

ACUTE TOXICITY TEST OF LOW CALCIUM OXALATE PORANG (*Amorphophallus muelleri* BLUME) FLOUR

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ABSTRACT

A field experiment Porang (*Amorphophallus muelleri* Blume) has the potential to be developed as a functional food ingredient because it contains high levels of glucomannan. Research on the acute toxicity test of macerated porang flour has been carried out. The results of research showed a toxic effect which was characterized by high SGOT and sodium levels. The purpose of this study was to find out the safety level of consuming porang flour with lowered calcium oxalate content. This research was an experimental study designed in one directional-pattern Completely Randomized Design using 5 treatments of porang flour administration with doses of 0; 5; 50; 500; 5000 and 15000 mg/kgbw and 6 repetitions for 60 days using Wistar-strain white rats (*Rattus norvegicus*) as laboratory animals. The results showed that during the treatment, the administration up to a dose of 500 mg/kgbw did not give a significant difference to all observed variables. The administration of 5000 and 15000 mg/kgbw gave a significant difference on the changes in body weight, the addition of the amount of water drunk, the levels of Calcium, Potassium and Sodium in the blood, SGOT and SGPT values, and observation on necrotic cells in the kidneys. The administration of the highest porang flour dosage, namely 15000 mg/kgbw did not cause any rat mortality and did not cause any real cell damage to the liver, but caused hyperactive behavior in female rats.

Key words: acute test, porang flour, oxalate

دونووارتي وآخرون

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اختبار السمية الحادة لأكسالات البورانج منخفضة الكالسيوم (*Amorphophallus muelleri* BLUME) الدقيق

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استاذ

يونين تا
استاذ

وايدجاناركو
استاذ

دونووارتي
باحث

المستخلص

تم إجراء بحث لنبات البورانج حول اختبار السمية الحادة لدقيق البورانج المتعفن. أظهرت نتائج البحث وجود تأثير سام يتميز بارتفاع مستويات SGOT والصوديوم. كان الغرض من هذه الدراسة هو معرفة مستوى الأمان لاستهلاك دقيق البورانج مع محتوى أكسالات الكالسيوم المنخفض. كان هذا البحث عبارة عن دراسة تجريبية مصممة بنمط اتجاهي واحد بتصميم عشوائي تمامًا باستخدام 5 معالجات من إدارة دقيق البورانج بجرعات 0 ؛ 5 ؛ 50 ؛ 500 ؛ 5000 و 15000 مجم / كجم من وزن الجسم و 6 مرات تكرار لمدة 60 يومًا باستخدام الفئران البيضاء كحيوانات مختبر. أظهرت النتائج أنه أثناء العلاج ، فإن الإغذاء حتى جرعة 500 مجم / كجم من وزن الجسم لا يعطي فرقاً معنوياً لجميع المتغيرات الملحوظة. أعطى إعطاء 5000 و 15000 ملجم / كجم من وزن الجسم فرقاً معنوياً في التغيرات في وزن الجسم ، وإضافة كمية الماء المشروب ، ومستويات الكالسيوم والبوتاسيوم والصوديوم في الدم ، وقيم SGOT و SGPT ، ومراقبة النخر. خلايا في الكلى. إن إعطاء أعلى جرعة من طحين البورانج ، وهي 15000 مجم / كجم من وزن الجسم ، لم يسبب أي وفيات للجرذان ولم يتسبب في أي ضرر حقيقي لخلايا الكبد ، ولكنه تسبب في سلوك مفرط النشاط في إناث الفئران.

كلمات مفتاحية : نبات البورانج و الصوديوم ، اكسالات الكالسيوم ، مستوى الامان .

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INTRODUCTION

Porang (*Amorphophallus muelleri* Blume) has the potential to be developed as a functional food ingredient because it contains high levels of glucomannan up to 67% (1). Glucomannan as fiber has the function of increasing the performance of the digestive system and immune system, reducing body weight, blood sugar and cholesterol levels (2). The main obstacle in using porang is its high calcium oxalate content (3), which can interfere with digestive and kidney health (4–6), namely about 61-65% in tubers and 31-35% in porang flour. However, many studies have been done to reduce calcium oxalate in porang flour (3).

Toxicity testing of a foodstuff is necessary for the safety of its consumption, especially foodstuffs that have benefits for human health in order to obtain a safe dose but optimal in its utilization. Research on the acute toxicity test of macerated porang flour has been carried out. The results of the 72-hour study showed that the administration of glucomannan flour did not show acute toxic potential in all groups, but had a significant effect on the level of potassium (7). The results of research showed that the administration of 250 and 1000 mg of porang flour for each kg body weight for 28 days has not had any toxic effect, but the administration of a dose of 4000 mg/kgbw for 28 days showed a toxic effect which was characterized by high SGOT and sodium levels (8). The purpose of this study was to find out the safety level of consuming porang flour with lowered calcium oxalate content.

MATERIALS AND METHODS

This study tested the toxicity level of porang flour with lowered oxalate content through gradual maceration. The research began with the making of flour from the porang tubers. Macerated porang flour was prepared according to the method of Faridah (3). The flour was made from chips of 1.2 kg porang tuber that were turned into flour using a stamp mill machine with a milling time of 17 hours 4 minutes 8 seconds and a speed of 19.23 mills. Then the flour was passed through a Retsch 5657's 30-mesh sieve and an air classifier made of PVC with a diameter of ± 7 cm and a blower. The remaining heavy fraction was then macerated with ethanol using the gradual

maceration method. As much as 25 g of porang flour from the heavy fraction was taken then macerated gradually with 40%, 60% and 80% ethanol, respectively, with stirring speed of 434.22 rpm for 4 hours 16 minutes with 233.77 mL of ethanol. The resulting flour dried and used in this study is known as low-oxalate porang flour. This research was an experimental study designed in a directional-pattern Completely Randomized Design using 5 (five) treatments and 6 (six) replications (9) and was conducted with the aim of finding out the effect of consuming macerated porang flour for 60 (sixty) days on the observed variables using Wistar-strain white rats (*Rattus norvegicus*) as laboratory animals. The animals were bred by Pharmacology Laboratory of the Faculty of Medicine, University of Brawijaya, Malang. As many as 30 male and 30 female rats aged 2-3 months old with 150-200 mg body weight were acclimated for one week and given standard feed and drinking water in *et libitum* way in order to adapt to their food and environment. Each rat was put into a colony cage, weighed by analytical scale. Rat that has been weighed was given a circle mark according to the serial number as identification of laboratory animal and Comfeed Pars standard feed which were carried out in the Pharmacology Laboratory of the Faculty of Medicine, University of Brawijaya, Malang-Indonesia. Porang flour was given by determining 6 (six) levels of porang flour dosage, namely 0; 5; 50; 500; 5000 and 15000 mg/kgbw on 6 (six) groups of white rats, in which each group consisting of 3 (three) male rats and 3 (three) female rats. The treatment of porang flour administration was done once and observations were made for the presence of dead rats. If there are no dead rats, the observation of the rat is continued for 6 (six) days. Observation and data collection were obtained by weighing the rats before, during the study and at the end of the study, recording the number of rat that died, changes in rat behavior, recording the amount of drinking and feed consumed by rats, faecal weight, analysis of blood electrolyte content at the end of the study and histopathological examination of the rats' kidneys. Calcium levels in the blood and urine of rats. The method of histopathological examination of

the rats' kidneys and livers was by terminating the rats then their kidneys and livers were taken and then fixated with 10% formalin buffer and processed according to the standard histological method with HE staining. Each preparation was observed under a microscope in 5 fields of view, namely at the four corners and the center of the preparation with magnifications of 100x and 400x. The target read was the lumen of the proximal tubule.

The results of each field of view were added up. The sum was representative of the value of proximal tubular damage for each rat. Observation on kidney and liver histopathology was also carried out through SEM.

RESULTS AND DISCUSSION

Porang flour that has been macerated has physico-chemical characteristics as presented in Table 1.

Table 1: Characteristics of Physical and Chemical Properties of Porang Flour After Maceration Treatment (Low Oxalate Porang Flour)

Parameters	Level (%)
Starch	1.32
Protein	1.05
Fat	0.84
Ash	2.26
Water	14.15
Ca-oxalate	0.09
Glucomannan	78.24
Viscosity	6300*
Total Fiber	8.9421
Dissolved fiber	1.8861
Insoluble fiber	7.0557
Starch digestibility index	42.8864
Protein digestibility index	70.4978
White degree	47.09**

Notes: * in c.Ps with a solution concentration of 1%; ** without unit, where the value 100 is assumed to be white

The 78.24% glucomannan content in porang flour is not much different when compared to commercial konjac flour which has a glucomannan content of up to 80%. The viscosity of glucomannan is 6300 cps which is much lower than commercial konjac flour which reaches 12000-14000 cps. This is influenced by several factors such as the particle size of porang flour which is still around 180–340 μm (10), while the size of commercial glucomannan flour granules has a smaller size ranging from 75.39–156.5 μm (11).

The oxalate content in porang flour in Table 1 is 0.09%, much lower than the oxalate content in fresh porang tubers that has not been processed, which is 65.65% (12). This result is reinforced by the results of the research by (13) which stated that washing porang flour with ethanol solution will be able to clean impurities that are on the surface of the glucomannan granule. The difference in the concentration of ethanol used aims to dissolve various polar and non-polar impurities.

Table 2: The Effect of Porang Flour Administration on the Mortality of Rats

Dosage (mg/kgbw)	The Number of Rat Mortality	
	Female	Male
0	0	0
5	0	0
50	0	0
500	0	0
5000	0	0
15000	0	0

The results of the observations in Table 2 show that there were no rats that died in the treatment of various doses of porang flour administration. Different levels of administration in each group of rats. According to (14), if there is no laboratory animal mortality for 24 hours, then porang flour is said to be safe for consumption. Observations were continued for 6 (six) days including rat behavior, body weight, feed weight, faecal weight, volume of drinking water, analysis of electrolyte levels (Ca, Na and K), SGOT and SGPT values in the blood and microscopic observations of the rats' liver and kidneys.

Behavior of Laboratory Rats for 6 (six) Observation Days

During the treatment, there were no dead rats, so the observation on the rats was continued for 6 days. Observation on the behavior of rats, both female and male, which was carried out on the observation of their response ability and liveliness in detail per day of observation as a whole is summarized in Table 3. The behavior of rat as laboratory animals was observed until the 6th day after treatment including their liveliness or movement and the speed of their response to external stimuli. Observation of this behavior was done descriptively and tabulated which would then be concluded descriptively as well.

Table 3: Effect of Porang Flour Administration on Rat Behavior

Dosage (mg/kgbw)	-th day of observation					
	1	2	3	4	5	6
0	N	N	N	N	N	N
5	AL	N	N	N	N	N
50	AL	AL	N	N	N	N
500	AL, RL	AL, RL	AL, RL	N	N	N
5000	AL, RL	AL, RL	AL, RL	27 N 9 RL	N (33) H (1J, 2B)	N (B), H (J)
15000	AL, RL	AL, RL	AL, RL	27 N 9 RL	N (1B) H (33)	N (J), H (B)

Notes: * The treatment was carried out on 6 groups of Wistar rats consisting of 3 males and 3 females, so that the total laboratory rats were 36

N (normal); AL (weak activity); RL (weak response); H (hyperactive); B (female); and J (male), the accompanying numbers indicate the number of rats.

Tabulation of descriptive behavior observation data is presented in Table 3. It can be seen that the rats that were fed porang flour with a dose of 0; 5 and 50 mg/kgbw behaved normally until the 6th observation day after treatment. On the other hand, the administration of 500 mg/kgbw made the rats looked weak, both in terms of their liveliness and response to stimuli, but gradually normalized on the 2nd day of observation and so on until the 6th day after treatment. At doses of 5000 and 15000 mg/kgbw, the rats showed different behavior compared to rats fed porang flour at a lower dose. On the rats with these two treatments showed a weak liveliness and response from the first day to the sixth day of observation, but at the end of the observation (day 6) showed hyperactive behavior characterized by more movement or activity than the others. Rats that experienced hyperactivity on the 6th day of observation, namely the treatment of 5000 mg/kgbw, were all male rats, while for

the administration of 15000 mg/kgbw were all female rats.

The Weight of Laboratory Rats

The body weight of laboratory rats was weighed before treatment, which was considered as day 0, and after being treated according to the treatments, the next day was weighed as day 1 and so on until day 6 after treatment. The results of the observation on changes in body weight of rat during observation are shown in Figure 1 for male rats and Figure 2 for female rats. It was found that the body weight of all rats treated at various treatment doses experienced changes in body weight. The results of statistical analysis of changes in body weight during treatment showed a difference on each observation day, meaning that the treated rats experienced a significant change in body weight during the observation time for each treatment, both for the male and female rats.

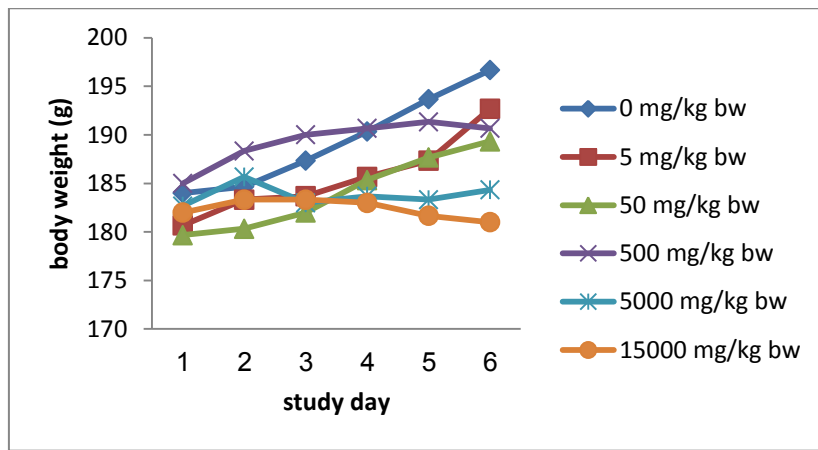


Fig. 1. Average body weight of female rats

Observations on body weight of rats are presented in Figure 20 for female rats and Figure 21 for male rats. It shows that, for both female and male rats, there are differences in body weight changes during observation. Body weight of both female and male rats has increased during observation after 0; 5; 50 and 500 mg administration of porang flour per kg body weight of rat, but not with the administration of 5000 and 15000 mg of porang flour, which actually showed a decrease in body weight.

Observation on the Volume of Water Drunk during Treatment:

The volume of water drunk by laboratory rats was measured based on the amount of water reduction observed every day at the same time until day 6 after treatment. When the treatment was considered as day 0, and after being treated according to treatment, the next day was considered as day 1 (first) and so on until day 6 (six) after treatment.

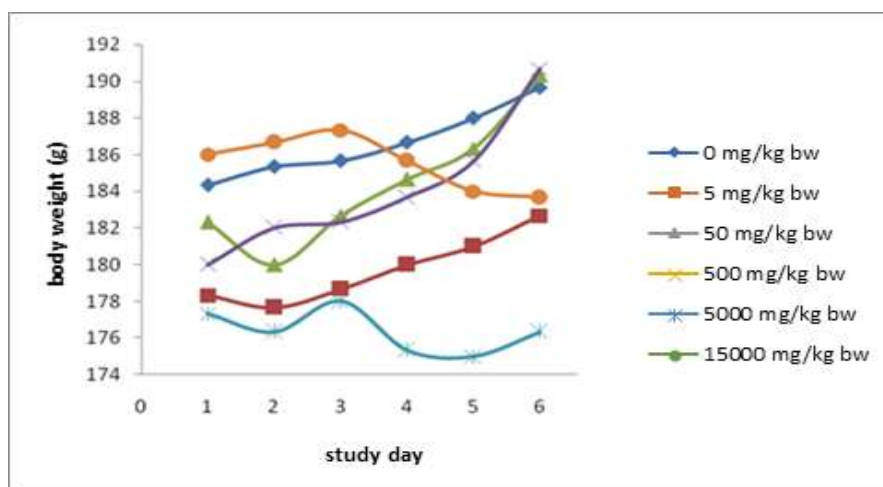


Fig. 2. Average body weight of male rats

The results of observation on water drinking volume in rats during observation period are shown in Figure 3 for female rats and Figure 4 for male rats. Obtained data that all rats treated with a dose of 0; 5; 50 and 500 mg/kgbw showed no different trends in drinking volume patterns. The difference in the volume of drinking water consumed was only seen in the 5000 mg/kgbw treatment and was very significant in the rats treated with 15000 mg/kgbw dosage. The drinking water volume of male and female rats tended to increase

after 5000 mg/kgbw treatment on the 1st to 3rd day of observation, but tended to decrease and was the same as the other treated rats after the 3rd day after treatment. Likewise, in the treatment of 15000 mg of porang flour for each kg body weight, the volume of drinking water for both types of rats decreased significantly after a very high increase on day 1 to 3 after treatment and after day 4 it decreased until the amount was the same as the others.

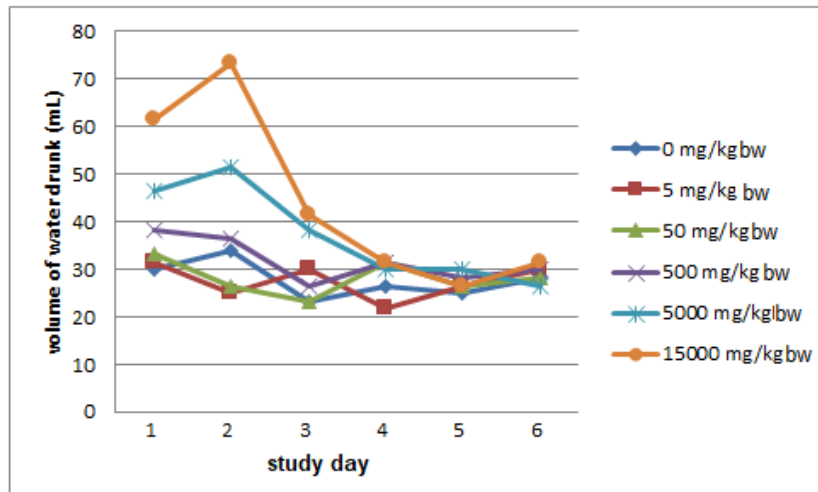


Fig. 3. Average Volume of Water Drunk by Female Rats

From the data obtained, it can be seen that the treatment of porang flour with a dose of 5000 mg/kgbw and 15000 mg/kgbw has a significant effect on the observed parameters. In the case of the volume of drinking water

and the weight of feed consumed, it gave an inversely proportional phenomenon in the treatment of 5000 and 15000 mg/kgbw, while in other treatments the values tended to be constant.

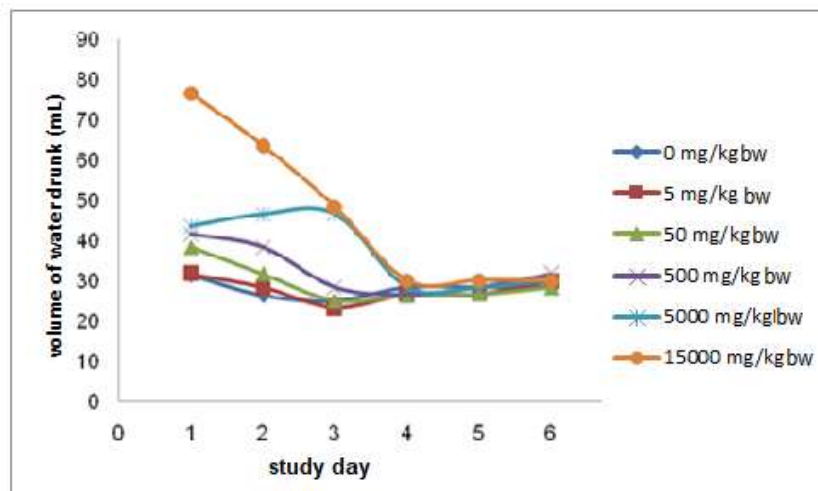


Fig. 4. Average Volume of Water Drunk by Male Rats

This can illustrate that the administration of porang flour at doses of 5000 and 15000 mg/kgbw when consumed can affect digestion leading to gastric fullness. Glucomannan has high viscosity properties, as evidenced by the behavior of laboratory animals that consumed high amount of drinking water but on the other hand, the feed consumed had a low weight value. As a result of this phenomenon, it is shown in the changes in body weight in rats treated as above, namely the increase in body weight was smaller and there was even a tendency to decrease, namely in rats treated with 15000 mg/kgbw, both female and male rats.

Observation on the Weight of Feed Consumed during the Treatment:

Observation on the weight of feed consumed

every day was carried out on the group of female rats and male rats by weighing the remaining feed every day which was carried out in the morning. In addition, the weight of the feed consumed was also calculated by reducing the weight of feed that has been given the day before, in which the initial weight has been known, with the weight of the remaining feed. The results of statistical analysis showed that changes in feed weight during treatment showed a significant difference in each observation day due to treatment. It means that there is at least one treatment with porang flour which caused the amount of feed consumed by rats to be significantly different from the rat group due to other treatments.

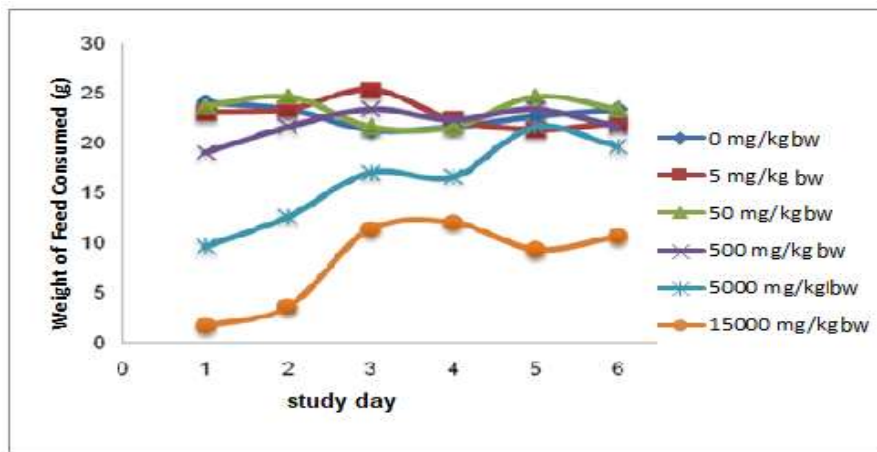


Fig. 5. Average Weight of Feed Consumed by Female Rats

Observations on feed weight of laboratory rats are presented in Figure 5 for female rats and Figure 6 for male rats. It can be seen that there is no difference between female and male rats in terms of the weight of feed consumed during the observation of the treatments with 0; 5; 50 and 500 mg/kgbw. However, this is not the case with the other two treatments, in the treatment of porang flour administration as much as 5000 and 15000 mg/kgbw. It can be seen that the amount of feed consumed was much less. This was because they have consumed large amounts of porang so that the

feed was not eaten. The feed weight of both female and male rats was seen to be constant or did not change during the observation on the administration of porang flour as much as 0, 5, 50 and 500 mg/kgbw. In the administration of porang flour of 5000 and 15000 mg/kgbw, the weight of the rats' feed was not constant. The feed weight of male and female rats tended to decrease after 5000 mg/kgbw treatment on the 1st day of observation, but tended to increase on the 3rd day of observation after treatment.

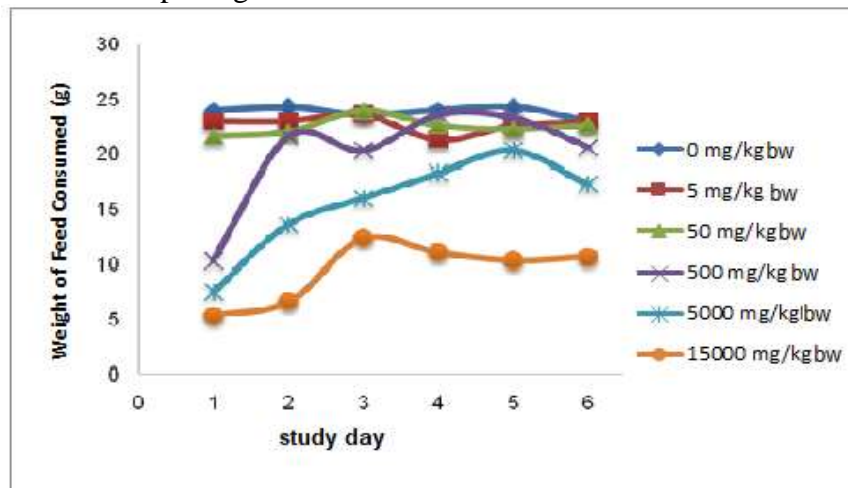


Fig. 6. Average Weight of Feed Consumed by Male Rats

In the treatment of 15000 mg/kgbw of porang flour administration, the feed weight of the two group of rats was significantly different from the other treatments and remained low until the last day of observation, namely the 6th day after treatment. The weight of rat feed between female and male rats was not significantly different during the observation period. The administration of porang flour which contains toxic substances, weight loss and feed intake were caused by several factors.

Sonde treatment is one of the factors that is thought to cause the rat's appetite to decrease. Based on the research of (15) about the in vivo testing on the hypocholesterolemic effects of porang-based green tea jelly, it showed that rat feed intake on the 7th-28th day continued to decline due to sonde treatment. Sonde caused discomfort in the mouth and throat of the rats, resulting in a low total feed intake. Another possibility is that the smell of porang caused the rats to experience a decrease in appetite.

According to (16), coarse porang flour has a distinctive aroma like fish. In addition, the stressed condition of the rats can cause their appetite and body weight to decrease. Stress conditions in rats arose after oral administration of porang flour. This treatment is thought to have been caused by the rat's stomach being forced to receive large amounts of food in an instant so that the rat's stomach became full in a short time. In studies concerning the toxicity testing of ethylene, continuous administration of high doses could have an impact on deteriorating renal tubular function. Kaya (16) added that kidney damage results in the body being unable to excrete substances that are not needed by the body and metabolic waste in the body. As a result, these substances will become toxic in the body. The longer and more these substances accumulate in the body, it can lead to a lack of appetite as well as kidney failure and heart failure in severe cases. Another factor that can cause behavioral changes in rat is the presence of toxic hepatitis. According to (17–20), toxic hepatitis arises from continuous exposure to toxic substances, causing inflammation of the liver. Toxic hepatitis in a severe condition can cause death.

Electrolyte Levels in Blood

The electrolyte elements in the body reflect a state of good balance in the body. A balanced proportion of electrolytes can lead to good health. If the proportions are not balanced, it is certain that there has been a disruption in the metabolism in the body. In toxicity testing, data on electrolyte levels of a research object is needed, so that the effect of the substance being tested can be known whether it has reached the level of disturbing metabolic activity (physiological) or not. This can provide an idea of the level of toxicity. In the toxicity test of porang flour, the levels of electrolytes, namely sodium (Na), potassium (K), chlorine (Cl) and calcium (Ca) were observed in the blood of rats after the administration of porang flour according to the treatment. Blood was analyzed, namely 6 (six) days after treatment. K element is an essential mineral that assists the kidneys in carrying out physiological functions. K is also an electrolyte that plays an important role in

maintaining the functions of heart, skeletal muscle and smooth muscle contraction for digestive function and movement. According to (21), K level in the blood of normal rat is 3.6 - 6 mEq/L. Hyperkalemia (high K level in blood) is a condition in which the blood potassium concentration is more than normal (for rat is 3.6–6 mEq/L). According to (22) and (8), hyperkalemia occurs when the kidneys are unable to excrete K properly. The most common cause of hyperkalemia is the use of substances (drugs or food) that block the kidney's disposal of K. According to (23), damaged kidneys with a decreased glomerular filtration rate are unable to excrete cellular K into body fluids, causing hyperkalemia. The results of the analysis of K and electrolyte levels in male and female rats are shown in Figure 7. It can be seen that the blood electrolyte levels measured on the seventh day after treatment showed an increase in line with the increasing dose of porang flour, especially the K electrolyte element, the administration of porang flour with a dose of 15000 mg/kgbw gave a significant and highest increase. Figure 7 shows the levels of Na, Cl, K and Ca that were analyzed on the seventh day after the administration of porang flour according to the treatment. On the seventh day after the administration of porang flour, the treatments with various doses did not cause the sodium electrolyte levels in the blood of female and male rats to be significantly different, except for the 15000 mg/kgbw treatment which caused the blood Na levels of rats to be significantly higher than other treatments. But overall, the same treatment did not cause significant differences between male and female rats. K levels measured on the seventh day after the administration of porang flour (Figure 7) showed that the various doses of treatment did not cause the K electrolyte levels in the blood of female and male rats to be significantly different, except for the treatment of 15000 mg/kgbw. At this dose, the blood K levels of male rats were significantly higher than other treatments. Overall, the same treatment did not cause significant differences between male and female rats, except at a dose of 15000 mg/kgbw.

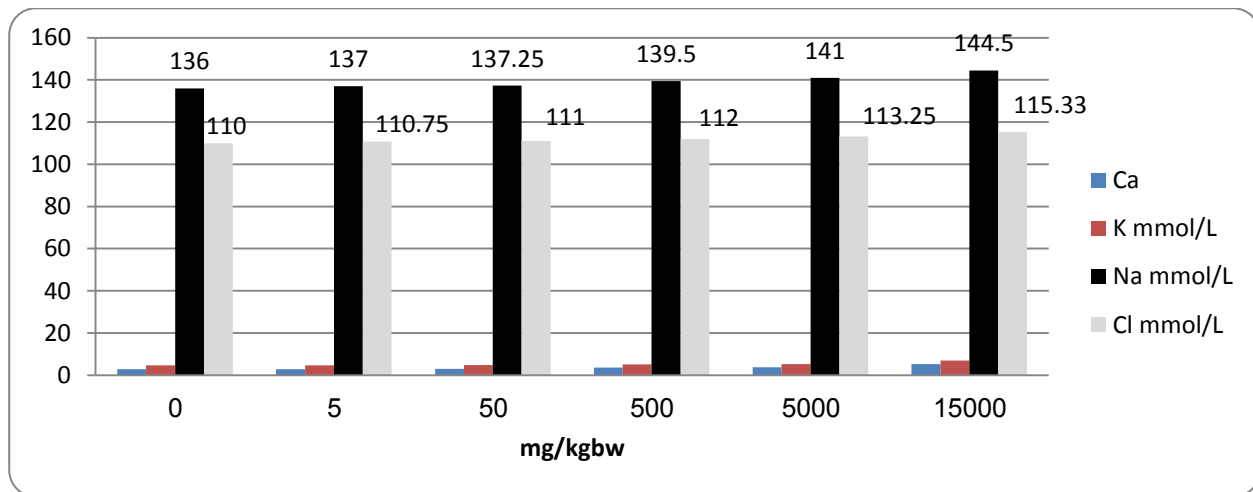


Fig. 7. Average Blood Electrolyte Levels of Rats after Porang Glucomannan Flour Administration According to the Treatment

Cl levels measured on the seventh day after the administration of porang flour, treatment with various doses did not cause the Cl electrolyte levels in the blood of female and male rats to be significantly different, except for the treatment of 15000 mg/kgbw that caused the blood Cl levels of male and female rats to be significantly higher than the other treatments, but overall, the same treatment did not cause significant differences between male and female rats. Ca levels measured on the seventh day after the administration of porang flour, treatment with various doses did not cause the Ca electrolyte levels in the blood of female and male rats to be significantly different, except for the treatment of 15000 mg/kgbw which caused the blood Ca levels of male and female rats to be significantly higher than the other treatments, but overall, the same treatment did not cause significant differences between male and female rats. When the Na levels in the extracellular fluid are high, the intracellular fluid (including K) is drawn out to normalize it. The increased extracellular fluid volume causes an increase in blood volume. The high volume of blood can result in high blood pressure and the heart has to work harder to pump blood (24). Exposure to toxic substances causes loss of volume regulation in cell parts. In order to maintain stability, cells must release metabolic energy to pump sodium ions out of the membrane and potassium ions into the membrane (25,26). If the cell membrane is damaged by toxic substances (microscopic calcium oxalate in porang flour and needle-shaped glucomannan flour which can injure the cell membrane) then the cell is

unable to pump sodium ions properly (27). This is what causes an increase in sodium concentration due to the accumulation of intercellular fluid and water influx into the extracellular region (28). Electrolytes and body fluids are needed by the body in the process of metabolism and fluid balance and electrolyte levels will affect physiological processes. In this toxicity study, the treatments did not have a significant effect on electrolyte levels in all groups of rats, both between treatments and between groups of male and female rats in the same treatment, except for the treatment of 15000 mg/kgbw of porang flour. In this treatment, the administration of porang flour at a dose of 15000 mg/kgbw has a significant effect on the levels of Ca, Na and K. This shows that simultaneous administration of porang flour at a dose of 15000 mg/kgbw will significantly increase the body's electrolyte elements, especially for Ca, K and Na which is likely to interfere with the body's physiological processes.

SGOT and SGPT Levels in the Blood

SGOT stands for Serum Glutamic Oxaloacetic Transaminase and SGPT is Serum Glutamic Pyruvic Transaminase. SGOT and SGPT are enzymes produced in the liver. Under normal circumstances, these enzymes stay in the liver cells, but if there is inflammation, the liver will release both enzymes in the blood. If the SGOT and SGPT in the blood are excessive, the blood test results will show the SGOT and SGPT values above the normal threshold (16). The research phase of this toxicity test, also analyzed the levels of SGOT and SGPT on the 6th (sixth) day after treatment. The function of

knowing the levels of SGOT and SGPT in blood is to find out the level of toxicity of a compound by knowing the degree of liver performance to the compound being tested. Figure 27 shows the SGOT and SGPT values taken from the average of the whole rats, both female and male. In Figure 8, it can be seen that the SGOT and SGPT levels have increased along with the increasing doses of porang flour, especially the SGOT value. Table 3 shows that the treatment with a dose

of 0; 5; 50 and 500 mg/kgbw did not give a significant difference in the SGOT value, but the treatment of 5000 mg/kgbw and 15000 mg/kgbw gave significantly different effects on the SGOT value, both on female and male rats. Different conditions on the SGPT value, all treatments did not have a significant effect on the SGPT value on all types of rats. Overall, all treatments did not have a significant effect on male and female rats in the same treatment (Figure 8).

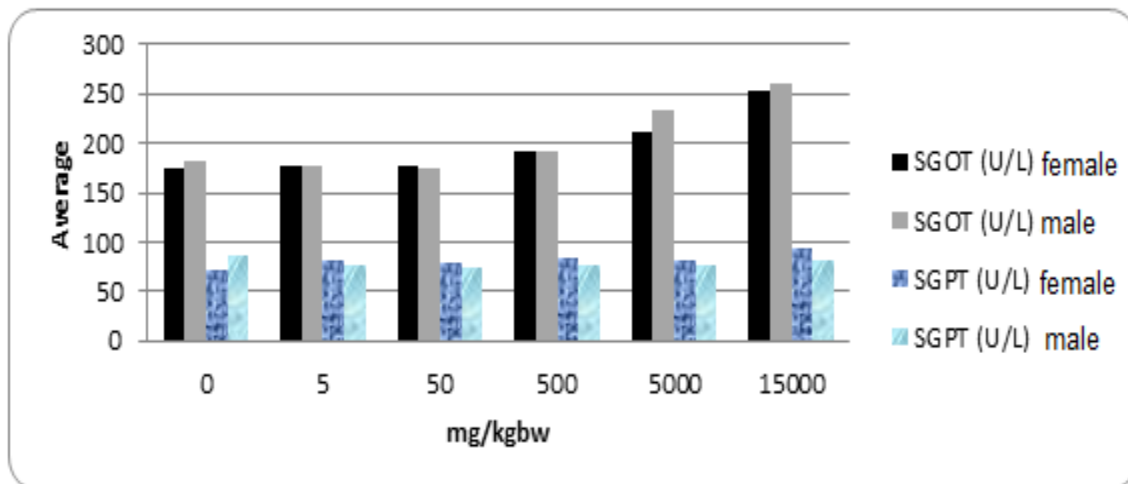


Fig. 8. Average SGPT and SGOT levels in the blood of male and female rats

In this toxicity study, the administration of porang flour at a dose of 0; 5; 50 and 500 mg/kgbw did not significantly affect the increase in SGOT and SGPT levels in all groups of treated rats, the treatment with a dose of 5000 mg/kgbw had a significant effect on increasing SGOT value, in both male and female rats, but not in terms of SGPT levels, the treatment of 5000 mg/kgbw did not have a significant effect on SGPT levels. In contrast, the treatment of 15000 mg/kgbw gave a significant increase in SGOT and SGPT levels, both in female and male rats. From this acute toxicity study, it can be concluded that the treatment of porang flour with a dose of 5000 mg/kgbw and 15000 mg/kgbw given at the same time can cause significant difference in blood electrolyte levels. Low weight gain and an increase in SGOT and SGPT which can indicate toxicity by glucomannan.

Microscopic Observations of the Liver

Organ:

The liver is the largest and most metabolically complex organ in the body. The liver is

involved in the metabolism of nutrients as well as most drugs and toxins. Structurally, the liver is composed of hepatocytes (liver parenchyma cells). Hepatocytes are responsible for the central role of the liver in metabolism (29,30). These cells are located between the sinusoids that fill with blood and the bile ducts. Kuffer cells cover the sinusoids of the liver and are an important part of the body's reticuloendothelial system. Blood is supplied through the portal vein and hepatic artery, and is channeled through the central vein and then the hepatic vein into the vena cava. The bile duct begins to act as tiny canaliculi formed by adjacent parenchyma cells. The canaliculi coalesce into the ductules, interlobular bile ducts, and the larger liver ducts (31–33). The main hepatic duct connects the cystic duct of the gallbladder and forms the common bile duct, which flows into the duodenum (34–38).

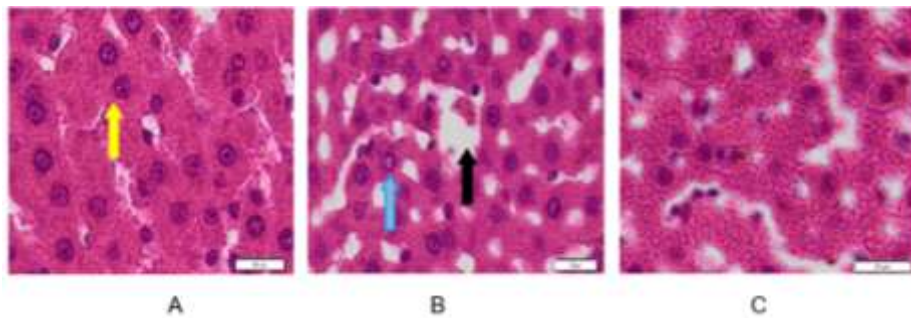


Fig. 9. Microscopic Observation on Laboratory Rats' Liver Organ after the Administration of Porang Flour according to Treatment

Notes : The Administration of Porang Flour as much as 0 mg/kgbw (A), The Administration of Porang Flour as much as 5000 mg/kgbw (B), The Administration of Porang Flour as much as 15000 mg/kgbw (C), yellow arrow = normal cells, blue arrow = necrotic cells and black arrow = central vein

Based on microscopic observations on liver cells close to the central vein and data on the number of necrotic cells per 100 hepatocyte cells was obtained, it was done 3 times (Figure 9). Necrosis is irreversible cell death that occurs due to long-term injury and the cells are

unable to repair themselves. Cells with necrosis are classified into three, namely karyorrhexis (broken cell nucleus), karyolysis (missing nucleus) and pyknosis (shrinking nucleus). Usually, necrosis is acute damage due to chemicals (39,40).

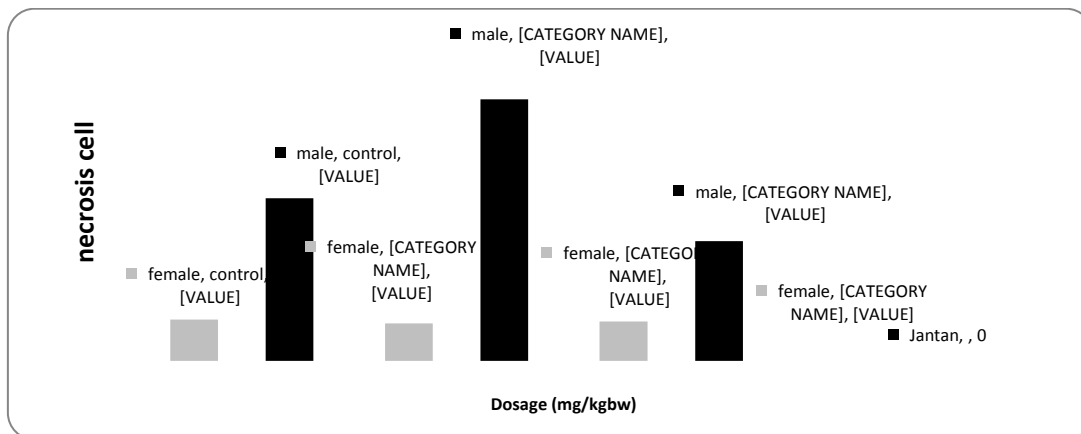


Fig. 10. Number of necrotic cells per 100 hepatocyte cells

The number of necrotic cells in female rats at a dose of 5000 and 15000 mg/kgbw is relatively the same as that of the control, which is about 0.3%. The number of necrotized hepatic cells in male rats at a dose of 15000 mg/kgbw was lower than the dose of 5000 mg/kgbw and control (Figure 10). The number of necrotic cells in female and male rats is relatively small, so it can be said that the use of porang flour up to a dose of 15000 mg/kgbw is safe for consumption.

Microscopic Observation of Kidney Organs

The kidneys are the main organ that plays a role in water and electrolyte homeostasis. The

kidneys are also the main organ that is exposed to toxic effects when the body is exposed to toxic substances. The main function of the kidneys is to remove metabolic waste, eliminate toxic substances, regulate salt fluid and acid-base balance, and regulate blood pressure (41,42). In addition, the kidneys function to concentrate the toxins on the filtrate and carry the toxins through the tubules. The kidneys also have a function as the release of waste products from normal metabolism and excrete xenobiotics and their metabolites (43,44).

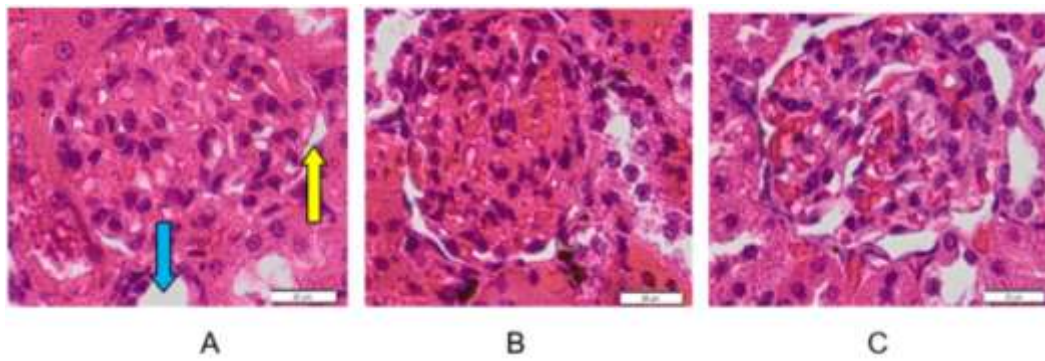


Fig. 11. Microscopic Observation on Laboratory Rat Kidney Organs due to the Administration of Porang Flour according to dosage

Notes: Administration of 0 mg of Porang Flour/KgBW (A), Administration of 5000 mg of Porang Flour/KgBW (B) Administration of 15000 mg of Porang Flour/KgBW (C)

The kidneys also have a function as endocrine organ that can produce erythropoietin, renin and prostaglandin hormones (45–47). The part of kidney which functions as a filter tool is the glomerulus which works based on hemodynamic and osmotic factors. The glomerulus is formed by a pile of capillary that pass through the arterioles afferens and flowed by the arterioles efferens (48). In normal conditions, the glomerulus cannot be passed by large molecular proteins, but in pathological conditions, these proteins can pass (49). In addition to reabsorbing function,

tubular cells also induce chemical substances such as iodine, ammonia and hippuric acid. In glomerular dysfunction, outside material arrives at the tubule at an abnormal rate through the Bowman's space. This will cause the tubular epithelial cells to degenerate and even die if too much materials have to be reabsorbed. Microscopic observation of the number of necrotic cells in the kidney was carried out by counting the necrotic cells around 6-12 distal tubules and proximal tubules with 3x repetitions (Figure 12).

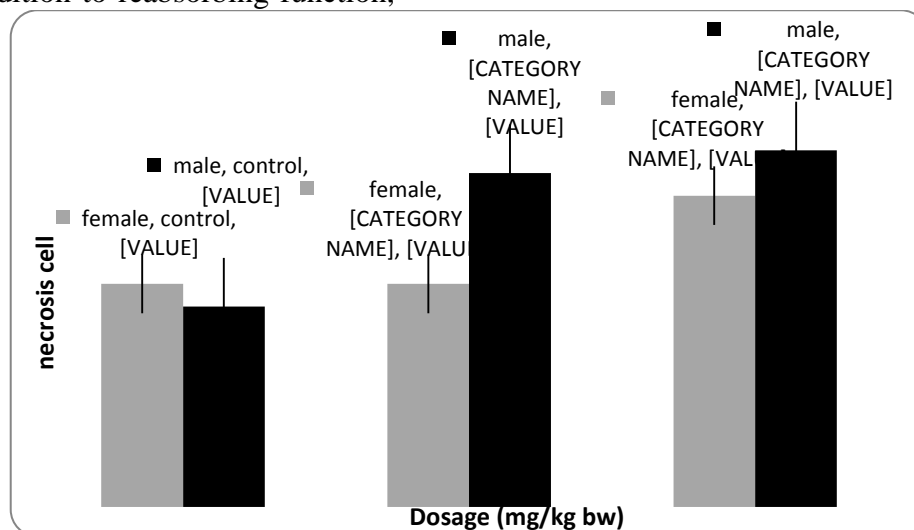


Fig. 12. The number of necrotic cells around 6-12 tubules of the kidney

Quantitatively, it can be seen that the number of cells experiencing necrosis in female and male rats is relatively similar against the control, which is about 1-3 cells per 6-12 tubules for doses of 5000 and 15000 mg/kgbw (Figure 12). This shows that at these dosages, porang flour is safe for consumption.

CONCLUSIONS

Based on the results, it can be conclude that (1) The administration of low-oxalate porang flour up to a dose of 500 mg/kgbw for 60 days

did not give a significant difference to all observed variables.; (2) The administration of low-oxalate porang flour at doses of 5000 and 15000 mg/kgbw for 60 days gave a significant difference to changes in body weight, increase in the amount of water drunk, levels of Calcium, Potassium and Sodium in the blood, SGOT and SGPT values, and observation on necrotic cells of the kidneys in all types of rats observed; (3) The administration of the highest dose of porang flour, 1500 mg/kgBW for 60

days did not cause any rat mortality and did not cause significant cell damage to the liver, but did cause hyperactive behavior on female rats

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