)


TPC yield (ug/ml)
0.035
0.03
0.025
0.02
0.015
30.00
28.00
26.00
24.00
22.00
20.00
A: Liquid Solid Ratio (ml/g)
70.00
65.00
60.00
55.00
50.00
C: Extraction Temperature (C)

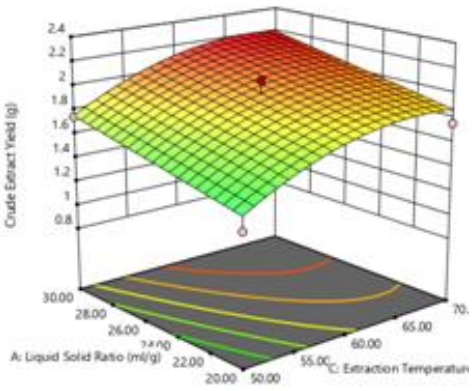
(7)


TPC yield (ug/ml)
0.035
0.03
0.025
0.02
0.015
30.00
28.00
26.00
24.00
22.00
20.00
A: Liquid Solid Ratio (ml/g)
60.00
55.00
50.00
45.00
40.00
D: Extraction Time (min)

(8)


Crude Extract Yield (g)
2.2
2
1.8
1.6
1.4
1.2
1
0.8
90.00
85.00
80.00
75.00
70.00
B: Ultrasonic Power (%)
30.00
28.00
26.00
24.00
22.00
20.00
A: Liquid Solid Ratio (ml/g)

(9)


Crude Extract Yield (g)
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
30.00
28.00
26.00
22.00
20.00
A: Liquid Solid Ratio (ml/g)
65.00
60.00
55.00
50.00
C: Extraction Temperatu

(10)

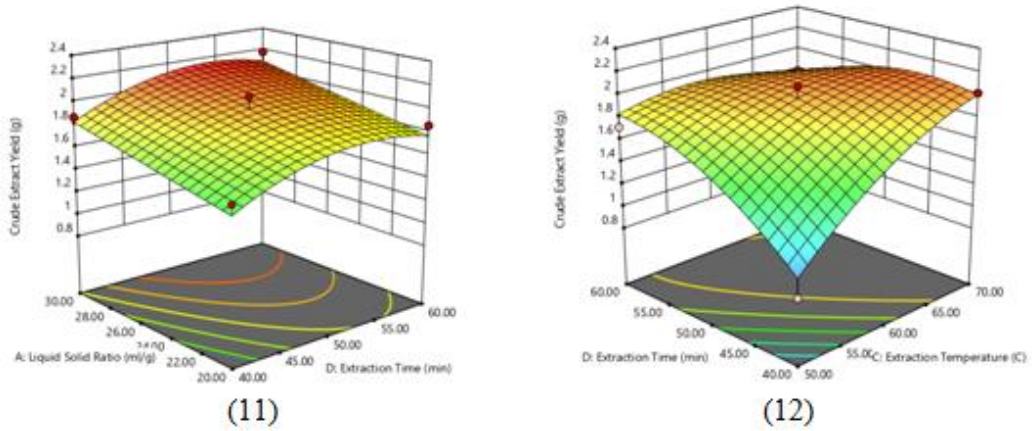


Figure 1 3D Response Plots of the Effect of Extraction Variables on (1-4) DPPH Antioxidant Activity, (5-8) TPC of Red Dates and (9-12) the Crude Extract Yield of Red Dates

3.3.2 Numerical Optimisation and Validation

Numerical optimization was adopted to optimize the independent extraction variables that displayed the highest antioxidant activity of red dates based on the three developed models. The optimum extraction condition was found to be at 58.55°C with power, ratio, and time of 70%, 30 ml/g, and 59.93 min. Further, the validity of models was tested using validation experiments. The experimental value of DPPH antioxidant activity under optimum condition was 90.59% whereas the predicted response was 98.24%. Since the difference between experimental and prediction antioxidant value was 8.44%, validation of the data was executed.

Besides, the RSM-optimised antioxidant activity of red dates was compared with two commercial synthetic antioxidants to validate their potential. Both BHT (84.71%) and BHA (77.73%) showed a significantly lesser antioxidant activity than red dates (90.59%). Thus, verifying as a strong alternate source of natural antioxidant in future industrial applications.

3.4 Comparison of Extraction Kinetic Models

The kinetic extraction process was investigated from the empirical model data of TPC obtained under the optimized condition. During the single factor study, it was observed that the trend of TPC followed that of the antioxidant activity. Therefore, the data of TPC was utilized during the kinetic study. The respective kinetic constants for each model are listed in Table 4. The first and second-order models were derived from Fick's diffusion laws, whereas, Page, Peleg, and Logarithmic models were derived based on moisture sorption curves (Cacace & Mazza, 2003).

Table 4 Difference between Experimental and Predicted TPC Yield (*RSME)*

Extraction time (min)	TPC yield (g GAE/g extract)		Difference (%)
	Experimental	Predicted	
45	0.0253±0.0002	0.0241	5.03
65	0.0250±0.0004	0.0261	4.51
85	0.0257±0.0002	0.0273	6.47

The validation of kinetic models was done by statistical correlations: R^2 (coefficient) and *RMSE* (root mean square error). The fittings of the mentioned models to the experimental extraction data of TPC were in the order of Second-order kinetic model > first-order kinetic model > Logarithmic model > Page's model > Peleg's model > Ponomaryov model. The second-order model was found to be the best to distinguish the extraction of antioxidants from red dates provided it had R^2 (0.9849) closest to unity and least RMSE (0.001028). The kinetic graphs of all of the models are presented in Figure 2.

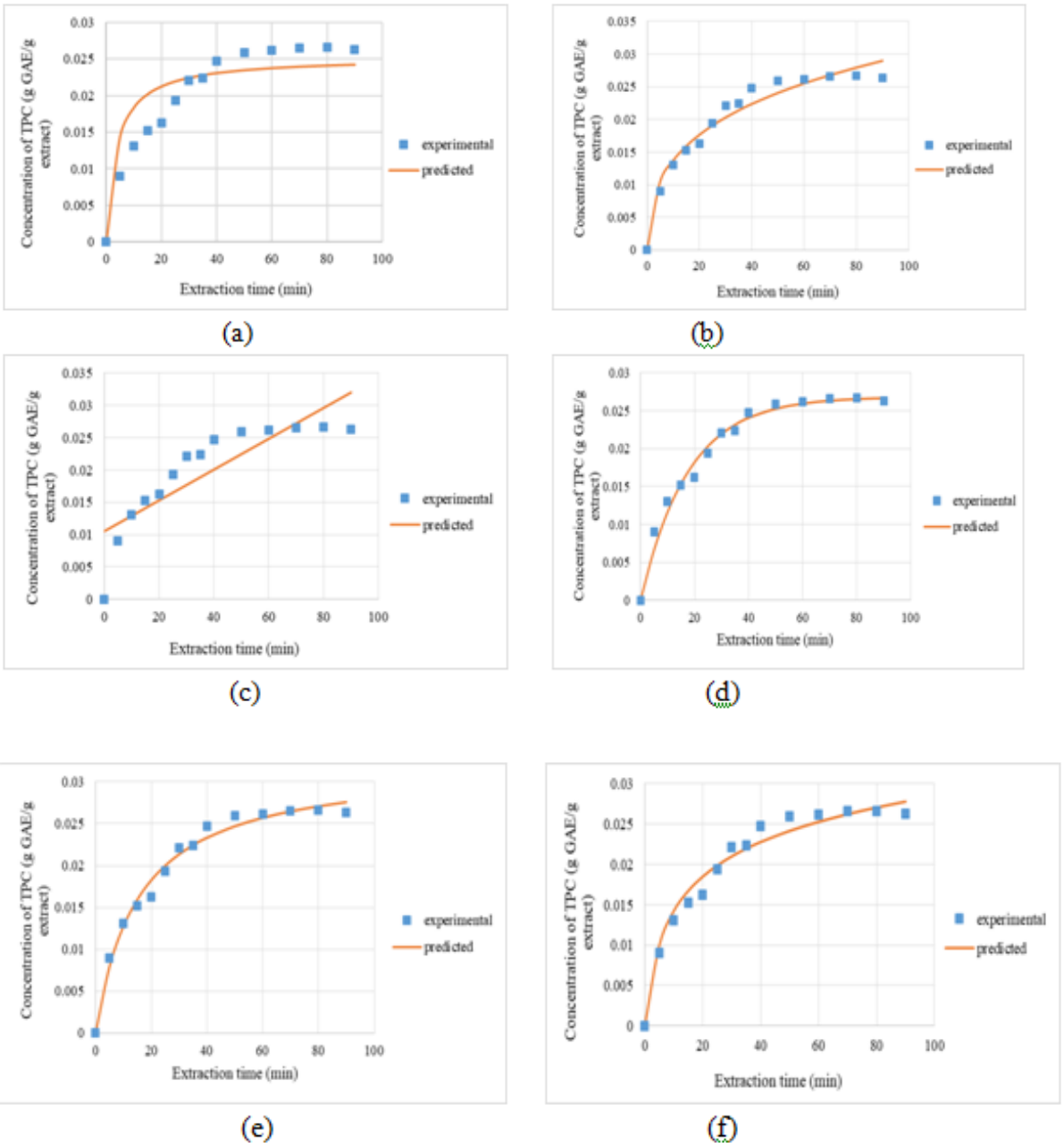


Figure 2 Kinetic Curves of Experimental and Predicted TPC of Red Dates (a) Peleg's Model (b) Page's Model (c) Ponomaryov's Model (d) First-Order Kinetic Model (e) Second-Order Kinetic Model (f) Logarithmic Model

3.4.1 Validation of Extraction Kinetic Model

Further endorsement of the most accurate kinetic extraction model was finalized to ensure its suitability and reliability by comparing the experimental with the predicted data of TPC under optimum extraction conditions for three different extraction periods of 45, 65, and 85 min (Table 5). Respective Kinetic Variables of Six Empirical Models showed that the differences between two sets of data at 45, 65, and 85 min were 5.03%, 4.51%, and 6.47%. Therefore, the second-order kinetic model was selected to predict the extraction TPC of red dates at different extraction periods.

Table 5 Respective Kinetic Variables of Six Empirical Models

Model name	Calculated model constants	R^2	*RMSE*
Peleg's	k_1 = 148.1 (min $g_{extract}$/g GAE) k_2 = 39.65 ($g_{extract}$/g GAE)	0.824	0.003506
Page's	k = 5.241 n = -0.08715	0.9663	0.001534
Ponomaryov	k_3 = 0.02623 (min^{-1}) b = 2.157 q_e = -0.009102 ($g_{extract}$/g GAE)	0.7160	0.004652
First-order	k_1 = 0.0568 (min^{-1}) C_e = 0.02684 ($g_{extract}$/g GAE)	0.9826	0.01103
Second-order	k_2 = 2.017 ($g_{extract}$/min g GAE) C_e = 0.03228 ($g_{extract}$/g GAE)	0.9849	0.001028
Logarithmic	a = 0.01422 b = 0	0.9740	0.0015

Conclusion

In this research, the preservation and extraction of natural antioxidants from red dates were investigated through optimum drying temperature, extraction optimization, and kinetic studies. An oven drying temperature of 60°C was found to be the optimum temperature that produced the highest crude extract yield, TPC, and DPPH antioxidant activity. The four-factor, three-level BBD design of RSM was used to optimize the UAE criteria for the extraction of antioxidants for parameters of solid-liquid ratio, temperature, ultrasound power, and time. The optimal extraction conditions were noted at 58.55oC with power, ratio, and time of 70%, 30 ml/g, and 59.93 min respectively. The high correlation coefficient (R^2) confirmed the validity of the developed second-order regression models for the responses of crude extract yield (0.8455), TPC (0.8042), and DPPH antioxidant activity (0.8120). The difference of DPPH antioxidant activity being less than 10% between the predicted (98.24%) and actual (90.59%) results obtained under the optimum extraction conditions further confirmed the validity of the proposed models. Additional comparisons with commercial synthetic antioxidants of BHT (84.71%) and BHA (77.73%) with optimized red dates (90.59%) solidified its antioxidant potential. Based on R^2 (0.9849) and RMSE (0.001028) value, kinetic studies revealed second-order model was the finest in describing the extraction of antioxidants from red dates. Overall, it can be concluded that red dates extract possesses strong antioxidant properties and can be taken into consideration as an alternate supply of potential herbal antioxidants in both food and pharma industries.

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FORMULATION OF SELECTED SOY PRODUCTS FOR WOMEN'S HEALTH

Anitha C[1*], Vasantha Esther Rani[2]

[1]Department of Home Science, Seethalakshmi Ramaswami College (Autonomous), Tiruchirappalli, Tamilnadu, India
[2]Research Department of Home Science, Fatima College ((Autonomous), Madurai, Tamilnadu, India

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ABSTRACT

Soybean differs from other cereals and legumes by containing the highest amount of complete protein. Tofu, soy milk, soy meat are nonfermented products of soybean. Soy contains chemical compounds are unique because of its high concentration of isoflavones, a type of plant estrogen. Women entering the menopausal stage are recommended to consume soya bean because this plant estrogen would benefit them. The study aims to inculcate the consumption of soy products through standardized recipes. The popular soy milk product is Tofu. It is prepared by curdling fresh hot soy milk with a coagulant. On milling, soybean yielded a nutritious product called Soy flour, which is available in two types namely full-fat soy flour (FFSF) and defatted soy flour (DFSF). When the soybean oil is extracted, the by-product called Soy Chunks is produced. In current study, soy products namely Tofu, Defatted Soy Flour, and Soy Chunks were used to standardize few recipes by incorporating them in commonly consumed recipes or using them wholly. Further, for this, Tofu with pasta and sandwich, Chunks in kurma and nuggets, DFSF with besan omelette, and cakes were incorporated and standardized. Along with this, organoleptic evaluations were carried out for the developed products and were standardized. The nutritive values for DFSF and storage stability of standardized cakes were also carried out.

* Corresponding author
E-mail: ceeanitha4@gmail.com (C. Anitha)

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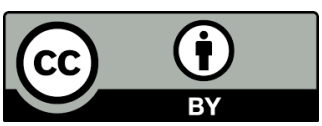

1 Introduction

Soybean (*Glycine max*) is known to be the world's widely cultivated and economically successful legume. Soybean is known as the "Golden bean", "Miracle crop", "Vegetable meat", and the "Cinderella crop" of the west because of its several uses. Soybean differs from other cereals and legumes by containing the highest amount of protein. Soybean has been known for long as versatile plant food that provides high-quality protein with only minimal saturated fat. Soybean is considered to be a good dietary source of protein (Singh et al., 2008). It is the only vegetarian food that contains all essential amino acids in the amounts needed for human health. It contains all the three-macro nutrients namely carbohydrate, protein, and fat required for good health and other nutrients like vitamins and minerals including calcium, folic acid, and iron. The soybean plant contains complete protein. Apart from the vegetable use of soybean, it is used by the oil industry and ranks first place in oil production. Soybean containing 43 percent protein and 20 percent oil has tremendous potential in the management of protein-calorie malnutrition among the ever increasing Indian population. Soy based food products are also suitable for diabetic patients as they contain less carbohydrate and low fat (Sharma et al., 2018). Soy protein is also good for people who are allergic to animal protein. Therefore, it is one of the most economical protein sources in the world for especially for those with lactose intolerance. Soy foods have several health benefits such as cancer prevention, cholesterol reduction, combating osteoporosis, and menopause regulation (Pabich & Materska, 2019).

Soybean is rightly praised as being a treasure trove of many nutrients. In current day to day life, soy can be used in many ways such as Soybean, Soybean oil, Soy milk, Soy flour, Soy paneer (Tofu), Soy pappad, Soy nuts, Soy chocolates, and Soy chunks. Traditional soy foods are classified into two categories: non-fermented and fermented. The non-fermented soybean products are soy milk, tofu, tofu skin, soy meat alternatives. The fermented products include soy sauce, fermented bean paste, natto, sufu, miso, onchom, and tempeh (Liu, 2008; Singh, 2019). Tofu, a staple food in Asia for 2000 years, is known for its extraordinary nutritional benefits (Pal et al., 2019). Tofu is more popular among all the soy milk products. Tofu is prepared by coagulating the soy proteins in soy milk. It is also known as bean curd. The curd is processed and whey is discarded. The curd is an excellent source of calcium, iron, and a good source of protein (Oboh et al., 2007). Soy flour is an excellent source of protein, iron, and calcium. Defatted soy flour is also a good source of fiber. Defatted soy flour is a common form in which soybean can be incorporated in various food preparations. The soybean meal can be used to manufacture a product with meat like texture, known as textured vegetable protein. The textured vegetable protein is similar to cooked meat; hence it can be frozen, canned, or dehydrated like meat. It proved to be a fortune crop in India, in terms of edible oil production, export earnings, and rural prosperity.

Soybean contains phytosterols which are structurally similar to cholesterol and steroid hormones, which inhibit cholesterol absorption by blocking the absorption sites. Soy contains different types of chemicals such as phytoestrogens and isoflavones. Soy isoflavones are daidzein and genistein. Women entering menopause are recommended to consume soy because of the phytoestrogen content. As the natural production of oestrogen stops and symptoms may start. The phytoestrogens act as a weak oestrogen and they may help to relieve symptoms by boosting their levels (Desmawati & Sulastri, 2019). Phytic acid content is high in soybean, which acts as an antioxidant and a chelating agent. Generally, people are aware of milk and its products but not soya and its products. Hence there is a need to create awareness and utilize these value added products. In this regard, in the current study selected soy products were used to standardize few recipes that can be applied in day-to-day cooking for consumption. These recipes aids in better health in the woman. Suitable recipes for each soy product namely Tofu, Defatted soy flour, and chunks were formulated. The study aims to enhance soy product consumption through normal diets for better health. Various recipes were formulated with soy products to ensure soy consumption at least in a meal.

2 Materials and Methods

2.1 Collection of the sample

Tofu, Defatted soy flour, and soy chunks were selected for the research purpose and they were collected from Sivakasi and Chennai, Tamilnadu, India. The other raw ingredients were collected from the local market. Analytical grade reagents and laboratory grade reagents were used for the biochemical analysis of the sample.

Soy milk is extracted from soybean by soaking 1kg of soybean overnight and washed well before grinding. Then it was ground to a fine paste with water and milk is strained with a nut milk bag. Seven liters of soy milk was obtained from 1kg of soybean. The soy milk was boiled and two grams of citric acid was added for every one liter of soy milk which means 14 grams of citric was used to ferment. The curdled part is filtered from soy whey water and washed with water. They were shaped and left pressed for 30 minutes and then cut into cakesto obtain tofu. The tofu was vaccum packed immediately. This tofu was used for product standardization whereas defatted soy flour and soy chunks were procured from the local market.

2.2 Tofu products

2.2.1 Tofu with pasta

2.2.1.1 Ingredients

Tofu - 25 gm; Pasta - 100 gm; Onion - 10 gm; Peas - 20 gm, French beans - 20 gm; Carrot - 20 gm; Green chilli - 5 gm; Oil – ½ Tsp.; Aniseeds – ¼ Tsp; Spices - 1 number each (Cardamom, cloves, cinnamon, star anise)

2.2.1.2 Method

The pasta was precooked with excess water and strained. The Tofu was cut into cubes, the peas shelled, and french beans and carrots were chopped. In a saucepan, oil was heated, seasoned with aniseeds and spices, chopped onion and green chillies were added and sautéed it for few minutes. To this, vegetables and Tofu were added the cooked with the required salt and water. This was followed by the addition of pasta was stirred. Tofu with pasta was ready. It was then subjected to organoleptic evaluation.

2.2.2 Tofu sandwich

2.2.2.1 Ingredients

Tofu - 50gm; Onion - 50 gm; Tomato - 50 gm; Green Chilli - 5 gm; Oil - 1 Tsp.; Pepper powder - 1 Tsp; Tomato ketchup - 1 Tbsp; Butter - 1 Tbsp; Cheese slices - 1 (Optional); Salt - To taste ; Bread - 4 slices.

2.2.2.2 Method

The tofu was shredded and kept aside. Onion, tomato, green chillies were finely chopped. Oil was heated in a saucepan, sautéed the chopped ingredients, pepper powder, and salt were added to the required taste. Finally, shredded tofu was added, garnished with coriander leaves, the stuffing was ready. The bread slices were greased with butter and tomato ketchup, the stuffing was spread, covered with another slice, and was toasted.

2.3 Soy chunks products

2.3.1 Soy Nuggets

2.3.1.1 Ingredients

Soy Chunks - 40 gm; Potato - 60 gm; Ginger Garlic paste – 4 gm; Red Chilli Powder - 4 gm; Garam Masala - 1 gm; Salt - As required; Corn Flour - 5 gm; Bread Crumps - 10 gm; Oil - To deep fry.

2.3.1.2 Method

Soy chunks were precooked for three to five minutes in boiling water and strained. They were then squeezed out to remove water and ground to crumbs. Potatoes were boiled, de-skinned, and mashed. Potatoes and soy chunk crumbs were mixed with the required amount of salt, chilli powder, and garam masala. They were rolled to balls and rolled in cornflour and bread crumbs and deep fried. It was then subjected to organoleptic evaluation.

2.3.2 Soy chunks kurma

2.3.2.1 Ingredients

Soy Chunks - 50 gm; Shallots - 15 gm; Garlic - 2 Pods; Tomato - 25 gm; Aniseeds - 2 gm; Jeera - 5 gm; Pepper - 15 gm; Coriander Seeds - 15 gm; Coconut - 15 gm; Green Chillies - 5 gm; Curry leaves - A sprig; Coriander leaves - To garnish.

2.3.2.2 Method

Soy chunks were precooked for three to five minutes in boiling water and strained. Aniseeds, jeera, pepper, coriander seeds, coconut, and green chillies were sautéed in oil and ground to a paste. In a sauce pan, oil was heated, chopped shallots, tomatoes, garlic were sautéed and curry leaves were added. The ground paste, turmeric powder, and salt were added, boiled with precooked chunks. Garnished with coriander leaves and served. It was then subjected to organoleptic evaluation.

2.4 Defatted soy flour incorporated products

2.4.1 DFSF omelette

2.4.1.1 Ingredients

Besan flour - 50 gm; DFSF - 50 gm; Onion - 50 gm; Green chilli - 5 gm; Chilli Powder - 5 gm; Turmeric Powder - 3 gm; Asafoetida - a pinch; Coriander leaves - 5 gm; Oil - 2 tsp; Water and Salt - as required.

2.4.1.2 Method

Besan flour and DFSF were made into pouring consistency with water and turmeric powder, chilli powder, asafoetida, and salt were added. In a sauce pan with little oil, chopped onion and green chillies were sautéed. The flour batter was poured into a thin spread sprinkled with coriander leaves and cooked on either side. It was then subjected to organoleptic evaluation.

2.4.2 DFSF incorporated Cake

2.4.2.1 Ingredients

Refined wheat flour - 200 gm; Defatted Soy Flour - 40 gm; Baking powder - 1 Tsp; Hydrogenated vegetable oil - 250 gm; Sugar - 250 gm; Egg - 6 Nos. (Medium Size); Pineapple essence - 1 Tbsp; Tutty fruity - 2 Tbsp.

Table 1 Organoleptic Evaluation of Tofu Products

Product	Appearance	Flavour	Texture	Taste	Overall Acceptability	Overall Mean Score	Std. Deviation
Tofu with Pasta	3.4	3.6	3.8	3.6	3.5	3.58	±0.1483
Tofu Sandwich	3.5	3.4	3.6	3.6	3.6	3.54	±0.0894

Table 2 Organoleptic Evaluation of Soy Chunk Products

Product	Appearance	Flavour	Texture	Taste	Overall Acceptability	Overall Mean Score	Std. Deviation
Soy Nuggets	3.6	3.8	3.6	3.8	3.8	3.72	±0.1095
Soy Kurma	3.5	3.6	3.6	3.6	3.6	3.58	±0.0447

Table 3 Organoleptic Evaluation of DFSF Incorporated Soy Omelette

Product	Appearance	Flavour	Texture	Taste	Overall Acceptability	Overall Mean Score	Std. Deviation
Soy Omelette	3.8	3.8	3.6	3.8	3.8	3.76	±0.0894

Table 4 Organoleptic Evaluation of DFSF Incorporated Cake at different levels of Incorporation

Product (Cake)	Appearance	Flavour	Texture	Taste	Overall Acceptability	Overall Mean Score	Std. Deviation
10% DFSF (A)	3.2	3.4	3.0	3.6	3.4	3.2	± 0.2280
20% DFSF (B)	3.4	3.3	3.1	3.8	3.6	3.4	± 0.2702
30% DFSF (C)	3	2.8	2.6	2	3.1	2.7	± 0.4358

Table 5 Microbial analysis of DFSF Cake during Storage Period

Sample	0^{th} Day		7^{th} Day		14^{th} Day	
	Bacteria (10^{-6})	Fungi (10^{-3})	Bacteria (10^{-6})	Fungi (10^{-3})	Bacteria (10^{-6})	Fungi (10^{-3})
P1	-	-	2	5	10	7
P2	-	-	1	3	4	6

P1 – Polythene packed cake; P2 – Airtight plastic container packed cake

Table 6 Nutritive value of DFSF

Nutrients	Value
Moisture %	7
Energy (kcal)	329
Protein (g)	51.28
Fat (g)	0.93
Carbohydrate (g)	10.50
Calcium (mg)	241
Iron (mg)	9.20

2.4.2.2 Method

Hydrogenated vegetable oil and sugar were creamed in a mixer until fluffy before adding the eggs. The defatted soy flour, wheat flour, and baking powder were thoroughly mixed together before folding into the creamed sugar and hydrogenated vegetable oil. Essence and garnishes were added. The batter was poured into a greased tray and baked at 200º F for 40 min.

Cakes were formulated by incorporating defatted soy flour in different proportions (10%, 20%, and 30%) along with other ingredients. The three samples of cakes namely A (10% DFSF incorporation), B (20% DFSF incorporation), C (30% DFSF incorporation) was developed, and one level of incorporation was formulated as per organoleptic evaluation. Defatted incorporated cakes were subjected for storage study and microbial analysis.

The dearth of information for the formulated recipes has motivated the author to prepare the products either with soy products as a base or as incorporation or on proportions. Based on the overall acceptability the proportion has been standardized in the recipes. The consumption of soy products would enhance the health of women. The recipes ensure the consumption of soy products in a meal per day.

2.5 Sensory evaluation of the developed products

The developed products were subjected to the hedonic scale of sensory evaluation by a panel of judges. Panel members of 15 were selected and each individual was provided with a score card to assess the appearance such as colour, taste, flavour, consistency, and overall acceptability of the developed tofu products, Defatted soy flour incorporated products, and Soy chunks products.

The scorecard contained 4 points hedonic scale rating, viz., colour was appetizing (4) or appealing (3) or good (2) or bad (1), flavor was excellent (4) or good (3) or fair (2) or bad (1), consistency highly acceptable (4) or acceptable (3) or moderately acceptable (2) or not acceptable (1), taste was excellent (4) or good (3) or fair (2) or bad (1) and overall acceptability was very good (4) or good (3) or fair (2) or bad (1). Scientific methods of sensory analysis of foods have become of increasing importance in assessing the acceptability of food products.

When the quality of a food product is assessed using human sensory organs, the evaluation is said to be sensory or subjective or organoleptic. Sensory quality is a combination of different senses of perception used in choosing and eating food**.** Appearance**,** which can be judged by the eye (eg): Colour, Size, Shape, uniformity, and absence of defects are of first importance in food selection (Srilakshmi, 2018).

3 Results

3.1 Standardization and sensory evaluation

3.1.1 Tofu products

3.1.1.1 Tofu with pasta

Table 1 shows the sensory evaluation scores of the formulated tofu with pasta and tofu sandwich. The organoleptic characteristics were evaluated using 15 judging panels and four points hedonic rating scale. The sensory attributes of tofu with pasta were as following - appearance was appealing (3.4), flavour was excellent (3.6), texture was highly acceptable (3.8), taste was excellent (3.6) and the overall acceptability was very good (3.5). Two grams of protein will be provided when 25gm tofu is consumed.

3.1.1.2 Tofu sandwich

The organoleptic characteristics for the formulated tofu sandwich show that the appearance was appetizing (3.5), flavour was good (3.4), texture was highly acceptable (3.6), taste was excellent (3.6) and the overall acceptability was very good (3.6) and it was standardized. Four grams of protein will be provided when 50gm of tofu sandwich is consumed (Table 1).

3.1.2 Soy chunks products

3.1.2.1 Soy nuggets

The organoleptic characteristics for formulated soy nuggets in table 2 show that colour was appetizing (3.6), flavour was excellent (3.8), texture was highly acceptable (3.8), taste was excellent (3.6) and overall acceptability was very good (3.8) and it was standardized. Twenty one grams of protein will be provided when 40grams of soy chunks are consumed (Table 2).

3.1.2.2 Soy chunks kurma

The organoleptic characteristics for the formulated soy kurma show that colour was appetizing (3.5), flavour was excellent (3.6), consistency was highly acceptable (3.6), taste was excellent (3.6) and the overall acceptability was very good (3.6) and it was standardized (Table 2). Twenty six grams of protein will be provided when 50grams of soy chunks are consumed.

3.1.3 Defatted soy flour incorporated products

3.1.3.1 Soy omelette

The organoleptic characteristics for the formulated soy omelette (table 3) show that colour was appetizing (3.8), flavour was excellent (3.8), texture was highly acceptable (3.6), taste was excellent (3.8) and the overall acceptability was very good (3.8) and it was standardized. Twenty five grams of protein will be provided when 50grams of defatted soy flour were consumed (Table 3).

3.1.3.2 DFSF incorporated cake

Cakes were formulated by incorporating DFSF in different proportions (10%, 20%, 30%). The developed cakes were subjected to the hedonic scale of sensory evaluation with 15 trained judges. Each individual was given a scorecard to assess the appearance such as colour, flavour, texture, taste, and overall acceptability of the developed cakes were evaluated and standardized.

Table 4 shows the organoleptic evaluation of DFSF (10%, 20%, 30%) cakes. The overall mean scores of 10 percent, 20 percent, and 30 percent incorporation were 3.2, 3.4, and 2.7 respectively.

Among the three formulations, 20 percent incorporation level was highly accepted and it was standardized. They were packed in polythene packet (250 gauges) (P_1) and airtight plastic container (P_2) to study the storage stability.

On storage, a slight decrease in all the quality attributes were observed due to a change in texture, flavour, and taste in both the packaging material. On 0 day of storage (Table 5) there was no bacteria and fungi growth in both P1 and P2. On 7th and 14th day of storage, there was an increase in bacterial and fungal growth in the cakes packed in P1and P2. The cake was highly acceptable on the 1st day. The cakes packed in air-tight plastic containers were good than the cakes packed in polythene packs.

Nutritive value of DFSF showed 7 percent of moisture content, 329 kcal of energy, 51.28 g of protein, 0.93 g of fat, 10.50 g of carbohydrate, 241 mg of calcium, and 9.20 mg of iron in Table 6. The nutritive content of the DFSF cake (20%) was analyzed during the storage period. It exhibited a slight decrease in the carbohydrate, protein, and fat content packed in P1and P2 respectively. The iron and calcium contents did not exhibit any change in storage.

4 Discussion

Vegetarians prefer soy and soy foods as their nutritional solutions because of their high protein content and functional property in the production of milk substitutes and meat like similarity. Hence soy products were taken for the study. Soy protein can be used as a source of high-quality protein. Soy proteins provide the necessary essential amino acids for physical and muscular development (Carbone & Pasiakos, 2019).

The phytoestrogens and bioactive compounds in soy namely isoflavones are polyphenols having estrogenic properties. These being rich in soy bean have remarkable characteristics which are noteworthy for vegetarians (Rizzo &Baroni, 2018). Soy bean meal can be used to manufacture a product with meat like texture, known as textured vegetable protein. The vegetable protein is extracted by the addition of an alkali and fibers are formed by extruding the protein through fine nozzles or spinerettes. The fibers are combined with fat, a protein binder along with colours and flavours. As the product is similar to cooked meat, it can be frozen, canned, or dehydrated (Anon, 2019).

Tofu is rich in B-vitamins and protein of high biological value. In vegetarian recipes, Tofu is used as a substitute for meat. It also contains nine essential amino acids and is also free of saturated fat or cholesterol. Further, Rizzo & Baroni (2018) also suggested that Tofu is rich in isoflavones, which will reduce the risk of osteoporosis. Tofu was introduced in Japan in the 8th century, where it was originally known as "Okabe", but was not called "tofu" until the 15th century. While serving as a traditionally made dish, tofu did not gain its great widespread popularity in the west until it was recognized as healthier foods. First, it was gaining more widespread attention during the 1960s, and skyrocketing in popularity ever since research has begun to reveal many significant benefits, this nutrient rich, plant-based food can provide.

In the present study, tofu was incorporated with the normally planned meals such as cereal based pasta and bread sandwich. The overall acceptability was very good for the formulated tofu products namely tofu with pasta and tofu sandwich with 3.5 and 3.6 scores in sensory evaluation. Diet rich in soy protein has many health benefits that are unique to women's needs.A diet rich in soy protein may alleviate certain symptoms associated with menopause.

It helps to reduce the risk of breast cancer, promoting heart health, and maintain bone health. It is a good source of fiber, iron, calcium, zinc, and B vitamins (Dudek 2001).

The effect of substituting 50 percent of the pulse fraction of Indian recipes with defatted soybean flour on quality and the nutritive value was found to have higher carbohydrate, calorie, calcium, phosphorus, iron, and protein contents (Kaveri & Bindhu, 2004). Hence defatted soy flour was incorporated with besan flour and refined wheat flour to develop soy omelette and soy cakes. Similarly, Chokkammal & Chitra (1999) also incorporated DFSF with bakery items namely bread, sweet bun, vegetable puffs, biscuits, teacake, and soymilk-incorporated desserts. Bakery products like biscuits formulated with soy were supplemented with B-complex and iron tablets in the diet of school children for 4 months resulted in a reduction of signs and symptoms of nutrient deficiencies in children.

In the present study different proportions (10%, 20%, 30%) of DFSF incorporated cakes were prepared. The quality attributes were found to be high at 20 percent incorporation and were standardized. Polythene packet (250 gauges) and airtight plastic container were used as packaging material to study the storage stability. On storage, the DFSF cake exhibited a slight decrease in the carbohydrate, protein, and fat content when packed P1and P2 respectively. The Iron and Calcium contents did not show any change in storage. On storage, a slight decrease in all the quality attributes were observed on 7th and 14th day of storage. There was an increase in bacterial and fungal growth in the cakes packed in P1and P2. It was concluded that the DFSF cake stored in a polythene packet and the airtight container was found to be acceptable.

Bakery product production is increasing and becoming popular in India, during the last 10 years. Soy flour does many things in bakery foods like increasing the protein content of a baked food

(Anon 2019). Further, Hoover (1979) stated that technology for the utilization of soy products in bakery foods is well established and reasonably simple. The baking industry can realize the benefits of soy protein. Soy flour helps to reduce the glycemic index of all baked food and provides a way to improve blood sugar control, reduce carbohydrate load improves foods protein content (Blair et al., 2006)

In the present study, soy chunks were used with standardizing suitable recipes incorporated and developed with the same. The products formulated with soy chunks namely soy nuggets and soy kurma had 3.8 and 3.6 overall acceptability score on sensory evaluation. Phytoestrogens are acting like synthetic estrogen which helps women to protect themselves from bone loss and maintain a healthy heart (Wardlaw, 2000). Soy protein balances the calcium by influencing the bone in postmenopausal women. It is significant for women who are not receiving hormone replacement therapy (Bhathena & Velasquez 2002).

Soy products mainly provide protein, energy, and vitamins, protect the heart, fight cancer, improve mood and mental health, improve mental state, reduce the symptoms of osteoporosis, managing diabetes, alleviate menopause problems, reduce Gastro-intestinal disorders (Itapu, 2000).

Conclusion

Soybean is an excellent health food and contains about 40 percent good quality protein and reasonable amounts of minerals and vitamins. Soy protein in the diet may alleviate certain menopausal symptoms in women. It also reduces the risk of breast cancer. Bone health will be maintained well and it promotes heart health also. To conclude the formulation of selected soy-based recipes for women's health will aid in combating the menopausal symptoms through their diet. The products were planned in such a way that women can consume soy atdifferent timing in different forms to ensure the intake of isoflavones through soy to overcome her symptoms.

Conflict of interest

The authors declare that they have no conflict of interest.

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FORMULATION OF STIRRED PROBIOTIC FRUIT YOGURT TO BOOST IMMUNITY

Poornima Jeyasekaran[1*], M. Deepa[2]

[1]Department of Food Science and Nutrition, The American College, Madurai, Tamil Nadu, India
[2]Department of Food Science and Nutrition, Periyar University, Salem, Tamil Nadu, India

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KEYWORDS

Stirred yogurt

Probiotic drink

Passion fruit

ABSTRACT

The food we eat plays a key aspect in determining our overall health and immunity. Improving our immunity during the Covid-19 pandemic is challenging for all age groups. So this study focused on formulating a ready to drink called probiotic fruit yogurt from less utilized passion fruits (*Passiflora edulis*), as a good option to build resilience in the body against infections and also to help the planters of Thandikudi hills, Tamil Nadu to promote their harvest into a valuable product. Passion fruits were procured and handled in a very hygienic manner. The formulation of stirred fruit yogurts was carried out in three different ratios (10%, 15%, and 20% pulp). These samples were standardized by sensory evaluation (9 points hedonic scale) and physicochemical parameters (pH). Fruit yogurt made from 20% passion fruit pulp scored the highest value in the mean score (8.5±0.17) for sensory evaluation except for texture. The pH value of the passion fruit yogurt was 3.5 found and it was more acidic compared to the plain yogurt value of 3.7 because of the addition of fruit pulp which was balanced by the addition of sugar/stevia. The acceptability of the stirred probiotic fruit yogurt with 20% pulp was mainly because of the flavoring compounds of the yellow passion fruit (*P. edulis Sims f. flavicarpa* Deg).

* Corresponding author

E-mail: poornimajeyasekaran@yahoo.co.in (Poornima Jeyasekaran)

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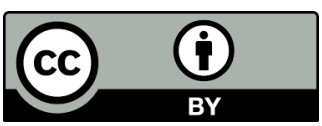

1 Introduction

The immune system is the complex network of cells and protein molecules that protect us from disease by surveilling our body and responding to any foreign (non-self) substances they recognize as threats, mainly infectious microbes. Our immune system has synchronized along with assorted gut flora, not only to create a defense against pathogens but also to develop some tolerance for constructive microbes (Ley et al., 2006). The importance of this interaction explores that 70–80% of the body's immune cells are found in the human gut (Abbas et al., 2017).

Probiotics are live microbes, which provided adequate amounts of health benefits to the host. The confirmation of the impact of probiotics on human health is intensifying and driving the profitable development of functional foods termed as "Probiotic food" (Sanders, 2019). Probiotics showed therapeutic potential for diseases, including several immune response-related diseases, such as allergy, eczema, viral infection, and potentiating vaccination responses (Fang & Polk, 2011).

Yogurt is the milk product formed from the culturing of milk with bacterial cultures that contain the lactic acid-producing bacteria, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus.* Furthermore, additional bacteria, such as strains of *Lactobacillus* and *Bifidobacterium* species, can be added for their health benefits (Hill et al., 2014).

Passion fruit (*Passiflora edulis Sims f. flavicarpa Deg*) grows in a perennial vine that is very vigorous in climbing. The woody vine yields round or ovoid fruits. The characteristics of the fruits are tough, smooth, and waxy with faint, fine white specks. Commonly, the rind is of two colors viz., dark purple and yellow. Fruit contains orange colored or pale pulpy juice with a large number of small, hard, pitted seeds which is either dark brown or black. The fruits are generally not used for table purpose (Sanders, 2009; Bakshi & Iqbal, 2019).

A combination of yogurt and fruit intake could provide high-quality protein, important fatty acids, a mixture of vitamins and minerals. This fruit yogurt bestows as both probiotic and prebiotic can to exert synergistic effects on health (Kailasapathy & Milchwissenschaft, 1998).

Improved probiotic viability was noted in fruit supplemented yogurt than plain yogurt due to fast consumption of phenolic compounds and organic acids such as citric acid by probiotic cultures. The inclusion of probiotic cultures and fruits in yogurt is recommended for enhancing the functional properties of traditional or home-made yogurt (Surajit, 2019). The current study was conducted to evaluate the sensory characteristics and physicochemical parameters of yogurt made from passion fruit.

2 Materials and Methods

2.1 Sample collection

A fresh pack of standardized milk was collected for the preparation of a thick yogurt formation because the addition of fruit pulp would dilute the stirred yogurt. Fully ripened yellow passion fruit was collected from the planter of Thandikudi hills - a Village in Kodaikanal Block in Dindigul District of Tamil Nadu State in India. The fruits were washed well and cut into two halves. Then the pulp along with the seeds was scooped out.

2.2 Formulation of Fruit yogurt

Milk sample was collected and boiled; this was followed by the cool down to a temperature of 37- 41ºC. Further, two spoons of the home-made yogurt culture were added and stir well. This mixture was covered and incubates in a warm place for 5-7 hours. Fruit pulp with seeds was added to the Yogurt and blended thoroughly till all the firm curd is broken down. The mixture was refrigerated at 0-5°C and this probiotic fruit yogurt can be used for 3 days of opening. The detailed procedure of probiotic fruit yogurt formulation is as below.

Standardized Milk (4.5 % fat, 8.5% SNF)
↓
Heated to 90°C/10 min
↓
Inoculation with culture (Probiotic culture from home-made starter @ 2 %)
↓
Incubation at room temperature for 5 hours
↓
Breaking the firm curd (120 ml) by blending for a min
↓
Addition of Stevia or sugar
↓
Addition of fruit pulp with seed (10%, 15% & 20 %)
↓
Stir well
↓
Fill in glass containers
↓
Probiotic fruit Yogurt (Refrigeration at 0-5°C)

Table 1 Mean score of 9-point hedonic scale

Quality Parameters	10% pulp yogurt	15% Pulp Yogurt	20 % Pulp Yogurt
Color and appearance	8.5±0.11	8.6 ±0.19	8.8±0.15
Flavor	8.1±0.17	8.2 ±0.16	8.4±0.25
Consistency	8.4±0.20	8.3 ±0.10	8.0±0.23
Taste	7.8±0.21	8.1 ±0.17	8.3±0.21
Overall acceptability	8.2±0.19	8.1 ±0.19	8.5±0.17

Table 2 Nutrient Analysis per 8 ounces / 227 g

Nutrients	Fruit Yogurt (Present Research)	Commercial Yogurt (USDA, 2019)
Energy	126kcal	138 kcal
Fat	5.7g	7.38g
Carbohydrates	14.2g	10.6g
sugars	14.2g	10.6g
Protein	4.3g	7.88g
Sodium	140mg	104mg
Calcium	157mg	275mg

2.3 Sensory Evaluation

The formulated stirred probiotic fruit yogurt was tested organoleptically using a 9-point hedonic scale. The primary purpose for the widespread acceptance of the 9-point hedonic scale is that its categorical nature and limited choices make it easy for both study participants and researchers to use. Its simplicity further makes the 9-point hedonic scale suitable for use by a wide range of populations without extensive training. Therefore, the primary concern of a study is measuring hedonic differences among foods, beverages, and consumer products and predicting their acceptance, the 9-point hedonic scale has proven itself to be a simple and effective measuring device (Lim, 2011).

2.4 Nutrient Content analysis

The nutrient analysis of the formulated 20% passion fruit pulp yogurt for the macronutrients and micronutrients followed the AOAC method 20th edition, 2016 (AOAC, 2016). The nutrients analyzed were Energy, Carbohydrate, Protein, Fat, Sodium, and Calcium.

2.5 Characterization of Microbial Culture

Overnight culture should be given for culture identification. One loopful of culture was taken and inoculated in peptone broth. Then after 24 hours, once confluent growth appeared, the culture was centrifuged. Taken in pellets and then sent to the biotechnology lab for identification (Dubey & Maheshwari, 2006). BLAST analysis is used for the identification of bacteria. This procedure helps to identify the strain of the bacteria present in the formulated yogurt to prove it to be a probiotic drink.

3 Results

3.1 Sensory attributes

The formulated fruit yogurts with three different ratios of fruit pulp (10%, 15%, and 20%) were analyzed organoleptic evaluation and tabulated in table 1. The samples were tested for their sensory attributes by 25 Semi trained panel members. The data gathered was statistically analyzed using mean ± standard deviation. The statistical analysis report revealed that the overall acceptability of 20 % passion fruit pulp incorporation was found to be highly acceptable. Whereas the overall acceptability of 10% pulp yogurt was found to be less acceptable and 20 % passion fruit pulp incorporation score high acceptability because of the good taste, excellent flavor, and better color.

Out of the three tested ratios of passion fruit pulp yogurt, the 20% pulp incorporated yogurt scored a maximum except for the consistency. To further scrutinize this finding the researcher planned to do a comparative test between the plain yogurt and 20% pulp fruit yogurt before standardizing the formulation. The results were analyzed statistically by mean and standard deviation which are graphically represented in Figure 1. The semi-trained panel members observed two aspects. One is the improved flavor from the aromatic compounds present in passion fruit pulp and the other one is the crunchy seeds that enhance the texture.

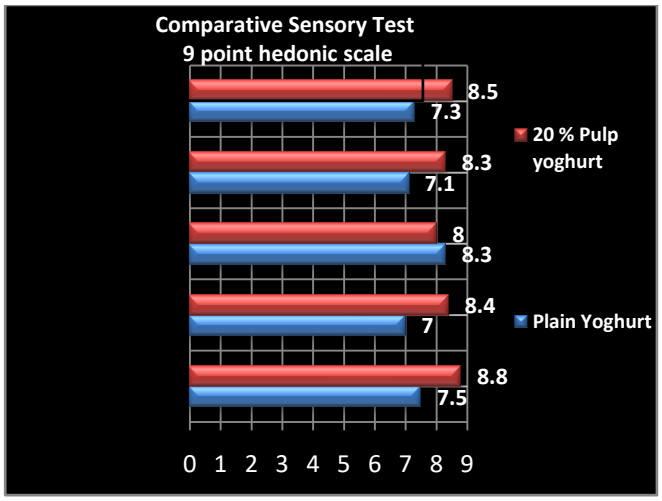

Figure 1 Comparative test graphical representation

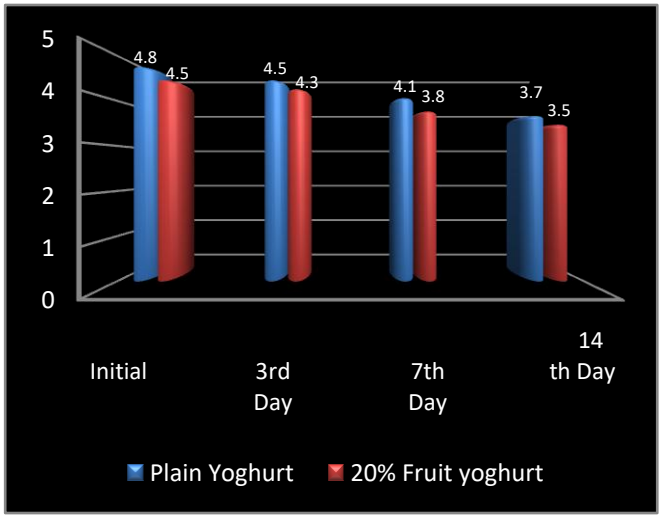

Figure 2 pH value of yogurts

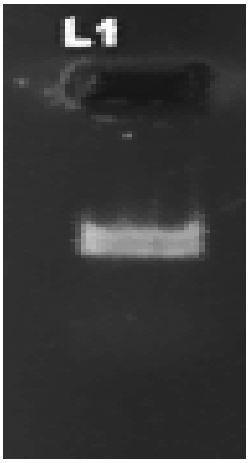

Figure 3 PCR products of 16s rRNA gene. (L1 – AM1)

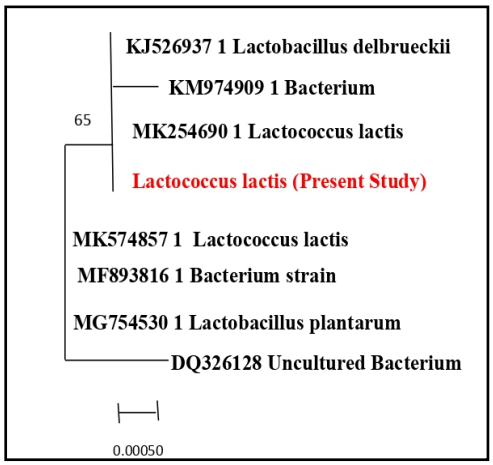

Figure 4 Phylogenetic tree

3.2 Changes in pH during storage

The researcher compared the pH score of the plain yogurt and 20% fruit yogurt to find out the physicochemical property. The stirred yogurts were refrigerated after fermentation and fruit pulp incorporation. The value was recorded by immersing the cathode of the pH meter into the sample. The pH value is higher in the formulated product when compared to the plain yogurt. The addition of fruit pulp also alters the pH and acidity of yogurt. Passion fruit pulp inclusion elevates the acidity due to the acidic character of the pulp. The result is graphically represented in figure 2. The pH reaches the minimum value of 3.5 for fruit yogurt on the 14^{th} day.

3.3 Nutrient Content

The nutrient analysis of the formulated 20% passion fruit pulp yogurt was analyzed for the macronutrients and micronutrients based on AOAC method 20^{th} edition, 2016. The values obtained were tabulated below in Table 2.

The content of calories, fat, and protein was a little lower compared to the commercial yogurt. Due to the addition of fruit pulp, there was an increase in the carbohydrates, and salt content of the stirred probiotic fruit yogurt.

3.4 Microbial identification of bacteria report

The prepared culture was sent for molecular identification of bacteria. Based on the higher percentage similarity against the reference species using BLAST analysis reported that the species that fermented the formulated fruit yogurt was *Lactococcus lactis.* Polymerase chain reactions were performed in the following temperature and timing conditions programmed in Applied Biosystems, thermal cycler.

Initial denaturation at 94 ºC for 5 min., number of cycles – 35, denaturation process carried out at 94 ºC for 30 sec, annealing took place at 56 ºC for 30 sec, extension passed at 72 º C for 1min and final extension process completed at 72 ºC for 10 minutes for amplification.

The amplified products were tested on 1.5% agarose gel electrophoresis and the molecular weight was checked using a molecular weight marker (100bp ladder). The amplified sequences belong to 16S rRNA figured in figure 3.

The amplified sequences belong to 16S rRNA were confirmed by similarity index built in the NCBI's BLAST program. Based on the higher percentage similarity against the reference species, the species utilized in this study were assigned as *L. lactis* species refer to figure 4.

4 Discussions

The 20% passion fruit pulp incorporated stirred yogurt scored a maximum in color and appearance, flavor, taste, and overall acceptability except for the consistency. The pH reaches the maximum value of 3.5 for 20% fruit yogurt on the 14^{th} day which is more acidic compared to plain yogurt. The nutrient content of the standardized fruit yogurt reveals that the 100 gram provides 126 kcal of energy, 14.2 g carbohydrates, 4.3 g of protein, 5.7g of fat, and 157 mg of calcium. The microbial identification of bacteria report confirms the species that fermented the formulated fruit yogurt was *L. lactis.*

The human gut is populated with as many as 100 trillion cells, whose collective genome, the microbiome, is a reflection of evolutionary selection pressures acting at the level of the host and the level of the microbial cell (Ley et al., 2006). The stirred yogurt fermented with *S. thermophilus* St-Body 1 at 37°C for 24 hours resulted in the highest score at each sensory evaluation category (Ho-Jin et al., 2005)

The higher the casein fraction and/or the fat level, the less hiatus in the network were observed. It is also evident that the addition of whey proteins strengthens the firmness properties of low-fat yogurts when compared to characteristics of full-fat yogurt (Sonne et al., 2011). On the other hand, increasing the level of whey proteins by modification of the whey protein: casein ratio in the milk base used for yogurt production led to a major increase in granular texture perception (Kailasapathy & Milchwissenschaft, 1998).

Nutritional facts of commercial yogurt given by the U.S. Department of Agriculture were, Calories: 138kcal, Protein: 7.88 g, Fat: 7.38g, Carbohydrates: 10.6g, Sugars: 10.6g, Calcium: 275mg, and Sodium: 104mg for plain yogurt (USDA, 2019). The content of calories, fat protein, and calcium were a little lower compared to the commercial yogurt. Due to the addition of fruit pulp, there was a mild increase in the carbohydrates, sugar, and sodium content of the stirred probiotic fruit yogurt.

Synergised health benefits may be exerted when yogurt and fruits are eaten together which provides potential prebiotic and their effects (Fernandez & Marette, 2017). The yogurts were flavored with 0.1% (wt/wt) strawberry flavoring containing 17 odorous compounds mixed with propylene glycol. The concentrations of the aroma compounds ranged from 1.01 to 32.53 mg/kg of yogurt (Saint-Eve et al., 2006).The pH values arrived by the researcher for stirred fruit yogurt agree with the findings of Tesfaye & Selvakumar (2015). The protective effects against the influenza virus were mostly derived from the cell components of *L. lactis* subsp. *cremoris* FC and its metabolites, such as exopolysaccharides. Many studies have shown that *L. lactis* can

support barrier function in terms of improved mucus, production of antimicrobial peptides, and secretion of soluble immunoglobulin IgA (Martín et al., 2014)

Conclusion

The research was conducted to formulate stirred fruit yogurt which has a probiotic effect that is very important for gut health that is a good sign to boost one's immune system. Fruit yogurt made from 20% passion fruit pulp scored the highest value in the mean score (8.5±0.17) for sensory evaluation except for texture and the pH range of the fruit yogurt (3.5) is more acidic compared to the plain yogurt (3.7) because of the addition of fruit pulp which is balanced by the addition of sugar/stevia. The acceptability of the stirred probiotic fruit yogurt with 20% pulp is mainly because of the flavoring compounds and the crunchy seeds of the yellow passion fruit. The BLAST analysis reveals that the species identified in the formulated fruit yogurt was *L. lactis.*

The shelf-life study to find out the growth of other microorganisms is under process. This research aims to be a corkscrew for the planters to convert their perishable produce into a valuable ready to drink convenient food with an extended shelf life and yields more profit for their produce, which also makes it available all through the year.

Recommendations and suggestion

Many locally available fruits can be converted in this way without the addition of class II preservatives thereby making them consumable for all age groups and any health conditions. Further, this study can be extended by incorporating passion fruit peel powder which is prebiotic into the stirred probiotic fruit yogurt. It can be analyzed for physicochemical characteristics and symbiotic effect.

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COMPARATIVE STUDY OF HAEMATOLOGICAL PARAMETERS IN FISHES FROM THE NATURAL HABITAT, MADURAI REGION

Malathi N*, Rino T, Jaya Supa Sooriya J, Shalini M

Fatima College, Mary Land, Madurai, Tamil Nadu, India

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KEYWORDS

Water quality parameters

Haemoglobin

Labeo rohita

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ABSTRACT

The heamatological profile of two freshwater fishes *Labeo rohita* (Rohu) and *Channa striatus* (Murrel) with a relationship to the sex and the water quality under natural habitat was analyzed. The freshwater fishes were collected from the pond located in Thodaneri, Vadipatti taluk, Madurai district, Tamil Nadu, India. In heamatology studies, no significant variation was observed in Heamoglobin content and found almost the same in both the fish irrespective of the sex and also falls in the range of control value. In differential Leucocyte count (DLC) the following blood components Lymphocyte, Eosinophil, and Monocyte count were significantly higher than the control values and the values are found maximum in Rohu when compared to Murrel.RBC (Erythrocyte) count was almost the same in both the fish and the value falls between the range of control value Packed Cell Volume (PCV) count was almost the same in both the fish and the value falls between the range of control value. The values of Hb and PCV were found higher in male fish as compared to female fish. The leucocyte count is also higher in the males when compared to the females of both Rohu and Murrel. These may be also influenced by physiological activity, feeding habits, infectious agents, water quality parameters, and the environmental stress on the organism.

* Corresponding author

E-mail: malathivishvaksenan@gmail.com ; malvish@rediffmail.com (N. Malathi)

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1 Introduction

The growing demands of urbanization cause a decrease in the amount of forest and, wetlands. This deplenishing green cover leads to variation in impervious-pervious which affect the quality of the storm water run-off, leading to poor watershed systems due to loads of pollutants carried by the storm water (Morisawa & La Flure, 1979). In addition to this, the pollutants that are released from the industries are also carried to the riverine system. The overall environment of the aqua system gets affected indefinitely based on the effects of the pollutants, and they directly or indirectly affect the organisms that habitat the wetlands. Fishes are considered valuable resources since they are an important source of food in the world. Fishes as a unit play an important role in maintaining the balance in the ecosystem.

Fishes in the pond are afflicted by the water quality parameters such as pH value, alkalinity, availability of oxygen, level of environmental stress, and hardness which eventually pose a great threat to fish health (Southamani et al., 2015). Rohu (*Labeo rohita*) is the predominant species used in carp polyculture systems. Rohu is a fast-growing species and thrives above 14 ℃ temperature. Spawning happens in the shallow water and the spawning season of Rohu generally coincides with the south-west monsoon. *Channa striatus* (Ng & Lim, 1990) generally lives in estuaries and can withstand the drought. It can breathe in an anaerobic way and can survive in the dry season (Qin & Fast, 1996; Pradhan et al., 2012). The Murrel involve in reproduction all-round the year (Ali, 1999), and suitable breeding habitat for this fish is the paddy field. Both these fishes are available in almost all seasons and hence chosen for the study.

The heamatology is considered an important indicator to oversee the physiological and pathological changes in the fishes. The total body weight of fish constitutes 1.3–7% of blood which exchanges gas between organism and environment. Studies of haematological parameters are based on health status, age, aquatic biotope, and fish species (Patriche et al., 2011; Kumar et al., 2019). It is greatly influenced by physiological stress, diseases, and the introduction of toxic pollutants in the aqua system. This study aimed to compare the haematological profiles of two freshwater fish in Rohu (*Labeo rohita*) and Murrel (*Channa striatus*) collected from the native environment.

2 Materials and Methods

The heamatological profile of the two freshwater fishes *L. rohita* and *C. striatus* in relationship with the sex and the water quality in a natural habitat is analyzed in this work. Ten healthy and disease-free male and female of each *L. rohita* and *C. striatus* species were collected for sampling from the pond located near Thodaneri, Vadipatti Taluk, Madurai, Tamil Nadu, India subjected to heamatological studies (Figure 1). Ten male and female fish of *L. rohita* and *C. striatus* served as a control. The studies include physicochemical parameters of the pond water and heamatology profile of the two selected fishes. The parameters taken for comparison are haematology and sex discrimination factors.

Figure 1 Satellite Map showing the catchment area where fish were collected from Thodaneri pond, Vadipatti Taluk, Madurai district

2.1 Water quality analysis

Pond water from the catchment area was collected in a sterilized PET bottle by immersing it completely in the middle of the pond and screw-capped tightly under the water without lifting the water bottle and it was transported immediately to the laboratory for physicochemical examination of water parameters as described by the standard protocols (APHA, 1989). The sampling was done between 10 am to 2 pm. Water samples in triplicates were collected at different intervals of time for the study. On-site analysis was done for pH and turbidity using a pH meter and turbidity meter. Other physicochemical parameters like dissolved solids, pH, total hardness, and bound minerals were carried out within 24 hrs of sample collection. It was preserved at 4○C. The water quality was analyzed by methods given in APHA (1989) and Trivedy & Goel (1986).

2.2 Collection of Blood

Blood was collected from the punctured caudal vein with the aid of 20 G×1.5 syringes and collected blood samples in falcon tubes containing EDTA from both the sexes of Rohu and Murrel separately. The experiment was repeated twice with ten samples of males and ten samples of the female of each fish. In haematological profile, the differential Leucocyte counts was determined with the help of a Neubauer hemocytometer. Nelson & Morris (1989) method was used to estimate hemoglobin concentrations and hematocrit. Packed cell volume was estimated by adopting the procedure of Wintrobe (1934). Further, erythrocyte indices such as MCV, MCH, and MCHC were calculated as mentioned below: MCH = hemoglobin x 10/erythrocyte count, MCV = PCV x 100/erythrocyte count and MCHC = hemoglobin x 100/PCV.

Sex was also determined for the hematological parameters.

2.3. Statistical analysis

Statistical analysis of hematological parameters in terms of Mean±SD in both sexes of fishes was employing ANOVA.

3 Results

The results are presented as comparative analyses between the species and sexes of Rohu (*Labeo rohita*) and Murrel (*Channa striatus*) the freshwater fish collected from the pond with that of the control.

In the physicochemical examination of water, total dissolved solids were very minimum (211mg/l) when compared to the acceptable limit, the electrical conductivity was 302µS/cm. The pH of the water was 7.10, which is falling under the recommended range.

In a chemical examination of water analysis through total hardness parameter was showing maximum (700 mg/l) the individual chemical components were found minimum when compared to an acceptable limit. Other physio-chemical parameters like calcium, magnesium, iron, ammonia, nitrate, chloride, fluoride, sulphate, and phosphates are below the recommended range (Table 1).

Table 1 Physico-chemical examination of pond water

S.No.	Physical examination	Acceptable limit	Pond Water (Sample)	Control water
1.	Colour	5	Clear &colourless	Clear &transparent
2.	Total Dissolved Solid (mg/l)	500	211	150
	Chemical Examination			
3.	P^H	6.5−8.5	7.10	6.70
4.	Total hardness (mg/l)	200	700	650
5.	Calcium (mg/l)	75	88	80
6.	Magnesium (mg/l)	30	35	33
7.	Iron (mg/l)	0.3	0	0.1
8.	Ammonia (mg/l)	0.5	0.5	0.1
9.	Nitrate (mg/l)	45	1	0.5
10.	Chloride (mg/l)	250	40	30
11.	Fluoride (mg/l)	1.0	0	0.5
12.	Sulphate(mg/l)	200	15	10
13.	Phosphate (mg/l)	0	0.5	0.1

Haematological profiles were compared between the sex of Rohu and Murrel (Table 2). No significant variation was observed in Hb content and found almost the same in both the fish (11±0.5) and also fell in the range of control value in Rohu female.

Mean corpuscular volume (MCV) were found significantly maximum (188±17) in Rohu male when compared with control range. Mean Corpuscular Heamoglobin (MCH) count was found significantly maximum (47±0.0) in Rohu males when compared to the control range.

In differential leucocytes count the following blood components Lymphocyte (87.0±3), Eosinophil (3.50±0.5) and Monocyte (1.75±1.5) count in Rohu male were significantly higher than the control values. Eosinophil(0.34−0.40) and Monocyte (0.14) and the values were found maximum in Rohu when compared to Murrel. Lymphocyte count was found maximum in both the sex of Rohu and Murrel when compared with control. RBC count was almost same for the both the fish and the value found minimum when compared to the range of control value. PCV count was almost the same in both the fish (40±1) and the value was found between the range of control value.

Table 2 Haematological Profiles of Rohu and Murrel Fish

S. No	Haematology parameters	Rohu		Murrel		Rohu (Control)		Murrel (Control	
		Male	Female	Male	Female	Male	Female	Male	Female
1.	HB	11±0.5	11.5±1	12±1	11±0.5	11±1	11±0.5	12±1	11±0.5
2.	PCV	40±1	38.5±0.5	41±1	38±1	42±1	40±0.5	43±1	41±2
3.	RBC	2.8±0.1	2.4±0.1	3±0	2.0±2	4±0.1	3.8±0.1	3.9±0	3.7±2
4.	MCV	188±17	122±2	158±12	156±3	93±3	85±2	94±1	81±2
5.	MCH	47±0.0	33±0.2	35±0.3	34±0.2	31±1	29±0.2	33±0.1	30±1
6.	MCHC	30±0.3	32±0.1	31±0.4	30±1.5	37±2	32±0.1	39±1	35±2.5
	Differential Leucocyte Count								
8.	Neutrophil	9.5±0.5	6.50±0.5	10±1	9±0.5	16±0.5	11±0.5	15±1	12±0.5
10.	Monocyte	1.75±1.5	1.00±0	1.00±1	1.00±1	0.13±0.5	0.1±0	0.13±0.01	0.11±0.1
11.	Eosinophil	3.50±0.5	2.00±0.5	1.5±0.5	1.75±0.5	0.3±0.1	0.2±0.2	0.4±0.1	0.1±0.2
12	Lymphocyte	87±2.5	82.5±3	86±2	81±2.5	79±2	78±1	78±1	77±1.5

The given values are Mean± SD

4 Discussion

Haematological results are presented as comparative analyses between Rohu *(L. rohita)* and Murrel *(C. striatus)* freshwater fish. The physico-chemical parameters taken for the study of pond water were above the control range except for iron content. The pH of the water is influenced by the dissolved minerals in the aquatic system, the pH of water influences the solubility of substances thus the presence of these substances influence the aquatic system. Furthermore, pH is additionally emphatically corresponded with electrical conductance and absolute alkalinity (Sahiti et al., 2018; Sarkar et al., 2020). The pH in the present study is alkaline and is also under the optimal range. Huq & Alam (2005) stated that electrical conductivity typically is shown for the dissolved concentration of free radicals in water. The total dissolved solids also influence the electrical conductivity of the water (McNeely et al., 1979).

The amount of chloride was below the optimal level. High Chloride level is an indicator of polluted water (Munawar, 1970). Phosphate was found 0.5 mg/l in the pond water. Phosphates are taken up by plants and are not found in high amounts in water. Phosphates in high quantities indicate pollution in water (WHO, 1993). Presently nitrate level was found to be influenced by the nitrification and denitrification process which is carried out by micro-organisms (Trivedy & Goel, 1986). The sulphates in water determine their use for public and industrial purposes, the sulphate content was 15 mg/l.

According to Svobodova (1994), stream hydrology and capacity are reliant on five significant factors viz., atmosphere, geography, soils, land use, and vegetation. These factors straightforwardly influence the elements of release and silt load, which thus affect the hydrology and morphology of the stream. Additionally, the pesticide and other chemicals leaches out and, in the end, influences the water quality, which thus impacts the miniature atmosphere of the lake and the life form which lives in it.

According to Southamani et al. (2015) the climate of the environment affects the hematology of fish. The specific distinction is seen in the hematological profile of both the species and also concerning the sex. Changes in the environment can induce changes in the hematological profile of fishes as they are very sensitive to their environment (Ahmed et al., 2020).

The values of Hb and PCV have been seen maximum in male fish which is due to the physiologically activeness of male fish. The variation in the erythropoietin is the indicator of higher hematological values in male fish influenced by the water quality of the pond. The leucocyte count is also higher in the males when compared to the females of both Rohu and Murrel. Leukocyte levels in the blood may also fluctuate according to environmental quality, nutritional status, and presence of infectious agents (Sunomonu & Oyelola, 2008).

The authors found that there is an increase in PCV level while decreasing in the erythrocytes due to habitat. Hypoxia results in swelling of RBC (Singh, 1995). Adrenaline stimulates membrane bound Na/K ATPase which is responsible for methemoglobin (Svobodova et al., 2008). This further leads to a decrease in the concentration of hemoglobin and an increase in cell volume. Increased accumulation of CO2 in the blood causes a decrease in Hb indicating poor oxygen transport. The unexpected occurrence of eosinophils in the blood may be the indicator of gomephysiological demands which is due to the internal and ecophysiological conditions (Qin & Fast, 1996; Tripathi et al., 2003)

Conclusion

The present work involves the comparative study of two freshwater fish Murrel and Rohu from the catchment area (Pond) subjected to haematological profiling of their blood samples. The results of the study showed almost similar data shared for most of the blood parameters among two species irrespective of their sex. On contrary, the values of Hb and PCV have been found maximum in male fish. The leucocyte count is also higher in the males when compared to the females of both Rohu and Murrel. These may be also influenced by the ecological niche and microclimate of the pond, physiological activity, feeding habit, infectious agents, water quality parameters, agricultural leachates, and the environmental stress on the organism. It should be once again evaluated more extensively as they possess many useful genes for fish sustained development.

Conflict of Interest

The authors declare that they have no conflict of interest.

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DOES PLAYING LOCATION-BASED AUGMENTED REALITY GAME INCREASES THE LEVEL OF PHYSICAL ACTIVITY?

Low Ann Gee[1], Ambusam Subramaniam[1]*, Sivaguru Muthusamy[1, 2], Rajkumar Krishnan Vasanthi[1]

[1]Physiotherapy programme, INTI International University, Nilai, Negeri Sembilan, Malaysia
[2]Department of Physical Education & Health Sciences, Alagappa University, Tamil Nadu, India

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Level of physical activity

Gamification

Location-based

ABSTRACT

Recently, there are an increasing trend in location-based augmented reality (AR) games that require players to move around physically to acquire the in-game features as well as game bonuses. The introduction of this location-based augmented reality (AR) games, specifically, Pokémon Go, has made the players physically move around to achieve higher levels and indirectly, improves the level of physical activity. Thus, the objective of the current study is to examine the association between the time spent playing location-based AR games specifically Pokémon Go, and the level of physical activity of the players in Malaysia. A self-administered questionnaire was circulated among Pokémon Go players and based on the inclusion and exclusion criteria, 47 players were recruited in the study. Global Physical Activity Questionnaire (GPAQ) was used to identify the level of physical activity. The association between time spent playing Pokémon Go and level of physical activity were examined using the Chi-square test. The results of the current study showed no significant association between days spent playing Pokémon Go and level of physical activity ($p = .14$), hours spent playing Pokémon Go and physical activity ($p = .516$), or between daily hours spent playing Pokémon Go and daily sedentary time ($p = .283$). Nevertheless, the mean of the study reported that the physical activity level of the players increased concurrently as the player's game frequency increases. Further studies are required to shed light on how location-based AR games can be implemented as potential strategies to engage an active lifestyle.

* Corresponding author
E-mail: ambusam_7@hotmail.com (Ambusam Subramaniam)

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1 Introduction

In today's generation, there is a growing concern over the consequences of sedentary lifestyle practice and physical inactivity. Physical activity refers to the involvement of bodily movement that requires the usage of energy in our daily life (WHO, 2020). According to the Global Health Observatory of WHO, the prevalence of physical inactivity among adults aged 18 and above in Malaysia was 38.8% in the year 2016 while females constitute the majority (42.8%) whereas males were 34.6% (WHO, 2018).

Moreover, a previous study has reported that campaigns to promote physical activity had only achieved limited success and yielded non-significant results (Leavy et al., 2011). Over the long run, physical inactivity will impact the population with major health repercussions especially non-communicable diseases (NCD) such as cardiovascular disease (CVD), hypertension, diabetes, and others (Zulkepli et al., 2019). Therefore, there is a crucial need of promoting the importance of physical activity among the public health to improve and sustain good health and quality of life.

The recent growth of smart phone obsession and mobile gamification are emerging rapidly among people regardless of their age, gender or status. The increased trends of mobile games are commonly linked with the practice of sedentary lifestyles and lack of physical activity.

However, the introduction of location-based (geolocation) augmented reality games have led to a turn of the tide. Augmented Reality (AR) is a software technology that capable of overlaying a real environment with a virtual 2D or 3D computer-generated image (Carlson & Gagnon, 2017). Location-based AR games use three features of the smart phone: Global Positioning System (GPS) to locate the device's location, the phone's compass to obtain the direction of the player is facing, and lastly, an accelerometer to obtain the orientation of the phone.

A popular example of location-based AR games is Pokémon Go. The game requires players to walk around the neighbourhood to obtain in-game features and bonuses. The players have no point staying at one place as the game's design is to get people moving. The players are required to walk, jog or run around the neighbourhood to catch Pokémons, interact with Pokéstops and PokéGyms to level up faster, and connect with other players. Thus, location-based AR games do the opposite of what mobile games do: to avoid players staying still at one place while playing the game.

Thus, this study was conducted to provide an insight on whether Pokémon Go is associated with the increase in physical activity level after the game was released since the year 2016. The objective of the current study is to examine the association between the time spent playing location-based AR games and the level of physical activity of the players.

2 Materials and Methods

2.1 Subjects

A cross sectional study was conducted to examine the association between the time spent playing location-based AR games and the level of physical activity of the players. Using convenience sampling, Pokémon Go Groups of different states on Facebook and WhatsApp were identified and the online questionnaires were distributed within the group. Questionnaires were also distributed to Pokémon Go Players on Pokémon Go's Community day at Pokémon Go active playing locations. The sample size of the current study was 84 participants.

Nevertheless, the total subjects recruited yielded 47 players based on the predefined inclusion and exclusion criteria. The participants in the current study were between 18 to 40 years old and both male and female were recruited in the study. Players that drive to play the game, already physically active before playing Pokémon Go, already vigorously active at work, or uses "GPS spoofing apps" were excluded from the study.

In the beginning, the author has informed the study procedure to the players and written informed consent was attained before the study. The Research Ethics Committee of INTI International University, Malaysia permitted to conduct the study (FHLS/RAC/JUL/12).

2.2 Procedure

The players were given a demographic questionnaire that includes age, gender, years of playing Pokémon Go, and time spent playing per hours/day and per days/week. The questionnaire was pre-tested by peers who played Pokémon Go to identify technical errors and misapprehension of vocabulary in the questionnaire.

Also, the Global Physical Activity Questionnaire (GPAQ) version 2.0 was used in the current study. The GPAQ V. 2.0 provides information in the physical activity level based on 4 domains "Work", "Transport", "Recreational Activity" and "Sedentary Behavior" in terms of "frequency" quantified as days per week and "duration" quantified as hours and minutes per day (Mum et al., 2017).

The data collected in GPAQ were analysed and expressed as Total Physical Activity in minutes per week or METs (Metabolic Equivalents) according to the physical activity level. The METs value can be categorized based on the following category; Inactivity with 1 MET, moderate and vigorous represented with 4 and 8 METs respectively (Herrmann et al., 2013).

2.3 Statistical Analysis

Data were analyzed using Statistical Software Package SPSS (Version 26.0, Chicago, IL, USA). Shapiro-Wilk test was used to

analyze the data and normal distribution was reported. Descriptive analysis were used to analyze the demographic details such as the age, gender, years started playing Pokémon Go as well as the time spent playing per hours/day and per days/week.

The data were not normally distributed, thus, the median score has been reported. Chi-square analyses were employed to analyze the days spent playing Pokémon Go and level of physical activity; days spent playing Pokémon Go and sedentary time with p-value of < 0.05.

3 Results

3.1 Demographics details of the subjects

Forty-seven participants were recruited in the current study. Most of the players were aged between 21-30 years (55.3%), among these majority are male (61.7%) and started playing Pokémon Go in the year 2016 (85.1%). The majority of the players (70.2%) spent 6 to 7 days in a week playing Pokémon Go and spent 1 to 4 hours a day (59.6%) playing Pokémon Go. Table 1 represents the demographic details of the players.

Table 1 Demographic details of the participants

Demographic variable	Number (n)	Percentage (%)
Age (years)		
18- 20	4	8.5
21-30	26	55.3
31-40	17	36.2
Gender		
Male	29	61.7
Female	18	38.3
Number of the year started playing Pokémon Go		
4 years	40	85.1
3 years	2	4.3
2 years	2	4.3
1 year	3	6.4
Days spent playing Pokémon Go in a week		
1-2 days	5	10.6
3-5 days	9	19.1
6-7 days	33	70.2
Duration spent playing Pokémon Go in a day		
Less than 1 hour	12	25.5
1-4 hours	28	59.6
5-8 hours	5	10.6
9-12 hours	0	0
More than 12 hours	2	4.3

3.2 Level of physical activity based on the Global Physical Activity Questionnaire (GPAQ)

Pokémon Go players spent a median (IQR) of 540 (300-1200) minutes/week being physically active and a median (IQR) of 2412 (1200-4800) MET-minutes/week. 0 (0-180.0) minutes/week were spent on the moderate intensity of work that involves brisk walking.

The median time spent walking or cycling to travel to from places was 210.00 (0-420.0) minutes/week.

A median of 60 (0-240.00) minutes/week was spent on vigorous recreational activity whereas 60 (0-240.00) minutes/week were spent on moderate intensity recreational activities. The median time spent sedentary was 300.0 (240.0-480.0) minutes/day. Among the 47 players, 40.4% were having a high level of physical activity, 27.7% were having a moderate level of physical activity and the remaining 31.9% were categorized as low in physical activity level. Table 2 represents the mean and standard deviation of the GPAQ variables and Table 3 represents the level of physical activity among the players.

Table 2 Mean and Standard deviation of Variables of GPAQ

Variables in GPAQ	Median (IQR)
Total time spent on moderate intensity during work per week (minutes)	0 (0-180.0)
Total time spent walking/cycling per week for travel (minutes)	210.00 (0-420.0)
Total time spent on vigorous recreational physical activity per week (minutes)	60 (0-240.00)
Total time spent on moderate recreational physical activity per week (minutes)	60.0 (0-240.00)
Total time spent sedentary per day (minutes)	300.0 (240.0-480.0)
Total PA in minutes/week	540.00 (300-1200)
Total MET-minutes/week	2412.00 (1200-4800)

IQR- Interquartile ranges

Table 3 Level of Physical Activity

Level of physical activity	Number (n)	Percentage (%)
Low	15	31.9
Moderate	13	27.7
High	19	40.4

3.3 Association between playing Pokémon Go and physical activity

Based on the cross-tabulation, those who played Pokémon Go for 6-7 days have shown to have High level of physical activity compared to those who played for only 1- 2 days (Table 4). Nevertheless, in chi-square test analysis, a p-value of 0.14 was obtained and the null hypothesis could not reject. Hence, there was no significant association between days spent playing Pokémon Go and level of physical activity among players of Pokémon Go in Malaysia, $\chi 2$ (4, N = 47) = 6.931, p = .140. There was also no association between hours spent playing Pokémon Go and physical activity, $\chi 2$ (6, N = 47) = 5.218, p = .516. Besides, no association between daily hours spent playing Pokémon Go and daily sedentary time, $\chi 2$ (8, N = 47) = 9.746, p = .283 were found.

Table 4 Association between days in a week playing Pokémon go and the level of physical activity

		Level of Physical Activity			Total
		Low	Moderate	High	
Average of playing Pokémon Go (Days/Week)	**1-2 days**	2	3	0	5
	3-5 days	2	4	3	9
	6-7 days	11	6	16	33
Total		15	13	19	47

4 Discussion

The result of the current study reported that the time spent playing Pokémon Go was not associated with the players' level of physical activity or sedentary time. Nevertheless, based on the mean, those who played the game 1-2 days/ week showed a lack of involvement in high physical activity levels and leaned towards the moderate level of physical activity. On the contrary, those who played more frequently (6-7 days/week) were more involved in a high level of of physical activity compared to those who played 1-2 days/week. Moreover, the number of players involved in high physical activity levels increased as the frequency of gameplay increased. The majority of the subjects played Pokémon Go 1-4 hours/day and the players had higher physical activity levels compared to those who played less than an hour/day. From this, there is a probability that those who played Pokémon Go more frequently tend to have a higher physical activity level but it is not associated with time spent gaming. Previous studies have reported that the main motive behind playing Pokémon Go is the players being Pokemon and game enthusiasts, followed by the motive of "staying active/walk more" (Marquet et al., 2017).

Therefore, there may be the possibility of other factors that might influence the increased level of physical activity among those who played Pokémon Go frequently. The small sample size of this study may also be a factorlimiting the statistical power and significance of the result.

Further, the findings of the current study were also similar to the previous study findings that stated the effects of Pokémon Go in motivating the physical activity levels lacks sustainability over some time (Althoff et al., 2016; Howe et al., 2016; Wattanapisit et al., 2018; Ni et al., 2019). Besides, another study conducted on the impact of Pokémon Go reported the effect didn't sustain beyond the first week (Ni et al., 2019) while another study discovered the effect only sustained for the first 30 days after the game is released (Althoff et al., 2016). Although, there is a significant increase in daily walking steps after playing Pokémon Go but the effect declines and stops at the 6th week post-game installation (Howe et al., 2016).

Thus, the current study was conducted approximately 3 years after the game was released and results showed there is no association between playing time spent playing Pokémon Go and physical activity. Based on the findings from the previous study, this could be possibly due to a lack of motivation and sustainability in playing the game in the long run (Althoff et al., 2016; Howe et al., 2016; Ni et al., 2019).

The results of this study were also comparable to the results of another correlation study that conducted among medical student in Thailand as there was no significant correlation between time spent playing Pokémon Go and the energy expenditure or sedentary behavior throughout their 3-month study period (Wattanapisit et al., 2018).

The limitation of the current study is the small sample size due to more than half of the players were excluded to minimize the influence of confounding variables. Secondly, self-administered questionnaire may be unreliable due to potential recall bias and reporting bias. Future study may involve a larger sample size and adopt devices such as accelerometers, pedometers, or other related health tracking applications to further provide greater insights on the virtual augmented game with a more precise, reliable physical activity measurement.

Conclusion

The current study revealed time spent playing Pokémon Go was not associated with the level of physical activity. Although this study showed there is a lack of association, the higher physical activity level is probable among frequent Pokémon players as it can be observed from the data those who spent more days playing Pokémon Go tend to have a higher level of physical activity. In contrary to the conservative approach of physical activity promotion such as awareness campaigns or health talks, incorporating technology and gaming enthusiasm may be more efficacious in motivating people to be physically engaging.

Integrating location-based AR games into fitness programs can be tempting to certain populations and an opportunity to foster a fun, active, and healthy lifestyle.

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IMPACT OF MUSIC AS MOTIVATION TO PERFORM EXERCISES AMONG UNIVERSITY STUDENTS – A SURVEY

Lai Yi Ying, Ambusam Subramaniam*

Physiotherapy Programme, INTI International University, Nilai, Negeri Sembilan, Malaysia

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ABSTRACT

The current study aimed to examine the association between music and motivation to do exercise among university students in Nilai, Negeri Sembilan, Malaysia. The demographics data, use of music during exercise, preferred type of music, and criteria to select music were assessed by a validated self-administered questionnaire. The motivation towards exercises was evaluated using the Behavioural Regulations in Exercise Questionnaire (BREQ-3) while the level of physical activity was assessed by Godin-Leisure Time Exercise Questionnaire (LTEQ). The demographics information, use of music during exercise, preferred type of music, and criteria to choose music were tabulated using descriptive statistics. A point-biserial correlation was used to analyze the relationship between music and motivation to do exercise while chi-square was utilized to determine the association between the use of music during exercise and physical activity level. The results of the current study showed that 80.3% of the participants listen to music during exercise. Energetic and rhythmic was the preferred type of music for exercise. Tempo/ speed/ bpm was the most popular factor to be considered during the exercise. Most of the participants prefer to listen to an individual music player during exercise rather than an open audio system.. Listening to music during exercise shown significant correlation with a motivation (p=0.006), external regulation (p=0.014), identified regulation (p=0.006), integrated regulation (p=0.002) and intrinsic regulation (p=0.015). There was a significant association between the use of music during exercise and physical activity level (p=0.003) in this study. Future research that involves the type of exercise performed with the music is encouraged to explore the significance of music as a motivational tool in exercise.

* Corresponding author

E-mail: ambusam_7@hotmail.com (Ambusam Subramaniam)

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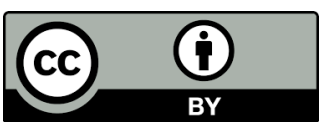

1 Introduction

Regular exercise has proven to improve physical capacity by enhancing muscular strength and endurance, thereby promotes the development of healthy bones and muscles (Baldari et al., 2010; Rajappan et al., 2015). Regular exercise plays an important role in decreasing the risk of premature mortality and non-communicable conditions such as type-2 diabetes, hypertension, osteoporosis, cancer, and cardiovascular disease (Duncan et al., 2010). Besides, engagement in exercise improves mental health through alleviation of symptoms of psychological discomfort such as anxiety and stress (Baldari et al., 2010; Rajappan et al., 2015).

Physical inactivity is the fourth primary cause of worldwide mortality (Rajappan et al., 2015). According to the report by the Malaysian Adult Nutrition Survey 2003, there was a 39.7% prevalence of physical inactivity and prevalence was higher in females (42.6%) than males (36.7%). World Health Organization (WHO) recommends adults aged 18-64 should perform at least 75 minutes of vigorous-intensity aerobic physical activity or at least 150 minutes of moderate-intensity aerobic physical activity per week to maintain health. However, WHO stated that approximately 60% of the global population do not meet the recommended daily minimum level of physical activity (Rhodes et al., 2017). This rate is concurrent with Malaysia as Malaysian youth claimed their physical activity level reduced after the age of 22 (Salamudin & Harun, 2013).

Music listening habits have been adopted as a strategy to achieve different emotional goals in everyday life (Laukka & Quick, 2013). Studies showed that music helps people to relax, regulate mood, heighten arousal, increase work output and divert attention from unhappy events (Karageorghis & Priest, 2008; Bigliassi et al., 2018). Music has been used during sport-related events to restrain mental and emotional fatigue, sustain motivation and possibly promote athletic performance (Bonnette et al., 2010). The use of music while exercising produces higher than expected levels of strength enhances academic performance for the reason that exercise improves the quality of sleep and reduces insomnia thus indirectly increases endurance, power, and productivity (Karageorghis & Priest, 2012b).

Individuals struggle with motivation to keep exercising regularly despite the well-known benefits of being active. Deficiency of motivation or dullness of exercise is a common cause for discontinuing exercise (Barney et al., 2012). Numerous types of motivation were found to influence intention in continuing exercise and effort to exercise (Duncan et al., 2010). Music as an environmental sensory cue has the potential to convey positive ergogenic and psychological effects across athletic training, widespread exercise or physical activity (Bonnette et al., 2010; Karageorghis & Priest, 2012b; Bigliassi et al., 2018) and even to overcome the monotony of activities such as jogging (Chamorro-Premuzic & Furnham, 2007). According to previous research, arousal regulation and fatigue dissociation are the fundamental concepts of impacts of music on exercise motivation and performance (Karageorghis & Priest, 2012a). Traditionally, the use of music motivates or inspires people in preparation for a critical event such as the pregame of an important competition (Bonnette et al., 2010). Also, music tends to suppress the awareness of fatigue-related symptoms by diverting the attention toward task-unrelated thoughts during exercise (Bonnette et al., 2010; Chow & Etnier, 2017; Bigliassi et al., 2018).Although many studies have been conducted in assessing the relationship between music and exercise in trained athletes, elderly or diseased population, there is limited research that has addressed the influence of music as a motivation among the current youth generation especially university students consisting of the current youth generation. Therefore, the current study aims to examine the association between music and motivation to do exercise among university students in Nilai, Negeri Sembilan, Malaysia.

2 Materials and Methods

2.1 Subjects

A cross-sectional study was used to determine the association between music and motivation to do exercise among university students in Nilai, Negeri Sembilan, Malaysia. Private universities located in Nilai, Negeri Sembilan, Malaysia were selected using convenient sampling. Electronics self-administered questionnaires were sent to students through e-mail and social media. The inclusion criteria of this study including private university students age from 18-30 years old that understand English in Nilai, Negeri Sembilan, Malaysia. 220 responses were collected but 17 responses were excluded according to exclusion criteria (physical challenge, injury in the last 6 months, auditory impairment, or medical conditions that contraindicated for exercise). The final sample number for this study was 203 (n=203). The response rate was 92.3% among the respondents. Ethical clearance was obtained from the committee board of INTI International University.

2.2 Procedure

A self-validated questionnaire was developed to determine the association between music and motivation to do exercise among university students based on a previous similar study (Hallett & Lamont, 2017). The questionnaire included demographic data and music usage during exercise (type of music, criteria to select music, etc.). Behavioral Regulation in Exercise Questionnaire-version 3 (BREQ-3) was used to assess the exercise motivation of participants as the questionnaire has shown a Cronbach's alpha reliability of more than 0.70 (Markland & Tobin, 2004). Whereas, the Godin-Leisure Time Exercise Questionnaire (LTEQ) for

participants was adopted to self-report their exercise intensity as the questionnaire reports an excellent test-retest reliability (r=0.86) (Eisenmann et al., 2002). Permissions were obtained to research private universities around Nilai, Negeri Sembilan, Malaysia. The electronics questionnaire was sent through email and social media. The explanation of the study procedure was informed before the survey and participants were required to submit the informed consent form before proceeding. The survey was terminated for those who selected electronics boxes of exclusion criteria. Data from the questionnaire was collected and analyzed.

2.3 Statistical Analysis

The data obtained from the questionnaire were analyzed using Statistical Package for the Social Sciences (SPSS window version 26.0). Descriptive statistics were used to summarize and report the demographic data as frequency and percentage for each section in the questionnaire. Association between physical activity level and preference of music during exercise were analyzed using Chi-square while the point-biserial correlation was adapted to determine the relationship between music and exercise motivation. The association or correlation is considered statistically significant if the p-value < 0.05.

3 Results

3.1 Demographics details of the subjects

Table 1 showed the general characteristic of the respondents. In the current study, 35.0% (n=71) male and 65.0% (n=132) female participated in this study. For the age distribution, most of the participants were in the age group of 18-23 years old (78.3%, n=129). Among female participants (n=132), 80.3% (n=106) listen to music during exercise while 80.3% (n=57) of male participants (n=71) listen to music during exercise. There is no statistically significant association between gender and the use of music during the exercise session (p=0.997). Table 3 shows a detailed preference of the music according to gender.

3.2 Use of music during exercise sessions and preference of music during exercise session according to gender and age

The analysis reported that 80.3% (n=163) of participants listen music during exercise and 19.7% (n=40) did not listen to music during exercise. The most common reasons for participants not listening tomusic were to stay focus on the activity (40.0%) followed by choosing to exercises in a quiet environment (30.0%). Table 2 shows the detailed frequency and percentage of not listening to music during exercise.

Both age groups have shown a high percentage of use of music during exercise. However, the association between age and listen to music during exercise is not statistically significant (p=0.318). Table 4 shows a detailed preference of the music according to the age group.

3.3 Importance of music as motivation, preferred type of music and factors influence the choice of music

The participants who listen the music during exercise (n =163) were questioned to rate how much music does motivate them during exercise sessions on a 6-point scale (1 = not at all important, 6 = very important).

Those who listen to music during exercise (n=163) were analyzed further for the preferred type of music during exercises (multiple responses were possible). Across 163 participants, energetic and rhythmic music (n=110, 67.5%) was the most popular to listen to during exercise, followed by upbeat and conventional music (n=77, 47.2%). Table 6 shows the details of the preferred type of music during exercise.

The mean importance rating was 5.03 (SD = 0.965). Most of the participants responded that music is an important motivation during exercise. Table 5 showed the detailed analysis of the importance of music as motivation during exercise.

Also, criteria for selecting music to exercise (multiple responses were possible) was surveyed among participants who listen to music during exercise (n=163). Speed/ tempo/ bpm (n=102, 62.6%) are the most important factor when choosing exercise music, followed by rhythm (n=99, 60.7%) and mood (n=93, 57.1%). The detailed factors were shown in Table 7.

There is a significant negative correlation between motivation and listen to music during exercise (r=-0.193,p=0.006), between external regulation and listen to exercise music (r=-0.173, p=0.014), between identified regulation and listen to music (r=-0.193, p=0.006), between integrated regulation and listen to music during exercise (r=-0.215, p=0.002). These results indicated that individuals who didn't listen to music during exercise score lower in motivation, external regulation, identified regulation, and integrated regulation.

3.4 Relationship between music and motivation to do exercise

The Behavioural Regulation in Exercise Questionnaire (BREQ-3) was used to determine the motivation for physical activity among participants.

Table 8 shows the results of mean scores for each subscale of BREQ-3 and its relationship with listening to music during exercise. Participants who listen to music (n=163) has a higher average score for each subscale when compared to the participants who did not listen to music during exercise (n=40).

Table 1 General characteristic of the respondents

Variable	Participants (N)	Percentage (%)
Gender		
Male	71	35.0
Female	132	65.0
Total	203	100.0
Age		
18-23	159	78.3
24-30	44	21.7
Total	203	100.0

Table 2 Reasons of not listening to music during exercise

Reasons	Participants (N)	Percentage (%)
Safety	7	17.5
Wish to focus on the activity	16	40.0
Prefer Quiet	12	30.0
Wanted to connect with the environment	5	12.5

Table 3 Preference of music during exercise session according to gender

Variables	Female		Male		Total	
	Frequency	Percentage (%)	Frequency	Percentage (%)	Frequency	Percentage (%)
Usage of music						
Yes	106	80.3	57	80.3	163	80.3
No	26	19.7	14	19.7	40	19.7

Table 4 Preference of music during exercise session according to age

Variables	Age groups			
	18-23		24-30	
	Frequency	Percentage (%)	Frequency	Percentage (%)
Usage of music				
Yes	130	81.8	33	75.0
No	29	18.2	11	25.0

Table 5 Importance of music as motivation during exercise

Rating	Participants (N)	Percentage (%)
2	2	1.2
3	10	6.1
4	31	19.0
5	58	35.6
6	62	38.0
Total	163	100.0

Table 6 Preferred Type of Exercise Music

Music type	Participants (N)	Percentage (%)
Reflective and Complex (classical, jazz, blues, folk)	31	19.0
Intense and Rebellious (Alternative, rock, heavy metal)	45	27.6
Upbeat and Conventional (Country, pop, religious, soundtracks)	77	47.2
Energetic and Rhythmic (Rap/ hip-hop, soul/funk, electronica/ dance, synchronous running music)	110	67.5
Spoken word (Podcasts and audiobooks)	9	5.5

Table 7 Factors When Choosing Exercise Music

Factor	Participants (N)	Percentage (%)
Speed/ tempo/ bpm	102	62.6
Style/ genre	62	38.0
Memories/ associations	22	13.5
Rhythm	99	60.7
Melody	66	40.5
Mood	93	57.1
Cultural fit	6	3.7

Table 8 Mean motivation scores for each subscale of BREQ-3 and its relationship with listening to music during exercise (p-value)

Subscales of BREQ-3	Mean scores	Listen to music during exercise		r =	p-value
		Yes	No		
Amotivation	2.867	2.928	2.619	-0.193	0.006
External Regulation	1.229	1.300	0.938	-0.173	0.014
Introjected Regulation	2.148	2.221	1.850	-0.131	0.062
Identified Regulation	2.867	2.928	2.619	-0.193	0.006
Integrated Regulation	2.477	2.564	2.119	-0.215	0.002
Intrinsic Regulation	2.883	2.948	2.619	-0.170	0.015

Table 9 Association between LTEQ scores and listen to music during exercise

LTEQ Scores	Listen to music during exercise				p-value
	Yes		No		
	Participants (N)	Percentage (%)	Participants (N)	Percentage (%)	
Active	118	72.4	18	45.0	0.003
Moderately active	35	21.5	15	37.5	
Insufficiently active	10	6.1	7	17.5	
Total	163	100.0	40	100.0	

The average score of intrinsic motivation was highest among the subscales for those who listen to music during exercise and there is a significant negative correlation between intrinsic motivation and listen to exercise music (r=-0.170, p=0.015). This indicates that the intrinsic motivation score was lower among individuals who didn't listen to music during exercise.

Although there was a negative correlation between introjected regulation and the use of music during exercise (r=-0.131), the correlation is not significant (p=0.062).

3.5 Association between music and physical activity level

Table 9 shows the association between Godin-Leisure Time Exercise Questionnaire (LTEQ) scores and listening to music during exercise. Across participants who listen to music (n=163), 72.4% (n=118) of participants are active, 21.5% (n=35) are moderately active and 6.1 % (n=10) are insufficiently active. Participants who did not listen to music are less active compared to those who listen to music while exercising.

4 Discussion

4.1 Use of music during exercise sessions

In this study, there was a higher percentage of participants who listen to music during exercise compared to those who did not listen to music while exercising. The choice of listening and not listening to music could be explained by the way an individual perceives the experiences of music based on their personality traits and cognitive ability (Chamorro-Premuzic & Furnham, 2007). Besides, the differences in response to music can be explained by an individual's aesthetic judgment, episodic memory, cultural background, or subjective experiences (Juslin, 2013; Clark et al., 2016).

Most of the participants reported that they wish to focus on the activity thereby choose not to listen to music during exercise. Music is reported as a dissociative factor for individuals that want to focus on their breathing and how they feel physically (Razon et al., 2009; Hutchinson & Karageorghis, 2013). Individuals with high training performance perceived music as distract or that have to be avoided (Hallett & Lamont, 2017).

The results of this study showed that females equally listen to music as males. However, other studies suggested women were more favoring to listen to music during exercise compared to men and the result was significant (Priest et al., 2004; Hallett & Lamont, 2017). Both age groups in this study have shown a higher percentage of listening to music while exercising. Younger individuals (<26 years old) feel more motivated and stimulated with music during exercise compared to older adults (>46 years old) (Priest et al., 2004).

Across participants who did not listen to music during exercise (n=40), 45.0% (n=18), 37.5% (n=15) are moderately active and 17.5% (n=7) are insufficiently active. There is a significant association between physical activity level (LTEQ) and listen to music during exercise (p=0.003).

Participants generally believed music plays an important role in motivation to do exercise as the mean importance rating of music was 5.03 on a 6-point scale. This result was in line with a previous study where participants reported a high mean importance rating (4.42) of music in sports and exercise (Laukka & Quick, 2013). According to the same study that surveyed emotional use of music in sports and exercise, participants reported intentions for listening to music during exercise including enhancing motivation, positive effect, endurance, and flow to make training more pleasurable and efficient (Edworthy & Waring, 2006).

Energetic and rhythmic was the most preferred type of music to listen during exercise among university students followed by upbeat and conventional and intense and rebellious. This result was similar to another study that concluded hip hop, rock, pop, and country music were popular types of music to listen to during exercise among college students (Barney et al., 2012). Besides, prioritization of speed/ tempo/bpm when selecting music as exercise motivation showed a high percentage in the current study whereby tempo of a song shown to improve the physical level during exercise (Edworthy & Waring, 2006; Waterhouse et al., 2010; Hallett & Lamont, 2017).

4.2 Relationship between music and motivation to do exercise

BREQ-3 has been adopted in the current study to determine the level of motivation to perform the exercise. The questionnaire consists of few components such as motivation, external regulation, introjected regulation, identified regulation, integrated regulation, and intrinsic regulation. The results of this study showed that each subscale of motivation component was higher among participants who exercised with the presence of music compared to those who did not listen to music while exercising.

There was a significant negative correlation between integrated regulation and exercise with music. This denotes the importance of music in improving the integrated regulation of university students during exercise. Integrated regulation was referred to as the behavior of an individual which represents their identity and personal values (Ryan & Deci, 2000) and plays as a key contributor for exercise frequency and duration among regular exercisers (Duncan et al., 2010). Identified regulation represents commitment in the behavior that is individually important and outcomes of the behavior appreciated by the individual (Ryan & Deci, 2000). Identified regulation has been shown to contribute to the increase in exercise frequency (Duncan et al., 2010). In this

study, there was a significant negative correlation between the use of music during exercise and identified regulation. Thus, individuals that listened to music while exercising are more likely to engage in exercise as identified regulation was higher.

The average score of intrinsic regulation of participants who listened to music during exercise was highest among all subscales. Intrinsic motivation is derived from individual satisfaction or interest that doesn't get affected by an external factor such as rewards (Duncan et al., 2010; Maloney, 2013). A significant negative correlation between intrinsic regulation and listen to music during exercise was found in the current study. Similarly, another study reported higher intrinsic motivation was associated with a higher frequency of exercise among university students (Li, 1999).

Motivation refers to when a person participates in behavior without any intention to perform it while extrinsic motivation comes from an external factor influenced by an outcome (Maloney, 2013). In this study, both external regulation and motivation were higher in those who listened to music during exercise and there is a significant correlation between listening to music during exercise and external regulation and between the use of music during exercise and motivation. Thus, music has been shown to influence an individual to perform the exercise while acting as an adjunct motivating tool. Introjected regulation is the longing to prevent self-imposed punishments or to accomplish intrapersonal achievements (Ryan & Deci, 2000). There was a significant influence of introjected regulation in improving exercise intensity (Edmunds et al., 2006). However, the result of this study shown there was no significant correlation between the use of music during exercise and introjected motivation.

Motivational music was recognized as music that controls arousal, decreases perceptions of exertion and enhances mood (Karageorghis & Priest, 2012a). Music can be used for the management of ideal arousal as well as the positive effect that is important for motivation and performance in sports (Hanin, 2007). Thus, motivational music can improve positive emotion thereby enhance psychological states even at a high intensity of exercise and indirectly, develop exercise adherence (Hanin, 2007). Theoretically, motivational stimuli such as music and video serve to decrease the harmful effects of fatigue on the efferent control of working muscles by readjusting electrical activity in the brain (Bigliassi et al., 2016). The findings of cerebral effects of music during exercise revealed that music altered attention toward unrelated thoughts and enhanced affective arousal thus reducing fatigue-associated symptoms and exercise perception (Bigliassi et al., 2018). Thus, music can be used as a prime tool to increase the level of motivation among students which indirectly enhances the performance, adherence, and mood towards exercise.

4.3 Association between music and physical activity level

Most of the participants who listened to music during exercise were categorized to be active (72.4%). The results of this study also showed there was a significant association between physical activity and the use of music during exercise. Motivational use of music during exercise improves commitment to exercise (Karageorghis & Priest, 2012a). Lowering perceptions of exertion is the crucial role of music in recreational exercise to increase the amount of effort performed by minimizing the negative feeling usually connected with high-intensity exercise (Karageorghis & Priest, 2008). Awareness of fatigue while exercising reduced with aid of music thereby provides a more enjoyable experience. Music has been proved to distract perceived exertion during physical activity thereby encourage a more pleasant experience of exercise (Bonnette et al., 2010). In a study that compared exercising in the presence of music, without music and noise situation, a significant improvement was seen in the heart rate, amount of work done and time spent exercising among participants who exercise with music (Thornby et al., 1995). Music enhances the positive feeling of exercise thus improves exercise adherence leads to an increased level of physical activity among those listening to music during exercise.

Secondly, music has proved to exert recuperative effects on health that fasten recovery from training, competition, or injury (Terry & Karageorghis, 2011). In a study that investigates the effects of music listening after stressful tasks among college students, there were significant improvements in neuroendocrine responses, immune function, and emotional states (Hirokawa & Ohira, 2003). When post-exercise fatigue was improved with the use of music during exercise, people are more likely to engage in the next exercise session thus improve overall physical activity level.

There are few limitations in this study such as the type of exercise performed by the participants was not included in this study as some of the exercises such as swimming is difficult to perform with music. In terms of sample collection, a smaller sample size of this study may misrepresent the study population.

Moreover, less response from male compared to female participants may affect the reliability of the study. Future studies may consider the type of exercise performed by the participants, larger sample size, and equal gender representation of the study population.

Conclusion

The current study explored the association of music as a motivation to perform exercise among university students. The result revealed that music can be a useful tool in maintaining the motivation level among students to engage in physical exercises.

Energetic and rhythmic music is the most popular music for exercise among university students while the most important criteria for choosing music to exercise was tempo/ bpm/ speed. Regular participation in exercises has shown to maintain good health, regulate mood, increase productivity, restrain mental and health emotion as well as improve sleep quality.

In summary, commitment to regular exercise encouraged by the use of music reduces the physical inactivity of university students as the motivational level were higher compared to those who don't listen to music.

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SENSORY EVALUATION OF CAROTENOID AND PALM JAGGERY INCORPORATED FUNCTIONAL DRINK JIGARTHANDA

Revathi P[1*], Vasantha Esther Rani[2]

[1]Assistant Professor, Department of Food Science and Technology, Arul Anandar College (Autonomous), Karumathur, Madurai, Tamil Nadu, India
[2]Associate Professor and Head, Research Department of Home Science, Fatima College (Autonomous), Madurai, Tamil Nadu, India

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Jigarthanda

Carotenoid

ABSTRACT

Functional foods are any fresh or processed food claimed to have a health-promoting or disease-preventing property beyond the basic function of the supply of nutrients. Functional foods like beverages offer potential health benefits that could enhance the well-being of consumers and reduce the economic and social costs of treating non-communicable diseases. Jigarthanda is one of the best tasting summer drink which has its origins in Madurai. The present study was carried out on the development of the functional drink Jigarthanda incorporated with carrot and palm jaggery using basic ingredients such as milk, Kova, and almond gum. The functional drink was prepared with three variations viz., VI, VII, and VIII in two proportions i.e. A and B. In proportion A, all the three variations were incorporated with 100 ml of carrot juice, and also VI, VII, and VIII contain 100 g, 150g, and 200g of palm jaggery respectively. In proportion B all the three variations were incorporated with 200 ml of carrot juice and also VI, VII and VIII contain 100 g, 150g, and 200g of palm jaggery respectively. Among the three variations, in Variation I, all the sensory factors were marked high with the mean value of 7 in both Proportion A and B. In Variation II and Variation III, all the factors in both proportions, A and B were marked high with the mean score of more than seven, and particularly in variation III, the taste factor got a high mean score of more than 8, and the overall acceptability was very high in Proportion B and the mean score was 7.3, which was more than the proportion A (7.27). Hence the functional drink Jigardhanda in VIII of Proportion B, which was made with 250gram palm jaggery, incorporated with 200 ml of carotenoid-rich carrot juice obtained the highest overall acceptability score and was regarded as the best sensory evaluated variation of Jigarthanda. The popular traditional beverage Jigarthanda has been value-added by incorporating carotenoid-rich functional food to the consumers.

* Corresponding author
E-mail: revathi.rpr@gmail.com (P. Revathi)

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1 Introduction

Jigarthanda is one of the famous cold beverages in the South Indian city of Madurai in Tamil Nadu. Jigarthanda Drink (*Jigar* means Heart and *Thanda means* Cool) is a chilled beverage or drink, meaning "Cool Heart" in English. Hence it is the best drink for the hot summer. Milk, almond gum, sarsaparilla syrup, sugar, and ice-cream are the main ingredients used for the preparation of Jigarthanda.

As it is a sweet drink, Jigarthanda energizes the body. It has potentially beneficial effects because it contains milk, which has a range of bioactive components (Mounika & Maloo, 2018). Further, almond gum and sarsaparilla syrup are natural body coolants. The almond gum is rich in protein and useful to boost male vitality, increase body weight, and gain muscle. The unique taste and health benefits of Jigarthanda is the only reason that makes this, a perfect cool drink to beat the scorching heat of summer (Jeyashri, 2019).

Functional foods are ingredients that offer health benefits that extend beyond their nutritional value it contains. The effective way of improving health status for all age groups is the regular intake of functional foods. At present, professionals are recognizing that some functional components of foods have a major role in health enhancement. Functional foods are any fresh or processed foods, which have a health-promoting or disease-preventing property other than the basic function of the supply of nutrients. Potential health benefits were offered by functional foods and it could enhance the well-being of consumers and reduce the economic and social costs of treating non-communicable diseases (Das et al., 2010).

Carrot is a root vegetable, which is rich in bioactive compounds like carotenoids and dietary fibers. These are rich in several other functional components having significant health-promoting properties like vitamin K1, potassium, and antioxidants. Since carrot has natural antioxidants and anti-cancer properties, the consumption of carrot and its products is increasing steadily (Sharma et al., 2012).

Palm Jaggery is made from palm juice, a traditional healthy product, prepared without using any chemicals. Minerals are rich in palm jaggery, especially iron (Le et al., 2020). The regular consumption of palm jaggery improves digestive health.

It has been observed that the sweetener present in jaggery activates the digestive enzymes, enhances the regular bowel movement, and cleanses the system. Palm Jaggery, also helps to relieve the most common cold and cough (Singh et al., 2013).

Jigarthanda is a milk-based sweet cool drink. Milk is a complex mixture of specific bioactive proteins, lipids, and saccharides and has several biologically active components (Donovan, 2006).

Except for iron and vitamin C, milk contains all the micronutrients like vitamins and trace elements. It also contains major nutrients like carbohydrates, protein, and fat.

The regular intake of functional components like carotenoids incorporated in food provides more health benefits. Carrot is a good source of carotenoids, vitamins, and dietary fiber (Nicolle et al., 2004). It is also a good source of minerals and antioxidants (Arscott & Tanumihardjo, 2010). Carotenoids are important micronutrients for human health. (Castermiller & West, 1998).

The edible portion of carrots contains carotenoids range from 6,000 to 54,800 µg /100 g (Simon & Wolff, 1987). The carotenoids are a precursor of vitamin A and it has been linked with the improvement of the immune system (Nocolle et al., 2003) and reduce risk of degenerative diseases like cancer, CVD, age-related macular degeneration, cataract formation (Ranganna, 1993), and Alzheimer's disease (Zaman et al., 1992).

Usually, to make Jigarthanda, a sweet drink, cane sugar is added with milk during Jigarthanda preparation. Instead of white cane sugar, the addition of palm jaggery provides the nutrient iron. Palm jaggery is a more nutritious sap based product providing numerous health benefits. It contains 1.04 % protein, 0.19 % fat, 76.86% sucrose, 1.66% glucose, 3.15% total minerals, 0.861 % calcium, 0.052 % phosphorus, 11.01 % iron and 0.767 % copper (Morton, 1988). Almond gum can be dissolved in water and is used in making jellies, health drinks, milkshakes, etc. Almond gum has the role of an emulsifier, stabilizer, and texturing additive which has been utilized by the food and pharmaceutical industries.

Since Jigarthanda has been one of the favorite delicious drinks for all the age groups, the present study focuses on the acceptability of the functional drink Jigarthanda with the incorporation of low cost, locally available functional food ingredients carrot and palm jaggery along with the basic ingredients. The present study was carried out with the aim of (i) formulation of value-added functional drink Jigarthanda and (ii) evaluation of the acceptability of the formulated functional drink Jigarthanda.

2 Materials and Methods

2.1 Procurement of raw materials

For the formulation of Jigarthanda, Fresh standard milk (Fat 4.5 & SNF 8.5), almond gum, and palm jaggery were procured from the departmental store located in Madurai. The carrot was purchased from the local market, Madurai. The ingredients used for preparing the Jigarthanda drink have a cooling effect on the system (Sailu, 2015).

Table 1 Proportions of the Ingredients used for the Functional drink Jigarthanda

Ingredients	Variation- I		Variation- II		Variation- III	
	Proportion A	Proportion B	Proportion A	Proportion B	Proportion A	Proportion B
Full Cream Milk	1 Liter	1 Liter	1 Liter	1 Liter	1 Liter	1 Liter
Palm Jaggery	100 g	100 g	150 g	150 g	250 g	250 g
Milk Kova	50 g	50 g	50 g	50 g	50g	50g
Carrot Juice	100 ml	200 ml	100 ml	200 ml	100 ml	200 ml
Almond Gum	200 g	200 g	200 g	200 g	200 g	200 g

Table 2 Sensory Evaluation of Formulated Functional Drink Jigarthanda

Functional Drink Jigarthanda	Factors	Proportion A (Carrot Juice-100 ml)		Proportion B (Carrot Juice-200 ml)	
		Percentage	Mean Value	Percentage	Mean Value
Variation I	Appearance	83.3	7.5	83.3	7.5
	Colour	85.0	7.65	85.5	7.7
	Consistency	84.4	7.6	82.2	7.4
	Taste	83.3	7.5	82.2	7.4
	Flavor	80.0	7.2	80.0	7.2
	Overall Acceptability	76.6	6.9	77.7	7.0
Variation II	Appearance	84.4	7.6	83.3	7.5
	Colour	85.0	7.65	86.1	7.75
	Consistency	83.3	7.5	81.6	7.35
	Taste	85.0	7.65	86.1	7.7 5
	Flavor	80.5	7.25	81.1	7.3
	Overall Acceptability	77.7	7.0	79.4	7.15
Variation III	Appearance	84.4	7.6	84.4	7.6
	Colour	86.1	7.75	87.2	7.85
	Consistency	83.3	7.5	82.2	7.4
	Taste	88.8	8.0	89.4	8.05
	Flavor	80.5	7.25	80.5	7.25
	Overall Acceptability	80.8	7.27	81.1	7.3

2.2 Preparation of carrot juice

Carrot Juice was prepared from orange color highly nutritious fresh carrots. It is rich in α and β-carotene, B-vitamins, folate, calcium, magnesium, potassium, copper, and iron (Schieber & Weber, 2016). The process starts with the carrots being scrubbed; washed and sorted (any damaged carrots are removed). The carrots are shredded and the juice was pressed out of the shredded carrots (Davis & Waldron, 2010) in the food processor or blender. A puree was made by adding small amounts of water to the carrots and giving them a nice stir. It is kept for 15-20 minutes and this will boost its nutrient properties. Finally, the juice was strained through a hand strainer (Karanreed, 2018).

2.3 Preparation of Almond gum

5 grams of almond gum was taken and soaked in 1 liter of water for 8 hours / overnight. It almost increased 10 times in volume. It absorbed all the water and turned transparent like a jelly. It was refrigerated until use.

2.4 Formulation & standardization of jigarthanda

Initially, three variations of the cold beverage Jigarthanda were prepared. The proportion of the various ingredients used for preparing the functional drink Jigarthanda is tabulated in Table 1. Jigarthanda has been prepared by the procedure given by Jeyashri (2019). One liter of full cream milk was taken in a stainless steel pan and boiled on high flame. To this, 100g/150g/ 250 g of palm jaggery were added in the variations combinations such as VI, VII, and VIII. It was stirred until dissolved. The full-fat milk was reduced by slowly boiling the milk until it thickened and reduced to almost half of its original quantity. The color of the reduced milk which was light brown gave Jigarthanda drink its unique color and flavor. The sides are scraped every 5 minutes to collect the cream from the milk. Then 50 grams of sweet less milk Kova was added into all three variations and then 100ml / 200 ml of carrot juice was added into VI, VII, and VIII. This was heated for just 5 to10 minutes and refrigerated until chilled for 4-5 hours. Then 200 ml of soaked almond gum was added into all the three Variations of prepared Jigarthanda and then stored at refrigerator temperature.

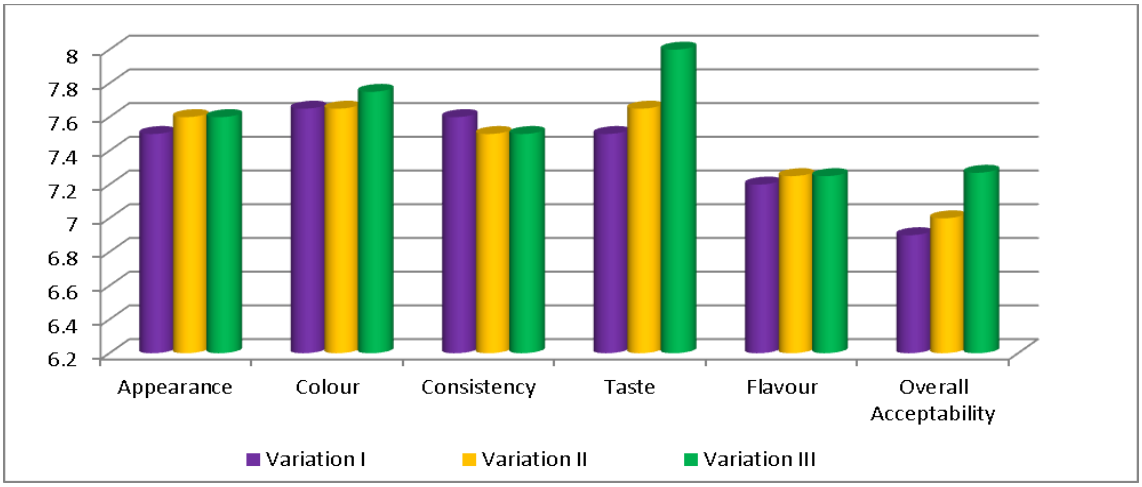

Figure 1: Sensory Evaluation of Formulated Functional Drink Jigarthanda
Proportion A (Carrot Juice-100 ml)

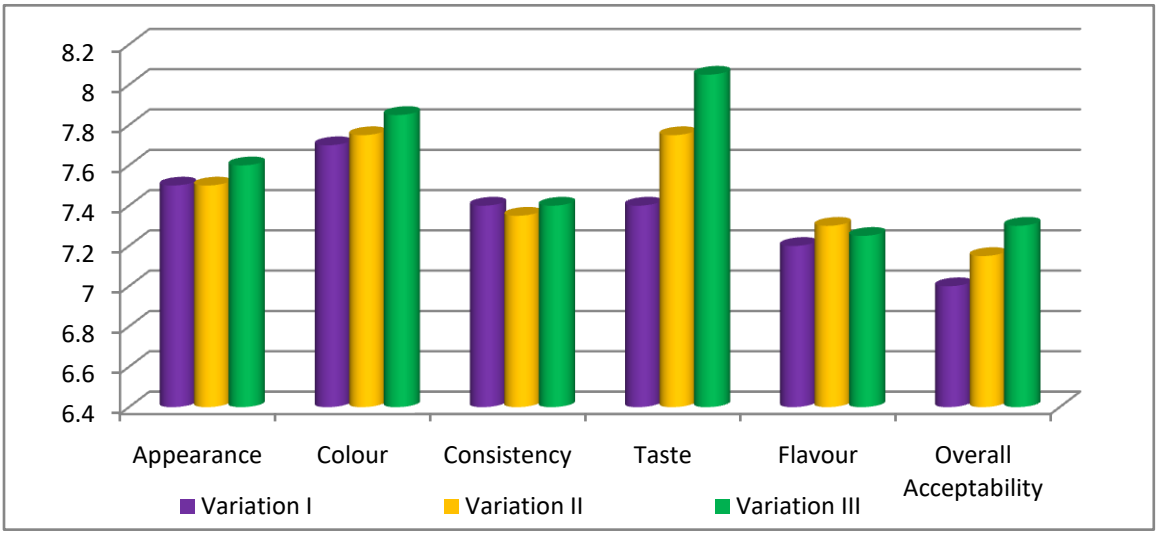

Figure 2: Sensory Evaluation of Formulated Functional Drink Jigarthanda
Proportion B (Carrot Juice-200 ml)

2.5 Sensory evaluation of formulated functional drink Jigarthanda

Sensory science is "a scientific discipline used to evoke, measure, analyze, and interpret reactions to meat sensory characteristics as perceived by the senses of sight, smell, touch, taste, and hearing" (Miller, 2001). The sensory attributes of the Jigarthanda namely appearance, color, consistency, taste, flavor, overall acceptability was evaluated using a nine-point hedonic scale as described by Ranganna (1993). The scoring was allotted from 9 to 1 respectively like extremely, like very much, like moderately, like slightly, neither like nor a dislike, dislike slightly, dislike moderately, dislike very much and dislike extremely.

Three variations were prepared separately and the products were subjected to the same methods of sensory evaluation. A nine-point hedonic scale scorecard was provided to the panelists to adjudge the quality of the product prepared by three different variations concerning the appearance, color, consistency, taste, flavor, and overall acceptability.

3. Results

Sensory Evaluation deals with analyzing and interpreting the qualities of food as they are perceived by the sense of appearance, color, flavor, consistency, taste, flavor, overall acceptability. Sensory evaluation is designed to reflect common preferences and to maintain the quality of food at a given standard condition. All three variations of the functional drink were prepared by using 100g, 150g, and 250 g of palm jaggery having two different concentrations of carrot juice viz., A (100ml) and B (200ml), and each was tested by a panel of 20 members.

The panel evaluated the formulated functional drink Jigarthanda for its sensory attributes namely appearance, color, consistency, taste, flavor, and overall acceptability. The findings are given in table 2 and also given in chart 1 & 2, which exhibits the overall ranking score for the formulated functional drink Jigarthanda in different proportions. Among the three variations, Variation I was marked high for all the studied factors with a mean value of 7 in both Proportion A and B, except the overall acceptability of Proportion A has a mean value below 7. While for Variation II and in Variation III, all the factors in both proportions A and B were marked as high with a mean score of more than 7 and particularly in variation III, the taste factor got a high mean score of more than 8 and the overall acceptability was very high in Proportion B and the mean score was 7.3, which was more than proportion A (7.27) but not statistically different. The results of the present study correlate with a similar study conducted on the acceptability of carrot powder and wheat flour mixed biscuit, evaluated in terms of color, flavor, texture, and overall acceptability by taste panel members. As per the sensory analysis, it was found that the biscuit incorporated with 6% carrot powder had maximum acceptability comparatively control (Mounika & Maloo, 2018). According to Phoebean et al. (2017), the biscuits incorporated with carrot powder and cowpea flour enrich the biscuit value and increasing the protein and carotenoid contents. Boland (2010) revealed that the incorporation of 20% carrot in paneer showed the highest acceptability by the panelists. The proximate analysis demonstrated that the formulated product was rich in nutritional values when compared to pure cow milk paneer. Also, it was suggested that the incorporation of natural products such as carrot in paneer can be used as a better source to enhance nutrition.

4 Discussion

Jigarthanda, being one of the delicious milk-based favorite drink of all age groups, was chosen to incorporate it with functional foods, to make it a value-added drink. The value addition in Jigarthanda seemed to be one of the best ways to improve human health status. Nutrient-rich generally available foods have been selected and incorporated to provide a functional drink to fulfill the nutrient demands of individuals. Carrot is rated tremendously high among vegetable foods when correctly utilized. It is a commendable source of a considerable quantity of carotenoids and other nutrients. Subsequently, in the current study, an attempt was made to formulate carrot juice and palm jaggery incorporated Jigarthanda to make it an enhanced nutritional value-added drink.

Sensory evaluation is a significant feature that reciprocates with taste, aroma, and visual appearance which includes the sensory attributes of foods. The only way to evaluate sensory characteristics by the grades of intuition experienced by a human being was by judging food which is attributed to the decision of the consumer. The study revealed that the functional drink Jigarthanda in VIII of Proportion B, which was made with 250 grams palm jaggery, incorporated with 200 ml of carotenoid-rich carrot juice obtained the highest overall acceptability score that was regarded as the best sensory evaluated variation of Jigarthanda.

Conclusion

In the present study, the sensory evaluation was analyzed ant it elicited that carrot and Palm Jaggery incorporated functional drink Jigarthanda was of high priority, in terms of sensory attributes like appearance, texture, and taste. Besides, it proved to be a nutrient-packed favorite milk-based drink Jigarthanda. Thus the popular traditional beverage Jigarthanda has been value-added to provide carotenoid, an irreplaceable nutraceutical, which can be commercialized to children and adults, the most likely consumers.

Recommendation

As Jigarthanda is a very popular delicious drink, more research on this product can make this nutritious drink available to the common man. Some suggestions for further studies (i) The formulated value-added Jigarthanda with the highest acceptability can be subjected to further study on shelf life, packaging methods, and cost analysis (ii) Analyze the nutritive value of the formulated functional drink Jigarthanda.

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