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The protective effect of celery ethanol extract on oxidative stress in chronic kidney disease rat model

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ABSTRACT

BACKGROUND

Chronic kidney disease (CKD) is a serious health problem in which oxidative stress plays an important role. Oxidative stress is an imbalance of reactive oxygen species (ROS) production and antioxidant defense, where antioxidants have the potential to inhibit CKD progression. Celery contains several substances that have an antioxidant effect. This study aimed to evaluate the administration of celery ethanol extract in the prevention of the progressive damage in CKD caused by oxidative stress in male rats.

METHODS

Twenty male Sprague-Dawley rats were randomly divided into 5 groups: sham operation (SO, n=4), subtotal nephrectomy (SN, n=4), SN+celery ethanol extract 200 mg/kg BW (SN+S1, n=4), SN+celery ethanol extract 250 mg/kg BW (SN+S2, n=4), SN+celery ethanol extract 300 mg/kg BW (SN+S3, n=4). The celery ethanol extract was given for 14 days before induction of CKD and 21 days after induction of the CKD rat model. Serum creatinine, malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH) were examined in this study. Data were analyzed by One way ANOVA followed by LSD test for creatinine, MDA, SOD, and Kruskal Wallis test for GSH.

RESULTS

There were significant between-group differences in serum creatinine, SOD, and MDA ($p < 0.05$), but not in GSH ($p > 0.05$). The administration of celery ethanol extract at 250 mg/kg BW was the most effective in preventing an increase in MDA and a decrease in SOD and GSH.

CONCLUSION

Celery ethanol extract has the potential to prevent oxidative stress in the CKD rat model.

Keywords: Celery, chronic kidney disease, oxidative stress, nephrectomy, rats

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INTRODUCTION

Chronic kidney disease (CKD) is a serious public health problem characterized by progressive loss in renal function that is growing in incidence. The worldwide prevalence of CKD is 11%-13%.⁽¹⁾ There are more than 2 million people with CKD globally and most are undergoing hemodialysis or need other forms of renal replacement therapy. Oxidative stress has an essential role in CKD.⁽²⁾ Oxidative stress, which is an overproduction of reactive oxygen species (ROS) and/or a reduction in antioxidant defense capacity,⁽²⁾ is known to occur in CKD patients and contributes to inflammation, endothelial dysfunction, risk of atherosclerosis, and progression of CKD. Oxidative stress causes oxidative damage and results in the production of various compounds such as malondialdehyde (MDA) which is a marker of oxidative injury. Antioxidants such as superoxide dismutase (SOD) and glutathione (GSH) decrease with the increase in renal dysfunction. Therefore, antioxidants have the potential for inhibiting CKD progression.⁽³⁾ Inhibition of CKD progression is important to prevent complications and mortality in CKD. Using natural ingredients as a preventive agent is one effort that can be done because it is widely available in the community.

Celery (*Apium graveolens* L.) is a vegetable that has many health benefits, functioning as antioxidant and anti-inflammatory agent and preventing cardiovascular disease.⁽⁴⁻⁶⁾ Celery contains phenols, flavonoids, alkaloids, steroids, furocoumarins, volatile oils, sesquiterpene alcohols, and fatty acids.⁽⁷⁾ Celery ethanol extract is known to prevent increased renal dysfunction.⁽⁸⁾ Li et al.⁽⁹⁾ investigated the in vivo and in vitro effect of apiin, an antioxidant flavonoid isolated from celery, and showed that apiin has significant inhibitory activity on MDA, and dramatically enhances the activity of SOD, glutathione (GSH) peroxidase, and catalase.⁽⁹⁾

Our previous study about the protective effect of celery ethanol extract at doses of 250,

500, and 1000 mg/kgBW, administered for 14 days before and 14 days after induction of 5/6 subtotal nephrectomy on anemia in the 5/6 subtotal nephrectomy rat model showed that celery ethanol extract at a dose 250 mg/kgBW is the most effective in preventing decreases in hemoglobin, red blood cells, and hematocrit and also in preventing decreases in serum creatinine. However, the prevention of anemia in that study was not significantly different between groups, therefore in the present study we try to extend the time after surgery. The administration of celery ethanol extract at doses of 500 mg/kgBW and 1000 mg/kgBW resulted in a higher serum creatinine level and in lower levels of hemoglobin, red blood cell count, and hematocrit.⁽⁸⁾ Other our previous study about protective effect of ethanol extract of celery (*Apium graveolens* L.) on kidney damage in an ischemia/reperfusion injury rat model showed that celery ethanol extract at a dose of 1000 mg/kgBW was the most effective in preventing kidney damage in the ischemia/reperfusion injury rat model. The difference between our previous study and our present study is that the present study uses a chronic model of kidney disease and longer administration times of celery ethanol extract, i.e. 14 days before and 21 days after operation. The present study investigated the effect of celery ethanol extract on oxidative stress in the CKD rat model.

METHODS

Research design

A study of experimental design with a posttest-only control group was conducted in the animal house of the Pharmacology Laboratory, Faculty of Medicine, Universitas Jenderal Soedirman, while laboratory measurements of the variables were performed in the Research Laboratory, Faculty of Medicine, Universitas Jenderal Soedirman from July until October 2020. The preparation of celery ethanol extract was conducted in the Lansida Herbal Technology laboratory, Yogyakarta.

Collection of plant material and extraction

Celery stems and leaves were collected from Pratin, Purbalingga, Central Java, Indonesia. The stems and leaves were washed and dried before being crushed in a disk mill with a sieve of 60 mesh. This material was then added to 70% ethanol in an extractor and extracted using an Ultra-Turrax® disperser at 1000 rpm and macerated for 24 hours. Filtration of the mixture was performed with a Buchner funnel attached to a vacuum pump. A vacuum rotary evaporator was used for evaporation of the filtrate at 45°C and 90 rpm. The extract was transferred into a porcelain dish and placed in a digital oven at 45°C, then dried 3 times to a constant weight. The extract was prepared with distilled water.

Animals and treatment

Twenty Sprague Dawley male rats, 190-210 g, 2-3 months old were used in this study. The minimum total number of subjects in this study was 15 based on the 'resource equation' approach.⁽¹⁰⁾ We used four animals per group in this study. The study subjects were selected through a simple random sampling technique. The rats were randomized into 5 groups: sham operation (SO, n=4), subtotal nephrectomy (SN, n=4), SN+celery ethanol extract 200 mg/kg BW (SN+S1, n=4), SN+celery ethanol extract 250 mg/kg BW (SN+S2, n=4), SN+celery ethanol extract 300 mg/kg BW (SN+S3, n=4). Celery ethanol extract was administered orally 14 days before and 21 days after induction of experimental CKD by 5/6 subtotal nephrectomy. The rats were housed in 12-h light-dark cycle, 25°C ambient temperature, 40-60% humidity with free access to water and food.

Induction of experimental chronic kidney disease

The 5/6 subtotal nephrectomy was performed to induce CKD. The procedure performed on the animals was either 5/6 subtotal nephrectomy or a sham operation, both under ketamine anesthesia (100 mg/kg BW). Nephrectomy was started on the left kidney

followed by the removal of the upper and lower poles of the right kidney. The peritoneum and skin were then closed and sutured using 3/0 silk.⁽⁸⁾ The rats were sacrificed by cervical dislocation.

Laboratory analysis

Blood serum was obtained from the retro-orbital vein for serum creatinine, SOD, GSH, and MDA level measurement. Serum creatinine levels, measured in the Medico Laboratory, Purwokerto, were used to evaluate renal function. The antioxidant parameters were the SOD and GSH levels. For SOD measurement serum samples and the WST-1 cell viability and proliferation assay (manufacturer, cat. no. 0000) were used. The absorbance was assessed using a spectrophotometer at 595 nm wavelength. Measurement of glutathione was carried out using an Elisa kit, prepared by the Research Laboratory, Faculty of Medicine, Universitas Jenderal Soedirman. The oxidant parameter was the measurement of the MDA level, using a colorimetric method with a spectrophotometer at 532 nm wavelength. The measurement was carried out in the Research Laboratory, Universitas Jenderal Soedirman.

Statistical analysis

We used the Shapiro-Wilk data normality test and Levene's test for homogeneity. The statistical analysis was performed using one-way ANOVA followed by LSD test for serum creatinine, SOD, and MDA, and the Kruskal-Wallis test for glutathione at a level of significance of 0.05.

Ethical clearance

This study has obtained ethical clearance from the Ethics Committee of the Medical Faculty, Universitas Jenderal Soedirman, Purwokerto, Indonesia, under No. 138/KEPK/VII/2020.

RESULTS

Serum creatinine levels after SN and administration of celery ethanol extract are

presented in Table 1. The level of serum creatinine in the SN group (0.95 ± 0.07 mg/dL) was higher than in the SO group (0.75 ± 0.08 mg/dL) ($p < 0.05$). This study used celery ethanol extract at 200, 250, and 300 mg/kg BW once daily for 14 days before and 21 days after 5/6 SN. Administration of celery ethanol extract at a dose of 200 mg/kg BW prevented an increase in serum creatinine level, but the effect was not significantly different from the SN group ($p = 0.268$). Administration of celery ethanol extract at 250 and 300 mg/kg BW for 14 days before and 21 days after 5/6 SN prevented an increase in serum creatinine level compared to the SN group ($p = 0.049$ and $p = 0.441$, respectively), but there were no significant differences between the 5/6 SN+celery 250 mg/kg BW group and the 5/6 SN+celery 300 mg/kg BW group ($p = 0.959$).

The mean MDA level in the SN group (33.41 ± 12.99 nmol/mL) was significantly higher than in the SO group (16.36 ± 0.94 nmol/mL) ($p < 0.021$) (Table 1), whereas the mean MDA level in the treatment groups was lower than that in the SN group. The MDA level after administration of celery ethanol extract at doses of 200, 250, and 300 mg/kg BW was 17.2 ± 1.48 nmol/mL, 18.21 ± 3.42 nmol/mL, and 28.64 ± 3.68 nmol/mL, respectively.

The mean glutathione and SOD levels in the SN group were lower than in the SO group (Table 1). The mean SOD levels in the treatment groups were 22.01 ± 1.22 U/mL, 22.91 ± 0.76 U/mL, and 23.92 ± 1.5 U/mL, after administration of celery ethanol extract at doses of 200, 250, and 300 mg/kg BW, respectively.

DISCUSSION

Induction of the CKD rat model by means of 5/6 subtotal nephrectomy in this study was successful, which can be seen from the mean serum creatinine level, this being significantly higher in the SN group than in the SO group. According to Sari et al.,⁽¹¹⁾ 14 days after 5/6 SN operation the serum creatinine level has increased significantly in the rats. The reduction in renal

mass in 5/6 SN led to decreased renal function and resulted in progressive hypertension, hypertrophy and focal segmental glomerulosclerosis. According to Afifah et al.,⁽⁸⁾ celery at a dose of 250 mg/kg BW can inhibit the increase in serum creatinine level for 14 days before and after 5/6 SN operation. However, although 14 days after SN operation there was an increase in serum creatinine, the operation was not able to cause anemia as a result of CKD. Therefore, the present study increased the time period after 5/6 SN operation to 21 days. Creatinine is produced from creatine, the important molecule in the energy production process in the muscle. This molecule is carried into the kidneys and filtered out in the urine, and is a reliable marker of renal function.⁽¹²⁾

Oxidative stress, the imbalance of ROS production and antioxidant defense, is an important mechanism in CKD. Even in the early stage of CKD, there is an increase in ROS production mainly caused by hyperactivation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and increased synthesis of oxidative stress markers and of uremic toxins.⁽¹³⁾ In line with Layal et al.⁽¹⁴⁾ the MDA level was significantly higher in the 5/6 SN rat model than in the control group. Malondialdehyde, which is the product of lipid peroxidation, is used as an indicator of the intensity of oxidative stress. The increase of MDA in CKD patients indicates increased oxidative stress and its progressive increase with worsening renal function implies that oxidative stress increases with disease progression.⁽³⁾

Oxidative stress arises when there is an imbalance between free radical production and antioxidant defense. Oxidative stress is involved in the progression of renal injury.⁽²⁾ The oxidative stress further causes oxidative damage to important biomolecules such as lipids, proteins, and nucleic acids resulting in the formation of various compounds such as MDA that have relatively longer half-lives and are used as general markers of oxidative injury.⁽³⁾ In the early stages of CKD, nutritional and pharmacological

Table 1. Distribution of creatinine, glutathione, MDA, and SOD level by treatment groups

Variables	Treatment groups				p value	
	SO	SN	SN+S1	SN+S2		SN+S3
Creatinine ^a (mg/dL)	0.75±0.08*	0.95±0.07 [#]	0.90±0.05 [#]	0.85±0.08*	0.85±0.03*	0.011
MDA ^a (nmol/mL)	16.36±0.94*	33.41±12.99 [#]	17.20±1.48*	18.21±3.42*	28.64±3.68 [#]	0.004
SOD ^a (U/mL)	22.64±0.5*	19.31±2.03 [#]	22.01±1.22*	22.91±0.76*	23.92±1.5*	0.002
Glutathione ^b (U/mL)	148.69 (141.13-191.53)	117.94 (102.82-135.08)	135.08 (126.01-153.23)	138.10 (137.10-186.49)	155.24 (125.00-167.34)	0.104

Note: MDA: malondialdehyde; SOD: superoxide dismutase; Data presented as mean ± SD, except glutathione [Median (Q1-Q4)]
^a=p<0.05 in ANOVA analysis. ^b: p>0.05 in Kruskal Wallis analysis. *p<0.05 vs SN, [#]p<0.05 vs SO. SO (Sham operation), SN (Subtotal nephrectomy), SN+S1 (Subtotal nephrectomy+celery 200 mg/kg BW), SN+S2 (Subtotal nephrectomy+celery 250 mg/kg BW), SN+S3 (Subtotal nephrectomy+celery 300 mg/kg BW)

intervention is needed to prevent the progressive damage in CKD.⁽¹³⁾ In our present study, the administration of celery ethanol extract at doses of 200, 250 mg/kg BW significantly prevents the increase in MDA level. Celery ethanol extract at 250 mg/kg BW is the most effective in preventing the increase in MDA level in the 5/6 subtotal nephrectomy rat model.

Chronic kidney disease increases ROS formation in the cells. The high production of ROS accompanies an increase in endogenous antioxidants. The increase in ROS production for an extended period will disturb the antioxidant activity against oxidative stress. Glutathione and SOD are the antioxidant enzymes that act as a defense against oxidative stress.⁽²⁾

Celery is a widely used plant in traditional medicine because of its content of phytochemical compounds.⁽¹⁵⁾ Celery ethanol extract has an antioxidant effect. Celery contains phenols, flavonoids, alkaloids, steroids, glycosides, fatty acids, and a wide range of trace elements, limonene, salinene, furocoumarine glycosides, vitamin A and vitamin C.^(16,17) Phenols and flavonoids have an antioxidant effect.⁽¹⁸⁾ The antioxidant capacity of flavonoids is derived from their ability to scavenge the ROS directly. Flavonoids can chelate free radicals quickly by donating a hydrogen atom or by single-electron transfer.^(12,19) Flavonoids can also stimulate antioxidant enzymes.⁽²⁰⁾ One type of flavonoids are the flavones, of which apigenin is the most commonly found in celery.⁽²¹⁾ In addition to flavonoids, celery also contains high amounts of vitamin C, which scavenges ROS and reactive nitrogen species by forming semidehydroascorbic acid and thus protects against oxidative damage.⁽¹³⁾

The limitation of this study is that it did not measure the antioxidant content of the celery ethanol extract. The clinical implication of this study is that celery has the potential to prevent the progressive damage in chronic kidney disease via its antioxidant effect. Further research is recommended to determine the contents of celery ethanol extract and examine other pathomechanisms in the CKD rat model.

CONCLUSIONS

This study demonstrated that celery ethanol extract has the potential to prevent oxidative stress in the CKD rat model. Celery ethanol extract at 250 mg/kg BW has the greatest effect in preventing oxidative stress in the CKD rat model.

CONFLICT OF INTEREST

The authors declared no conflict of interests.

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CONTRIBUTORS

AF, FWP interpreted the data and prepared the manuscript for publication. AF, AS, DHA, ZK, RAF collected the data and conducted the study. All of the authors have read and approved this manuscript. 

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