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Effect of *Coffea canephora* Bean Extract on Lead Acetate-Induced Rats

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ABSTRACT

Lead (Pb) is a non-essential heavy metal that is toxic and has no known uses. Most cases of poisoning in livestock are caused by lead poisoning. This study aims to determine the effect of *Coffea canephora* bean extract as an antioxidant on the heart histopathological features of white rats (*Rattus norvegicus*) induced by lead acetate. This study used 25 male Wistar rats aged between eight and ten weeks and weighing between 200 and 250 grams. The rats were divided into five treatment groups, namely negative control (C-), positive control (C+), treatment one (T1), treatment two (T2), and treatment three (T3). The results suggested that the administration of 200 mg/kg BW, 400 mg/kg BW, or 800 mg/kg BW of *Coffea canephora* bean extract was not effective in improving the heart histopathological features, including cell degeneration, hemorrhage, and necrosis, of rats induced with lead acetate at a dose of 2 mg/kg BW.

Keyword: heart, histopathology, lead acetate, *Coffea canephora* bean extract

INTRODUCTION

Lead (Pb) is a non-essential heavy metal that is toxic and has no known uses (Rondi et al., 2021). It is commonly found in the environment due to the increasing amount of industrial waste and the rapid development of the industrial sector (Gebretsaldik et al., 2020). Charkiewicz and Backstrand (2020) stated that lead is often found in objects in the environment of living organisms, such as battery plates, cable protectors, soldering materials, paints, ceramics, primary inks, and fuels. In addition, automotive fuel produces waste in the form of lead, specifically tetraethyl lead (TEL) or tetramethyl lead (TML), which are additives in fuel (Mielke et al., 2019).

Lead has several compounds, including lead acetate, which can enter the body through the digestive, respiratory, and dermal routes. Once in the bloodstream, it can spread to the aortae, liver, lungs, brain, and spleen (Carocci et al., 2016). It can be absorbed 1.5 times faster than other compounds and, if accumulated in the human or animal body above the limit, can have a significant impact on health. Lead poisoning is the most common cause of poisoning in livestock (Kadhum, 2019).

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Heavy metals in the body can affect biochemical and metabolic processes, for instance, a decreased in the performance of enzyme and the disruption of the interaction between calcium and other biological molecules (Charkiewicz & Backstrand, 2020).

Lead exposure can cause damage to various organs, including the heart. The heart plays a crucial role in pumping blood throughout the body. Therefore, any exposure to lead can result in decreased heart function due to the toxic effects of lead on the structure of cardiac muscle (Boarescu et al., 2019). Free radicals can damage the cardiac muscle cell membrane by altering its fluidity, structure, and function. Lead is lipophilic, which means that it binds to lipids in the cell membrane and causes lipid peroxidation. This, in turn, affects permeability and blocks the distribution of ions in the body due to lead binding (Rems et al., 2019).

Antioxidants can suppress the production of reactive oxygen species (ROS) that occurs as lead circulates in the bloodstream and reaches the organs. Robusta coffee (*Coffea canephora*), which contains chlorogenic acid, is a potential source of exogenous antioxidants that can suppress free radical activity (Harahap, 2017). In addition to chlorogenic acid, it contains other antioxidant compounds, including caffeine, tannin, minerals, theophylline, protein, proanthocyanidin, coumarin, tocopherol, alkaloids, flavonoids, polyphenols and terpenoids. These compounds can counteract free radical activity and prevent oxidation processes in the body (Hasanah et al., 2017). Chlorogenic acid exhibits antioxidant activity by preventing lipoprotein oxidation caused by various oxidants, scavenging free radicals, and interrupting chain reactions (Rojas-González *et al*., 2022). Based on the aforementioned explanation, this study aims to determine the effect of *Coffea canephora* bean extract on the heart histopathological features of Wistar rats (*Rattus norvegicus*) induced with lead acetate.

MATERIALS AND METHODS

This study used 25 Wistar rats (*Rattus norvegicus*) aged between eight and ten weeks and weighing between 200 and 250 grams, with no abnormalities or illnesses. The rats were provided with commercial feed and water ad libitum. Cages and cage mats were lined with sterile sawdust. Food and water containers were kept sterile. This study used lead acetate at a dose of 2 mg/kg BW, *Coffea canephora* bean extract at doses of 200 mg/kg BW, 400 mg/kg BW, and 800 mg/kg BW using the Soxhlet extraction, distilled water, physiological NaCl, CMC-Na 1%, formalin buffer 10%, ketamine and xylazine. Meanwhile, the research equipment included an oral sonde, a trinocular microscope (Nikon Eclipse E200), microscope slides, and minor surgical instruments for necropsy.

Preparations for the heart histopathology using the hematoxylin-eosin (HE) staining were carried out at the Research and Diagnostic Laboratory of the Satwa Sehat Veterinary Clinic in Malang. Prior to treatment, an adaptation test was conducted for seven days by monitoring the weight of the rats. Subsequently, the rats were randomly divided into five treatment groups, each of which consisted of five rats.

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The negative control group (C-) did not receive lead acetate and *Coffea canephora* bean extract, while the positive control group (C+) was induced with lead acetate at a dose of 2 mg/kg BW orally. The treatment one group (T1) was induced with lead acetate at a dose of 2 mg/kg BW orally and administered with *Coffea canephora* bean extract at a dose of 200 mg/kg BW. The treatment two group (T2) was induced with lead acetate at a dose of 2 mg/kg BW orally and administered with *Coffea canephora* bean extract at a dose of 400 mg/kg BW. Finally, the treatment three group (T3) was induced with lead acetate at a dose of 2 mg/kg BW orally and administered with *Coffea canephora* bean extract at a dose of 800 mg/kg BW.

According to Roni (2019), the dose of lead acetate was 2 mg/kg BW for 14 days. Meanwhile, according to El-Nouri and Jankeer (2009), the doses of *Coffea canephora* bean extract were 200 mg/kg BW, 400 mg/kg BW, and 800 mg/kg BW administered four hours after the administration of lead acetate was administered for gastric emptying. On day 15, the rats were weighed for their final body weight and euthanized using a combination of 8 mg/kg BW of ketamine HCL and 1 mg/kg BW of xylazine for anesthesia, followed by cervical dislocation. Subsequently, the hearts of the rats were removed and stored in a 10% buffered formalin solution for histopathology using the HE staining.

Heart histopathological features were examined using a trinocular microscope at 400x magnification. Each specimen was examined for changes in five different visual fields to performe the modified scoring developed by Klopfleisch's (2013), which includes the following scores: 0 (normal), 1 (cell degeneration), 2 (hemorrhage), and 3 (necrosis, including pyknosis, karyorrhexis, and karyolysis). The data on cardiac muscle cell damage were analyzed using the Kruskal-Wallis and the Mann-Whitney post hoc tests with the SPSS Statistics 20 for Windows computers.

RESULTS AND DISCUSSION

The scoring results showed varying degrees of cardiac muscle cell damage. The C+ group, which was induced by lead acetate at a dose of 2 mg/kg BW, had the highest score of 2.08 ± 0.36. This suggested that lead acetate affected cardiac muscle cells. The microscopic examination of heart cells revealed necrosis and hemorrhage in the cells. In comparison, the C- group had the lowest scoe of 0.24 ± 0.17. This suggested that the cardiac cells were almost normal because the C- group did not receive any treatment. Instead, it was used as a representation of normal cardiac muscle cells for comparison with the treated groups. Furthermore, the T1 group showed had a score of 1.48 ± 0.92, which is lower than that of the C+ group. The T2 and T3 groups also had lower scores than the C+ group, which are 0.92 ± 0.46 and 0.56 ± 0.52, respectively. These results suggested that the administration of *Coffea canephora* bean extract affected the heart histopathological features of rats induced with lead acetate.

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Table 1. Mean and standard deviation of heart cell damage

Group	Mean ± SD
C-	0.24 ± 0.17
C+	2.08 ± 0.36
T1	1.48 ± 0.92
T2	0.92 ± 0.46
T3	0.56 ± 0.52

Notes: mean = average heart cell damage; SD = standard deviation

According to the results of the Mann-Whitney post hoc test in Table 2, the C- group differed significantly from the C+, T1, and T2 groups, but did not differ significantly from the T3 group. On the other hand, the C+ group differed significantly from the T2 and T3 groups, but did not differ significantly from the T1 group. In addition, the T1 group showed a significant difference from the C- group, but did not show a significant difference from the C+, T2, and T3 groups. The T2 group also showed a significant difference from the C- and C+ groups, but did not show significant difference from the T1 or T3 groups. Finally, the T3 showed similar results to the T1 group, that is, a significant difference from the C- and C+ groups, but not from the T1 or T2 groups.

Table 2. Results of the Mann-Whitney post hoc test

Group	p-value
C- and C+	0.008*
C- and T1	0.008*
C- and T2	0.008*
C- and T3	0.390
C+ and T1	0.141
C+ and T2	0.011*
C+ and T3	0.009*
T1 and T2	0.278
T1 and T3	0.074
T2 and T3	0.203

Note: *$p < 0.05$ (significantly different)

The histology of cardiac muscle cells in the C- group showed no evidence of degeneration, hemorrhage, or necrosis. These results are consistent with the findings of Hakimah et al. (2021) who reported minimal necrosis of cardiac muscle cells in the C- group. In contrast, the C+ group, which received lead acetate at a dose of 2 mg/kg BW, exhibited necrosis, hemorrhage, and degeneration in cardiac muscle cells.

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The damage caused by lead induction is consistent with the findings of Aksu et al. (2017) who reported hemorrhage, inflammatory cell inflation, and necrosis in cardiac muscle cells. Lead-induced damage to cardiac tissue results in the production of superoxide anion radicals, leading to an increase in malondialdehyde (MDA) levels, which reflect the overall levels of lipid peroxidation (Javorac et al., 2022).

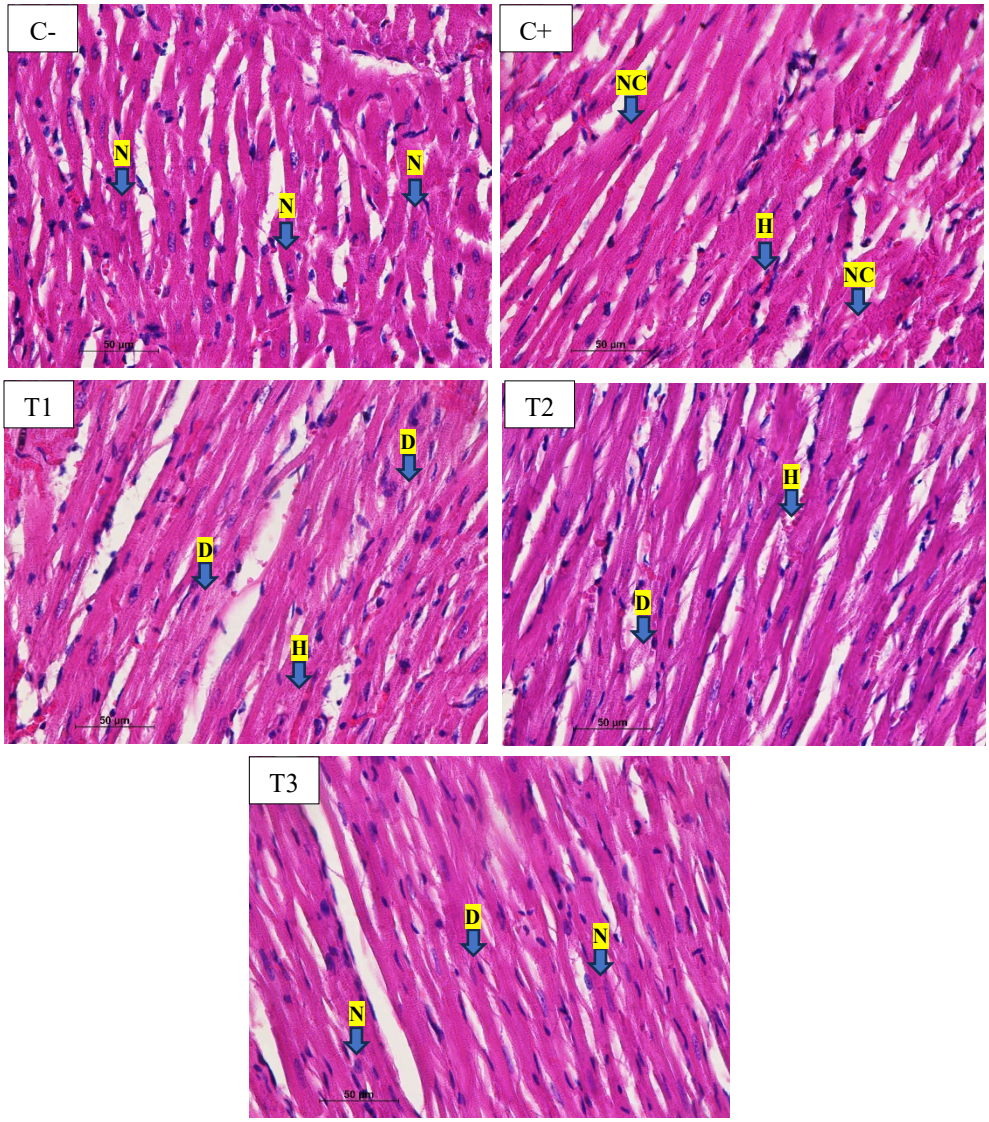

Figure 1. Heart histopathology of Wistar rats at 400x magnification. Description: C- (no treatment); C+ (2 mg/kg BW of lead acetate); T1 (2 mg/kg BW of lead acetate and 200 mg/kg BW of *Coffea canephora* bean extract); T2 (2 mg/kg BW of lead acetate and 400 mg/kg BW of *Coffea canephora* bean extract); T3 (2 mg/kg BW of lead acetate and 800 mg/kg BW of *Coffea canephora* bean extract); N = normal; D = degeneration; H = hemorrhage; NC = necrosis.

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The T1 group showed no significant difference from the C+ group. The dose of coffee bean extract administered to the T1 group, 200 mg/kg BW, did not result in an increase in the repair response of cells, suggesting that it was not effective in repairing lead-induced damage to cardiac muscle cells. *Coffea canephora* bean extract contains chlorogenic acid, which acts as an antioxidant by donating H+ atoms to free radicals. However, if the dose is too low, this mechanism can have adverse effects by promoting oxidation (Miao & Xiang, 2020).

Furthermore, the T2 group showed no significant difference from the C+ group but showed significant difference from the C- group. The dose of coffee bean extract administered to the T2 group, 400 mg/kg BW, did not result in a significant improvement in lead-induced cardiac muscle cell damage, which is similar to the T1 group. In contrast, the T3 group showed a slight improvement in the heart histopathological features. Therefore, the T3 group showed significant difference from the C- and C+ groups.

Genes that can encode antioxidants are induced by transcription factors with polyphenols, one of which is chlorogenic acid. Chlorogenic acid can activate nuclear-factor-erythorid-2-related-factor-2 (NRF2) and regulate the transcription of genes that encode cytoprotective proteins, including superoxide dismutase (SOD). The inactive state of NRF2 is typically stored in the cytosol and binds to its inhibitor protein, Kelch-like ECH-associated protein-1 (Keap1). Similarly, nuclear factor kappa-B (NF-kB) is stored in the cytoplasm and binds to its protein inhibitor, I-kB. NRF2 plays a role in inhibiting NF-kB in the inflammatory process. If activated, NRF2 translocates to the nucleus and leads to increased transcription of various antioxidants, including SOD (Layal, 2016; Tazuyyun, 2020).

The antioxidant mechanism of *Coffea canephora* bean extract administered orally was unable to counteract the effects of lead-induced free radicals due to the decomposition of its chlorogenic acid content in the gastrointestinal tract (Wisudawati et al., 2023). In addition, the lack of prolonged administration of the extract may also contribute to the lack of difference between the treatment and control groups. Sasmita et al. (2017) argued that bioactive compounds in *Coffea canephora* bean extract are already effective at normal concentrations in the control group. However, the doses of 200 mg/kg BW, 400 mg/kg BW, and 800 mg/kg BW were found to be ineffective in improving cardiac muscle cells induced with lead acetate at a dose of 2 mg/kg BW.

CONCLUSION

This study concluded that the administration of *Coffea canephora* bean extract at a dose of 200 mg/kg BW, 400 mg/kg BW, and 800 mg/kg BW did not improve the heart histopathological features of Wistar rats (*Rattus norvegicus*) induced with lead acetate.

APPROVAL OF ETHICAL COMMISSION

This study received ethical approval from the Animal Ethics Committee of the Faculty of Veterinary Medicine, Universitas

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