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Evaluation of Subacute Toxicity of Flavonoid from Phaleria Macrocarpa Fruit Extract in Mice

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Abstract

Background: *Traditional medicine and supplements including Phaleria macrocarpa or Mahkota Dewa are extensively utilised. Rape fruit of phaleria macrocarpa contains flavonoid as a secondary metabolite that has a beneficial effect on endometriosis treatment and other illnesses. The purpose of this study was to look into the subacute toxicity of flavonoid from phaleria macrocarpa fruit extract on the brain and heart of BALB/c female mice.* ***Method:*** *The BALB/c female mice aged 6-8 weeks with a body weight of 20-30gr were divided into four groups and treated for 28 days. The control group received simply water, whereas the treatment group received flavonoid at doses of 500mg/kg/day, 1000mg/kg/day, and 2000mg/kg/day. At the end of the experiment, the mice were sacrificed and important organs were extracted and assessed. The organ weight was assessed using macroscopy, and the brain and heart were assessed using microscopy (histopathological abnormalities).* ***Result:*** *The BALB/c female mice treated with oral administration flavonoid from phaleria macrocarpa fruit extract showed no clinical abnormalities and no change in organ weight compared to the control groups (p>0,5) in subacute toxicity experiments. There were no abnormalities in the organs of the treated mice at a dose of 500mg/kg/day, according to histopatological testing.* ***Conclusion:*** *As a result, flavonoid had no harmful impact in brain and heart mice after subacute therapy up to a level of 500 mg/kg/ body weight. High doses of 1000 and 2000 mg/kg/day are not recommended for long-term use.*

Keywords: *Subacute Toxicity, Flavonoid, Phaleria Macrocarpa*

1. Introduction

Herbal products are increasingly being used as an alternative to chemical medications to treat a variety of ailments just because no side effect and low cost (1–3). Phaleria macrocarpa is a plant that is utilised as a traditional herbal medicine due to its several benefits(4,5). Phaleria macrocarpa is a member of the thymelaeceae family that originated in Papua and is commonly found in tropical places (4,6,7) The presence of secondary metabolites such as flavonoids, alkaloids, fophenols, saponins, lignans, and phalerins was revealed by the screening results of the phytochemical test Phaleria macrocarpa (6,8). The fruit of Phaleria macrocarpa contains the greatest flavonoids, such as Eriodictyol, Glykitin, 5-O-Methylgenistein, 4-(8-Isopropenyl-3, 4,8, 9-tetrahydro-2H-furo [2,3-H] chromen-3 yl) -1, 3-benzenediol 3,5-dihydroxy - 2-(4-hydroxyphenyl)4- oxo-3, 4-dihydro-2H chromen 7 yl hexopira nosida, (+) - catechin 7-O-beta-D-xyloside, (-) -8-prenylnaringenin -naringenin, Apigenin trimethyl esters(9). Secondary metabolite Phaleria macrocarpa has biological activity that includes antibacterial, anti-inflammatory, and anti-allergenic, anti-tatogenic, anti-thrombotic, cardioprotective, and

vasodilator properties, and it plays a role in slowing the progression of endometriosis by boosting SOD and lowering VEGF (4,10–13)

Despite their numerous and diverse applications, prior investigations on flavonoid from fruit extracts Phaleria macrocarpain did not include toxicity assessments(14,15). The incorporation of a component into herbal products must adhere to safety guidelines for exposure limits and risk assessment (16–18). The subacute toxicity test is a type of toxicity test used to assess the safety and effects of long-term herbal medicine usage (19). The purpose of this study is to assess the subacute toxicity of flavonoids derived from fruit extracts of Phaleria macrocarpain in BALB/C female mice. The findings of this study are expected to provide safety information on the usage of flavonoids from Phaleria macrocarpaso fruit extracts, which can be utilised as a guideline for future research.

2. Materials And Methods

The research design employs a real experimental post test only control group design. The research protocol was carried out in accordance with a letter of approval from the Ethics Committee of the Medicine Faculty, Brawijaya University, with the reference number 38/EC/KEPK/03/202. This study was carried out in the Polinema Malang Laboratory, the Embryology Laboratory, the Faculty of Veterinary Medicine, Airlangga University, and the Pathology Anatomical Laboratory, Brawijaya University, Malang.

Flavonoids from Phaleria macrocarpa fruit extract are obtained by extracting and partitioning the fruit Phaleria macrocarpa maturity acquired from Aceh Besar District. Up to 20 kg of ripe Phaleria Macrocarpa fruit is cleaned, dried in an oven at 80C° and pulverised to make simplicia powder. 2500 g simplicial powder soaked in 30 litres of 96% ethanol for 30 minutes, agitated for 30 minutes until fully mixed, then allowed for 5 nights to precipitate. Filter through filter paper. Flavonoids are produced by separating n-hexane and n-butanol. Flavonoid content was determined by mixing 0,5 gr of the supernatant with 5 mg of magnesium powder and 1 ml of concentrated Hcl. Shaking is done, if a red color is found it means it contains flavonoids in it.

Adult BALB/c female mice weighing 20-30 grammes were used in the study, and they were procured from the East Java Veterinary Centre. Before being treated, the mice were acclimatized for 1 week in the experimental cage at the Faculty of Veterinary Medicine, Airlangga University. The test animals were fed ordinary commercial diet and allowed unlimited access to water. The test animals were divided into four groups at random and subjected to four repeats each. The first group received simply aquabides, while the second group received flavonoids at a level of 500 mg/kg body weight/day, the third 1000 mg/kg of weight/day, and the fourth group 2000 mg/kg body weight/day. Toxicity symptoms, weight fluctuations, and death were all observed during the 28-day therapy period. The body weight of the mice was measured once a week, namely days 0, 7, 14, 21, 28. On the 29th day, the mice were terminated using ketamine and sylazine. Mice surgery was carried out to take the brain and heart organs and then examined macroscopically, organ weights and microscopic examination, namely histopathological view.

3. Results and Discussion

Microscopic Image of Mice Brain and Heart Weight

The brain and heart weight data of mice were acquired with a normal and homogenous distribution ($p>0,05$) as a result of data analysis. The statistical analysis Annova One Way indicate valuep-value 0,129 is greater than 0,05, indicating that there is no significant influence of flavonoids from fruit extracts Phaleria macrocarpa at various doses on mouse brain weight. Nonetheless, there was an increase in brain weight in all treatment groups, namely groups P1, P2, and P3, as compared to the control group, which had an average brain weight of 396,25±45,5mg. The highest mean increase in brain weight occurred in the P2 group, namely the group of mice given flavonoids from fruit extracts phaleria macrocarpa with a dose of 1000 mg/kg body weight/day which has an average brain weight of 457±37,76 mg.

According to statistical analysis using Annova One Way demonstrated that p-value 0,756 is greater than 0,05, indicating that there is no significant influence of flavonoids from fruit extractsPhaleria macrocarpa at different doses on the heart weight of mice. However, according to Table 2, the average heart weight of mice in all treatment groups was lower than the control group of 157,25±25mg. The P3 group, which received flavonoids from Phaleria macrocarpa fruit extract at a dose of 2000 mg/kg body weight/day and had an average heart weight of 141,25±17,55mg, had the highest drop in mean heart weight.

Table 1. Annova One Way Mice Brain Weight with Flavonoid Administration from Fruit Extract Phaleria macrocarpa Test Results

Group	Mean SD/mg	P-Value
K	396,25±45,5	0,129
P1	457±37,76	
P2	451,5±25,85	
P3	419,74±42,12	

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

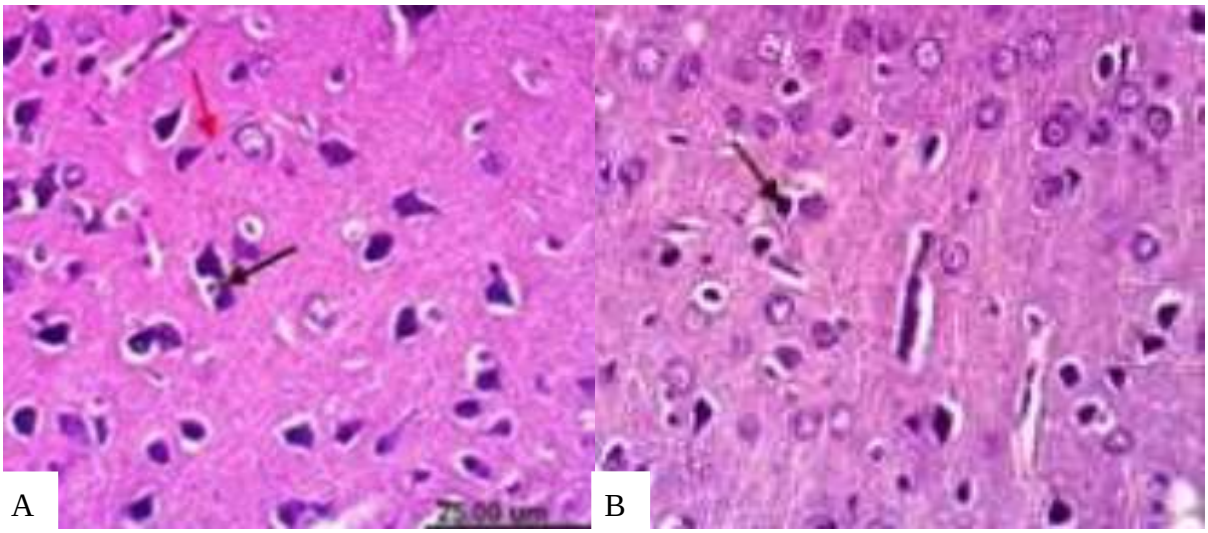
Table 2. Test Results Annova One Way Mice Heart Organ Weight with Flavonoid Administration from Fruit Extract Phaleria macrocarpa

Group	Mean SD	P-Value
K	157,25±25	0,756
P1	149,50±7,72	
P2	151,25±18	
P3	141,25±17,55	

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

Microscopic View of Mice Brain and Heart Histopathological Structure

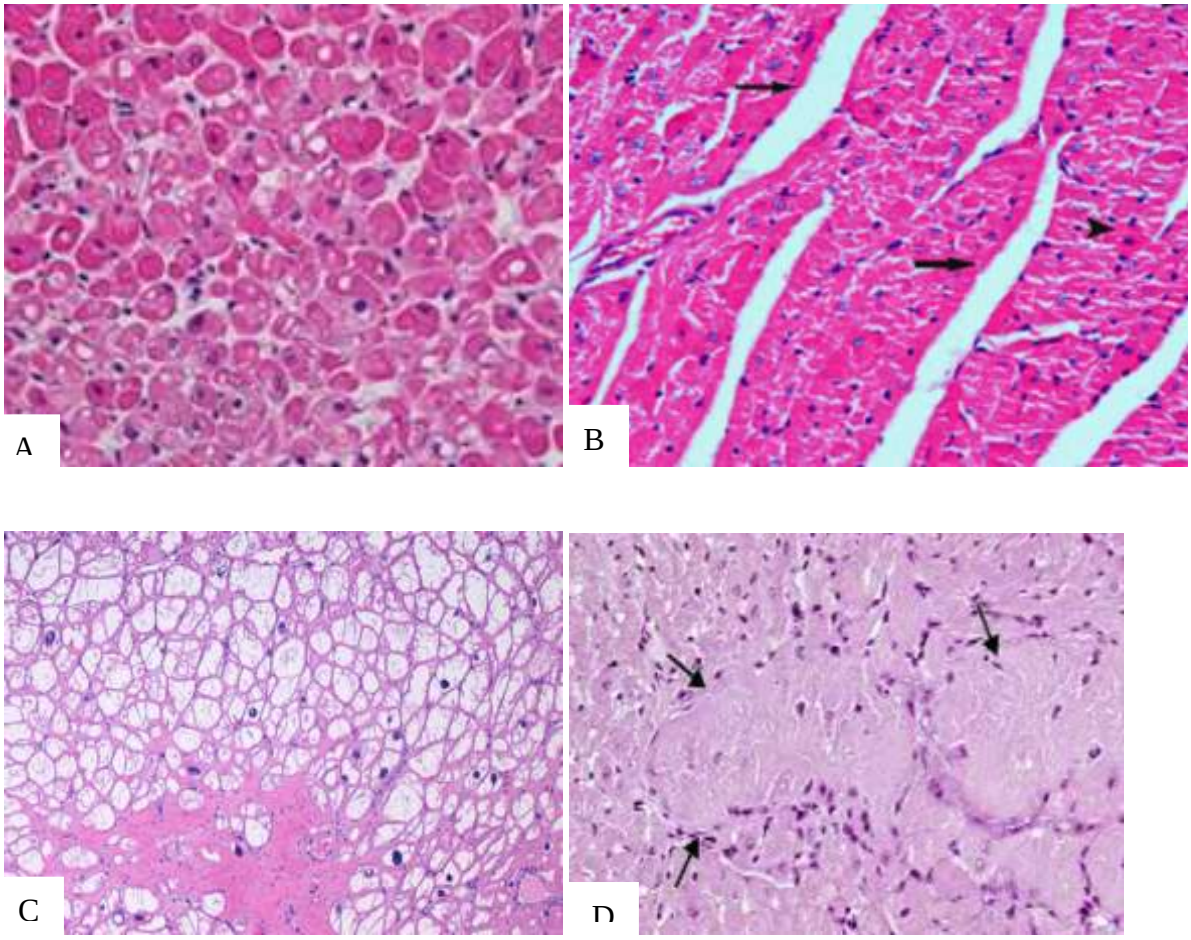
Figure 1. Microscopic view of the mouse brain histopathological structure



Information: Figure A is a histopathological picture of the control group with normal neuron cells. Figure B Histopathological appearance in the treatment group with necrosis of neuron cells.

The microscopic view of the histological structure of the mice's heart in the control group, which consisted of four replications, did not alter, nor did it in the P1 group. One sample in the P2 group or the group administered Flavonoids 1000 mg/kg body weight/day demonstrated parenchymal degeneration. One sample in the 2000 mg/kg body weight/day group had hydropic degeneration and one sample had necrosis. Figure 2 shows a microscopic image of normal heart histopathological structure, parenchymal degeneration, hydropic degeneration, and necrosis.

Figure 2. Microscopic view of the histopathological structure of the heart of BALB/c female mice



Information: A Picture of normal heart muscle cells, B Picture of parenchymal degeneration in heart muscle cells, C Picture of hydropic degeneration in heart muscle cells, D Picture of necrosis in heart muscle cells

The results of the histological investigation of the brain and heart organs of mice in each group exhibited a p-value larger than 0,05 in all data. This shows that the data is regularly distributed and homogeneous.

Table 3. Test Results Annova One Way Histopathological Scores of Mice Brains with Flavonoid Administration of Fruit Extracts Phaleria macrocarpa

Group	Mean SD	P-Value
K	0 ± 0^{a}	0,00
P1	$0,15\pm0,1^{a}$	
P2	$0,7\pm0,2^{b}$	
P3	$0,95\pm0,25^{b}$	

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.

Table 4. Test Results Annova One Way Histopathological Scores of Mice Hearts with Flavonoid Administration of Fruit Extracts Phaleria macrocarpa

Group	Mean SD	P-Value
K	0 ± 0^{a}	0,00
P1	$0,125\pm0,09^{ab}$	
P2	$0,27\pm0,15^{b}$	
P3	$1,25\pm0,19^{c}$	

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

The outcomes of the tests Annova One Way Show that p-value 0,00 is less than 0,05, indicating that there is a significant relationship between the administration of flavonoids from phaleria macrocarpa fruit extract at various doses in several groups and the histological scores of the brain and heart of mice. The average histopathological score of mouse brain and heart has increased. The average brain histopathology score of mice in the control group was 0±0 and P1 0,15±0,1, indicating an increase but no significant difference. Likewise, the average histopathological score of the brain of mice in the P2 group 0,7±0,2 given a dose of 1000 mg/kg body weight/day and P3 0,95±0,25 given a dose of 1000 mg/kg body weight/day showed an increase, but between the two no significant difference. The mean histopathological score of the control group mice 0±0 and P1 0,125±0,09 showed an increase, but there was no significant difference. The average cardiac histopathological score of mice in the P1 and P2 groups showed an increase, but there was no significant difference. There was a significant increase in the cardiac histopathological score of the control group mice with P2 0,27±0,15 and P3 1,25±0,19.

The goal of this study was to assess the subacute toxicity of flavonoids derived from the fruit extract Phaleria macrocarpa on the brain and heart organs of BALB/c female mice in terms of body weight and microscopic histopathological structure. According to the findings of statistical analysis, administration of flavonoids from fruit extracts of Phaleria macrocarpa had no significant effect on the weight of the brain and heart of mice. The brain weight of the control group, on the other hand, increased. This is consistent with a 14-day study utilising watermelon ethanol containing flavonoids, which was demonstrated to increase rat brain weight (20). A similar study using kolaviron, a flavonoid dose of 200 mg/kg body weight/day for 56 days in mice also made weight gain in mice (21). Another study stated that there was no effect of giving sweet potato ethanol extract containing flavonoids for 90 days on the heart of Rattus norvegicus mice (16). Subacute toxicity study (22) in mice using Rhamnus prinoides leaves which contains flavonoids at a dose of 1000 mg/kg body weight/day does not affect the weight of the brain and heart.

The administration of flavonoids from extracts Phaleria macrocarpa fruit considerably exhibited changes in the histopathological score data of the brain and heart of mice, according to the results of a statistical analysis using Annova One Way. A microscopic study of the histopathological structure of the brain and heart revealed no significant difference when flavonoids from fruit extracts Phaleria macrocarpa were administered at a level of 500 mg/kg body weight/day compared to the control group. This demonstrates that providing flavonoids from Phaleria macrocarpa fruit extracts at a level of 500 mg/kg body weight/day has no harmful effect.

This is consistent with study showing that using ethanol extract Phaleria macrocarpa fruit with good processing in rats for 28 days with doses up to 5000 mg/kg body weight/day does not induce harmful effects (23). Subchronic investigation utilising total flavonoids from Rosa Laevigata fruit revealed that at doses of 500 mg/kg body weight/day and 1000 mg/kg body weight/day, no toxic effects were seen (24). Subchronic toxicity test studies utilising flavonoids in Parkinson's therapy evaluating body weight, haematology, clinical biochemistry, and histopathology of rats concluded that the use of flavonoids from safflower at a dose of 100,300,500 mg/kg does not induce harmful consequences (25). Toxicity test using licorice flavonoid oil as obesity therapy for 90 days at a dose of 800 mg/kg/day in female mice and 400 mg/kg/day in male mice did not cause toxic effects (26).

Neuron cells in the treatment group necrosed, according to microscopic examinations of the histological structure of the brain. This can be produced by flavonol exposure or other factors such as stress, temperature, food, and so on (Yustisia *et al.*, 2020). The presence of pycnotic, karyorexis, and karyolysis is a sign of necrosis. The pycnotic is distinguished by nuclear shrinkage as a result of a homogenised and more eosinophilic cytoplasm. Karyorexis is the destruction of a cell nucleus, which results in chromatin fragments distributed throughout the cell. The absence of a nucleus is referred to as karyolysis; if it is painted, the colour would seem faded (29). Because the brain has a fat composition of more than 80%, it is prone to free radicals (30,31). The mechanism of neurotoxicity occurs due to increased calcium levels, excitotoxicity, mitochondrial dysfunction, oxidative stress and neuroinflammation (32,33). Flavonoids are able to protect the brain from neurotoxicity, but if exposure to flavonoids is carried out continuously for a long time and at high doses, it will result in oxidative stress(34,35).

Microscopic examinations of cardiac muscle cells in the control group revealed that they were not harmed. However, in the therapy group, parenchymal degeneration, hydropic degeneration, and necrosis were discovered. Parenchymal degeneration is characterised by expanded cell size, nuclei pushed to the margins, and cell cavities that seem empty and granular. Cells in hydropic degeneration appear brighter, with vacuoles loaded with water but no fat in the cytoplasm, whereas cells in necrosis

exhibit picnotic, karyorexis, and karyolysis (36,37). Long-term exposure to flavonoids at high concentrations causes oxidative damage and systemic inflammation. ROS play a significant function in the necrosis of cardiac histopathological structures(38). ROS can promote aberrant gene expression and alterations in signal transmission, resulting in increased cardiac muscle necrosis (39).

4. Conclusion

The study concluded that using flavonoids from fruit extracts of Phaleria macrocarpa at doses of 500, 1000, and 2000 mg/kg body weight/day did not create any changes in the macroscopic appearance of the weight of the brain and heart of mice. Provision of flavonoids from fruit extracts of Phaleria macrocarpa at a dose of 500 mg/kg body weight/day did not cause microscopic changes in the histopathological structure of the brain and heart of mice, or in other words, did not cause subacute toxicity effects on the histopathology of the brain and heart of mice.

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Conflict of Interest

There is no conflict of interest in this research

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