



Secondary Metabolites Screening and Acute Toxicity Test of *Peperomia pellucida*(L.) Kunth Methanolic Extracts

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Abstract : *Peperomia pellucida* (L.) Kunth. is a wild plant that can be found in various shaded and damp habitats in tropical and subtropical climate. Aerial part of this plant is traditionally used as a medicine in Indonesia such as dizziness, headache, fever, and stomach ache. This study aims to determine the content of secondary metabolites and acute toxicity of methanolic extracts of *Peperomia pellucida* (L.) Kunth herb. The methanolic extract obtained by gradual maceration using n-hexane and methanol. Secondary metabolites analysis by appropriate reagents showed that methanol extract contains flavonoids, tannins, and steroids. Acute toxicity tests performed in DDY mice showed that methanol extract LD₅₀ is greater than 4000 mg/kgBW. There was no sign of toxicity on the skin and hair, respiration system, defecation, feed intake and behavior up to 4000 mg/kgBW. At that dose, the extract did not cause mortality, influence growth, and liver damage.

Keywords : *Peperomia pellucida* (L.) Kunth, acute toxicity, methanol extract, LD₅₀.

Introduction

Peperomia pellucida(L.)Kunth is a wild plant from tropical America that has been widely grown in many countries. This herb grows in shaded and damp hard surfaces, such as walls, rock, steep gullies, and in flower pots as a weed¹. Many countries in tropical America used this plant as medicine, such as to stop hemorrhages (Bolivia), hypercholesteremic agent (northeastern Brazil), cough suppressant (Guyana), treatment proteinuria (Amazon). In Asian countries, this plant also used as traditional medicine. In Philippine, this plant is used to treat acne, renal problem, and decreasing uric acid levels². In India, the whole plant is used to treat a cough, the renal problem (kidney disorder, dysuria, urinary retention, urinary tract infections), and general weakness³. In Indonesia, the crumpled leaves are used to treat dizziness or headaches if the patient has a fever¹.

Peperomia pellucida(L.)Kunth from many studies have been known to have secondary metabolite i.e. alkaloid, saponin and tannin², secolignans, tetrahydrofuran lignans, and methoxylated dihydronaphthalenone⁴, xanthon⁵, flavon, steroid and terpenoid⁶. Pharmacological study of *Peperomia pellucida*(L.)Kunth showed that this plant has antidiabetic activity, antioxidant⁷, antibacterial⁸, analgesic^{9,10}, antiinflammation¹⁰ and antihypertension^{11,12}.

Toxicity study of ethanolic extract showed that *Peperomia pellucida*(L.) Kunth has LD₅₀ 15.13 g/kg BW male mice and 11.87 g/kg BW female mice; meanwhile, LD₅₀ for aqueous extract is more than 5000 mg/kg BW¹³. *Peperomia pellucida* aqueous extract categorized as low toxicity (>5000 mg/kg BW)¹⁰.

This study aims to investigate phytochemical and acute toxicity (LD₅₀) of *Peperomia pellucida*(L.) Kunth methanolic extract.

Experimental

Plant materials

Peperomia pellucida(L.)Kunth was collected from Bogor, West Java, Indonesia. The species was identified and determination by Plant Conservation Botanic Gardens Center, Indonesian Institute of Sciences, Bogor. The voucher specimen was deposited in Herbarium of Pharmacognosy-Phytochemistry Laboratory, Faculty of Pharmacy, Universitas Indonesia. The plant was dried in open air then in the drying cabinet. Dry sortation the plant to separate unwanted material such as other plants, rocks, soils, and other impurities. Powdered the dried plant then keep in sealed bag.

Extract preparation

Peperomia pellucida(L.)Kunth powder was extracted by gradual maceration process with n-hexane, continued with methanol (6 x 24 hours each). Liquid extract was then concentrated with rotary vacuum evaporator at 40°C, and then was dried with vacuum the oven at 40°C. The final extract put in the sealed bottle and stored in a refrigerator for further analysis.

Phytochemical screening

Secondary metabolite presence analysis is done by phytochemical screening. The *Peperomia pellucida* methanolic extract was qualitatively tested for the phytochemical screening using standard established method. Secondary metabolite tested for flavonoid, alkaloid, saponin, tannin, quinon, steroid, and triterpenoid.

Animals

The DDY mice male and female were purchased from Animal Production and Technology Department, Faculty of Animal Science, Bogor Agricultural University. The animals weighed 20-40 g, housed five mice per cage and maintained at a constant 12-hour light/12-hour dark cycle. Feed and water were available *ad libitum*. The animals were examined and weighed and kept in their cages for 7 days prior to the first day of study then allowed to for acclimatization to the laboratory environment. Ethical approval for the protocol testing was received from Health Research Ethics Committee – Universitas Indonesia and Cipto Mangunkusumo Hospital.

Doses

The acute toxicity of methanol extract was studied by preparing four different doses, i.e. 500, 1000, 2000 and 4000 mg/kg body weight. Before administered the test dose orally, animals fasted (only feed not water for 3-4 hours). Following the period of fasting, the animals weighed and then administered the extract. The feed may be withheld after 1-2 hours of extract administration.

Toxicity symptoms observation

Observations of the animals were done individually after extract administered for the first 30 minutes, periodically during the first 24 hours and special attention given during the first 4 hours, and daily thereafter, for a total of 14 days. Toxicity symptoms observation include changes in skin and fur, eyes, respiratory, salivation, diarrhea, urination, and the central nerve effects including tremors, and behavior pattern.

Body weight and feed consumption

Individual body weight was measured and recorded prior to the administration of extracts for 14 days. Weight changes were also calculated and recorded. The amount of feed consumption was measured daily from the food quantity remaining after 24 hours for 14 days of the study period.

Organ weight and histopathology

Animal organs were collected at the end of the acute toxicity study (14 days) using standardized surgical procedures. Each abdominal cavity of the animal was dissected for their organs namely the heart, liver, and kidney. Then, they were quickly removed, cleaned with saline, weighed and preserved in 10% formalin for histopathology analysis. Macroscopic examination from heart, liver and kidney is done by observing the color, size and morphological abnormalities of organ. Histopathology slides of the liver, heart and kidney prepared using hematoxylin and eosin staining (HE).

Blood Biochemical

Blood serums were collected at the end of the toxicity study (14 days). Animals were anesthetized and then dissected for collecting blood from heart aorta valve. Collected blood centrifuged 3500 rpm for 15 minutes to have clear serum. Serum stored at $\sim -20^{\circ}\text{C}$ for further analysis. In this study, the liver function test is done by analyzing Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) from serum.

Statistical analysis

The results are expressed as the mean \pm SD for each group. Statistical differences were evaluated using one-way analysis of variance (ANOVA) followed by Post hoc Test. The results were statistically significant at $p>0.05$.

Result

Phytochemical Screening

Phytochemical screening showed that *Peperomia pellucida*(L.) Kunth methanol extract contains flavonoid, tannin, and steroid. Alkaloid, saponin, quinon and triterpenoid observed to be absent.

Acute toxicity study

Mortality and toxicity symptoms observation

There was no mortality of animal until the end of the study in all group (14 days). Observation of the toxicity symptoms between normal and doses group showed no differences in behavior, skin effect, loss of hair, breathing, defecation, and impairment in feed intake consumption.

Body weight and feed consumption

Body weight of each animal was recorded every day for 14 days. An increased body weight significantly is shown in male mice, while in female mice not occur ($>0,05$).

Feed consumption of all groups has a significant correlation with the body weight. As we can see in the Table 1, that normal female mice group has the highest feed consumption but not correlated to body weight gain significantly.

Table 1 Body weight (g) and food consumption (g/mice)

	Normal		500 mg/kg BW		1000 mg/kg BW		2000 mg/kg BW		4000 mg/kg BW	
	BW	Food	BW	Food	BW	Food	BW	Food	BW	Food
Male	38.75 ± 3.46	8.22 ± 1.07	33.72 ± 3.19	7.11 ± 1.05	35.18 ± 5.02	8.58 ± 1.25	35.40 ± 6.50	6.53 ± 1.28	36.20 ± 4.77	7.89 ± 1.01
Female	27.93 ± 2.08	9.0 ± 0.84	25.60 ± 5.72	5.58 1.51	27.89 ± 2.36	6.417 ± 1.79	29.40 ± 1.89	5.24 ± 1.14	28.96 ± 3.90	7.18 1.49

Organ weight

At the end of experiment for acute toxicity test period (14 days), organ weights of mice were measured. Absolute organ weight on all groups showed no significant differences (Table 2 and Table 3). Relative organ weight is a comparison of absolute organ weight to the body weight. There are also no significant differences in relative organ weight for all groups ($p>0.05$). Macroscopic observation of liver, heart and kidney showed no difference between normal groups and dose groups.

Table 2 Male absolute and relative organ weight

Organ	Normal	500 mg/kg BW	1000 mg/kg BW	2000 mg/kg BW	4000 mg/kg BW
Liver	2.28 ± 0.27	2.01 ± 0.201	2.07 ± 0.591	1.97 ± 0.501	2.20 ± 0.277
RW	5.67 ± 0.16	5.96 ± 0.38	5.67 ± 0.80	5.62 ± 0.61	5.89 ± 0.58
Kidney	0.73 ± 0.075	0.48 ± 0.132	0.66 ± 0.167	0.498 ± 0.136	0.576 ± 0.121
RW	1.82 ± 0.22	1.4 ± 0.2	1.82 ± 0.22	1.42 ± 0.15	1.52 ± 0.13
Heart	0.26 ± 0.095	0.184 ± 0.03	0.134 ± 0.05	0.194 ± 0.06	0.23 ± 0.065
RW	0.66 ± 0.27	0.54 ± 0.05	0.37 ± 0.10	0.55 ± 0.11	0.60 ± 0.122

RW = Relative weight (%)

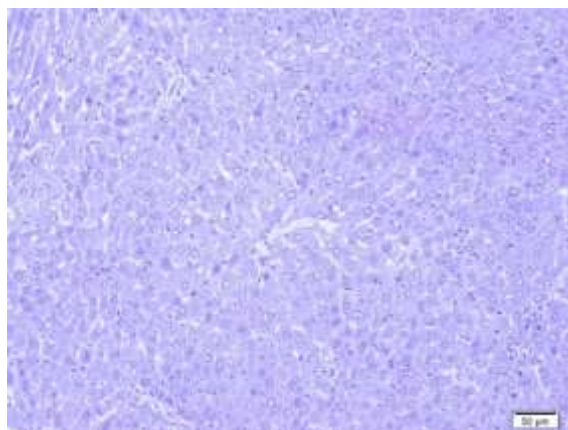
Table 3 Female absolute and relative organ weight

Organ	Normal	500 mg/kg BW	1000 mg/kg BW	2000 mg/kg BW	4000 mg/kg BW
Liver	1.88 ± 0.22	1.56 ± 0.36	1.72 ± 0.30	1.76 ± 0.36	1.84 ± 0.15
RW	5.99 ± 0.76	6.12 ± 0.87	6.20 ± 1.32	5.86 ± 1.09	6.25 ± 0.86
Kidney	0.37 ± 0.11	0.46 ± 0.21	0.41 ± 0.10	0.45 ± 0.07	0.45 ± 0.14
RW	1.18 ± 0.35	1.82 ± 0.79	1.46 ± 0.40	1.56 ± 0.27	1.47 ± 0.36
Heart	0.22 ± 0.03	0.13 ± 0.06	0.13 ± 0.05	0.23 ± 0.07	0.18 ± 0.05
RW	0.70 ± 0.11	0.55 ± 0.32	0.45 ± 0.18	0.78 ± 0.24	0.62 ± 0.21

RW = Relative weight (%)

Histopathology

Histopathological observation showed no specific abnormalities in heart, liver and kidney between normal groups and dose groups. Color difference in organ slide due to the ability of the tissue to stained (Figure 1, 2, and 3).



Normal

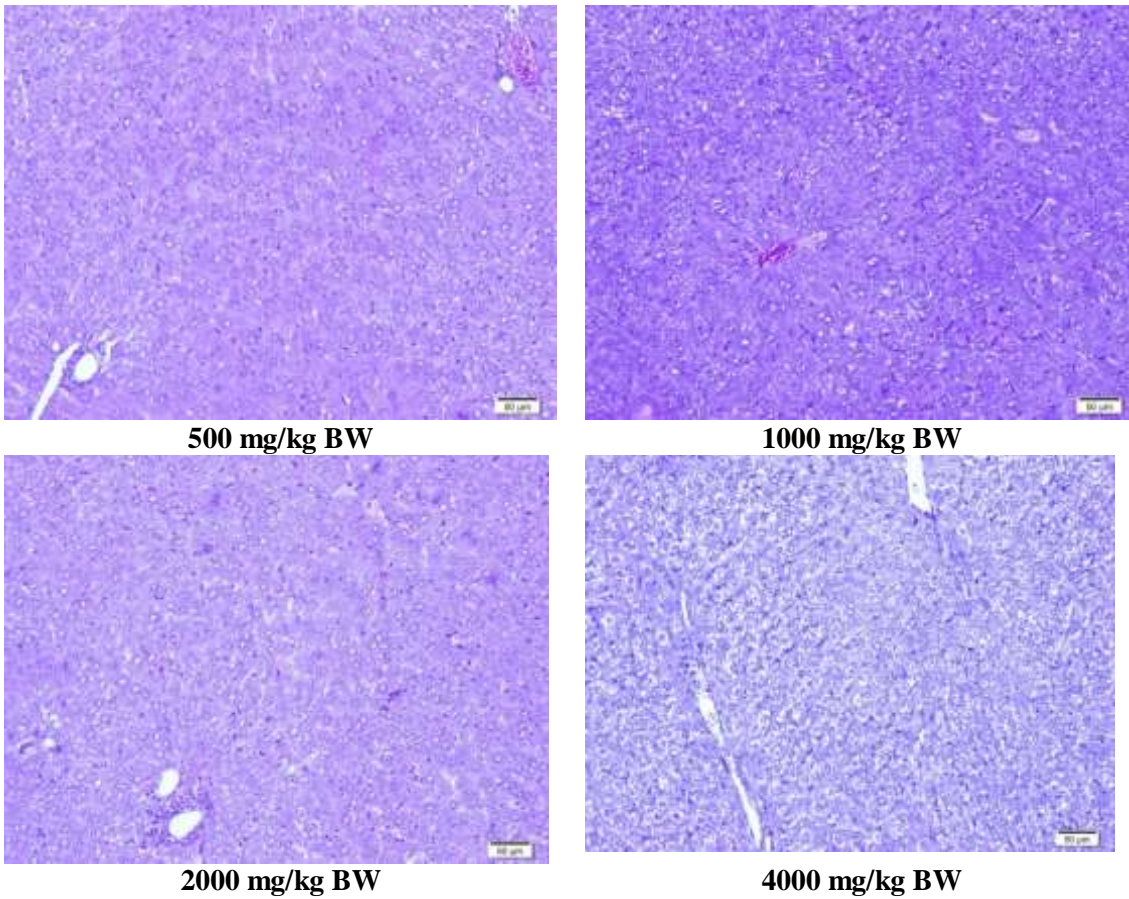
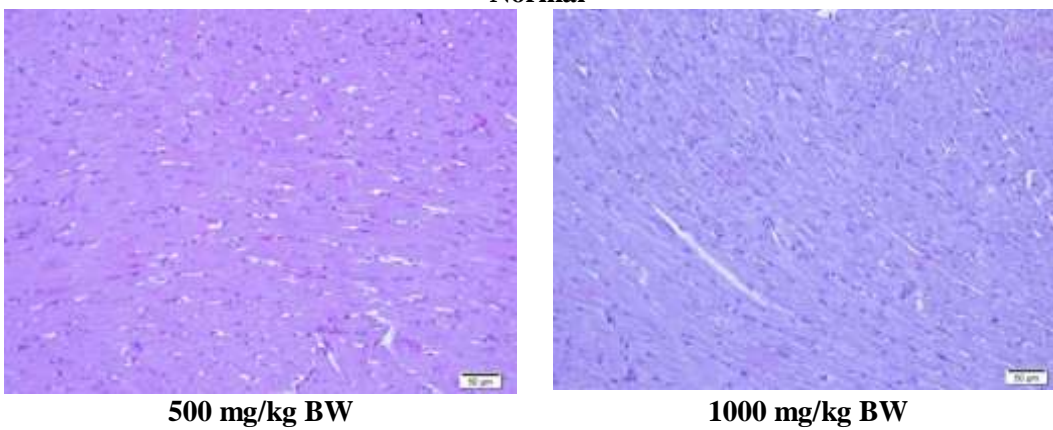
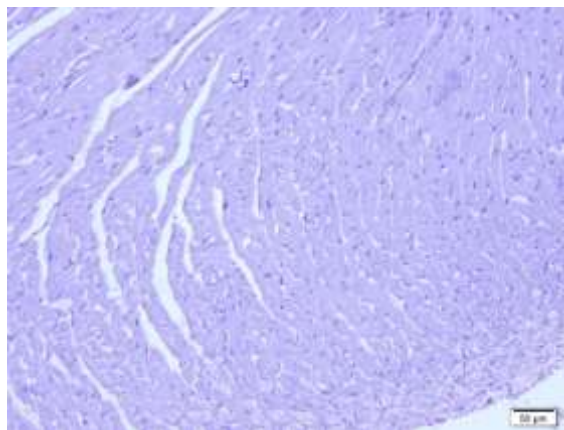
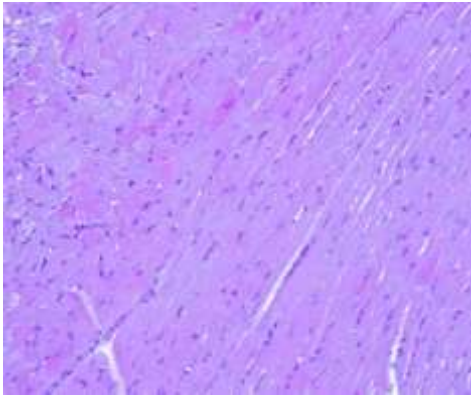
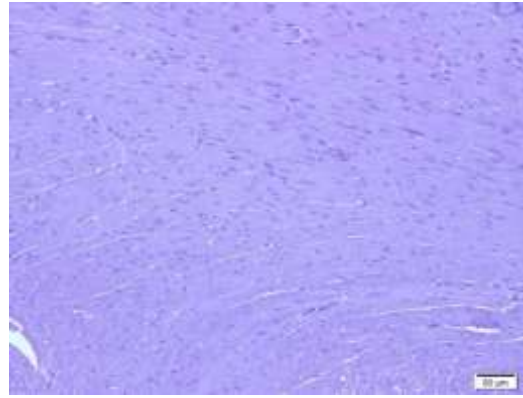


Figure 1. Liver tissue (HE stained,200x)



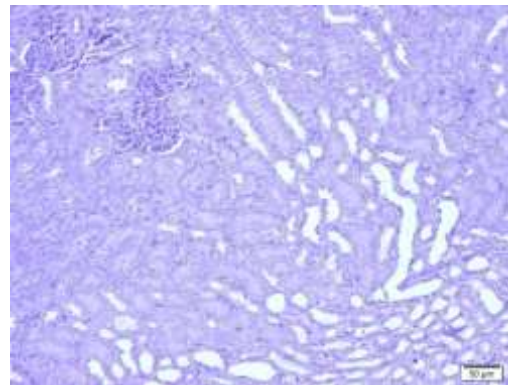


2000 mg/kg BW

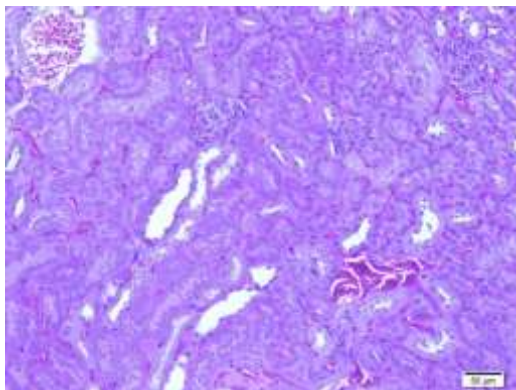


4000 mg/kg BW

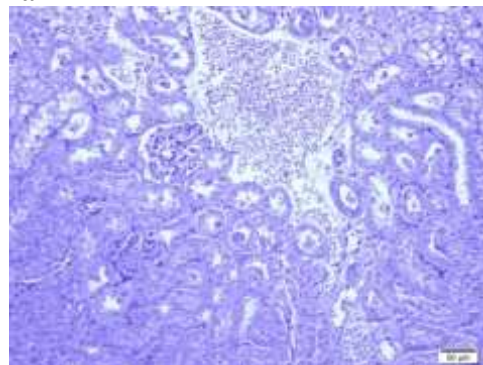
Figure 2. Heart Tissue (HE stained,200x)



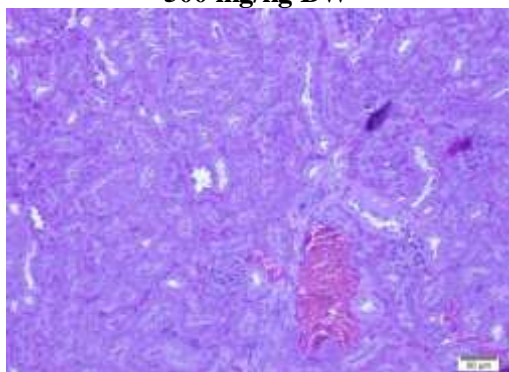
Normal



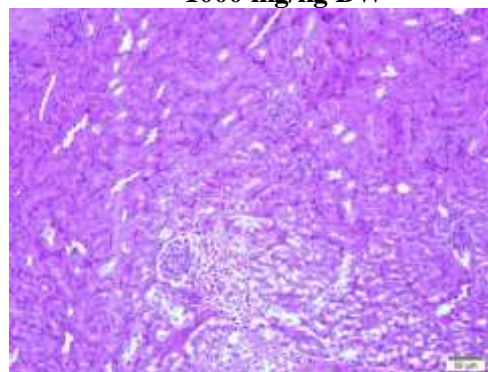
500 mg/kg BW



1000 mg/kg BW



2000 mg/kg BW



4000 mg/kg BW

Figure 3. Kidney tissue (HE stained,200x)

Blood biochemical

ALT level for all normal groups showed no significant difference between all groups. Male groups 1000 mg/kg BW showed significant differences ALT level with 2000 mg/kg BW and 4000 mg/kg BW group, and for AST level showed significant differences with all groups. ALT and AST level for all female groups showed no significant differences (0.05). The result of AST and ALT level as showed in Table 4.

Table 4 AST and ALT level

		Normal	500 mg/kg BW	1000 mg/kg BW	2000 mg/kg BW	4000 mg/kg BW
AST	Male	58.70+8.46	62.85+14.41	36.14+22.34	66.76+13.12	67.68+3.08
	Female	34.30+5.42	24.86+13.413	23.48+15.06	38.90+9.91	37.35+3.39
ALT	Male	26.24+11.88	26.70+18.40	7.37+8.36	44.66+7.52	43.28+21.19
	Female	29.01+15.28	24.86+15.49	19.11+11.55	20.72+2.57	35.68+13.34

Discussion

Peperomia pellucida is a native plant of South America but has been widely used as a traditional medicine in some countries. Many studies regarding to the pharmacological activities of this plant, but a lack of data on toxicity and adverse effect. Toxicity study is important to have safety guarantee for the use of natural material for human health.

Our study of *Peperomia pellucida* methanol extract showed the presence of flavonoid, tannin and steroid. In the previous study, we have isolated quercetin (flavonoid) that have ACE inhibitor (antihypertension) activity from methanol extract¹². Raman (2012) study on phytochemical screening on methanol extract exhibit the presence of flavonoid, saponin and alkaloid in sequential maceration with dichloromethane, ethyl acetate and methanol¹⁴. The presence of secondary metabolites will most likely differ influenced by environment conditions¹⁵. A secondary metabolite is the characteristic feature of plants against a wide variety of microorganisms (viruses, fungi, bacteria) and herbivores (vertebrates, arthropods). Plant biological activities might be related to human biological activities. Flavonoid in the plant can become antibacterial, this function in plant showed correlation with *Peperomia pellucida* activity as antimicrobial activity^{8,14,15}. Secondary metabolite function in the plant can be the basis of the biological activity¹⁵. The use of different solvent will produce different secondary metabolites. Polarity level of the solvent affect the withdrawal of a secondary metabolites in plants¹⁴.

Observation of toxicity test for 14 days of *Peperomia pellucida* methanol extract dose 500, 1000, 2000, and 4000 mg/kg BW showed no mortality and no symptoms of toxicity. Therefore, the LD₅₀ value is greater than 4000 mg/kg BW¹⁶. OECD guideline mentions that the highest dose level for acute toxicity is 5000 mg/kg BW, for reason of animal welfare concern¹⁷.

Acute toxicity test observation on bodyweight parameter showed no significant weight gain in female while male group showed significant weight gain. Feed consumption for all group has a significant correlation with the body weight. This result showed that *Peperomia pellucida* extract did not influence food appetite and growth. Organ weight analysis showed that there were no significant changes in the absolute and relative organ weight of the liver, heart, and kidney in all group. Body weight changes, feed intake and organ weight result showed no significant differences, it is suggested that oral administration of *Peperomia pellucida* extract did not influence normal growth and metabolism¹⁸.

Blood collecting from heart aorta valve is to obtain an optimum blood sample. ALT and AST level were parameters for reviewing liver function. ALT level of male group dose 1000 mg/kg BW showed the lowest level. For male normal group showed no significant difference with other groups. The liver disorder can be seen when ALT and AST level is increasing. ALT is an enzyme which can be found only in the cytoplasm, while AST in mitochondria and cytoplasm. ALT level can be used to determine liver and heart damage. AST values on chronic hepatotoxic conditions can be increased 5 to 10 times the normal value¹⁹. ALT and AST level of single administered *Peperomia pellucida* extract showed no differences between normal group and dose groups. This results showed that there is no liver damage and heart damage of oral administered *Peperomia pellucida* extract dose less than 4000 mg/kg BW.

Histopathology as a microscopic analysis for tissue organ support blood chemistry analysis. From histopathology Figure 1 and 2, showed that no differences between normal groups and dose groups in liver and heart tissue. Color difference in organ slide due to the ability of the tissue to stained¹⁶.

Based on blood and tissue data, we can conclude that *Peperomia pellucida* extract does not cause damage to liver and heart at doses less than 4000 mg/kg BW. Further study needed to determine the compounds contained in *Peperomia pellucida* extract and further analysis for blood biochemistry parameters.

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