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Original article

Effect of white guava (*Psidium guajava* L.) fruit juice on the quality of lead acetate induced rats (*Rattus norvegicus*) spermatozoa

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ABSTRACT

This study aims to determine the effect of the administration of white guava (*Psidium guajava* L.) fruit juice on spermatozoa plasma membrane integrity (PMI), morphological abnormality, viability, and motility of lead acetate induced rats (*Rattus norvegicus*). Twenty-five male rats were divided into five groups: NC (negative control) group, rats were administered with distilled water twice daily at four-hour intervals; T0 (positive control) group, rats were administered daily with lead acetate 50 mg/kg bw and distilled water four hours later; T1, T2, and T3 groups, rats were administered daily with lead acetate 50 mg/kg bw and 0.5 mL of 25, 50, and 100% white guava fruit juice four hours later. The treatment of the rats was conducted for 14 days, and on day 15, all rats were sacrificed to assess the spermatozoa quality. Data was analyzed using ANOVA followed by Duncan's multiple range test at a confidence level of 95%. The results showed that exposure to lead acetate (T0) caused lower spermatozoa PMI, viability, and motility as well as higher spermatozoa morphological abnormalities ($p < 0.05$) compared to those of the T0 group. Administration of white guava fruit juice starting at a dose of 25% (T1) resulted in higher spermatozoa motility, viability, and PMI as well as lower spermatozoa morphological abnormalities ($p < 0.05$) compared to rats in the T0 group. It could be concluded that white guava fruit juice maintained the spermatozoa quality of lead acetate induced rats.

Keywords: abnormality, guava, motility, spermatozoa plasma membrane integrity, viability

INTRODUCTION

Heavy metals are one of the elements contained in waste that pollute the environment. High levels of heavy metals in the surrounding environment result in food, water, and air contamination. Lead acetate is one of these heavy metals (Das *et al*., 2023). Lead acetate is the second most dangerous toxic substance after arsenic. More than 80% of daily sources of lead

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acetate exposure come from food, including drinks, soil, motor vehicle exhaust, and ingested dust. The entry route for lead acetate into the body is through the skin, breathing, and digestion (Kumar *et al*., 2020). Exposure to lead acetate caused poisoning depending on how much of the metal is bound to the body. Lead acetate had the ability to work as a barrier to enzyme action so that metabolic processes were disrrupted (Assi *et al*., 2016), which ultimately caused allergies, mutagens, teratogens, or carcinogens (Dasharathy *et al*., 2022). Several body organs that are targets of lead acetate exposure, are the circulatory system, nervous system, endocrine system, urinary tract, reproductive organs, and heart (Wani *et al*., 2015). Lead acetate affects the reproductive systems of animals and humans. In animals, lead acetate caused sterility, abortion, changes in the estrous cycle, teratogenic effects, abnormalities, decreased spermatozoa production, reduction in diameter, and edema of the seminiferous tubules (Tirpák *et al*., 2021).

In the reproductive system, lead caused abnormalities in the testicles, which work through testicular and testicular mechanisms (Dimitriadis et al., 2017). Damage to the testicles affected the quality of spermatozoa (El-Magd *et al*., 2017). Lead acetate could also reduce antioxidant levels, increased the production of free radicals in the body, and increased reactive oxygen species (ROS) (Sudjarwo *et al*., 2017). Excessive amounts of ROS caused oxidative stress and changed cell membranes so that they lost their main function, which was called lipid peroxidation. Molecules that ROS took up would lose one of their electrons, resulting in cell damage (Su *et al*., 2019). ROS could directly damage spermatozoa DNA by attacking purine pyrimidine bases. ROS-induced caspase enzymes damaged DNA and apoptosis of spermatozoa (Wagner *et al*., 2017). The spermatozoa plasma membrane, which is composed of unsaturated fatty acids, was susceptible to oxidative damage due to free radicals. Damage to the spermatozoa plasma membrane was detrimental to spermatozoa viability, abnormalities, and motility (Alahmar, 2019).

Spermatozoa can neutralize oxidant compounds with the enzyme glutathione peroxidase, uric acid, and the enzyme catalase, which work to neutralize the oxidant hydrogen peroxide. However, if the levels of free radicals are excessive, antioxidants need to be added. Antioxidants are substances or agents that scavenge reactive oxygen metabolites, inhibit their formation or enhancing endogenous antioxidant capabilities (Chaudhary *et al*., 2023). Natural antioxidants that could balance the scavenging system could be obtained from fruits and vegetables, which contained lots antioxidants (Rahaman *et al*., 2023). White guava fruit (*Psidium guajava* L.) is a fruit that has high antioxidant content. Guava is famous for its high vitamin C content. Apart from that, guava contains other chemicals that can affect antioxidants, such as flavonoid compounds, a combination of saponins with oleic acid, guajavarin, and quercetin (Tousif *et al*., 2022), and several researchers found high concentrations carotenoids (lycopene, β-carotene, β-cryptoxanthin) which functions as an antioxidant, anti-hyperglycemic, and anti-neoplastic (Lok *et al*., 2023).

Therefore, this study aims to determine the effect of administering white guava (*Psidium guajava* L.) fruit juice on the plasma membrane integrity, abnormalities, viability, and motility of lead acetate induced rats (*Rattus norvegicus*) spermatozoa.

MATERIALS AND METHODS

This study used 25 healthy male rats (*Rattus norvegicus*) aged 12 weeks, with a body weight of 200 grams. Rats were adapted to plastic cages measuring 31cm x 28cm x 9cm and covered with wire mesh. At the bottom of the cage, 0.5-1 cm thick husk is placed and replaced every two days. Commercial pellets were given twice daily, and drinking water was provided ad libitum. The lead used was Lead (II) acetate trihydrate (Merck Millipore CAS 6080-56-4), which was dissolved in distilled water. The dose of lead acetate was

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50 mg/kg bw orally once a day (Septiani *et al*., 2022). Guava fruit was peeled, cut and mashed using a blender and then squeezed out the juice (100%), which was further diluted in water to a concentration of 50% and 25%.

This research proposal has been approved by the ethical commission 555/HRECC.FODM/XII/2020. Rats were divided randomly into five groups: CN, T0, T1, T2, and T3. In the NC group, rats were administered 0.5 mL of distilled water twice daily at four-hour intervals as a negative control. In goup T0, as a positive control, rats were administered 0.5 mL of lead acetate solution 50 mg/kg bw orally once a day and distilled water four hours later. In groups T1, T2, and T3, rats were administered 0.5 mL of 50 mg/kg bw lead acetate solution and 0.5 mL of white guava fruit juice with concentrations of 25, 50, 100% respectively four hours later. Treatment was given for 14 days, and on day 15, all rats were anesthetized with an intraperitoneal injection of 1 mL/kg bw ketamine and 0.25 ml/kg bw xylazine. Rats were sacrificed by decapitation to remove the testicles and cauda epididymis. The cauda epididymis of rats was separated by cutting the proximal part of the corpus epididymis and the distal part of the vas deferens. Cauda epididymis was cut and stirred in physiological NaCl in a petri dish and spermatozoa that swim out were collected (Sari *et al*., 2023) to be assessed the motility, viability, abnormalities, and integrity of the spermatozoa plasma membrane.

The integrity of spermatozoa plasma membrane

The hypoosmotic swelling (HOS) test method was used to evaluate the integrity of the spermatozoa plasma membrane. The hypoosmotic solution was prepared using 1.35 g fructose and 0.73 g sodium citrate dissolved in double distilled water to reach a volume of 100 mL. As much as 0.1 mL spermatozoa suspension was put into a microtube containing 0.9 mL of hypoosmotic solution. After a gentle stir mixture was then incubated at 37°C for 30 minutes. Plasma membrane integrity was evaluated using a light microscope with 400x magnification (Nikon Eclipse E100). Intact plasma membrane of spermatozoa was characterized by a curved tail, while the tail of spermatozoa whose plasma membrane was damaged remained straight (Susilowati *et al*., 2021).

Spermatozoa abnormalities

In examining spermatozoa abnormalities, the spermatozoa preparations were observed under a microscope with 400x magnification (Nikon Eclipse E100). Spermatozoa abnormalities include morphological abnormalities in the head, neck and tail (Pahlevy *et al*., 2022).

Spermatozoa viability

One drop of spermatozoa suspension and one drop of 2% Eosine nigrosine solution were mixed homogeneously on a glass slide, and then smeared thinly on another glass slide. The slides were observed on a microscope with 400x magnification (Nikon Eclipse E100). Live spermatozoa were transparent on the head because they did not absorb the Eosin nigrosine dye, while dead spermatozoa were reddish on the head because they absorbed Eosin nigrosine dye (Sari *et al*., 2023).

Spermatozoa motility

One drop of spermatozoa suspension was put on a glass slide, covered with a cover slip, and examined under a microscope (Nikon Eclipse E100) with 400x magnification. Progressively moving spermatozoa are calculated as a percentage of 100 spermatozoa (Sari *et al*., 2023).

Data analysis

Data on spermatozoa plasma membrane integrity, abnormalities, viability, and motility were analyzed using one-way Anova, followed by Duncan's multiple range test at a confidence level of 95%. Statistical analysis was conducted using Statistical Product and Service Solutions version 25 for Windows software.

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RESULTS

The results of this study showed that exposure to lead acetate (T0) caused lower PMI, viability, and motility as well as higher spermatozoa morphological abnormalities ($p < 0.05$) compared to rats in the negative control group. The intactness of the spermatozoa plasma membrane, spermatozoa viability, and spermatozoa morphological abnormality were shown in Figures 1, 2, and 3, respectively. Administration of white guava fruit juice starting at a dose of 25% (T1) resulted in higher motility, viability, and PMI as well as lower spermatozoa morphological abnormalities ($p < 0.05$) compared to rats in the T0 group. There were no significant differences ($p > 0.05$) in spermatozoa motility and viability of rats in the T1, T2, and T3 groups. Spermatozoa morphological abnormalities were the lowest compared to other group rats ($p < 0.05$). There was no significant difference ($p > 0.05$) in spermatozoa PMI in rats in the T2 and T3 groups (Table 1).

Table 1 Spermatozoa plasma membrane integrity, morphological abnormality, viability, and motility of lead acetate induced rats (*Rattus norvegicus*) treated with white guava juice

	PMI	abnormality	viability	motility
NC	73.80 ± 2.39 ^{d}	9.00 ± 2.45 ^{b}	79.60 ± 2.70 ^{c}	79.00 ± 1.58 ^{c}
T0	42.60 ± 6.27 ^{a}	19.40 ± 2.70 ^{c}	37.40 ± 8.08 ^{a}	30.60 ± 4.04 ^{a}
T1	54.00 ± 6.04 ^{b}	8.00 ± 2.92 ^{b}	55.60 ± 9.45 ^{b}	52.40 ± 8.02 ^{b}
T2	64.40 ± 3.36 ^{c}	8.40 ± 1.14 ^{b}	58.00 ± 7.58 ^{b}	56.60 ± 7.30 ^{b}
T3	69.40 ± 1.52 ^{c}	4.00 ± 1.00 ^{a}	60.00 ± 12.25 ^{b}	59.40 ± 13.99 ^{b}

Different superscripts in the same column indicate significant differences ($p < 0.05$). NC: rats were administered 0.5 mL of distilled water twice a day at four-hour intervals, T0, T1, T2, and T3: rats were exposed to 0.5 mL of 50 mg/kg bw of lead acetate solution followed by the administration of 0.5 mL of distilled water, 25, 50, and 100% white guava fruit juice four hours later.

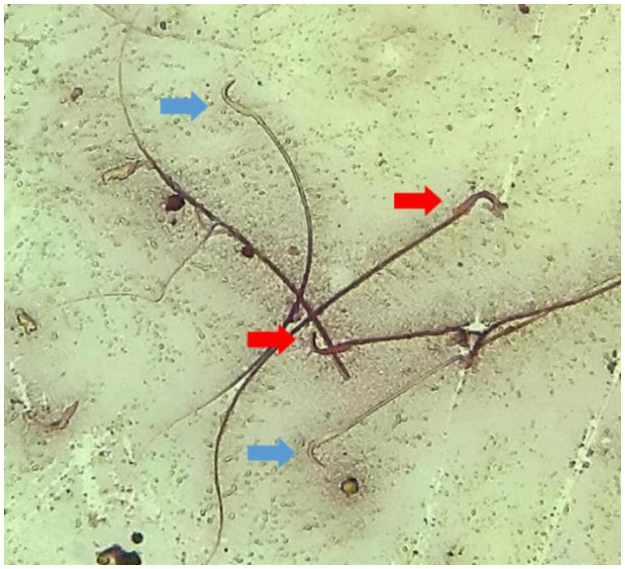

Figure 1 Live and dead spermatozoa of lead acetate induced rat (*Rattus norvegicus*) and treated with white guava (*Psidium guajava* L.) fruit juice; Eosin nigrosine staining under a light microscope 400x magnification (Nikon Eclipse E100); blue arrow: transparent live spermatozoa; red arrow: reddish dead spermatozoa.

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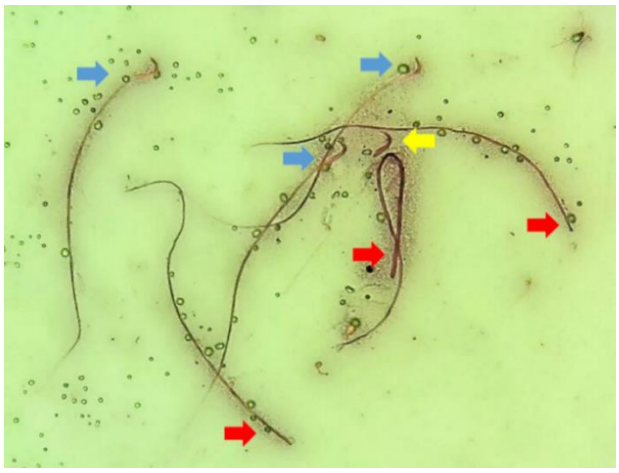

Figure 2 Spermatozoa morphological abnormality of lead acetate induced rat (*Rattus norvegicus*) and treated with white guava (*Psidium guajava* L.) fruit juice; Eosin nigrosine staining under a light microscope with 400x magnification (Nikon Eclipse E100); blue arrow: normal spermatozoa; red arrow: headless spermatozoa; yellow arrow: abnormal spermatozoa without neck and tail.

Figure 3 Spermatozoa plasma membrane integrity of lead acetate induced rat (*Rattus norvegicus*) treated with white guava (*Psidium guajava* L.) fruit juice; hypoosmotic swelling test under a light microscope with 400x magnification (Nikon Eclipse E100); blue arrow: spermatozoa with intact plasma membrane; red arrow: spermatozoa with damaged plasma membrane.

DISCUSSION

Rats induced by lead acetate alone showed lower PMI, viability, and motility and higher spermatozoa morphological abnormalities. Exposure to lead acetate induced the formation of ROS. The antioxidant defense system could not neutralize excessive ROS production in spermatozoa. Polyunsaturated fatty acids in the plasma membrane of spermatozoa were sensitive to lipid oxidation by ROS. Damage to the spermatozoa plasma membrane revealed a straight tail after the addition of hypoosmotic solution (Pitaloka *et al*., 2023). Spermatozoa plasma membrane physiologically functioned as a control of the transport system (Check *et al*., 2023). Spermatozoa plasma membrane is sensitive to oxidative damage because it contained large amounts of polyunsaturated fatty acids and a relative lack of antioxidant enzymes in the spermatozoa cytoplasm. Lipid peroxidation on the spermatozoa plasma membrane resulted in increased membrane permeability and damage to the integrity (Agarwal *et al*., 2014).

The high level of spermatozoa abnormalities may be caused by high levels of free radicals, which triggered defects in the head, neck, and tail. Rats were administered lead acetate, which decreased testicular weight and increased spermatozoa morphological abnormalities (Adamkovicova *et al*., 2016; Abdel-Emam and Ahmed, 2021). Spermatozoa morphological abnormalities are divided into primary and secondary defects. Primary defects are spermatozoa abnormalities in the testicles due to failure of spermatogenesis or spermiogenesis. It was characterized by spermatozoa with heads that are too large or too small, short heads, elongated flat heads, double heads, and double tails. Several factors caused the spermatozoa's primary defect, such as heredity, diseases, and harmful environmental influences. Secondary spermatozoa defects are abnormalities after spermatozoa leave the seminiferous tubules, characterized by broken tails, heads without tails, and broken heads (Gatimel *et al*., 2017). This study revealed that an increase in the percentage of morphological abnormalities in spermatozoa is caused by lead-free radicals. The toxic effect of lead on male reproduction is that it affects spermatogenesis, resulting in a decrease in semen quality in terms of the number, morphology, motility, and abnormal morphology of spermatozoa (Kumar, 2018). Free radicals damaged spermatozoa DNA directly by attacking purine and pyrimidine bases, initiating apoptosis by activation of caspase enzymes (Wagner *et al*., 2017) and morphological abnormalities (head, neck, and

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tail) of spermatozoa (Adamkovicova *et al*., 2016).

The decrease in the percentage of spermatozoa viability is preceded by damage to the plasma membrane. Membrane permeability was closely related to nutrient transport, which has a vital role in cell metabolism. If the permeability of the spermatozoa membrane was disturbed, then nutritional needs would be disturbed, resulting in the spermatozoa dying (Alahmar, 2019). Lipid peroxidation due to lead exposure caused DNA and protein damage, decreasing the number of viable spermatozoa (Dutta *et al*., 2019). Some living spermatozoa are motile and some are non-motile. Rats without lead acetate induction had the highest motility due to the absence of obstacles to the metabolic activity of the spermatozoa plasma membrane. The spermatozoa plasma membrane would protect the energy production in mitochondria for spermatozoa motility. Spermatozoa motility is the standard of healthy spermatozoa and a determining factor for fertilization. The motility of spermatozoa came from the pushing movement of the tail (Adamkovicova *et al*., 2016). Meanwhile, the rats induced by lead acetate only had the lowest spermatozoa motility. It was in line with the decrease in the percentage of spermatozoa motility due to administration of lead acetate reported by Sudjarwo and co-workers (2017). Excessive ROS from lead acetate exposure damaged spermatozoa membranes and mitochondria, disrupting oxidative phosphorylation and Na, K, and Ca ATPase activity (Castellini *et al*., 2021). This could lead to decreased spermatozoa motility due to loss of intracellular ATP, leading to axonemal damage, increased midpiece morphology abnormalities, deleterious effects on spermatozoa capacitation and acrosome reaction (Chianese and Pierantoni, 2021).

This study indicated that white guava fruit juice maintained the integrity of spermatozoa plasma membrane of rat exposed to lead acetate. The administration of 25% white guava fruit juice increased the percentage of spermatozoa plasma membrane integrity optimally. This revealed that white guava fruit juice inhibited the lipid peroxidation chain reaction induced by lead acetate. The lipid peroxidation chain reaction could be stopped by antioxidants to break the chain reaction (Ayala *et al*., 2014). The antioxidant content in white guava fruit juice inhibited lipid peroxidation due to its ability to capture free radicals by releasing hydrogen ions with one electron. The antioxidant content in white guava fruit, such as vitamin C, quercetin, and flavonoids, was able to fight free radicals (Anggraini *et al*., 2021). Flavonoids could act as antioxidants, inhibiting oxidative stress against free radicals and increasing spermatogenesis. Flavonoids provided a competitive substrate for unsaturated fats in cell membranes, increasing regeneration by counteracting free radicals and accelerating the repair of damaged cell membranes. The quercetin compound protected cells from free radical damage by increasing endogenous antioxidants: Superoxide Dismutase and Catalase. Due to its antioxidant content, it could be used as a free radical scavenger. If antioxidants could capture excessive free radicals in the body, then cells damaged by free radicals can regenerate themselves (Rahaman *et al*., 2023).

The administration of 25% white guava fruit juice optimally reduced the percentage of spermatozoa abnormalities. The reduction in the percentage of spermatozoa abnormalities was due to antioxidants, which could reduce oxidative stress due to exposure to lead acetate and maintained the quality of spermatozoa by binding free radicals and preventing oxidative stress in fat, protein, and DNA from spermatozoa (Reisinta *et al*., 2018). According to Sabeti *et al*. (2016), the reduction in abnormalities could be caused by achieving a balanced condition of antioxidants in the cells, thus affecting spermiogenesis. The processes of spermiogenesis and spermatozoa maturation are very vulnerable to ROS interference. The antioxidants administered reduced ROS and increased the number of spermatogonia, spermatids, and normal spermatozoa morphology.

Rats exposed to lead acetate and treated with white guava fruit juice increased spermatozoa

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viability compared to those treated with lead acetate only. These results are in line with research by Anggraini *et al*. (2021) which showed that guava fruit improved the viability of rats, which declined due to oxidative stress due to free radicals exposure. Exogenous antioxidants in white guava fruit juice provided a preventive effect on the viability of rats induced by lead acetate. Antioxidants could inhibit and reduce oxidative stress activity caused by excessive ROS, resulting in an increased presentation of live spermatozoa. The way antioxidants work was to give one or more compounds electrons to become an oxidant, then changed the oxidant into a more stable compound. Antioxidants could eliminate free radicals (Meles *et al*., 2021). The plasma membrane protected the internal organs of cells and filtered intracellular and extracellular surfaces (Tapia *et al*., 2012). Guava fruit contains vitamin C, vitamin B1, vitamin A, protein, fat, carbohydrates, water, calcium and phosphorus. Guava fruit also contains polyphenols: tannin, manganese, saponins, flavonoids, guajavarin, and quercetin. Vitamin C and quercetin, one of the dominant active compounds, play a role in warding off free radicals. Quercetin is a compound that can inhibit oxidative stress by regulating the balance between oxidants and antioxidants. Quercetin effectively protected cells from radical damage by increasing endogenous antioxidant levels and suppressing oxidative stress caused by lead acetate. In addition, vitamin C, as an antioxidant, acted as a hydrogen ion donor (Rahaman *et al*., 2023).

This study showed that the administration of 25% white guava fruit juice effectively maintained spermatozoa motility of rats exposed to lead acetate. This increase in spermatozoa motility is in line with research by Anggraini *et al*. (2021), which proved that giving guava fruit could increase spermatozoa motility of rat exposed to free radicals. Increased spermatozoa motility was caused by antioxidants, which defended the plasma membrane from lipid peroxidation caused by lead acetate so that the integrity of the spermatozoa plasma membrane remained intact. The plasma membrane is vital for spermatozoa motility (Susilowati *et al*. 2021). Providing adequate antioxidants could also increase the body's metabolism and accelerate the formation of ATP which is used as an energy source to move flagella so that spermatozoa motility would increase (Park and Pang, 2021). Antioxidants in guava could stabilize free radicals by completing free radicals' lack of electrons and inhibiting chain reactions from forming free radicals, which could cause cell damage. Several studies showed that providing antioxidants from guava could stabilize free radicals and is preventive (Chechani *et al*., 2023).

The combination of quercetin and vitamin C compounds could reduce excessive ROS production. Excessive ROS could inactivate Superoxide Dismutase and catalase. Vitamin C contained in guava fruit is an antioxidant needed for the survival of spermatozoa. Vitamin C could capture free radical activity, which prevented free radical activity and radical chain reactions, thereby avoiding peroxidative damage affecting spermatozoa quality. Vitamin C is a water-soluble vitamin that protected spermatozoa from damage by oxidative stress by neutralizing hydroxyl, superoxide, and hydrogen peroxide radicals and preventing spermatozoa agglutination. Vitamin C had a vital role in protecting spermatozoa lipids from oxidation reactions, which would reduce spermatozoa motility (Fanaei *et al*., 2014). White guava fruit also contained flavonoids, which can act as antioxidants that could inhibit oxidative stress against the dangers of free radicals and could increase spermatogenesis. Flavonoids could also increase regeneration by destroying free radicals, providing competitive substrates for unsaturated lipids in membranes, and accelerating repair mechanisms for damaged cell membranes (Rahaman *et al*., 2023).

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CONCLUSION

Administration of white guava juice (*Psidium guajava* L.) improved motility, viability, morphological abnormalities, and membrane integrity of spermatozoa in lead acetate induced rats (*Rattus norvegicus*).

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AUTHOR'S CONTRIBUTIONS

Annisa Suci Alifia (ASA), Wurlina Wurlina (WW), Soeharsono Soeharsono (SS), Tatik Hernawati (TH), Sri Agus Sudjarwo (SAS), Budi Utomo (BU), Sri Mulyati (SM), Muhammad Thohawi Elziyad Purnama (MTEP).

ASA: conceived the idea, designed the mainframe of this manuscript, acquisition, analysis and interpretation of data, and manuscript drafting under the supervision of WW and SS. SM, MTEP, TH, ASA, and BU: critically read and revised the manuscript for intellectual content. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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REFERENCES

Abdel-Emam RA, Ahmed EA. 2021. Ameliorative effect of L-carnitine on chronic lead-induced reproductive toxicity in male rats. Vet Med Sci. 7: 1426-35.

Adamkovicova M, Toman R, Martiniakova M, Omelka R, Babosova R, Krajcovicova V, Grosskopf B, Massanyi P. 2016. Sperm motility and morphology changes in rats exposed to cadmium and diazinon. Reprod Biol Endocrinol. 14: 42.

Agarwal A, Virk G, Ong C, du Plessis SS. 2014. Effect of oxidative stress on male reproduction. World J Mens Health 32: 1-17.

Alahmar AT. 2019. Role of oxidative stress in male infertility: An updated review. J Hum Reprod Sci. 12: 4-18.

Anggraini EJ, Wurlina W, Rahmahani J. 2021. Effect of Guava fruit (*Psidium guajava L.*) extract to the motility and viability spermatozoa of rats (*Rattus novergicus*) exposed to cigarette smoke. Poll Res. 40: 335-40.

Assi MA, Hezmee MN, Haron AW, Sabri MY, Rajion MA. 2016. The detrimental effects of lead on human and animal health. Vet World 9: 660-71.

Ayala A, Muñoz MF, Argüelles S. 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014: 360438.

Castellini C, D'Andrea S, Cordeschi G, Totaro M, Parisi A, Di Emidio G, Tatone C, Francavilla S, Barbonetti A. 2021. Pathophysiology of mitochondrial dysfunction in human spermatozoa: Focus on energetic metabolism, oxidative stress and apoptosis. Antioxidants 10: 695.

Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, Modu B, Calina D, Sharifi-Rad J. 2023. Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. Front Chem. 11: 1158198.

Chechani B, Roat P, Hada S, Kumar Yadav D, Kumari N. 2023. *Psidium guajava*: An insight into ethnomedicinal uses, phytochemistry, and pharmacology. Comb Chem High Throughput Screen 26: 10.2174.

Check JH, Check DL, Bollendorf A. 2023. Hypo-osmotic swelling test and male factor. Reprod Med. 4: 118-32.

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Chianese R, Pierantoni R. 2021. Mitochondrial reactive oxygen species (ROS) production alters sperm quality. Antioxidants 10: 92.

Das S, Sultana KW, Ndhlala AR, Mondal M, Chandra I. 2023. Heavy metal pollution in the environment and its impact on health: Exploring green technology for remediation. Environ Health Insights 17: 11786302231201259.

Dasharathy S, Arjunan S, Maliyur Basavaraju A, Murugasen V, Ramachandran S, Keshav R, Murugan R. 2022. Mutagenic, carcinogenic, and teratogenic effect of heavy metals. Evid Based Complement Alternat Med. 2022: 8011953.

Dimitriadis F, Adonakis G, Kaponis A, Mamoulakis C, Takenaka A, Sofikitis N. 2017. Pre-testicular, testicular, and post-testicular causes of male infertility. In: Simoni M, Huhtaniemi IT (Eds). Endocrinology of the testis and male reproduction. Endocrinology. Springer, Cham. 1-47.

Dutta S, Majzoub A, Agarwal A. 2019. Oxidative stress and sperm function: A systematic review on evaluation and management. Arab J Urol. 17: 87-97.

El-Magd MA, Kahilo KA, Nasr NE, Kamal T, Shukry M, Saleh AA. 2017. A potential mechanism associated with lead-induced testicular toxicity in rats. Andrologia 49: 10.1111.

Fanaei H, Khayat S, Halvaei I, Ramezani V, Azizi Y, Kasaeian A, Mardaneh J, Parvizi MR, Akrami M. 2014. Effects of ascorbic acid on sperm motility, viability, acrosome reaction and DNA integrity in teratozoospermic samples. Iran J Reprod Med. 12: 103-10.

Gatimel N, Moreau J, Parinaud J, Léandri RD. 2017. Sperm morphology: Assessment, pathophysiology, clinical relevance, and state of the art in 2017. Andrology 5: 845-62.

Ilmi LUN, Meles DK, Wurlina W. 2021. The effect of guava fruit (*Psidium guajava L.*) extract to the amount of Leydig cells of male rats (*Rattus novergicus*) exposed to cigarette smoke. Poll Res. 40: 255-8.

Kumar A, Kumar A, Cabral-Pinto MMS, Chaturvedi AK, Shabnam AA, Subrahmanyam G, Mondal R, Gupta DK, Malyan SK, S Kumar S, A Khan S, Yadav KK. 2020. Lead toxicity: Health hazards, influence on food chain, and sustainable remediation approaches. Int J Environ Res Public Health 17: 2179.

Kumar S. 2018. Occupational and environmental exposure to lead and reproductive health impairment: An overview. Indian J Occup Environ Med. 22: 128-37.

Lok B, Babu D, Tabana Y, Dahham SS, Adam MAA, Barakat K, Sandai D. 2023. The anticancer potential of *Psidium guajava* (Guava) extracts. Life. 13:346.

Meles DK, Wurlina W, Mustofa I, Rachmawati M, Suwasanti N, Putri DKSC. 2021. The effect of Guava (*Psidium guajava* L.) fruit extract on pulmonary tissue and Malondialdehyde levels of rat (*Rattus novergicus*) exposed to cigarette smoke. Int J Pharm Res. 13: 130-8.

Pahlevy JR, Ratnani H, Fikri F, Restiadi TI, Saputro AL, Agustono B. 2022. The addition of vitamin C in tris–egg yolk extender maintained Sapera goat semen quality in 5° C storage. Ovozoa: J Anim Reprod. 11: 1-8.

Park YJ, Pang MG. 2021. Mitochondrial functionality in male fertility: From spermatogenesis to fertilization. Antioxidants 10: 98.

Pitaloka DG, Hariadi M, Hestianah EP, Akeju SI, Susilowati S, Utomo B, Hernawati T. 2023. The effect of addition of melon (*Cucumis melo* L.) flesh juice into Lactated Ringer's-egg yolk extender on spermatozoa plasma membrane integrity and spermatozoa morphological abnormalities of semen of native rooster. Ovozoa: J Anim Reprod.12: 48-56.

Rahaman MM, Hossain R, Herrera-Bravo J, Islam MT, Atolani O, Adeyemi OS, Owolodun OA, Kambizi L, Daştan SD,

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Calina D, Sharifi-Rad J. 2023. Natural antioxidants from some fruits, seeds, foods, natural products, and associated health benefits: An update. Food Sci Nutr. 11: 1657-70.

Reisinta D, Lokapirnasari WP, Hernawati T, Meles DK, Madyawati SP, Suprayogi TW. 2018. The effect of watermelon's inner rind extract (*Citrullus lanatus*) towards the integrity of the plasma membraneand spermatozoa morphology abnormality of male norway rats (*Rattus norvegicus*) after being exposed to hot temperature. Ovoza : J Anim Reprod. 7: 137-42.

Sabeti P, Pourmasumi S, Rahiminia T, Akyash F, Talebi AR. 2016. Etiologies of sperm oxidative stress. Int J Reprod Biomed. 14: 231-40.

Sari NM, Yuliani GA, Yimer N, Hernawati T, Herupradoto EBA, Hidayatik N. 2023. Red dragon (*Hylocereus polyrhizus*) fruit peel extract increased the motility and viability of spermatozoa of hypercholesterolemic rats (*Rattus norvegicus*). Ovozoa : J Anim Reprod. 12: 33-41.

Septiani RA, Hamid IS, Sabdoningrum EK, Ma'ruf A, Hestianah EP, Mafruchati M. 2022. Tomato (*Lycopersicon esculentum* Mill.) juice restored the number of Leydig cells, and the diameter of the seminiferous tubules of mice (*Mus musculus*) exposed to lead acetate. Ovozoa : J Anim Reprod. 11: 123-9.

Su LJ, Zhang JH, Gomez H, Murugan R, Hong X, Xu D, Jiang F, Peng ZY. 2019. Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. Oxid Med Cell Longev. 2019: 5080843.

Sudjarwo SA, Sudjarwo GW, Koerniasari. 2017. Protective effect of curcumin on lead acetate-induced testicular toxicity in Wistar rats. Res Pharm Sci. 12: 381-90.

Susilowati S, Mustofa I, Wurlina W, Triana IN, Utama S, Rimayanti R. 2021. Effect of insulin-like growth factor-1 complex of Simmental bull seminal plasma on post-thawed Kacang buck semen fertility. Vet World 14: 2073-84.

Tapia JA, Macias-Garcia B, Miro-Moran A, Ortega-Ferrusola C, Salido GM, Peña FJ, Aparicio IM. 2012. The membrane of the mammalian spermatozoa: Much more than an inert envelope. Reprod Domest Anim. 47 Suppl 3: 65-75.

Tirpák F, Greifová H, Lukáč N, Stawarz R, Massányi P. 2021. Exogenous factors affecting the functional integrity of male reproduction. Life 11: 213.

Tousif MI, Nazir M, Saleem M, Tauseef S, Shafiq N, Hassan L, Hussian H, Montesano D, Naviglio D, Zengin G, Ahmad I. 2022. *Psidium guajava* L. An incalculable but underexplored food crop: Its phytochemistry, ethnopharmacology, and industrial applications. Molecules 27: 7016.

Wagner H, Cheng JW, Ko EY. 2017. Role of reactive oxygen species in male infertility: An updated review of literature. Arab J Urol. 16: 35-43.

Wani AL, Ara A, Usmani JA. 2015. Lead toxicity: a review. Interdiscip Toxicol. 8: 55-64.

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