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The Effect of Celery Extract on Caspase-3 and TNF-α Gene Expression in Lead Poisoning-Induced Renal Injury in Rats

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ABSTRACT

Background: Mesenchymal stem cells (MSCs) are known for their immunomodulatory properties, particularly their ability to secrete anti-inflammatory cytokines such as interleukin-10 (IL-10). Enhancing the secretion of IL-10 by MSCs could have significant therapeutic potential for treating inflammatory diseases. **Objective:** To determine the effect of Apium graveolens L (Celery) extract in the TNF-α and caspase-3 gene expression on the lead poisoning-induced renal injury rats' model. **Methods** This study is experimental research with post test only control group design. Twenty male Wistar rats were randomly allocated into four groups (n=5). The control group was orally administrated with distillate water. The second group received lead acetate 200mg/kg body weight orally for 14 days, the third group were orally administered lead acetate 200 mg lead acetate/kg body weight and vitamin E 50IU/kg body weight for 14 days. The fourth group was administrated with leas acetate like second group and celery extract 300mg/kg body weight for 14 days. The TNF-a and caspase-3 gene expression was analyses under qRT-PCR. **Results** The phytochemical analysis of APE indicated the presence of alkaloids, flavonoids, tannins, saponins, and steroids. Leads acetate increased the TNF-α (3.87±0.09) and caspase-3 (7.95±0.23) gene expression. The celery extract was significantly decrease in the TNF-α (3.13±0.34) and caspase-3 (2.48±1.23) gene expression. **Conclusion:** In conclusion, the presence of celery extract inhibited the renal injury-induced lead acetate toxicity by inhibition of TNF-α proinflammation protein and caspase-3 proapoptosis protein

Keywords : Apium graveolens, TNF-α, caspase 3, lead acetate poisoning

INTRODUCTION

Lead is one of the harmful environmental pollutants in the world and carries high non-carcinogenic risks for human [1,2]. Lead can be absorbed by humans via respiratory, alimentary track, and skin. Furthermore, firstly the lead intake of the body into bloodstream, and distributed to the kidney, causing kidney injury [3]. Lead accumulation in the body contributes to oxidative stress that causes cellular DNA damage, triggering inflammation characterized by an increase in inflammatory cytokines, including TNF-α [4]. Furthermore, prolonged inflammation in the kidney induces cell death characterized by overexpression of caspase-3 leading to impaired renal function [5,6]. To date, chelation is still a well-known treatment for lead poisoning. Unfortunately, chelate agents have disadvantages such as neurotoxicity, hepatotoxicity, and some serious mucocutaneous reaction [7]. Therefore, it is of great theoretical value and practical significance to seek effective and low-side-effect drugs to protect against lead-induced renal injury.

In recent years, natural alternatives have attracted much attention due to their affordability, availability, and minimal side effects [8–10]. *Apium graviolens L.* (Celery) extract was shown to contain alkaloid, saponin, tannin, flavonoid, and steroid compounds that have anti-inflammatory effects and prevent oxidative stress [11]. In this study, the lead acetate (PbAc2) was used to establish a kidney injury model, and doses of celery extract 300mg/kgBW were administrated to rat with lead toxicity. We also used vitamin E in dose 50IU/kg BW for positive control. Vitamin E functions as an antioxidant and protects the body from polyunsaturated fatty acids (PUFAs) such as oleic acid, linoleic acid, linolenic acid, and arachidonic acid [12]. In addition, vitamin E also functions as an antidote to free radicals such as in lead poisoning [13]. However, the effect and mechanism of celery extract against lead-induced kidney injury have not been investigated to date. In this study, aims to evaluate the effect of celery extract on TNF-α and caspase-3 gene expression on the lead poisoning-induced renal injury rats' model.

MATERIAL AND METHODS

Material and study design

This post-test only control group study design was conducted in Stem Cell and Cancer Research (SCCR) Laboratory, Faculty of Medicine, Sultan Agung Islamic University, Semarang. The study was approved by the Ethic Committee of Sultan Agung Islamic University (No. 278/VIII/2022/Komisi Bioetik).

Extraction of Apium graveolens extract

Apium graveolens herbs were collected from Tawangmangu in Central Java Indonesia in August 2022 (Latitude -7.665158 and Longitude 111.129500). They were rinsed with tap water followed by distilled water to remove the dirt on the surface. The dried *Apium graveolens* herbs was blended until small pieces and sieved with a mesh size of 120 mesh. The 100 g of *Apium graveolens* herbs was extraction in a maceration apparatus with 1000 mL 98% ethanol for 24 h. The filtrated was then evaporated under rotary vacuum evaporator (IKA) and the crude extract west kept in refrigerator 4℃ [9,14]. The *Apium graveolens* crude extract was dissolved in CMC-Na suspension based until the extract concentration was 60mg/mL. The formulations were stored at 4℃ until further analysis [15–17].

Phytochemical screening of Apium graveolens extract

The *Apium graveolens* crude extract (APE) were tested for the presence of flavonoids, alkaloids, tannins, steroids, terpenoids, and saponins. The qualitative results are expressed as (+) for the presence and (−) for the absence of phytochemicals. The flavonoids were test using Wilstater's test according to [18]. Briefly, 2 mg of APE was mixed with HCl 500µL and 0.02 mg magnesium. The presence of flavonoids is characterized by the occurrence of discoloration. The presence of alkaloids indicated with a brown colored precipitate that determined under Wagner's test, 15 mg of APE was stirred with 1% HCl (6 mL) on water bath for 5 minutes and filtered. The filtrate was added with few drops of Wagner solution (2 gram of Potassium iodide and 1,27 g of Iodine in 95 mL of distilled water) [19]. Furthermore, Tannins content was analysis the APE with 1% ferric chloride, the black or blue coloration was taken a positive result of tannins [20]. Liebermann-Burchard test was used to determine the presence of steroids and terpenoids, briefly 100 mg of APE was shaken with chloroform and added the few drops of acetic anhydride was added to the test tube and boiled in a water bath and rapidly cooled in iced water. Concentrated H_2SO_4 (2 mL) was added alongside of the test tube. Formation of a brown ring at the junction of two layers and turning the upper layer to green shows the presence of steroids while formation of deep red color indicates the presence of triterpenoids [21]. The saponin presence was analysis under Forth's test, 500 mg of APE was shaken with 10 mL of distilled water. The formation of frothing, which persists on warming in a water bath for 5 min, shows the presence of saponins [21].

Total flavonoid content of Apium graveolens extract

Total flavonoid content was determined using the aluminum colorimetric method [22] with some modifications using quercetin as the standard. A calibration curve of quercetin was prepared in the range of 200 - 700µg/mL. Briefly, extract (0.5 mL) and standard (0.5 mL) were placed in different test tubes and to each 10% aluminum chloride (0.1 mL), 1 M potassium acetate (0.1 mL), 80% methanol (1.5 mL) and distilled water (2.8 mL) were added and mixed. A blank was prepared in the same manner where 0.5 mL of distilled water was used instead of the sample or standard, and the amount of aluminum chloride was also replaced by distilled water. All tubes were incubated at room temperature for 30 min. The absorbance was taken at 415 nm. The concentration of flavonoid was expressed as mg quercetin equivalent (QE) per gram of extract [10,23].

Lead acetate poisoning animal model

Twenty male Wistar rats weighing (200-250) g were used for this study. The animals were housed in a temperature (25 ± 1 °C), humidity controlled room and a 12-h light-dark cycle. Rats were allowed free access to tap water and standard pellet diet. The institutional Animal Ethics Committee approved all experimental protocols. The animals were classified into 4 groups, each of 5 as follows:

(i) Control groups (Healthy): Rats received distilled water.

(ii) Lead acetate-treated group (Control -): Rats were orally administered lead acetate at a dose of 200 mg lead acetate/kg body weight, by stomach tube once daily for 14 days.

(iii) Lead acetate and vitamin E 50IU/kg body weight (reference drug) treated group (Control +): Rats were orally administered lead acetate at a dose of 200 mg lead acetate/kg body weight and vitamin E 50IU/kg body by stomach tube once daily for 14 days.

(iv) Lead acetate and 300mg/kg body weigh APE treated group (T): Rats were orally administered lead acetate at a dose of 200 mg lead acetate/kg body weight and 300 mg/kg APE/body weight by stomach tube once daily for 14 days.

At the end of experiment, fasting blood samples were withdrawn from the retro-orbital vein of each animal using a glass capillary tube after fasting period of 12 h. The blood samples allowed to coagulate and then centrifuged at 3000 rpm for 20 min. The separated sera were used for the estimation of serum protein of TNF- α by using ELISA kits (Invitrogen, USA). Total serum protein was evaluated using kits from Biodiagnostic, Egypt. The brains was excised and

Analysis of TNF- α and Caspase-3 Gene Expression

Total RNA from rat kidney tissue was extracted with TRIzol (Invitrogen, Shanghai, China) according to the manufacturer's protocol. Briefly, first-stranded cDNA was synthesized with 1 g of total RNA using Super-Script II (Invitrogen, Massachusetts, USA). SYBR No ROX Green I dye (SMOBIO Technology Inc, Hsinchu, Taiwan) was used for reverse-transcription in a real-time PCR instrument (PCR max Eco 48), and mRNA levels of TNF- α and caspase-3 genes were measured using the respective primers (Table 1). The thermocycler conditions used were as follows: initial step at 95°C for 10 minutes, followed by 50 cycles at 95°C for 15 seconds, and 60°C for 1 minute. The gene expression was recorded as the Cycles threshold (Ct). Data were obtained using Eco Software v5.0 (Illumina Inc, San Diego, CA, USA). All reactions were performed in triplicate, and data analysis used the $2^{-\Delta\Delta}$ Ct method (Livak method).

Table 1. primer sequences for TNF- α, Caspase-3 and GAPDH genes

Gene symbol	Primer sequence 5' → 3'
TNF- α	Forward TNF- α 5'- CTCTTCTGCCTGCTGCACTTTG -3'
	Reverse TNF- α 5'- ATGGGCTACAGGCTTGTCACTC -3'
Caspase-3	Forward Caspase-3 5'- CCTCAGAGAGACATTCATG-3'
	Reverse Caspase-3 5'- GCAGTAGYCGCCTCTGAAG -3'
GAPDH	Forward GAPDH 5'- GTCTCCTCTGACTTCAACAGCG-3'
	Reverse GAPDH 5'- ACCACCCTGTTGCTGTAGCCAA-3'

Statistical analysis

Statistical analyses were accomplished with software SPSS 26.0 (SPSS Inc., Chicago, IL, USA). All data are presented as mean ± standard deviation (SD). Data analysis used Kruskal-Wallis and continued with the Man-Whitney test with p-value <0.05. P < 0.05 was considered to be statistically significant.

RESULTS

Phytochemical screening of Apium graveolens extract

The phytochemical screening of celery extract revealed the presence of some secondary metabolites such as alkaloids, saponins, tannins, flavonoids, and steroids (Table 2). The total flavonoid content of celery extract was 14.30 ± 0,55 QE/g. These values are higher than total flavonoid content of the same family with methanol solvent [24].

Table 2. Phytochemical screening of secondary metabolites from *Apium graveolens* extract

Chemical componenet	Name of the test	CTFE
Alkaloids	Wagner test	++
Flavonoids	Wilstater test	+++
Tannins	Braemer's test	+
Saponins	Forth test	+
Steroids	Lieberman Burchardt test	+
Terpenoids	Lieberman Burchardt test	-

Effect of Celery Extract Administration on TNF-α gene expression

TNF-α gene expression is a type of pro-inflammatory cytokine that is excreted and secreted by immune cells including monocytes, macrophages, and T cells in response to increased oxidative stress, such as in lead poisoning conditions. In this study, celery extract (T) significantly decrease the TNF-α gene expression (3.13±0.34) compared to control – groups (3.87±0.09). The control + group (2.13±0.26) had a lower trend than the treatment (Figure 1).

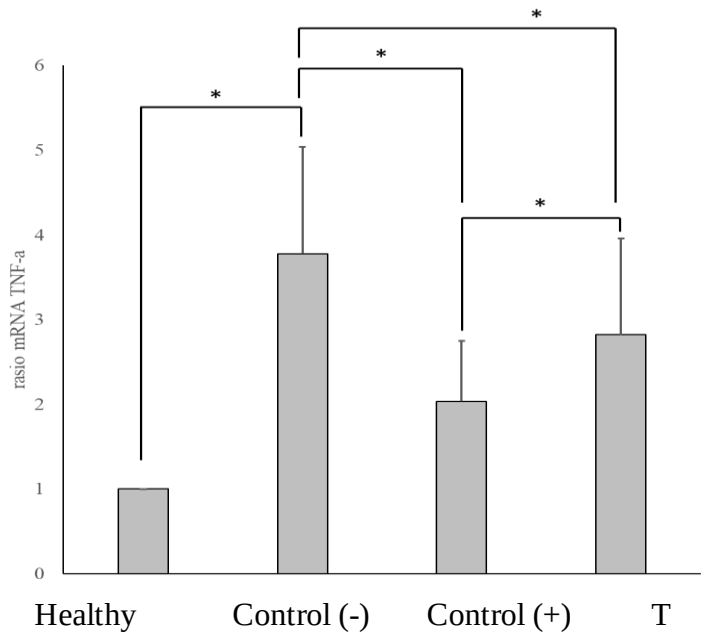

Figure 1. The effect of *Celery extract* on TNF-α gene expression on lead poisoning-induced renal injury rats' model. Data are presented as fold change in gene expression relative to untreated unexposed group *Differences were considered statistically significant at $p < 0.05$. ns were considered statistically non-significant different.

Effect of Celery Extract Administration on Caspase-3 gene expression

The results of caspase-3 gene expression in the T group were lower (2.48±1.23) compared to the control - group (7.95±0.23) and control + group (7.05±0.28) (Figure 2).

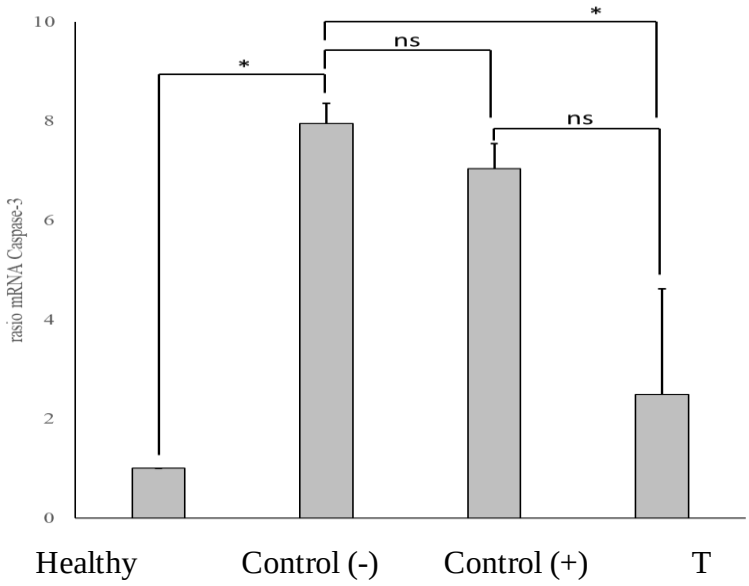

Figure 2. The effect of *Celery extract* on caspase-3 gene expression on lead poisoning-induced renal injury rats' model. Data are presented as fold change in gene expression relative to untreated unexposed group *Differences were considered statistically significant at $p < 0.05$. ns were considered statistically non-significant different.

DISCUSSION

It is public knowledge that lead exposure or lead poisoning can cause a series of physiological, biochemical, and behavioral disorders in experimental animals and humans [25,26]. The kidney is considered one of the target organs affected by lead toxicity owing to its storage in the kidney after lead exposure [27,28]. This study shows that celery extract can reduce the level of TNF alpha gene expression and caspase 3 gene expression in kidneys exposed to lead acetate. Lead exposure was increased the oxidative stress and activate the Nf-kB pathway, increasing the expression of pro-inflammatory cytokines, such as TNF-α [29,30]. This can activate the phosphatidyl triinositol (PI3K) pathway through increased expression of p38 which increases the expression of caspase- 3 [31]. Previous study reported that compounds contained in celery extract such as flavonoids were shown to be antioxidants that can reduce the inflammatory process by increasing the level of nrf2 to suppress oxidative stress [32]. The flavonoid could reduce inflammation and activate antioxidant responses to protect from kidney injury in septic rats by regulating proinflammatory cytokine levels and the Keap1-Nrf2/antioxidant response element (ARE) signaling pathways [31,33]. Previous experiment showed that flavonoid alleviated ethanol-induced liver injury by reducing hepatic apoptosis, oxidative stress, and NOD-like receptor 3 (NLRP3) inflammasome [34–37].

The ability of flavonoid compounds as antioxidants has been shown to reduce oxidative stress conditions leading to inhibition of TNF-α levels [38,39]. The low level of TNF-α inhibits the apoptosis of brain cell by inhibition Fas-associated proteins with death domain (FADD) signaling, resulting in caspase-3 inactivation [40]. Previous study also reported that the blocking of TNF-α production or TNF-α signaling pathways with caspase-3 inhibitors reduces TNF-α-induced hepatic injury [41–43]. Taken together, the presence of celery extract inhibited the lead acetate toxicity by inflammation and apoptosis inhibition. The limitation of this study is that the dose of celery extract used is not better than vitamin E to suppress TNF alpha gene expression and caspase 3 gene expression in rat kidneys exposed to lead acetate. This study also did not measure ROS levels, which is a major factor in the effects of lead acetate.

Conclusion

In this study, we concluded that the celery extract have ability to prevent kidney injury in lead acetate toxicity by antiinflammation and antiapoptotic activity. Overall, the finding of this study could be beneficial for research on accelerating and directing the screening of possible targets and identifying the molecular mechanism of celery extract to prevents lead acetate toxicity.

Authors' contributions

HP, TS, CC and AP contributed to the conception of the work. NDA, SAH, SP, and MF contributed to the acquisition of the work. HP and NDA contributed to the analysis and interpretation of data. AP, CC and NDA contributed to drafting the work. HP, MF and SAH contributed to revising the work critically. NDA contributed to the revising of the manuscript. TS and AP is responsible for giving the final approval of the manuscript.

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Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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