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PROPHYLACTIC EFFECT OF CAMEL MILK ON PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN CCL_4-INTOXICATED RATS

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Rats

ABSTRACT

Present study has been carried out evaluate prophylactic effect of Camel milk on physiological and biochemical changes in CCl4-intoxicated rats. A total of 36 male albino rats were randomly divided into 6 groups viz. control group (G1), silymarin (SM) group (G2), camel milk (CM) group (G3), CCl_4-intoxicated group (G4), silymarin prophylactic group (G5) and camel milk prophylactic group (G6). Each group has six rats. After completion of the study (8 wks), blood samples were collected and hematological parameters, liver contents of glutathione (GSH), malondialdhyde (MDA) , superoxide dismutaseactivity (SOD), serum urea, uric acid, creatinine, testosterone, estradiol were measured. The results of this study revealed that CCl_4 toxicity significantly reduced Hb level, RBCs count and PCV, whereas it significantly increased the WBCs count with respect to normal control. Intoxication of CCl_4 in G4 rats led to microcytic hypochromic anaemia, increased erythrocyte fragility and, leukocytosis was accompanied neutrophils increases and a decrease in lymphocyte counts. Also, CCl_4 produced significant increase in serum urea, uric acid, creatinine and estradiol level and decrease in circulating testosterone level compared with normal rats. Pre-treatment with CM and SM brought significant restoration in hematological, renal function parameters and sexual hormones disturbance. Moreover, lipid peroxidation and oxidative stress were obviously noted in CCl_4 intoxicated (G4) organism where a

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significant increase in liver MDA and decrease in its content of GSH and SOD activity. Contrarily, pre-treatment with CM and SM did not only decrease liver content of MDA but also increased hepatic GSH and SOD activity, suggesting that CM and SM attenuated CCl_4-induced oxidative stress. In conclusion, CM and SM had a considerable prophylactic effectiveness against hematotoxicity, oxidative stress, lipid peroxidation, renal impairment and sexual hormones disturbances developed by intraperitoneal injection of rats withCCl_4.

1 Introduction

Liver is the largest body organ and plays a vital role in detoxification of deleterious materials. It regulates numerous metabolic functions and maintains body homeostasis (Mayuren et al., 2010). Liver disorders are one of the most common problems throughout the world. Liver injury can be induced by certain xenobiotics and microbialin filtration via ingestion or infection (Hai et al., 2011). Carbon tetrachloride (CCl_4) is well-known as xenobiotic agent. Liver is not the only the target organ of CCl4 but it also affects several body organs such as lungs, heart, testes, kidneys and brain (*Oz*turk et al., 2003). Evidence demonstrated that CCl_4 activated highly reactive trichloromethyl radical in liver which initiates free radical mediated lipid peroxidation of cell membrane phospholipids (rich with polyunsaturated fatty acids) which are vulnerable to peroxidation. Accordingly, various functional and morphological changes are developed in liver cell membrane which caused an accumulation of lipid-derived oxidants and finally liver injury encountered (Singh et al., 2008). CCl_4 is rapidly absorbed by liver tissue in humans and animals. Once it absorbed, it is widely spread among tissues, especially those with high lipid content, reaching peak concentrations in <1–6 hours, depending on exposure dose or its duration time (U.S. EPA. IRIS, 2010).

Oxidative stress and membrane damage in hepatocytes, mainly caused via CYP2E1 (Manibusan et al., 2007). Also, CCl_4 alter the antioxidant profile of the liver by reacting with sulfhydryl groups of glutathione (GSH) and thiols group of protein, including the antioxidant enzymes as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione transferase (GST) (Knockaert et al., 2012; Yang et al., 2015).

Camel milk (CM) is an excellent source of well-balanced nutrients. It exhibits a range of biological activities that influence digestion, metabolism, growth and development of specific organs. These biological properties are mainly due to the presence of certain peptides and proteins in milk (Yagil et al., 1984; Korhonen & Pihlanto, 2001). Camel milk is different from other ruminant milk; it has low cholesterol, sugar and protein but high minerals such as sodium, potassium, iron, copper, zinc and magnesium. Besides this, presence of vitamins A, B2, C and E was also reported in camel milk. The presence of high insulin concentration was also reported in camel milk (Knoess, 1979). Along with this it can be consumed by lactase-deficient individuals because of non-allergic properties of camel milk. A series of metabolic and autoimmune diseases can be successfully cured by camel's milk. In India, camel's milk widely used therapeutically against dropsy, jaundice, problems of the spleen, tuberculosis, asthma, anemia, piles and diabetes (Rao et al., 1970). Further, antibacterial and antiviral activities of CM were also studied by El-Agamy et al., (1992).

Silymarin (SM), a polyphenolic flavonoid confined from milk and this is another antioxidant that has been found affective against liver injuries induced by various hepatotoxins including CCl_4 (Shaker et al., 2011; Bektur et al., 2016). SM also prevents lipoperoxidation of membranes and scavenges ROS, thus increases GSH availability (Parveen et al., 2011; Vargas-Mendoza et al., 2014). The aim of this study was to investigate the prophylactic effect of camel milk and silymarin on hemotoxicity, oxidative stress, lipid peroxidation, renal function and sex hormone disturbances in CCl_4-intoxicated rats.

2 MATERIALS AND METHODS

2.1 Chemicals

Carbontetrachloride (CCl_4) obtained from LobaChemie, India. Solution of CCl_4 prepared by dissolving in 50% olive oil V:V and injected intraperitoneally in to the experimental rats at a dose of 1 ml/kgb.w, once daily, 3 times weekly for four weeks to induce toxicity as described by Abdel-Moneim et al. (2015).

Raw silymarin was obtained from ElobourMedern Pharmaceutical Industries Co., Egypt. Rats were given silymarinorally at a dose of 150 mg/kgb.w suspended in distilled water (Chen et al., 2012). Recommended dose of silymarin were given once daily, 5 times in a week for 2 weeks and 3 times in a week for next 4 weeks.

Early morning, hand milking camel milk (CM) was collected daily from western desert camel farm in sterile screw capped

containers and transported to the laboratory in cool boxes. CM was given to rats in a dose of 5 ml/ rat according to El Miniawy et al. (2014), once daily, 5 times in a week for 2 weeks and 3 times in a week for next 4 weeks.

2.2 Experimental animals

Total thirty six (36) adult male albino rats weighing about 180g in average were used for this study. They were selected among the animals bred in the small Animal House of the Nuclear Research Center, Atomic Energy Authority, Egypt. The animals were acclimatized for two weeks under ambient environmental conditions and housed in well aerated cages.

2.3 Animal grouping and treatment

Animals were randomly assigned into six equal groups viz. Normal control animals, without any treatment (G1), SM ingested (G2), CM drenched (G3), CCl_4 intoxicated (G4), SM prophylactic group (G5 – SM treatment for first six weeks + CCl_4 treatment started from fifth week of SM treatment and continue for the next 4 weeks) and CM prophylactic group (CM treatment for first six weeks + CCl_4 treatment started from fifth week of CM treatment and continue for the next 4 weeks) and they were fed on a balanced rodent diet. They had free access to feed and drinking water from the beginning of the experiment until its termination

At the end of each treatment and after overnight fasting, animals were decapitated and trunk blood samples were collected in tubes with or without anticoagulant for assaying hematological indices, serum levels of renal function tests and sex hormones. Simultaneously liver was excised from each scarified rat. Promptly liver samples were rinsed in 0.1 M cold phosphate buffer (PH 7.4) and homogenized using a Teflon pestle to prepare 10% homogenates used for assessment of oxidative stress and peroxidation biomarkers.

2.4 Hematological Measurements

Blood samples were collected in EDTA coated tubes and used for determination of hematological parameters included hemoglobin (Hb) level, erythrocytes (RBCs), platelets (PLT) and leucocytes (WBCs) counts which were evaluated according to Dacie & Lewis (1993). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from the values of PCV, Hb and RBCs count as described by Jain (1986). For differential leucocyte counts, blood smear were stained with Giemsa.

2.5 TBARS and antioxidant enzymes assays

Lipid peroxidation biomarkers were expressed as malondialdhyde (MDA) and determined according to Satoh (1978). Reduced glutathione (GSH) was assayed as described by Beutler et al. (1963) and superoxide dismutase was measured by the procedure given by Nishikimi et al. (1972).

2.6 Levels of serum testosterone and estradiol estimation

Levels of serum testosterone and estradiol were assayed by RIA kits (10227-Czch Republic purchased from IMMUNOTECH Company) by following manufacturer's instructions.

2.7 Renal function tests

Renal function tests were determined by following the method of Fawcett & Scott, (1960) for urea, Barham &Trinder, (1972) for uric acid and Larsen, (1972) for creatinine.

2.8 Statistical Analysis:

The obtained results were expressed as means ± standard errors. The data were subjected to F test one way analysis of variance (ANOVA) according to Snedecor & Cochran (1982) followed by Duncan's multiple range test (Duncan, 1955) to determine the significance of difference ($P \leq 0.01$) between means of treated groups.

3 RESULTS

Result of study presented in table 1, revealed that administration of CCl_4 had negative effect on all studied hematological parameters. Significant reduction ($P < 0.01$) was reported in Hb level, RBCs numbers, PCV, MCHC, PLT and lymphocyte counts. While, an improvement ($P < 0.01$) was reported in MCV, MCH, WBCs and neutrophil counts in CM, SM treated animals as compared the control group. Similarly, when CM and SM were used as prophylactic agents against CCl4 toxicity, they nearly succeeded to bring back the values of the above mentioned parameters toward the basal figures of normal control groups.

Regarding to lipid peroxidation and oxidative stress, data presented in table 2 denoted that IP injection of rats with CCl_4 (G4) led to a significant increase ($P < 0.01$) in MDA content of liver. While, concentration of GSH and SOD activity were significantly ($P < 0.01$) reduced as compared with their corresponding values in control group. Pre- treatments of CM and SM remarkably repaired the negative effects of CCl_4 on hepatic MDA and GSH whereas the activity of SOD was completely restored and the level of GSH was partially returned close to the normal values of control group.

Table 1 Effect of camel milk and silymarin on hematological parameters of normal CCl_4 intoxicated rats

Group	Hematological parameters									
	Hb (g/dl)	RBCs (10^6/mm^3)	PCV %	MCV (fl)	MCH (Pg)	MCHC (g/dl)	PLT (10^3/mm^3)	WBCs (10^3/mm^3)	Neutrophils (%)	Lymphocytes (%)
G1	13.70^{b}±0.03	6.86^{b}±0.09	41.10^{cd}±0.22	60.01^{bc}±1.04	19.98^{b}±0.28	33.32^{a}±0.12	467.2^{bc}±33.6	11.82^{b}±0.40	24.17^{bc}±0.60	67.0^{ab}±0.73
G2	14.25^{a}±0.14	7.07^{ab}±0.14	44.0^{b}±0.40	62.68^{b}±0.88	20.28^{b}±0.27	32.45^{b}±0.14	386.2^{cd}±55.7	10.88^{b}±1.84	19.33^{c}±0.56	64.2^{bc}±0.60
G3	13.82^{b}±0.15	7.39^{a}±0.08	45.6^{a}±0.54	61.82^{b}±0.23	18.75^{c}±0.07	30.37^{d}±0.14	485.5^{abc}±29.7	12.32^{b}±0.53	21.83^{bc}±0.83	71.2^{a}±1.64
G4	10.29^{e}±0.19	4.23^{c}± 0.14	35.47^{e}±0.27	84.37^{a}±2.90	24.40^{a}±0.59	29.00^{e}±0.50	330.1^{d}±27.8	20.61^{a}±0.63	31.00^{a}±2.44	58.60^{c}±2.70
G5	13.12^{c}±0.06	7.20^{ab}±0.13	42.02^{c}±0.22	58.33^{bc}±1.13	18.38^{c}±0.42	31.23^{c}±0.11	599.3^{a}±31.4	12.53^{b}±1.19	22.33^{bc}±1.28	61.8^{bc}±0.87
G6	12.70^{d}±0.17	7.07^{ab}±0.06	40.40^{d}±0.44	57.25^{c}±0.36	18.03^{c}±0.25	31.43^{c}±0.30	517.8^{ab}±54.4	13.35^{b}±0.97	26.33^{ab}±2.81	61.3^{bc}±3.17

Data are expressed as Mean ± S.E, Mean values with different letters in the same column are significantly different at (P< 0.01)

Table 2 Effect of camel milk and silymarin treatment on hepatic lipid peroxides markers as (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD) of normal rats and CCl_4 intoxicated rats

Group	Liver Functioning Parameters		
	MDA (nmol/g.tissue)	GSH (mg/g. issue)	SOD (U/g. tissue)
G1	11.89^{b}±0.36	61.99^{a}±0.56	12561.5^{a}±367.7
G2	8.04^{bc}±0.94	58.40^{a}±1.38	12357.9^{a}±190.5
G3	6.69^{c}±1.40	59.20^{a}±2.12	11735.2^{ab}±263.0
G4	20.38^{a}±2.19	22.87^{c}±0.77	10517.9^{c}±364.4
G5	22.31^{a}±1.62	40.08^{b}±1.23	10864.5^{bc}±443.8
G6	19.75^{a}±1.49	38.60^{b}±1.38	11517.5^{abc}±179.5

Data are expressed as Mean ± S.E, Mean values with different letters in the same column are significantly different at P< 0.01

Table 3 Effect of camel milk and silymarin treatment on serum urea, uric acid and creatinine of normal and CCl_4 intoxicated rats

Group	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
G1	41.64^{c}±1.60	3.13^{a}±0.11	1.07^{bcd}±0.05
G2	49.92^{b}±1.81	2.38^{b}±0.15	0.96^{cd}±0.05
G3	48.92^{b}±1.10	2.18^{b}±0.06	0.92^{d}±0.09
G4	56.76^{a}±1.65	3.56^{a}±0.25	1.47^{a}±0.06
G5	48.03^{b}±1.15	3.00^{a} ±0.23	1.14^{bc}±0.07
G6	48.00^{b}±2.31	3.20^{a} ±0.32	1.23^{b}±0.06

Data are expressed as Mean ± S.E, Mean values with different letters in the same column are significantly different at P< 0.01

Table 3 visualized that CCl_4 intoxication induced obvious renal impairment as the levels of serum urea and creatinine were significantly (P< 0.01) higher. While, insignificant increase of uric acid was observed in G3 as compared to control group. Clearly, the prophylactic effects of CM and silymarin was evident when they were given to CCl_4 intoxicated rats. This allegation is confirmed by significant (P< 0.01) decrease noted in the level of urea, uric acid and creatinine in pre CM and SM treated groups as compared with those injected with CCl_4.

The data cited in table 4 revealed that administration of CCl_4 to male albino rats led to disturbances in the levels of serum sex hormones. Where testosterone was significantly (P< 0.01) decreased and estradiol was significantly (P< 0.01) increased in comparison to normal control group. Alternations of these hormones were significantly (P< 0.01) reversed by pre administration of CM and SM to CCl_4 intoxicated rats. Unsurprisingly, the results of this investigation revealed that administration of CM alone to normal male rats led to significant (P< 0.01) increment of estradiol level.

4 Discussion

In the present study, reduction was noted in the levels of blood Hb, RBCs count and PCV in CCl_4 intoxicated rats than healthy control, CM and SM group may attributed to the cytotoxic effect and suppression of the erythropoiesis caused by CCl_4. Similar results were reported by Mandal et al. (1998). In this study, negative alternations was reported in Hb, RBCs, PCV, MCV, MCH and MCHC in CCl_4 group which indicated that CCl_4 intoxication caused microcytic hypochromic anemia. Tung et al. (1975) referred these effects to disturbed hematopoiesis, destruction of erythrocytes and reduction in the rate of their formation and/or their enhanced removal from circulation. Moreover, Makni et al. (2012) added that peroxidation developed

Table 4 Effect of camel milk and silymarin treatment on serum testosterone and estradiol levels of normal and CCl_4 intoxicated rats

Group	Testosterone (ng/ml)	Estradiol (pg/ml)
G1	$6.30^{b} \pm 0.31$	$67.25^{c} \pm 2.62$
G2	$3.52^{c} \pm 0.18$	$85.51^{c} \pm 2.37$
G3	$7.34^{a} \pm 0.23$	$105.56^{b} \pm 0.72$
G4	$0.52^{f} \pm 0.08$	$134.36^{a} \pm 12.11$
G5	$1.55^{e} \pm 0.15$	$72.63^{c} \pm 2.23$
G6	$2.51^{d} \pm 0.24$	$83.39^{c} \pm 2.22$

Data are expressed as Mean ± S.E, Mean values with different letters in the same column are significantly different at $P< 0.01$

by CCl_4 led to a destruction of membrane protein, alternation of membrane bound enzymes as well as erythrocyte osmotic fragility. The significant increase in WBCs noticed herein CCl_4 group which may be due to the release of marginal neutrophils and other neutrophil pool into the circulation which produced the observed neutrophilia in those rats which were under the influence of stress hormones and catecholamine (Swenson, 1993).

Results of this study revealed that CM ingestion can mitigate all the adverse effects of CCl_4 on hematological parameters by the virtue of its high content of vit E which play a major role in maintaining the flexability of RBCs and reduce the fragility and damage as a result of the oxidation of membrane phospholipids of RBCs (Kraus et al., 1997). This vitamin also has a direct impact on the formation of RBCs in the bone marrow (McDowell, 2000). Likewise CM, SM possesses hematopoietic activity since it is nothematotoxic. According to Kumarappan et al. (2010) camel milk also has antioxidative properties. Therefore, when CM or SM was used as prophylactic agents in combination with CCl_4, they can almost reverse negative features of hemtoxicity.

GSH is the major thiol in mammalian cells which prevents interaction of ROS with critical cellular constituents. It is the master non enzymetic antioxidant that can directly conjugate with free radical and its status may consider a good measure of antioxidant. MDA is the major oxidative degeneration product of membrane unsaturated fatty acids which is the end product of lipid peroxidation.

In the present study, administration of CCl_4 was accompanied by increased lipid peroxidation, reduced SOD activity and depletion in GSH in liver. Treatment with SM protects liver from GSH depletors such as paracetamol (Videla & valenzela, 1982). Increased lipid peroxidation is evidenced in this study by the elevated level of MDA in hepatic tissue of CCl_4 treated rats. These results are in agreement with the findings of Kim et al. (2003). The decreased level of MDA, increased content of GSH and elevated activity of SOD in CCl_4 intoxicated group after giving CM indicated that lipid peroxidation was inhibited. This action could be attributed to the high content of CM with vitamins C, A, E, zinc and magnesium. All these constituents are potent antioxidants can protect liver cells from injury (Barbagallo et al., 1999; Althnaian, 2012). In accordance with the results of the current study, Ibrahim et al. (2017) showed that CM has hepatoprotective action against acetominophenon induced hepatotoxicity in mice, possibly in part through antioxidative effects. El Miniawy et al. (2017) ascribed this positive effect of CM to the improvement observed in SOD activity and reduction of the lipid peroxidation. The effect of CM similar to the effect of SM in comparison with CCl_4-intoxicated rats.

Kidney function tests include blood urea, uric acid and serum creatinine. Among these, serum creatinine is a useful indicator of regular filtration in kidney. It is the end product of creatine metabolism in muscle. Urea and uric acid are the principal waste products of protein catabolism. They are synthesized in the liver from the ammonia which produced as a result of amino acids de-amination (Young et al., 1972). In the kidney, creatinine is distilled by the glomerulus and excreted by the tubules and only free creatinine appears in the blood serum (Baker et al., 1979).

In the present study, the increased levels of serum urea, creatinine was reported in CCl_4 treated group and this was significantly higher than the control one which reflected the impairment in renal functions alongside with oxidative stress in kidney. These results are in accordance with findings of Churchill et al. (1983), Perez et al. (1987), Khan et al. (2009) and Hamed et al. (2012). It is believed that CCL treatment causes glomerular hypercellularity, moderate to severe necrosis and tubule interstitial alterations which cause alteration in the capacity for tubular absorption thus bringing about functional overload of nephrons with subsequent renal dysfunction (Adewole et al., 2007).

High levels of urea and creatinine indicates sever disturbances in kidney functioning (Maxine & Benjamin, 1985). Supplementation of CCl_4 injected rabbits with CM significantly reduced the rise in serum creatinine levels, compared to CCl4–induced rabbits by enhancing the renal function that is generally impaired in CCl4–induced rabbits (Omar & Hmmam, 2014). Further, Venkatanarayana et al. (2012) and Yacout et al. (2012) showed that administration of CC14 causes nephrotoxicity which significantly elevated level of urea and creatinine inside the serum which attributed the damage of nephron structural integrity (Khan et al., 2012). The increased creatinine level suggests that muscular wasting occurred during CCl_4 intoxication since creatinine production has a direct relationship with muscle mass (Banfi& Del Fabbro , 2006).

The presence of vit C can significantly minimize blood urea nitrogen (BUN) level in diabetic rats (Al-Shamsi et al., 2006). CM have to three times higher vit C level than dairy milk (Farah et al., 1992). Hence it is postulated that the higher content of vit C in CM may act as scavenger of free radicals developed as a result of CCl_4 toxicity (Cecen et al., 2010).

Present study revealed that when SM or CM were ingested together with CCl_4, kidney function tests were significantly improved as compared to CCl_4 group. It is well known that gonadal activity relies on normal liver function. However, specific clinical signs of hypogonadism are commonly encountered in patients suffering from liver cirrhosis (Youssef & Mullen, 2002; Zietz et al., 2003). It is well established that liver plays an essential role in synthesis of all compounds sharing in the maintenance of anadaquate endocrine homostasis such as hormones, their transport proteins and conversion enzymes (Youssef & Mullen, 2002). Serum testosterone (the male sex hormones), FSH and LH were considerably decreased; While, estradiol and prolactin concentrations were remarkably raised in CCl_4 intoxicated rats (Sahreen et al., 2013). In this manner, results of present study are concord with the findings of Youssef & Mullen (2002) and Sahreen et al. (2013). The reduction in serum testosterone indicates either a direct effect of CCl_4 on the number of leyding cells or as indirect impact by disturbing the hormonal environment at hypothalamopitutary axis (Latif et al., 2008). The enhancement of serum estradiol noted herein in CCl_4 intoxicated group over control one could be ascribed to the conversion of androgens to estrogens under the effect of aromatase enzyme and deactivation of sex hormones by specific enzymes in CCl_4 intoxicated liver. Thus, increased serum estradiol may be liable for the origin of hypogonadism observed in the current study. This result are in agreement with those obtained by Abdel-Rahman & El-Nahary (2004) and Abdel Kader (2017) those who recorded a reduction in testosterone hormones after i.p. injection of CCl4 (1 mg/kg b. wt.). Administration of CM to CCl_4 intoxicated rats resulted in increased level of testosterone hormone significantly compared to CCl_4 intoxicated rats. Soliman et al. (2016) showed that CM has an effective therapeutic role in improvement the altered gene expression luteinizing hormone receptor (LHR) using the semi-quantitative polymerase chain reaction (RT-PCR) in diabetic rats. LH acted on testicular leydig cell and stimulated the synthesis of testosterone, so the plasma level of testosterone is increased. The level of testosterone was significantly increased in CM group than SM group in CCl_4-intoxicated rats. Meanwhile, no difference in the levels of estradiol between CM and SM groups in comparison to CCl_4 group.

In this investigation, treatment of rats with CCl_4 in combination with CM ameliorated the level of testosterone but this rise was not great enough and significantly different from CCl_4. This improvement may be attributed to the high content of CM with vit C which scavenge superoxide, H_2O_2 and hydroxyl radicals, hence prevents lipid peroxidation (Curney et al., 1996; Veldink et al., 2007). CM is rich in zinc and magnesium which helps in absorption and metabolism of other powerful antioxidants such as vit E and C (Yousef et al., 2006).

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