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Effect of Robusta Coffee Extract (*Coffea canephora*) on Mice (*Mus musculus*) on Gastric Histopathology and Anxiety Level

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Abstract

Robusta coffee (*Coffea canephora*) contains bioactive compounds, such as caffeine that have the potential to affect the digestive system and anxiety levels. This study aims to evaluate the effects of robusta coffee extract on gastric histopathology and anxiety levels in mice (*Mus musculus*). The method used involved 4 treatments, K1: negative control (without treatment), K2: positive control with aspirin 10.84 mg/20gr body weight of mice, K3: coffee extract 6.72 mg/20gr body weight of mice, K4: coffee extracts 13 mg/20gr body weight of mice. The results showed that robusta coffee extract impacted the histological structure of the stomach, with indications of changes in the gastric mucosa. In addition, there was a tendency for changes in the level of anxiety in mice analyzed through the behaviour test, the Elevated Plus Maze test. This study provides insight into the potential physiological effects of robusta coffee consumption and its implications for gastric health and psychological conditions.

Keywords: *Coffea canephora*; *Mus musculus*; Gastric; Histopathology; Anxiety Level.

INTRODUCTION

Coffee is one of Indonesia's favorite drinks from time to time. This is supported by Indonesia's ability to produce coffee as a leading commodity in the plantation sector (Haryani et al., 2022). Indonesia is ranked second in the Asia and Oceania region in coffee production. In 2022-2023, coffee production in Indonesia increased by 2.4% or around 12 million bags (International Coffee Organization, 2023). One type of coffee widely circulated on the market and is often used is robusta coffee (*Coffea canephora*) (Lumaksono et al., 2021). The caffeine in robusta coffee is almost twice as high as that of arabica coffee, which is 2.4% compared to that of arabica coffee. Arabica coffee contains 1.3% of caffeine in dry conditions.

According to (Sri & Rubiyanti, 2020), excessive caffeine consumption can stimulate the heart and muscles, and increase gastric acid secretion. The caffeine in coffee can accelerate the process of stomach acid formation. The formation of gastric acid causes excessive gas production in the stomach, so people often complain of a bloating sensation. Consumption of caffeine can damage the tissue that makes up the stomach, especially the mucosal tissue. If the gastric mucosa is damaged, there is diffusion of HCl to the gastric mucosa and HCl will damage the mucosa. The presence of HCl in the gastric mucosa stimulates the change of pepsinogen to pepsin which stimulates the release of histamine from mast cells. Histamine will increase capillary permeability, resulting in fluid movement from the intracellular to the extracellular space, leading to edema and capillary damage, which in turn causes gastric bleeding. When the stomach continues to interact with irritants, the tissue will become inflamed, leading to the loss of the gastric mucosal layer and atrophy of gastric mucosal cells (Azer et al., 2025).

Another effect of caffeine is that it can affect a person's anxiety levels. This compound target is adenosine receptor antagonists (1,3,7-trimethylxanthine) which can stimulate the central nervous system. It is said that caffeine has anxiogenic and panicogenic effects in both animals and humans (Meurling, 2020). In some sensitive individuals, such as patients with panic disorder (PD), anxious behavior can be seen. The core symptoms of excessive anxiety are recurrent panic attacks, fear, or discomfort in some parts of the body, such as palpitations, shortness of breath, numbness, and dizziness. Panic attacks can also occur in other anxiety disorders as well as depressive disorders, and/or even sometimes in healthy individuals (Klevebrant & Frick, 2022). Identification of behavior in rodents can be seen using the elevated plus maze (EPM), the most used test to measure anxiety-like behavior in rodents. The use of EPM is based on the behavior of animals avoiding open

areas and their tendency to move towards more protected places (Florén Lind et al., 2023)

MATERIALS AND METHODS

Study area

This study used mice as experimental animals, with samples taken randomly using the Completely Randomized Design (CRD) method. The study lasted for 37 days, consisting of 7 days of acclimatization and 30 days of treatment. The sample criteria included male mice aged 3–4 months with a body weight of 25–35 grams, having a healthy physical condition, and showing no anatomical defects. This study was conducted at the Biology Education Laboratory, FKIP, Sebelas Maret University and Lab Pusat UNS. This study requires various tools and materials to support its implementation. The tools used include Elevator Plus Maze (EPM), mouse cages, analytical scales, beakers, erlenmeyers, stirrers or spatulas, grinders, and sieves. In addition, filter paper, aluminum foil, black plastic, rotary evaporator, oven, mice drink bottle, mice food container, oral sonde, latex, paraffin surgical pad, scissors, scalpel, cutter, pins, and object glass are required. The materials used include 3–4-month-old male mice, robusta coffee bean extract, 96% ethanol, mouse feed, water, and wood dust as additional media. All these tools and materials are used systematically to ensure the research was conducted according to the designed method.

Procedures

Robusta coffee exctract

Extraction is obtained by the maceration method. The extraction process by the maceration method is most often used to prevent damage to the content of certain compounds in robusta coffee. Coffee beans are roasted and ground to the same size. The coffee bean powder is stored in a container and tightly closed. Coffee bean samples are ground by pounding and blending which aims to increase the surface area of the sample so that the interaction of caffeine withdrawal in coffee occurs optimally. Robusta coffee bean extraction by the maceration method begins by inserting 120 g of coffee bean powder into an Erlenmeyer flask. The powder is soaked in 225 mL of 96% ethanol and the container is covered with aluminum foil. This process is carried out for 5 days with occasional stirring. After that, the mixture is filtered using filter paper to obtain the first filtrate and dregs. The dregs left are then soaked again (remaceration) with 75 mL of 96% ethanol for 2 days while stirring occasionally. Like the previous process, the mixture is filtered using filter paper to obtain the second filtrate. The two filtrates obtained were combined and evaporated using a rotary evaporator at 40-50°C to produce a thick extract (Tanauma et al., 2016).

Preparation of animals

The research sample criteria included male mice, aged 3-4 months, weighing 25-35 grams, in a healthy physical condition without anatomical defects. Animals were adapted a week before treatment (1 week) excluding 30 days of test treatment.

Treatment of animals

The treatment was conducted for 30 days, each group was given orally to 6 test animals which were divided into 4 groups:

- Negative control (K1): No treatment
- Positive control (K2): Aspirin 10.84 mg/20gr body weight of mice to induce gastritis
- Dose I (K3): 6.72 mg/20gr body weight of mice
- Dose II (K4): 13 mg/20gr body weight of mice

Data analysis

Anxiety level

In the EPM method, mice are placed in the center of the maze facing one of the closed compartments and are allowed to move freely to both sides. The duration and frequency spent by the experimental animals on the open and closed arms are recorded. Anxious animals will tend to be on the closed arm, while animals affected by anti-anxiety compounds will spend more time on the open arm.

This maze has two dimensions, the dimensions of the open arm: length 50 cm × width 10 cm. The dimensions of the closed arm: length 50 cm × width 10 cm with a wall height of 40 cm. The entire maze is elevated 50 cm above the floor. The anxiety level of the treatment effect was evaluated using the parameters (a) percentage of time spent on the open arm ([seconds on the open arm] / [300 s] × 100), (b) percentage of open arm entries ([open entries] / [total entries] × 100), (c) percentage of closed arm entries ([closed entries] / [total entries] × 100) (Guillén-Ruiz et al., 2021).

Histopathology

After 30 days of treatment, the mice were prepared for histological observation by removing their abdominal organs. Before removing the organs, the mice were euthanized by inhaling chloroform (AVMA, 2013)Next, the lower abdomen of the mice was cut to the chest. Next, the lower abdomen of the mice was cut to the chest, then the organs were removed from the mice's body. The abdominal organs must be separated from the attached intestines, liver, and gallbladder. After the mice died, a laparotomy was performed to remove the mice's stomach for microscopic preparations. Histological examination of the rat stomach was carried out by observing the gastric mucosal layer using an OPTILAB IRIS-4 microscope based on the Wattimena score (Lumaksono et al., 2021) which was then analyzed using the Kruskall-Wallis statistical test.

Wattimena score:
1 : No erosion found
2 : Erosion only on the surface epithelium
3 : Erosion reaches a depth of 1/3 of the upper gastric glands
4 : Erosion reaches a depth of 1/3 of the middle gastric glands
5 : Erosion reaches a depth of 1/3 of the lower gastric glands
6 : Erosion reaches the depth of the lamina muscularis mucosa

RESULTS AND DISCUSSION

Body weight of mice

This study was conducted using four different treatments on mice to observe their effects on changes in body weight, histopathology, and anxiety levels. The results of the observations showed variations in changes in body weight between treatment groups. The data obtained were analyzed using Analysis of Variance (ANOVA) and Tukey's advanced test in the study of the effect of robusta coffee extract on the body weight of mice shown in Table 1.

The results showed that the average between groups did not differ ($p>0.05$) with a significance level of 0.59 (body weight in week 1) and 0.29 (body weight in week IV). The treatments did not significantly affect the body weight of mice, despite a decrease observed in K2, K3, and K4 (Table 1). There was no significant difference in body weight changes between treatment groups. The decrease that occurred showed the effect of the treatment, but the effect was too small to be detected by the statistical test used.

Table 1. Average body weight (grams) of mice during treatment (30 days).

Treatments	Initial treatment X±SD	Final treatment X±SD	Body Weight Difference
K1 (Negative control)	31.13 ± 1.11	35.63 ± 1.11	+4.5
K2 (Aspirin 10.84 mg/20g BW of mice)	32.13 ± 2.98	31.25 ± 2.25	-0.88
K3 (Coffee extract 6.72 mg/20g BW of mice)	33.50 ± 3.72	31.00 ± 5.12	-2.5
K4 (Coffee extract 13 mg/20g BW of mice)	34.50 ± 5.52	33.63 ± 4.80	-0.87

Note: Data presented as mean (X) ± standard deviation (SD)
BW = Body Weight

Anxiety levels in mice

The anxiety level of mice was analyzed using the Elevated Plus Maze (EPM) method. This method is one of the standard tests for evaluating anxiety behavior, which is based on the tendency of mice to choose open or closed areas. Data were tested using Anova and Tukey's advanced test.

The results of this observation are expected to provide an overview of the anxiety response that arises due to the treatment given. There is a significant difference ($p <0.05$) in each treatment group, which is interpreted as mice's anxiety level. From the data presented K2, K3, and K4 significantly showed anxiety activity (Figure 1). Meanwhile, K1 (Negative control) did not show anxiety activity. The average percentage of time spent in the open arm position was highest in the negative treatment group (K1) which showed a low level of anxiety. On the other hand, K4 showed the lowest frequency of entering the open area compared to other groups. This can indicate a high level of anxiety compared to other groups. This is due to K1 not receiving any treatment. Giving coffee extract to mice affected the anxiety of mice, in the form of a low entry time and entry frequency when mice entered the open arm.

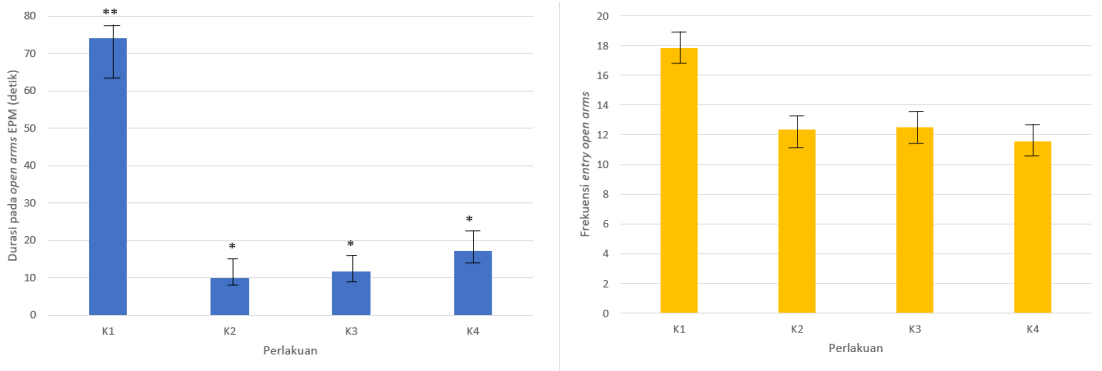

Figure 1. Average duration (left) and frequency (right) of entries in open arms during elevated plus maze (EPM) testing. Data shown as mean values and standard deviation (SD). Significant differences indicated by * and ** (one-way ANOVA test with Post Hoc LSD).

Table 2. Data on the effect of robusta coffee extract on anxiety levels.

Treatment	Time in open arms X±SD	Entry to open arms X±SD	Entry to close arms X±SD
K1 (Negative Control)	$74.2^{b} \pm 13.93$	$17.8^{a} \pm 12.8$	$15.5^{a} \pm 11.4$
K2 (Aspirin 10.84 mg/20g BW of mice)	$9.89^{a} \pm 0.84$	$12.34^{a} \pm 7.7$	$20.98^{a} \pm 7.7$
K3 (Coffee extract 6.72 mg/20g BW of mice)	$11.66^{a} \pm 6$	$12.5^{a} \pm 4.17$	$20.83^{a} \pm 8.3$
K4 (Coffee extract 13 mg/20g BW of mice)	$17.22^{a} \pm 5.66$	$11.56^{a} \pm 3.12$	$21.7^{a} \pm 11.2$

Note: Data presented as mean (X) ± standard deviation (SD)
BW= Body Weight

Gastric Histopathology

Table 3. Kruskal-Wallis Test.

	Histopatology
Kruskal-Wallis	7.500
df	3
Asymp. Sig.	0.58

Data analysis showed that the dose 2 group had the highest histopathology score, followed by the positive and dose 1 groups (with the same Mean Rank value), then the negative group with the lowest Mean Rank. The p value (0.058) exceeds the significance level (α = 0.05). Thus, there was no significant difference in the gastric histopathology score between treatment groups. Although there was a difference in Mean Rank between groups (especially at the higher dose 2), the Kruskal-Wallis test results showed that the difference was not statistically significant.

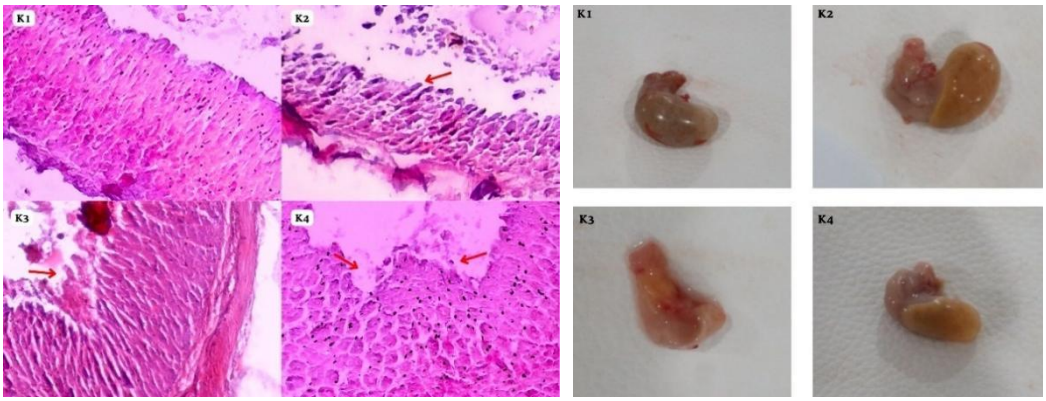

Figure 2. Microscopic histological picture of mice stomach. 40x magnification (left) and morphology of mice stomach (right). Red arrows indicate erosion on the surface epithelium.
Note: (a) K1: Negative control, Wattimena Score 1; (b) K2: Aspirin 10.84 mg/20g BW mouse, Wattimena Score 2; (c) K3: Coffee extract 6.72 mg/20g BW mouse, Wattimena Score 2; (d) K4: Coffee extract 13 mg/20g BW mouse, Wattimena Score 2.

In the negative group, the epithelium on the surface of the intact mucosal layer (score 1), without erosion. This is due to the absence of gastritis triggers such as caffeine in coffee drinks compared to other groups. Compared to other groups, the morphological picture of the stomach in the negative group shows a normal and healthy stomach. In group K2: Aspirin 10.84 mg/20gr of mouse body weight, K3: Coffee extract 6.72 mg/20gr of mouse body weight, and K4: Coffee extract 13 mg/20gr of mouse body weight showed erosion on the surface epithelial layer (score 2).

Morphologically, the negative group shows a healthy and normal stomach, shaped like red beans or J-shaped (Chen et al., 2016), the color of the stomach shows an even red. Group 2 shows changes in color and texture in the form of lumps indicating irritation due to aspirin administration. K3 and K4 show changes in color and shape of the stomach, in the form of shrinkage, where the normal shape of the stomach is like red beans or the letter J.

Discussion

The results of the study showed that the treatment given did not have a significant effect on changes in the weight of mice. Although there was a decrease in body weight in several treatment groups, the effect was too small to be detected by the statistical test. The insignificant difference was likely due to the relatively short duration of the study. According to Wijayanti & Kadita (2017), weight loss can be felt through long-term coffee consumption.

At the level of anxiety, giving coffee extract to the mice affected the anxiety of mice. This is indicated by the low time and frequency of entry when mice entered

with open arms. The caffeine content in coffee can have a significant negative impact on mood and work performance if consumed continuously. Caffeine is known to trigger feelings of restlessness, anxiety, and anger which have the potential to hurt the body (Purdiani, 2014). As a psychostimulant drink, coffee is considered to be able to reduce fatigue and drowsiness, as well as provide additional energy. The structure of caffeine is similar to that of adenosine, allowing it to bind to adenosine receptors in the brain, thereby increasing cortical activity through the ARAS (Ascending Reticular Activating System) pathway and stimulating nerve activity (Dewanti & Tadjudin, 2023).

In histopathological observations, although there was a difference in Mean Rank between groups (especially at a higher dose of 2), the results of the Kruskal-Wallis test showed that the difference was not statistically significant. This shows that the administration of coffee extract at the tested dose did not provide significant changes in the histological structure of the stomach of mice between treatment groups.

Under normal conditions, the defense mechanism of the preepithelial gastric mucosa, namely mucus, can protect the stomach from endogenous and exogenous damage. The normal stomach lining is due to the absence of gastritis triggers, such as caffeine and others. Giving aspirin to mice aims to provide pain relief from gastritis. Aspirin is a non-steroidal anti-inflammatory drug (NSAID) that can cause discomfort because it is induced by aspirin which causes nausea and vomiting (Farhan et al., 2022). This drug has two mechanisms of action, namely irritating the gastric epithelium and/or inhibiting prostaglandins. Then, gastric ulcers (inflammation of the gastric mucosa) are formed (Oktrinorma & Indriyanti, 2020).

The effect of erosion on the mucosal layer is caused by the caffeine content in robusta coffee. In Lumaksono et al (2021), 100 grams of Coffea canephora seeds contain 6.1-11.3 grams of chlorogenic acid, 1.5-2.5 grams of caffeine, and 0.2-0.8 grams of diterpene. The caffeine content in coffee can increase stomach acid production if consumed continuously. Excessive stomach acid can cause inflammation of the stomach wall, gastritis. In addition, caffeine can also weaken the esophageal sphincter (valve muscle between the esophagus and stomach) (Haryani et al., 2022)

In addition to internal factors, mouse epithelial erosion can also be influenced by uncontrolled external variables, such as psychological stress experienced by mice or initial stomach conditions not analyzed in this study. Psychological stress can trigger the release of angiotensin II which impacts decreasing blood flow to the gastric mucosa. As a result, reactive oxygen species are formed which trigger oxidative stress, ischemia, and damage to the gastric mucosal layer (Lumaksono et al., 2021).

CONCLUSIONS

The use of robusta coffee extract in this study had varying effects on several parameters. In the parameter of weight, there was no significant effect between treatments, although there was a tendency for weight loss in mice. In the histopathology of the stomach of mice, there was an effect of the treatment with robusta coffee extract. Coffee extract caused damage in the form of erosion on the surface epithelium of the stomach. In addition, there was a significant effect too on the observation of anxiety levels. This is indicated by the frequency of mice in the open area being lower, compared to the closed area.

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Competing Interests: There are no competing interests.

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