The increase of blood creatinine levels and the gastric histopathology of rat after feeding of porang (Amorphophallus oncophyllus) flour treated with Strobilanthedes crispa

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ABSTRACT

Background: Porang (Amorphophallus oncophyllus) tuber is the original Indonesian tuber containing glucomannan. Glucomannan is utilized as food additives and food supplements for people who have problems with diabetes, high blood pressure, constipation and weight loss. However, it contains calcium oxalate which causes itchy if it is consumed therefore itness appropriate preparation. Soaking of S. crispa in vivo has proven lowering the levels of calcium oxalate. However, the excess consumption of oxalate calcium can cause renal function disorders, especially at the glomerular filtration rate (GFR) and affects the kidneys work, ie absorption and filtration creatinine.

Objectives: The objective of this study is to know the influence of the porang flour with S. crispa (keji beling) on the level of blood creatinine and gastric histopathology of rats (Rattus norvegicus) Wistar on acute toxicity test.

Methods: This research used experimental with one test group, without control group design. The subjects were 20 white female Wistar rats (Rattus norvegicus) with the weight of 110-180 grams, ages of 8 - 12 weeks, healthy, and normal. Rats were divided into. Native porang flour (TPM) and porang flour treated...
Consumption of porang flour had the affect on the digestive organs such as mouth, tongue, esophagus and stomach. The stomach is a mixed endocrine and exocrine organ that digests food and hormones. It also destroy the food and drink consumed, the stomach wall can also absorb certain substances through its walls. This causes the stomach to be susceptible to exposure to toxic substances directly and make it as one of the toxic effect targets (7).

In addition, excessive consumption of oxalic acid over long periods can lead to its accumulation of the kidneys and cause the malfunction of this organ especially at the glomelurus filtration rate (LFG), so that it can affect the kidney work ie absorption and creatinine filtration (8). Creatinine was excreted by kidney through a combination of filtration and excretion with relatively constant concentration in blood plasma. The amount of creatinine that exceeds the normal value indicates decreased of excretion caused by impaired renal function (10). A 2-fold increase in the amount of creatinine indicates a 50% reduction in kidney function.

The oxalic acid removal technique from porang has been widely practiced before this studies by cooking or by drying (3). Study of salt concentrations, in soaking treatment, stamp mill, and fractionation (blower) or grinding by surface response method were also proved to remove the oxalic acid (11-13). In addition, another way that is expected to be used is by immersion of keji beling leaf in vivo which is proven to reduce calcium oxalate levels (14). The studies of toxicity test has proved that S. crispa showed no death on animal (15).
The aims of study were to evaluate the effect of soaking porang with ethanol extract of *S. crispa* on the level of calcium oxalate levels. Its influence on the creatinine levels and histopathology levels were also studied.

**MATERIALS AND METHODS**

This research was a part of a joint research about porang tuber coordinated by *Alma Ata Center for Health Life and Food (ACHEAF)*. This was an experimental research with *one group test without control group design*. It was conducted in May-July 2017. The subjects were white Wistar rat (*Rattus Norvegicus*) as many as 20 rats aged 8-12 weeks, weighing 110-180 grams, healthy, and not pregnant. Rats were divided to group of TPM (native porang flour) dose of 2000 and 5000 mg/kg body weight (BW) and TPK (treated porang flour) dose of 2000 and 5000 mg/kg BW. TPM was contained 3.27% of calcium oxalate. The maceration process was performed using 3.2 % of *S. crispa* extract. Creatinine levels were analyzed from blood rat after feeding of porang at 24th and 72th hours. In the end of study, gastric histopathology was also observed.

**RESULTS**

**Porang flour preparation**

Porang flour was used for feeding after treating with *S. crispa* extract. The effect of type of solution, duration of soaking and rinsing frequency on the level of porang calcium oxalate were studied.

**Table 1. The effect of solution type on ca-oxalate levels**

<table>
<thead>
<tr>
<th>Type of Solution</th>
<th>Calcium oxalate level (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>4.03 ± 0.09</td>
<td>0.057</td>
</tr>
<tr>
<td>Ethanol + <em>S. crispa</em></td>
<td>2.99 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

Notes: p<0.05 showed significant value with *Paired Sample T-test*

**Table 2. The effect of period of soaking on ca-oxalate levels in porang flour**

<table>
<thead>
<tr>
<th>Period of soaking</th>
<th>Calcium oxalate level (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours</td>
<td>2.74 ± 0.08</td>
<td>0.065</td>
</tr>
<tr>
<td>72 hours</td>
<td>2.99 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

Notes: p<0.05 showed significant value with *Paired Sample T-test*

**Table 3. The effect of rinsing frequency on calcium oxalate levels**

<table>
<thead>
<tr>
<th>Rinsing Frequency</th>
<th>Calcium Oxalate Level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>0 times rinse</td>
<td>5.28 ± 0.04</td>
</tr>
<tr>
<td>1 times rinse</td>
<td>3.87 ± 0.04</td>
</tr>
<tr>
<td>2 times rinse</td>
<td>2.74 ± 0.07</td>
</tr>
</tbody>
</table>

Information: Superscript within the same column with the same letters are not significantly different (p>0.05).

**Table 4. Normality test of initial blood creatinine content data**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Shapiro-Wilk Statistic</th>
<th>Df</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPM</td>
<td>0.90</td>
<td>10</td>
<td>0.20</td>
</tr>
<tr>
<td>TPK</td>
<td>0.89</td>
<td>10</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Notes: TPM, native porang flour. TPK, treated porang flour.

**Table 4** showed that baseline blood creatinine data were not significant different (p>0.05). The *p-value* of TPM group was 0.202 TPK group was
0.186. It meant’t that distribution of data were categorized normal.

Table 5 showed that after a 24-hour treatment, blood creatinine levels of rat were significantly different in the administration of the same type of porang flour with different doses. While in the different type of porang flour, there was no significant difference after 24 hours treatment within the same dose. Therefore, dosage had the role on the increase of creatinine levels. The decrease in blood creatinine levels of rat in all groups after treatment at 24 hours were ranged from 0.036-0.136 mg/dL. Blood creatinine levels of normal mice were 0.20-0.80 mg/dL (16). So it can be concluded that at the observation of the 24th hour treatment of blood creatinine levels were normal.

Table 6 shows that at the 72 hours of observation, creatinine levels of blood rat increased with the increased of dosage, both in TPM and TPK groups (p<0.05). The decrease of blood creatinine levels after 72 hours of treatment were ranged from 0.01 to 0.20 mg/dL. Blood creatinine levels of normal rat were 0.20-0.80 mg/dL (916). It meant that blood creatinine levels of rat in all treatment groups were normal.

The difference in the creatinine level of rat blood compared between the 24th hour and the 72nd hour after treatment was used to see the changes in blood creatinine levels after the porang administration. The results show that generally, creatinine levels increased at the observation of 24th and 72nd hours. The increased was consistent with the higher of dosage.
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Figure 2 shows that inflammation was found at the lowest degree in the treatment of TPM 2000 mg/kg of body weight. The addition of *S. crispa* increased the degree of inflammation.

### DISCUSSION

**Porang flour preparation**

Porang flour was made from porang tuber. Porang has high enough calcium oxalate content, leading to irritation and itching in the throat when eaten (17). Therefore, it is necessary to find the alternative treatment to reduce calcium oxalate levels.

**Creatinine levels of rat blood**

Preliminary test was conducted to obtain a *starting dose*. It used 1 rat for each dose. The doses used were 300 mg/kg BW because there was no previous research was found using similar test materials and female rats had high sensitivity level compared with male rats (20). The results did not show symptoms of toxicity or death at a dose of 300 mg/kg BW, so the *starting dose* used was 2000 mg/kg and 5000 mg/kg BW with a single dose for 24.

Kidneys are susceptible organs against the presence of toxic compounds. Creatinine is the final product of cretin metabolism in the muscle. Metabolically, creatinine is an inactive component that diffuses into the plasma and
is excreted through the urine. Increased blood creatinine levels and the amount of creatinine in the urine can be used to improve glomerulos filtration rate. In this study, creatinine of rat was measured using Jaffe method. This method is quite specific, simple, widely used and can relatively eliminate the intervention of the blood components that can react with reagents on the Jaffe method without modification.

Measurements of blood creatinine levels were performed at the 0th hour before treatment, at 24 hours and 72 hours after treatment. The result of the initial measurement is analyzed by homogeneity of data using Shapiro-wilk test to know the homogeneity of data. The test results revealed that the spread of early blood creatinine data (0 hour) was normal or there was no significant difference between TPM group and TPK group either at the same dose or different dose. It because at the hour 0, rat had not given the treatment yet.

At the 24 hours creatinine observation results presented in Table 5 shows that there was a difference in creatinine levels between the same administration and the different doses (p <0.005). At higher doses, blood creatinine levels also increased. It was due to the higher content of protein and calcium oxalate in the increase of dosage that was leading to higher protein metabolism. In the body, especially the liver, the protein will undergo a deamination process that will produce nitrogen and non nitrogen metabolite. One of them is creatinine which is the final product of nitrogen. And the presence of oxalate will produce free radicals that trigger the occurrence of oxidative stress conditions. This condition is initiated by the release of vasoactive mediators with effect vasoconstriction of blood vessels, in this case the kidney blood vessels and result in a decrease glomerulos filtration rate (FLG) (21).

The average range of blood creatinine levels of rat in all groups after treatment at 24 hours was 0.0060-0.0280 mg/dL. And the creatinine content of rat normal blood was 0.20-0.080 mg/dL (16 ). So it can be concluded that the observation of the 24th hour after treatment of blood creatinine levels in all groups were classified as normal. Therefore, large doses of TPM and TPK have the same effect on the increase of blood creatinine level, so that the soaking treatment of ethanol extract in vermicular calcium oxalate did not affect the increase of blood creatinine level. Some of the factors that can affect blood creatinine levels include sex, famine and muscle tissue size. Increase of blood creatinine levels can also be caused by some of the circumstances such as tissue hypoxia, decreased glomerulos filtration rate, the presence of metabolic disease and toxic chemicals (22).

At the 72nd hour of observations, TPM and TPK administration had significant effect on creatinine levels of rat on the same type of porang with different dosage (Table 6). The mean of blood creatinine level in all groups were ranged between 0.0080-0.2 mg/dL that was still within the normal range (0.20-0.80 mg/dL). Furthermore, the change in blood creatinine levels at 24 and 72 hours showed no difference except for the treatment of dosage of 2000mg/kgBW. It may be influenced by rat muscle mass. The rat are in growth mass, so that muscle mass was also slightly increased. The greater the muscle mass, the higher the creatinine level from the metabolism. The decrease in blood creatinine levels of rat was influenced by the dose and duration of administration.

In this study, the dose gave no impact on the given. This was consistent with the study of acute toxicity test using corn hair extract, its effect on Lethal Dose (LD) 50, liver and kidney function was in observed dose of 3.84; 7.68; 15.36; 30.27 g/kg. It showed that the extract of corn hair water in the highest dose did not result in death and did not affect the liver and kidney function of the rat (23). Other results showed that Kare Bulbs contained prostratin as a potent anti-HIV drug tested in 40 rats of swiss strains given intra-mus snake (0.28, 0.42, 0.56 mg/kg BW ) in the negative control group and intraperitoneally administered (0.01, 0.015, 0.020 mg/kg BW) had sub-lethal discharging effects. The percentage of ALT levels in peroral treatment groups decreased on 14th day by 1.46-18.76%. While presentation of creatinine levels in the control group and peroral treatment (intramusc snake and intraperitoneal)
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increased on the 14th day by 20-100%. Statistically it did not show significant difference (p = 0.335), but the creatinine level activity had significant difference between control group and treatment (p=0.00). Liver organ damage in the form of picnosis, cariorrexis and cariolysis on organ ren was degenerated on proximal and distal tubulus (24).

In addition, a decrease in mean creatinine levels was possible due to the effect of the use of ethanol extract of S. crispa leaf and long exposure of test preparation. S. crispa contains flavonoid compounds, namely natural phenolic compounds that have potential as antioxidants and anti-inflammatory (25). Flavonoids exhibit both in vitro and in vivo anti-inflammatory activation. Some of the cellular flavonoid action mechanisms show such activation. This compound may have multiple cellular mechanisms, but its contribution as the most important anti-inflammatory agent was its effect on the activation of the eicosanoid enzyme. Inhibition of eicosanoid enzymes activity will decrease prostaglandin concentration, which will be the blood flow decereation to the kidneys (vasoconstriction). It lead to the reduction of inflammatory mediators (26).

Gastric Histopathology

Histopathologic examination provides information about the target organs of damaged from chemical compounds. It may be observed from the alternation or abnormality in that organs. The stomach is a mixed endocrine-exocrine organ that digest food and hormones. In addition to destroying food and beverages consumed, stomach can also absorb certain substances through the walls. This causes the stomach to be susceptible to exposure to the toxic substances directly and make it one of the targets of the toxic effect (7).

The results of microscopic examination of gastric organs, showed that there were of inflammation and mast cells in the sub mucosa were consecutively in degrees of two (++) and three (+++). In the presence of inflammation triggers hemorrhage, that was occurred in gastrointestinal, hem can increase serum creatinine levels without negative effects on kidney which is in the main spotlight (27). The inflammatory reactions can increase the number of possible mast cells as a key mechanism to protect the digestive tract from injury. It is occurred in the mucosal immune system that can detect strange things into the mucosa and regulate the inflammatory response that occurs. In the gastrointestinal tract, mast cells biologically activate process, including the regulation of blood flow, the epithelial and endothelial permeability, can be the mucosa, gastrointestinal motility (28). The results of another study showed the preparation of combination test of phenolic leaf extract of live and ethanolic extract of Chinese teak leaves leave dose of 2000 mg/kg and 5000 kg mg/kg between 14 days in rats wistar strain affects PKPB (Puritan Increase Weight For Day) and showed no symptoms of toxic and therewere also no spectrum toxic effects were significant at the macroscopic observation as well as the results of the histology of the organ that shows the damage a cellular (including the stomach) (28).

The use of porang flour is worried to cause allergies. The cause of the allergy in people maybe come from calcium oxalate content in the porang tuber, leading to itching or heat in the mouth. However, food allergies are usually proteins that do not change during the digestive process. There are four types of calcium oxalate crystals: druse, rafida, prism and sand. Various proteins play a role in the formation of calcium oxalate. The protein was called the matrix protein that includes Asp-rich acidic proteins (many proteins that contain many amino acids asparagin) and Ser-rich glycoproteins (glycoproteins contain many amino serine acids). The protein has a very strong ability to bind calcium. From this description, the subgroup of a lergen on the tuber is the matrix protein in the crystal rather than the calcium oxalate rabbit (29). Thus the presence of this inflammation from the reaction of the lambung organ to the treatment of TPM and TPK although it does not directly affect the kidney and blood creatinines.
CONCLUSIONS AND RECOMMENDATION

The frequency of rinsing affected the decrease of calcium oxalate content in porang, while the type of solution and the period of soaking did not. Rat blood creatinine level slightly increased after TPM and TPK dose 5000 mg/kg BW. Giving TPM dose of 5000 mg/kg BW significantly effected on the increase of blood creatinine level but not on giving of TPK. Gastric histology examination contained inflammatory cells in the gastric sub mucosa on TPM and TPK doses of 5000 mg / kg, but did not directly affect blood levels of creatinine.

The addition of ethanol extract of *S. crispa* with washing frequency 2 of times each 100 ml can be used to reduce the calcium oxalate content in pure porang so it does not affect kidney function. Consumption of porang flour with the addition of ethanolic extract of *S. crispa* leaves to a dose of 5000 mg/kg BW rat or equivalent premises 26 grams/day safe for consumption.

Acknowledgments

Acknowledgments of the authors was delivered to ACHEAF (Alma Ata Center for Healthy Life and Food) for their supports and technicians of PSPG Laboratory (Mr. Yuliyanto). UGM Veterinary Pathology Laboratory (Dr. drh Yuli Purwandari K, MP), which has provided a lot of assistance so that this research can be completed as planned.

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