

Effect of Ethanol Extract of Celery (*Apium graveolens* L) against Urea and Creatinine Level in Male Wistar Rats on Ethylene Glycol Induced Nephrolithiasis

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Abstract: The use of celery in Indonesia was known as cooking spice to enrich the taste of food, has not been widely used as herbal medicine either in the form of juice or extract. The Pre-clinical testing of celery herbs in addition to diuretics it is also to treat sore eyes, sprains, rheumatism, hypertension, and as a hair tonic. The celery Herb is rich in the content of flavonoids and potassium that can be used to dilute kidney stones. The aim of this study was to investigate effect of ethanol extract of celery (*Apium graveolens* L) against urea and creatinine level in male wistar rats on ethylene glycol induced nephrolithiasis. In vivo test was performed male wistar which divided into 6 groups. Group I as normal control without any treatment, Group II as negative control was induced ethylene glycol, Group III as positive control was given Batugin Elixir, Group IV, V and VI were given ethanol extract of celery a dose of 50, 100 and 150 mg/kgBW respectively. Blood was collected by cardiac puncture then measured level of urea and creatinine. The data were analyzed statistically using ANOVA (analysis of variance). The results showed that ethanol extract of celery has an effective activity at a dose of 150 mg / kgBW ($P < 0.05$) to negative group in decreasing level of urea and creatinine male wistar rats on ethylene glycol induced nephrolithiasis and it showed a dose-dependent manner.

1 INTRODUCTION

Urolithiasis is a formation of crystal stone that formed in the kidney and known as nephrolithiasis, its stone also found in the urinary tract, bladder and ureters (Selvam et al., 2001). The prevalence of urolithiasis >10% with expected recurrence rate of >50%. The incidence of nephrolithiasis in worldwide is high, and around 80% of nephrolithiasis cases are found as form of calcium oxalate or mixed with stone of calcium phosphate (Brikowski et al., 2008). Epidemiological studies reported that the cases of nephrolithiasis disease are higher between the ages of 20–40 in both in men and women, men is more prevalent as 12% than in women as 6% (Lopez et al, 2010).

Various study regarding to nephrolithiasis disease has been investigated, such as radiological treatment and laboratory determination. Both are not efficiently to determine the mechanisms and causes of stone deposite in the kidney and urinary tract (Tiselius et

al., 1980). However, some factors that would be responsible induced kidney stone formation have been studied recently (Curhan et al, 1997). It is reported that the urine is saturated with material compound which is a insoluble substance, it leads to formation of crystals and aggregate into stone form (Sakhaee et al, 1993). The nephrolithiasis needs curative and preventive treatment. Nowadays, there are no good modern medicine, that can dissolve stone form in the part of kidney or urinary tract, therefore the physicians is considering to alternative medicine or treatment to heal the nephrolithiasis disease (Coe at al, 1992). The other medical treatment of nephrolithiasis was surgical removal treatment of the stones (Uribarri et al, 1989). Recently treatment including extracorporeal shock wave lithotripsy (ESWL) and percutaneous nephrolithotomy (PCNL) are not guarantee the recurrence of the disease (Srisubat et al., 2009). Moreover, it can cause various side effects such as bleeding, increasing blood

pressure and also kidney fibrosis (Wadhwa et al, 2007). In the traditional cure, most of the sources were taken from plants which has been proved empirically in community that it is believe to heal many disease. Herbal medicines are known have lower side effects and efficacious than modern medicine and it also reduce the prevalent of the recurrence cases. Phytochemical properties such as flavonoids spread widely in plants, and its biological effect such as antioxidant have been reported in various studies such as, cancer, coronary heart diseases and diabetes (Hopper et al., 2008). Herbal Medicine have lower side effects compare to chemical modern drugs because its antioxidant activity has a role in lowering the toxicity of drugs (Bent et al, 2004). Today herbal medicine are used as an alternative treatment to chemical drugs including in treating nephrolithiasis (Butterweck and Khan, 2009). Recent studies reported that the high content of flavonoids plant could effectively inhibit the formation of kidney stone in form of CaOx stones in vivo and in vitro, correlating with its biological activity such as anti-inflammatory, diuretic, antioxidant, antibacterial properties and other protective activity (Rad et al, 2011). Based on the explanation, it showed that the flavonoids or flavonoid-rich plant extracts has potential activity as anti-nephrolithiasis.

Celery (*Apium graveolens* L) is a apiaceae family plant, and grow in Europe and the tropical and sub tropical regions of Africa and Asia. Beneficial compounds are found in celery such as flavonoids vitamins A and C, furocoumarin glycosides, limonene and, selinene (Sowbhagya, 2014). Flavonoids content of celery are the reason that celery has potential as anti-urolithiasis activities

2 MATERIAL AND METHODS

2.1 Chemical

Ethanol 96%, aqudest, ethylene glycol, ammonium chloride, Creatinine kit, Ureum kit

2.2 Plant Collection

Celery herb (*Apium graveolens* L) was collected from local market at Padang Bulan, Northern Sumatra, Indonesia. The plant samples authenticated by Indonesian Institute of Science, Research Center of Biology, Bogor, Indonesia.

2.3 Extraction of Celery Herb

An amount of 500 g dried Celery herb were crushed in a blender, then macerated in ethanol 80 % for 5 days thereafter continue to remacerated for 2 days. The solvent was evaporated at low pressure with a temperature of not more than 40 ° C using a Rotary evaporator, then dried using freeze dryer (Nugraha et al, 2018).

2.4 Phytochemical Screening of Ethanol Extract Celery Herb

Phytochemical screening carried out on ethanol extract celery herb includes examining the chemical secondary metabolites of alkaloids, flavonoids, glycosides, tannins, saponins, triterpenoids, and steroids

2.5 Animals and Blood Sample

An amount of 30 male wistar rats used in this study, weighing around of 180-220 g. The blood sample was collected by cardiac puncture.

2.6 Experimental Design

The animals were given solution of 0.75% ethylene glycol and also solution of 2% ammonium chloride as much as 1% body weight orally to induce Nephrolithiasis for 14 days.

Group I : as normal control

Group II : as negative control (Na-CMC 0,5 %)

Group III : as positive control. Rats were received Batugin Elixir 0.5 ml/ 200 g BW

Group IV : Rats were received ethanol extract of celery herb at a dose of 50 mg/kg BW

Group V : Rats were received ethanol extract of celery herb celery at a dose of 100 mg/kg BW

Group VI : Rats were received ethanol extract of celery herb at a dose of 150 mg/kg BW

Treatment was given orally after ethylene glycol induced. Treatment were given as long as 10 days, than in the day of 11, the blood was collected from cardiac to determine the level of urea and creatinine. Determination level of urea and creatinine were measured using "Cobas Integra".

2.7 Statistical Data Analysis

Data were analyzed using ANOVA and continue to Tukey's Multiple Comparison Test. Significance *P* values were set at 0.05. Values for all measurements are expressed as the mean ± SD

3 RESULT

3.1 Phytochemical Screening

Qualitative analysis of the various phytochemical properties in the celery herb were done by qualitative determination methods. the celery herb contains of glycosides, alkaloids, steroids, tannins and flavonoids compounds.

3.2 Level of Urea and Creatinine

Level of urea and creatinine was illustrated the effectiveness of ethanol extract of celery herb to inhibit nephrolithiasis that caused by ethylene glycol induction. The level of urea and creatinine showed in table 1 and table 2. Some study showed that ethylene glycol induced the formation of kidney stone form in albino Wistar rat. The process of supersaturation in the urine which is contact to various forming compound is the major factors in the kidney stone deposition (Brikowski et al., 2008). Using ethylene

glycol as an inducer of kidney stone formation is the most suitable method and it is similar to kidney stone that found in humans which cause the kidney damage (Brikowski et al., 2008). Degradation of renal function characterized by elevated levels of urea and creatinine (Meier et al, 2004). In the present study, we investigated the effect of celery (*Apium graveolens* L) ethanol extract against urea and creatinine level in male wistar rats on ethylene glycol induced nephrolithiasis.

Urea is the final product of protein metabolism and amino acids containing nitrogen One of the important roles of the kidneys is to eliminate these potentially toxic substances from the body. If there is a decrease in kidney function, the blood urea nitrogen level (BUN) increases (Meier et al., 2004). BUN measurements was one of kidney health marker. Measurement of blood serum urea level of rats was done to determine the effect of induction of ethylene glycol 0.75% and 2% ammonium chloride to kidney damage, and the effect of variation dose ethanol extract celery herb on blood serum urea level of rat.

Table 1: Level of urea

No	Group	Level of urea					Mean \pm SD
1	Normal control	40	41	41	42	39	40.6 \pm 1.1*
2	Negative control	57	56	58	55	56	56.4 \pm 1.1#
3	Positive control	43	44	42	42	43	42.8 \pm 0.8*
4	Extract 50 mg/kgbw	47	48	50	49	47	48.2 \pm 1.3**
5	Extract 100 mg/kgbw	47	45	45	46	44	45.4 \pm 1.1**
6	Extract 150 mg/kgbw	39	42	43	41	37	40.4 \pm 2.4**

Where

* (Significantly different to negative control) P<0.05

(Significantly different to positive control) P<0.05

Table 1: Level of creatinine

No	Group	Level of creatinine					Mean \pm SD
1	Normal control	0.26	0.27	0.27	0.25	0.24	0.25 \pm 0.013 *
2	Negative control	0.45	0.49	0.48	0.45	0.48	0.47 \pm 0.018 #
3	Positive control	0.24	0.23	0.24	0.25	0.24	0.24 \pm 0.07*
4	Extract 50 mg/kgbw	0.37	0.44	0.45	0.42	0.45	0.42 \pm 0.033 #
5	Extract 100 mg/kgbw	0.37	0.40	0.39	0.35	0.37	0.37 \pm 0.019_**
6	Extract 150 mg/kgbw	0.33	0.30	0.28	0.29	0.30	0.30 \pm 0.018 *

Where

* (Significantly different to negative control) P<0.05

(Significantly different to positive control) P<0.05

Creatinine was a metabolism molecule product which is produced from the muscle metabolism. Creatinine was an important molecule in the process of energy production in the muscle, it is produced from creatine. Every day, about 2% of the creatine in the body, it was converted into creatinine form (Horio, 2014). This molecules are transported into the kidneys through blood vessel. The kidneys filter out and remove of the creatinine in the urine. The creatinine was a reliable indicator to determine the function of kidney.

The results of rat blood serum testing in each group were statistically analyzed by spss to compare the differences between treatment groups to negative control and positive control. Based on Table 1, it showed that the effective dose of ethanol extract celery herb was 150 mg/kg BW and it showed dose dependent manner. Level of urea in treatment group of ethanol extract 150 mg/kgBW was 40.6 showed there was no different to positive control ($P>0.05$) and significant different to negative control ($P<0.05$).

Table 2 showed that effective dose of ethanol extract celery herb to creatinine level of rat induced ethylene glycol was 150 mg/kgBW with level of creatinine was 0.30. Level of creatinine was not different to positive control ($P>0.05$) and significant different to negative control ($P<0.05$). Content of flavonoids in ethanol extract celery herb prevents oxidative stress in the kidney by increasing glutathione s-transferase (GSH) antioxidant activity, increasing GSH synthesis and trapping directly ROS formed by donating H atoms to free radicals resulting in non-reactive free radical compounds and non-reactive radical flavonol compounds that can improve kidney function (Kang et al, 2016). In this research, elevation of urea and creatinine were decreased by treatment with ethanol extract celery herb and thereby improved kidney functions in nephrolithiatic condition.

4 CONCLUSION

Ethanol extract of celery herb at the dose of 50,100 and 150 mg/kgbw have effect in decreasing level of urea and creatinine. Treatment dose which has effective activity was 150 mg/kgbw and it showed dose dependent manner.

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