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 around 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/kgBW + 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 galanga*; 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 studies have proven that lead does not have a safe limit 

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Lead enters the body as toxins. One of the most common heavy metals is lead 4. Lead can increase the production of ROS in the body, disrupt the balance between oxidants and antioxidants in the body, resulting in oxidative stress, resulting in a decrease in endogenous antioxidant levels. Several studies have proven that lead does not have a safe limit 5 when ingested by the body. One of the toxic and accumulative properties of lead can affect the animal’s reproductive system 6. 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 7. 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 8.

The body parts that are most affected by lead exposure are the nervous system, kidney system, reproductive system, endocrine system, and heart 6. 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 male 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 (Fe²⁺ and Cu²⁺) 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 Pathology 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 administrated 1% CMC-Na/day, C(+): Mice were administrated lead acetate 20 mg/KgBW/day (starting on day 4 for 21 days) and CMC-Na 1%/day, P1: Mice were administrated 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 administrated lead acetate 20 mg/KgBW/day (starting on day 4 for 21 days), and AG Extract 400 mg/KgBW/day, P3: Mice were administrated 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 sacrificed with dislocation cervix, abdomen 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 cation. The roundest seminiferous tubules in each group C(+) were compared with C(-) group as a significant difference (p<0.05). 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(+) compared with group C(-), P1, 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

<table>
<thead>
<tr>
<th>Group</th>
<th>Thickness Mean ± SD</th>
<th>Diameter Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(-): CMC-NA</td>
<td>59.64± 1.83</td>
<td>180.71± 1.37</td>
</tr>
<tr>
<td>C(+): CMC-NA + lead acetate</td>
<td>52.42± 1.24</td>
<td>172.87± 1.97</td>
</tr>
<tr>
<td>P1: P3: galangal extract 200 mg/kgBB + lead acetate</td>
<td>53.76± 2.22</td>
<td>175.47± 0.89</td>
</tr>
<tr>
<td>P2: P3: galangal extract 400 mg/kgBB + lead acetate</td>
<td>59.83± 0.49</td>
<td>180.89± 0.85</td>
</tr>
<tr>
<td>P3: galangal extract 800 mg/kgBB + lead acetate</td>
<td>57.28± 1.13</td>
<td>177.78± 1.04</td>
</tr>
</tbody>
</table>

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

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

REFERENCES


