The Effect of Celery Ethanol Extract on Proteinuria in Unilateral Ureter Obstruction Rat Model

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Abstract. Chronic kidney disease (CKD) is a condition of decreased kidney function that occurs for more than three months. Proteinuria is one of the markers of impaired kidney function, especially in the renal filtration process. Unilateral ureter obstruction causes impaired kidney function, including the filtration process. Celery (*Apium graveolens* L.) contains phenolic compounds that can prevent the decrease of kidney function. This study aims to determine the effect of celery ethanol extract on proteinuria in the UUO rat model. This study was an experimental study with a post-test only with control group design. A total of 25 rats were assigned to 5 groups. Group 1: sham control, 2: negative control (UUO), 3: UUO with 125 mg/kgBW celery ethanol extract, 4: UUO with 250 mg/kgBW celery ethanol extract, and 5: UUO with 500 mg/kgBW celery ethanol extract. The treatment of celery ethanol extract was given for 14 days. Urine samples were taken on the 15th day for examination of proteinuria. The results were analyzed by the Oneway ANOVA test and showed a significant difference between the control group and the treatment groups ($p<0.05$). A dose of 250mg/kgBW is the most effective dose of celery ethanol extract in this study.
Keywords: *Celery, Proteinuria, Renal function, Unilateral ureteral obstruction*

1. Introduction

Obstructive nephropathy is a common clinical condition that can have long-term irreversible consequences. it can cause renal insufficiency or interfere with renal maturation. Obstructive nephropathy causes oxidative stress and inflammation as a result of ischemia and hypoxia. The unilateral urological obstruction (UUO) model is widely used to study obstructive nephropathy. The experimental procedure entails ureteral ligature, usually with a silk thread, most commonly on the left. Proteinuria is one of the two primary biomarkers in renal function disturbances [1,2].

The UUO animal model is used for a variety of purposes. UUO in adults serves as a model for accelerated renal fibrosis, which is a feature of progressive renal disease. The UUO in adult mouse allows researchers to study cell death, inflammation, and extracellular matrix synthesis. The UUO in adult mice is an experimental renal lesion model that leads to tubulointerstitial fibrosis. The last common pathway of many renal diseases leads to renal insufficiency with dialysis or renal transplantation, because no effective treatment is currently available. UUO allows for the rapid study of various stages of fibrosis development, such as inflammatory cell infiltration, tubular cell death, extracellular matrix (ECM) deposit, and tubular atrophy [3].

The management of chronic kidney disease now are hemodialysis and giving of angiotensin-converting enzyme (ACE) inhibitor preparate to prevent the progressivity of renal damage. The treatment of chronic kidney disease is expensive cost and long time to manage the chronic kidney disease. There are need an

effort to prevent progressive damage of chronic kidney disease. Adapting the culture of the Indonesian people who use the surrounding plant to treat the disease, we can use plants that have often been used to prevent further renal damage [4].

Celery (*Apium graveolens* L.) is a plant that has been widely used by the community. Celery contains essential fatty acids (alinine and allicin), flavonoids, proteins, vitamin A, vitamin C, vitamin B, iron, calcium, sulfur, and phosphorus. Human flavonoids are used as anti-allergens, anti-inflammatories, antivirals, and anticancer agents. Flavonoids are antioxidants that have the potential to prevent the formation of free radicals. Occasionally, flavonoids are found in the cuticle cells. It has been demonstrated that flavonoids exist in the celery in the four articles used. Flavonoids are antioxidants that can prevent the formation of free radicals [5].

The damage that free radicals can inflict on cells may be prevented by one of the substances referred to as antioxidants. These antioxidants can prevent cellular damage by stabilizing free radicals. The non-appearing free radical electrons will have their electrons completed by antioxidants. In addition to foods, antioxidants can be obtained from plants that contain secondary metabolic products such as flavonoid compounds. These flavonoids can directly reduce oxygen-free radicals like the superoxide produced by the reaction of the enzyme xanthine oxidase. The celery (Apium graveolens L.) is one of the plants whose flavonoid content and antioxidant activity have been established. The amount of total flavonols influences the antioxidative activity; the higher the number of flavonols in an extract, the higher the antioxidative activity [6].

In the diagnosis and treatment of primary and secondary renal diseases, proteinuria plays a crucial role. Today, proteinuria and its treatment rank among the most recent advancements in the treatment of renal diseases, serving as one of the two primary biomarkers in nephrology together with serum creatinine. Proteinuria, and its treatment, are now at the forefront of recent advances in kidney disease management, both as one of the two central biomarkers. Proteinuria is significant because of its association with progressive loss of kidney function and eventual kidney failure. Furthermore, changes in proteinuria are strongly related to kidney outcomes. The prognosis worsens with increasing proteinuria in almost all kidney diseases, including diabetes and non-diabetic CKD. Proteinuria drives the pathophysiological process of deteriorating kidney function and has a direct impact on cardiovascular risk [2].

The pathophysiology of proteinuria and kidney function loss is complex and still poorly understood, but a clear picture is emerging that focuses on the tubular epithelial cell response to filtered protein. Proximal tubular epithelial cells reabsorbed filtered albumin. With heavy or persistent proteinuria, however, intracellular pathways involving lysozymes and the endoplasmic reticulum, which are designed to handle reabsorbed albumin, become stressed or overwhelmed. Tubular cells respond by producing cytokines, which promote inflammation and attract inflammatory cells like macrophages. Albumin also causes changes in tubular cell function, which can lead to apoptosis. Additionally, albumin-bound free fatty acid (particularly oleic/linoleic acid) and metabolites of non-essential fatty acid long-chain acyl-CoA stimulate tubular cell lipoapoptosis. More broadly, the changes in stressed renal tubular cells represent a transition to a partial mesenchymal phenotype, which includes the production of profibrotic mediators like transforming growth factor-beta 1 (TGF- β1). A subset of renal tubular cells continues their migration to the interstitium, where they, along with existing interstitial fibroblasts, contribute directly to the production of extracellular matrix and fibrosis [2]. Against this background, the aim of this study was to determine the effect of celery ethanol extract on proteinuria levels as a biomarker of kidney function in rats model of unilateral ureteral obstruction.

2. Methods

2.1. Method Types of Research

This was an experimental study with a post-test only control group design. The goal was to determine the effect of the celery extract on proteinuria in unilateral ureteral obstruction by using white rats Sprague dawley as an experimental animal.

2.2. Research Time and Place

The research was conducted in June-September 2022 at the Research Laboratory and Pharmacology Laboratory, Faculty of Medicine, Universitas Jenderal Soedirman.

2.3. Tools and Materials

The tools used in this study were a syringe injection, minor surgical instrument, sterile gloves, sample pot, and pipette of hematocrit. Whereas, the materials used in this study were Sprague dawley rats, celery, 70% ethanol, ketamine, AD2 mouse feed, 3/0 silk suture, and povidone-iodine.

2.4. Research Design

This research is a quantitative study using laboratory experimental methods. This research protocol already approved by the Medical Research Ethics Committee, Universitas Jenderal Soedirman, Purwokerto, Indonesia with the number 007/KEPK/PE/VIII/2022. The sample size for this study was 25 rats referred to the Ferderer formula which is $(t-1)(n-1)>15$ with the value of t as the number of treatments is 5. The value of $n>4.75$ is rounded up to 5, so that each group of the model consists of 5 rats. The rats used in this research were 150-250 g male Sprague Dawley rats aged 2-3 months. Rats were divided into five groups (A, B, C, D, E) but before that, they were adapted for 7 days and given ad-libitum feed. Group A was the sham control group, the rats were only exposed to the skin and muscles without induced unilateral ureteral obstruction and without being given celery extract. Group B is a negative control, rats only induced unilateral ureteral obstruction without being given celery extract. Group C was the treatment group, rats were induced with unilateral ureteral obstruction and were given ethanol extract of celery at a dose of 125 mg/KgBW for 14 days. Group D was the treatment group, rats were induced with unilateral ureteral obstruction and were given celery ethanol extract at a dose of 250 mg/KgBW for 14 days. Group E was the treatment group, rats were induced with unilateral ureteral obstruction and were given celery ethanol extract at a dose of 500 mg/KgBW for 14 days. After 14 days, the rats' urine was taken and collected. After that, the rats were terminated via cervical dislocation. Before termination, the rats were given ketamine anesthesia. All of these processes were carried out at the Pharmacology Laboratory, Faculty of Medicine, Universitas Jenderal Soedirman.

2.5. Celery Extraction

The celeries (*Apium graveolens* L.) were collected from Pratin, Purbalingga, Central Java. Celeries were washed thoroughly and then dried for a few days. Celery then oven-dried at 60°C to maximize drying. After drying, the celery was blended to become powder. Celery powder was then added to 70% ethanol in a ratio of 1:10 for the maceration process for 72 hours. The ethanol was replaced with a new ethanol solution every 24 hours. To get a pure extract, the ethanol extract of celery was evaporated using a rotary evaporator.

2.6. Evaluation of Kidney Function

To evaluate the rat kidney function, the proteinuria examination was used in this study. Examination of proteinuria was measured with a dipstick stick. The examination was carried out right before the termination of the rats after 14 days of treatment and the observations were completed.

2.7. Data Analysis and Summarizing

The data obtained from the proteinuria examination were then analyzed using IBM SPSS Statistic Base 22.0 software. This study used Oneway ANOVA test followed by post-hoc LSD test to analyze differences in proteinuria due to different treatments between groups. First, the normality and

homogeneity tests were carried out using the Shapiro-Wilk test and the Lavene test, then the bivariate analysis was carried out. After that, the study was concluded based on the statistical data analysis. The result of the proteinuria average was considered significant when the p-value <0.05.

3. Result and discussion

The Marker of the progression of chronic kidney failure damage is an increase in protein levels in the body. The normal level of protein in the human body is 60 ml/minute/1.73 m2. Chronic renal failure can cause a decrease in the glomerular filtration rate. The glomerular filtration rate begins with the filtration of plasma through the walls of the glomerular capillaries. Glomerular filtration results in the form of electrolytes, glucose, urea, creatinine, and certain proteins. So an increase in protein levels can indicate a decrease in kidney function. Decreased glomerular filtration rate can be caused by inflammation of the kidneys resulting in structural functional damage to the kidneys [7,8]. Protein levels in rats' urine were examined to assess renal function after treatment. The difference in mean proteinuria levels in sham control, pain control, and administration of celery ethanol extract 125mg/kgBW, 250mg/kgBW, and 500mg/kgBW obtained by One Way ANOVA test showed a significant difference ($p<0.05$). The results of the comparison test for proteinuria levels can be seen in figure 1.

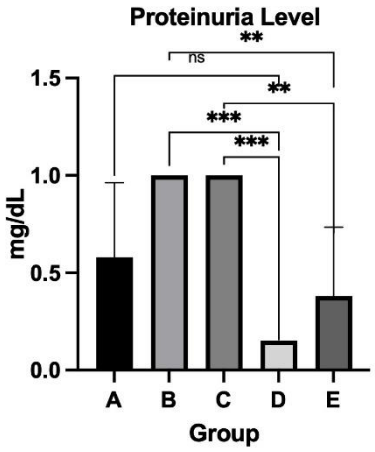

Figure 1. Comparison of proteinuria levels between treatment groups. Group A: false control; Group B: pain control; Group C: 125mg/kgBW celery ethanol extract treatment; Group D: 250mg/kgBW celery ethanol extract treatment, Group E: 500mg/kgBW celery ethanol extract treatment. ns : non-significant; *: significant.

The result above shows that there is a significant difference in the mean proteinuria levels in the control group and the treatment group, especially in group B (disease control) and group D (treatment with celery ethanol extract 250 mg/kgBW) with results in group B being 1mg/dL and in group D being 1mg/dL and in group B being 1mg/dL. In Group B, the result was 1mg/dL. Group D is 0.15 mg/dL. While in group A as control with group D (treatment with celery ethanol extract 250 mg/KgBW) there was no significant difference with the values in group A, namely 0.58 mg/dL and 0.15 mg/dL in group D. Urine protein levels in rats group A as a sham control, group B as a negative control, the treatment group giving celery ethanol extract 125 mg/kgBW, 250 mg/kgBW and 500 mg/kgBW respectively were 0.58 mg/dL, 1 mg/dL, 1 mg/dL, 0.15 mg /dL, and 1.38 mg/dL. Giving celery extract serves as a prevention of progression in chronic kidney failure. Celery contains bioflavonoid apigenin, flavone glycogen, and aglycone which have anti-inflammatory functions. The bioflavonoid apigenin inhibits inflammation by inhibiting COX-2. Anti-inflammatory in celery prevents a decrease in renal function [9]. Celery also contains phenolic which acts as an antioxidant. Phenolic can capture free radical activity so that the increase in ROS can be stopped and prevent damage to kidney function [3,10]. Group D (treatment with celery ethanol extract 250 mg/kgBW) showed a decrease in protein levels in rats. These results indicate that the administration of celery ethanol extract can prevent the progression of kidney damage in rats.

The flavonoids in celery extract act as an antioxidant. Decreased antioxidant activity can cause oxidative stress and damage to kidney function. Antioxidants may intervene early in the pathogenesis of kidney injury by directly scavenging ROS or oxidant sources. Studies in terminally ill sepsis patients with varying degrees of renal dysfunction and renal insufficiency have shown elevated biomarkers of circulating protein and lipid oxidation, which are the inflammatory and oxidative marker [11]. Antioxidants play an important role in maintaining the physiological and pathophysiological homeostasis of cells and tissues. When pathological tissue damage occurs, their neutralizing capacity is exceeded by oxidative stress, causing the dysregulation of antioxidants. Oxidative stress causes

oxidative damage, production of malondialdehyde (MDA) as the marker of oxidative damage, decreasing the antioxidants such as superoxide dismutase (SOD) and glutathione (GSH), and increasing the renal impairment condition [12]. Furthermore, studies suggest overproduction of ROS and reduction of SOD and other antioxidants, may initiate the development of hypertension. As the effect of hypertension continues to increase, kidney damage develops and kidney function decreases [13].

When the kidneys are damaged, kidney function decreases, and high levels of protein are detected in the urine. This is reflected in group B proteinuria level as a negative control. High histogram plots indicate proteinuria levels in this group as well as a progressive decline in renal function in Group B rats. Group D, treated with celery ethanol extract at 250 mg/kgBW, had the lowest levels of proteinuria compared to the other treatment groups. The histogram of group D shows the highest significance of group B as a negative control, this indicates that celery has a protective effect on renal function in group D rats at a dose of 250 mg/kgBW. A previous study found that a dose of 250 mg/kg body weight of celery was an effective dose to provide protective effects by preventing increases in MDA and decreases in SOD and GSH [12]. At high concentrations, the antioxidants contained in phenolic compounds can be lost or even become pro-oxidants [14]. This was shown on the histogram graph of the proteinuria score for Group E, which increased the proteinuria score. This is the scientific basis that the dosage of the drug is very important to achieve a therapeutic effect and prevent the occurrence of toxicity due to overdose.

The detection of protein in the urine of group A rats is a normal condition. Normal urine protein levels range from 40-80 mg per day, with a composition of 10-15mg albumin, Tamm-Horsfall protein, and other small proteins. [15]. The proteinuria condition is suspected if urine protein exceeds 40-80 mg daily. Untreated proteinuria is strongly associated with progressive loss of renal function and renal failure. This condition activates various inflammatory and fibrotic pathways, leading to interstitial fibrosis and glomerulosclerosis [2]. In conclusion, celery ethanol extract at a dose of 250 mg/kgBW showed a protective effect in the low-proteinuria treatment group (group D). This statement is supported by statistical analysis showing significant differences between group B and group D. A celery extract dose of 250 mg/kgBW for 14 days in UUO rats was the most effective dose compared to other doses.

The limitation of this study is that it did not measure the phytochemistry content of the celery ethanol extract. The clinical significance of this study is that celery may prevent progressive damage due to decreased renal function through its antioxidant and anti-inflammation properties. Further research is recommended to measure the phytochemistry content of celery.

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