Renal Function Parameter on Acute Toxicity Test of Kapulaga (Amomum cardamom) Seed Extract in Rat

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Abstract. Kapulaga (Amomum cardamom) is one of herb which is commonly used for spices or medicine, especially the seeds. Previous studies showed that the essential oil of cardamom have antimicrobial, antinflammatory, analgesic and antispasmodic activities. There was also evidence that A.cardamom has antiatherogenic and anti diabetic activity in rat. Despite the medicinal benefits of A.cardamom, this herb is not yet standardize for herb medicine. For its standardization, A.cardamom has to pass the preclinical and clinical studies to ensure its efficacy and safety profile. The aims of this study was to examine the safety profile of A.cardamom seed extract based on renal parameter function (ureum and creatine levels).

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 preliminary test, initially with 300 mg/kg BW dose of A.cardamom seed extract, followed by 2000 mg/kg BW. The main test consists of control and treatment group and each group used 5 rats. The rats in both groups were given 2000 mg/kg BW in a single dose of A.cardamom seed extract. The ureum and creatinine levels were assessed at day 14th using an enzymatic-photometric method. The data were analyzed by independent sample test. The results revealed that the ureum and creatinine levels in control and treatment groups were not statistically different. The mean of ureum levels in the control and tretment groups were 47.24±6.18 and 43.98±6.78 (p=0.45) and the mean of creatinine levels were 0.43±0.04 and 0.36±0.12 (p=0.31) respectively. These results show that a high single dose of A.cardamom seed extract (2000 mg/kgBW) did not toxic in rat based on renal function parameters.

1. Introduction
The health-promoting properties of many herbs and spices are numerous and well recognized. Moreover, herbs and spices have a traditional history of use with strong roles in cultural heritage [1,2]. One of herb which is commonly used for spices or medicine is cardamom. Cardamom is a famous aromatic spice and widely used in Eastern, Arab, and Scandinavian culinary. Moreover, this herb is also common as ingredients of Indian ayurveda and Chinese traditional medicine [3]. In Indonesia, cardamom not only use for culinary purposes but also commonly use as medicinal herb [4]. Cardamom belongs to the ginger family Zingiberaceae and consist of two genus, Elletaria and Amomum. Both forms are small spindle-like seedpod, but they are difference in size and appearance.
Elettaria is smaller and light green in colour but Amomum is bigger and its appearance is blackish grey [3].

Many studies have shown that cardamom plays an important wide range of health promoting roles against various pathologic conditions such as constipation, colic, diarrhea, dyspepsia, vomiting, headache, epilepsy, and cardiovascular diseases [1]. Previous studies revealed that Amomum cardamom leaf extract have an activity to decrease the atherogenic index and blood sugar levels in diabetic rats model [5]. Whilst, Elletaria cardamom was reported have many properties such as antibacterial, antiviral and antifungal [6]. Others studies reported that the essential oil of cardamom have antimicrobial, antiinflammatory, analgesic and antispasmodic activities [7]. All this collected evidences support the potential development of A. cardamom as standard herb medicine. Despite the medicinal benefits properties of A. cardamom, this herb is not yet standardize for herb medicine. For its standardization, A. cardamom has to pass the preclinical and clinical studies to ensure its efficacy and safety profile.

The kidney (renal) is a major target for drug-induced toxicity due to its function as the major organ of excretion. Kidney are naturally exposed to a greater proportion of circulating drug and chemicals compound and plays an important role as the primary eliminator of exogenous drugs and toxin. It makes the kidney is vulnerable to develop various form of injury. Renal toxicity has been reported for various agents including a large number of drugs and leads to acute kidney injury which is associated with high morbidity and mortality [8,9]. An important and unregulated source of potentially renal toxic substances is the alternative/complementary products, include herbal remedies, natural products and nutritional supplements that are commonly used in community and widely available at most health food stores. Moreover, interaction of herbal products with conventional drug is also potential source of renal toxicity [9]. The development of drug with minimal potential renal toxic effect would be one strategy to prevent these problem [8].

The uses of A. cardamom as medicinal plant are common, but there was lack of a proven studies on the toxicity of these treatment, to our knowledge. This study aims to examine the safety profile of A. cardamom seed extract based on renal parameter function (ureum and creatine levels). This study was conducted in order to obtain scientific evidence related to A. cardamom toxicity, needed for its development as standardized herbal medicine as well as for developing herbal medicines with less potential of renal toxic effect.

2. Experimental

2.1 Acute Toxicity Test

Pharmacological and toxicological evaluations of medicinal plants are essential for drug safety and development. Toxicity study of medicinal plants is important in predicting their safety. The main types of toxicological evaluations include: acute toxicity, subacute toxicity, subchronic toxicity, and chronic toxicity studies. Acute toxicity of medicinal plant extract refers to those adverse effects occurring following oral or dermal (topical) administration of a single dose of a substance or multiple doses given within 24 hour. There are some methods in acute toxicity test, and one of them is fixed dose procedure[10].

The acute toxicity test in this study was conducted based on the OECD 420 fixed dosed procedure that consist of two test steps, preliminary and main test. OECD 420 fixed dose procedure is one of OECD guidelines for testing chemical compound which avoided death of animal as endpoint to asses the acute toxicity and relied instead on the observation of clear signs of toxicity at one of a series of fixed dose levels. The preliminary test used initial dose of cardamom seed extract 300 mg/kg body weight because based on this procedure, if there was no information from any in vitro or in vivo study for standard dose which expected to show toxicity evidence of the testing compound, the preliminary dose use 300 mg/kg body weight as initial dose [11]. Using this initial dose, the rat survived, so after 24 hours from the first treatment, the dose was increased to 2000 mg/kg body weight as maximal dose in OECD 420 fixed dose procedure [11]. The main test consists of two groups, control group (5 rats)
and treatment group (4 rat and 1 rat from preliminary test). Treatment group was given high single dose of cardomom seed extract based on preliminary test (2000 mg/kg BW).

2.2 Renal Function Parameters
In the 14th day, the renal function parameters (ureum and creatinine) were assessed. Blood samples were collected from rat’s orbital vein (3 cc) of the control and treatment groups. Ureum and creatinine level were measured using enzymatic-photometric method. All the collected data were statistically analyzed with shapiro wilk and independent sample t-test.

3. Results and Discussion
In recent times, plant origin drugs play a vital role in the management of various chronic disease and have received a great attention by researcher as alternative source to pharmaceutical drugs. Moreover, a wide range of natural products isolated from herbs and spices have been shown to posses immunomodulatory effect that potentially have beneficial against many diseases. Whilst, there is a lack of proven scientific studies on the toxicity and adverse effect of these treatments [1,12].

As mentioned before, cardamom is one of the common ingredients and well-known aromatic spice that widely used as herbal medicine for many condition such as for improving digestion, stimulating metabolism and exhibit antioxidant and antiinflammatory effects [3,13]. The kidney is routinely assessed during preclinical safety evaluation due to its important role as a central detoxification organ. It leads an extraordinary exposure of renal tissues to drugs, reactive metabolites or environmental compounds [14,15]. In this present study, we conducted an acute toxicity test to observe the safety profile of cardamom seed extract based on renal function parameter (ureum and creatinine).

Table 1. showed that A.cardamom seed extract which was given to rats as a high single dose (2000 mg/kgBB) may not be toxic based on renal function parameter. This was assessed from ureum and creatinine levels between the control and treatment group which were not statistically different (p > 0.05) and mortality was not observed in any of the experimental groups.

| Renal Function Parameters | A.cardamom seed extract |
|---------------------------|-------------------------|
|                          | Control Group (0 mg/kgBW) | Treatment Group (2000 mg/kgBW) | P |
| Ureum(U/L)               | 47.24 ± 6.18             | 43.98 ± 6.78                  | 0.45 |
| Creatinine (U/L)         | 0.43 ± 0.04              | 0.36 ± 0.12                  | 0.31 |

Results are expressed as the mean ± SD (n=5)

Ureum and creatinine are two important chemical substances in blood which can be evaluated to help assessing the Glomerular Filtration Rate (GFR) and renal function. The elevated level of ureum and creatinine in blood can be used to assess the progression of kidney damaged. Ureum is an organic compound and plays a vital role in the metabolism of nitrogen-containing compounds. Urea is filtered by the kidneys into urine as a waste product from dietary protein. Healthy kidney remove this compound from blood and its level will increase when the kidney failure occurs [14]. Creatinine is the breakdown product of creatinine phosphate in muscle and is excreted through the kidney along with other waste products. The balance of creatinine concentration between its generation and excretion is maintained by the kidneys at a fairly constant rate. The quantity of creatinine in serum depends on their generation, glomerular filtration and tubular secretion of this compound. Both serum ureum and creatinine are widely accepted as important biomarkers to assess renal function due to its pivotal role in the diagnosis and follow up of kidney failure [14,16,17].
Increased creatinine levels and blood urea nitrogen simultaneously indicate impaired renal filtration [18]. Blood urea nitrogen levels indicate urea levels in the blood. As mentioned above, urea is one of the body's disposal products. Urea is produced when the liver metabolizes proteins and eliminates through the kidneys. The body will maintain urea levels in the blood to stay normal so that the liver and kidneys should function normally [19].

Table 1 demonstrated that, urea level between control (47.24 ± 6.18) and treatment group (43.98 ± 6.78) were not statistically different (p=0.45). Moreover the creatinine level in both control (0.43 ± 0.04) and treatment (0.36 ± 0.12) groups were still in normal range based on study by Derelanko[18] which reported that the normal creatinine level in rat is in range 0.3-0.8 mg/dl. Cretinine is considered as one of the most reliable indicators of renal function and the increasing of blood creatinine is strongly related with renal damaged [20]. These evidence supported that acute high single dose of A. cardamom did not alter the renal function, since no changed observed in urea level and the creatinine level is still in normal range.

This study revealed that acute high single dose of A. cardamom did not alter the blood urea and creatinine level compared to control. Whist, Table 1 showed that both urea and creatinine level in the treatment group (43.98 ± 6.78 and 0.36 ± 0.12) were slightly lower than those in control group (47.24 ± 6.18 and 0.43 ± 0.04), although there were not statistically difference. It might be due to the antioxidant properties of A. cardamom. Chemical analysis of cardamom showed, it contains antioxidant flavonoid which observed have effect to induce glutathione S-transferase (GST) and to reduce the lipid peroxidation in Swiss Albino Mice [21]. In accordance with this, Lim et al.[22], reported that ethyl acetate fraction of A. cardamom has a high content of polyphenol and flavonoid compounds, which was proven plays a role in decreasing lipid peroxidation in liver tissues.

In addition, several studies demonstrated that most of the components of cardamom act as antioxidants such as limonene, cineole, linalool, pinene and borneol. Cardamom also contains Cu and Mn that are required for the activation of superoxide dismutase (SOD) enzyme which play an important role in the reducing of lipid peroxidation process [3]. This antioxidant activities of cardamom is also likely to occur in the kidneys and protect them from damage.

4. Conclusion
This present study suggested the safety profile of a high single dose of A. cardamom seed extract (2000 mg/kgBW) in rat based on renal function parameters.

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