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# Acute Oral Toxicity and Histophatological Study of Ethanol Extract and Fractions of *Etlingera elatior* Flowers in Mice

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***Abstract***

***Background:*** *Etlingera elatior (Jack) R.M. Sm or Kecombrang Flower had been used traditionally to enhance the taste of food. Some studies reported its pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, and anticancer. However, its safety has not yet been reported explicitly.* ***Objective****: To investigate the acute oral toxicity, macropathological and histopathological changes of 96% ethanol extract, n-hexane, ethyl acetate and methanol-water fractions of Etlingera elatior flowers in Balb/C mice.* ***Methods****: The 96% ethanol extract, n-hexane, ethyl acetate and methanol-water (3:7) fractions were given to mice with 4 dose levels (75, 150, 300, and 600 mg/Kg body weight). Single oral administration of them was done on the first day of the test and the mice were then observed in 14 consecutive days. The control group received Na-CMC 0,3%. Changes in behavior, mortality rate, body weight, macropathology and histopathology of kidneys and liver were assessed.* ***Results****: No signs of toxicity or mortality were observed when mice were exposed to the 96% ethanol extract, n-hexane, ethyl acetate and methanol-water fractions. The were no significant changes in the body weight. Macropathological examination of the liver and kidneys showed normal results with a brownish red color, smooth surface and rubbery consistency. Histopathological examination revealed mild, moderate, and severe damage to the liver and kidneys of mice, however the level of damage was not followed by an increase in dose. The oral lethal dose was higher than 600 mg/Kg.* ***Conclusion****: Etlingera elatior (Jack) R.M. Sm did not produce toxic effects in mice after acute treatment.*

***Keywords:*** *acute toxicity, Etlingera elatior, kecombrang*

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## INTRODUCTION

Herbal medicines are usually considered safe or have low toxicity based on their long history of use by humans. However, recent studies showed that many herbal medicines have side effects (Bent, 2008). The safety issues are major problem in the use of medicinal plants, so it is important to carry out studies on toxicity to ensure their safety profile. In the context of developing and using standardized herbal medicines, ensuring effectivy and toxicity are necessasy issues. Therefore, evaluating the toxicological effects of each medicinal plant extract is an important aspect before the drug is used for humans.

*Etlingera elatior* (Jack) R.M. Sm, commonly known as Kecombrang, is one of the medicinal plants. It is a shrub that grows annually and has fronds similar to banana plants, it forms rhizomes and is green in color. This plant produces flowers, fruit and seeds, and is useful as a vegetable. The flower is a hump-shaped, top-shaped compound flower with a stem length of between 40 and 80 cm. This plant belongs to the Zingiberaceae family (Saudah et al., 2022).

*Etlingera elatior* flowers are usually used as a spice for food flavouring and for ornamental purposes. In traditional medicine, *Etlingera elatior* was used to treat fever by roasting or burning the shoots, and then consuming the inner side or contents. This plant could also be used as a medicine for skin-related diseases, including measles (Lachumy et al., 2010). Previous studies have demonstrated the pharmacological effects of *Etlingera elatior* flowers as an antioxidant (Jackie et al., 2011), anti-inflammatory (Juwita et al., 2020; Nurhayatun et al., 2023), antibacterial (Ferreira et al., 2023), and anti-cancer (Zan et al., 2011). According to Naufalin et al. (2021), the secondary metabolite compounds of *Etlingera elatior* flowers were alkaloids, flavonoids, tannins, terpenoids, saponins, steroids, polyphenols and essential oils. The diverse pharmacological activities are linked to these secondary metabolites such as phenolics and flavonoids, diarylheptanoids, terpenoids, and curcumin (Juwita et al., 2018).

The safety and side effects of medicinal plants can be determined through toxicity tests, one of which is the acute toxicity test. Acute toxicity is interpreted as Lethal Dose 50 ($LD_{50}$), the dose of a substance that causes death in 50% of a test population (usually rodents) after a single exposure. The higher the $LD_{50}$ value, the lower the toxicity of the active ingredient (Ayun et al., 2021). The main purposes of acute oral toxicity testing are to obtain the presence of toxic effects on the tested animals after administration of a single dose or repeated doses within 24 hours. When a substance is suspected of causing harm, histopathological examination is crucial to evaluate its toxic effects on cells and tissues. Histopathology is the examination that aims to see the structure of damaged tissues and cells under a microscope (BPOM, 2022).

Acute toxicity evaluation of ethanol extract and methanol fraction of *Etlingera elatior* flowers had been studied. The acute toxicity test of the ethanol extract of *Etlingera elatior* flowers in mice at doses of 1000, 1500, 2000 mg/KgBW showed that the extract was not toxic (Sungthong & Srichaikul, 2018). The acute toxicity test of methanol extract of *Etlingera elatior* flowers using the Brine Shrimp Lethality Test (BSLT) method also showed no toxicity (Lachumy et al., 2010). Meanwhile, the evaluation of the ethyl acetate and hexane fraction have not yet been conducted by any research group. This study, thus, was aimed at examining the acute toxicity of *Etlingera elatior* flowers in Balb/C mice and histopathological examination of the liver and kidneys, so that the maximum limit for consuming these flowers safely could be determined.

## MATERIALS AND METHODS

### Materials

*Etlingera elatior* flowers were harvested from Pangandaran, West Java. Mature flowers were collected from plants that took around two years to flower. The flowers sample was authenticated by a botanist at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret with a certificate of determination with the letter number 131/UN27.9.6.4/Lab/2022.

Male BALB/C mice (2-3 months old, weighing 20-25 grams) were acclimated for 7 days before testing. Prior to the test, mice were fasted for 3-4 hours, during which water remained available. After the test substance was administered, food was withheld for an additional 1 to 2 hours. This short period allowed for the absorption and initial effects of the administered substance without interference from food intake. This study protocol had obtained Ethical Clearance from the RSUD dr. Moewardi Solo with the letter number 1.726/XII/HREC/2022.

### Method

#### Collection and processing of plant material

The flowers that were still in bud and pink were cut crosswise with a thickness of 1-2 cm and then washed with tap water to clean off the extraneous materials. After sorting, they were dried in the oven at temperature

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of 50º C. The dried flowers were then ground in a blender to fine powder and sieved using a 60 mesh sieve to get size uniformity.

**Extraction and fractinations**

*Etlingera elatior* flowers powder was extracted for 24 hours in 96% ethanol by maceration technique. The powder and solvent ratio was 1:5. The extract was then filtered through the filter paper. Remaceration with fresh solvent was carried out twice with powder and solvent ratio of 1:3. The remaceration step used a smaller solvent-to-material ratio compared to the initial maceration to maximize the extraction of remaining active compounds from the plant material. By using a more concentrated ratio in the second step, the gradient for diffusion was improved, allowing for further extraction and a higher overall yield of the desired compounds.

The filtrate of maceration and remaceration was evaporated using a rotary evaporator and followed by a water bath to concentrate. Fractination was carried out with liquid-liquid extraction. Ten grams of thick ethanol extract was dissolved in 100 mL of methanol-water (3:7), then liquid-liquid partition was carried out using n-hexane which formed two layers (methanol-water and hexane phases). The n-hexane solution was separated, while the remain filtrate was partitioned with ethyl acetate. The ethyl acetate solution was separated, and remain methanol-water solution. The three fractions obtained were methanol-water, ethyl acetate and hexane fractions which were then evaporated and concentrated to achieve the thick extract. To ensure all solvent had evaporated, the sample was heat in a controlled way to a constant weight by repeatedly heating and weighing the sample until the weight no longer changes.

**Acute toxicity test**

The number of tested animals was 5 mice in each 4 dose levels, so that 20 mice were needed. There were 4 extract treatment group (96% ethanol extract, n-hexane, ethyl acetate, and methanol-water fraction), so that 80 mice were needed. The negative control group (Na-CMC 0.3%) consisted of 5 mice, so a total of 85 mice were used in this study. Each extract was given orally once with dose I (75 mg/KgBW), dose II (150 mg/KgBW), dose III (300 mg/KgBW), and dose IV (600 mg/KgBW). The extract and fractions were given to mice at fixed dose levels according to OECD Guideline 423: *Acute Toxic Class Method*. This study protocol had obtained Ethical Clearance from the RSUD dr. Moewardi Solo with the letter number 1.726/XII/HREC/2022.

**Observation of animal behavior, body weight, macropathology and histopathology of liver and kidneys**

Toxic symptoms were observed in the first 30 minutes and continued for 4 hours after administration of the extract. Observations were continued for 14 days, once a day (BPOM, 2022). Animal behavior observed was fast heartbeat, decreased or increased breathing, excessive licking, skin (itching), changes in fur color, hair loss, body shaking, convulsions, excessive salivation (salivation), diarrhea, sleep, weakness or decreased activity, and death (Fithria et al., 2018); (BPOM, 2022). On the 15$^{th}$ day, surgery was performed on 1 mouse for each dose, then the liver and kidneys were observed macropathologically and histopathologically.

Body weight was monitored at least once a week after administration of the test preparation (BPOM, 2022). In this study, weighing was carried out 3 times: 0 day, 7$^{th}$ day, and 13$^{th}$ day.

Observations were made using the H and E (Hematoxcylline and Eosin) staining method and observed using a microscope at 400x magnification. Observation of liver damage was carried out thoroughly in the hepatocyte cells near the central vein and those that far from the central vein. Observation of kidney damage was carried out thoroughly in the cortex and medulla of the kidney. Fatty degeneration and inflammation in the liver and kidneys of mice were scored, score 0 if there was no damage, score 1 (mild damage) if there was local or focal inflammation or fatty degeneration, score 2 (moderate damage) if there was multifocal inflammation or fatty degeneration, score 3 (severe damage) if there was even or diffuse inflammation or fatty degeneration (Darmayanti et al., 2020).

**Data analysis**

Body weight was analyzed statistically using One Way Anova, followed by Tukey test. Toxic symptoms, macropathology and organ histopathology were analyzed qualitatively. The $LD_{50}$ was calculated using the Thomson and Weil method. According to Siswadi & Saragih (2018), the Thomson and Weil formula is:

$\text{Log } LD_{50} = \text{Log } D + d\ (f + 1)$

Note: D: smallest dose, d: logarithm of dose multiples, f: a factor in Weil's $LD_{50}$ calculation list.

## RESULTS AND DISCUSSION

**Extraction and fractinations**

*Etlingera elatior* flowers used in this study were still in bud and pink in color with the aim of ensuring

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that the secondary metabolite content, especially flavonoids, was optimal. The flowers were dried at a temperature of 50º C because this temperature was considered safe to prevent damage to flavonoid compounds. The extraction was carried out by maceration with 96% ethanol as a solvent because it can dissolve flavonoid and polyphenol compounds found in *Etlingera elatior* flowers (Suwarni & Cahyadi, 2016).

The thick ethanol extract was then continued to the fractionation process. The purpose of fractionation was to separate secondary metabolites based on their polarity, with each fraction enriched in compounds of similar chemical properties, such as nonpolar or semi-polar compounds. This is crucial for identification of which specific compounds or groups of compounds were responsible for the toxic effects observed. Thick ethanol extract might contains a complex mixture of hundreds of compounds, while a single fraction might contain a compound or a mixture of compounds that is more potent (e.g., more toxic or more active) than the original whole extract, as it concentrates the active molecules. The yield of ethanol extract and fractions can be seen in Table 1.

**Behavioral responses and mortality rate**

Mice that were given Na CMC, 96% ethanol extract, and fractions at all doses did not die during 14 days of observation (Table 2). In this study, the highest dose (600 mg/Kg body weight) did not cause death. Therefore, if the highest dose given did not cause death for 14 days, then the preparation was declared non-toxic (BPOM, 2022). The $LD_{50}$ value in this experiment could not be calculated because there were no deaths in the tested animals.

The results of toxicological observations were compared between the negative control and the treatment group. After administration of ethanol extract and fractions, several toxic symptoms were observed, including sleepiness, decreased activity, excessive licking, and rapid heartbeat (Table 3). The mice in the negative control group, ethanol extract and fractions treatment were experienced sleeping behavior. This was normal behavior because mice are nocturnal animals that are active at night. According to Lestari et al. (2019), if these symptoms also occur in the control group, they cannot be concluded as symptoms of poisoning or toxic symptoms.

**Table 1.** Yield of ethanol extract and fractions of *Etlingera elatior* flowers

| Extraction / Fractination solvent | Yield (% *w/w*) |
|---|---|
| 96% ethanol extract | 14.55 |
| Methanol:water (3:7) | 7.14 |
| Ethyl acetate | 2.08 |
| n-Hexane | 57.9 |

**Table 2.** The number of deaths in mice given Na CMC, extract, and fraction of *Etlingera elatior* flowers

| Treatment | Number of mice | Number of dead mice |
|---|---|---|
| Negative control (Na CMC 0.3%) | 5 | 0 |
| Ethanol extract dose 75 mg/KgBW | 5 | 0 |
| Ethanol extract dose 150 mg/KgBW | 5 | 0 |
| Ethanol extract dose 300 mg/KgBW | 5 | 0 |
| Ethanol extract dose 600 mg/KgBW | 5 | 0 |
| Methanol-water dose 75 mg/KgBW | 5 | 0 |
| Methanol-water dose 150 mg/KgBW | 5 | 0 |
| Methanol-water dose 300 mg/KgBW | 5 | 0 |
| Methanol-water dose 600 mg/KgBW | 5 | 0 |
| n-Hexane dose 75 mg/KgBW | 5 | 0 |
| n-Hexane dose 150 mg/KgBW | 5 | 0 |
| n-Hexane dose 300 mg/KgBW | 5 | 0 |
| n-Hexane dose 600 mg/KgBW | 5 | 0 |
| Ethyl acetate dose 75 mg/KgBW | 5 | 0 |
| Ethyl acetate dose 150 mg/KgBW | 5 | 0 |
| Ethyl acetate dose 300 mg/KgBW | 5 | 0 |
| Ethyl acetate dose 600 mg/KgBW | 5 | 0 |

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**Table 3.** Toxic symptoms after administration of Na-CMC, extract and fractions

| Treatment group | Behavioral responses |
|---|---|
| Negative control (Na-CMC 0.3%) | Sleeping |
| 96% ethanol extract | Sleeping, decreased activity, excessive licking |
| Methanol-water fraction | Sleeping, decreased activity, faster heart beats |
| N-hexane fraction | Sleeping, decreased activity |
| Ethyl acetate fraction | Sleeping, decreased activity, excessive licking, faster heart beats |

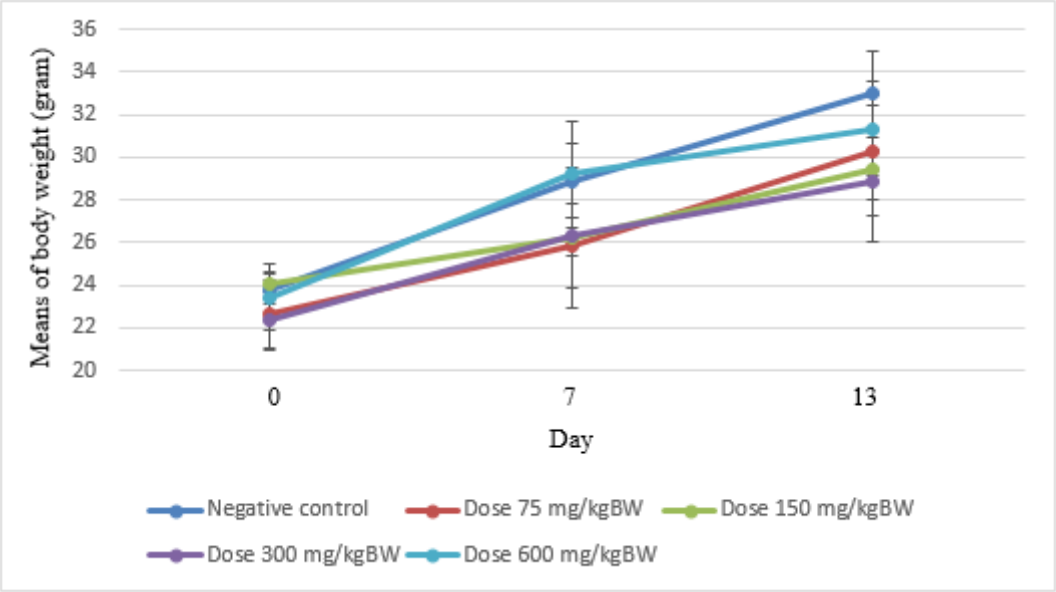


**Figure 1.** Effect of 96% ethanol extract on mice's body weight

During 14 days of observation, the behavior was evaluated such as excessive licking, decreased activity, and a faster heartbeat. Faster heart beat in a mouse was observed visually. After administering the extract, the mouse's chest was observed for increased visible heart pulsation. These symptoms were not found in all mice and were not found after day 2 to day 14, so this behavior could be caused by the different response abilities of mice. According to Rauf (2018), mice could experience stress which was characterized by changes in behavior such as frequently licking their bodies, their hearts beating faster, and being often silent. Apart from that, changes in mouse behavior that did not occur in all mice could be caused by other factors; different physiological conditions in each mouse (Fithria et al., 2018).

Previous studies have shown that ethanol extract of *Etlingera elatior* flower were not acutely toxic, with oral administration of ethanol extracts at doses 1000, 1500, and 2000 mg/KgBW to mice showing no fatalities (Sungthong & Srichaikul, 2018). According to Lachumy et al. (2010) methanol extract of *Etlingera elatior* flowers using the BSLT method also found the extract to be non-toxic to *Artemia salina*, showed an $LD_{50}$ value was 2.52. This recent study also found that the methanol:water, ethyl acetate, and n-hexane fractions did not cause toxicity in mice. Therefore, the results of this study complement previous studies. These consistent results from different studies and testing models support the conclusion that *Etlingera elatior* flower extracts are safe in terms of acute toxicity at the tested doses.

**Body weight measurement**

Mice treated with 96% ethanol extract at doses of 75, 150, 300 and 600 mg/KgBW showed an increase in body weight at the 1st, 2nd, and 3rd weighing. The 96% ethanol extract did not affect mouse growth, as both the extract-treated groups and the negative control group showed weight gain after the 1st, 2nd, and 3rd weighings. In the One Way Anova test, a Sig. value of > 0.05 was obtained, which means there was no significant difference in body weight of mice between the negative control group and ethanol extract-treated group. The findings in this study, that 96% ethanol extract of *Etlingera elatior* did not cause toxic effects, as shown by increa sing body weight in experimental animals that was not significantly different from the control group.

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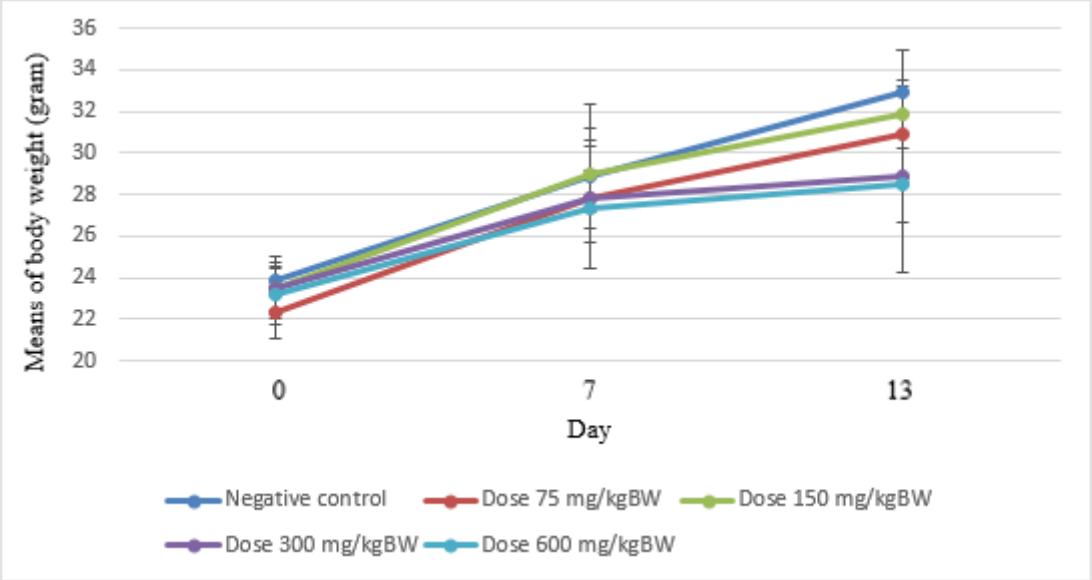


**Figure 2.** Effect of methanol-water fraction on mice's body weight

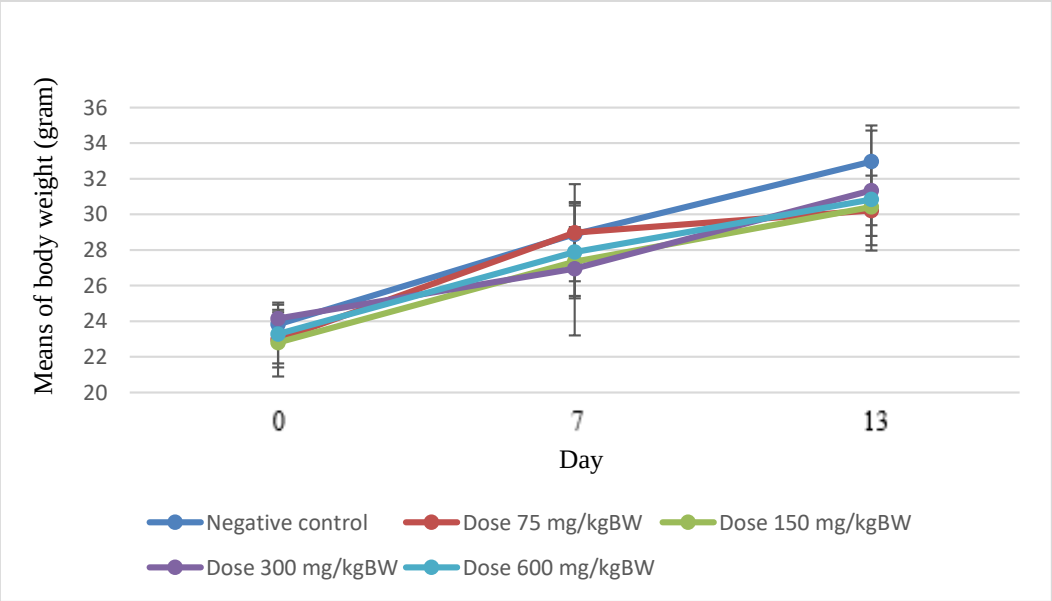


**Figure 3.** Effect of n-hexane fraction on mice's body weight

Mice treated with methanol-water fraction at doses of 75, 150, 300 and 600 mg/KgBW showed an increase in body weight at the 1st, 2nd, and 3rd weighing. The methanol-water fraction did not affect mouse growth, as both the fraction-treated group and the negative control showed weight gain after the 1st, 2nd, and 3rd weighings. In the One Way Anova test, a Sig. value of > 0.05 was obtained, which means there was no significant difference in body weight of mice between the negative control and methanol-water group. This indicated that methanol-water fraction of *Etlingera elatior* did not cause toxic effects, as shown by increasing body weight in experimental animals that was not significantly different from the control group.

Mice treated with n-hexane fraction at doses of 75, 150, 300 and 600 mg/KgBW showed an increase in body weight at the 1st, 2nd, and 3rd weighing. The n-hexane fraction did not affect mouse growth, as both the n-hexane fraction treated and the negative control group showed weight gain after the 1st, 2nd, and 3rd weighings. In the One Way Anova test, a Sig. value of > 0.05 was obtained, which means there was no significant difference in body weight of mice between the negative control and n-hexane group. This indicated that n-hexane fraction of *Etlingera elatior* did not cause toxic effects, as shown by increasing body weight in experimental animals that was not significantly different from the control group.

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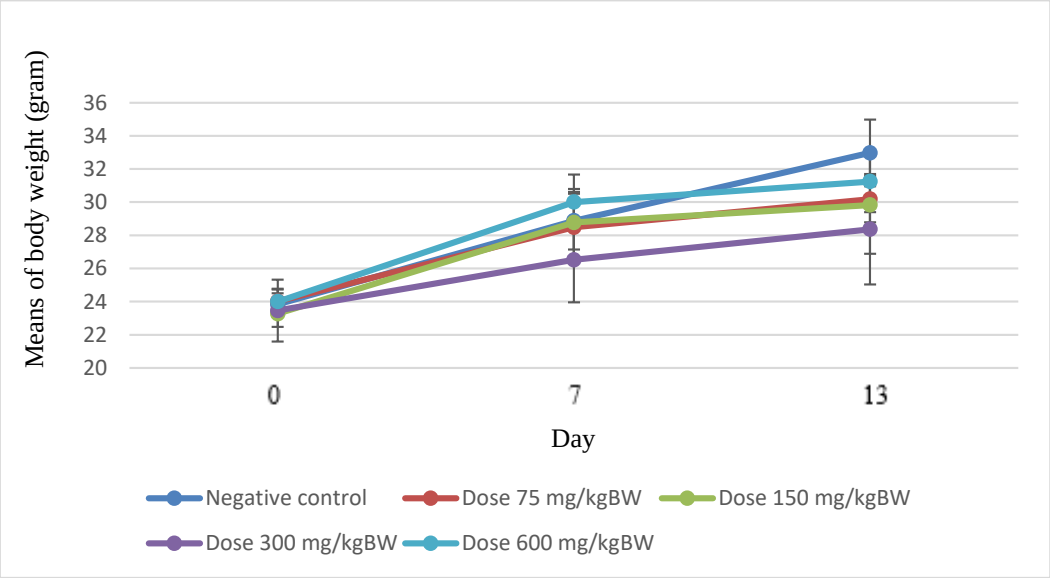


**Figure 4.** Effect of ethyl acetate fraction on mice's body weight

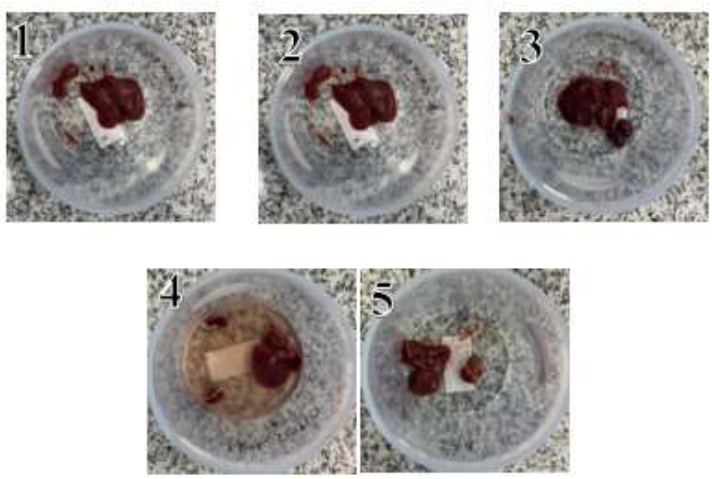


**Figure 5.** Liver macropathology after administration of Na CMC, extract and fractions of *Etlingera elatior* flowers
Notes: (1). Negative control Na-CMC 0.3%, (2). Ethanol extract dose-I (75 mg/KgBB), (3). Methanol-water fraction dose-I (75 mg/KgBB), (4). N-Hexane fraction dose-I (75 mg/KgBB), (5). Ethyl acetate fraction dose-I (75 mg/KgBB)

Mice treated with ethyl acetate fraction at doses of 75, 150, 300 and 600 mg/KgBW showed an increase in body weight at the 1st, 2nd, and 3rd weighing. The ethyl acetate fraction did not affect mouse growth, as both the fraction treated and the negative control group showed weight gain after the 1st, 2nd, and 3rd weighings. In the One Way Anova test, a Sig. value of > 0.05 was obtained, which means there was no significant difference in body weight of mice between the negative control and ethyl acetate fraction group. This indicated that ethyl acetate fraction of *Etlingera elatior* did not cause toxic effects, as shown by increasing body weight in experimental animals that was not significantly different from the control group.

After administration of ethanol extract and fractions of *Etlingera elatior* flowers, mice experienced weight gain which was marked by an increase in the graph. According to Tangkere et al. (2022), if the tested animals experience weight loss, this could be due to the administration of the extract. While an increase in body weight indicates that the mice are healthy, which is characterized by eating regularly. In toxicological studies, a healthy animal will either maintain or increase its body weight. If a substance is toxic, it will typically show a significant decrease in body weight.

Mice in all treatment groups experienced increased body weight. These increases were not statistically significant when compared to the negative control group ($p>0.05$), meaning there were no significant differences between the weight gain in the treatment groups and the control group at the end of the experiment. Based on this statistical results, it could be concluded that

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administration of ethanol extract and fractions did not affect the body weight of mice. The evidence suggested that the ethanolic extract and fractions of *Etlingera elatior* flower were safe and did not induce harmful effects, as shown by these physiological markers.

**Macropathology of liver**

Macropathological observations were used to determine toxic effects visibly or visually. Organ damage can be seen from changes in color, consistency and surface of the organ. The results of observations of the liver and kidney organs of mice in the negative control group and the treatment groups can be seen in Figure 5. Based on Figure 5, it can be seen that the liver and kidney organs of mice are brownish red in color, have a smooth surface, and have a chewy consistency, so that the liver and kidneys of mice are normal. According to Takapaha et al. (2022), a normal liver has a brownish red color, while an abnormal liver changes color. Apart from that, a normal liver has a surface that has a smooth texture and is somewhat hard when pressed (Dorland, 2002), whereas a normal kidney is characterized by a smooth surface, springy consistency, has a bean shape, and is brownish red in color because it receives 22% of the blood volume pumped by the heart (Guyton & Hall 2007).

**Histological features of liver**

The scoring for liver damage in mice can be seen in Table 4. Observations of the liver after administration of ethanol extract and fractions resulted in fatty degeneration and inflammation in hepatocyte cells. Normal liver cells are characterized by polyhedral cells arranged radially around the central vein (Surasa et al., 2014). Fatty degeneration in hepatocytes is characterized by small vacuoles in the cytoplasm, which can then develop into larger vacuoles, resulting in the nucleus being compressed to the periphery (Andreas et al., 2015). Further damage, inflammation, is characterized by cells that are very purple in color, have an indeterminate size, and lack of distance between the cytoplasm and the nucleus (Nazarudin et al., 2017).

Liver damage can be seen in Figure 6-10. The negative control group showed no damage in the form of inflammation or fatty degeneration. The choice of Na CMC was based on its non-toxic or inert nature and its inability to react with the extract. Administration of 96% ethanol extract caused moderate liver damage in the form of inflammation and fatty degeneration. Inflammation occurred at all doses, while fatty degeneration only occurred at dose 2 (150 mg/KgBW).

Administration of the methanol-water fraction caused mild liver damage in the form of inflammation. Fatty degeneration did not occur with the methanol-water fraction, indicating that this fraction did not disrupt fat metabolism in the liver. Inflammation occurred only at dose 3 (300 mg/KgBW).

The ethyl acetate fraction caused mild inflammation in the livers of mice, but no fatty degeneration occurred after administration of the ethyl acetate fraction. Inflammation occurred only at dose 1 (75 mg/KgBW), while no inflammation occurred at doses 2 (150 mg/KgBW), 3 (300 mg/KgBW), and 4 (600 mg/KgBW).

Administration of the n-hexane fraction caused mild to moderate liver cell damage in the form of fatty degeneration and inflammation. Dose 1 (75 mg/KgBW) resulted in more inflammation and fatty degeneration than dose 4 (600 mg/KgBW), while dose 2 (150 mg/KgBW) only caused fatty degeneration and in greater amounts than dose 4 (600 mg/KgBW). Dose 3 (300 mg/KgBW) only resulted in inflammation, while dose 4 (600 mg/KgBW) resulted in both fatty degeneration and inflammation. The level of liver damage that occurred was not accompanied by an increase in dosage.

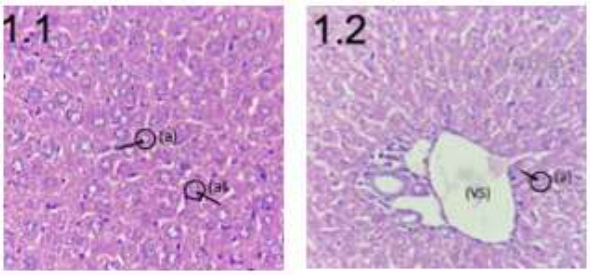


**Figure 6.** Liver histpathology after administration of Na CMC 0.3%

Note: (a) Normal hepatocytes, (vs) central vein, (1.1). Far from central vein, (1.2). Near central vein

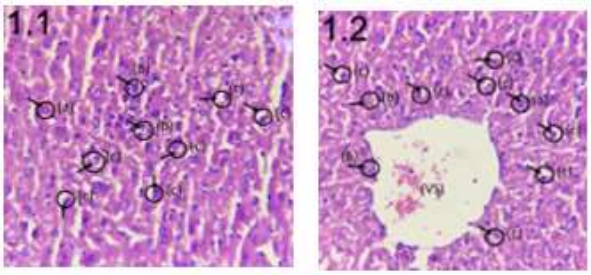


**Figure 7.** Liver histpathology after administration of 96% ethanol extract

Note: (a) Normal hepatocytes, (b) Inflammation cells, (c) Fatty degenerations, (vs) central vein, (1.1). Far from central vein, (1.2). Near central vein

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**Table 4.** Scoring of liver damage in mice after administration of Na-CMC, extract and fractions

| Preparate code | Fatty degeneration score | Number of locations | | Inflammation score | Number of locations | |
|---|---|---|---|---|---|---|
| | | Near central vein | Far from central vein | | Near central vein | Far from central vein |
| Negative control (Na CMC 0.3%) | 0 | 0 | 0 | 0 | 0 | 0 |
| Ethanol extract dose 75 mg/KgBW | 0 | 0 | 0 | 2 | 2 | 0 |
| Ethanol extract dose 150 mg/KgBW | 3 | Difuse | Difuse | 2 | 2 | 1 |
| Ethanol extract dose 300 mg/KgBW | 0 | 0 | 0 | 2 | 4 | 0 |
| Ethanol extract dose 600 mg/KgBW | 0 | 0 | 0 | 2 | 2 | 1 |
| Methanol-water dose 75 mg/KgBW | 0 | 0 | 0 | 0 | 0 | 0 |
| Methanol-water dose 150 mg/KgBW | 0 | 0 | 0 | 0 | 0 | 0 |
| Methanol-water dose 300 mg/KgBW | 0 | 0 | 0 | 1 | 1 | 0 |
| Methanol-water dose 600 mg/KgBW | 0 | 0 | 0 | 0 | 0 | 0 |
| n-Hexane dose 75 mg/KgBW | 0 | 0 | 0 | 1 | 0 | 1 |
| n-Hexane dose 150 mg/KgBW | 0 | 0 | 0 | 0 | 0 | 0 |
| n-Hexane dose 300 mg/KgBW | 0 | 0 | 0 | 0 | 0 | 0 |
| n-Hexane dose 600 mg/KgBW | 0 | 0 | 0 | 0 | 0 | 0 |
| Ethyl acetate dose 75 mg/KgBW | 2 | 3 | 0 | 1 | 1 | 0 |
| Ethyl acetate dose 150 mg/KgBW | 2 | 4 | 0 | 0 | 0 | 0 |
| Ethyl acetate dose 300 mg/KgBW | 0 | 0 | 0 | 1 | 0 | 1 |
| Ethyl acetate dose 600 mg/KgBW | 1 | 1 | 0 | 1 | 1 | 0 |

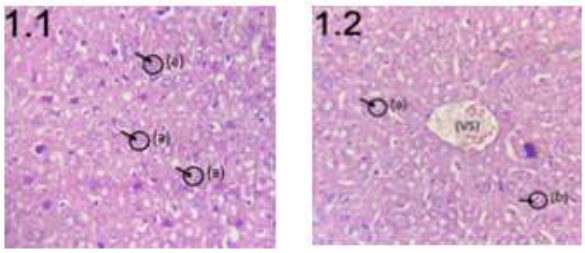


**Figure 8.** Liver histpathology after administration of methanol-water fraction

Note: (a) Normal hepatocytes, (b) Inflammation cells, (vs) central vein, (1.1). Far from central vein, (1.2). Near central vein

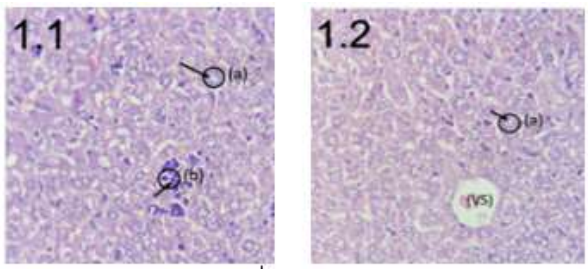


**Figure 9.** Liver histpathology after administration of ethyl acetate fraction

Note: (a) Normal hepatocytes, (b) Inflammation cells, (vs) central vein, (1.1). Far from central vein, (1.2). Near central vein

**Histological features of kidneys**

The scoring for kidney damage in mice can be seen in Table 5. In this study, the kidney damage in mice showed inflammation, characterized by very purple cells, an uncertain size, and no distance between the cytoplasm and the cell nucleus. Inflammation is a crucial defense mechanism for the body against various hazards and compounds that can disrupt its balance. Tissues experiencing resistance will show signs of inflammatory cell infiltration, such as an accumulation of white blood cells and immune cells like lymphocytes, plasma cells, and macrophages, in a histopathological examination. This infiltration is a typical sign of the inflammatory response to tissue damage or infection and can be observed and quantified under a microscope after staining tissue samples.

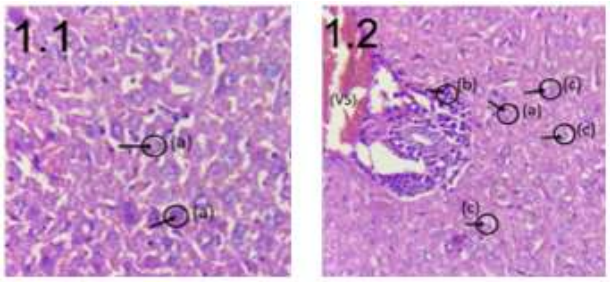


**Figure 10.** Liver histpathology after administration of n-hexane fraction

Note: (a) Normal hepatocytes, (b) Inflammation cells, (c) Fatty degeneration, (vs) central vein, (1.1). Far from central vein, (1.2). Near central vein

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E-ISSN: 2580-8303

**Table 5.** Scoring of kidneys damage in mice after administration of Na-CMC, extract and fractions

| Preparate code | Fatty degeneration score | Number of locations | | Inflammation score | Number of locations | |
|---|---|---|---|---|---|---|
| | | Cortex | Medula | | Cortex | Medula |
| Negative control (Na CMC 0.3%) | 0 | 0 | 0 | 0 | 0 | 0 |
| Ethanol extract dose 75 mg/KgBW | 0 | 0 | 0 | 3 | Difuse | Difuse |
| Ethanol extract dose 150 mg/KgBW | 0 | 0 | 0 | 3 | Difuse | Difuse |
| Ethanol extract dose 300 mg/KgBW | 0 | 0 | 0 | 3 | Difuse | Difuse |
| Ethanol extract dose 600 mg/KgBW | 0 | 0 | 0 | 3 | Difuse | Difuse |
| Methanol-water dose 75 mg/KgBW | 0 | 0 | 0 | 2 | 3 | 0 |
| Methanol-water dose 150 mg/KgBW | 0 | 0 | 0 | 0 | 0 | 0 |
| Methanol-water dose 300 mg/KgBW | 0 | 0 | 0 | 0 | 0 | 0 |
| Methanol-water dose 600 mg/KgBW | 0 | 0 | 0 | 2 | 3 | 0 |
| n-Hexane dose 75 mg/KgBW | 0 | 0 | 0 | 3 | Difuse | Difuse |
| n-Hexane dose 150 mg/KgBW | 0 | 0 | 0 | 3 | Difuse | Difuse |
| n-Hexane dose 300 mg/KgBW | 0 | 0 | 0 | 3 | Difuse | Difuse |
| n-Hexane dose 600 mg/KgBW | 0 | 0 | 0 | 3 | Difuse | Difuse |
| Ethyl acetate dose 75 mg/KgBW | 0 | 0 | 0 | 2 | 6 | 4 |
| Ethyl acetate dose 150 mg/KgBW | 0 | 0 | 0 | 2 | 2 | 2 |
| Ethyl acetate dose 300 mg/KgBW | 0 | 0 | 0 | 2 | 4 | 5 |
| Ethyl acetate dose 600 mg/KgBW | 0 | 0 | 0 | 2 | 12 | 8 |

Kidney damage can be seen in Figure 11-15. Administration of 96% ethanol extract at all doses caused severe inflammation in the kidneys of mice, but did not cause fatty degeneration.

Administration of the methanol-water fraction caused inflammation in the kidneys of mice. Moderate inflammation was observed in the kidneys of mice at doses 1 (75 mg/KgBW) and 4 (600 mg/KgBW), while no inflammation was observed in doses 2 (150 mg/KgBW) and 3 (300 mg/KgBW).

Administration of ethyl acetate fraction at all doses caused severe inflammation, while administration of n-hexane fraction at all doses caused moderate inflammation in the kidneys of mice.

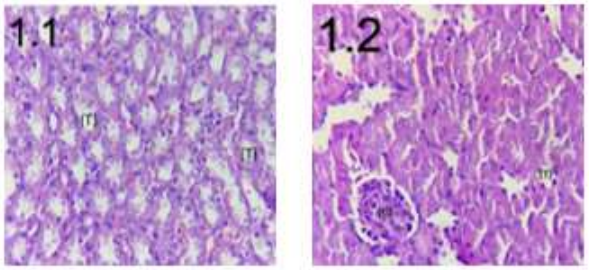


**Figure 11.** Kidneys histopathology after administration of Na-CMC 0.3%

Note: (T) Tubulus, (G) Glomerulus, (1.1). Medula, (1.2). Cortex

Histopathological examination was carried out to determine the presence of organ damage that was not visible macroscopically due to the administration of the test preparation. In this study, histopathological observations were carried out on the liver and kidneys of mice. This is because the liver is the place for drug metabolism, while the kidneys are a site for excretion of metabolites. The liver is the major place where a drug is metabolized when it enters the body. The results of metabolism are called metabolites, where the resulting metabolites can be toxic even though the initial compound is not toxic (Rollando, 2017). In addition, a histopathological examination of the kidneys was carried out, which functions to filter and excrete metabolic waste. A toxic compound that enters the body will undergo several processes before being excreted in the body, the process of entering the compound is the exposition, toxokinetic and toxodynamic phase (Rahayu & Solihat, 2018).

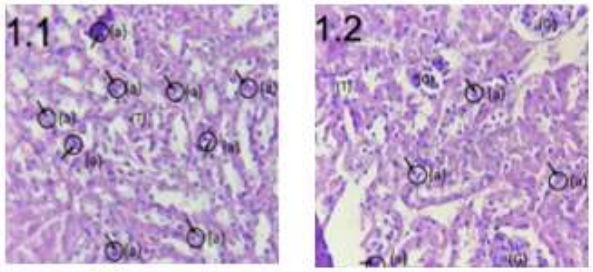


**Figure 12.** Kidneys histopathology after administration of 96% ethanol extract

Note: (a) Inflammation cell, (T) Tubulus, (G) Glomerulus, (1.1). Medula, (1.2). Cortex

The damage that occured to the mice's liver was mild to moderate inflammation and fatty degeneration, while the damage to the mice's kidneys was moderate to severe inflammation. Mild or focal damage (score 1) indicated minor or localized problem where only one

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inflammatory lesion or fatty degeneration was found in one location. A score of 2 indicates moderate or multifocal damage, if more than one inflammatory lesion or fatty degeneration was found. A score of 3 indicates severe or diffuse damage, if inflammation or fatty degeneration was found evenly in several locations.

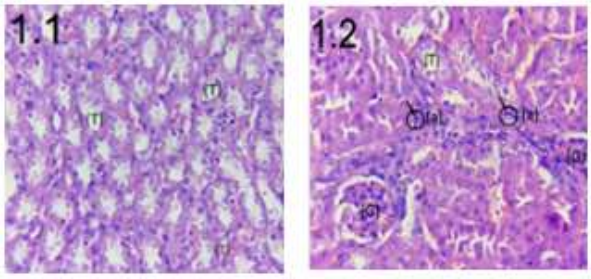


**Figure 13.** Kidneys histopathology after administration of methanol-water fraction
Note: (a) Inflammation cell, (T) Tubulus, (G) Glomerulus, (1.1). Medula, (1.2). Cortex

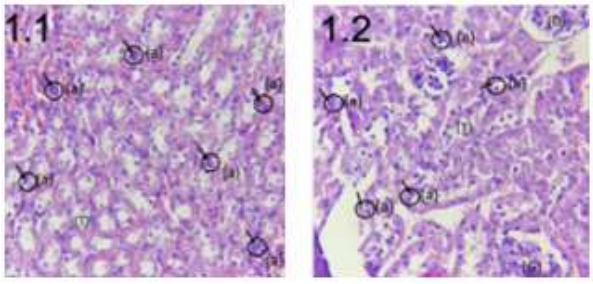


**Figure 14.** Kidneys histopathology after administration of ethyl acetate fraction
Note: (a) Inflammation cell, (T) Tubulus, (G) Glomerulus, (1.1). Medula, (1.2). Cortex

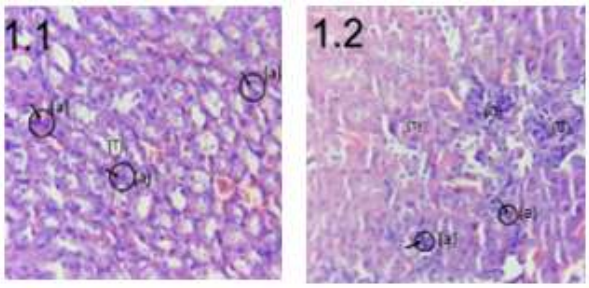


**Figure 15.** Kidneys histopathology after administration of n-hexane fraction
Note: (a) Inflammation cell, (T) Tubulus, (G) Glomerulus, (1.1). Medula, (1.2). Cortex

The inflammation in the kidney tent to be higher than in the liver. The kidneys are highly susceptible to toxic damage due to high blood flow, concentration of toxicants during filtration and secretion, high metabolic activity, the ability to metabolize chemicals, and prolonged residence time of substances within the tubules. Kidney's renal tubules are responsible for reabsorbing and secreting substances, which can concentrate toxins in the kidneys, unlike the liver where biotransformation can detoxify them. Toxic substances are filtered from the blood and processed in the liver, then re-enter the blood to be excreted by the kidneys (Lee et al., 2018).

Inflammation is characterized by cells having a very purple color, there is no distance between the cytoplasm and the cell nucleus, and the size of the cells is uncertain (Nazarudin et al., 2017). Fatty degeneration is characterized by cells having small vacuoles, which can then become larger so that the nucleus is compressed and pushed to the edge (Andreas et al., 2015). Scoring of damage that occurred to the liver and kidneys of these mice was not followed by an increase in dose. This could happened by various factors. Different metabolic processes, internal infections, nutritional deficiencies, cell aging, and lack of oxygen can significantly impact mice's health and physiology, contributing to varied responses in experimental studies. These factors can trigger physiological shifts like chronic inflammation, altered immune function, and oxidative stress, affecting diverse biological processes and ultimately complicating scientific interpretations of mouse models (Li et al., 2023). According to Apriliani et al. (2015), this also might be occured because each mouse has a different metabolic process and response, resulting in significant fatty degeneration even at lower doses. This results also could be due to the mice's endurance and stress factors, which vary from individual to individual. These factors can trigger increased oxidative stress, leading to the production of free radicals or reactive oxygen species exceeding the body's defenses. The resulting free radicals, which the body cannot neutralize, can cause cell damage (Ervina & Sukarjati, 2017).

The inflammation and fatty degeneration that occured could also be caused by the secondary metabolite content of the flowers. According to Naufalin et al. (2021), *Etlingera elatior* flowers contain alkaloids, flavonoids, tannins, polyphenols, terpenoids, steroids, saponins, and contain essential oils. The methanol-water fraction is polar and contains flavonoid and tannin compounds, while the ethyl acetate fraction is semi-polar and contains saponin, tannin, flavonoid and steroid compounds. The hexane fraction is non-polar and contains terpenoid compounds, namely dodecanal, 1-dodecanol, dedecanoic acid, 1-hexadecanol, 1-hexadecene, and 17-pentatriacontene which are volatile

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compounds resulting from GC-MS analysis of the n-hexane fraction of *Etlingera elatior* plants (Maimulyanti & Prihadi, 2015). The flavonoid compounds contained in *Etlingera elatior* flowers are quercetin, apigenin, kaempferol, luteolin. Rutin, quercetin, kaempferol, and kaempferol-3-O-glucoside, are polar flavonoids (Ghasemzadeh et al., 2015).

Inflammation and fatty degeneration in the liver and kidneys of mice were thought to be caused by the compounds kaempferol, quercetin and tannins in *Etlingera elatior* flowers. This was in accordance with several previous studies. In the study of Su et al. (2018), *Etlingera elatior* flowers contained kaempferol and quercetin, thus causing death in *Spodoptera litura*. Apart from that, in research by Suryanto et al. (2018), the kaempferol compound in *Etlingera elatior* leaves caused death in *Culex quinquefasciatus* mosquito larvae. According to Koraag et al. (2016), *Etlingera elatior* flowers contained tannins, which caused death to *Aedes aegypti* larvae. The mechanism of inflammation is that plasma protein fluid and leukocytes will go to the area experiencing infection, tissue that fights inflammatory cells will be characterized by infiltration of inflammatory cells (Baratawidjaja, 2002). Fatty degeneration can occur due to an increase in free fatty acids, then a decrease in triglyceride export due to a deficiency in fat-binding apoproteins, and a reduction in free fatty acid oxidation (Dancui et al., 2009).

The result of histopathology examination of liver and kidney in this study confirmed the results of toxicity result. If a high dose of a substance results in death in mice, histopathological analysis of tissues can confirm whether the death was due to specific damage (e.g., liver necrosis, renal failure). Even if no obvious symptoms of toxicity were observed, histopathology might reveal microscopic organ damage (such as cell death, inflammation, or fatty degeneration) that would otherwise go unnoticed. In this study, there were no toxic symptoms or death in mice treated with ethanol extract and fractions of *Etlingera elatior* flowers. But the administration of ethanol extract and fractions of *Etlingera elatior* flowers caused mild to moderate damage to the liver, and moderate to severe damage to kidney. This suggested the ethanol extract and its fractions caused internal organ harm despite the absence of outward signs of toxicity or mortality in the animal model. Futher research is needed to identify the toxic components, elucidate the exact mechanisms of organ damage, determine the dose-response relationship for toxicity, investigate potential interactions with other substances, and establish safe and effective dosage limits for human use of the ethanol extract and its fractions.

## CONCLUSION

Administration of 96% ethanol extract, n-hexane, ethyl acetate and methanol-water (3:7) fractions of *Etlingera elatior* flowers did not cause toxic symptoms and death in mice, so they were categorized as non-toxic. Macropathological examination of the liver and kidneys showed normal results with a brownish red color, smooth surface and rubbery consistency. Effect of administration of ethanol extract and fractions of *Etlingera elatior* flowers in the liver of mice caused mild and moderate damage, while in the kidneys of mice, it caused moderate and severe damage.

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## AUTHOR CONTRIBUTIONS

Conceptualization, I.S.; Methodology, I.S., N.H.; Software, S.B.N.; Validation, I.S.; Formal Analysis, S.B.N.; Investigation, I.S., N.H., S.B.N.; Resources, N.D.I.; Data Curation, I.S., S.B.N.; Writing - Original Draft, I.S., S.B.N.; Writing - Review & Editing, I.S., S.B.N.; Visualization, S.B.N.; Supervision, N.H.; Project Administration, N.H.; Funding Acquisition, N.H.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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