EFFECT OF PORANG POWDER CONSUMPTION ON RAT (*RATTUS NOVERGICUS* WISTARSTRAIN) BODY WEIGHT AND KIDNEY HEALTH

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ABSTRACT

This study aimed to determine the effect of macerated porang powder consumption to rat kidney's health through five treatment groups including control, 300 mg/kg BW and 500 mg/kg BW of macerated porang powder, 300 mg/kg BW and 500 mg/kg BW of non macerated porang powder. Each group consisted of six male rats (*Rattus novergicus* Wistar strain). Rats were treated for 60 days and weighed on the first and last treatments. Calcium level in the blood was also measured and histopathology analysis of kidney was conducted by using microscope observation. Consumption of macerated porang powder with dosage 300 mg/kg BW for 60 days lead to the kidney defect but the defect did not observe at non macerated porang powder treatment. The defect was shown with necrosis at the cell of tubules. Body weight gain was affected by the difference of applied dosage but not affected by maceration treatment.

Keyword: Porang Powder, Calsium Oxalate, Kidney, Body Weight, Rat.

INTRODUCTION

Functional foods is growing rapidly and usually used by people to meet their daily needs. Glucomannan powder derived from porang (*Amorphophallus muelleri* Blume) is one of foods that can be developed as a source of new functional food. Glucomannan powder is produced from powdering process of porang corm, grouped in Araceae, which is widely cultivated as intercrops in protected forests area as an implementation of the cooperation between the forester with forest communities.

Porang powder contained high glucomannan content ranged from 70-80% (Syaifulloh, 1990), and it was also followed by high levels of oxalate up to 6.11% in chip porang (Faridah et al., 2012) which may risk for the kidney health. One of technology for powdering process using stamp mill with multistage ethanol maceration method able to produced porang powder with 0.073% oxalate level and glucomannan content reached 80% (Faridah et.al., 2012). However, low levels of oxalate is still feared due to its potential cause health problems and according to quality standards of WHO which required that the functional foods must meet some criteria's, including quality, safety and efficacy (Anonymous, 2000). So, it need to be conduct a research about consumption effects of porang powder to kidney health and body weight.

Kidney is one of the vital organs in the body, due to its function to excrete metabolism remnants from the body. Increased excretion of metabolites remnants can cause kidney defect because of poisoned by contact with such materials.If tissue damage did not get medical treatment, it would cause kidney failure that ended with death (Wilson, 2005).

Kidney defect was observed onrat with calcium carbonat ($CaCO_3$) treatments. Because high Ca absorption given to rat caused toxic effect on the kidney. Microscopic observation showed that kidney with toxin exposure possessed degeneration even necrosis on the cells. These results corroborated by Cotran (2007), which stated that the damage occured on tubular epithelial cells, that directly contacted with the reabsorbed materials, can caused cell degeneration and necrosis of kidney cells.

MATERIALS AND METHOD

Porang powder was used in this study that derived from porangchip.Thisporang chip was taken from farmers in Nganjuk. Rats (*Ratusnorvegicus*Wistar strain) were used for in vivo necessary and obtained from Laboratorium Pharmacology, Faculty of Medicine, University of Brawijaya, Malang.

Macerated porang powder was prepared according to Faridah et al (2012) method, that it was made from porang chip with 1.2 kg in weight, then it was powdered with a stamp mill machine for 17 hours 4 minutes 8 seconds, speed of 19.23. Then porang powder was filtered using Retsch 5657 30 mesh and passed to air classifier built from PVC pipe ± 7 cm in diameter and blower. Filtrate was macerated with ethanol using multistages maceration method. Porang powder was taken as much 25 g from deposit then macerated with 40%, 60%, and 80% of ethanol. The mixture was stirred with speed 434.22 rpm for 4 hours 16 minutes, volume of ethanol 233.77 ml. The deposit was dehydrated and used for next stages.

Six rats was acclimatized at a cage with size of 45 cm x 30 cm x 25 cm in the laboratory. Metal rack is provided for the placement of rat cage. The husk was put on inside of cage and a rat drink was prepared from a glass bottle.

In this study want to determine the consumption effect of macerated porang powder to rat kidney. The treatment was divided into five groups including control, 300 mg/kg BW non macerated porang powder, 500 mg/kg BW non macerated porang powder, 300 mg/kg BW macerated porang powder and 500 mg/kg BW macerated porang powder. Each group consisted of six male rats Winstar strain. Rats were treated for 60 days and weighedat first and last of the treatments. Blood serum was also analysed after the treatment time and hispatology analysis of rats kidney was carried out using microscope. Kidney slides were prepared using hemotoxilin eosin (HE) staining.

Blood serum analysis was conducted by using Random Access Auto AnalyserBiosystem A15. Serum sample was calibrated using NaCl 0.9% in C.f.a.s (Calibrator for automated system). The calibrator was dissolved with aquabidest and mixed untill totally homogeneous. The mixture was put into 200 µl cups and stored in the freezer with temperature 2 – 8^0C. After sample was calibrated, “quality control” was carried out by using Bio-Rad. As much 300 µl of Bio-Rad was pipetted into serum cup and put in control rack that has been determined, then parameter interest was measured. Macerated with ethanol by gradient polarity method. The mixture stirred with speed 434.22 rpm for 4 hours 16 minutes and the volume of ethanol was 233.77 ml. The deposit materials were dehydrated and were use for the next step.

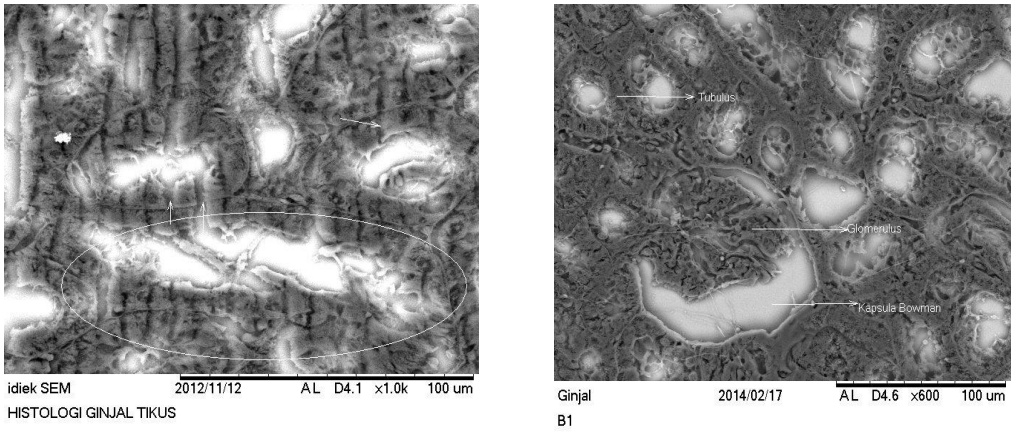
The rats were acclimatized at a cape at a size of 45 cm x 30 cm x 25 cm in the laboratory. Metal rack is provided for the placement of rat rage. The husk putted on inside of cage and a rat drink prepared from a glass bottle et libitum. These rats divided into five groups including

control, 300 and 500mg/kg BW macerated porang powder, 300 and 500 mg/kg BW non macerated porang powder, respectively. Each group consisted of six male rats Wistar stain. Rats treated for 60 days and tarred at first and last the treatments. Blood serum was analyzed after the treatment and kidney was sliced and put in a glass slide and covered with glass slip, then stained using hemotoxilin eosin (HE). Histopathology analysis of kidneys rat were carried out by using scanning electron microscophy (SEM).

Blood serum analysis was conducted by using Random Access Auto Analyser Biosystem A15. Serum sample was calibrated using NaCl 0.9% in Calibrator for automated system (CAS). The calibrator was dissolved with aquabidest and mixed until homogeneous. The mixture putted into 200µl cups and stored in the freezer at 2 – 8ºC temperature. The quality control was carried out by using 300 µl of Bio-Rad and poured into serum cup and put onto control ract that has been determined, and the parameter interest was measured.

RESULTS

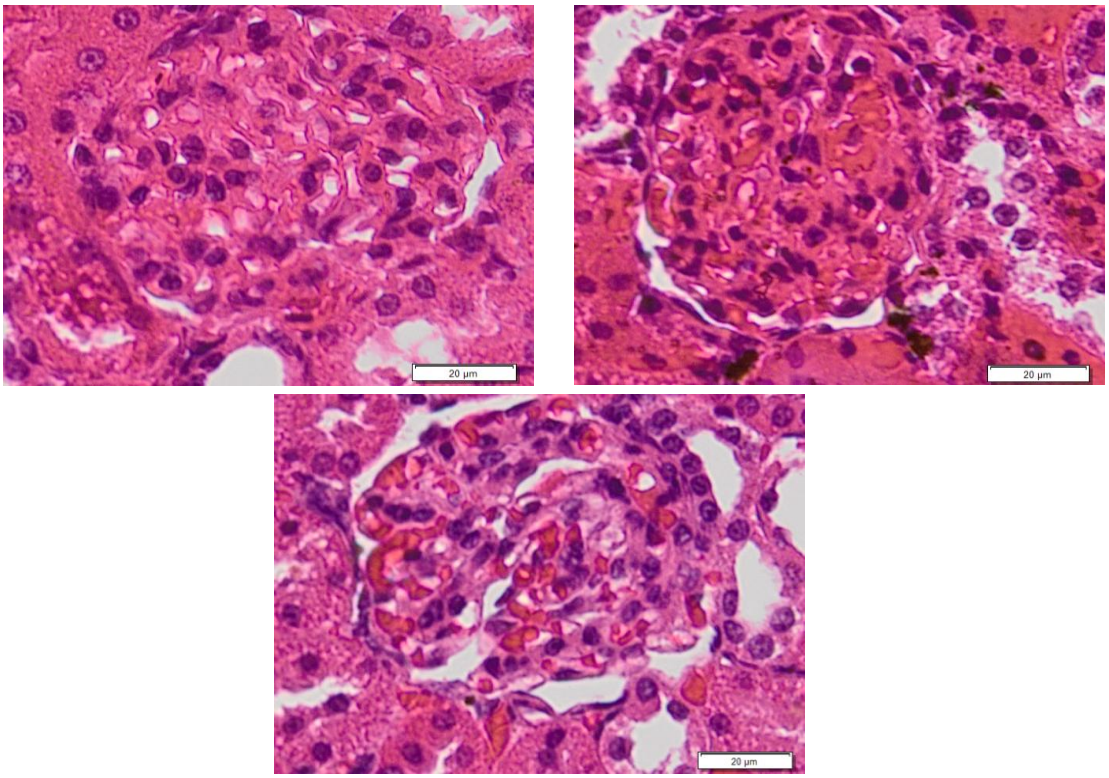
The measurement of tubular damage level can be seen in the narrowing of proximal convoluted tubules, necrosis of the proximal convoluted tubule epithelial. Renal tissue of treated rat encountered tubules cell damage shown by cell separation (Figure.1)



Note: non macerated porang powder with dosage 300mg/kg BW/day (A) and macerated porang powder with dosage 300mg/kg BW/day (B), masnification 1000x

Figure 1. Scanning Electron Microscopy (SEM) of rat kidney defect after treatment

The observation showed that rats were treated by non macerated porang powder contained 2.33% oxalate (A) and macerated porang powder contained 0.99% oxalate (B) in their feed for 60 days (Figure 2). Non macerated porang treatments significantly caused kidney tissue defect compared to the macerated porang. 500 mg/kg BW/day macerated porang powder treatment generated no different kidney defect with 300mg/kg BW/day macerated porang powder. Histopatology observation result is shown at Figure 2. There have been seen minimum defect on kidney tubules with macerated porang powder treatment and non macerated porang powder. Thus, in the process of data collection, the damage to renal tubules can only be seen from the number of tubules, that was shrinkage or even closed. Kidney defect also had been seen at non macerated porang powder treatments. The worst damage occurred when rats were supplemented with Porang powder A, mom-macerated powder with dosage of 500/kg BW/day.



Notes: control (K); (with no porang flour, A1 (porang flour A1 0,3 g/kgBB/day dose) A2 (porang flour A 05 g/kgBB/day dose), B1
(porang flour B 03 g/kgBB/day dose), B2 (porang flour b 05 g/kgBB/day dose)

Picture 2 The wistar rat kidney microcospic histopalogy effect by Porang powder treatment

Table 1. Description of rat kidney observation by used SEM after porang powder treatment

Porang powder (mg/kgBB)	Maceration	Description of observation
Control	------	No tubules constriction
300,	without	Many tubules construction
500	without	More tubules construction
300,	with	Little tubules construction
500	with	Little tubules construction

Oxalate levels in the rat blood were presented in Table 2. Turkey test showed that the control group had the lowest oxalate levels on average and was significantly different from other treatments. Treatment with macerated porang powder dosage of 300 mg/kg BW/day was not significantly different from non-macerated Porang powder with same dosage, but it was significantly different from the other treatments. Consumption of non macerated porang powder dosage 500 mg/kg BW/day generated the highest oxalate level and was significantly different from other treatments.

Table 2. Avarage of oxalate level in blood after porang powder treatment

Porang powder (mg/kgBB)	Maceration	Oxalate level in blood(mg/l)
Control	, ------	2,25 a
300,	without	4,57 c
500	without	5,35 d
300,	with	3,19b
500	with	3,34 b
BNJ 0,05		0,69

Note: Number with same letter in the column not significantly different on BNJ 0.05 test

Body weight of rats have changed due to Porang powder treatment. The rat standard feeding was shown at Figure 2. Based on the ANOVA test, there was not significantly different of body weight between before and after treatment. Increasing induced dosage, from 300 mg to 500 mg/day/rat, would reduce the body weight of rats both of with macerated and without macerated porang powder. It indicated that the control group had the largest body weight increase. Adding dosage leads to the decrease of body weight.

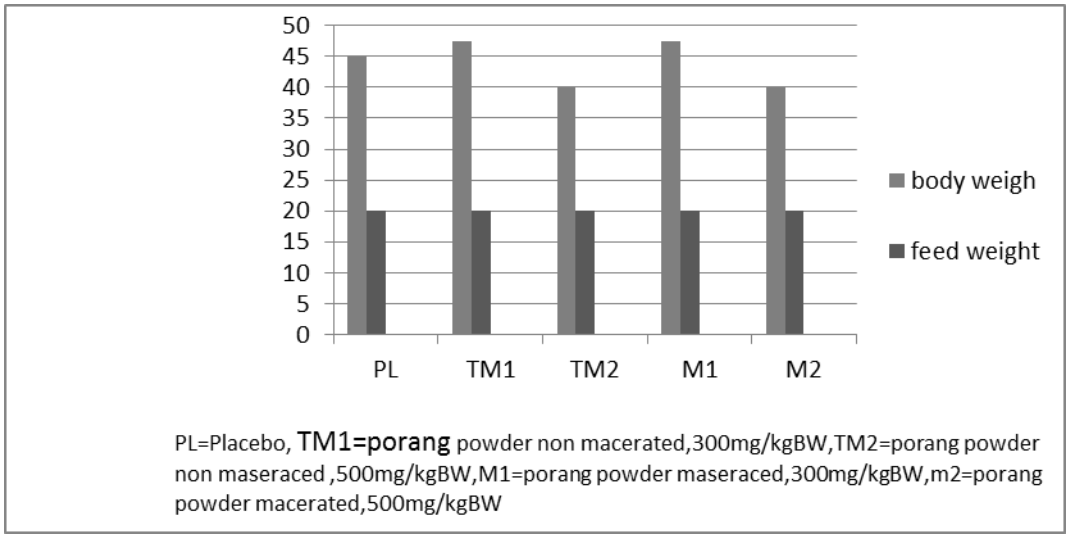


Figure 3. The increase of rats body weight after porang powder treatment

DISCUSSION

Calcium is one of the important mineral that is needed by the body. Some functions of Ca are playing important a role in the formation process of bones and teeth which influenced by vitamin D, protecting from radioactive substances, supporting cardio muscle, nervous system, and brain activity, blood clotting process, and enzyme activation. The amount of Ca was requirement for adults, children, pregnant women, nursing mothers are 0.8 g/day, 1.4 g/day, 1.5 g/day, 2.0 g/day, respectively. According to Li *et al* (2010), most of calcium filtered in kidney would be reabsorbed (98.99%). About of 60% calcium was reabsorbed in tubulus contortus proximalis, remains reabsorbed in the ascended loop of Henle and tubulus contortus distalis. Reabsorption occurred in tubulus contortus distalis and was a transport active process regulated by parathyroid hormone whereas Ca reabsorbtion in tubulus contortus proximalis was indirectly affect by parathyroid hormone. Process of calcium reabsorption had determined point (Tm Ca) in tubulus distal parathyroid that circulated at the blood system (Rao, 2009).

Bhandari *et al* (2002) reported that an excessive cheminal substances contained in the kidney would lead to a cell damages as pycnosis and congesti. Pycnosis or shrinkage a cytoplasm homogenation and eosinophyl enrichment. Pycnosis could be occurred due to inner cell damage such as cell membrane leakage, mitochondria and Golgi apparatus damage so cell did not able to eliminated water and triglyceride resulting accoumulation in the cytoplasm. In the Kidney, most of pycnosis occurred at tubulus proximal because reabsorbtion process was taken place in this tubules. Hence, risk of damage was so high in these parts when exposed with toxin.

The damage that arises to the kidney in the treatment of a rat being nurtured by calcium carbonate, because of the so high calcium intake will cause toxic effects on the kidneys. In the microscopic observation on the kidneys experiencing toxicity, the presence of degeneration or necrosis of the cells was found. The result of this research was supported by Sharage (2005), who mentioned that any damage to the tubular epithelial cells that come in direct contact with the reabsorben material may result in cell degeneration and necrosis of the kidneys,. Changes in the nucleus necrosis cell in the pycnosis basophils. The increasing levels of calcium in the kidney cortex or medulla caused by nephrocalcinosis. Mohammaden *at al* (2013) stated that nephrocalcinosis was defined as an increase in the renal calvium levela, mainly because of hypercalsemia an hyperdalsiuria, triggering deposition of calcium in the parenchyma or renal medulla. Calcium metabolism disorders, such as hypercalcemia and hypercelcalciuria can tiger the formation of calcium stones in the kidneys and the deposition of calcium salts in the renal parenchyma. Widespread deposition can cause chronic tubulointerstitial disease and renal insufficiency. Early signs of damage due to hypercalcemia seen in the intracellular levels in the tubular epithelial cell. This mitochondria distortion, condition will cause a mitochondria distortion, cytoplasm and membrane abasalisis. Consumption Porang powder macerad with a dose of 1000 mg/kg BW to rats for 4 weeks caused damage to the kidney and histopathology changes in the kidneys as the calcium Oxalate intake is too high, so that exceed the ability of the kidneys to excrete and absorb the calcium caused sitoplasma defect and some cell degeneration (krystanti dkk, et al 2014).

The loss of weight in rats caused by the increasing doses, presumably due to the increasing levels of oxalate in the flour given, from a dose of 300 mg to a doses of 500 mg will also oxalate in the degistive system of rats until it affect metabolism reaction in its bidy. This will also affect the increasing of the body weigth, according to Dianawati (2012) who stated that supplementing 18 mg calcium in the diestary of a Wistar rat will affect the weight of a controlled rat. Zemal *et al* (2005) and Schrager *et al*, (2005), reported that high calcium intake would reduce the concentration of dehydroxy 1,25 vitamin D3 and further affected role of fatty acid synthase anzyme, lipogenesis and increase of lipolysys responsible for fat storing in adipose tissue.

In the other hand, due to increase of porang powder consumption increase levels of glucomannan it was thought that it would decrease the body weight (Vuksab *et al*, 2000, Zhang *et al*, 2005, Alonso *et al*, 2009). Viscous properties of glucomannan is supposed to lead more severe disgestive process so it will pass through small intense faster, resulting in reduce of food become hard and quickly excretedescribeed that glucomannan treatment leads to weight loss may be caised by viscous properties of glucomannan that can swelling which is abait 200 times in the water absorbtion that caused full stomach effect this reducing appetite.

CONCLUSION

Conclusion of macerated Porang powder with dosage 300 mg/kg BW for 60 days lead to the kidneys defect but the was not detected in non meceratorang powder treatment. The defect is shown with cell necrosis at the tubules. The Highest oxalate level in blood was 5,35% reached by the 500 mg/kg BW porang powder treatment, whreras in the same dosage by porang powder macerated can obtained and seems to affected by the difference of applied dosage but not affected by maceration nor non macerated treatment.

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