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Research Article

ACTIVITY OF ETHYL ACETATE FRACTION OF CELERY HERB (*Apium graveolens* L.) ON CREATININE AND UREA LEVEL IN ETHYLENE GLYCOL INDUCED RATS

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ABSTRACT

The utilization of celery by the community in Indonesia was known as a seasoning, particularly in soups to enrich the taste of food, the potential effect of celery herb especially as herbal medicine both in preventive or curative therapy are not widely known yet. The celery herb is rich in flavonoids content which has potential effect to dissolve kidney stones.The purpose of this study was to determine the effect of ethyl acetate (*Apium graveolens L*) fraction on urea and creatinine levels in male wistar rats induced by ethylene glycol. The in vivo test was conducted on male Wistar rats that divided into 6 groups. Group I as a normal control without treatment, Group II as a negative control induced by ethylene glycol, Group III as a positive control was given Batugin Elixir, Group IV, V and VI were given ethyl acetate fraction of celery at 50, 100 and 150 mg / kgbw respectively . Blood sample was collected through cardiac puncture method. The results showed that ethyl acetate fraction of celery herb had an effective activity at a dose of 150 mg / kgbw which was significantly different to the negative control group ($P < 0.05$) measured by a reduction of urea and creatinine level on male wistar rats induced by ethylene glycol.

Keyword: ethyl acetate fraction, *Apium graveolens* L, creatinine, urea, ethylene glycol

INTRODUCTION

The kidney is an important organ in humans that has many functions such as regulating water balance, mineral concentration in the blood and excretion of waste materials. However, many kidney problems may occur, including kidney stones, urinary tract inflammation and kidney inflammation [1-2].

Hyperoxaluria is a condition of oxalate excess in the body that will form a calcium oxalate crystal deposition. Kidney stones generally contain of calcium oxalate, calcium phosphate, uric acid, xanthine, cystine, silicates or other compounds. About 70-80% of kidney stone disease are formed by calcium oxalate and calcium phosphate[3]. The ethylene glycol is an appropriate model for formating a kidney stone on animal study, it induces the formation of calcium oxalate[4]. The Calcium formed can lead to permanent renal failure through nephron infarction called an acute cortical necrosis. Hyperoxaluria condition is formed due to ethylene glycol intoxication that can induce a renal tubular damage and nephrolithiasis [5].

Previous researchers have conducted research using several varieties of plants to prevent and dissolved a kidney stones, such as *Orthosiphon stamineus*, *Strobilanthes crispa, Ceiba pentandra*, corn silk, and *Cucumis sativus*[6-10]. Some study reported that rich flavonoid plant has an activity in dissolving a kidney stones by forming a chelate complex with calcium kidney stones[11-12]. The Celery herb is rich in flavonoids plant[13]. Celery herb also contains various compounds, namely caffeic acid, p-coumaric acid, ferulic acid, apigenin, luteolin, tannin, saponin, and kaempferol[13]. It is necessary to conduct research on celery herbs as nephroprotective agent in hyperoxaluria conditions. This study was conducted to determine the effect of ethyl acetate fraction of celery herb (*Apium graveolens L.*) on creatinine and urea level of rats induced by ethylene glycol.

MATERIAL AND METHODS

Materials and Plant Collection

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

Extraction of Celery Herb

Dried Celery herb as many as 500 g were crushed in a blender, then macerated in ethanol 80 % for 5 days and continue to re-meceration for 2 days. The solvent were evaporated at low pressure with a temperature of not more than 40 °C using a Rotary evaporator, then dried using freeze dryer[14].

Fractionation of celery herb extract

Ethanol extracts of celery herbs were fractionated by liquid-liquid extraction model. Ethanol extract was fractionated by n-hexane solvent and continue to fractionate using ethyl acetate solvent[15].

Phytochemical screening of ethyl acetate fraction celery herb

Phytochemical screening carried out on ethyl acetate fraction celery herb includes examining the chemical secondary

metabolites of alkaloids, flavonoids, glycosides, tannins, saponins, steroids and triterpenoids.

Animals and blood sample

Thirty male wistar rats were used in this study, weighing around of 180-220 g. The blood sample was collected by cardiac puncture. The studies were carried out in accordance to the institutional ethical guidelines based on National Guidelines on Health Research Ethics 2005 Indonesia.

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 ethyl acetate fractionof celery herb at a dose of 50 mg/kg bw
Group V **:** Rats were received ethyl acetate fraction of celery herb celery at a dose of 100 mg/kg bw
Group VI **:** Rats were received ethyl acetate fraction of celery herb at a dose of 150 mg/kgbw

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

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

RESULT

Phytochemical screening

The ethyl acetate fraction of celery herb contains of glycosides, alkaloids, tannins and flavonoids.

Level of urea and creatinine

Level of urea and creatinine was illustrated the effectiveness of ethyl acetate fraction 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.

Table 1: Level of urea

No	Group	Level of urea					Mean ± SD
1	Normal control	40	41	41	42	39	40.6 ± 1.1*
2	Negative control	57	56	58	55	56	56.4 ± 1.1#
3	Positive control	43	44	42	42	43	42.8 ± 0.8*
4	ethyl acetate fraction 50 mg/kgbw	46	47	49	50	48	48 ± 1.5*#
5	ethyl acetate fraction 100 mg/kgbw	46	45	43	44	44	44.4± 1.14*#
6	ethyl acetate fraction 150 mg/kgbw	39	42	42	43	37	40.6 ± 2.5*

Where, * (Significantly different to negative control) P<0.05, # (Significantly different to positive control) P<0.05

Table 2: Level of creatinine

No	Group	Level of creatinine					Mean ± SD
1	Normal control	0.26	0.27	0.27	0.25	0.24	0.25 ± 0.013 *
2	Negative control	0.45	0.49	0.48	0.45	0.48	0.47 ± 0.018 #
3	Positive control	0.24	0.23	0.24	0.25	0.24	0.24 ± 0.007*
4	ethyl acetate fraction 50 mg/kgbw	0.38	0.45	0.46	0.43	0.45	0.43 ± 0.0320*#
5	ethyl acetate fraction 100 mg/kgbw	0.36	0.38	0.38	0.36	0.39	0.37 ± 0.0134#*
6	ethyl acetate fraction 150 mg/kgbw	0.31	0.29	0.28	0.30	0.30	0.29 ± 0.0114#*

Where, * (Significantly different to negative control) P<0.05, # (Significantly different to positive control) P<0.05

DISCUSSION

Several study reported that the ethylene glycol induced kidney stone form in albino Wistar rat by process of supersaturation in the urine and forming a kidney stone deposition [16]. ethylene glycol is used as an inducer of kidney stone formation, it was the most appropriate method and the forming stone is similar to kidney stone that found in humans which cause the kidney damage[16]. The condition of renal function could characterized by elevated levels of urea and creatinine[17]. In the present study, we investigated the effect of celery (*Apium graveolens* L) ethyl acetate fraction against urea and creatinine level in male wistar rats on ethylene glycol induced nephrolithiasis.

Urea is the metabolism product of protein and amino acids, it is 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[17]. 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 ethyl acetate fraction celery herb on blood serum urea level of rat.

The creatinine level could be a predictable marker in kidney health. It was a metabolism result of muscle. Creatinine is produced from creatine, it was an important molecule in the process of energy production in the muscle. Every day, about 2% of creatine were converted into creatinine [18]. 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 ethyl acetate fraction celery herb was 150 mg/kg BW and it showed dose dependent manner. Level of urea in treatment group of ethyl acetate fraction 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 ethyl acetate fraction celery herb to creatinine level of rat induced ethylen glycol was 150 mg/kgBW with level of creatinine was 0.29. 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 ethyl acetate fraction 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 [19]. In this research, elevation of urea and creatinine were decreased by treatment with ethyl acetate fraction of celery herb and thereby improved kidney functions in nephrolithiasis condition.

CONCLUSION

Ethyl acetate fraction of celery herb at the dose of 50,100 and 150 mg/kgbw decreased the level of urea and creatinine in dose dependent manner. Treatment dose with effective activity was found to be150 mg/kgbw

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