The Effect of Calculusol™ to the Plasma Free Radical and Serum Creatinin in *Mus Musculus* Nephrolithiasis Model

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ABSTRACT

Calculusol™ is a traditional medicine (jamu) made from Tempuyung (*Sonchus arvensis*) extract and is usually used for cure kidney stone disease. However, there has not been any studies which investigate the its mechanism. The aim of this study is to know the effect of Calculusol™ to the plasma free radical and serum creatinin of *Mus musculus* model for renal calcium-oxalate accumulation. This study is carried out by administration of Porang (*Amorphophallus muelleri*) tuber flour for 3 months with the doses 6 mg/100g BW to induce renal calcium oxalate accumulation and Calculusol™ treatment for 7 days with the doses of 3.3mg/g BW. Group I was used as a control group. Group II was only given porang every day for 3 months. Group III was only given Calculusol™ for 7 days. Group IV were given porang for 3 months then given Calculusol™ for 7 days. Group V were given porang and Calculusol™ simultaneously for 3 months. Porang and Calculusol™ is administrated orally. Blood was collected from the tail of the animal for serum creatinin test and plasma free radical test using TBARS method. The data was analyzed using ANOVA followed by Tukey HSD to compare the means employing SPSS 16.0 for windows. The result of the research shows that the treatment Calculusol™ on mice model for renal calcium oxalate accumulation. The content of MDA at group I, group II, group III, group IV and group V, respectively is 0.81 ± 0.5 mg/ml; 2.63 ± 0.8 mg/ml; 0.56 ± 0.5 mg/ml; 2.09 ± 0.9 mg/ml and 0.17 ± 0.17 mg/ml. The content of serum creatinine are 0.15 ± 0.04 mg/dL; 0.13 ± 0.03 mg/dL; 0.12 ± 0.08 mg/dL; 0.11 ± 0.016 mg/dL and 0.14 ± 0.015 mg/dL at group I, group II, group III, Group IV, and group V respectively. This indicates that Calculusol™ decreases plasma free radical production during renal stone formation, while serum creatinin reduced but not significantly changed. Calculusol™ has an effect to decrease free radical during renalstone formation in mice model for calcium oxalate accumulation and also decrease serum creatinin.

Keywords: *Calculusol™, creatinin, free radical*

INTRODUCTION

Nephrolithiasis (kidney stone disease) is one of the diseases that is characterized by calcium deposits in kidney especially in renal tubules, pelvis, ureter and urinary bladder [1]. The number of kidney stone disease cases has increased in the last five decades in this world. Most of kidney stone is caused by calcium oxalate accumulation [2]. Kidney stone formation

consists of several stages including supersaturation, nucleation, growth, aggregation, and retention within renal tubules [3].

Urine supersaturation condition allows for interaction between oxalate and renal tubular cells, that can lead to cell damage caused by free radical or reactive oxygen species (ROS) activities. The formation of free radicals due to the interaction between oxalate and renal tubular cells will result in some lipid signaling that is responded by mitochondrion with the increase of free radical [4]. The increase of free radicals is followed by the increase of lipid peroxidation

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product, malondyaldehyde (MDA), and the decrease of antioxidant enzyme activity such as SOD, catalase, and GPx [5]. The involvement of free radicals at the calcium oxalate accumulation creates cell debris of dead cells for nucleation that triggers calcium oxalate accumulation [6]. The presence of calcium oxalate in kidney that caused by administration of porang (*A. muelleri*) tuber flour can trigger an increase of MDA in kidney, in which thin free radicals cause cell damage characterized by necrosis [7, 8]. This accumulation also increases the plasma and hepar of MDA [9].

Calcium oxalate accumulation in the kidney also affects blood and urine creatinine level, in which calcium oxalate accumulation can increase blood creatinine level, and decrease urine creatinine level. However, treatment using herbal agent that contains antioxidants can reduce blood creatinine level and increase urinary creatinine levels in mice with accumulate calcium oxalate level [2]. This suggests that creatinine may be associated with renal physiology function [10].

Individuals who have formed a kidney stone are more likely to form another, so we need a treatment to prevent formation of another stones. Treatment using herbal agent to break down kidney stones is suggested since the medicine has no side effect. Calculosol™ is a traditional medicine or jamu that has been used to break down kidney stones. Calculosol™ is made from tempuyung (*Sonchus arvensis*) leave extract. However, the cellular mechanisms of decaying kidney stones remain unknown. Based on the research by dr. Sardjito (1964), the action of Calculosol™ goes directly on kidney stones. However, the mechanism of Calculosol™ action on breaking down kidney stones is still unknown. Therefore, in this study we want to examine the physiological mechanism of Calculosol™ in reducing kidney stones. In the present study, we examine the way Calculosol™ decreases plasma MDA or free radicals and blood creatinine levels of mice (*Mus musculus*) induced with calcium oxalate accumulation.

MATERIALS AND METHODS

Mouse Model Induced by CaOx accumulation

This experiment used mice (*Mus musculus*) as the animal model. Twenty-five Swiss male mice (8 weeks old), weighing 25-30 g were divided into 5 groups, each group consisted of 5 mice. Mice were obtained from LPPT Gajah Mada University. To induce CaOx accumulation in mice, porang (*A. muelleri*) was orally administered in mice for 3 month with doses of 6mg/100 g BW [8]. The occurrence of nephrolithiasis is characterized by calcium oxalate accumulation in kidney.

Calculosol™ Treatment

The animals were divided into 5 groups. Group I was as a control. Group II was orally given porang every day for 3 months. Group III was given Calculosol™ for 7 days. Group IV were given porang for 3 months then given Calculosol™ for 7 days. Group V were given porang (*A. muelleri*) and Calculosol™ simultaneously for 3 months. Calculosol™ were orally given to mice at doses 3.3 mg/g BW for 7 day.

Serum Creatinine Assay

At the end of experiment, blood was taken from tail mice, and then put in a tube *ependof*. Blood samples were incubated at 37°C for 30 minutes and centrifuged at 7500 rpm for 10 minutes at 4°C. Supernatant was used for creatinine assay in Saiful Anwar Hospital Malang by enzymatic method using Kobas 501 Kit.

Measurement Plasma Free Radical using TBARS Method

Malondyaldehyde (MDA) content was determined using thiobarbituric acid reactive (TBARS) method [2]. Mice blood was centrifuged at 7250 rpm. Plasma were taken as 100 µL and dissolved in 900 µL tris buffer pH 7 and added 50 µL TCA 100% and 125 µL of 1N HCl. The samples were centrifuged at 1800 rpm for 20 min at 4°C. The supernatant was added 50 µL Na-Thio 1, incubated 100 ° C for 30 minutes using water bath, then cooled in room temperature. The samples were measured at a wavelength of maximum absorbance (533 nm) using spectropho

tometer UV-Vis.

Analysis Data

The data was analyzed using a test of variance (ANOVA) technique with $P < 0.05$ and the Tukey. HSD test was used to compare the means. All the tests employed SPSS 16 for windows.

RESULTS AND DISCUSSION

MDA is one of lipid peroxidation products that is usually used as a biomarker of oxidative stress in the cells. Figure 1 shows statistically significant reduction of plasma MDA level at the Calculosol™ treatment to control group. The contents of MDA are 0.81 ± 0.5 mg/ml; 2.63 ± 0.8 mg/ml; 0.56 ± 0.5 mg/ml; 2.09 ± 0.9 mg/ml and 0.17 ± 0.17 mg/ml in group I, group II, group II, group IV, and group V, respectively. There is no significant difference of MDA level at group V, group II and group I.

From this result, it is known that the treatment of Calculosol™ on mice model of calcium oxalate accumulation can reduce MDA (malondyaldehyde) as an indicator of stress oxidative in cells caused by lipid peroxidation. Figure 2 shows the blood creatinine level at all groups in this experiment. The levels of blood creatinine are 0.15 ± 0.04 mg/dL; 0.13 ± 0.03 mg/dL; 0.12 ± 0.08 mg/dL; 0.11 ± 0.016 mg/dL and 0.14 ± 0.015 mg/dL in group I, group II, group II, group IV, and group V respectively. There was no significant increase in the blood creatinine levels between all groups.

The oxalate induced membrane peroxidation leads to membrane integrity loss, renal cell damage and finally, calcium oxalate crystal deposition. In this study, the Calculosol™ treatment decreased the lipid peroxidation and reverse the effects of oxalate on oxidative stress parameters. Accumulation of calcium oxalate in the body, especially in kidneys [11], such as porang (*A. muelleri*) consumption tuber flour for a long time (three months) may cause calcium oxalate accumulation in kidneys and also increase of free radicals [8]. Before entering the kidneys, calcium oxalate will be absorbed by the intestinal cells, and intact through passive transport pathways, and further carried by the blood

circulatory system and further carried by the blood circulatory system [12]. When calcium oxalate enters kidneys, the activity of oxalate binding protein will increase. This protein plays role in the nucleation process in the kidneys crystallization [3]. This condition generates interaction between oxalate with renal cells. Besides that, urine supersaturation condition allows for interaction between oxalate and renal tubular cells that leads to crystallization process. The super saturation urine is a condition in which urine have high amount of calcium, phosphate, and oxalate will cause the formation of calcium oxalate crystal. Thus, the oxalate crystal will bind the renal tubules cell and generate interaction between renal tubular cells and oxalate [13].

Exposure to oxalate elicits will cause changes in renal plasma membrane that can lead to cell injury including endocytosis and retribution of phosphatidylserine membrane from the inner layers of the cells to the surface of the cells. This condition promotes crystal attachment to the renal cell. The interaction between oxalate and renal tubular cells, leading to membrane changing include endocytosis of crystal and retribution of phosphatidylserine generating lipid signaling. This signaling will be responded by the mitochondria with the increase of ROS and cytochrome C, reduced GSH activity, and decrease potential membrane. The net response to oxalate exposure can be cell death or apoptosis [14].

Free radicals are one of the molecules having free electron, and are generally short lived, and thus inflict damage only in the local environment where they are produced. The cytotoxic aldehydes are extremely active; they can diffuse within or even escape from the cell and attack targets far from the site of the original free radical initiated event, resulting in cell damage [15]. Malondialdehyde (MDA) is a product of lipid peroxidation and its measurement serves as an indicator of free radical damage. From the analysis of this experiment, high level of MDA is seen only in nephrolithiasis mice. This suggests that the potential ability of Calculosol™ to reduce the greater impact of free radical damage by interacting with hydroxyl radical and enhancing the suppression of free radical

Figure 1. Malondialdehyde content after supplemented with Calculusol™

Figure 2. Creatinine Level after supplemented with Calculusol™

mediated lipid peroxidation. Free radicals are produced in kidney will diffuse into the blood vessels. Thus, the content of MDA in the mice blood is high by the accumulation of calcium oxalate compared with controls. [9] Suggests that the accumulation of calcium oxalate that caused by the consumption of porang tuber can increase the MDA level of blood plasma. In other studies, it is also noted that increasing levels of MDA will happen not only in the kidney, but also in the blood plasma [16, 17]. However, the Calculusol™ treatment can reduce free radicals, characterized by the decrease of MDA levels compared with mice that accumulate calcium oxalate ($p < 0.05$). Calculusol™ is possible to have antioxidant that can neutralize free radicals formed in the kidney because Calculusol™ is made from herbal agent of Tempuyung (*Sonchus arvensis*) extract.

The assumption of oxidative stress as a mechanism in oxalate induced renal damage suggests that antioxidants might play a beneficial role against oxalate toxicity. Creatinine is one of breakdown product from creatin. At the normal condition, creatinine will be discarded from the body by the kidneys through urine. When the physiological function of kidney impaired, the renal creatinine will be distributed in the blood. So, the level of creatinine in the blood will increase [10]. This indicates that blood creatinine levels describe the physiological function of the kidney. Plasma or serum creatinine is the

routinely used marker for the assessment of renal function [18]. Super saturation of urine with CaOx, the most common component of kidney stones is an important factor in crystallization [19] and enhanced urinary creatinine levels are indicators of renal impairment [20]. In the present study, porang tuber administration does not show such significant difference between all group including the treatment using Calculusol™. However, in other studies, rat on ethylene glycol administration experience an increase in the level of creatinine [21]. Other studies show that glyoxylate administration at mice is capable to increase blood creatinine levels and also reduce urine creatinine level. The accumulation of calcium oxalate in the kidneys causes a decrease in the physiological function, and affects the metabolism of creatinine in the body. This suggests that the accumulation of calcium oxalate influences kidney physiological condition in creatinine clearance. When creatinine is excreted in the urine should actually be distributed in the blood resulting in decreasing urine creatinine levels. Nevertheless, the treatment with herbs can be seen as an effort to prevent the formation of calcium oxalate accumulation in kidneys which can restore creatinine level to its original state [2].

CONCLUSIONS

Calculusol™ can decrease free radical levels during renal stone formation in mice model of

calcium oxalate accumulation. It is characterized by the decrease of MDA levels. This suggests that the potential ability of Calcosol™ is to reduce the greater impact of free radical damage. Calcosol™ is possible to have antioxidant that can neutralize free radicals formed in the kidney, because Calcosol™ is made from herbal agent of Tempuyung (*Sonchus arvensis*) extract. There was no significant increase in the blood creatinine levels between all groups in the experiment.

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