

THE EFFECT OF *AMORPHOPHALLUS MUELLERI* BLUME AND *MORINGA OLEIFERA L* LEAVES ON BODY WEIGHT, FEED INTAKE, AND HEPATIC HISTOPATHOLOGY IN MICE

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ABSTRACT

Objective: This study aims to determine the effect of *Amorphophallus muelleri* Blume and *Moringa oleifera L* leaf on body weight, food intake, and hepatic histopathology in mice.

Methods: The mice were divided into five groups according to their diet, which includes *porang*, wheat, *porang-moringa*, wheat-moringa, and control diet. Each group consists of 5 males and 5 females, which were fed for 28 d, and then analyzed for their body weight, total food intake, alanine aminotransferase (ALT) as well as aspartate aminotransferase (AST) levels in plasma, and hepatic histopathology.

Results: The result showed that the group of *porang* and *porang-moringa* has lower body weight and feed intake, which is significantly different compared to the others. Furthermore, an increase was observed on plasma AST/ALT activities in 30% *porang* and 20% *porang-moringa* group. Also, one of the mice of *porang* group has inflammatory cell infiltration (++) on histopathology results.

Conclusion: It was therefore concluded that feeding containing *porang* causes low food consumption. Furthermore, weight loss increases AST/ALT and leukocyte infiltration even though a mouse consistently deteriorates.

Keywords: *Amorphophallus muelleri* Blume, *Moringa oleifera L*, Hepatic histopathology, Functional food

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INTRODUCTION

Indonesia's wealth is very diverse, therefore nutrition and safety, need to be taken into consideration when developing plants into foodstuff to ensure that they are non-toxic. *Porang* tuber (*Amorphophallus muelleri* Blume) is a source of carbohydrate containing glucomannan, which is easy to digest and has a low glycemic index [1]. Also, *moringa oleifera L* leaves contain nutrients that are essential for the body and therefore possess good prospects for food [2].

About 43.99% glucomannan, which is a specific carbohydrate in the form of processed flour is found in *porang* tubers [3]. This flour is used as an alternative food ingredient of carbohydrates since it prevent the absorption of blood glucose and increases its levels, therefore it is suitable for diabetics [4].

Previous studies on the combination of *porang* tuber flour and *Moringa* leaf extract showed the effectiveness of the glycemic index, triglycerides, and cholesterol in the experimental animals [5], therefore it is proposed as a functional food.

As a functional food ingredient, its safety needs to be tested, *porang* flour in particular contains calcium oxalate which is toxic to the body when consumed in large quantities [6]. Consequently, it is necessary to conduct safety testing with the experimental animals for 28 d by administering repeated doses orally [7].

Furthermore, observations were made on the body weight, total feed intake, increase in AST and ALT, and the liver histopathology of mice, which were given treatment with a single feeding of *porang* flour, as well as combination with *moringa* leaves for 28 d. This study aims to determine the toxicity and effect of single feed with *porang* flour as well as a combination with *moringa* leaves on the body weight parameters, total feed intake, liver function, and organs of the mice.

MATERIALS AND METHODS

Samples

The materials include dry slices of *porang* tubers (obtained from farmers in the Silo Sanen Jember plantation area, East Java), *Moringa*

leaf powder (obtained from the Jatikuwung Kelor plantation, Karanganyar, Central Java). The tubers and leaves were determined by Clara Erlin Zuliarty as Botanist at the Botanical Research and Development Center "Herbarium Bogoriense", Center for Biological Research, LIPI, Bogor, West Java, Indonesia, (No: 782/IPH.1.01/If.07/IV/2018).

Chemical and reagent

The reagents used include Aspartate transaminase (AST) and Alanine transaminase (ALT) were purchased from Biolabo (Maize, France). 10% neutral buffered formalin (NBF) as a fixative was purchased from Merck KGaA (Darmstadt, Germany). The chemical for histological preparations: Haematoxylin Eosin staining (physiological 0.9% NaCl, alcohol, xylol, liquid paraffin, hematoxylin solution, eosin, adhesive media, and distilled water) were purchased from Merck KGaA (Darmstadt, Germany). Standard food Broiler Finisher (BR2) was purchased from JAPFA Comfeed (Jakarta, Indonesia) contain rough protein (19-20%), fat (5%), rough fiber (5%), ash (7%), water content (12%), calcium (0.8-1.1%), phosphor (0.45%), and ME (3050-3150 Kcal/kg). Wheat flour, meat oil and fish flour were purchased from the Giant Supermarket (Jakarta, Indonesia).

Apparatus

The tools used are maintenance equipment for mice, animal and analytical scales, capillary and eppendorf tubes, surgical tools (knives, scissors, tweezers), cotton, glass, 300 microlab, and centrifuge devices. Also, the equipment used for making histological preparations with Eosin Hematoxylin staining include glass beaker, slide and cover glass, embedding tissue console, microtome, water bath, incubator, staining jar, timer, and light microscope equipped with a camera (Nikon Eclipse 80i DS Fi1, Japan).

Feed preparation

5 types of mice feed were prepared, these include *porang* (P) and wheat flour (T), combination of *porang* and *moringa* (PK), wheat flour and *moringa* (TK), and BR2 (table 1).

Table 1: Feed composition calculation

Material	Feed group composition (%)				
	P	T	PK	TK	BR2
Porang flour	60	-	30	-	-
Wheat flour	-	60	-	30	-
Moringa Leaf Powder	-	-	30	30	-
Meat Oil	5	5	5	5	-
Fish flour	20	20	20	20	-
Standard Feed BR2	15	15	15	15	100

Note: P= Porang,T= wheat flour, PK=combination of porang and moringa, TK=combination of wheat flour and moringa, and BR2=standard food as control

The composition of each feed is mixed homogeneously and placed in a pellet molding machine, and then cooled for 1 h. This production takes place at the Feed Industry Laboratory, Department of Animal Nutrition and Feed Technology, Faculty of Animal Husbandry, Bogor Agricultural University.

Preparation of experimental animals

The experimental animal (*Mus musculus*) ddY were obtained from the Faculty of Animal Husbandry, Universitas IPB, Bogor, West Java, Indonesia. The experimental animals used were healthy male and female ddY mice aged 6-8 w. They were divided into 5 groups of feed, each consisting of 5 males and 5 females, therefore there are 50 mice in total [8]. The experimental animals that fulfilled the criteria are acclimatized for 7 d at the Laboratory of Pharmacology and Toxicology, Faculty of Pharmacy, Pancasila University. They were weighed before and after the acclimatization [7]. Ethical permission to conduct the study was approved by the Health Research Ethics Committee, Universitas Pembangunan Nasional Veteran, Jakarta, Indonesia (No. 2781/IX/2020/KEPK).

Feeding treatment

The feed was given as much as 5 grams per day for 28 d and the remainder was weighed to calculate the amount of feed consumed. Furthermore, the mice were weighed once a week.

Blood draw

A blood sample was drawn on the last day and the plasma was taken for AST and ALT measurements.

Assignment of ALT and AST levels in plasma

The compositions for AST measurements are Ethylenediaminetetraacetic acid (EDTA), 2-Oxoglutarate, L-Asparate, malate dehydrogenase (MDH), lactate dehydrogenase (LDH), nicotinamide adenine dinucleotide (NAD)+hydrogen (H) (NADH), Tris Buffer, temperature at 30 ° C, and preservative. Also, preparation of 1 ml reagent plus 100 µl of the specimen (plasma) is homogenized for 1 minute, then measured at λ 340 mm. Furthermore, the compositions for ALT measurement are 2-Oxoglutarate, L-Alanine, LDH, NADH, Tris Buffer, pH at 30 ° C, and preservative with the same preparation as AST.

Liver organ taking and anatomical observation

The organs were washed with physiological NaCl solution, then macroscopic observations were carried out to determine the color and weight measurements of the organs. Subsequently, it was added to a 10% NBF fixative for histological preparations.

Liver histopathological preparations

The fixed organ tissue is dehydrated with a stratified concentration of alcohol, therefore it no longer contains water. Furthermore, it was purified 3 times with xylol I, II, and III, each for 1 h, afterwards, embedding and blocking were carried out.

The paraffin blocks tissue is made, therefore it is sliced into thin using a Rotary Microtom with a thickness of 4 microns. The slices were placed on a glass object and observed under a microscope, then dried with a hot plate at 37 °-40 ° C and labeled. Furthermore, the preparations are stored and incubated in an oven for at least 24 h.

The staining begins with defarization by immersing the preparation in xylol 3 times to ensure that the paraffin dissolves in the tissue. Furthermore, rehydration was conducted to return water to the tissue using alcohol with a grade of 100% to 70%, it was then rinsed with normal and distilled water. Subsequently, it was dripped with dye and covered with a glass lid and then labeled. Afterwards, the preparations were observed under a microscope for diagnosis and image capture.

Data analysis

The data on the difference in body weight of mice, the amount of feed consumption, AST and, ALT were tested for statistical results. Descriptive observations and semi-quantitative analyses were conducted on liver tissue by comparing mice in the treatment and standard groups.

RESULTS AND DISCUSSION

This study proves the presence or absence of unwanted effects from feeding porang flour and Moringa leaf powder. One of which is due to the calcium oxalate content in porang flour. Reference was made to the methods listed in the Head of Indonesian Food and Drug Administration Regulation No. 7 of 2014 on Guidelines for *In vivo* Non-clinical Toxicity Test.

Evaluation of mice body weight

This evaluation determines the increase or decrease in the body weight of mice during different feeding treatments (table 2). Furthermore, weight loss is the initial factor indicating a toxic effect from the given test material, as well as with other parameters. Based on the results, mice given porang feed had a lower body weight compared to mice given flour and standard feed. This is due to the glucomannan contained in porang, which expands when mixed with water [8]. Therefore, glucomannan is absorbed by the body longer than glucose contained in flour, which results in a longer feeling of fullness in the experimental animals.

Table 2: Average weight difference (g) day 0 and day 28

Group	Weight (g) on day 0		Weight (g) on day 28		Difference in body weight (g)		Difference in body weight (g) per group
	Male	Female	Male	Female	Male	Female	
P	32.40±4.57	28.70±2.51	28.13±1.92	25.86±3.64	-4.27±4.10	-2.84±3.82	3.56±1.01
T	31.86±4.13	28.30±2.56	36.53±4.22	33.77±5.16	4.67±6.47	5.47±4.24	5.07±0.56
PK	27.12±1.44	28.20±3.36	24.50±3.96	25.30±2.69	-2.63±3.96	-2.90±2.91	-2.76±0.19
TK	28.00±2.55	27.50±1.27	33.12±3.14	28.92±2.99	5.12±3.58	1.4±2.59	3.27±1.61
BR	27.02±3.52	26.70±0.97	35.00±3.87	27.99±3.47	7.98±2.15	1.29±3.10	4.64±4.73

Note: The data were given in mean±SD, n=5; Note: P= Porang,T= wheat flour, PK=combination of porang and moringa, TK=combination of wheat flour and moringa, and BR2=standard food as control.

Evaluation of mice feed consumption

The group feed was given to experimental animals on a standard diet (table 3). The amount of feed containing porang was consumed less than the average amount of consumption. This is consistent with the explanation in point A where the weight of experimental

animals has decreased within 28 d. This is because the glucomannan in porang expands due to water absorption, which gives a feeling of fullness when consumed [9]. In general, the low feed consumption in porang and porang-moringa groups has no toxic effect on other parameters as tested, even in other studies.

Table 3: Average total of feed consumption

Group	Average amount of feed intake (g) at week 4		Average amount of feed intake (g) at week 4 per group
	Male	Female	
P	3.40±1.21	2.85±0.86	3.12±1.03
T	4.57±0.43	4.12±0.88	4.34±0.70
PK	2.58±0.55	2.87±0.56	2.86±0.62
TK	3.66±0.70	3.75±0.79	3.71±0.70
BR	4.63±0.50	4.43±0.40	4.53±0.44

Note: The data were given in mean±SD, n=5; P= Porang, T= wheat flour, PK=combination of porang and moringa, TK=combination of wheat flour and moringa, and BR2=standard food as control

Liver function parameters

Another toxicity assessment is by observing liver function through AST and ALT parameters. AST is an enzyme found in the heart,

liver, skeletal muscle, and kidneys. The presence of liver damage is interpreted by measuring AST levels in the blood [10] because an increased AST is a marker of disease. The result can be seen in table 4.

Table 4: Average levels of AST (Aspartate aminotransferase)

Group	AST (IU/l) Average per sex		AST (IU/l) Average per group
	Male	Female	
P	121.08±132.2	81.02±48.58	101.05±96.24
T	24.13±13.68	79.18±54.92	51.66±47.60
PK	2017.5±140.79	82.63±30.44	138.13±110.58
TK	55.83±18.04	50.63±30.61	53.23±23.85
BR	64.08±20.53	57.46±20.52	60.77±19.66

Note: The data were given in mean±SD, n=5; P= Porang, T= wheat flour, PK=combination of porang and moringa, TK=combination of wheat flour and moringa, and BR2=standard food as control

Furthermore, the combined feed obtained a higher average AST content compared to the standard BR2 group. Subsequently, some of the related individuals need to be confirmed on the ALT value.

ALT is a more specific parameter made in liver cells than others and an inflammation caused by viral hepatitis and bile duct disease result in its increase [11]. The average ALT levels in experimental animals are shown in table 5.

Table 5: Average levels of ALT (Alanine aminotransferase)

Group	ALT (IU/l) Average		ALT (IU/l) Average per group
	Male	Female	
P	70.95±61.41	40.05±37.14	55.5±50.54
T	22.86±9.91	22.98±2.57	22.92±6.82
PK	78.84±71.79	87.41±89.45	83.60±77.16
TK	23.49±9.64	26.47±12.68	24.98±10.73
BR	30.89±8.95	27.26±10.26	29.08±9.27

Note: The data were given in mean±SD, n=5; P= Porang, T= wheat flour, PK=combination of porang and moringa, TK=combination of wheat flour and moringa, and BR2=standard food as control

Based on AST and ALT data for the individual experimental animal, several mice consistently experienced an increase in AST and ALT

(table 6). This increase implies that these mice are indicated to have damaged liver function [12].

Table 6: Consistent mice recap increases AST and ALT levels

Group	Mice to-	AST (IU/l)	Normal Score	ALT (IU/l)	Normal Score	Heart Weight Ratio (%)
P	Male 1	104.9	41.11-80.43	62.3	19.81-38.35	5.18
	Male 4	352.3		178.95		4.42
	Female 1	165.5		106.2		4.98
PK	Male 4	389.7	180	3.35		
	Female 3	116.25	63.4	6.11		

Note: P= Porang; PK=combination of porang and moringa

Anatomy and histopathology examination

The results of liver histopathology were carried out descriptively on selected mice samples based on macroscopic observations of organs and abnormal liver ratios (table 7). Furthermore, a mononuclear inflammatory cell infiltration was found to have a score (+to+++). This research focuses on histopathology having a value greater than (+)

and in the group of female flour with an inflammation score (+++), the liver shows nodules of the bacteria which is a response to the infection (hepatitis). Furthermore, the table shows that 4 male porang groups had an inflammatory score (++), morphologically, the anatomy remains normal, however, AST and ALT were much higher (fig. 1 and fig. 2). Also, the Infiltration of inflammatory cells in each group is caused by the parent or broodstock of the test animals.

Table 7: Liver histopathological interpretation results

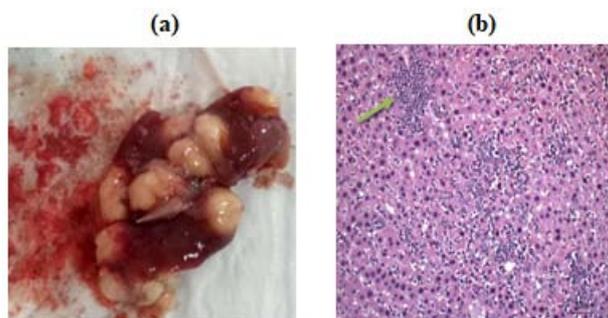
Group	Sex	Mice to-	Inflammatory cell infiltration
I	Male	1	(+)
		4	(++)
		5	(+)
	Female	2	(+)
		3	(+)
II	Male	4	(++)
		1	(+)
		3	(+)*
	Female	4	(+)
		2	(+)
III	Male	3	(+++)
		5	(+)
		1	(+)
	Female	2	(+)
		4	(+)
IV	Male	1	(+)
		3	(+)
		4	(+)
	Female	1	(+)
		2	(+)
V	Male	4	(+)
		1	(+)
		3	(+)*
	Female	4	(+)
		1	(+)
		3	(+)
		4	(+)

Description: (+) = A small number infiltration of mononuclear inflammatory cells in multifocal area of parenchyma; (++) = Moderate amounts Infiltration of mononuclear inflammatory cells in multifocal area of parenchyma; (+++) = Large number infiltration of mononuclear inflammatory cells in multifocal area of parenchyma; * = Degeneration and Hepatcellular Coagulent Necrosis.

The indicators of impaired liver function damage are seen from macroscopic, microscopic, biochemical, and clinical blood profiles. It appears that the tendency of feed containing porang causes the AST and ALT values in some mice to be higher above the normal range. This is evident in the existence of a mouse in the porang group showing increased AST, ALT, and liver inflammation scores (++) . Furthermore, in the group of macro-anatomic observation flour, there were 2 mice experiencing fat around the liver. Also, 3 mice had fat in the wheat-moringa group, while there was none for the porang and standard groups.

Furthermore, no mouse showed consistent toxic effects across all parameters, however, an increase in AST and ALT is not always accompanied by the abnormality of anatomical and histopathological macro (fig. 1 and 2).

In the fig. 1 can be seen that liver anatomy of the 3rd female wheat flour groups, fig. 1(a) shows many nodules on the surface of organ. Fig. 1(b) A large number infiltration of mononuclear inflammatory cells in multifocal parenchyma area (arrow) with a normal (++) AST/ALT level score.

**Fig. 1: Anatomy and histopathic morphology of of 3 female mice liver in wheat group. (a) organ and (b) parenchima cell**

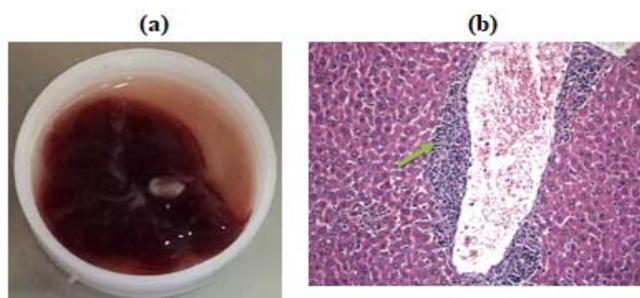


Fig. 2: Anatomical morphology and liver histopathic of male mice-4 *porang* group. (a) normal liver and (b) parenchyma cells

The liver anatomy of 4th male mice groups of *porang* can be seen in fig. 2. In the fig. 2(a) shows the normal liver anatomy. Fig. 2 (b) shown a large number infiltration of mononuclear inflammatory cells in multifocal parenchyma area (arrow) with a score (++), AST/ALT was significantly higher than the standard.

The relative weight of the liver

The relative weight of the liver is the ratio of its weight to that of the body. It is also one of the parameters used in determining the toxic level of the test substance. This is calculated by comparing the liver with the body weight of mice on day 29 (fig. 3).

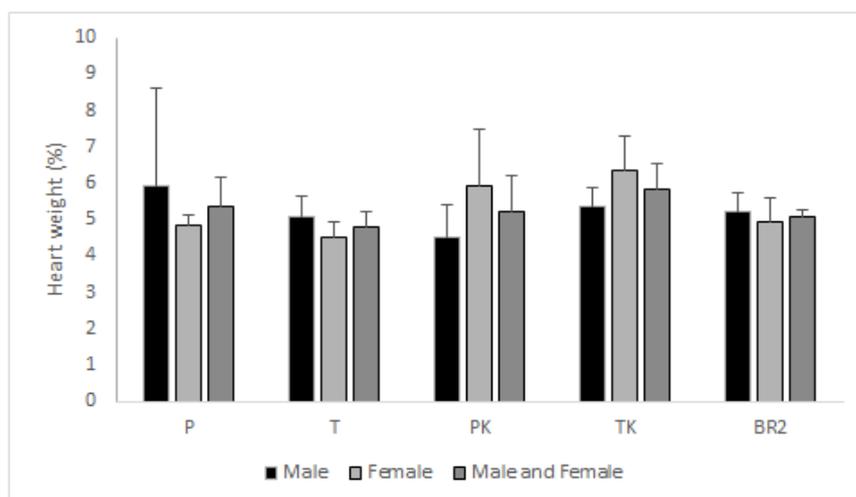


Fig. 3: Average relative weights of liver

Note: the data were given in mean+SD, n=5; P= Porang, T= wheat flour, PK=combination of porang and moringa, TK=combination of wheat flour and moringa, and BR2=standard food as control

The average relative weight of liver is higher in wheat-moringa group (5.85%) followed by *porang* (5.38%). Furthermore, some types of liver damage affect its relative weight, this includes inflammation which increases its value. This is because the liver is one of the important organs for detoxification and it is responsible for the biotransformation of harmful into harmless substances [13, 14].

The hepatotoxic effect of medicinal plant and supplement food must be our awareness. The Porang tuber (*Amorphophallus muelleri* Blume) is potential supplement food due to one of glucomannan source with a low digestive and glycemic index [15]. Also, *Moringa oleifera* L leaf is a valuable nutrient source that is important for good health, especially for diabetics [16, 17].

CONCLUSION

Based on the results, it was concluded that mice in the porang and porang-moringa feed groups experienced a decrease in body weight and the level of feed consumption was significantly lower than the standard group. A total of 30% of porang-feed and 20% porang-moringa experienced an increase in AST and ALT which is significantly different from the standard group. However, an increase in AST and ALT is not always accompanied by anatomical and histopathological macro abnormalities.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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