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Acute Toxicity Study of Porang (*Amorphophallus oncophyllus*) Flour Macerated with *Strobilanthes crispus* in Wistar Rats

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Running title

Acute Toxicity Study of Porang Macerated with *Strobilanthes crispus*

Abstract

The acute oral toxicity study of porang (*Amorphophallus oncophyllus*) macerated with *Strobilanthes crispus* based on renal and hepatic function such as urinary protein, SGOT, and SGPT levels of Wistar rat *(Rattus norvegicus*) has been investigated. An acute toxicity test was conducted based on the OECD 420 Fixed-Dose Procedure Guideline that consists of two test steps, preliminary and main tests. For the preliminary study, eleven rats were used. Each of them was represented as a treatment group based on the dose of oral feeding, i.e. control (distilled water treatment), NPF1 (natural porang flour), NPF2, NPF3, NPF4, NPF5, and SPF1 (*S. crispus*-macerated porang flour), SPF2, SPF3, SPF4, SPF5 for) for the dose of 50, 300, 2000, 5000 mg/kg BW, respectively. For the main study, twenty rats were divided into 4 groups, i.e. NPF6 (dose of 2000 mg/kg BW), NPF7, SPF6, ad SPF7. i.e. Levels of urinary protein and blood serum SGOT and SGPT levels were measured at 0, 24, and 72 after treatment. The study showed that the rats did not show any remarkable toxic signs or mortality in the acute toxicity study. It was forced by biochemical examination that found the urinary protein, SGOT, and SGPT levels increase, but they were still in normal ranges.

Keywords: *Amorphopahallus oncophyllus*, *Strobilanthes crispus*, acute toxicity, urinary protein, SGOT, SGPT

Introduction

Porang or iles-iles is included in the genus of *Amorphophallus*. These plants wildly grow in the jungle, under the bamboo tree, along the riverside, and mountain slope [1]. Porang tuber (*Amorphophallus oncophyllus*) contains glucomannan or known as mannan. Glucomannan is a water-dissolved food fiber that is low in calories and has a special function for diet, so that it has been widely used for food products, such as nuggets, noodles, ice cream, etc [2–4]. The food containing glucomannan reduced cholesterol level, blood sugar, and hypertension [5]. Its relatively low glycemic index, which was 20.6, could decrease blood sugar level and made this tuber was good to be consumed by diabetic patients [6].

Several studies had proven that there was a functional compound in glucomannan that affected several mechanisms in organism bodies. Haihong Chen in his study showed that konjac glucomannan as a nutraceutical can be used to boost the therapy of diabetes type 2 through lipid metabolism improvement [7], while Jialin Zheng reported that glucomannan worked in synergy with metformin to increase its hypoglycemia effect [8].

Even though it had been proven that there were many benefits of glucomannan, the utilization of porang tuber as the source of glucomannan is still low. This is caused by the homemade product of porang still has the weakness, that is itchy when consumed. This itchiness is caused by the content of oxalate [9–12]. Oxalate acid consumption in a high number increased blood creatinine and urea levels [9,13]. It also decreased the bioavailability of calcium inside the body, formed kidney stones, can cause corrosion of the mouth and gastrointestinal tract, kidney failure, and hematuria [14]. Various efforts had been carried out to reduce the level of calcium oxalate level in porang tuber, both mechanically using stamp mill and blower, ball mill, also chemically using NaCl, ethanol, and aluminum sulfate [11,12,15–17]. The research using natural or herbal ingredients in reducing oxalate calcium content is still limited. In a previous study, ethanol extract of *Strobilanthes crispus* leaves reduced the level of calcium oxalate in porang flour [18], but it still needs to be proved before consumption for its safety. Therefore, in vivo toxicity test is needed to be carried out [19].

A toxicity test is a set of analyses to detect the toxic effect of a substance on the biological system and to obtain typical dose-response data from the test preparation [20]. In addition, a toxicity test can be carried out to determine the toxic effects on the vital organs of the animal, such as the kidneys and liver. One of the kidney functions is to excrete foreign compounds such as drugs, food, pesticides, and other non-nutritional exogenous materials that enter the body [21]. The study of the level of porang toxicity was reported on porang tubers [22,23], but its combination with *S. crispus* needs to be studied further.

In this study, an acute toxicity test was conducted by observing the mortality rate (LD50) and the changes in behavior during 72 hours. To confirm the kidney and liver function, the urea protein tests, kidney histopathology, and biochemical tests of aminotransferase enzyme activity (SGOT and SGPT) were also done.

Materials and methods

Plant materials and sample preparation

Porang was purchased from the farmer in Madiun, East Java. It was directly processed into flour at the laboratory. Porang flour macerated with *S. crispus* was then produced based on the Patent Application No. S00202006668 [19].

Experimental animals

Non-pregnant female Wistar (*Rattus Norvegicus*) rats weighing 110-180 grams with the age of 8-12 weeks were used in this acute study. For the preliminary study, eleven rats were used. Each of them was represented as a treatment group based on the dose of oral feeding, i.e. control (distilled water treatment), NPF1 (natural porang flour), NPF2, NPF3, NPF4, NPF5, and SPF1 ((*S. crispus*-macerated porang flour), SPF2, SPF3, SPF4, SPF5 for) for the dose of 50, 300, 2000, 5000 mg/kg of bodyweight (BW), respectively. For the main study, twenty rats were divided into 4 groups, i.e. NPF6 (dose of 2000 mg/kg BW), NPF7, SPF6, ad SPF7.

An acute toxicity study was conducted based on the Organization of Economic Co-Operation and Development (OECD) Guideline 420 for testing chemicals. The procedures consisted of two steps, those were preliminary and main analysis. For preliminary analysis, the rats were orally administered with porang flour or *S. crispus*-macerated porang flour with doses of 5, 50, 300 mg/kg BW. If there was no mortality, the dose was increased up to 2000 mg/kg BW. For the main analysis, the doses used were 2000 mg/kg BW and 5000 mg/kg BW [24].

The acclimatization of animals was 5 days and fed with free access to standard laboratory diet and ad-libitum water. They were then fasted for 18 hours before administered with the flours. The mortality, any injury or illness, physical appearance, behavior changes (step backward, walk with the stomach,

sleepy), diarrhea were observed visually after the first 30 minutes and every 1 hour for 24 hours. If there was no sign of toxicity, the test was completed until 72 hours. The bodyweight of rats was monitored at the beginning and the end of the analysis. The urine and blood samples were collected before treatment, 24 hours and 72 hours after treatment for determining the urinary protein levels, SGOT (serum glutamic oxaloacetic transaminase), and SGPT (serum glutamic pyruvic transaminase) levels, respectively. At the end of the study, rats were euthanized by decapitation, the kidney organs were excised carefully, then preserved in 10% buffered formalin before the histopathological study.

Urinary protein levels were analyzed by the pyrogallol red-molybdate method. SGOT and SGPT were analyzed by Optimized UV-test according to IFCC (International Federation of. Clinical Chemistry and Laboratory Medicine) [20].

Statistical analysis

Data were performed by mean values, followed by statistical analysis used SPSS ver. 16. The differences between samples were tested by analysis of variance (ANOVA) with Duncan's multiple range test. Differences were significant when $p<0.05$.

Results and discussion

Acute oral toxicity study

The first acute oral toxicity study was preliminary. This study was important to find the starting dose that must be done in the main study because there was still no study about the toxicity of porang with the combination of *S. crispus*. The study showed that there was no mortality (LD50), injury, physical appearance, or behavior changes from the first 30 minutes until 72 hours' observations, for both NPF and SPF groups. The data were used as the reason to conduct the main study with the dose of 2000 mg/kg BW and 5000 mg/kg BW.

The main study was also showed no toxicity sign and mortality in all groups. Because the maximum dose did not cause mortality, LD50 was stated as apparent LD50. Based on The Hodge and Sterner Toxicity Scale, porang both natural and macerated with *S. crispus* were categorized as practically non-toxic due to no mortality (LD50) at the administration dose of 5000-15000 mg/kg BW [25].

Bodyweight of rats

Figure 1 showed the bodyweight of rats during 72 hours of preliminary study. The bodyweight of rats in this preliminary test did not show the weight loss that was in line with the results of the main study [13]. The absence of weight loss indicated that their growth was normal or there was no indication of impaired absorption of nutrients due to the oral administration of porang flour both natural and macerated with *S. crispus*.

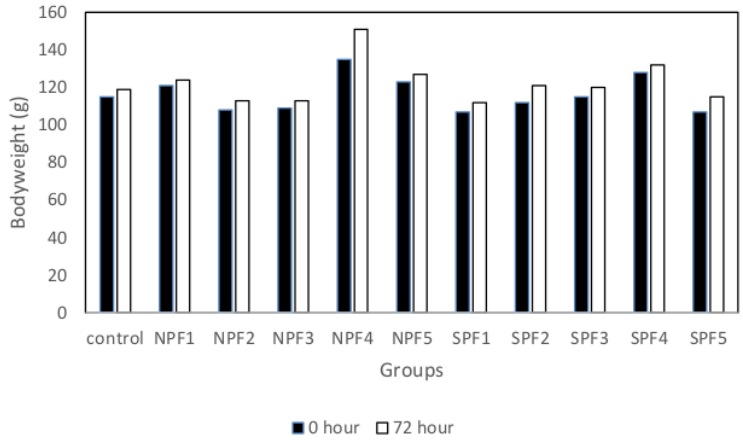

Figure 1 Bodyweight of rats in acute toxicity study of natural porang and *S. crispus*-macerated porang flour (control=distilled water treatment, NPF=natural porang flour, SPF= *S. crispus*-macerated porang flour. NPF1, NPF2, NPF3, NPF4, NPF5 and SPF1, SPF2, SPF3, SPF4, SPF5 for the dose of 50, 300, 2000, 5000 mg/kg BW in preliminary study, respectively).

Urinary protein levels and histopathological examination of kidney organ

The effect of the administration of porang flour on urinary protein levels was shown in Table 1. All groups showed urinary levels under 200 mg/l that categorized as normal levels [26]. However, there was an increase observed in NPF7 and SPF7 both in 24 hours and 72 hours of administration and NPF6 after 72 hours of administration ($p<0.05$). It indicated that the elevation of protein levels may be seen at a shorter time when a higher dose was applied.

The increase of protein levels in the urine indicated a progression to a reduction in renal performance [27]. This study was relevant to the previous publication of the same study which showed an increase in urea and creatinine levels which can be used as an indicator of kidney function/performance [9,13]. In another study, the administration of the herbal medicine of galohgor also showed an increase in urea level, creatinine, and urinary protein [28]. In this study, the decrease was still within the normal range, therefore it is necessary to confirm with further toxicity tests of sub chronic and others to find out whether in a longer period of studies there will be a gradual reduction in kidney performance.

In this study, there was no difference in urea protein levels between the NPF and SPF groups, which indicated that immersion in *S. crispus*, which is one of the herbal medicines, did not affect increasing urea protein levels. However, porang flour contains components that can cause the risk of damage to the kidneys, namely calcium oxalate. Calcium oxalate had an impact on mechanical abrasion of the gastrointestinal tract and renal smooth tubules [29]. In another study, the administration of herbal medicine caused acute tubular necrosis or fibrotic interstitial nephritis that occurred rapidly and progressively. This syndrome was characterized by progressive renal failure, the discovery of a lot of urine sediment, shrinkage of kidney size with mild proteinuria, and was associated with the incidence of urothelial cancer [30,31].

Table 1 Urinary protein levels of rats in acute toxicity study of porang and *S. crispus* macerated porang flour

Urinary Protein (mg/l)	NPF6 (mean±SD)	SPF6 (mean±SD)	NPF7 (mean±SD)	SPF7 (mean±SD)
24 hours				
Pre	62.32 ± 7.23	59.45 ± 4.78	60.33 ± 6.03	60.33 ± 2.29
Post	62.74 ± 6.83	59.29 ± 3.90	77.77 ± 2.11	74.92 ± 4.73
P-value[1]	0.235	0.783	0.004*	0.005*
Δ	0.42 ± 0.68	-0.16 ± 1.18	17.43 ± 6.68	14.59 ± 5.68
P-value[2]	0.370		0.489	
72 hours				
Pre	62.32 ± 7.23	59.45 ± 4.78	60.33 ± 6.03	60.33 ± 0.29
Post	65.91 ± 5.60	60.13 ± 4.21	80.50 ± 1.99	76.48 ± 5.21
P-value[1]	0.009*	0.217	0.002*	0.005*
Δ	3.59 ± 1.68	0.68 ± 1.04	20.17 ± 6.41	16.15 ± 6.30
P-value[2]	0,011*		0.346	

*Significant ($p<0.05$) with [1] paired t-tests to compare pre- and post- result; [2] independent t-tests to compare NPF (natural porang flour) and SPF (*S. crispus* macerated porang flour). NPF6, NPF7 or SPF6, SPF7 were administered with the dose of 2000 and 5000 mg/kg BW, respectively.

As a confirmation test for the effect of oral administration of porang flour, this study also conducted a kidney histopathology test (Figure 1). Histopathology of the kidney showed that all renal organs changed in terms of anatomical pathology in the form of congestion, except for the NPF7 group which was in the form of hemorrhage. Mild congestion was found in the control, NPF6, and SPF6 groups, while the most severe congestion was seen in SPF7.

Congestion and hemorrhage in this study may be excluded from histopathological parameters, because it was included in a natural process that usually occurs after decapitation. Decapitation leads to tissue injury which causes an increase in blood flow to organs, one of which is the kidneys. In kidney congestion, there is an increase in venous blood pooling in the renal vascular that may be due to physiological conditions, passive blood pressure, and secondary effects to hypovolemic shock, insufficiency, and hypostatic cardiac. In this condition, capillary dilation occurs due to vasodilator stimulation so that the vascularization at the site of the injury widens and contains stagnant blood. Meanwhile, hemorrhage can occur due to the breakdown of blood vessels after congestion or intolerable congestion [32].

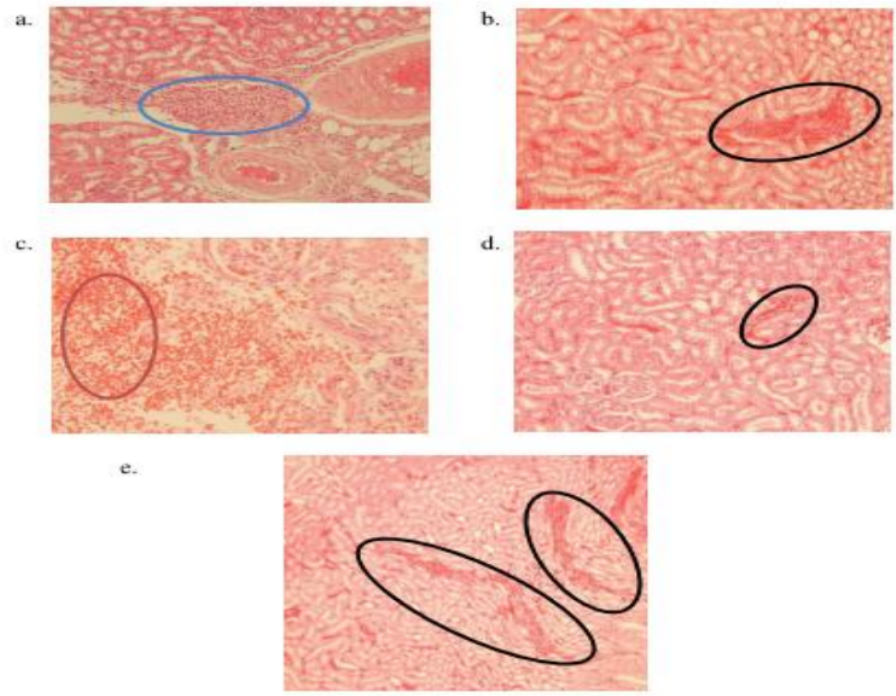

Figure 1 Histopathological examination of kidney organ of the rat in acute oral toxicity study of porang and *S. crispus* macerated porang flour: a) control group, b) NPF6, c) NPF7, d) SPF6, e) SPF7. Blue, red, and black circles describe inflammation, hemorrhage, and congestion, respectively

SGOT levels of blood serum

SGOT blood serum levels increased significantly in NPF7 and SPF7 groups, both in the observation of 24 hours and 72 hours after oral administration ($p<0.05$) (Table 2). It meant that the high dose of porang and *S. crispus*-macerated porang flour had an impact on SGOT levels. However, the increase was still in the normal range between 36.99-42.62 units/liter [33]. This study was in line with other subacute toxicity studies that used glucomannan flour with the dose of 4000 mg/kg BW and porang flour in acute toxicity study at a dose of 5000 mg/kg BW (21,17).

Table 2 also showed that there were no differences between NPF and SPF groups in the dose of 2000 and 5000 mg/kg BW. It indicated that *S. crispus* maceration did not affect the SGOT levels. The increase in SGOT levels affected by the calcium oxalate content which has the risk of damaging the liver cell membrane so that its permeability was impaired, resulting in the SGOT enzyme leaving cells freely, entered the extracellular space and blood vessels beyond normal conditions [23,35].

Table 2 SGOT blood serum levels of rats in acute toxicity study of porang and *S. crispus* macerated porang flour

SGOT (units/liter)	NPF6 (mean±SD)	SPF6 (mean±SD)	NPF7 (mean±SD)	SPF7 (mean±SD)

24 hours				
Pre	37.38 ± 0.77	37.67 ± 0.55	37.38 ±0 .34	36.61 ± 1.22
Post	37.58 ± 0.55	39.03 ± 0.74	45.54 ± 0.93	43.50 ± 0.55
P-value[1]	0.704	0.009*	0.000*	0.000*
Δ	0.19 ± 1.06	1.36 ± 0.63	8.16 ± 1.16	5.83 ± 0.97
P-value[2]	0.068		0.158	
72 hours				
Pre	37.38 ± 0.77	37.67 ± 0.55	37.38 ± 0.34	36.61 ± 1.22
Post	37.67 ± 0.77	36.99 ± 1.34	42.14 ± 0.41	42.62 ± 0.63
P-value[1]	0.552	0.245	0.000*	0.001*
Δ	0.29 ± 1.01	-0.68 ± 1.12	4.76 ± 0.53	6.02 ± 1.63
P-value[2]	0.189		0.138	

*Significant ($p<0.05$) with [1] paired t-tests to compare pre- and post- result; [2] independent t-tests to compare NPF (natural porang flour) and SPF (*S. crispus* macerated porang flour). NPF6, NPF7 or SPF6, SPF7 were administered with the dose of 2000 and 5000 mg/kg BW, respectively.

In addition, psychological factors occurred due to repeat blood sampling in a relatively short time, leading to stress which also triggered the release of the SGOT enzyme in the blood and increased SGOT levels [36]. Several studies proved that the increase of SGOT enzyme levels did not specifically indicate liver dysfunction, because the enzyme was also found in the skeletal muscles, pancreas, heart, blood vessels of the brain, lungs, and testes [37,38]. Thus, the increase in SGOT levels was not only caused by damage to the liver cells but also other organs.

SGPT levels of blood serum

SGPT levels of blood serum had a similar result to SGPT, which increased significantly after oral administration of porang in NPF7 and SPF7 groups ($p<0.05$). However, there was no significant difference between NPF6 and SPF6 groups ($p>0.05$). These results explain that at higher doses (up to 5000 mg/kg BW), porang both with or without maceration affected the increase of SGPT.

SGPT levels in this study were classified as normal (18.16-24.96 units/liter) [33]. However, an increase in SGPT needs attention, because it is an indicator of active hepatocellular damage [39]. Several studies conducted in France, North America, and the Pacific Islands had proven the occurrence of hepatitis in patients who consumed herbal ingredients in high doses for long period [40–42]. Therefore, it needs further studies to know the effects of consuming porang for a longer period on the liver. The tendency of SGPT to increase in this study could be caused by the content of needle-shaped calcium oxalate crystals that may dissolve in the blood and scratch or damage the liver cells. In addition, toxicity may occur as the interaction between components in porang containing calcium oxalate and *S. crispus* containing alkaloids, saponins, flavonoids, potassium, and polyphenols [43,44].

Table 3 SGPT blood serum levels of rats in acute toxicity study of porang and *S-crispus* macerated porang flour

SGPT (units/liter)	**NPF6 (mean±SD)**	**SPF6 (mean±SD)**	**NPF7 (mean±SD)**	**SPF7 (mean±SD)**
24 hours				
Pre	18.74 ± 0.26	18.45 ± 0.34	18.45 ± 0.59	18.06 ± 0.63
Post	18.16 ± 0.65	18.64 ± 0.43	24.96 ± 0.55	23.88 ± 0.41
P-value[1]	0.109	0.178	0.000*	0.000*
Δ	-0.58 ± 0.63	0.20 ± 0.27	6.51 ± 0.27	5.83 ± 0.97
P-value[2]	0.035*		0.170	
72 hours				
Pre	18.74 ± 0.26	18.45 ± 0.34	18.45 ± 0.59	18.06 ± 0.63
Post	18.45 ± 0.34	18.35 ± 0.53	23.40 ± 0.40	23.40 ± 0.40

P-value[1]	0.209	0.800	0.000*	0.001*
Δ	-0.29 ± 0.43	-0.10 ± 0.80	4.95 ± 0.80	5.34 ± 0.49
P-value[2]	0.643		0.378	

*Significant ($p<0.05$) with [1] paired t-tests to compare pre- and post- result; [2] independent t-tests to compare NPF (natural porang flour) and SPF (*S. crispus* macerated porang flour). NPF6, NPF7 or SPF6, SPF7 were administered with the dose of 2000 and 5000 mg/kg BW, respectively.

Conclusions

Based on the acute toxicity study, it can be concluded that porang and porang macerated with *S. crispus* were not toxic until the highest dose of 5000 mg/kg BW. It was proved by the absence of LD50, no change in behavior, no weight losses, and also the results of biochemical tests, such as urea protein, SGOT, and SGPT which were still in the normal range. It is necessary to carry out further toxicity studies, including sub chronic to determine the safety level of porang and *S. crispus* consumption for long period.

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