SUB CHRONIC EFFECT OF ETHANOL EXTRACT OF NUTMEG (*myristica fragrans* Houtt) SEED IN RAT KIDNEY

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Abstract

Background. *Nutmeg (Myristica fragrans) is original plant from Indonesia has sedative, hepatoprotective, and anti-seizure activities.*

Objective. *The aim of this study was to determine the toxic effects of ethanol extract of nutmeg seed on the rat kidney.*

Methods. *Male rats grouped into 4 groups and each group consists of 7 animals. Group I as the control group 0.5% CMC-Na. Group II, III, and IV were given ethanol extract of nutmeg dose of 50 mg / kg, 100 mg / kg, and 200 mg / KgBW respectively. Samples were administered for 31 days, on the 32th day was taking blood from the orbital sinus eye for BUN, creatinine assay and histopathology examination.*

Outcome measured. *BUN, creatinine, and histopathological profile*

Results. *The results showed all doses of ethanol extract of nutmeg seed causes an increase BUN levels. While on the creatinine levels between the control group and the treatment group showed no significant difference. Histopathological examination showed no change in renal morphology was given ethanol extract of nutmeg seeds.*

Conclusion. *The conclusions this research that the ethanol extracts of nutmeg (Myristica fragrans Houtt) seed did not cause toxic effects on the kidney.*

Keyword : *Biji Pala (**Myristica fragrans** Houtt.), BUN, Creatinine, Histopathological morphology*

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INTRODUCTION

Nutmeg (Myristica fragrans) is original plant from Indonesia, which originated from the archipel Malaise Banda and Maluku islands then spread and grow into islands such as Sumatera, North Sulawesi, and Papua. Nutmegs including Family Myristicaceae grow to five genuses of 250 species (Drazat, 2008). Olaleye *et al.* (2006) reported nutmeg seed has antioxidant activity, anti diarrhea, carminative and stimulant. Also has activity as sedative (Sonavane *et al.*, 2001), anti microba (O'Mahony *et al*., 2005), anti depressant (Dhingra and Sharma, 2006), anti diabetic (Han *et al*., 2008), aphrodisiaca (Tajuddin et al., 2005), *cytotoxicity* (Lee *et al*., 2005), hepatoprotective (Morita et al., 2003). Nutmeg seed induces apoptosis in leukemia cells (Chirataworn *et al*., 2007), anti inflammatory (Lee and Park, 2011), anti hyperlipidemia (Kareem *et al.,* 2009).Sonavane *et al*. (2002) proved nutmeg seed has anticonvulsant activity. Ethanol extract of nutmeg has anti-seizure activity in mice-induced pentylenet etrazoles (Saiful bachri,M.and yuliani,S., 2011). Nutmeg has pharmacological activity, also has toxic effect. Aromatic compounds myristicine, and safrole elimycine are found 2-18% in seeds and are inducing hallucinations. Maximum consumption of 5 grams of powder or nutmeg oil lead poisoning is characterized by vomiting, headache and dry mouth (Nurdjannah, 2007). Myristicine and elimycine have toxic effect (Jukic *et al.*, 2006).

Materials or medicinal compounds of plant or animal must go through a series of pre-clinical tests such as pharmacology tests, toxicology testing and clinical trials for use in formal treatment. One of the toxicology tests are subchronic toxicity tests to get information of potential toxicity when used in long term. Among much toxicity evaluation parameters are biochemical parameters such as creatinine and blood urea nitrogen are useful to evaluate the condition of the kidney (Henry JB. 2001,).

It is necessary to study sub-chronic administration of ethanol extract of nutmeg and investigated the kidney histopathology.

MATERIALS AND METHODS

Animals

Male wistar (SD) (200±10 g) rats were purchased from Gadjah Mada university. All animals were maintained in the institutional animal facility. Animals were acclimatized for a week before starting the experiments with condition, light/dark cycle: 12 hr, in university animal room and with free access to rodent food and water ad libitum throughout the experimental period.

Preparation of the extract

Nutmeg seed powder was purchased from a Beringharjo market in Yogyakarta Indonesia. Samples were processed into extract in Biology Pharmacy Department of faculty of Pharmacy, Ahmad Dahlan University Yogyakarta, Indonesia. The 3 kg of powder dried samples was dissolved three times in 6 liters of ethanol for 3 days, filtered, and evaporated to obtain the crude ethanol extract (25 g). The crude ethanol extracts freeze dryer for 3 days to get 15 g dried powder.

Animal Groups and experimental treatment

Animals were divided into four groups with seven animals in each group; Group I, Control rats treated with the vehicle only (CMC-Na 0.5%); Group II, rats treated with EtOH extract of Nutmeg Seed (50 mg/kg); Group III, rats treated with EtOH extract of Nutmeg seed (100 mg/kg); Group IV, rats treated with EtOH extract of Nutmeg seed (200 mg/kg). On day 32th, take the blood from orbital sinus for analysis of creatinine and BUN activities with spectrophotometer. Then the rats sacrificed, take the kidney for histopatological examination. Histopathologic examination was performed in Pathology Laboratory Animal of Veterinary medicine faculty, Gadjah Mada University to

determine the possibility of damage to the kidney.

RESULT AND DISCUSSION

BUN dan Creatinine Analysis

Table I showed the average BUN levels between the control group and the treatment group there is a difference or increasing. Increased BUN levels can be caused by loss of extracellular fluid and plasma volume (such as bleeding, shock, excessive vomiting, lack of water, salt), which decreased glomerular filtration rate but no kidney disease. BUN levels can also be increased with an increase in protein catabolism as occurs in the gastrointestinal tract bleeding or tissue (Aslam 2003). Another factor that led to increased BUN levels, the lack of nutrients and hepatotoxicity are common effects of some toxicant (Lu, 2010).

Increased levels of BUN in nutmeg ethanol extract at a dose of 100 mg / kg and 200 mg / kg body weight may be due to damage to the kidneys according to existing studies showed elevated levels of BUN at a dose of 400 mg / kg - 1000 mg / kg (Eweka, *et al.*, 2010; Olaleye *et al*., 2006). Whereas at a dose of 50 mg / kg had no increasing effect when compared with controls.

Urea is the final product of amino acid catabolism. In the process of solving the amino acids will form ammonia compounds that are toxic to the human body. Furthermore ammonia will be converted into non-toxic compounds, namely in the form of urea through the urea cycle formation. Urea in the blood will be reabsorbed into the renal medulla and immediately excreted through the urine (Poedjiadi *and* Supriyanti 2006). The presence of urea in the blood (calculated as Blood Urea Nitrogen, BUN) and urea in the urine can be used to determine the effectiveness of renal function (Lu, 2010). On the condition of impaired renal function, plasma urea concentration increases because of the decrease in glomerular filtration process (Anonymous, 2006b).

Table I show that the average creatinine levels between the control group and the treatment group showed no significant difference. Normal levels of creatinine in mice are about 0.2-0.8 mg / dl (Malole and Pramod, 1989). The result on creatinine, the average levels of creatinine showed all the groups are still in the normal range. Creatinine is a metabolite of creatine and excreted entirely in the urine via glomerular filtration. Thus, increased levels of creatinine in the blood are an indication of damage to kidney function (Lu, 2010). Serum creatinine is considered to be more sensitive and specific indicator of kidney disease compared with BUN test. The increase in creatinine levels themselves are not affected by the intake of food or drink (Kee, 2008).

The results of this research of BUN and creatinine levels can be seen that the ethanol extract of nutmeg seed cause elevated levels of BUN in the dose group 100 mg / kg and a dose of 200 mg / kg compared with the control group (CMC-Na), whereas serum creatinine levels did not increase or decrease that considered normal. Increased levels of BUN with normal creatinine levels usually become clues to the cause of non-renal uremia. Increased levels of BUN values could be due to loss of extracellular fluid and plasma volume as lack of water or salt and can also occur due to increased protein catabolism that occurs in the gastrointestinal tract or body tissues (Aslam, 2003).

Synthesis of creatinine is relatively constant, therefore the blood creatinine levels can describe of renal creatinine clearance. Creatinine excretion was excreted through glomerular filtration process of kidney. If serum creatinine levels rise mean creatinine clearance decreased (Wildmann, 1995).

The research shows that the value of normal creatinine levels, not an increase, so that the ethanol extract of nutmeg is subchronic for 31 days did not affect renal function, seen from the blood chemistry parameters levels of urea and creatinine values, because the normal creatinine levels indicate that the kidneys are also normal. creatinine clearance suggests that

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Table I. Mean ± SD of BUN dan Creatinin Level caused nutmeg seed administration

Group	Dose (mg/KgBW)	BUN (mg/dl)	creatinine (mg/dl)
Kontrol	CMC-Na	39.47 ± 4.24	0.8 ± 0.15
EtoH extr.	50	42.95 ± 3.92	0.8 ± 0.16
	100	45.54 ± 2.12 *	0.7 ± 0.12
	200	44.88 ± 3.84 *	0.8 ± 0.14

* different compare with control group p<0.5

the glomerular filtration rate. The Circumstances where the normal creatinine levels while increasing BUN levels can be caused by lack of fluids in the body or dehydration (hypovolemia) (Kee, 2008).

Histopathological examination showed no change in renal morphology was given ethanol extract of nutmeg seeds (picture not shown).

The Conclusions this research that the sub chronic administration of ethanol extracts of nutmeg (Myristica fragrans Houtt) seed did not cause toxic effects on the kidney.

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