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Effect of Giving *Jamblang* (*Syzygium cumini*) Leaf Extract on Testosterone Serum Level and Concentration of Spermatozoa in Male Rats Intoxicated with Lead Acetate

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ABSTRACT

Lead is a source of free radicals in the environment. Lead accumulation in the blood generates increasing reactive oxygen species (ROS) in the body and various health problems, including male infertility. One effort that can be made to prevent this problem is to use antioxidants from natural sources such as jamblang (*Syzygium cumini*) leaf extract.

This study is experimental with a randomised posttest-only control group design method. The object of this study was 30 white rats (*Rattus norvegicus* strain Wistar) which were divided into five groups (K-, K+, P1, P2, and P3). Lead acetate was given as much as 40 mg/kgBW in groups K+, P1, P2, P3 orally for 30 days. Jamblang (*Syzygium cumini*) leaf extract was given 50 mg/kgBW in the P1 group, 150 mg/kgBW in the P2 group, and 300 mg/kgBW in the P3 group orally for 30 days. Testosterone levels were examined using blood serum taken through the retro-orbital vein and examined using the Enzyme-Linked Immunosorbent Assay (ELISA) method. Spermatozoa were taken from the vas deferens and counted using the Neubauer counting chamber.

The average level of testosterone serum was at K-2.47 ± 0.59 nmol/ml, K+ 2.23 ± 0.45 nmol/ml, P1 2.35 ± 0.65 nmol/ml, P2 2.56 ± 0 .73 nmol/ml, P3 1.96 ± 0.59 nmol/ml, there was no significant difference statistically ($p > 0.05$). The average concentration of spermatozoa at K-32.06 ± 2.66 million/ml, K+ 19.45 ± 8.07 million/ml, P1 27.05 ± 1.89 million/ml, P2 28.65 ± 1.10 million/ml, P3 27.11 ± 5.64 million/ml, statistically there was a significant difference ($p < 0.05$).

There was no effect of giving jamblang (*Syzygium cumini*) leaf extract on testosterone serum levels and there was an effect of giving jamblang (*Syzygium cumini*) leaf extract on the concentration of spermatozoa of male *Rattus norvegicus* rats intoxicated with lead acetate.

Keywords: lead acetate, jamblang leaf extract, testosterone, spermatozoa

INTRODUCTION

Lead is a heavy metal that can generate environmental pollution and health problems in human (WHO, 2021). Humans are mostly exposed to lead from gasoline, industrial processes such as lead smelting and burning, the pottery industry, the shipbuilding industry, lead-based painting,

pipes containing lead, battery recycling, book printing, and others (Wani, Ara and Usmani, 2015). The Institute for Health Metrics and Evaluation (IHME) estimates that in 2017, lead exposure caused 1.06 million deaths and 24.4 million disabilities (WHO, 2021). The Centers for Disease Control and Prevention (CDC) set a limit for blood lead levels in adults ≤10 µg/dL and children ≤5 µg/dL (CDC, 2021). The level of lead in the body is affected by the length of exposure time. Lead accumulation in the blood and tissues can generate some complications of health problems such as kidney and liver damage, anemia, cardiovascular problems, immune system complications, neurological problems (Mehrpour *et al.*, 2020), and infertility (Famurewa and Ugwuja, 2017).

Infertility is defined as a non-contraceptive couple that is sexually active and not being able to get pregnant within one year. About 25% of couples do not being able to get pregnant within one year, 15% of these couples seek medical treatment for it and less than 5% remain childless (WHO, 2019). The 2017 Global Burden of Disease (GBD) data estimates that the burden of global, regional and national infertility from 1990 to 2017 in 195 countries, including Indonesia shows the prevalence rate of infertility based on age increases by 0.370% per year for women and 0.291% per year for men (Sun *et al.*, 2019). Male factors account for 50% of infertility cases (WHO, 2019). The most important parameters indicating male factor infertility are low sperm count, poor sperm motility, and abnormal sperm morphology, which are described as oligospermia, asthenospermia, and teratozoospermia (Ghafouri-Fard *et al.*, 2021). Research in the last decade shows that the pathology of infertility in 30-80% of infertile men is oxidative damage to spermatozoa due to oxidative stress (Huang *et al.*, 2018).

Increasing oxidative stress in the body can be generated by heavy metals exposed, such as lead (US EPA, 2013). Lead toxicity effects arise due to the tendency to catalyze oxidation reactions resulting in Reactive Oxygen Species (ROS) formation. This causes disruption of intracellular homeostasis, including damage to lipids, proteins, enzymes, and Deoxyribose Nucleic Acid (DNA) through the production of free radicals. These free radicals can be one of the main contributors to the pathophysiology of various diseases, including infertility (Jan *et al.*, 2015).

Various efforts have been developed to overcome lead poisoning, including efforts to develop natural ingredients such as plant extracts. This effort was made because plants containing various molecules that can bind free radicals such as phenolic and flavonoids which have strong antioxidant activity (Ahmed *et al.*, 2019). One type of plant that is a source of natural antioxidants is jamblang leaf (*Syzigium cumini*). The myricetin content in jamblang leaf has activity as a prevention of DNA damage and quercetin acts as a free radical scavenger, which can be prevent damage to cell components caused by free radicals. Myrtenol, quercetin are compounds belonging to the flavonoid group which also act as additional antioxidants from outside the body (Ramya, Neethirajan and Jayakumararaj, 2012).

The research results of Margaret, et al. explained that the total phenolics and flavonoids in jamblang leaf were greater than those in the fruit and seeds (Margaret, Shailaja and Rao, 2015). The results of quantitative tests of antioxidant activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method for the ethanol extract of jamblang leaf were IC50 12.84 bjp, while the antioxidant activity of jamblang fruit was IC50 319.89 bjp. Based on these studies, the antioxidant activity of jamblang leaf is categorized as very active, while the antioxidant activity of jamblang fruit is categorized as weak. The antioxidant activity of jamblang leaf extract is equivalent to the value of the antioxidant activity of Vitamin C (Marliani, Kusriani and Sari, 2014).

Based on the description above, it appears that lead has a bad effect on health, including infertility problems. This is caused by an increase in free radicals due to accumulation of lead in the body. Therefore, it is necessary to conduct research to determine the effect of jamblang leaf extract on male infertility. This assessment was carried out by assessing the levels of the hormone testosterone and the number of spermatozoa of male rats.

MATERIALS & METHODS

This study was an experimental study with a randomized post-test only control group design which was carried out in October 2020-August 2022. The population for this study was male white rats (*Rattus novegicus* strain wistar) with a body weight of 150-250 grams. Based on WHO criteria, the minimum sample size is five mice for each test group (WHO, 2000). The inclusion criteria in this study were male white rats aged around 2-3 months, body weight ranging from 150-250 grams, the rats were not physical disability, and actively moving. While the exclusion criteria in this study were mice that looked sick (inactive movements, refusal to eat, dull and falling hair, exudate from the eyes, mouth, genitals and anus). To prevent drop out in the implementation of the research, a sample size correction of at least six rats was carried out in each treatment group. The independent variables in this study were the dose of ethanol extract of jamblang leaf and the dose of lead acetate (PbAc). Meanwhile, the dependent variables are the concentration of spermatozoa and testosterone hormone levels.

Mice were acclimatized during the first week of research to adapt to the environment. Lead acetate was administered orally using a probe at a dose of 40 mg/kgBW. The ethanol extract of jamblang leaf was administered orally to three treatment groups at doses of 50 mg/kg, 150 mg/kg, and 300 mg/kg. This treatment was carried out for 30 days. The day after treatment was stopped, the experimental animals were then given inhalation anesthesia using ether. Furthermore, surgery was performed to collect spermatozoa samples and free testosterone levels were examined using blood serum taken from the orbital vein using the ELISA method.

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Spermatozoa samples were taken immediately after the animal was attempted to be dissected. Samples were taken from the epididymis to the end of the vas deferens of the experimental animal. The vas deferens was cut at both ends and placed in a cup containing 0.9% NaCl. Sperm is obtained by massaging the vas deferens tract until there are no spermatozoa left in it. The concentration of spermatozoa was counted by means of homogeneously stirred sperm sucked with a erythrocytes pipette of 0.5 mL. The homogeneous solution was dripped into the Improved Neubauer counting chamber, then the amount was counted. The data from this research were processed statistically using the One-Way Anova method by first carrying out a normality test to assess the distribution of data in each treatment group. This research was conducted after obtaining approval and being declared to have passed the ethical test by the ethics committee of the Faculty of Medicine, Andalas University, which was written in the Certificate of Passing the Ethical Test Number: 550/UN.16.2/KEP-FK/2021.

RESULT

The results of the research on the effect of jamblang leaf extract on testosterone hormone level and the number of spermatozoa in male rats intoxicated with lead acetate can be seen in the following figures:

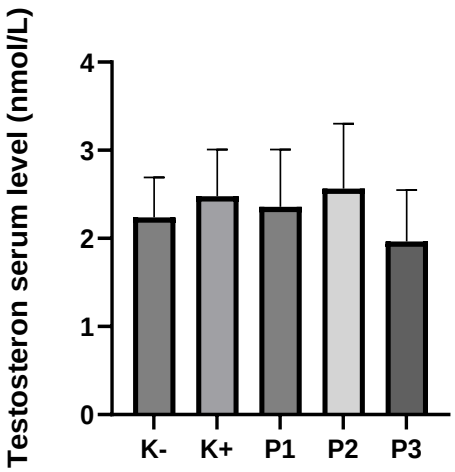

Figure 1. The Average of Testosterone Hormone Level

The results of research regarding the effect of administering jamblang leaf extract on testosterone hormone levels are depicted in Figure 1. The results of statistical tests using One Way ANOVA for testosterone hormone levels show that there are no significant differences (*p* 0.469) between the five groups. Even though there was no statistically significant difference, there was still a difference in the mean levels of the testosterone hormone in the five experimental groups. The mean testosterone level in the negative control group was 2.47 ± 0.59 nmol/ml. There was a decrease in the mean testosterone hormone levels in the positive control group compared to the negative control group, namely 2.23 ± 0.45 nmol/ml. Then an increase in testosterone hormone levels occurred in treatment group one and treatment group two, namely 2.35 ± 0.65 nmol/ml and 2.56 ± 0.73 nmol/ml. The decrease in the average level of the hormone testosterone again occurred in the three treatment groups, namely 1.96 ± 0.59 nmol/ml. This shows that the administration of jamblang leaf extract has an effect on testosterone hormone levels and provides protection against damage caused by free radicals due to exposure to lead acetate.

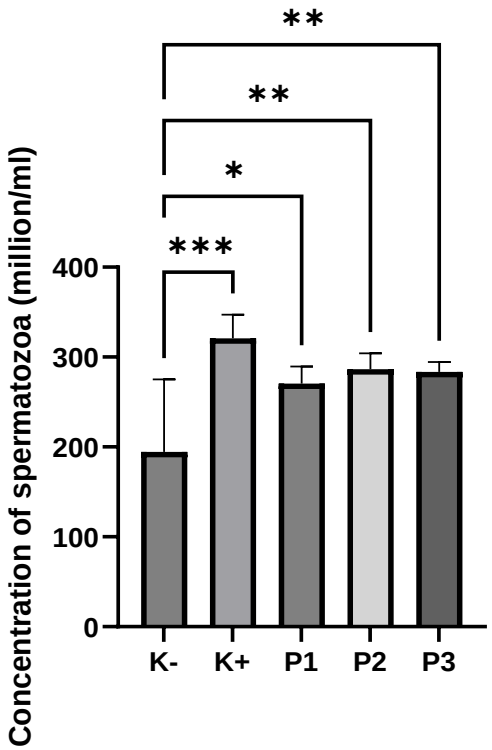

Figure 2. The Average of Concentration of Spermatozoa

Figure 2 describes the average concentration of spermatozoa in male rats intoxicated with lead acetate and given jamblang leaf extract. The results of the One-Way Anova statistical test showed that there was a significant difference (*p* 0.001) between the control and treatment groups. The mean concentration of spermatozoa in the negative control group was 32.06 ± 2.66 million and decreased in the positive control group to 19.45 ± 8.07 million. The treatment group was given jamblang leaf extract for 30 days with three dose levels, namely 50 kg/kg, 150 mg/kg, and 300 mg/kg. The number of spermatozoa from the three treatment groups was 27.05 ± 1.89, 28.65 ± 1.10, and 27.11 ± 5.64 respectively. Based on these results it appears that there was an increase in the number of spermatozoa after being given jamblang leaf extract compared to the positive control group.

DISCUSSION

The effect of giving jamblang leaves and lead acetate on testosterone hormone levels

The production of testosterone is driven by the hypothalamic-pituitary-gonadal (HPG)-axis. Hypothalamic gonadotropin releasing hormone (GnRH) stimulates the secretion of gonadotropins by the pituitary gland, namely luteinizing hormone (LH) and follicle- stimulating hormone (FSH). LH regulates the secretion of testosterone by the Leydig cells, whereas FSH supports spermatogenesis (Ide, Vanderschueren and Antonio, 2021).

Lead-induced toxicity occurs through the mechanism of oxidative stress which can affect a variety of target cells (Rajendran and Selvaraj, 2018). In the male reproductive system, increased production of Reactive Oxygen Species (ROS) due to lead toxicity causes a decrease in the synthesis of the hormones testosterone, LH and FSH, disrupts the blood-testis-barrier (BTB), interferes with sperm parameters, apoptosis of Sertoli cells and Leydig cells so that it has an impact in the form of infertility (Ghafouri-Fard *et al.*, 2021). Oxidative stress decreases antioxidant activity and increases lipid peroxidation in Leydig cells which causes a decrease in testosterone synthesis (Soleimanzadeh *et al.*, 2019). Giving jamblang leaf antioxidants can reduce the negative effects of lead exposure by suppressing the formation of ROS so that the synthesis of the hormone testosterone increases.

Research by Tutkun et al., who conducted research on the relationship between blood lead levels and reproductive hormones in workers exposed to lead in the workplace. Fifty-eight workers had blood lead levels > 5 g/dL and no infertility problems, included in the study as a case group. The control group was 63 healthy office workers without exposure to heavy metals. The results of this study showed that there were significant differences in the levels of total testosterone (TT), free testosterone (FT), prolactin (PRL), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) in the study group and control group (Tutkun *et al.*, 2018).

From the results of this study, it was seen that there was a decrease in testosterone levels in the third treatment group (P3), namely at the dose of jamblang leaf extract 300 mg/kg BW, with lower results compared to the negative control group. This can happen because the dose in treatment 2 (P2) is probably the optimum dose. Another possibility of this is the prooxidant effect of jamblang leaf extract (*Syzygium cumini*). Providing natural antioxidants, including polyphenols, flavonoids, anthocyanins and carotenoids, can also act as pro-oxidants (Eghbaliferiz and Iranshahi, 2016) (Jomová *et al.*, 2019) which trigger oxidation reactions in the body when used in excessive doses and cause toxic effects on the body (Sotler *et al.*, 2019).

The effect of giving jamblang leaves and lead acetate on the concentration of spermatozoa

Spermatogenesis occurs in the seminiferous tubules due to stimulation of the anterior pituitary gonadotropin hormone. In the first stage of spermatogenesis, spermatogonia migrate among the Sertoli cells toward the central lumen of the seminiferous tubules. Spermatogonia that enter the Sertoli cell layer are gradually modified and enlarge to form primary spermatocytes. The spermatocyte then undergoes mitosis to form two secondary spermatocytes. Secondary spermatocytes divide several times to become spermatids which will eventually be modified into spermatozoa (Hall, 2016).

Hassan et al.'s study on 32 male albino rats that were given lead acetate as much as 20 mg/kgBW for 56 consecutive days showed a significant reduction in testicular and epididymal weight, sperm count, sperm motility and viability, FSH, LH, testosterone and estradiol levels. serum, as well as a significant decrease in testicular antioxidant molecules, and a significant increase in

sperm abnormalities, oxidative biomarkers (Malondialdehyde and Nitric oxide) compared to the control group (Hassan *et al.*, 2019).

Significant changes in the concentration of spermatozoa can be caused by the effect of lead which directly works to damage the integrity of sperm DNA. Experiments on male rats that were given high concentrations of lead acetate (0.5% and 1%) in drinking water for six weeks affected sperm motility and increased the percentage of spermatozoa with abnormal morphology, and showed DNA damage and chromatin structure damage that increased significantly (Li *et al.*, 2018).

Other studies have also shown striking degenerative changes in testicular histopathology due to lead exposure. Giving lead acetate to rats orally for 150 days can change the structure of the seminiferous tubules and spermatozoa, ultrastructural changes in the form of vacuolization of the Sertoli cell cytoplasm and an increase in the size and number of lysosomes. Chronic exposure to lead can change the function of Sertoli cells (Rajendran and Selvaraj, 2018). Androgen-binding Protein (ABP) is synthesized by Sertoli cells and functions as a binder for the hormone testosterone and brings it to the epididymis via the seminiferous tubules in the process of spermatogenesis. However, there has not been much research on the effect of lead exposure on ABP.

Giving jamblang leaf extract to the treatment group showed positive results. An increase in the number of spermatozoa occurred in the three treatment groups compared to the positive control group. Lead exposure induces oxidative stress, which can be reduced by Syzygium cumini's leaves (Rita and Sy, 2021) The phenolic and flavonoid content found in jamblang leaf can inhibit the formation of free radicals caused by exposure to lead acetate. The myricetin content in jamblang leaf has activity to prevent DNA damage and quercetin which acts as a free radical scavenger which prevents damage to cell components caused by free radicals (Ramya, Neethirajan and Jayakumararaj, 2012).

In this study, the concentration of spermatozoa in treatment group three (P3) tended to show lower results compared to treatment group one (P1) and treatment group two (P2), as was the case with examination of testosterone hormone levels. The researcher's assumption is that this can happen because the dose of jamblang leaf extract given to the P2 group has reached the optimum dose. The decrease in the number of spermatozoa in the third treatment group (P3) did not exceed that of the positive control group, which was different from the results of examining testosterone hormone levels in this study.

Jamblang plants contain polyphenols and flavonoids which can act as antioxidants and prooxidants which influence ROS concentrations depending on the reaction conditions (Chobot and Hadacek, 2011). The prooxidant properties of flavonoids can cause oxidative damage when they react with various biomolecules, such as lipids, proteins, and DNA (Procházková, Boušová and Wilhelmová, 2011). Hormones are amino acids that are present in very low concentrations in the body, so they can experience changes in concentration when there is a pro-oxidant effect, as happened in this study. On the other hand, the effects of lead toxicity can occur systemically, it is possible that the effects of lead toxicity have an influence on other spermatozoa formation pathways, so that the concentration of spermatozoa is reduced even though in this study the administration of lead acetate did not affect testosterone hormone levels.

This study proves that administration of jamblang leaf extract has an effect on the concentration of spermatozoa of male rats that have experienced lead intoxication. However, this study still has limitations, including the infertility parameters examined in this study were limited to examining testosterone levels and the concentration of spermatozoa.

CONCLUSION

Based on the results of research on the administration of jamblang (*Syzygium cumini*) leaf extract on testosterone levels and the number of spermatozoa in male rats intoxicated with lead acetate, it can be concluded that administration of jamblang (*Syzygium cumini*) leaf extract increased the number of spermatozoa in male rats intoxicated with lead acetate.

Declaration by Authors

Ethical Approval: The Ethics Commission of the Faculty of Medicine, Universitas Andalas, accepted this study with the designation number 550/UN.16.2/KEP-FK/2021.

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Conflict of Interest: The authors declare no conflict of interest.

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