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Evaluation of Robusta Coffee Bean Extract (Coffea canephora) Efficacy as an Antioxidant in Mitigating Lead Acetate (Pb) Induced Alterations in Villi Epithelial Height and Duodenum Crypts Depth in Rats (*Rattus norvegicus*)

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Abstract

Exposure to lead acetate in the long term can cause health problems and cell damage. This study was conducted to determine the effectiveness of post-administration of lead acetate with robusta coffee bean extract (*Coffea canephora*) on the histotoxicity of the duodenum in25male Wistarrats (*Rattus norvegicus*) Wistar strain. The study included five treatment groups: K(-) as the negative control, K(+) with oral exposure to 2 mg/kg body weight of lead acetate, and P(1), P(2), and P(3) receiving 2 mg/kg body weight of lead acetate followed by robusta coffee bean extract (*Coffeacanephora*) at doses of 200 mg/kg, 400 mg/kg, and 800 mg/kg, respectively. Each group comprised five rats. The investigation aimed to assess the dose-dependent protective effects of the coffee bean extract on villi epithelial height and duodenum crypts depth in rats (Rattus norvegicus) exposed to lead acetate and the data were analyzed statistically using the Kruskal-Wallis test. Measurement of villi height and duodenal crypt depth in this study showed that the antioxidant effect of robusta coffee bean extract (Coffea canephora), given in doses of 200, 400, and 800 mg/kg BW that caused by lead acetate showed an increase in mean villi height and an increase in mean crypt depth although not significant in reducing duodenal damage.

Key words: *Coffea canephora*, duodenum, health, lead acetate

Heavy metals can increase levels of harmful pollutants that can cause toxicity or poison disrupt life processes and after reaching certain levels can kill pets (Zulfahmi *et al*., 2018). Non-essential heavy metals such as Pb, Cd, and Hg are still unknown the beneficial to living things and can even be very harmful to living things (Asati *et al*., 2016). Lead (Pb) is one of the heavy metals used by humans and is most commonly found in the environment. Pb has an atomic number of 82 and a density of 11.34 g/cm^3 (Bhardwaj *et al*, 2021). It is a transition metal from the carbon group in the periodic table that has stability, high density, corrosion resistance, and a very long half-life (Sembel, 2015). Lead acetate is a combination of lead (II) oxide with acetic acid which is soluble in water, concentrated sulfuric acid, nitric acid, and acetic acid (Riwayati *et al*., 2015).

The most common causes of lead poisoning are consuming lead-contaminated food and water, or accidentally ingesting lead-contaminated soil (Bergeson, 2008). Exposure to lead can trigger increased production of free radicals, leading to oxidative stress characterized by a decrease in antioxidants in the body (Sari *et al*., 2018). Rapid bloodstream absorption of lead

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results in a variety of morphological, biochemical, and physiological alterations in different body organs (Sansar *et al*., 2011). The formation of cellular damage through the mechanism of membrane lipid peroxidation is a protective factor that reduces antioxidants, namely the superoxide dismutase (SOD) and catalase (CAT) enzymes (Kham, 2010).

Lead can damage cells in the body and disrupt the biochemical and physiological functions through the formation of reactive oxygen species (ROS) that induce oxidative stress (Santoso, *et al*., 2021). One of the damages caused by lead is damage to the submucosa and mucosa of the duodenum (Elsenhans, 2011). According to Cunningham and Klein (2007), the duodenum is an organ that has a function for the absorption of nutrients and water. The villi are the most responsible part for nutrient absorption because they have absorptive cells in the form of cylindrical layered epithelium with striated borders in the villi mucosa. One of the segments of the small intestine where most absorption takes place is the duodenum. Damage to the duodenum will result from excessive ROS production affecting the process of nutrient absorption.

Lowering ROS levels can be done by increasing the bodydefensesystem. The body has a defense system, one of which is antioxidants. Antioxidants are molecules that have the ability to overcome the effects of free radicals. The role of antioxidants is to reduce or stop chain reactions by removing free radicals or inhibiting other oxidation reactions (Elsayed and Azab, 2019). Antioxidants can be endogenous antioxidants, which are found in the body, and exogenous antioxidants, which come from outside the body, such as from food and supplements (Parwata, 2016).

Robusta coffee (*Coffea canephora*) can be an exogenous antioxidant that plays a role in reducing free radicals or inhibiting other oxidation reactions (Saputra *et al*., 2021). Robusta coffee (*Coffea canephora)* has chlorogenic acid or chlorogenic acid is the most polyphenolic compound. Chlorogenic acid is known to inhibit the action of xanthine oxidase when oxidizing xanthine (Farhaty and Muchtaridi, 2016). Robusta coffee (*Coffea canephora*) contains many polyphenolic compounds in the form of caffeoylquinic acids (CQAs), feruloyl quinic acids (FQAs), dicaffeolyquinic acids (diCQAs), and chlorogenic acid (CGA) contained in more than 14% (Ayelign and Sabally, 2013; Farah and Donangelo, 2006). Robusta coffee (*Coffea canephora*) contains alkaloid compounds, flavonoids, saponins, tannins, caffeine, and phenols (Wigati *et al*., 2018).

Taking into consideration the aforementioned background, it is commonly observed that lead poisoning generally occurs through oral ingestion of contaminated food or water. The duodenum is particularly susceptible to harm as it serves as a crucial site for nutrient and water absorption. Consequently, the cells within the duodenum are adversely affected by oxidative stress induced by the accumulation of reactive oxygen species, primarily caused by the presence of lead. currently, it is imperative to investigate the efficacy of external antioxidants extracted from robusta coffee beans (*Coffea canephora)* to treat the toxic effects of lead acetate induction, as observed through duodenal histopathological changes.

Materials and Methods

This study was conducted at the Laboratory of Animal Experiments, School of Health and Life Sciences, Banyuwangi, Universitas Airlangga. Histopathological preparations of the duodenum of rats were made at the Research and Diagnostic Laboratory of the Healthy Animal Clinic.

The experimental animals used in this study were 25 rats (*Rattus norvegicus*) male Wistar strain aged three months, weighing 200 - 250 grams and healthy. Rats (*Rattus norvegicus*) were obtained from the UD Tiput Abdi Jaya Animal Husbandry Test, Jogjakarta with a certificate (No.1.KEH.052.01.2023).

The materials used in this study were lead acetate at a dose of 2 mg/kg BW robusta green coffee bean extract, commercial feed, drinking water, physiological NaCl, CMC Na 1%, 10% buffered formalin, ketamine, and xylazine.

The tools used were rat cages, oral sonde, 1 cc syringe, analytical balance, Trinocular

microscope (Nikon Eclipse E200).

Extraction of robusta coffee beans (*Coffea canephora*) was carried out using the maceration method. The dried and ground robusta coffee (*Coffea canephora*) bean extraction sample was pre-extracted with of ethanol 96% and left for 48 hours. The ethanol fraction was discarded to obtain maximum results, while the ethanol extract was evaporated to dryness under reduced pressure in a rotary evaporator at 50°C. Solutions were filtered through a filter (Ayanlowo *et al*., 2020).

In order to conduct the experiment on the treatment of male Wistar strain (Rattus norvegicus), a total of 25 white rat samples (Rattus norvegicus) were selected. The sample size was determined using the Federer formula, resulting in five treatment groups (K-, K+, P1, P2, P3), each consisting of five replicates. The body weight of rats was measured prior to undergoing treatment. The rats were randomly allocated into five distinct research groups, with each group comprising five rats. The animals were acclimated to the conditions of the cage for a period of seven days. The animals were provided with ad libitum access to bottled drinking water and were also given commercial feed.

Details of the treatment of robusta coffee bean extract (*Coffea canephora*) and lead acetate, namely:K(-): without lead acetate exposure),K(+): given 2 mg/kg BW lead acetate exposure orally, P(1); P(2); and P(3): given2 mg/kg BWlead acetate exposure then given robusta coffee bean extract (*Coffea canephora*) at a dose of 200 mg/kgBW; 400 mg/kg BW; and 800 mg/kg BW. The treatment was carried out for 14 days.

On the 15th day, the final body weight of rats (*Rattus norvegicus)* was calculated. Then rats were euthanized using a combination of ketamine HCl8 mg/kgBWand xylazine 1 mg/kg BWvia intramuscular injection. After that, rats were fixed and dissected to separate the intestine organs from the digestive system tract, then take a small pieces of the duodenum for histological examinations. Washed the organ in normal saline, and placed in 10% buffered formalin. Using the ascending ethhyl alcohol (70%, 90%, and 100%) for dehydration of fixed tissue and then cleared with xylene. Infiltration with paraffin wax at 60 and followed by embedding. Paraffin blocks were cut at 4µm thick from all specimens using mikrotome, then affixed to the slides and stained with Haematoxylin and Eosin (HE) for the coloring of histopathologycal slides.

The act of examining the organs in the duodenum pertains to the study conducted by Rahayu (2023), where the focus was on the examination of villi structures. This examination was conducted by an objective lens magnification of 100 times to size up the villi and duodenal crypts within five specified fields of view. Subsequently, the height of the villi and the depth of the crypts were assessed through the assistance of a trinocular microscope equipped with the NIS-Elements D ver 5.41.00 software application. The assessment involved determining the proportion between the height of the epithelial villi and the depth of the duodenal crypts.

The results of quantitative data obtained from damage to villi epithelial height andduodenalcrypt depth will be analyzed using the SPSS (Statistical Product and Service Solution) program version 23. Data were tested using the One Way ANOVA test followed by the PostHoctest.

Results and Discussions

The data obtained showed that the duodenal villi height was not significantly different between treatments K(-), K(+), P(1), P(2), and P(3) because the significance value of Asymp. significance (p) > 0.05but there was an increase in the average villi height. The significance value (p) through ANOVA test on villi height is 0.349 (p>0.05). The average obtained in the ANOVA test on duodenal villi height of treatment groups K(-) 493 µm, K(+) 543 µm, P(1) 618 µm, P(2) 554 µm, and P(3) 553 µm (Fig 1).

The results of the data obtained in the ANOVA test showed that the depth of the duodenum was significantly different because of the significance value of Asymp. significance 0.003 (p < 0.05) and there is an increase in the average of each treatment. The post hoc follow-up test showed that the K(+) and P(3) treatments had a significance value of 0.034 (p<0.05). The mean obtained in the ANOVA test on the height of

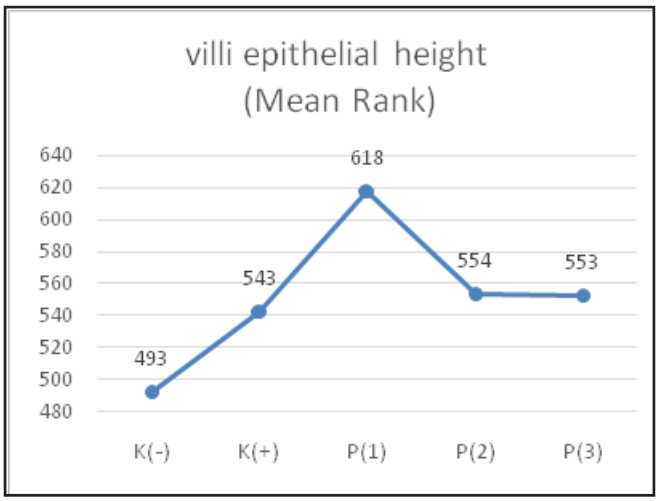

Fig 1. Mean villi height

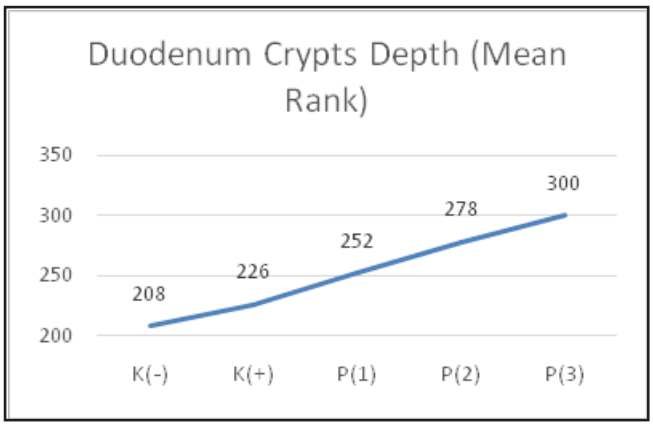

Fig 2: Mean depth of duodenal crypts

duodenal villi in treatment groups K(-) 208 µm, K(+) 226 µm, P(1) 252 µm, P(2) 278 µm, and P(3) 300 µm (Fig 2).

Based on the statistical results of the effect of robusta coffee bean extract (Coffea canephora), with 800 mg/kgBW able to give optimal results to increase the depth of duodenal crypts. While the effect of robusta coffee bean extract (Coffea canephora), 200 mg/kgBW, 400 mg/kgBW, and 800 mg/kgBW had no effect on increasing villus height and duodenal crypt depth and tended to decrease as the dose increased.The results of this study are in accordance with the research of Rosyidi *et al.* (2020) on the effect of giving robusta coffee from Lampung (*Coffea canephora*) on the relative amount of Ho-1, Nrf2 that the measurement of villi height shows a decrease as the dose of coffee extract increases.

Research by Chen *et al.* (2018) states that villi height and the ratio of villi height to duodenal crypt depth are considered criteria to reflect the morphology of the intestinal mucosa and the absorption capacity of the small intestine, thus, an increase in villi height, villi: crypt ratio depth corresponds to an increase in nutrient digestion and absorption.

The addition of robusta coffee bean extract (*Coffea canephora*) at doses of 200 mg/kgBW, 400 mg/kg BW, and 800 mg/kg BW did not affect the morphology of the height of the villi and the depth of the duodenal crypts, because damage to the intestinal villi can cause substances not to be absorbed in the duodenum. It happens as a result of the xenobiotics that can interfere with the process of food absorption (Natalia, 2007). The toxic compound caused by the administration of lead acetate (Pb) at a dose of 2 mg/kgBW is used as a model of damage to the duodenal tissue. Pb will produce free radicals where free radicals can produce reactive oxygen compounds (ROS) that induce oxidative stress (Santoso, *et al.*, 2021). The most dangerous ROS compound is the hydroxyl radical which will have a negative impact on cell membranes. Hydroxyl radicals can form imprints on cells through the mechanism of membrane lipid peroxidation as a protective factor that reduces antioxidants inside the body (Kham, 2010). Cell damage in the duodenum due to lipid peroxidation causes the intestinal mucosa layer to be unable to absorb incoming food nutrients so that metabolism in the body is disrupted (Hariono, 2006). As a result, the content of chlorogenic acid as an antioxidant in robusta coffee bean extract (*Coffea canephora*) has not been able to overcome the damage caused by lead acetate exposure.

Conclusion

Based on this study it can be concluded that treatment for 14 days in rats (Rattus norvegicus) given robusta coffee bean extract (Coffea canephora) at a dose of 200 mg / kgBW, 400 mg / kgBW, and 800 mg / kgBW has no significant effect but there is an increase in the average histopathological picture of villi height in each treatment group. For the results of the depth of duodenal crypts in the administration of robusta

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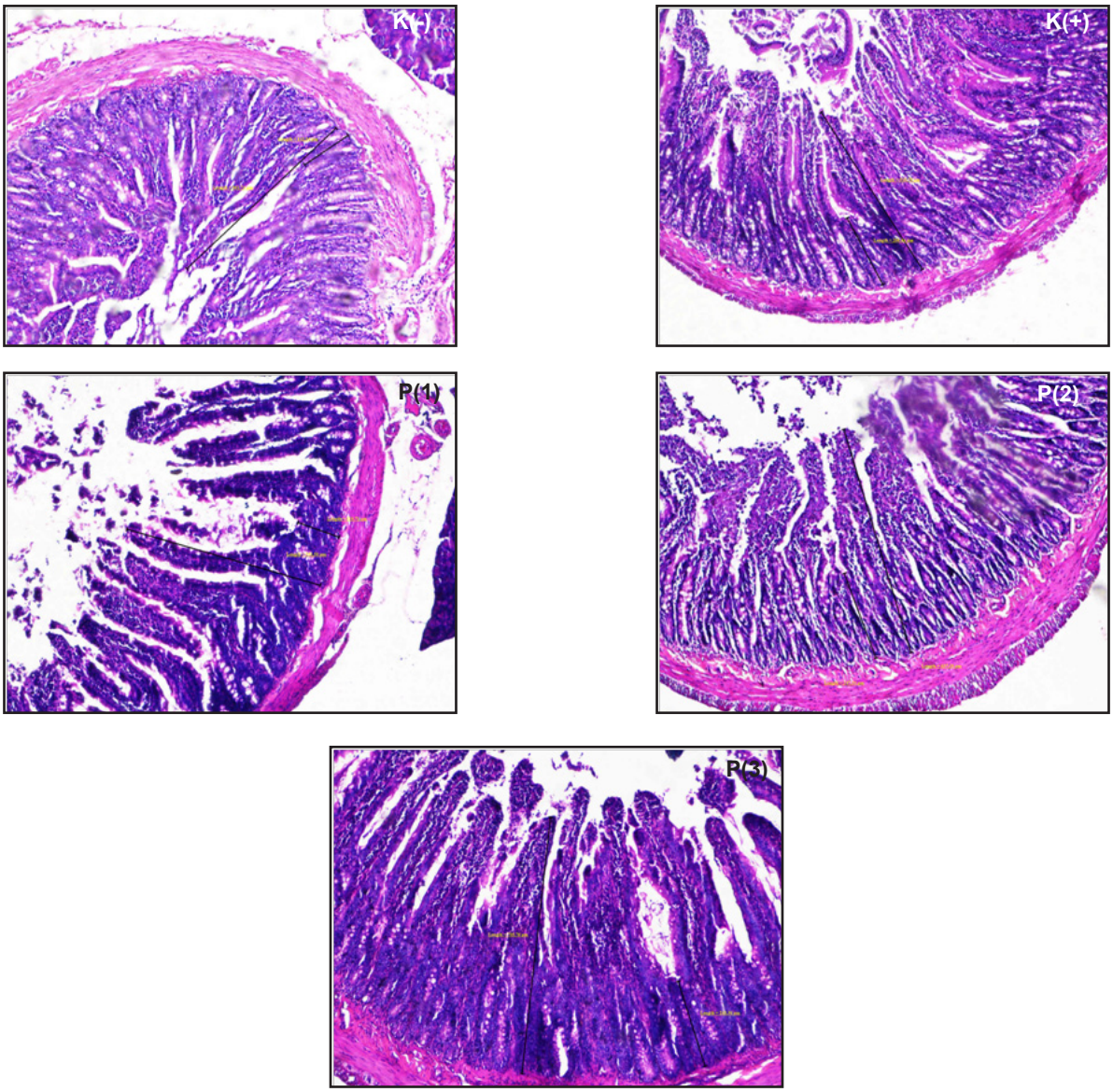

Fig 3. Histopathology of the duodenum with HE stain and 100x objective lens magnification. Description: Blue arrows indicate villi and red arrows indicate crypts.

coffee bean extract (Coffea canephora) at a dose of 800 mg / kgBW which was given exposure to lead acetate (Pb) 2 mg / kgBW gave results that had a significant effect and the average results increased in the treatment group on the depth of duodenal crypts.

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