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Ethanol extract of galangal (*Alpinia Galanga*) administration on degeneration, necrosis, and inflammatory cells in the proximal tubules of male mice (*Mus musculus*) exposed to lead acetate

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Abstract

Introduction: This study aimed to investigate the preventive effect of the ethanolic extract of galangal (*Alpinia galanga*) on degeneration, necrosis, and inflammatory cells in the kidney proximal tubules of male mice (*Mus musculus*) exposed to lead acetate.

Objective: This was a pure experimental laboratory study using a completely randomized approach/randomized posttest only control group design. Twenty-five male mice (*Mus musculus*) were divided into five treatment groups: negative control (K-) received 0.5% CMC Na and aquades, positive control (K+) received induction of 20 mg/kgBW lead acetate for 20 days, and P1, P2, and P3 groups received oral administration of galangal ethanol extract from day 1 to day 24 as preventive measures with successive doses of (P1) 200 mg/kgBW, (P2) 400 mg/kgBW, and (P3) 800 mg/kgBW, respectively. Lead acetate administration at a dose of 20 mg/kgBW started from day 4 to day 24. On the 25th day, all groups were sacrificed, and kidney tissues were harvested for histopathological examination using the scoring method. Histopathological data analysis was performed using the Kruskal-Wallis test followed by Mann-Whitney U test to compare differences between groups.

Results: The Mann-Whitney U test showed a significant increase in degeneration, necrosis, and infiltration of inflammatory cells in the kidney proximal tubules of male mice (*Mus musculus*) exposed to lead acetate ($p < 0.05$). Administration of galangal ethanol extract significantly reduced degeneration, necrosis, and infiltration of inflammatory cells ($p < 0.05$). The highest dose of galangal ethanol extract (800 mg/kg BW) exhibited a significant decrease in degeneration, necrosis, and infiltration of inflammatory cells ($p < 0.05$), comparable to the normal condition (K-).

Conclusion: Administration of ethanol extract of galangal (*Alpinia galanga*) effectively prevented lead acetate-induced degeneration, necrosis, and infiltration of inflammatory cells in the kidney proximal tubules of male mice (*Mus musculus*).

Keywords: Degeneration; Galangal; Male Mice; Necrosis; Lead Acetate; Healthcare.

1. Introduction

Lead or Plumbum (Pb) is one of the most abundant elements on Earth. The sources of Pb generally come from mining pollutants, agriculture, coal production, and the combustion of motor vehicles and industrial emissions. Additionally, lead is often found in pesticides, fertilizers, gasoline, battery materials, cosmetics, and metal products such as ammunition, solder, and pipes [1]. Entry of Pb into the body occurs through inhalation of air or dust, consumption of contaminated food and water. Animals are also at risk of exposure to heavy metal Pb, thus animal-derived products must also be free from lead contamination.

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The kidney is a vital organ that functions to maintain the body's acid-base balance and regulate hydro-electrolyte balance in the disposal of waste products from the blood [2, 3]. The kidney is the primary route of Pb excretion and can accumulate Pb higher than other organs. Clinically induced high concentrations of Pb lead to nephrotoxic effects due to the accumulation of Pb in the epithelial cells of the proximal tubules [4]. Lead accumulates in tubule epithelial cells leading to the loss of brush border epithelial cells and resulting in acute tubular necrosis, apoptosis, and massive proteolysis [5].

The mechanism of nephrotoxicity due to Pb induction involves the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) resulting in oxidative stress. Excessive ROS production causes kidney damage through lipid membrane peroxidation, protein denaturation, and DNA damage [6]. Nephrotoxicity also involves the activation of inflammatory processes, apoptosis, and decreased antioxidant defenses [7, 8]. Various studies using antioxidant and anti-inflammatory agents have been proven to prevent nephrotoxic conditions caused by Pb induction [9].

One plant with natural antioxidants is red ginger (*Alpinia galanga*). Red ginger as a herbal medicine has the potential to be anti-inflammatory and also reduce lipid peroxidation [10]. Red ginger contains flavonoid compounds, tannins, quinones, steroids, triterpenoids, and alkaloids. The antioxidant activity of red ginger rhizome extract has very high antioxidant power as indicated by the IC50 value (500 ppm). Red ginger extract (*Alpinia galanga*) can be used as an anti-inflammatory, antitumor, antioxidant, antibacterial, gastroprotective, hypoglycemic, and hypolipidemic agent. Ginger contains flavonoids, sitosterol terpinen-4-ol, alkaloids, saponins, terpenoids, phenolates, and carbohydrates [11].

Based on the above description, research is needed to determine the effectiveness of red ginger extract (*Alpinia galanga*) on degeneration, necrosis, and inflammatory cells in the proximal kidney tubules of male mice (*Mus musculus*) exposed to lead acetate.

2. Material and methods

This research is a pure experimental laboratory study (true experimental) with a research design using a completely randomized approach/randomized posttest only control group design.

2.1. Materials

The experimental unit in this study used 25 healthy male white mice (*Mus musculus*), Balb/C strain, with an average weight of 25-30 grams and aged 2.5-3 months. The equipment used included mouse cages, feeding and drinking troughs, digital weighing scales, syringes, probes, gloves. The tools used for kidney histopathology sampling were surgical scissors, surgical knives, tweezers, organ containers, microscope, glass slides, cover glass, water bath, staining jar. The tools used for galangal extraction were knives, knife stands, blenders, containers, filter paper, cloth, rotary evaporator. The materials used were galangal, mouse feed, ethanol, lead acetate suspension for pro analysis (Merck, Indonesia) (Catalogue Number: 1073750250), 10% Neutral Formalin Buffer, 70%, 80%, 90%, 95% alcohol concentration, and absolute alcohol, xylene solution, liquid paraffin, and hematoxylin eosin dye.

2.2. Methods

The mice (*Mus musculus*) were randomly assigned and divided into five groups, each group consisting of six mice. The mice underwent acclimation or adaptation in the environment for seven days with appropriate temperature, humidity, and light. Feed was given once a day and drinking bottles were filled daily, feed and water containers were cleaned twice a week.

2.2.1. Preparation of lead acetate

Lead acetate powder was dissolved in aquadest and administered orally at a dose of 20 mg/kg BW to mice (*Mus musculus*) after the fourth day of Galangal ethanol extract administration [12].

2.2.2. Preparation of galangal ethanol extract

One kilogram of Galangal rhizome (*Alpinia galanga*) was washed thoroughly with running water then thinly sliced. Drying was done directly under sunlight with a black cloth cover for three days. After the drying process, the grinding process was carried out with a blender and sifted using a 25 mesh sieve [13]. Fine powder of Galangal rhizome totaling 500 grams was extracted using 1000 ml of pro-analysis ethanol solution for the maceration process. The ethanol used was 75% concentration ethanol. Maceration was carried out for 2 x 24 hours at room temperature with stirring every 6 hours. Filtration with filter cloth was done at the end of the maceration process. The filtrate was then evaporated

using a Buchi evaporator to evaporate the ethanol solvent to obtain a concentrated extract. Galangal rhizome ethanol extract was stored at 0-5°C.

2.2.3. Treatment stage

After a seven-day acclimation process, the research continued for 21 days. This research consisted of five treatment groups, namely: Group (K-): mice only given CMC-Na, starting on day 1 for 24 days. Group (K+): mice given lead acetate dissolved in aquadest at a dose of 20 mg/kg BW. Group (P1): mice given ethanol extract of Galangal at a dose of 200 mg/kg BW and lead acetate at a dose of 20 mg/kg BW. Group (P2): mice given ethanol extract of Galangal at a dose of 400 mg/kg BW and lead acetate at a dose of 20 mg/kg BW. Group (P3): mice given ethanol extract of Galangal at a dose of 800 mg/kg BW and lead acetate at a dose of 20 mg/kg BW. All treatment groups with lead and ethanol extract of Galangal were given from day 4 to day 24 at a rate of 0.2 ml/head orally.

Sampling was carried out on the 25th day on 25 mice (*Mus musculus*) and euthanized by cervical dislocation. The peritoneal area of the mice was dissected using surgical equipment to remove a pair of kidneys. The harvested kidneys were stored in containers filled with 10% formalin solution. The formalin-fixed kidneys were embedded in paraffin and stained with Hematoxylin eosin (HE). The stained sections were examined under a microscope at 400x magnification [12].

2.2.4. Measured variables

The observed variables were the histopathological images of mouse kidneys using a light microscope. Then, the degree of kidney damage was scored, including degeneration of proximal tubule epithelial cells, necrosis of proximal tubule epithelial cells, and infiltration of inflammatory cells in the kidney interstitium [14]. The scoring value of the degree of damage in each sample was the average of all types of lesions (Table 1).

Table 1 Parameters for kidney damage scoring (Klopfleisch, 2013) [14].

Lesion Form	Skor	Information
Degeneration of Proximal Tubular Epithelial Cells	0	No degenerative changes occur
	1	If the number of degenerative cells is <25% of the visual field(VP)
	2	If the number of degenerative cells is between 26-50% of the VP
	3	If the number of degenerative cells is between 51-75-% of the VP
	4	If the number of degenerative cells is >76% of the VP
Proximal Tubular Epithelial Cell Necrosis	0	No necrotic changes occurred
	2	If the number of necrotic cells is <25% of the visual field (VP)
	4	If the number of necrotic cells is between 26-50% of the VP
	6	If the number of necrotic cells is between 51-75-% of the VP
	8	If the number of necrotic cells is >76% of the VP P
Inflammatory Cell Infiltration in the Renal Interstitial	0	If no inflammatory cells are found in the renal interstitium
	1	If there are sporadic inflammatory cells in the renal interstitium
	2	If there is focal inflammatory cell infiltration in the renal interstitium
	3	If there is multifocal inflammatory cell infiltration in the renal interstitium
	4	If there is evenly distributed (diffuse) inflammatory cell infiltration in the renal interstitium

2.2.5. Data Analysis

Data obtained based on scoring the degree of kidney damage was processed by assessing the mean scoring. All quantitative data were analyzed using the non-parametric Kruskal Wallis test method and Mann-Whitney qualitative data as well as statistical methods using SPSS software.

3. Results and discussion

3.1. Degeneration of Proximal Tubule Epithelial Cells in Kidneys

Observations of degeneration in the histopathological images of the kidneys analyzed using the Kruskal-Wallis test showed significant differences ($p<0.05$) among the treatment groups. Degeneration was characterized by enlarged and cloudy cytoplasmic changes. These results indicate that lead acetate induction and administration of ethanol extract of Galangal significantly affect the degeneration of proximal tubule epithelial cells in the kidneys. Differences between treatment groups were determined by further analysis using the Mann-Whitney test. The mean scoring results can be seen in Table 2.

Table 2 Mean scoring of cell damage in the form of degeneration, necrosis, and infiltration of inflammatory cells in the proximal tubule epithelial cells of mouse kidneys given Galangal and lead acetate.

Group	Mean Scoring		
	Degeneration	Necrosis	infiltration of inflammatory
K (-)	1.68 ± 0.74^{a}	3.04 ± 1.35^{a}	1.72 ± 0.72^{ab}
K (+)	3.32 ± 1.31^{c}	6.38 ± 1.85^{c}	3.30 ± 0.83^{d}
P1	2.48 ± 1.31^{b}	5.04 ± 1.52^{b}	2.42 ± 0.83^{c}
P2	2.10 ± 0.74^{b}	4.56 ± 1.51^{b}	1.90 ± 0.70^{b}
P3	1.56 ± 1.12^{a}	3.44 ± 1.34^{a}	1.60 ± 0.60^{a}

Different superscripts in the same column indicate significant differences ($p<0.05$). Group (K-): CMC-Na, (K+): lead acetate dose of 20 mg/kg BW, (P1): ethanol extract of Galangal dose of 200 mg/kg BW and lead acetate dose of 20 mg/kg BW, (P2): ethanol extract of Galangal dose of 400 mg/kg BW and lead acetate dose of 20 mg/kg BW, and (P3): ethanol extract of Galangal dose of 800 mg/kg BW and lead acetate dose of 20 mg/kg BW.

The measurement results of degeneration level analyzed using the Mann-Whitney test showed that there was no significant difference ($p>0.05$) between the K(-) group and the P3 group, while the P3 group was significantly different from the P2 group ($p<0.05$), but all three treatments were significantly different ($p<0.05$) from the K(+) group. Further comparisons showed significant differences ($p<0.05$) between the K(+) group and the P1, P2, and P3 groups. The comparison between the P1 group showed significant differences ($p<0.05$) with the P2 and P3 groups (Table 2). The histopathological images of degeneration of proximal tubule epithelial cells in the kidneys given Galangal ethanol extract due to lead acetate induction can be seen in Figure 1.

3.2. Necrosis of Proximal Tubule Epithelial Cells in Kidneys

Observations of necrosis in the histopathological images of male mouse kidneys were analyzed using the Kruskal-Wallis test and the results showed significant differences ($p<0.05$) among the treatment groups. Necrosis was characterized by the presence of cell nuclei undergoing pyknosis, karyorrhexis, or karyolysis, and was accompanied by infiltration of inflammatory cells around the necrotic tissue. These results indicate that the administration of lead acetate and Galangal ethanol extract significantly affects the necrosis of proximal tubule epithelial cells in male mouse kidneys. Differences between treatment groups were determined by further analysis using the Mann-Whitney test. The mean scoring results can be seen in Table 2.

The measurement results of necrosis level analyzed using the Mann-Whitney test showed that there was no significant difference ($p>0.05$) between the K(-) group and the P3 group, and the P1 group with P2, while the mean scoring of necrosis in the P2 and P3 groups showed significantly different results ($p<0.05$), but all four treatments were significantly different from the K(+) group ($p<0.05$) (Table 2). The histopathological images of necrosis of proximal

tubule epithelial cells in the kidneys given Galangal ethanol extract due to lead acetate induction can be seen in Figure 2.

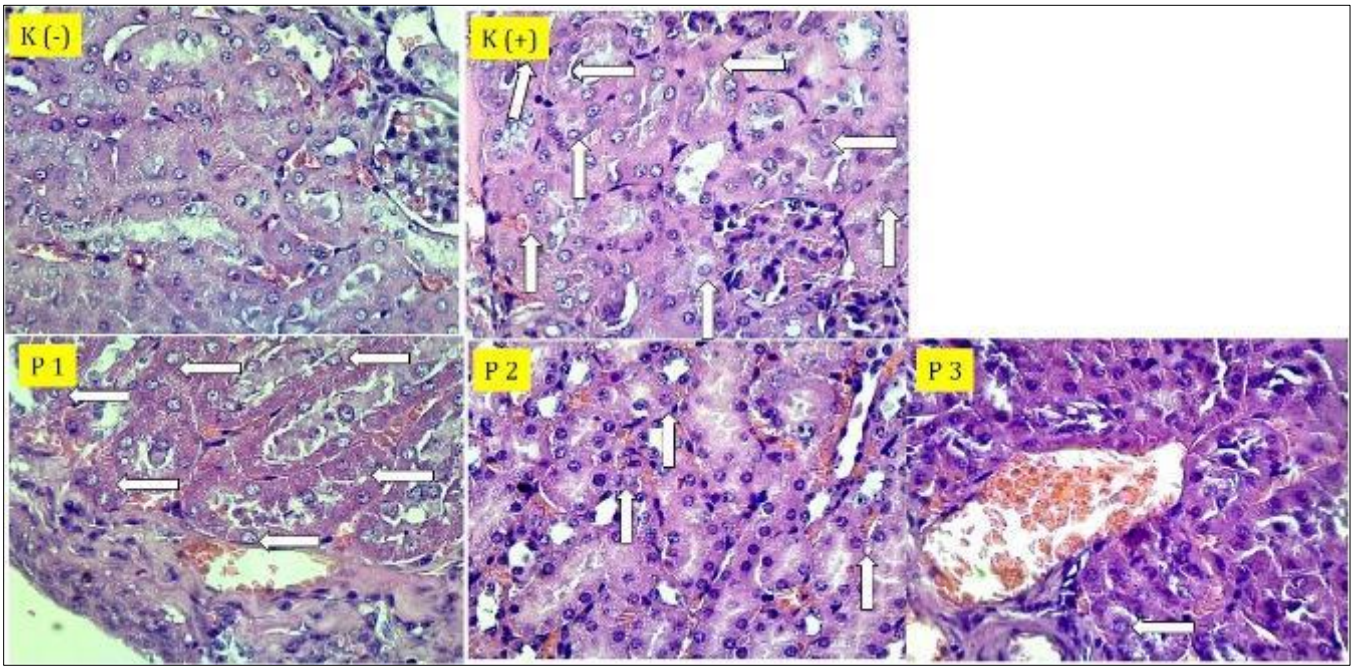

Figure 1 Comparison of histopathological images of degeneration of proximal tubule epithelial cells in kidneys from groups K(-), K(+), P1, P2, and P3, stained with H.E, magnification 400X. White arrowheads indicate degeneration of cells with enlarged and cloudy cytoplasm. The K(-) group shows no degenerative changes, thus has a score of 0, the K(+) group shows maximum degeneration with a score of 4, the P1 group shows degeneration with a score of 3, the P2 group shows degeneration with a score of 2, the P3 group shows mild degeneration with a score of 1 and is close to the K(-) group.

3.3. Infiltration of Inflammatory Cells in the Kidney Interstitium

The observation results of the number of inflammatory cells in the histopathological images of male mouse kidneys were analyzed using the Kruskal-Wallis test, showing significant differences ($p<0.05$) among the treatment groups. Infiltration of inflammatory cells can be seen in the kidney interstitium. These results indicate that the administration of lead acetate and Galangal ethanol extract significantly affects the infiltration of inflammatory cells in the kidney interstitium of male mice. Differences between treatment groups were determined by further analysis using the Mann-Whitney test. The mean scoring results can be seen in Table 2.

The measurement results of the level of infiltration of inflammatory cells in the kidney interstitium using the Mann-Whitney test showed that there was no significant difference ($p>0.05$) between the K(-) group and the P3 group, but the K(-) group showed significant differences ($p<0.05$) compared to the other treatment groups. The scores of infiltration of inflammatory cells between the P1 group and the P2 group and between the P2 group and the P3 group showed no significant differences ($p>0.05$), but all three treatment groups were significantly different from the K(+) group (Table 2). The histopathological images of infiltration of inflammatory cells in the kidney interstitium given Galangal ethanol extract due to lead acetate induction can be seen in Figure 3.

4. Discussion

Degeneration is a cellular response to injury that is still reversible. If the injury is not too severe and can be stopped, the cells can recover and return to normal structure and function [15]. Based on the results and data analysis of the study, the K(+) group showed the highest level of degeneration of proximal tubule epithelial cells in the kidney. The high level of degeneration in the K(+) group is due to degeneration being the first manifestation of damage resulting from lead acetate administration. Excessive accumulation of lead acetate can further cause oxidative stress, which can lead to cell damage [16]. Inside the kidney organ, lead acetate will bind to megalin, which is then transported to the cell organelles. In the process of endocytosis, excessive accumulation of lead acetate will be released into the cytoplasm along with calpains and cathepsins. This can activate mitochondrial damage. Damaged mitochondria will trigger the production of

ROS and lead to a decrease in ATP, which can disrupt cellular osmotic balance [17]. Decreased ATP and excessive ROS production will cause lipid peroxidation, disrupting the sodium-potassium pump, leading to sodium (Na^+) retention in the cell and calcium (Ca^{2+}) and H_2O entering the cell. This results in cell degeneration, where the cells appear cloudy, enlarged, and the cytoplasm is filled with water vacuoles [18].

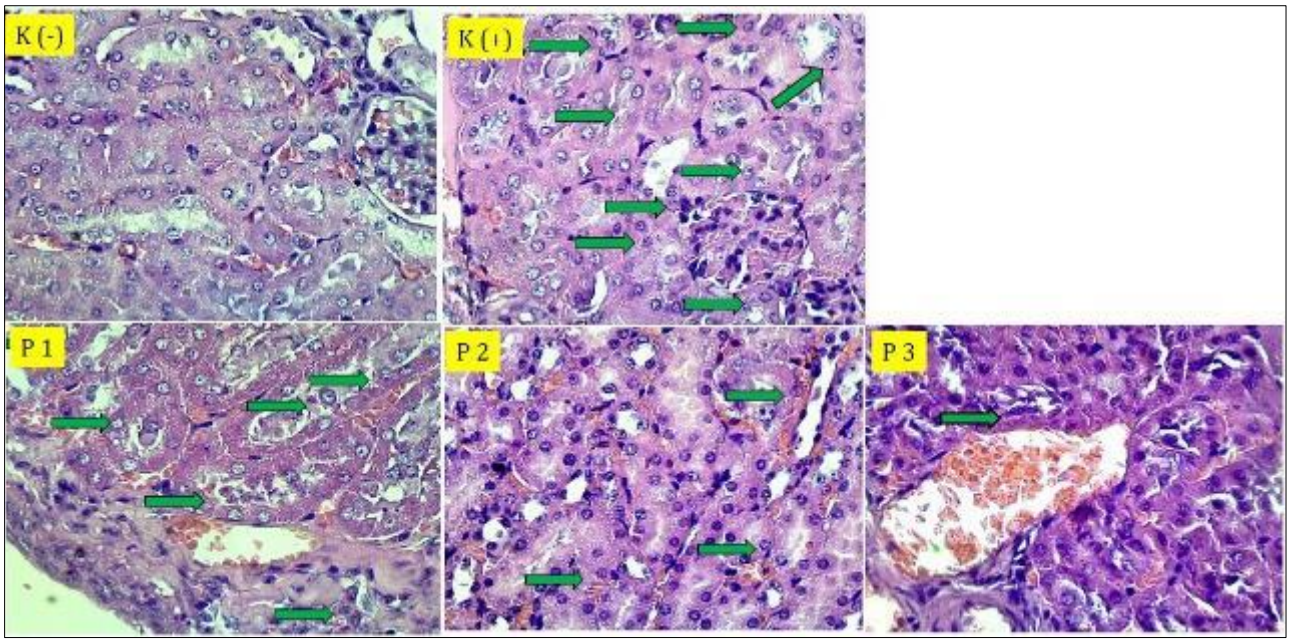

Figure 2 Comparison of histopathological images of necrosis of proximal tubule epithelial cells in kidneys from groups K(-), K(+), P1, P2, and P3, stained with H.E, magnification 400X. Green arrowheads indicate necrotic cells. The K(-) group shows no necrotic changes, thus has a score of 0, the K(+) group shows severe necrosis with a score of 8, the P1 group shows severe necrosis with a score of 6, the P2 group shows moderate necrosis with a score of 4, the P3 group shows mild necrosis with a score of 2 and is close to the K(-) group.

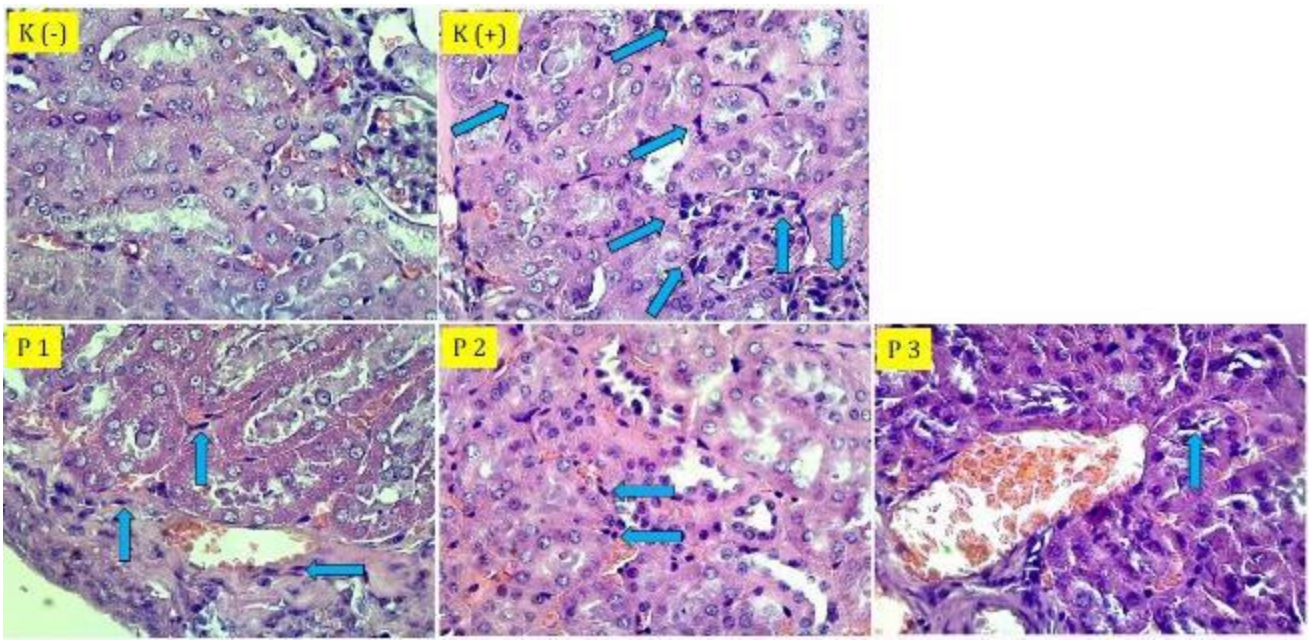

Figure 3 Comparison of histopathological images of infiltration of inflammatory cells in the kidney interstitium from groups K(-), K(+), P1, P2, and P3 stained with H.E, magnification 400X. Blue arrowheads indicate infiltration of inflammatory cells. Inflammatory cells appear clustered in the kidney interstitium. The K(-) group has a score of 1, the K(+) group has a score of 4, the P1 group has a score of 3, the P2 group has a score of 2, the P3 group has a score of 1 with sporadically more leukocyte cells than the K(-) group.

The P1, P2, and P3 treatment groups showed a decrease in the level of degeneration of proximal tubule epithelial cells in the kidney. The most effective results in reducing the number of cell degenerations were obtained from the P3 group with a dose of 800 mg/kg BW, which showed a mean scoring value approaching the K(-) group. The P2 group showed a low level of degeneration, as indicated by the mean scoring value approaching the P3 group, while the P1 group still showed a relatively high level of degeneration. The high level of degeneration in the P1 group may be due to insufficient antioxidant supply to neutralize continuously formed ROS, thus unable to optimally reduce the level of degeneration.

The administration of Galangal ethanol extract as a preventive measure resulted in a decrease in the level of degeneration of proximal tubule epithelial cells in male mouse kidneys. This supports the research by Muhartono et al. [19] where it was observed that the dose of Galangal ethanol extract had a significant effect on reducing the scoring of kidney cell degeneration.

Necrosis is an advanced condition of degeneration where cells experience severe injury to the point of irreversible "point of no return" state [18]. Necrosis is the premature death of living cells and tissues caused by external factors such as infection, exposure to substances accompanied by inflammatory cell infiltration, while cell death or apoptosis is programmed cell death of living cells and tissues without inflammation. Based on the results and data analysis of the study, the K(+) group showed the highest level of necrosis of proximal tubule epithelial cells in the kidney. This is consistent with the study by Kandemir et al. [9] that lead acetate administration at a dose of 20 mg/kg BW intraperitoneally for 20 days resulted in severe kidney damage indicated by high levels of necrosis. The high level of necrosis in the K(+) group is caused by ROS that emerge due to continuous lead acetate induction, leading to oxidative stress conditions that cause cell necrosis [20].

Cell death due to lead acetate induction can occur in the form of apoptosis and necrosis. Apoptosis can be caused by the activation of pro-apoptotic proteins such as B-cell lymphoma 2 (BCL-2) due to mitochondrial damage [17, 21], while necrosis is caused by cells that cannot maintain their condition due to increased cytoplasmic influx of Ca^{2+}. In microscopic observations, necrosis can be observed by the infiltration of inflammatory cells around tissues undergoing necrosis [20].

Necrosis can be caused by mitochondrial damage, leading to decreased ATP production and increased ROS production. The production of radical compounds such as superoxide radical anion (O_2^-), nitric oxide (NO), and hydrogen peroxide (H_2O_2) can directly cause cell necrosis [22]. Additionally, necrosis can also be caused by protein damage, DNA structural changes, and lipid peroxidation due to high levels of ROS, leading to oxidative stress. Oxidative stress further triggers ROS production and increases malondialdehyde (MDA) production, resulting in inhibited activity of natural antioxidants in the body [16]. Natural antioxidants in the body function to neutralize radical compounds by donating electrons to these radicals. Inhibited natural antioxidants in the body, such as glutathione, superoxide dismutase, catalase, glutathione S-transferase, and glutathione peroxidase, will result in uncontrollable ROS increase [23]. ROS will further induce mitochondrial damage, leading to decreased ATP until the cell reaches a severe enough state where it cannot maintain its condition, leading to necrosis. Subsequent cell necrosis can trigger inflammatory cell infiltration, where worsening necrosis conditions can lead to acute tubular necrosis, ultimately resulting in acute kidney injury or kidney failure [20].

The P1, P2, and P3 treatment groups showed a decrease in the level of necrosis in the proximal tubule epithelial cells of the kidneys compared to the K(+) group, but the P1 group still showed a relatively high level of necrosis. The high level of necrosis in the P1 group may be due to insufficient antioxidant supply to neutralize the increasing levels of ROS due to lead acetate induction. The antioxidant level in Galangal ethanol extract at a dose of 200 mg/kg BW is still not effective in inhibiting the progression of irreversible necrosis. The results of the P2 group showed a moderate level, indicated by the same superscript as P1, but also the same as P3. This indicates that in the P2 group, necrosis experienced a decrease compared to the P1 group, but the results were not better than P3.

The P3 group showed the same superscript as the K(-) group and had a mean scoring value close to the K(-) group. This indicates that Galangal ethanol extract at a dose of 800 mg/kg BW is the most effective dose in reducing the number of necrotic cells, although microscopically, the P3 group still shows a higher level of necrosis and is not better than the K(-) group. The microscopic appearance of the P3, which still shows a higher level of necrosis than K(-), may be due to lead acetate induction, which can damage kidney cells, still showing higher necrosis levels in proximal tubule epithelial cells, resulting in differences in statistical data compared to clinical conditions in male mice. The decrease in the level of necrosis shown by the P2 and P3 groups is consistent with the research by Adedeji et al. [24], which shows that Galangal at doses of 400 and 800 mg/kg BW can effectively protect the kidneys from nephrotoxicity due to Gentamicin induction, as indicated by decreased creatinine and urea concentrations.

The administration of Galangal ethanol extract as a preventive measure resulted in a decrease in the level of necrosis of proximal tubule epithelial cells in the kidney. Some antioxidants we know of include flavonoids, one of which can be produced from products [25] One plant that contains flavonoids with antioxidant properties is Galangal. Galangal rhizome (*Alpinia galanga*) contains approximately 1% greenish-yellow essential oil mainly consisting of methyl cinnamate 48%, cineole 20%, eugenol, camphor 1%, sesquiterpenes, galangin, a resin called galangol, yellow crystals called kaemferida, cadinenes, hexahydrocadelen hydrate, kurestin, kaempferol, aluminum, and several other flavonoid compounds [26].

According to Samarghandian et al. [26], Galangal rhizome contains two phytochemical compounds, namely acetoxy chavicol acetate (ACA) and hydroxy chavicol acetate (HCA), which have antitumor, anti-inflammatory, antimicrobial, and antioxidant effects [27]. The ACA content in Galangal rhizome extract can inhibit the inflammatory process by inhibiting nitric oxide (NO) and cyclooxygenase-2 (COX-2), thus preventing cell damage.

Inflammatory cell infiltration is a tissue response to damage caused by various pathogens, dead or damaged cells, foreign bodies, physical injury, irritation, radiation, or toxic substances [18]. Based on the results and analysis of research data, the K(+) group showed the highest level of inflammatory cell infiltration in the interstitial area of the kidney. This is consistent with the high level of necrosis in the K(+) group. According to Randjelovic et al. [20], tissue necrosis caused by lead acetate induction can induce an inflammatory reaction by triggering the migration of monocytes and macrophages to damaged tissues. In addition to necrosis, inflammatory cell infiltration can be triggered by oxidative stress caused by ROS such as superoxide and hydrogen peroxide. Cells undergoing necrosis along with ROS activate Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kb), which then induces various cytokine expressions such as tumor necrosis factor alpha (TNF-α) and Interleukin 6 (IL-6), adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1), and growth factors. ICAM-1 increases leukocyte adhesion to the endothelium and promotes leukocyte migration into the interstitial space, leading to inflammatory cell infiltration [28].

The P1, P2, and P3 treatment groups showed a decrease in the level of inflammatory cell infiltration in the kidney interstitium compared to the K(+) group, but the P1 group still showed a relatively high level of inflammatory cell infiltration. The P2 group showed a mean scoring value that was not significantly different ($p<0.05$) from the P1 group, and also not significantly different from the P3 group ($p<0.05$). This is in line with the research by Primadina et al. [29], which states that the healing process with inflammatory cell infiltration involves early and late phases of inflammation, where this inflammatory phase begins immediately after injury until day five, and on day seven, the regeneration phase begins. Inflammation aims to remove and destroy dead tissue debris through phagocytosis.

The P3 group showed a mean scoring value that was not significantly different ($p>0.05$) from the K(-) group. This indicates that Galangal ethanol extract at a dose of 800 mg/kg BW is the most effective dose in inhibiting inflammatory cell infiltration in the kidney interstitium.

In this study, exposure to lead acetate caused histopathological changes in the proximal tubules of the kidneys. Degenerative changes are the initial process of kidney cell damage. Degeneration results in cell swelling, as seen in the treatment groups (K+). Exposure to lead acetate also causes irreversible damage to the proximal tubules of the kidneys, namely cell necrosis and inflammatory cell infiltration. This occurred in all groups exposed to lead acetate.

5. Conclusion

Based on the results of this study, it can be concluded that the administration of Galangal ethanol extract (*Alpinia galanga*) can inhibit degeneration, necrosis, and inflammatory cell infiltration in the proximal tubules of male mice kidneys (*Mus musculus*) exposed to lead acetate, with the best results at a dose of 800 mg/kg BW.

Compliance with ethical standards

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Disclosure of Conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The study was approved by the Faculty of Veterinary Medicine Animal Ethics Committee of Universitas Airlangga. All variables were considered in accordance with the Ethics Committee related to the animal handling to ensure no discomfort or pain was caused to the animals during sampling(certificate registration number: 1.KE.118.10.2021.

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