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Effect of *Psidium guajava* Juice on The Seminiferous Tubules Diameter and Epithelium Thickness in *Rattus norvegicus* Exposed by Lead Acetate

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Rats

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ABSTRACT

Lead is one of humans and animals' most common and hazardous heavy metals. This study aimed to investigate the effect of white guava (*Psidium guajava*) fruit juice on the seminiferous tubule diameter and epithelium thickness in rats (*Rattus norvegicus*) exposed to lead acetate. The research design was a completely randomized design (CRD). A total of 25 male rats with an average weight of 200 grams were used for the study, divided into 5 treatment groups, each consisting of 5 rats. The treatments were as follows: the control group (C) was given distilled water orally; the T0 group was induced with lead acetate (50 mg/Kg BW) orally; the T1, T2, T3 groups were induced with lead acetate (50 mg/Kg BW) and then given 25%, 50%, and 100% concentration of white guava fruit juice, respectively. All the treatments were conducted for 14 days. The histopathology slides of the testis were made with HE staining, and the seminiferous tubule diameter and epithelium thickness were measured. The data were analyzed using One Way ANOVA and Duncan test ($p<0.05$). The results showed that the control group (C) which was given distilled water only had a seminiferous tubule diameter and epithelium thickness of 336.24±23.32 µm and 66.46±4.39 µm, respectively. The T0 group which was induced with lead acetate only showed a reduction in the diameter and epithelium thickness of seminiferous tubules (243.38±49.35 µm and 44.08±14.45 µm). The members of the T1, T2 and T3 groups showed positive effects on the diameter (323.49±22.82 µm; 314.41±13.04 µm; 325.04±16.88 µm, respectively) and epithelium thickness (56.36±3.36 µm; 60.50±3.81 µm; 66.744±9.50 µm, respectively). There was no significant difference reported between each group. The administration of guava juice to rats induced with lead acetate can positively affect the diameter and epithelium thickness of seminiferous tubules.

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1 Introduction

Heavy metal pollution is an unavoidable problem due to the rapid growth of the population (Yang et al. 2018). This issue is crucial because heavy metals are toxic, persistent in the environment, and bioaccumulative (Ali et al. 2019). Among the heavy metals, lead (Pb) is one of the most common pollutants found in the environment (Hansda et al. 2014). Lead (Pb) is known for its widespread presence and is considered one of the most hazardous heavy metals for human and animal health (Hansda et al. 2014; Song and Li 2015; Assi et al. 2016).

In recent years, studies have reported a decline in male fertility worldwide (Xu et al. 2019). This decline has been observed in humans and animals vulnerable to lead exposure (Assi et al. 2016). Lead has been found to have spermicidal potential in both humans and animals, as reported by Wang et al. (2013). Previous research has shown that exposure to lead in male rats can damage the reproductive system, such as changes in cell morphology, increased apoptosis, hormonal disruption, and impaired semen quality parameters (Lovaković 2020). Additionally, lead exposure in male reproduction causes the overproduction of Reactive Oxygen Species (ROS), which leads to the inhibition and alleviation of the activity of antioxidant enzymes such as alkaline phosphatase, superoxide dismutase, glutathione peroxidase, and catalase (El-Magd et al. 2017). This high ROS level caused by lead toxicity can lead to oxidative stress, which in turn can lead to testicular impairment (Sudjarwo et al. 2019).

Oxidative stress can damage cell membranes and disrupt hormone receptors that play a role in spermatogenesis, reducing germ cells (Al-Olayan et al. 2014). This decrease in spermatogenic and Sertoli cells can result in a thinning of the seminiferous epithelium and a reduction in the diameter of the seminiferous tubule (Tripathi et al. 2015). The diameter and thickness of the seminiferous tubules are often used as indicators of testicular toxicity (Vidal and Whitney 2014).

It has been found that antioxidants can protect the seminiferous tubules of testes from lead-induced toxicity by inhibiting free radicals (Abarikwu et al. 2020). Animal studies have demonstrated that herbal plant extracts containing various antioxidants can help to reduce oxidative stress induced by lead and thus protect against lead-induced damage (Diana et al. 2017).

Due to its delicious taste and flesh, white guava (*Psidium guajava*) is a popular fruit in Indonesia. It contains various bioactive compounds such as flavonoids, carotenoids, terpenoids, triterpenes, and more, which give it medicinal properties (Zhang et al. 2020). White guava is rich in antioxidants such as quercetin, lycopene, vitamin C, vitamin E, and vitamin A (Naseer et al. 2018), which help balance the antioxidant and oxidant levels in the body. This makes it beneficial for infertile males as it can improve sperm production (Naseer et al. 2018). This study focuses on the effect of white guava (*P. guajava*) fruit juice with different doses on testicular seminiferous tubules' diameter and epithelium thickness in rats exposed to Lead acetate.

2 Materials and Methods

2.1 Experimental Animals

The research utilized a completely randomized design (CRD). Twenty-five male rats (*R. norvegicus*) aged 10-12 weeks and weighing 200 grams were housed in the Faculty of Veterinary Medicine experimental animal laboratory at Universitas Airlangga in Indonesia.

2.2 Lead Acetate Preparation

Lead acetate ($Pb(CH_3COO)_2$) suspension was made from lead acetate powder diluted in distilled water. The dosage that was used in this research was 50 mg/KgBW.

2.3 Guava Juice Preparation

Around two kilograms of fresh white guava were used to make the juice. First, the guava was washed with clean water and then directly ground without any added solvent. The resulting juice was filtered with a fruit juice filter and stored in a container. For 100% concentration of white guava juice, no solvent was added. For 50 and 25% concentrations, distilled water was added to dilute the juice to the required dosage (Chin et al. 2020). White guava juice was prepared every two days and stored in a refrigerator at a temperature of about 5^{o}C. The oral dosages of white guava juice for rats in this study were 100%, 50%, and 25% concentration.

The rats were given lead acetate to induce toxicity, and after four hours, they were administered guava juice for 14 days. The treatment groups were as follows: distilled water only (C), rats induced with 50 mg/kg BW lead acetate (T0), rats induced with 50 mg/kg BW lead acetate and 25% concentration of white guava juice (T1), rats induced with 50 mg/kg BW lead acetate and 50% concentration of white guava juice (T2), and rats induced with 50 mg/kg BW lead acetate and 100% concentration of white guava juice (T3).

2.4 Testis Histology Sample Preparation

After 14 days of treatment, all the rats were anaesthetized with a combination of ketamine (100mg/kg BW) and xylazine (10mg/kg BW) given intraperitoneally. They were then humanely sacrificed by cervical dislocation, following the guidelines set out by Flecknell (2015). The rats' abdomen was surgically opened to collect their testes, which were subsequently stored in a container filled with 10% formalin solution.

Table 1 Effect of the various imposed treatments on the diameter and the epithelium thickness of seminiferous tubules in rats

Treatment Groups	Seminiferous Tubules Diameter (µm)	Epithelium Thickness of Seminiferous Tubules (µm)
C	336.24[b] ± 23.32	66.46[b]± 4.39
T0	243.38[a] ± 49.35	44.08[a] ± 14.45
T1	323.49[b] ± 22.82	56.36[b] ± 3.36
T2	314.41[b] ± 13.04	60.50[b] ± 3.81
T3	325.04[b] ± 16.88	66.74[b] ± 9.50

Data are mean of five replicates; ± Standard Deviation of the mean; Values without common letters differ significantly at LSD P<0.05

2.5 Examination and Data Analysis

The diameter of seminiferous tubules and the thickness of the epithelium will be measured by examining testis histology slides stained with hematoxylin-eosin (HE) using a microscope with 200X magnification. The resulting data will be analyzed using One-way ANOVA, followed by Duncan's test for multiple comparisons to compare each group respectively. A statistically significant difference will be considered if P < 0.05. The statistical analysis will be performed using the Statistics Product and Service Solution (SPSS) 20.0 software (Al-Arif 2018).

3 Results

Results presented in Table 1 revealed the effect of various imposed treatments on the seminiferous tubule diameter and epithelium thickness. The rats given lead acetate (T0) showed a reduction in the mean value diameter of seminiferous tubules (243.38 ± 49.35 µm), while this value was reported 336.24 ± 23.32 µm for the control group (C). The group that was administered with 25% concentration (T1) of white guava juice shows a tubule diameter of 323.49 ± 22.82 µm, while it was reported 314.40 ± 13.04 µm and 325.04 ± 16.88 µm mean value in case of 50% (T2) and 100% (T3) concentration of white guava juice respectively and this was significantly (P<0.05) higher than T0 group but no significant differences was reported in the various treatment groups.

Similar findings have been reported regarding the mean value of the seminiferous tubule epithelium thickness (Table 1). Among the imposed treatments, the lowest mean value was reported in the T0 group (45.79 ± 15.65 µm), while the highest mean value (66.45 ± 4.38 µm) among all groups was reported from the only water control treatment. In the case of guava juice treatments, seminiferous tubules epithelium thickness is increased with the increasing concentration of guava juice and the highest thickness was reported from the treatment group T3 (64.72 ± 12.18 µm), and it was followed by the mean value of T2 (60.50 ±3.81 µm) and T1 (59.21 ± 4.63 µm) groups but this difference was not significantly different (P<0.05).

4 Discussion

The study's findings showed that treating a group of rats with lead acetate (T0) decreased the mean value of their seminiferous tubule diameter and epithelium thickness. These results align with the findings of Dorostghoal et al. (2020) and Widawati et al. (2017), demonstrating that lead toxicity can reduce the seminiferous tubule diameter and epithelium thickness in rats' testes. Lead exposure can cause oxidative stress at the testicular level due to increased reactive oxygen species (ROS). This, in turn, triggers the process of germ cell apoptosis (Nurkarimah et al. 2017). When there is an imbalance between ROS and antioxidants in the body, the mitochondrial pores become oxidized. As a result, the mitochondrial membrane permeability is disrupted, allowing free radicals and cytochrome c to leak into the cytosol (Abdrabou et al. 2019). Once cytochrome c enters the cytosol, it binds to another protein, activating the caspase cascade and initiating the mitochondria-mediated apoptosis pathway (Selvakumar et al. 2013). Lead exposure also alters cytochrome c release and Bcl-2/Bax signaling, ultimately resulting in caspase-3-dependent death (Kiran Kumar et al. 2009; Corsetti et al. 2017).

According to recent research, exposure to lead may cause reproductive toxicity, leading to a higher rate of cell apoptosis. Lead exposure also induces oxidative stress, which results in the peroxidation of membrane lipids, causing a loss in membrane integrity and a decrease in membrane potential. This results in ATP depletion and DNA fragmentation, ultimately leading to cell necrosis (Zachary and McGavin 2012). Additionally, lead exposure causes a decrease in endogenous antioxidants such as catalase (CAT), glutathione peroxide (GPx), and glutathione (GSH), leading to a high level of ROS and oxidative stress (Vigeh et al. 2011). Moreover, lead toxicity can cause hypothalamic-pituitary-testicular axis disorders, which can reduce the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Apriliani et al. 2013). This reduction in FSH and LH leads to a disruption of spermatogenesis, causing a decrease in spermatogenic cells and a reduction in the epithelium's thickness and the seminiferous tubules' diameter (Ramu and Jeyendran 2013).

The results presented in Figure 1 indicate that the T1 group of rats had a higher epithelium thickness than the T0 group. Similar results were observed for the T2 and T3 treatment groups. Additionally, significant differences were observed between the control group and the T0, T1, T2, and T3 groups. The results

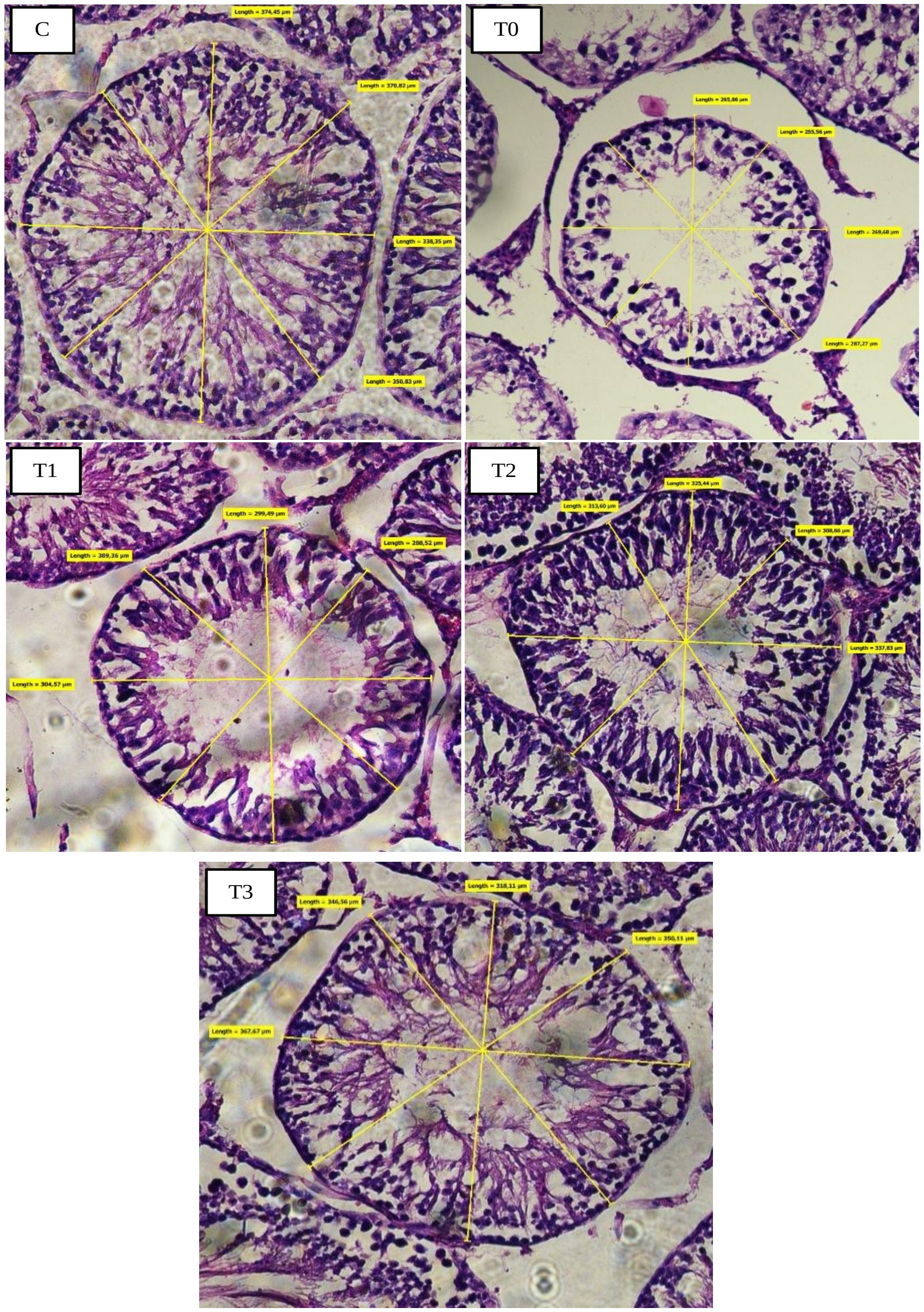

Figure 1 Histopathology and diameter of seminiferous tubules in groups C, T0, T1, T2, and T3 (200x, hematoxylin-eosing staining)

suggest that a 25-100% concentration range produced positive results and maintained normal diameter and epithelium thickness values similar to the control group (C).

Antioxidants are compounds that eliminate and scavenge the formation of ROS. Various studies have evaluated the effect of antioxidants on male fertility (Susanti et al., 2020). Many phytonutrients, such as lycopene, quercetin, vitamin C, and vitamin A, act as antioxidants and are found in white guava (Naseer et al. 2018). In cases of lead toxicity, quercetin blocks oxidative stress to protect male reproductive health and inhibits apoptosis by acting as a natural antioxidant and metal chelator (Meles et al. 2021). Several studies have shown that lycopene protects testes by reducing apoptosis and enhancing the scavenging of ROS. It also increases Bcl-2 expression, which means that lycopene is crucial in modulating and reducing the apoptotic process (Antonuccio et al. 2020; Trejo-Solis et al. 2013). Vitamin C, another antioxidant found in white guava, maintains the oxidation cycle of vitamin E.

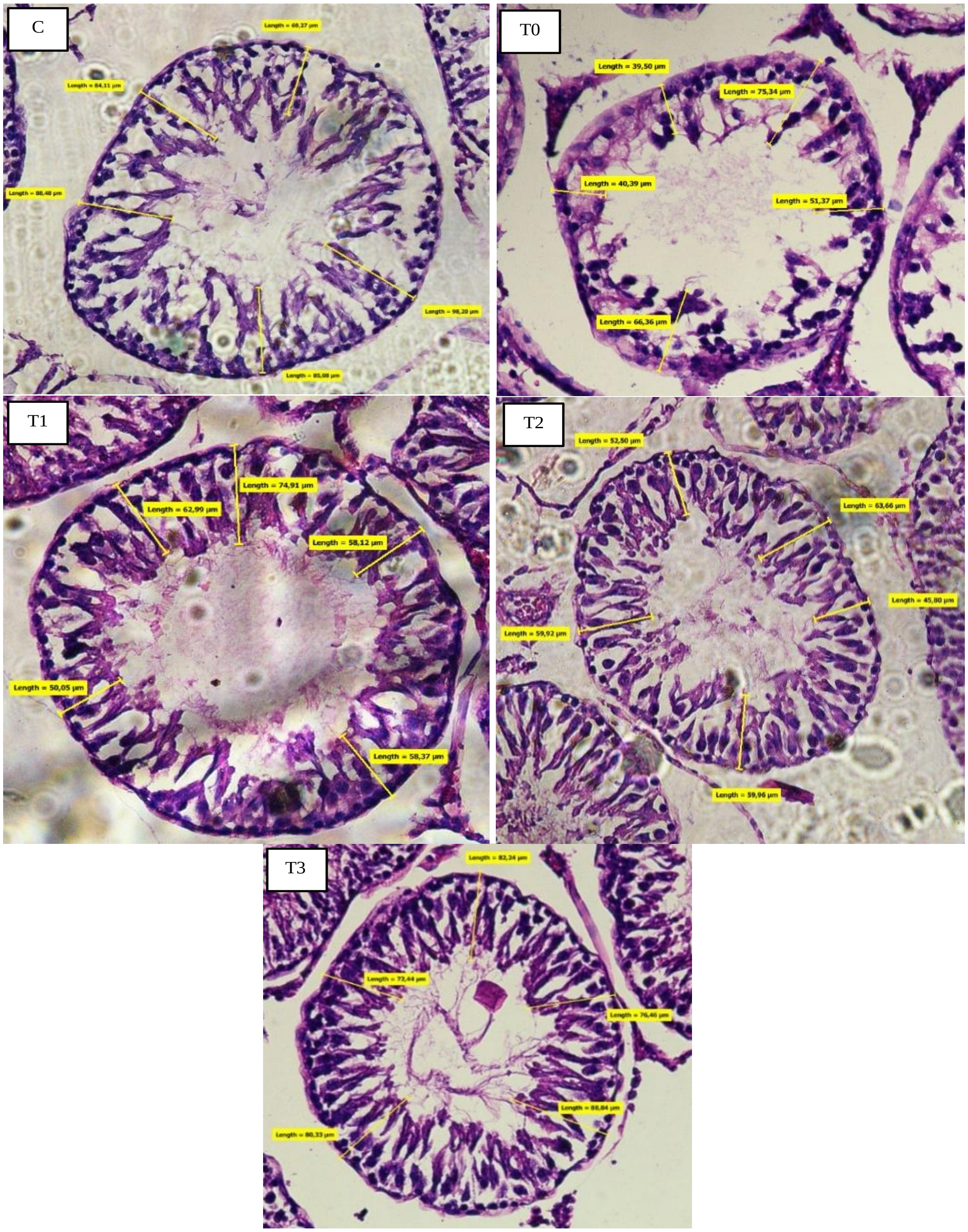

Figure 2 Histopathology and epithelium thickness of seminiferous tubules in groups C, T0, T1, T2, and T3 (200X, hematoxylin-eosing staining)

It neutralizes ROS and helps protect spermatogenic cells from oxidative damage (Freitas and de Oliveira 2018). Vitamin A, also present in white guava, can counteract free radicals (Hogarth and Grisworld 2010). Due to the presence of vitamins A and E, white guava fruit could positively affect the diameter of the seminiferous tubules in rats' testes induced with lead acetate.

In this study, no significant difference was found in the mean value of the diameter of seminiferous tubules between the negative control group (T0) and groups T1, T2 and T3 (Figure 2). These results are consistent with the findings of Wardani et al. (2019), where a higher concentration of extract led to a greater effect. Guava fruit juice contains a significant amount of moisture (water), crude fiber, protein, fat, ash, and carbohydrates such as phytonutrients, which have an antioxidant effect (Dakappa et al., 2013). However, the composition of white guava fruit juice can lead to poor bioavailability (McClements et al., 2015), and the blood-tissue barriers actively increase the impermeability of phytonutrients from the white guava juice (Mao et al. 2020). As a result, the potential benefits of many of the antioxidant phytonutrients may not be optimally effective.

Conclusions

The study's results suggest that different white guava fruit juice concentrations significantly affect rats exposed to lead acetate. The fruit juice plays an essential role in maintaining the diameter and epithelium thickness of the seminiferous tubules. While no concentration-dependent effect on the diameter and epithelium thickness of the seminiferous tubules in rats induced with lead acetate was reported, the value of these two parameters increased with the increasing concentration of white guava juice.

References

Abarikwu, S. O., Onuah, C. L., & Singh, S. K. (2020). Plants in the management of male infertility. *Andrologia*, *52*(3), e13509. https://doi.org/10.1111/and.13509

Abdrabou, M. I., Elleithy, E. M. M., Yasin, N. A. E., Shaheen, Y. M., & Galal, M. (2019). Ameliorative effects of *Spirulina maxima* and *Allium sativum* on lead acetate-induced testicular injury in male albino rats with respect to caspase-3 gene expression. *Acta histochemica*, *121*(2), 198–206. https://doi.org/10.1016/ j.acthis.2018.12.006.

Al-Arif, M. A. (2018). *Rancangan Percobaan.* Surabaya: Lentera Jaya Madina.

Ali, H., Khan, E., & Ilahi, I. (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of chemistry, 2019*. Article ID 6730305 | https://doi.org/10.1155/2019/6730305

Al-Olayan, E. M., El-Khadragy, M. F., Metwally, D. M., & Abdel Moneim, A. E. (2014). Protective effects of pomegranate (*Punica granatum*) juice on testes against carbon tetrachloride intoxication in rats. *BMC complementary and alternative medicine*, *14*, 164. https://doi.org/10.1186/1472-6882-14-164

Antonuccio, P., Micali, A., Puzzolo, D., Romeo, C., Vermiglio, G., et al. (2020). Nutraceutical Effects of Lycopene in Experimental Varicocele: An "In Vivo" Model to Study Male Infertility. *Nutrients*, *12*(5), 1536. https://doi.org/10.3390/nu12051536

Apriliani, M., Nurcahyani, N., & Busman, H. (2013). Efekpemaparankebisinganterhadapjumlahsel-sel spermatogenic dan diameter tubulus seminiferous mencit *(Mus musclus L)'*. Seminar Nasional Sains&Teknologi V, Lampung, Lembaga Penelitian, Universitas Lampung.

Assi, M. A., Hezmee, M. N., Haron, A. W., Sabri, M. Y., & Rajion, M. A. (2016). The detrimental effects of lead on human and animal health. *Veterinary world*, *9*(6), 660–671. https://doi.org/10.14202/vetworld.2016.660-671

Chin, J. H., Wong, K.H., & Yeong, S.O. (2020). Gastroprotective Effect of Chinese Cabbage (*Brassica oleracea* L. var. pekinensis) Juice in Sprague Dawley Rats. *The Natural Products Journal*, *10*(5), 587-594. https://doi.org/10.2174/2210315509666190902111029

Corsetti, G., Romano, C., Stacchiotti, A., Pasini, E., & Dioguardi, F. S. (2017). Endoplasmic Reticulum Stress and Apoptosis Triggered by Sub-Chronic Lead Exposure in Mice Spleen: a Histopathological Study. *Biological trace element research*, *178*(1), 86–97. https://doi.org/10.1007/s12011-016-0912-z

Dakappa, S. S., Adhikari, R., Timilsina, S.S., & Sajjekhan, S. (2013). A Review On The Medicinal Plant *Psidium Guajava* Linn. (Myrtaceae). *Journal of drug delivery and theraupetic*, *3*(2). https://doi.org/10.22270/jddt.v3i2.404

Diana, A. N., I'tishom, R., & Sudjarwo, S.A. (2017). *Nigella Sativa* Extract Improves Seminiferous Tubule Epithelial Thickness in Lead Acetate-Exposed Balb/c Mice. *Folia Medica Indonesiana*, *53*(3), pp.180-184. DOI:10.20473/fmi.v53i3.6444

Dorostghoal, M., Seyyednejad, S. M., & Nejad, M. N. T. (2020). Cichorium intybus L. extract ameliorates testicular oxidative stress induced by lead acetate in male rats. *Clinical and experimental reproductive medicine*, *47*(3), 161–167. https://doi.org/10.5653/ cerm.2019.03496

El-Magd, M. A., Kahilo, K. A., Nasr, N. E., Kamal, T., Shukry, M., & Saleh, A. A. (2017). A potential mechanism associated with lead-induced testicular toxicity in rats. *Andrologia*, *49*(9), 10.1111/and.12750. https://doi.org/10.1111/and.12750

Flecknell, P. (2015). *Laboratory Animal Anasthesia* (4th ed.). The Boulevard, Langford, Kidlington Oxford: Academic Press.

Freitas, M.L., & de Oliveira, R.A. (2018). Nutraceutical in male reproduction. *Brazilian Journal of Veterinary Medicine*, *40*(1), e220118-e220118. https://doi.org/10.29374/2527-2179.bjvm220118

Hansda, A., Kumar, V., Anshumali, & Usmani, Z. (2014). Phytoremediation of heavy metals contaminated soil using plant growth promoting rhizobacteria (PGPR): A current perspective. *Recent Research in Science and Technology*, *6(1), 131-134.* doi: 10.29374/2527-2179.bjvm220118.

Hogarth, C.A., & Griswold, M.D. (2010). The key role of vitamin A in spermatogenesis. *The Journal of clinical investigation*, *120*(4), pp.956-962. DOI: 10.1172/JCI41303

Kiran Kumar, B., Prabhakara Rao, Y., Noble, T., Weddington, K., McDowell, V. P., Rajanna, S., & Bettaiya, R. (2009). Lead-induced alteration of apoptotic proteins in different regions of adult rat brain. *Toxicology letters*, *184*(1), 56–60. https://doi.org/10.1016/j.toxlet.2008.10.023

Lovaković, B. T. (2020). Cadmium, arsenic, and lead: elements affecting male reproductive health. *Current Opinion in Toxicology*, *19*, 7-14. https://doi.org/10.1016/j.cotox.2019.09.005

Mao, B., Bu, T., Mruk, D., Li, C., Sun, F., & Cheng, C. Y. (2020). Modulating the Blood-Testis Barrier Towards Increasing Drug Delivery. *Trends in pharmacological sciences*, *41*(10), 690–700. https://doi.org/10.1016/j.tips.2020.07.002

McClements, D. J., Li, F., & Xiao, H. (2015). The Nutraceutical Bioavailability Classification Scheme: Classifying Nutraceuticals According to Factors Limiting their Oral Bioavailability. *Annual review of food science and technology*, *6*, 299–327. https://doi.org/10.1146/annurev-food-032814-014043

Meles, D. K., Mustofa, I., Wurlina, W., Susilowati, S., Utama, S., Suwasanti, N., & Putri, D. K. S. C. (2021). The Restorative Effect of Red Guava (*Psidium guajava* L.) Fruit Extract on Pulmonary Tissue of Rats (*Rattus norvegicus*) Exposed to Cigarette Smoke. *Veterinary medicine international*, *2021*, 9931001. https://doi.org/10.1155/2021/9931001

Naseer, S., Hussain, S., Naeem, N., Pervaiz, M., & Rahman, M. (2018). The phytochemistry and medicinal value of *Psidium guajava* (guava). *Clinical Phytoscience, 4*(1), 1-8.https://doi.org/10.1186/s40816-018-0093-8.

Nurkarimah, D.A., Hestianah, E.P., Wahjuni, R.S., Hariadi, M., Kuncorojakti, S., & Hermadi, H.A. (2017). Effect of Propolis on Spermatogenic Cells Number and Diameter of Seminiferous Tubules in Male Mice *(Mus musculus). KnE Life Sicences, 3(6)*: 677 – 683. https://doi.org/10.18502/kls.v3i6.1197

Ramu, S., & Jeyendran, R.S. (2013). The hypo-osmotic swelling test for evaluation of sperm membrane integrity. In Totowa, N.J. (Eds.) *Spermatogenesis* (pp. 21-25). Humana Press. https://doi.org/10.1007/978-1-62703-038-0_3

Selvakumar, K., Krishnamoorthy, G., Venkataraman, P., & Arunakaran, J. (2013). Reactive oxygen species induced oxidative stress, neuronal apoptosis and alternative death pathways. *Advances in Bioscience and Biotechnology*, *4*(1). Article ID:26913. DOI:10.4236/abb.2013.41003

Song, Q., & Li, J. (2015). A review on human health consequences of metals exposure to e-waste in China. *Environmental pollution (Barking, Essex : 1987)*, *196*, 450–461. https://doi.org/10.1016/j.envpol.2014.11.004

Sudjarwo, S. A., Anwar, C., Wardani, G., & Eraiko, K. (2019). Antioxidant and anti- caspase 3 effect of chitosan-Pinus merkusii extract nanoparticle against lead acetate-induced testicular toxicity in rat. *Asian Pacific Journal of Reproduction*, *8*(1), 13-19. DOI: 10.4103/2305-0500.250418

Susanti, N.F., I'tishom, R., & Khaerunnisa, S., (2020). Potensiekstrak Solanum betaceumterhadappeningkatanselspermatogenik pada mencit (Mus musculus) yang dipapar timbal asetat. *RisetInformasi Kesehatan*, *9*(1), 87-91. https://doi.org/10.30644/rik.v9i1.377

Trejo-Solís, C., Pedraza-Chaverrí, J., Torres-Ramos, M., Jiménez-Farfán, D., Cruz Salgado, A., et al. (2013). Multiple molecular and cellular mechanisms of action of lycopene in cancer inhibition. *Evidence-based complementary and alternative medicine : eCAM, 2013*, 705121. https://doi.org/10.1155/2013/705121

Tripathi, U. K., Chhillar, S., Kumaresan, A., Aslam, M. K., Rajak, S. K., et al. (2015). Morphometric evaluation of seminiferous tubule and proportionate numerical analysis of Sertoli and spermatogenic cells indicate differences between crossbred and purebred bulls. *Veterinary world*, *8*(5), 645–650. https://doi.org/10.14202/vetworld.2015.645-650

Vidal, J. D., & Whitney, K. M. (2014). Morphologic manifestations of testicular and epididymal toxicity. *Spermatogenesis*, *4*(2), e979099. https://doi.org/10.4161/21565562.2014.979099

Vigeh, M., Smith, D. R., & Hsu, P. C. (2011). How does lead induce male infertility?. *Iranian journal of reproductive medicine*, *9*(1), 1–8.

Wang, H., Ji, Y. L., Wang, Q., Zhao, X. F., Ning, H., et al. (2013). Maternal lead exposure during lactation persistently impairs testicular development and steroidogenesis in male offspring. *Journal of applied toxicology : JAT*, *33*(12), 1384–1394. https://doi.org/10.1002/jat.2795

Wardani, G., Ernawati, Eraiko, K., & Sudjarwo, S. A. (2019). The Role of Antioxidant Activity of Chitosan-*Pinus merkusii* Extract Nanoparticle in against Lead Acetate-Induced Toxicity in Rat Pancreas. *Veterinary medicine international*, *2019*, 9874601. https://doi.org/10.1155/2019/9874601

Widawati, T., Sudjarwo, S.A., & Hermadi, H.A. (2017). Protective Effect of Propolis Extract Against Lead Acetate Toxicity in Mice

(*Mus musculus*) Testes. *KnE Life Sciences*, *3*(6), 557-565. https://doi.org/10.18502/kls.v3i6.1183.

Xu, D., Hu, M. J., Wang, Y. Q., & Cui, Y. L. (2019). Antioxidant Activities of Quercetin and Its Complexes for Medicinal Application. *Molecules (Basel, Switzerland)*, *24*(6), 1123. https://doi.org/10.3390/molecules24061123

Yang, H., Ma, M., Thompson, J. R., & Flower, R. J. (2018). Waste management, informal recycling, environmental pollution and public health. *Journal of epidemiology and community health*, *72*(3), 237–243. https://doi.org/10.1136/jech-2016-208597.

Zachary, J.F., & McGavin, M.D. eds., (2012). *Pathologic Basis of Veterinary Disease 5*. Elsevier Health Sciences.

Zhang, W., Wang, J., Chen, Y., Zheng, H., Xie, B., & Sun, Z. (2020). Flavonoid compounds and antibacterial mechanisms of different parts of white guava (*Psidium guajava* L. cv. Pearl). *Natural product research*, *34*(11), 1621–1625. https://doi.org/10.1080/14786419.2018.1522313