Purslane (Portulaca oleracea) Ethanol Extract decreases hs-CRP levels and Total Score of Renal Tubular Degeneration in Rat Model of Gentamicine-Induced Renal Damage

Ragil Saptaningtyas¹, Shofa Chasani², Taufiqurrachman Nasihun³
1 Masters in Biomedical Sciences, Faculty of Medicine, Sultan Agung Islamic University, Semarang, Indonesia
2 Department of Internal Medicine, Faculty of Medicine, Diponegoro University, Semarang, Indonesia
3 Department of Biochemistry, Faculty of Medicine, Sultan Agung Islamic University, Semarang, Indonesia

*Corresponding author email: ragilsapta92@yahoo.com

ABSTRACT

Introduction: Injection of intraperitoneal gentamicin at a dose of 60 mg/kg BW intra-peritoneal in rats for 7 days has been shown to trigger renal tubular degeneration and increase in high sensitivity C-reactive protein (hs-CRP) level. The use of purslane (Portulaca oleracea) to reduce hs-CRP levels and total score of renal tubular degeneration has not been reported. Objective: to determine the effect of purslane ethanol extract on hs-CRP levels and renal tubular degeneration score in gentamicin induced-renal damage in rats.

Methods: This was a study using posttest only control group design. Twentyfive male Wistar rats aged 8-12 weeks, weighed 150-200 grams were allocated into 5 groups: normal (Nor-G), without treatment; negative (Neg-G), gentamicin (60 mg/kg BB), intraperitoneally; P-200; P-300 ; P-400 (gentamicin plus purslane extract at a dose of 200, 300, and 400 mg/kg BB/hari respectively) for 7 days. The hs-CRP was evaluated using the ELISA method. Total score of renal tubular degeneration was evaluated using modified score of Sarjadi.

Results: there was a significant difference in mean hsCRP level and total score of renal tubular degeneration between groups (p <0.05). Posthoc analysis showed that hsCRP level and total score group of P-200, P-300 and P-400 were significantly lower than those of Neg-G, p <0.05. Meanwhile, the levels of hsCRP and total score of renal tubular degeneration in P-200, P-300, P-400, and Nor-G groups were not significantly different (p> 0.05).

Conclusion: the administration of purslane ethanol extract at doses of 200, 300, and 400 mg/kg BB for 7 days improve hs-CRP level and total score of renal tubular degeneration similar to normal.

Keywords: purslane ethanol extract, gentamicin, hs-CRP levels, total score of renal tubular degeneration.

INTRODUCTION

Acute kidney Injury (AKI) is a condition in which the process of renal glomerular filtration rate decreases rapidly causing nitrogen retention, especially creatinine and blood urea nitrogen (BUN). The causes of AKI are divided into 3 categories namely prerenal, renal, and postrenal. The most common causes of AKI is the renal category comprising 35% (Ostermann and Joannidis, 2016). Degeneration of renal tubular epithelial cells falls into the renal category that can be...
triggered by nephrotoxic drugs, including gentamicin (Basile, Anderson and Sutton, 2012). The incidence of AKI due to aminoglycosides varies ranging from 5% to 25% (Dutta et al., 2017). A multinational study involving 4683 patients showed that 1261 had AKI, and 543 required hemodialysis (Kaddourah et al., 2017). Purslane (Portulaca oleracea), as an anti-inflammation agent, has been shown to inhibit production of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells compared to indomethacin (Young Ock et al., 2015). In addition, purslane ethanol extract at a dose of 400 mg has been shown to reduce carrageenan-induced rat paw edema by 30.2% (Andayani, Suprihartini and Astuti, 2015). However, it is unknown whether purslane extract can reduce hsCRP and total score of renal tubular degeneration in renal tubules induced by gentamicin.

Gentamicin, one of aminoglycoside, is the most active antibiotic against gram-negative bacteria. However, the irrational use of gentamicin can cause AKI (Grill and Maganti, 2011). A study conducted by Lintong showed that the histopathologic appearance of kidney of Wistar rats injected with gentamicin at a dose of 60 mg/kg BW for 7 days showed swelling, necrosis, and apoptosis (Lintong, Kairupan and Sondakh, 2012). Kidney damage occurs due to the accumulation of gentamicin in the epithelial cells of the proximal tubule and integrity disruption of the lysosome membrane leading to the release of protease and gentamicin enzymes into the cytoplasm. This can trigger inflammation mediated by nuclear factor kappaB (NF-kB) followed by the formation of reactive oxygen species (ROS) (Wang et al., 2016). ROS then damages proximal tubular endothelial cells and various self-defense cells such as natural killer (NK) cells, neutrophils, macrophages, and dendritic cells in damaged tissue. These leads to a release of proinflammatory cytokines such as TNF-α, IL-1 and IL-6, followed by the synthesis and secretion of CRP by hepatocytes cells (Duann et al., 2016). High sensitivity-CRP (hs-CRP) is a CRP measurement parameter in a very low concentration (less than 0.2 ng/mL).

Purslane (Portulaca oleracea) contains tocopherol, ascorbic acid, beta carotene, and flavonoids capable of being scavengers against ROS. Also capable of reducing cell damage and inflammation in the kidneys due to exposure to gentamicin followed by a decrease in CRP and total score of renal tubular degeneration induced by gentamicin (Jayaprakasha, Jagan Mohan Rao and Sakariah, 2013; Uddin et al., 2014; Sudaryati and Nusandari, 2017). The objective of this study was to evaluate whether the administration of purslane extract improve hs-CRP and total score of renal tubular degeneration induced by gentamicin in rats.

**METHODS**

This was an experimental study with a post test only control group design. A total 25 male Wistar rats aged 8-12 weeks weighing 150-200 grams were divided into 5 groups. Groups of normal (Nor-G) received distilled water. The negative control (Neg-G) received intraperitoneal injection of gentamicin at a dose of 60 mg/kg BW for 7 days. The treatment groups consists of P-200, P-300, and P-400, given an intraperitoneal injection of gentamicin at a dose of 60 mg/kg BW for 7 days and ethanol extract of purslane at dose of 200 or 300 or 400 mg/kg BW orally for 7 days. The study was concluded on day 8 for the Nor-G group and day 15 for the Neg-G, P-200, P-300, and P-400. The blood sample was obtained from orbital sinus of the eye. Data on hs-CRP levels were analyzed using the ELISA method. The kidney was removed. The total score of renal tubular degeneration was analyzed using a modified scoring of Sarjadi. The research was conducted after obtaining ethical approval from the Ethics Committee of FK UNISSULA (Decree no. 285/VII/2018/Bioethics Committee).

**Tabel 1. Mean level of hs-CRP and renal tubular degeneration**

| Variable                          | Nor-G (n=6; 5.2±0.4472) | Neg-G (n=5; 5.2±0.4472) | P-200 (n=5; 5.2±0.4472) | P-300 (n=5; 5.2±0.4472) | P-400 (n=5; 5.2±0.4472) | p (ANOVA) |
|----------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-----------|
| hs-CRP (ng/ml)                   | 0.0927 ± 0.0045         | 0.6990 ± 0.0055         | 0.1250 ± 0.0055         | 0.1269 ± 0.0055         | 0.0788 ± 0.0055         | <0.001    |
| Total score of renal tubular degeneration (Σ) | 3.1667 ± 0.0045         | 8.000 ± 0.0055          | 5.200 ± 0.0055          | 4.600 ± 0.0055          | 4.400 ± 0.0055          | <0.004    |
Purslane (Portulaca oleracea) Ethanol Extract

Purslane was obtained from the Bandungan area, Semarang Regency. All parts of purslane were dried then macerated with 96% ethanol, and evaporated with a rotary evaporator to obtain a pure purslane extract. The purslane ethanol extract was mounted into a bottle and stored in the freezer. The ethanol extract of purslane was then weighed to obtain doses of 200, 300, and 400 mg/kg BW of male Wistar rats. The weighed purslane ethanol extract was then dissolved in distilled water to 40 mL, homogenized, and kept in a tightly closed brown bottle. Each treatment group (P-200, P-300 and P-400) was given ethanol purslane of 1 mL per day using a feeding tube for 7 days.

Figure 1. Kidney morphology in Nor-G (a control rat), Neg-G (rat treated with gentamicin alone), P-200 (rat treated with gentamicin + purslane extract at the dose of 200 mg/Kg BW), P-300 (rat treated with gentamicin + purslane extract at the dose of 300 mg/Kg BW), P-400 (rat treated with gentamicin + purslane extract at the dose of 400 mg/Kg BW).

DH: Hydropic degeneration, DP: parenchymatous degeneration, DL: lipid degeneration (Haematoxylen & Eosin × 400).

Figure 2. A. Level of hs-CRP; B. Total score of renal tubular degeneration in each group. Post Hoc Analysis p<0.05; ns: not significant.
Measurement of hs-CRP level

hs-CRP level was evaluated using ELISA kit started with adding 100 uL of standard, sample in each well, closed and incubated for 90 minutes at a temperature of 37º C. Then, the liquid in wells was disposed. After that, 100 uL Biotinylated Ab detection was added to each of the wells, incubated for 1 hour at 37 ºC. The next process is the washing the wells by adding a wash buffer of 350 uL 3 times. Then 100 µL of HRP conjugate working solution was added to each well, covered and incubated for 30 minutes at 37 ºC. The washing was carried out as in the previous washing process for 5 times adding 90 uL of substrate reagent to each well until homogeneous, and incubated for 15 minutes at 37º C and it was keep away from light. Next, a stop solution of 50 µL was added to each well. The absorbance was read on the ELISA reader with a wavelength of 450 nm. The standard absorbance data obtained were then curved and the hsCRP concentration of the sample was calculated in units of ng/ml.

Examination of Total Score of Renal Tubular Degeneration

The total score for tubular degeneration was calculated by modified score of Sarjadi. The degeneration was graded as follows: 0= normal tubule, 1= parenchymal degeneration, 2= hydropic degeneration, 3 = fatty degeneration.

Statistical Analysis

The data were statistically analyzed using one-way ANOVA test and posthoc LSD test. p< 0.05 was accepted as statistically significant value.

RESULTS

The administration of ethanol extract of purslane doses of 200, 300, and 400 mg/kg body weight in gentamicin-induced renal damage for 7 days resulted in mean hs-CRP and total score of tubular degeneration as shown in Table 1.

Table 1 shows that the highest mean hs-CRP level was in the Neg-G group followed by the P-300, P-200, Nor-G, and the lowest was found in P-400. ANOVA test showed that the levels of hs-CRP between groups were significantly different (p <0.05).

The highest total score of renal tubular degeneration was found in the Neg-G group followed by P-200, P-300, P-400, and the lowest was found in the Nor-G group (figure 1). ANOVA test showed that the total score of renal tubular degeneration was significantly different (p <0.05). To determine significant differences between groups, Posthoc LSD was applied as follows:

hs-CRP levels

The results of the Post-Hoc analysis showed that the hs-CRP level in the Neg-G group was significantly higher than the Nor-G group (p <0.05). The levels of hs-CRP at P-200, P-300, and P-400 were significantly higher than those of Neg-G (p< 0.05), not significantly different than those of Nor-G (p> 0.05). Whereas between P-200, P-300, and P-400 there was no significant difference, p>0.05 (Figure 2). The results of this study indicate that administration of purslane ethanol extract at doses of 200, 300, and 400 mg/kg BW male Wistar rats can reduce hs-CRP levels in gentamicin induced renal damage.

Total score of Renal Tubular Degeneration

The post hoc LSD test showed that the mean total score of renal tubular degeneration (TSRTD) in the Neg-G group was significantly higher than that of the Nor-G group (p <0.05). The mean TSRTD at P-200, P-300, P-400 was significantly lower than Neg-G (p <0.05). The mean TSRTD at P-200, P-300, P-400 did not differ significantly compared to that of Nor-G (p> 0.05). Similarly, mean TSRTD among the P-200, P-300 and P-400 (p> 0.05) (Figure 2). The results of this study indicate that administration of purslane ethanol extract at doses of 200, 300, and 400 mg/kg BW was able to reduce TSRTD in gentamicin induced renal damage.

DISCUSSION

The results of this study indicate that administration of purslane ethanol extract at doses of 200, 300, and 400 mg/kg BW/day affect hs-CRP levels and the total score of renal tubular degeneration in gentamicin induced renal damage. Administering gentamicin at a dose of 60 mg/kg BW in rats intraperitoneally for 7 days was proven to cause renal tubular degeneration indicated by an increase in TSRTD and hs-CRP in the negative control group compared to the normal group. The finding of this supports that of Lintong’s study, showing that the administration of gentamicin 60 mg/kg BW causes inflammation and fatty degeneration in tubular epithelial cells (Lintong, Kairupan and Sondakh, 2012). The results of other studies also showed that gentamicin is a drug that plays an important role in the incidence of kidney toxicity because it is actively eliminated by glomerular filtration. Furthermore, 3-5% of the gentamicin entering the glomerular filtration is actively reabsorbed by the
proximal tubular cells and causes necrosis of the proximal segment of the tubule. In addition, damage to the renal tubules due to gentamicin, is also caused by changes in the main components of cells involved in the transport of water and compounds dissolved in it. The results of animal studies indicate that gentamicin administration can cause necrosis and apoptosis in tubular epithelial cells (Randjelović et al., 2017).

Necrosis and apoptosis can trigger an inflammation characterized by an increase in TSRTD and hs-CRP, the sensitive markers of inflammation. The increase in hs-CRP levels is caused by the accumulation of gentamicin in the epithelial cells of the proximal renal tubules, which causes disruption of membrane permeability causing lysosomal rupture. The lysosomal rupture then lead to increased levels of pathogen associated molecular pattern molecules (PAMPs) and damage associated molecular pattern molecules (DAMPs), which then triggers activation of toll like receptors (TLRs). Toll like receptors will increase levels of NF-kB leading to increased levels of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α so that hs-CRP levels increase (Duann et al., 2016; Mulay, Linkermann and Anders, 2016).

The results of this study also showed that the levels of TSRTD and hs-CRP, the parameters of acute inflammation, can be detected in low concentrations, were shown to decrease after administration of purslane extract at doses of 200, 300, and 400 mg/kg BW/day for 7 days. This illustrates that purslane extract containing tocopherol, ascorbic acid, beta carotene, and flavonoids has an anti-inflammatory activity. These result supports that of study conducted by Kim showing that purslane extract was able to significantly reduce IL-6 and TNF-α levels in RAW 246.7 macrophage cell culture (Young Ock et al., 2015). Since hs-CRP formation in hepatocyte cells is induced by IL-6 and TNF-α, therefore the decrease in IL-6 and TNF-α levels will be followed by a decrease in hs-CRP. In addition, the purslane ethanol extract containing tannins, saponins, tocopherols, omega 3 fatty acids, and flavonoids was shown to reduce the level of inflammation by becoming scavengers against ROS. The vitamin and mineral in purslane ethanol extract was also shown to able to improve chemotaxis and improve overall phagocytosis. The decrease in macrophage activity will reduce the levels of proinflammatory cytokines such as IL-1, IL-6, and TNF-α leading to decreased hs-CRP (Trevor, Katzung and Kruidering-Hall, 2014; Uddin et al., 2014).

The study also indicates that intra-peritoneal injection of gentamicin at 60 mg/kg BW for 7 days can cause parenchymal degeneration, hydropic degeneration, and lipid degeneration, but not necrosis of tubular epithelial cells. Parenchymal degeneration is the lowest degree of degeneration. Parenchymal degeneration occurs because the mitochondria and endoplasmic reticulum are impaired due to oxidation. This causes cell swelling and turbidity in cytoplasm. Cell swelling occurs because the injury is unable to eliminate water causing water retention in cell (Fahrimal, Rahmiwati and Aliza, 2016; Almunawati, Budiman and Aliza, 2017). Meanwhile, hydropic generation is degeneration that is more severe than parenchymal degeneration. The histologic appearance showed that there was a water-filled vacuolization in the cytoplasm. Lipid degeneration occurs due to an abnormal accumulation of fat in the various cytoplasm which then pushes the nucleus to the edge (Suhita, Sudira and Winaya, 2013).

Gentamicin enters the cell by pinocytosis through the megalin and cubilin receptors on the epithelial cell membrane of the renal tubules, and then most of it will accumulate in the lysosomes. The accumulated gentamicin causes lysosome aggregation and interferes the permeability of the cell membrane, hence the lysosomes rupture. The rupture of lysosomes causes gentamicin and the enzyme preotease to enter into the cytoplasm. This process triggers PAMPs and DAMPs) which can activate pro-inflammatory cytokines such as IL-1, IL-6, and TNF- α via the NF-kB pathway. Other than NF-kB pathway, cytokine activation is also triggered by the inflammosome complex leading to the increase in levels of proinflammatory cytokines. The presence of gentamicin that is released causes renal tubular injury (Quiros et al., 2011; Wang et al., 2016). Renal dysfunction begins when Na + K + -ATPase activity is disrupted due to transport barriers within cells. This causes the cells to lack ATP and an imbalance of sodium and potassium so that the cells undergo degeneration, both parenchymal, hydropic, and lipid degeneration. Cell damage and inflammation can also increase levels of ROS.

The results of this study indicate that administration of purslane extract at doses of 200 mg, 300 mg, and 400 mg for 7 days can improve renal damage induced by gentamicin. This occurs because the content of purslane ethanol extract such as antioxidants, vitamins and minerals can be scavengers against free radicals formed during cell injury leading to suppression of effect of lipid peroxidation and decreased renal tubular damage. Antioxidants will induce tissue proliferation and regeneration of cells, and therefore will replace damaged cells and restore kidney function. The vitamin and mineral content in the purslane ethanol extract improves chemotaxis and phagocytosis resulted in
suppression of inflammation and the lower level of total score of renal tubular degeneration (Trevor, Katzung and Kruidering-Hall, 2014; Uddin et al., 2014).

CONCLUSION
The administration of purslane extract at doses of 200 mg, 300 mg, and 400 mg for 7 days improve hs-CRP level and total score of renal tubular degeneration in rats.

CONFLICT OF INTEREST
There is no conflict of interest in this publication

ACKNOWLEDGMENT
We highly appreciate and thank the laboratory staffs of the Center for Food and Nutrition Studies, Gadjah Mada University for their hard work and guidance in maintaining and testing samples during this research.

REFERENCES
Almunawati, Budiman, H. and Aliza, D. (2017) ‘Histopatologi Ginjal Tikus Putih (Rattus novergicus) yang Diinjeksi Formalin’, JIMVET, 1(3), pp. 424–431.

Andayani, D., Suprihartini, E. and Astuti, M. (2015) ‘Antiinflamasii Ekstrak Etanol Krokok (Portulaca oleracea, L.) pada Udema Tikus yang diinduksi Karagenin’, Molecules, p. 20.

Basile, D. P., Anderson, M. D. and Sutton, T. A. (2012) ‘Pathophysiology of Acute Kidney injury’, National Institute of Health, (2), pp. 1303–1353. doi: 10.1002/cphy.c110041.

Duann, P. et al. (2016) ‘Autophagy, innate immunity and tissue repair in acute kidney injury’, International Journal of Molecular Sciences, 17. doi: 10.3390/ijms17050662.

Dutta, R. K. et al. (2017) ‘Beneficial Effects of Myo-Inositol Oxynegenase Deficiency in Cisplatin-Induced AKI’, Journal of American Society of Nephrology, pp. 1421–1436. doi: 10.1002/jn.17050662.

Fahrimal, Y., Rahmiwati and Aliza, D. (2016) ‘Gambaran Histopatologis Ginjal Tikus Putih (Rattus novergicus) Jantan yang Diinfekskian Trypanosoma evansi dan Diberi Ekstrak Daun Sernai (Wedelia biflora)’, Jurnal Medika Veterinaria, 10(2), pp. 166–170. doi: 10.21157/j.med.vet.v10i2.4386.

Grill, M. F. and Maganti, R. K. (2011) ‘Neurotoxic effects associated with antibiotic use: Management considerations’, British Journal of Clinical Pharmacology, 72(3), pp. 381–393. doi: 10.1111/j.1365-2125.2011.03991.x.

Jayaprakasha, G. K., Jagan Mohan Rao, L. and Sakariah, K. K. (2013) ‘Chemistry and biological activities of Flavonoids: An Overview’, The Scientific World Journal. doi: 10.1016/j.tifs.2005.08.006.

Kaddourah, A. et al. (2017) ‘Epidemiology of Acute Kidney Injury in Critically Ill Children and Young Adults’, New England Journal of Medicine, 376(1), pp. 1–23. doi: 10.1056/nejmoa1611391.

Lintong, P. M., Kairupan, C. F. and Sondakh, P. L. N. (2012) ‘Gambaran Mikroskopik Ginjal Tikus Wistar (Rattus Norvecicus) Setelah Diinduksi Dengan Gentamisin’, Jurnal Biomedik(jbm), 4(3), pp. 185–192. doi: 10.35790/jbm.4.3.2012.800.

Mulay, S. R., Linkermann, A. and Anders, H. J. (2016) ‘Necroinflammation in Kidney Disease’, Journal of the American Society of Nephrology, 27(1), pp. 27–39. doi: 10.1681/ASN.2015040405.

Ostermann, M. and Joannidis, M. (2016) ‘Acute kidney injury: Diagnosis and Diagnostic Workup’, Critical Care, 20, p. 229. doi: 10.1186/s13054-016-1478-z.

Quiros, Y. et al. (2011) ‘An Integrative Overview on The Mechanisms Underlying The Renal Tubular Citotoxicity of Gentamicin’, Toxicological Sciences, 119(2), pp. 245–256. doi: 10.1093/toxsci/kfq267.

Randjelovi, P. et al. (2017) ‘Gentamicin Nephrotoxicity in Animals: Current Knowledge and Future Perspectives’, EXCLI Journal, 16, pp. 388–399. doi: 10.17179/excli2017-165.

Sudaryati and Nusandari, R. (2017) ‘Karacteristik Fitokimia dan Antimikroba Krokok (Portulaca oleracea L.)’, in Prosiding Seminar Nasional FKPT-TPI.

Suhita, N. L. P. R., Sudira, I. W. and Winaya, I. B. O. (2013) ‘Histopatologi Ginjal Tikus Putih Akibat Pemberian Ekstrak Pegagan (Centella asiatica) Peroral’, Buletin Veteriner Udayana, pp. 71–78.

Trevor, A. J., Katzung, B. G. and Kruidering-Hall, M. (2014) Basic and Clinical Pharmacology 13th
Saptaningtyas, et al.

Edition, Basic and Clinical Pharmacology. doi: 10.1007/978-1-4614-1174-1_13.

Uddin, M. K. et al. (2014) ‘A Perspective Plant Source of Nutrition, Omega-3 Fatty Acid, and Antioxidant Attributes’, The Scientific World Journal. doi: 10.1155/2014/951019.

Wang, S. et al. (2016) ‘Necroptosis in Acute Kidney Injury: a shedding light’, Austin Journal of Nephrology and Hypertension, 7. doi: 10.1038/cddis.2016.37.

Young Ock, K. et al. (2015) ‘Anti-inflammatory effects of Portulaca oleracea L. on the LPS-induced RAW 264.7 cells’, Journal of Medicinal Plants Research, 9(12), pp. 407–411. doi: 10.5897/jmpr2012.1183.