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The Effect of *Alpinia Galanga* Extract to Seminiferous Tubular Epithelial Thickness and Seminiferous Tubular Diameter of Male Mice Induced to Lead Acetate

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ABSTRACT

The purpose of this research was to determine the effect of Alpinia galanga extract for seminiferous tubular epithelial thickness, and seminiferous tubular diameter of mice induced to lead acetate. This study was experimental, 25 male mice (Mus musculus) strain Balb/C with average arround 30 gram were divided into 5 groups in a completely randomized design with different treatments for 24 days. The negative control group C (-) was given CMC-NA, the positive control group C(+) was given CMC-NA + lead acetate, groups P1, P2, and P3 were given galangal extract 200, 400, and 800 mg/kgBB + CMC-NA + lead acetate. Mice were sacrificed at a predetermined time and then histopathological preparations of testicular organs were made for epithelial thickness and seminiferous tubule diameter. Data analysis used ANOVA test and continued with LSD test. The result of the research galangal extract administration showed an increase in epithelial thickness and diameter significantly by the three treatment groups against the control (+) ($p<0.05$). group P2 which given galangal extract 200 mg/kgBw/day is the most effective to prevent epithelial thickness (59.83 ± 0.48; $p<0.05$) and diameter of seminiferous tubules (180.89 ± 0.85; $p<0.05$) because the result not significantly different from the control group (-) where epithelial thickness (59.64 ± 1.83; $p<0.05$) and seminiferous tubule diameter (180.71 ± 1.37; $p<0.05$) were given CMC- NA 0.2 ml/day. It can be concluded that giving galangal extract to male mice exposed to lead acetate was proven to maintain thick epithelium and seminiferous tubule diameter.

Keywords: *Alpinia galangal;* epithelium thickness of seminiferous tubules; diameter of seminiferous tubules; lead acetate; reproductive health

INTRODUCTION

Heavy metals often cause environmental pollution. These heavy metals can accumulate in the body and remain for a long time as toxins. One of the most common heavy metals is lead [1]. Lead can increase the production of ROS in the body, disrupt the balance between antioxidants and oxidants in the body, resulting in oxidative stress, resulting in a decrease in endogenous antioxidant levels. several many studies have proven lead does not have a safe limit when ingested by the body. One of the toxic and accumulative properties of lead can affect the animal's reproductive system [2]. Lead that enters the body can reduce antioxidant levels and increase the production of free radicals such as reactive oxygen species (ROS) resulting in oxidative stress and lipid peroxidation [3]. ROS are compounds produced during metabolic processes and are involved in enzymatic reactions, electron transport, mitochondrial respiration, signal transduction, activation of nuclear transcription factors, gene expression, and antimicrobial action of neutrophils and macrophages [4].

The body parts that are most affected by lead exposure are the nervous system, kidney system, reproductive system, endocrine system, and heart [5]. In the reproductive system, increased oxidative stress causes seminiferous tubular cells to become oxidized, so that Sertoli cells, Leydig cells, and other spermatogenic cells can experience damage and decrease the number of cells [6]. The toxic effects of lead can result in impaired spermatozoa motility, increase the percentage of abnormal morphology, DNA damage, and show adverse effects on the physiological parameters of spermatozoa as a whole [7]. Germ cells and sperm cells are susceptible to oxidative stress because they are composed of high unsaturated fat bonds, making them susceptible to lipid peroxidation and cell damage [8]. Damage to these cells will cause the thickness of the epithelium and the diameter of the seminiferous tubules to decrease so that the make lumen diameter looks wide.

Antioxidants are compounds that can prevent oxidative stress and free radicals. Antioxidant mechanism in inhibiting oxidation reactions and preventing cell damage by binding to free radicals and reactive molecules [9]. Antioxidant mechanism works by donating hydrogen ions (H^+) to ROS so that the activity of these oxidant compounds is inactivated [10]. One of the plants that contain natural antioxidant activity is Alpinia galanga (AG). AG has strong antioxidant activity, the antioxidants contained in galangal are flavonoids (galangin, kaempferol, galangal acetate) which have various benefits for the body, one of which is to prevent oxidative stress by preventing the formation of ROS [11]. Flavonoids prevent the formation of ROS by inhibiting the work of xanthine oxidase and Nicotinamide

Adenine Dinucleotide Phosphate (NADPH) oxidase, and binding metals (Fe^{2+} and Cu^{2+}) so as to prevent redox reactions that can produce free radicals [12]. Testing the antioxidant levels of galangal obtained a value of 69.06 ppm which is a classification of a strong antioxidant group (50-100ppm). Besides containing flavonoids, galangal also contains alkaloids, phenolic compounds, tannins, and terpenoids [13].

METHODS

The experimental design used in this study is completely randomized design. This research was conducted at the Laboratorium of Experimental Animal at Faculty of Veterinary Medicine in University Airlangga, from October 2021 till December 2021. The study was approved by Faculty of Veterinary Medicine Animal Ethics Committee. The testes samples were collected from the animals after considerations in accordance to Faculty of Veterinary Medicine Animal Ethics Committee related to animal handling were observed to ensure no discomfort or pain to animal during sampling (No: 1.KE.118.10.2021)

This study used equipment on experimental animals, namely: mice cages, places to eat and drink, balance, syringe 1ml, sonde. Tools for organ harvesting and histopathology are: sterile surgical instruments, light microscopes, object glass, cover glass, storage containers, calculators, stationery, water baths, staining jars. The tools needed for extraction are knife, placemat, blender, filter paper, cloth, rotary evaporator (Tang et al., 2018). The materials needed in this study were: mice feed, mineral water, ethanol 96% pro analysis, lead acetate suspension pro analysis, Phosphate Buffer Formalin, 70%, 80%, 90%, 95% alcohol solution and absolute alcohol, xylol solution , liquid paraffin, and Hematoxylin Eosin dye [14].

The AG rhizome were collected from traditional market Surabaya, East Java, Indonesia. They were washed with tap water, chopped, and dried at room temperature and normal humidity. The rhizome were then weighed and blended to a fine powder to obtain the rhizome extract. The extraction process was performed at the Patology Clinic Laboratory, Faculty of Veterinary Medicine, Airlangga University, Surabaya. The powder (1 Kg) was macerated with ethanol 96% for 3 days. Then, the filtrate was collected and allowed to evaporate using a rotary evaporator until a half-condensed ethanol extract of the AG rhizome was produced [14]. The extract was stored at 2-4°C and used for the experiment mixed with CMC-Na 1% suspension.

The dose lead acetate 20 mg/kg BW. Preparation done by dissolving 0,02 mg lead acetate in 100 ml aquadest. Lead acetate were given after one hour of AG extract. Setiyowati et al [15] stated that the administration of lead acetate at a dose of 20 mg/Kg BW can reduced testicular weight, epithelial thickness and seminiferous tubule diameter.

This research carried out on 25 male mice Mus musculus strain Balb/c aged around 2-3 months, have an average body weight 25-30 gram obtained from Pharmacy Veterinary Center (PUSVETMA) Surabaya and were then fed and underwent environmental adaptation for 7 days. A total of 25 male mice were divided into five equal groups for the 24-day testing period. The animals were housed in plastic cages, fed standard commercial mice chow, and provided drinking water ad libitum. Twenty five mice were divided into 5 treatment groups, each group consisted of 5 mice, and all treatments were given orally divided: C(-): Mice were administred 1% CMC-Na/day, C(+): Mice were administred lead acetate 20 mg/KgBW/day (starting on day 4 for 21 days) and CMC-Na 1%/day, P1: Mice were administred lead acetate 20 mg/KgBW/day (starting on day 4 for 21 days), and AG Extract 200 mg/KgBW/day (24 days),

P2: Mice were administred lead acetate 20 mg/KgBW/day (starting on day 4 for 21 days), and AG Extract 400 mg/KgBW/day, P3: Mice were administred lead acetate 20 mg/KgBW/day (starting on day 4 for 21 days), and AG Extract 800 mg/KgBW/day.

At the day 25, mice were sacrified with dislocation cervix, abdoment were dissected to collect a organ testis. The collected testis then stored in Phosphate buffer formalin 10% as fixation solution. Tissue processing and histopathological examination was done in Pathology Laboratory, Division of Pathology, Faculty of Veterinary Medicine, Universitas Airlangga.

The epithelium thickness of seminiferous tubules were measured by using micrometer from software (Image raster) with 400x magnification for total 15 random lumen views, and 4 times measure thickness epitel at same lumen but different location. The epithelium thickness was measured from spermatogonium near basement membrane of seminiferous tubules until the spermatid. The diameter of seminiferous tubules were measured by using micrometer from software with 400x magnification for total 15 random lumen views, and 2 times measure diameter at the same lumen but different location. The roundest seminiferous tubules in each view was measured.

RESULTS AND DISCUSSION

The results on Table 1, showed that there was a significant difference in the average thickness of the seminiferous tubular epithelium of male mice in group C(+) compare with group C(-), P2, and P3 ($p<0.05$). However, there was no significant difference mean thickness of epithel between group C(+) and P1 dose 200 mg/KgBW. The result on Table 1, showed a significant difference in the average diameter of seminiferous tubules of male mice in group C(-), P1, P2, and P3 as compared with C(+) group ($p<0.05$).

TABLE 1: The effect of Alpinia galanga on the thickness of the epithelium and the diameter of the seminiferous tubules of mice (Mus musculus) exposed to lead acetate

Group	Mean ± SD	
	Thickness	Diameter
C(-): CMC-NA	59.64^{c} ± 1.83	180.71^{d} ±1.37
C(+): CMC-NA + lead acetate	52.42^{a} ± 1.24	172.87^{a} ±1.97
P1: P3: galangal extract 200 mg/kgBB + lead acetate	53.76^{a} ± 2.22	175.47^{b} ±0.89
P2: P3: galangal extract 400 mg/kgBB + lead acetate	59.83^{c} ± 0.48	180.89^{d} ±0.85
P3: galangal extract 800 mg/kgBB + lead acetate	57.28^{b} ± 1.13	177.78^{c} ±1.04

Different alphabetical superscripts in the same column represent a signification difference $p<0.05$.

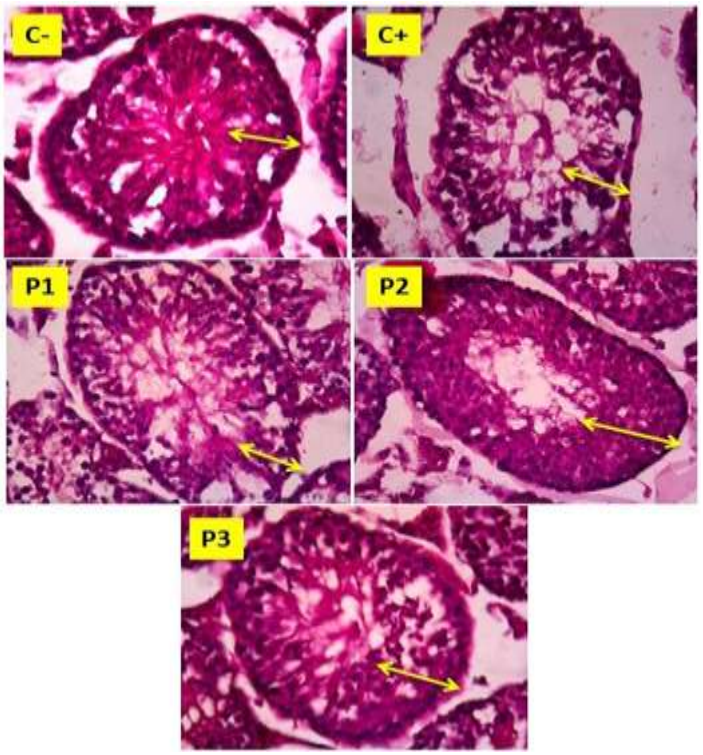

FIGURE 1: The thickness of the epithelium of the seminiferous tubules of mice (*Mus musculus*) after administration *Alpinia galanga* extract exposed to lead acetate (HE, 400x magnification).

In Figure 1 can be observed regarding the condition of the histopathological preparations of the testes. C- group with the intact appearance, dense collection of spermatogenic cells up to the lumen, and no tissue damage was found at group. The C+ group with the appearance of scattered epithelial cells, the lumen area is getting bigger and the spermatogenic cell collection doesn't seem to be densely filled with the epithelium.

The P1 group had a less dense appearance of epithelial tissue than the C- group, the lumen of the seminiferous tubules was larger. The P2 group with the appearance of intact seminiferous tubules, a collection of spermatogenic cell that looks tight until the lumen is getting narrower like group C-. The group 3 with the appearance of the interstitial space resembling normal, almost intact like normal tissue, spermatogenic cells almost tightly filling the lumen, and the lumen getting narrower than C+ group.

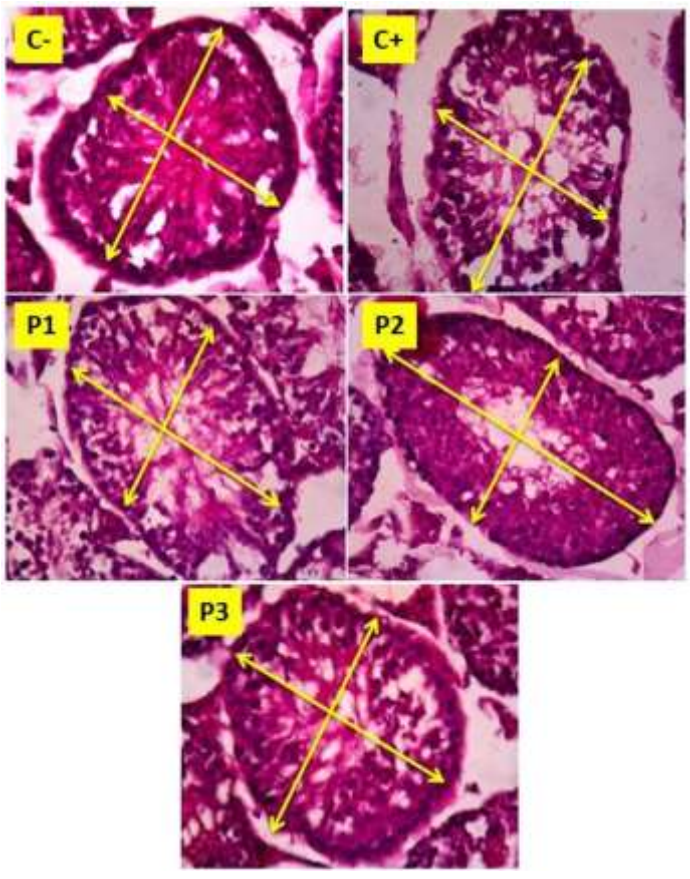

FIGURE 2: The diameter of the seminiferous tubules of mice (*Mus musculus*) after administration *Alpinia galanga* extract exposed to lead acetate (HE, 400x magnification).

In this study, the administration AG extract had a significant effect on epithelial thickness and seminiferous tubule diameter of male mice induce to lead acetate. lead exposure can decrease the thickness of seminiferous tubular epithelium and diameter of seminiferous tubular in mice. This is consistent with with the result of a study by Gayatri et al [16] which showed that administration of lead acetate to mice showed a decrease in testosterone level, diameter and thickness of seminiferous tubule epithelium. Patra et al [17] state that exposure lead in the long time can result in the formation of excess ROS in the body especially in testis, cause oxidative stress. Oxidative stress is formed by an imbalance of ROS and antioxidant in the body which can cause lipid peroxidation, formation of active compounds and cell damage [14].

ROS can cause cell apoptosis, resulting in a decrease in the number of spermatogenic cells, leydig cells and sertoli cells in the testes. Apoptosis is caused by the release of cytochrome-C protein (as result of ROS invade in inner and outer membranes of mitochondria). Cytochrome-C is secreted by an increase Ca^{2+} [18]. The increased in Ca^{2+} is caused by membrane permeability failure due to reactions occuring in ROS towards lipids and protein membranes [19]. Beside released cytochrome-C protein, lead induction can increase caspase-3 expression. Increased expression caspase-3 provides an indication that testicular cells undergo excessive apoptosis. The decrease in the number of spermatogenic cells due to apoptosis has an impact on the inhibited spermatogenesis process, thinning of the thickness of the epithelium and the diameter of the seminiferous tubules.

In this study, the treatment group P2 which was given AG extract at a dose 400 mg/KgBw/day for 24 days had the most effective ability to maintain the thickness of epithelium and diameter of the seminiferous tubule in mice compared to other treatment groups (P1 and P3).

The treatment group P2 with a dose of 400 mg/kgBW, and P3 at a dose of 800 mg/kgBW were proven to significantly maintain the thickness of the seminiferous tubule epithelium ($p<0.05$). The Pl treatment group with a dose of 200 mg/kgBW did not significantly affect the C+ group without AG extract ($p<0.05$). The treatment group P1 with a dose of 200 mg/ kgBW, P2 with a dose of 400 mg/ kgBW, and P3 at a dose of 800 mg/ kgBW were proven to significantly maintain the diameter of the seminiferous tubules compared to the C+ group without galangal extract ($p<0.05$). But in P3 treatment there was a significant decrease in tubular diameter compared to P2 ($p<0.05$). This activity occurs due to a decrease in antioxidants in cells due to excess antioxidant molecules in the body and become pro-oxidants and can cause poisoning [20], and is often referred to as the antioxidant paradox [21].

Chan et al [22] said that damage to cell membranes due to free radicals can be inhibited using antioxidants, so that the spermatogenesis process is more optimal and there is an increase in spermatogenesis cells. Flavonoids contained in AG extract can increase regeneration cells by describing free radicals and accelerating the repair mechanism of damaged cell membranes [23]. Flavonoids also inhibit oxidative stress, fight free radicals and increase spermatogenesis. This is reinforced by the statement of Diana et al [19] and Gayatri et al [16] which states that flavonoids are able to maintain the thickness of epithelium and the diameter seminiferous tubular.

CONCLUSION

Based on this research, it could be concluded that giving Alpinia galanga extract to male mice exposed to lead acetate was proven to maintain thick epithelium and seminiferous tubule diameter with efective dose 400 mg/KgBW.

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