



Original Research

Hepatotoxicity Oral Administration of Flavonoids-Rich Extract from *Phaleria macrocarpa* in MiceSiskanita Nur Fitriana^{1*}, Sri Andarini², Sutrisno Sutrisno³, Gita Nawangtantrini⁴, Maharani Maharani⁵¹ Magister of Midwifery, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia² Department of Public Health, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia³ Department of Obstetric and Gynecology, Faculty of Medicine, Universitas Brawijaya/ Saiful Anwar General Hospital, Malang, East Java, Indonesia⁴ Division of Pathology Anatomy, Department Pathology Anatomy Margono Soekarjo General Hospital/ Faculty of Medicine, Jendral Soedirman University⁵ Department of Midwifery, Polytechnic of Health-Ministry of Health, Aceh, Indonesia

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CORRESPONDING AUTHOR

Siskanita Nur Fitriana

nitasiska@gmail.com

Magister of Midwifery, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia

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Hepatotoxicity; Flavonoid; *Phaleria macrocarpa*This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>)

ABSTRACT

Introduction: Herbal medications are increasingly being used to treat a wide range of illnesses. *Phaleria macrocarpa*, often known as the God's Crown Fruit, is a plant rich in flavonoids that is frequently used in herbal therapy. The goal of this study was to see what effect a flavonoid-rich extract from *Phaleria macrocarpa* has on the livers of mice.**Material and Methods:** This study used adult female mice aged 6-8 weeks and weighing 20-30 gram as experimental animals, which were divided into four groups: the control group was given distilled water, group 2 was given a flavonoid dose of 500 mg/kg BW/day, group 3 was given a flavonoid dose of 1000 mg/kg BW/day, and group 4 was given a flavonoid dose of 2000 mg/kg BW/day. The flavonoid-rich extract of *Phaleria macrocarpa* was administered for 28 days. On the 29th day, the mice were necropsied, followed by surgery and examination of the mice's livers.**Results:** The flavonoid-rich extract of *Phaleria macrocarpa* at a dose of 500 mg/kg BW/day resulted in no changes in the weight or histological structure of the liver ($p > 0.05$). Hydropic degeneration and necrosis were observed in the group administered the flavonoid-rich extract of *Phaleria macrocarpa* at doses of 1000 and 2000 mg/kg BW/day.**Conclusion:** The administration of a flavonoid-rich extract from *Phaleria macrocarpa* at a level of 500 mg/kg BW/day caused no harm in the liver of mice. The use of more than 500 mg/kg BW/day over an extended period of time has hazardous effects and is not recommended.**Cite this as:** Fitriana SN, Andarini S, Sutrisno S, Nawangtantrini G, Maharani M (2023) Hepatotoxicity Oral Administration of Flavonoids Rich Extract from *Phaleria macrocarpa* in Mice. *Asian J Heal Res.* 2 (2): 36–41. doi: <https://doi.org/10.55561/ajhr.v2i2.109>

INTRODUCTION

The use of herbal medicines as an alternative to chemical treatments is expanding as a result of the increasingly loud slogans of "back to nature," the abundance of materials available, the low cost, and the public's assumption that herbal medicines have no negative effects [1,2]. There are around 250,000 to 500,000 plant species with therapeutic characteristics, yet their safety is still debatable due to the fact that their use is not always safe [3]. *Phaleria macrocarpa* is a plant

that grows in the tropics and is frequently used in herbal medicine due to its high antioxidant content, including flavonoids [4-6].

The use of *Phaleria macrocarpa* phytochemicals, specifically flavonoids, shows potential activity as an anti-microbial, anti-bacterial, anti-fungal, anti-allergic, anti-teratogenic, anti-inflammatory, antioxidant, anti-thrombotic, cardioprotective, neuroprotective, hepatoprotective, and vasodilator in cancer treatment [4,6-12]. In addition to its therapeutic characteristics, consumption of *Phaleria macrocarpa* in large quantities and for a long time makes toxic substances and resulted

harmful to the liver. The liver is the body's metabolic center, and it is where practically all medicines and foreign chemicals are metabolized (13). Liver damage can occur due to toxic substances through the mechanism of inhibition of metabolism enzymes, oxidative stress, and mitochondrial damage (14-15). The presence of injury in the liver can be determined by measuring the levels of ALT and AST and observing hydropic degeneration, inflammatory cell infiltration, and necrosis of liver cells (16-19). The purpose of this study was to see the effect of giving flavonoid-rich extract from *Phaleria macrocarpa* fruit to the liver of mice.

MATERIAL AND METHODS

This study used an experimental post-test only control group design. This study was carried out at a number of laboratories, including the Biology Laboratory at Brawijaya University Malang, the Embryology Laboratory at Airlangga University's Faculty of Veterinary Medicine, and the Pathology Anatomical Laboratory at Brawijaya University.

Ripe *Phaleria macrocarpa* fruit is washed, the seeds are removed, and the fruit is oven-dried at 80 °C. Simplicia powder is created by blending dried *Phaleria macrocarpa*. Simplicia powder is steeped in 96% ethanol for 30 minutes, agitated thoroughly, and allowed to stand for 5 nights to settle. Using a buncher funnel, filter the liquid. To get the flavonoid-rich extract, the ethanol extract was partitioned using polar and non-polar solvents, namely n-hexane and n-butanol.

The mice used were mature female BALB/c mice weighing 20-30 grams and aged 6-8 weeks. Mice were acclimatized for 7 days in the Airlangga University Faculty of Veterinary Medicine's experimental cage. During acclimatization, the mice were fed A2 chicken pellets twice a day and given free access to water. On the eighth day, the mice were randomly separated into four groups, one control, and three treatment groups. The control group was only given a drink as usual, treatment group 1 was given flavonoids at a dose of 500 mg/kg BW/day, treatment group 2 was given flavonoids at a dose of 1000 mg/kg BW/day, treatment group 3 was given flavonoids at a dose of 2000 mg/kg BW/day. Weighing is done once each week. On the 29th day, the mice were necropsied, then surgery was conducted, and the livers of the mice were taken to be weighed and the histological structure was inspected. Creating histopathological slides with Hematoxylin and Eosin staining. Histopathological score assessment using a binocular microscope with magnification 400, at 5 different fields of view using a score on Simanjuntak research, which normal hepatocyte cells have a score of 0, parenchymatous degeneration has a score of 1, hydropic degeneration has a score of 2 and necrosis has a score of 3 (16).

Ethics

The ethics committee of the Faculty of Medicine at Brawijaya University approved this study with the number 38/EC/KEPK/03/202.

RESULTS

Assessment of Mice Liver Weight

The data normalcy test uses Saphiro-Wilk in the hepatic weight variable, while the data homogeneity test employs the l test wean off. The results of normality and homogeneity tests on mouse liver revealed ep-values greater than 0.05. This indicates that the information is typically distributed and homogeneous. The test is conducted out ANOVA one manner at the next stage.

Table 1. One-Way ANOVA Test Results for Mice Liver Weight with Oral Administration Flavonoid-Rich Extract from *Phaleria macrocarpa*

Group	Mean $\pm$ SD /mg	p-Value
K	1616.24 $\pm$ 372.48	0.129
P1	1500.25 $\pm$ 290.83	
P2	1726.25 $\pm$ 317.31	
P3	1719.25 $\pm$ 356.6	

If the p-value <0.05 then there is a significant difference, if the p-value >0.05 then there is no significant difference

In Table 1, the liver in group P1 with a dose of 500 mg/kg BW/day had an average weight of 1719.25 $\pm$ 356.6 mg and P2 had an average weight of 1726.25 $\pm$ 317.31 mg, both groups increased compared to the control group, but not the P3 group, namely mice with the highest dose of 2000 mg/kg BW/day, 1500.25 $\pm$ 290.83 mg, which decreased compared to the control group with an average liver weight of 1616.24 $\pm$ 372.48 mg. In the liver, a p-value of 0.129 was found to be greater than the significance value of $\alpha = 0.05$ in a parametric statistical test utilizing the test ANOVA One Way. This can be interpreted that there is no significant effect between the administration of flavonoid-rich extract from *Phaleria macrocarpa* with the weight of the mouse liver.

Mice Histopathological Structure Assessment

The data normality test Saphiro-Wilk was used to measure the histopathological structure score of mice, and the Lavenne test was utilized to test data homogeneity. The p-value for the Shapiro-Wilk test is greater than 0.05, indicating that the data is regularly distributed. Because the p-value for the test coming mark was less than 0.05, it was decided that the liver histopathology score data was not homogeneous. The following data analysis will compare the effect of administering a Flavonoid-rich extract from *Phaleria macrocarpa* using the ANOVA One Way test. Games Howel is then used in a post-hoc analysis to identify noteworthy changes.

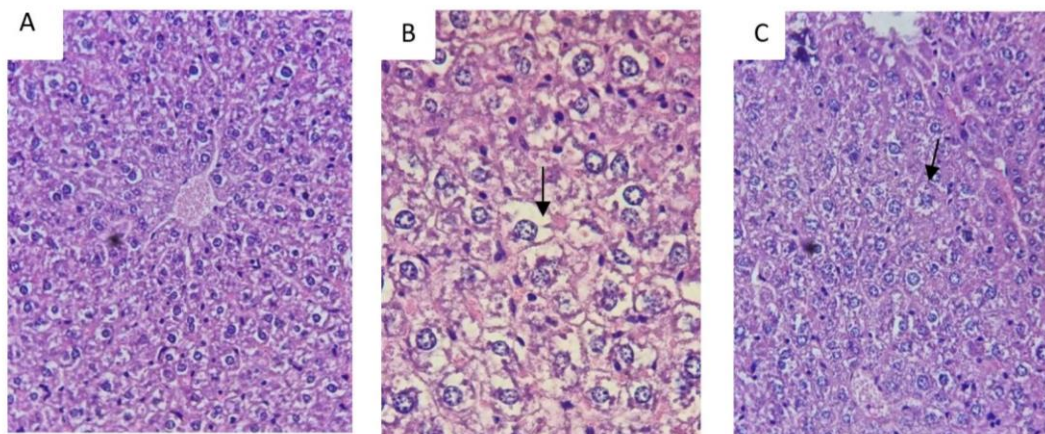


Fig. 1. Histopathological of the Mice Liver. Histopathological images of normal hepatocyte cells were identified in the control and P1 groups (A), histopathological images of hydropic degeneration (B) were detected in group P2, and images of necrosis (C) were observed in treatment group 3/P3. 400x magnification image with HE staining

The mean hepatic histopathology score of mice P1 1.5 ± 0.57 increased from the control group 0 ± 0 , although there was no significant difference, as shown in Table 2. The mean hepatic histopathology score of mice in the P2 group was 2 ± 0.18 , which was a rise and a significant difference when compared to the control group. The mean liver histopathology score in the P3 group was 3 ± 0.18 , up from 0 ± 0 in the control group indicating a significant difference.

Table 2. Test Results ANOVA One Way Hepar Histopathological Score of Mice With Flavonoid-Rich Extract From *Phaleria macrocarpa*

Sample group	N	Mean liver histopathological score	p-value
Control	4	$0 \pm 0a$	0.00
P1	4	1.5 ± 0.57^a	
P2	4	2 ± 0.18^b	
P3	4	3 ± 0.18^c	

Description: If p-value < 0.05 means there is a significant difference, if the p-value is > 0.05 then there is no significant difference. If it contains the same letter it means that it has no significant difference, if it contains different letters it means it has a significant difference

DISCUSSION

Based on the ANOVA one-way analysis There was no significant difference in the average liver weight of the control mice and the animals in the treatment group given 500, 1000, and 2000 mg/kg BW/day flavonoid-rich extract from *Phaleria macrocarpa*. The p-value is more than 0.05 to support this. Organ weight is a sensitive measure of the test substance's effect on the liver, which serves as the target organ. There was no

significant difference between the control and treatment groups, which could be attributed to the process of hepatocyte cell regeneration [20].

A comparable investigation utilizing sipatah-patah stem extract containing flavonoids found no significant variation in the liver weight of mice [21]. Another study utilizing nano herbs from *Phaleria macrocarpa* in rats with doses up to 1608.1 mg/kg BW/day found no significant variation in the hepatic weight of mice [16]. This is consistent with subchronic experiments in mice that used ethanol extract nanoparticles from the plant *Phaleria macrocarpa* at dosages of 85, 170, and 340 mg/kg BW/day. The results of this study stated that there was no significant difference in the macroscopic appearance of the liver between the control and treatment groups, the liver looked normal, had a rubbery texture, was dark red, consisted of several lobes, and did not show any morphological abnormalities [17].

The findings of a statistical analysis using ANOVA One Way revealed that the administration of flavonoid-rich extract of *Phaleria macrocarpa* resulted in significant variations in the data of the mice's liver histopathology score. On microscopic analysis of histopathological structures, there was no significant difference between the treatment group and the control group when flavonoid-rich extract of *Phaleria macrocarpa* was administered at a level of 500 mg/kg BW/day. This demonstrates that providing flavonoid-rich extract at a level of 500 mg/kg BW/day has no harmful effect. This is consistent with research utilizing *Phaleria macrocarpa* fruit ethanol extract with good treatment in rats for 28 days at doses up to 5000 mg/kg BW/day that does not induce harmful effects [22].

Subchronic investigation utilizing total flavonoids from *Rosa Laevigata* fruit revealed that at doses of 500 mg/kg BW/day and 1000 mg/kg BW/day, no toxic

effects in the liver were observed [23]. This is conceivable because the liver is a critical organ in the body's homeostatic processes, metabolism, and toxin detoxification, as well as its involvement in regulating endogenous and external xenobiotics [24]. The liver contains Kupfer cells, which phagocytize poisons and other foreign substances/objects [21,25]. Subchronic toxicity tests on rats using flavonoids in Parkinson's therapy evaluating body weight, haematology, clinical biochemistry, and histopathology found that using flavonoids from Safflower at doses of 100, 300, and 500 mg/kgBW/day did not create toxic consequences [26]. A toxicity test using licorice flavonoid oil as obesity therapy for 90 days at a dose of 800 mg/kg/day in female rats and 400 mg/kg/day in male rats did not cause toxic effects [27].

In the therapy group at doses 1000 and 2000 mg/kg BW/day, histological examination of the liver revealed hydropic degeneration and hepatic necrosis. The cells seem brighter in hydropic degeneration, and there are vacuoles packed with water but no fat in the cytoplasm [28,29]. Flavonoids, through active transport abnormalities, cause oxidation failure. Cells are unable to pump Na⁺ out of the cell, causing the intracellular concentration of Na⁺ to rise and disturb the osmosis process. The influx of water causes the cells to expand. Hydropic degeneration and parenchymal degeneration are types of damage that can be reversed if exposure to harmful substances is halted; else, necrosis will occur [30-32]. Cells undergoing necrosis are characterized by the presence of pycnotic, karyorexis, and karyolysis and these are irreversible [19].

In mice, administering a dosage of 1608.1 mg/kg BW/day nano herbals of *Phaleria macrocarpa* had toxic effects on the liver in the form of parenchymal degeneration, hydropic degeneration, and necrosis [16]. A subchronic investigation of ethanol extract nanoparticles *Phaleria macrocarpa* in mice at a dose of 85 mg/kg BW/day revealed hydropic degeneration, a dose of 170 mg/kg BW/day revealed inflammatory cell infiltration, whilst a dose of 340 mg/kg BW/day caused necrosis in the liver. The higher the dose, the greater the damage to the liver cells [17]. The use of flavonoids from the leaves of *Croton blanchetianus* members in mice at a concentration of 1000 mg/kg BW/day induced liver damage in the form of alterations in transaminase and leukocyte infiltration [33].

The administration of an ethanol extract of *Phaleria macrocarpa* in Javanese quails for two months at doses of 50, 100, and 200 mg/kg BW/day induced liver damage [9]. Giving Madiensis olive extracts, which include flavonoids, to pigs for 28 days at a dose of 1000 mg/kg/day caused liver damage in the form of reduced ALT and AST [34]. It was discovered that prolonged usage of high doses may cause liver damage [35] as in the group with 1000 and 2000 doses of flavonoids from

Phaleria macrocarpa fruit extract. Hepatotoxicity occurs due to interactions with important enzymes in the body thereby inhibiting hepatic activity, such as quercetin which is included in the flavonoid group which will inhibit the CYP450 enzyme which is responsible for the metabolism of certain drugs, if the drug is not metabolized properly it will result in toxicity and damage to the body [14].

Another mechanism for toxic substances in the liver is through reactions that damage mitochondrial homeostasis in both function and structural mitochondria, oxidative stress, specifically an imbalance of oxidants and antioxidants, apoptosis, including mitochondrial apoptosis, endoplasmic reticulum and receptor regulation that stop, and reactions in special conditions, specifically immune suppression. anomalies and polymorphisms in genetic code [15]. Giving a dose of 500 mg/kg BW/day of flavonoids from *Phaleria macrocarpa* fruit extract for 28 days is still well metabolized in the liver so it does not result in oxidative stress, mitochondrial damage, or disruption of enzymes that play a role in liver metabolism. Giving flavonoids at the right dose will protect the liver from damage, such as flavonoid which have an antioxidant effect that will act as hepatoprotectors [36].

CONCLUSION

A dose of 500 mg/kg BW/day of a flavonoid-rich extract from *Phaleria macrocarpa* had no effect on the liver ($p>0.05$), this means that it is not toxic. Giving flavonoid-rich *Phaleria macrocarpa* extract flavonoids at 1000 and 2000 mg/kg BW/day caused a change in the liver, specifically hydropic degeneration and necrosis. Giving the liver 1000 and 2000 mg/kg BW/day of flavonoid-rich extract from *Phaleria macrocarpa* flavonoids proved harmful.

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CONFLICT OF INTEREST

There is no conflict of interest in this research.

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