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Protective Effect of Ethanol Extract of Celery (*Apium graveolens* L) on Kidney Damage in Ischemia/ Reperfusion Injury Rats Model

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ABSTRACT. Ischemia/ reperfusion injury (IRI) is marked by the sudden decrease of blood supply to the kidney followed by restoration of blood flow. Ischemic acute kidney injury (AKI) is the leading cause of morbidity and mortality. Therefore, the prevention strategy for decreasing the damage due to IRI is required. Celery (*Apium graveolens* L) is often consumed as food. Celery has antioxidant and anti-inflammatory effects. This study aimed to investigate the protective effect of the celery on kidney damage in the kidney ischemia/ reperfusion injury rat model. Twenty-five rats male, 2-3 months old Sprague Dawley were divided into 5 groups: Group 1 was sham operation, group 2 was ischemia/ reperfusion injury (IRI), group 3, 4, 5 were IRI and 250 mg/kgBW, 500 mg/kgBW, 1000 mg/kgBW of ethanol extract of celery respectively for 14 days before and 3 days after operation. A blood serum sample was taken on 3 days after the operation for measuring urea, creatinine, superoxide dismutase (SOD) and nitrite oxide (NO). Hematoxylin-eosin (HE) staining was utilized to examine kidney tubular injury score. Data were analyzed using One-way ANOVA ($p<0.05$). The ethanol extract of celery at dose 1000 mg/kgBW prevented the increased of urea, creatinine serum, kidney tubular injury score and prevented the decrease of SOD, NO in the kidney ischemia/ reperfusion injury rat model ($p<0.05$). In conclusion, the ethanol extract of celery has a protective effect on kidney damage in the ischemia/ reperfusion injury rat model.

Keyword: Acute kidney injury, Celery, Ischemia/ reperfusion injury

INTRODUCTION

Ischemia/ reperfusion injury (IRI) is characterized by the sudden decrease of blood supply to an organ followed by restoration of blood flow. In the kidney, IRI contributes to pathological conditions called acute kidney injury (AKI) (Malek & Nematbakhsh, 2015). AKI is one of the most concerning health problems. The prevalence of AKI in the world is increasing between 9% - 67% (Mehta et al., 2007). AKI prolonged hospitalization duration of the patient at the hospital so that increase the cost (Kerr, Bedford, Matthews, & O'donoghue, 2014). The decrease of renal blood flow and then reperfusion caused severe damage to the kidney. Kidney damage caused by ischemia/ reperfusion called ischemia/ reperfusion injury (Eltzschig & Eckle, 2011). Ischemic AKI is a major cause of morbidity and mortality (Chertow, Burdick, Honour, Bonventre, & Bates, 2005). Therefore, require the prevention strategy for decreasing damage due to IRI. The mechanism of renal IRI is very complex, but the main pathological pathways are inflammation and release oxygen species (ROS) (Malek & Nematbakhsh, 2015). Blocking either inflammation or release ROS effects were new potential for therapy and protection in the kidney IRI.

Celery is often consumed as food. Most people use stems and leaves of celery as food additives. These ingredients consist of carbohydrates, flavonoids, alkaloids, steroids, glycosides, phenols, furocoumarins, volatile oils, sesquiterpene alcohols, fatty acids, and the wide range of trace elements (Al-snafi, 2014). Celery has been known as anti-oxidative stress (Chonpathompikunlert, Boonruamkaew, Sukketsiri, Hutamekalin, & Sroyraya, 2018; Sameh, Ibtissem, Mahmoud, Boukef, & Naceur, 2011; Sukketsiri, Chonpathompikunlert, Tanasawet, & Choosri, 2016) and anti-inflammatory (Atta & Alkofahi, 1998; Taylor et al., 2012). Several researchers have been used the effect of anti-inflammatory and anti-oxidative stress of celery to protect and decrease the severity of several disease models such as rheumatoid arthritis (Sukketsiri et al., 2016), liver injury induced by carbon tetrachloride (Popović, Kaurinović, Trivić, Mimica-Dukić, & Bursać, 2006), Parkinson's like symptoms (Chonpathompikunlert et al., 2018), prediabetic in elderly (Yusni, Zufry, Meutia, & Sucipto, 2018). The study of celery in ischemia/ reperfusion injury has never been done. Considering the main pathomechanism in ischemia/ reperfusion injury and the potential effect of celery as antioxidant and anti-

inflammatory, it interests the researcher to examine whether they might have a protective effect on kidney damage in ischemia/ reperfusion injury rat models. For this purpose, we prepared the celery ethanol extract in various doses and examined their protective effect on kidney function, NO, SOD and histopathological assessment.

EXPERIMENTAL SECTION

Animal and kidney IRI model

Twenty-five Sprague Dawley male rats (200-250 g, 2-3 months of age; obtained from Pharmacology and Therapy Laboratory, Universitas Gadjah Mada, Yogyakarta). The rats were housed in 12-h light and dark cycle with free access to water and food. The Ethics Committee of Medical Faculty Universitas Jenderal Soedirman, Purwokerto, Indonesia approved this experimental protocol and animal care methods in the experiment through the number of certificate 1628/KEPK/IV/2017.

The rats were anesthetized using intravenous ketamine (100 mg/ kgBW). The abdomen was opened. Kidney ischemia/ reperfusion model was performed by clamping both of renal pedicles, using a non-traumatic vascular clamp (Hammacher®) for 45 minutes. Then, both clamps were released and followed by reperfusion. The incision site then closed using silk surgical thread 3/0 (One Med®). Sham-operated (SO) mice (n = 5) underwent similar procedures except for renal pedicles clamping. All groups were sacrificed on day-3 after the operation.

For sacrifice, the rats were anesthetized with ketamine 100 mg/kgBW). Blood sample for renal function test was taken from the retro-orbital vein. The serum from non-heparinized blood was carefully collected for creatinine, urea, NO, and SOD level. The animals were sacrificed by cervical dislocation. Necropsies of all animals were done after the blood collection. Perfusion of the organ was done from the left ventricle with NaCl 0.9%. The left kidney was harvested and fixated into buffer normal saline for histopathological examination. The tissues were embedded in paraffin, and then sectioned, stained with hematoxylin and eosin then were examined microscopically. The process of preparation and staining conducted in Research Laboratory, Medical Faculty, Universitas Jenderal Soedirman.

Tools and materials

The tools used in this study were rat cages, bottled drinks, digital scales, evaporator, rubber gloves, water bath, vortex, buldock clamp (Hammacher®), rat oral gavage, minor surgical tools, microscope, glass jar, cover glass, tip pipette 10-100 µL (yellow), tip pipette 0.5-10 µL (white), silk surgical thread 3/0 (Onemed®) and other tools that were used as needed. Materials used in this research were rats, pellets, celery plant, ketamine, ethanol 96%, distilled water, alcohol 70%, Hematoxylin-eosin reagent, NaCl 0.9% (Otsu-NS®), and 1% NaCMC solution.

Extraction

Celery plant was collected from Pratin, Purbalingga, Central Java, and it was identified in Taxonomy Laboratory in the Faculty of Biology, Universitas Jenderal Soedirman. The celery plants (stem and leaves) were air-dried to a constant weight and blend to a powder. The dried powder of celery plant (500 g) was soaked and macerated by 2 liters of ethanol (96%) for 72 hours and every 24 hours were replaced with new solvents at room temperature. The macerate was collected and then evaporated using a rotary evaporator.

Treatment

The rats separated into five groups: (1) sham-operated group; (2) untreated ischemia/ reperfusion injury (IRI); (3) IRI pretreated with celery ethanol extract (250 mg/kgBW) orally for 14 days before and 3 days after operation; (4) IRI pretreated with celery ethanol extract (500 mg/kgBW) orally for 14 days before and 3 days after operation; (5) IRI pretreated with celery ethanol extract (1000 mg/kgBW) orally for 14 days before and 3 days after operation.

Evaluation of kidney function

Serum creatinine and urea level were used to evaluate the renal function. To evaluate these factors, serum samples and related kits (prepared by the Pharmacology and Therapy Laboratory, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto, Indonesia) were used.

Measurement of NO

NO is a vasodilator that produces by eNOS in endothelial cell. Measurement of NO was carried out using the Griess method. We used serum sample and NO kit (prepared by Biochemistry Laboratory, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto, Indonesia). The absorbance was assessed using a spectrophotometer 530 nm wavelength.

Measurement of SOD

Antioxidant was estimated by measuring the SOD. At the end of the study, serum sample was used, and SOD kit (prepared by the Biochemistry Laboratory, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto, Indonesia) was used. The absorbance was assessed using a spectrophotometer Ransod 595 nm wavelength.

Kidney histopathology

The kidney was embedded in the paraffin block and cut into the thickness of 4 µm with the microtome. Paraffin sections were deparaffinized and rehydrated using xylene and alcohol serial. The samples staining was performed using hematoxylin and eosin (H&E) to determine tubular injury score. Scoring was done by grading tubular injury and dilatation, intra-luminal cash, and brush border loss. Quantification was measured from 20 fields for each sample with 400x magnification. The lesions were graded on a scale 0 to 4: 0: normal; 1; the injury involves less than 25%; 3: the injury involves 50-75%; 4: the injury involves> 75%.

Statistical analysis

The result was expressed as the mean ± standard deviation. Statistical analysis was performed using One-Way ANOVA followed by post hoc least significance (LSD) test. Differences were considered to be significant at $p< 0.05$.

RESULTS AND DISCUSSION

This experimental study determined the protective effect of celery ethanol extract on kidney damage caused by ischemia/ reperfusion injury. It has been done at the Laboratory of Pharmacology and Therapy, Faculty of Medicine, Universitas Jenderal Sudirman. We used the celery stems and leaves from the local area of Pratin, Purbalingga, Central Java. Kidney function, kidney tubular injury, NO and SOD was assessed to evaluate the damage of the kidney caused by ischemia/reperfusion injury.

Urea and serum creatinine were assessed to evaluate the kidney function. The protective effect of celery ethanol extract on the decrease of kidney function known through the level of urea and serum creatinine. Data of urea and serum creatinine level in SO group, IRI group, IRI + ethanol extract of celery at the doses 250 mg/kgBW, 500 mg/kgBW, 1000 mg/kgBW can be seen in **Table 1**. Saphiro Wilk normality test was performed to determine data distribution of urea and serum creatinine. Levene's test was used to assess the homogeneity of data. The urea was homogenous ($p>0.05$), but not normally distributed ($p<0.05$). We used Log10 for urea data transformation, and the result was normally distributed and homogenous ($p>0.05$). While serum creatinine showed normally distributed ($p>0.05$) and homogenous ($p>0.05$) after data transformation used log10. Normality and homogeneity test of data urea and serum creatinine showed normal and homogenous then continued data analysis using parametric tests.

Tabel 1 shows that the average of urea level, the SO group is 70.00 ±6.28 mg/dL, IRI group is 261.20 ±121.81 mg/dL, IRI+250 mg/kgBW of celery ethanol extract group is 191.60 ±50.38 mg/dL, IRI+500 mg/kgBW of celery ethanol extract group is 169.80 ±41.31 mg/dL, IRI+1000 mg/kgBW of celery ethanol extract group is 145.60±79.50 mg/dL. The average of serum creatinine level of the SO group is 2.236 ±0.26 mg/dL, IRI group is 3.033 ±0.65 mg/dL, IRI+250 mg/kgBW of celery ethanol extract group is 2.477±0.47 mg/dL, IRI+500 mg/kgBW of celery ethanol extract group is 1.799±0.14 mg/dL, IRI+1000 mg/kgBW of celery ethanol extract group is 1.717 ±039 mg/dL. The successful of ischemia/ reperfusion injury model in this study can be seen on urea and serum creatinine level in the IRI group. Urea and serum creatinine level in the IRI group higher than SO group and significantly difference ($p<0.05$). It was consistent with the research conducted by Wu et al., (2014), Younan et al., (2012), Chen et al., (2004), which was serum creatinine level increase in ischemia/ reperfusion injury (Chen et al., 2004; K. Wu, Lei, Tian, & Li, 2014; Younan, Shawky, & Rashed, 2012).

One-way ANOVA test was conducted to determine the difference of average of urea and serum creatinine level between groups after administration of celery ethanol extract 14 days before until 3 days after the operation. One-way ANOVA test proved that there was a significant difference in urea ($p=0.000$) and serum creatinine level ($p=0,000$) between groups ($p<0.05$). Post hoc test was performed using the least significance (LSD). LSD results of urea showed that there were significant differences between SO group to IRI group ($p=0.000$) and also between IRI group to IRI+1000 mg/kgBW of celery ethanol extract group ($p=0.010$). The result of the LSD test of serum creatinine level showed that there were significant differences between SO group to IRI group ($p=0.015$), IRI group to IRI+500 mg/kgBW of ethanol extract of celery group ($p=0.000$) and IRI group to 1000 mg/kgBW of ethanol extract of celery group ($p=0.000$). In this study showed that ethanol extract of celery prevented the increase of urea and serum creatinine level significantly at dose 1000 mg/kgBW compare to IRI group ($p<0.05$).

Tabel 1. Average of urea and serum creatinine level

Groups	SO	IRI	IRI+Ethanol extract of celery (mg/kgBW)			*P Value*
			250	500	1000	
Urea (mg/dL)	70.00 ±6.28*	261.20 ±121.81	191.60 ±50.38	169.80 ±41.31	145.60±79.50*	0.000
Creatinine (mg/dl)	2.236 ±0.26*	3.033 ±0.65	2.477 ±0.47	1.799 ±0.14*	1.717 ±039*	0.000

*P<0.05 significant difference compared with IRI group.

Tabel 2. Average of NO level and tubular injury score

Groups	SO	IRI	IRI+Ethanol extract of celery (mg/kgBW)			*P Value*
			250	500	1000	
NO (µmol/L)	115.59±7.27*	17.62±3.67	96.25±16.88*	111.10±5.93*	113.78 ±8.99*	0.000
Tubular injury score	0.58*	2.78	2.50	2.42	2.08*	0.000

*P<0.05 significant difference compared with IRI group.

In normal condition, creatinine is filtered by glomerulus but not absorbed. About 10-20% creatinine is excreted to proximal tubules. Thus, any damage in tubules will affect the process, and consequently, the serum creatinine level becomes higher. The first change induced by ischemia is associated with decreased oxygen delivery. Decreased oxygen levels will induce the switch from aerobic to anaerobic glucose metabolism. Anaerobic metabolism is insufficient to meet the demand of aerobic tissues. The lack of oxygen will further enhance ATP consumption in the mitochondria, then the cell function will be disturbed and damaged. Serum creatinine and urea have typically been used to diagnose AKI (Peres et al., 2010). Serum creatinine is an inexpensive and readily available physiological biomarker of kidney function and remains the standard gold test for diagnosing AKI (Okusa et al., 2013).

The principle of IRI is a sudden decrease in blood supply to an organ followed by restoration of blood flow. According to main pathological pathways on kidney IRI that are inflammation and release ROS caused endothelial damage. Endothelial dysfunction decreases eNOS activation so that decrease NO production. NO is vasodilator produced by the endothelial cell. Several studies with the goal of increasing renal NO activity have demonstrated protective effects in AKI. The decrease of NO caused vasoconstriction so that injury more severe (Basile & Yoder, 2014). Celery ethanol extract prevented the decrease of NO in IRI can be seen in **Table 2**. The consequence of ischemia continued by reperfusion is a tubular injury, especially showed in proximal tubules. The tubular injury was characterized by tubular dilatation, loss of brush border of proximal tubules, depletion of the tubular epithelial cell and the accumulation of intraluminal cast (**Figure 1**) (Bonventre & Yang, 2011). The tubular injury score data can be seen in **Table 2**.

Tabel 2 shows that the average of NO level, the SO group is 115.59±7.27 µmol/L, IRI group is 17.62±3.67 µmol/L, IRI+250 mg/kgBW of celery ethanol extract group is 96.25±16.88 µmol/L, IRI+500 mg/kgBW of celery ethanol extract group is 111.10±5.93 µmol/L, IRI+1000 mg/kgBW of celery ethanol extract group is 113.78 ±8.99 µmol/L. One way ANOVA test proved that there was a difference in NO level between group (p=0,000). LSD test showed that there was a significant difference between SO group compare to IRI group, IRI group compare to all of the doses ethanol extract of celery (p=0.000). The higher dose of celery ethanol extract showed a higher NO level. The ethanol extract of celery at a dose of 1000 mg/kgBW is the significantly highest of NO level. According to Webb et al. (2004), administration NO protect myocardium from ischemia/ reperfusion injury (Webb et al., 2003). In addition to protect the decrease of NO, celery is a source of nitrate (Lidder & Webb, 2013).

Table 2 presents data on the protective effect of celery ethanol extract to tubular injury score. The results showed IRI causes a significant increase in tubular injury score. Tubular injury score in IRI+250 mg/kgBW of celery ethanol extract group is 2.50, IRI+500 mg/kgBW of celery ethanol extract group is 2.42, IRI+1000 mg/kgBW of celery ethanol extract group is 2.08. One way ANOVA test proved that there was a significant difference in tubular injury score between group (p=0.000). LSD test showed significant differences in the normal group of rats and the ethanol extract of celery 1000 mg/kgBW compare to IRI group (p=0.000). That means, celery ethanol extract at dose 1000 mg/kgBW protected tubular injury in this study. According to Wu et al. (2007), ischemia/ reperfusion injury causes damage to the kidneys, especially the kidney tubules start on day 1-9 with peak damage occurring on the first day and decreasing until the 9th day (H. Wu et al., 2007).

In this study, ethanol extract of celery at a dose of 1000 mg/kgBW prevented kidney damage. The protective effect produced by the ethanol extract of celery is suspected because of the content of apiin and phthalide glycoside as anti-inflammatory (Mencherini et al., 2007; Zhu, Bao, Deng, Li, & Chen, 2017) and caffeic acid, p-coumaric acid, ferulic acid, apigenin, luteolin, tannin, saponin, kaempferol as powerful antioxidant (Kooti & Daraei, 2017).

Ischemia/ reperfusion injury produced an excessive amount of ROS cause of oxidative stress (Malek & Nematbakhsh, 2015). Antioxidant such as SOD decreased oxidative stress by eliminated superoxide, hydrogen peroxide, and hydroxyl radicals. The data of SOD in this study can be seen in **Table 3**.

Table 3 shows that the average of SOD, the SO group is 74.27±7.27 U/mL, IRI group is 47.44±5.18 U/mL, IRI+250 mg/kgBW of celery ethanol extract group is 66.71±3.73 U/mL, IRI+500 mg/kgBW of celery ethanol extract group is 73.79±2.86 U/mL, IRI+1000 mg/kgBW of celery ethanol extract group is 81.97±2.79 U/mL.

The administration of celery ethanol extract prevented the decrease of SOD in IRI rat model. We used a one-way ANOVA test because data were normally distributed and homogenous. One-way ANOVA proved that there was a difference in SOD level between groups (p=0.000). LSD test was conducted, there was a significant difference between SO group compare to IRI group, IRI group compare to all of the doses of celery ethanol extract (p=0.000). The higher dose of celery ethanol extract showed a higher SOD level. The ethanol extract of celery at a dose of 1000 mg/kgBW is the significantly highest of SOD level. SOD level in IRI group lower than SO group significantly. The previous study reported that ischemia did not disturb of SOD, but reperfusion following ischemia led to a significant decrease in antioxidant enzymes, so that SOD in IRI was low (Malek & Nematbakhsh, 2015). The administration of celery ethanol extract increases the SOD in IRI rat model. Therefore, reducing free radical production is the strategy to protect the tissue damage during IRI.

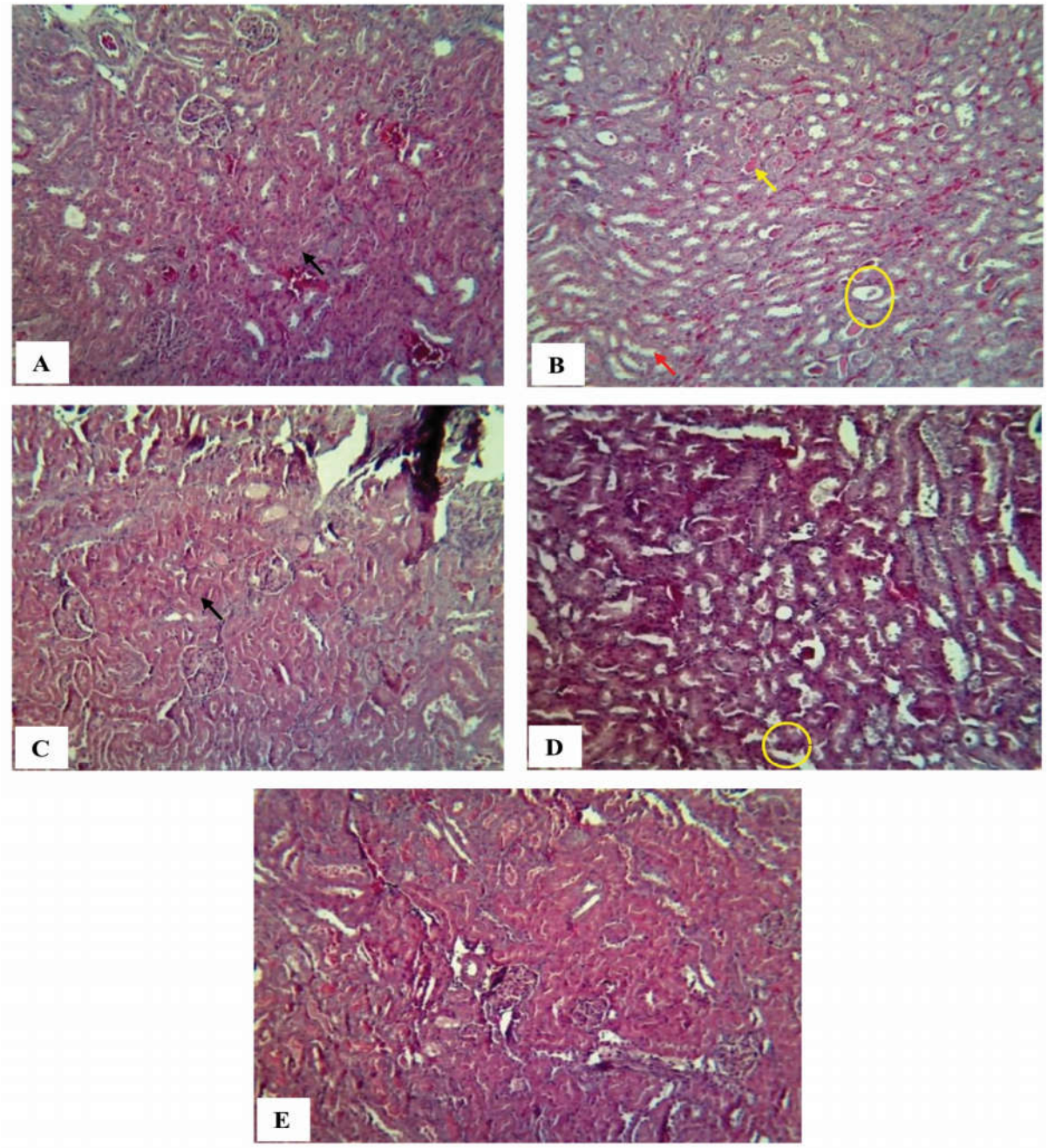

Figure 1. The renal histological picture of H&E staining to show tubular injury. (**A**) Group 1 (Sham Operation), (**B**) Group 2 (IRI), (**C**) Group 3 (IRI+ethanol extract of celery 250 mg/kgBW), (**D**) Group 4 (IRI+ethanol extract of celery 500 mg/kgBW), (**E**) Group 5 (IRI+ethanol extract of celery 1000 mg/kgBW)). Black arrows showed the brush border. Red arrows showed depletion of the epithelial cell. Yellow arrows showed intraluminal cast. Yellow circle showed tubular dilatation.

Tabel 3. Average of SOD level

Groups	**SO**	**IRI**	**IRI+Ethanol extract of celery (mg/kgBW)**			***P Value***
			250	**500**	**1000**	
SOD (U/mL)	74.27±7.27*	47.44±5.18	66.71±3.73*	73.79±2.86*	81.97± 2.79*	0.000

*P<0.05 significant difference compared with IRI group.

CONCLUSION

Ischemia/ reperfusion injury causes kidney damage marked by the increased urea, serum creatinine level, tubular injury score, and decrease of NO and SOD level. Oral administration of celery ethanol extract at a dose of 1000 mg/kgBW protected kidney damage in the kidney ischemia/ reperfusion injury rat model.

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REFERENCES

Al-snafi, A. E. (2014). The Pharmacology of Apium graveolens-A Review. *International Journal for Pharmaceutical Research Scholars (IJPRS)*, *3*(1–1), 671–677.

Atta, A. ., & Alkofahi, A. (1998). Anti-nociceptive and anti-inflammatory effects of some Jordanian medicinal plant extracts. *Journal of Ethnopharmacology*, *60*(2), 117–124. https://doi.org/10.1016/S0378-8741(97)00137-2

Basile, D. P., & Yoder, M. C. (2014). Renal endothelial dysfunction in acute kidney ischemia reperfusion injury. *Cardiovascular & Hematological Disorders Drug Targets*, *14*(1), 3–14.

Bonventre, J. V., & Yang, L. (2011). Cellular pathophysiology of ischemic acute kidney injury. *The Journal of Clinical Investigation*, *121*(11), 4210–4221. https://doi.org/10.1172/JCI45161.4210

Chen, C., Liu, Z., Wang, H., He, W., Wang, Y., & Wu, W. (2004). Effects of ulinastatin on renal ischemia-reperfusion injury in rats, *25*(10), 1334–1340.

Chertow, G. M., Burdick, E., Honour, M., Bonventre, J. V, & Bates, D. W. (2005). Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *Journal of the American Society of Nephrology : JASN*, *16*(11), 3365–3370. https://doi.org/10.1681/ASN.2004090740

Chonpathompikunlert, P., Boonruamkaew, P., Sukketsiri, W., Hutamekalin, P., & Sroyraya, M. (2018). The antioxidant and neurochemical activity of Apium graveolens L. and its ameliorative effect on MPTP-induced Parkinson-like symptoms in mice. *BMC Complementary and Alternative Medicine*, *18*(1), 1–12. https://doi.org/10.1186/s12906-018-2166-0

Eltzschig, H. K., & Eckle, T. (2011). Ischemia and reperfusion—from mechanism to translation. *Nature Medicine*, *17*(11), 1391–1401. https://doi.org/10.1038/nm.2507

Kerr, M., Bedford, M., Matthews, B., & O'donoghue, D. (2014). The economic impact of acute kidney injury in England. *Nephrology Dialysis Transplantation*, *29*(7), 1362–1368. https://doi.org/10.1093/ndt/gfu016

Kooti, W., & Daraei, N. (2017). A Review of the Antioxidant Activity of Celery (Apium graveolens L). *Journal of Evidence-Based Complementary and Alternative Medicine*, *22*(4), 1029–1034. https://doi.org/10.1177/2156587217717415

Lidder, S., & Webb, A. J. (2013). Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate-nitrite-nitric oxide pathway. *British Journal of Clinical Pharmacology*, *75*(3), 677–696. https://doi.org/10.1111/j.1365-2125.2012.04420.x

Malek, M., & Nematbakhsh, M. (2015). Renal ischemia/reperfusion injury; from pathophysiology to treatment. *Journal of Renal Injury Prevention*, *4*(2), 20.

Mehta, R. L., Kellum, J. a, Shah, S. V, Molitoris, B. a, Ronco, C., Warnock, D. G., & Levin, A. (2007). Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Critical Care (London, England)*, *11*(2), 1–8. https://doi.org/10.1186/cc5713

Mencherini, T., Cau, A., Bianco, G., Loggia, R. Della, Aquino, R. P., & Autore, G. (2007). An extract of *Apium graveolens* var. dulce leaves: structure of the major constituent, apiin, and its anti-inflammatory properties. *Journal of Pharmacy and Pharmacology*, *59*(6), 891–897. https://doi.org/10.1211/jpp.59.6.0016

Okusa, M. D., Jaber, B. J., Doran, P., Duranteau, J., Yang, L., Murray, Patrick T., … Ince, C. (2013). Physiological Biomarkers of Acute Kidney Injury: A Conceptual Approach to Improving Outcomes. *Contrib Nephrol*, *182*, 1–17. https://doi.org/10.1159/000349967

Peres, L. A. B., Cunha Júnior, A. D. Da, Schäfer, A. J., Silva, A. L. Da, Gaspar, A. D., Scarpari, D. F., Oliveira, T. F. T. De. (2010). Biomarkers of acute kidney injury. *Jornal Brasileiro de Nefrologia : 'orgão Oficial de Sociedades Brasileira e Latino-Americana de Nefrologia*, *35*(3), 229–236. https://doi.org/10.5935/0101-2800.20130036

Popović, M., Kaurinović, B., Trivić, S., Mimica-Dukić, N., & Bursać, M. (2006). Effect of celery (Apium graveolens) extracts on some biochemical parameters of oxidative Stress in mice treated with carbon tetrachloride. *Phytotherapy Research*, *20*(7), 531–537. https://doi.org/10.1002/ptr.1871

Sameh, B., Ibtissem, B., Mahmoud, A., Boukef, K., & Naceur, A. (2011). Antioxidant Activity of Apium graveolens Extracts. *Journal of Biologgically Active Product from Nature*, *1*(5–6), 340–343. https://doi.org/10.1080/22311866.2011.10719102

Sukketsiri, W., Chonpathompikunlert, P., Tanasawet, S., & Choosri, N. (2016). Effects of Apium graveolens Extract on the Oxidative Stress in the Liver of Adjuvant-Induced Arthritic Rats. *Prev. Nutr. Food Sci*, *21*(2), 79–84.

Taylor, P., Baananou, S., Borgi, W., Mahmoud, A., Boukef, K., & Chouchane, N. (2012). Anti-inflammatory and Analgesic Activities of Tunisian Apium graveolens L . Leaves Extracts in Rats. *Journal of Biologgically Active Product from Nature*, *4*(August 2013), 37–41. https://doi.org/10.1080/22311866.2012.10719129

Webb, A., Bond, R., Mclean, P., Uppal, R., Benjamin, N., & Ahluwalia, A. (2003). Reduction of nitrite to

nitric oxide during ischemia protects against myocardial ischemia–reperfusion damage, 2–7. https://doi.org/10.1073/pnas.0402927101

Wu, H., Chen, G., Wyburn, K. R., Yin, J., Bertolino, P., Eris, Jj. M., … J, C. (2007). TLR4 Activation Mediates Kidney Ischemia/Reperfusion Injury. *The Journal of Clinical Investigation*, *117*, 2847–2859. https://doi.org/10.1097/00007890-200607152-00905

Wu, K., Lei, W., Tian, J., & Li, H. (2014). Atorvastatin treatment attenuates renal injury in an experimental model of ischemia – reperfusion in rats. *BMC Nephrology*, *15*(14).

Younan, S. M., Shawky, H. M., & Rashed, L. A. (2012). Effect of Ischemic Postconditioning on Renal Ischemia-Reperfusion Injury in Male Rats. *Kasr EL Aini Medical Journal*, *18*(2), 23–38.

Yusni, Y., Zufry, H., Meutia, F., & Sucipto, K. W. (2018). The effects of celery leaf (Apium graveolens L.) treatment on blood glucose and insulin levels in elderly pre-diabetics. *Saudi Medical Journal*, *39*(2), 154–160. https://doi.org/10.15537/smj.2018.2.21238

Zhu, L.-H., Bao, T.-H., Deng, Y., Li, H., & Chen, L.-X. (2017). Constituents from Apium graveolens and their anti-inflammatory effects. *Semiconductor Science and Technology*, 2–9. https://doi.org/10.1080/10286020.2017.1381687