The Role of Chlorogenic Acid on Mice with Unilateral Ureteral Obstruction Model: A Study toward Tubular Injury

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Abstract: Chronic kidney disease is a global public health problem with a poor prognosis of renal fibrosis. Injury to tubular epithelial cells is the beginning of the occurrence of renal fibrosis. Unilateral Ureteral Obstruction (UUO) is a representative method for obtaining models of renal fibrosis. Chlorogenic acid (CGA) is known to have a renoprotective, anti-inflammatory and antioxidant effect. This study aims to examine the role of CGA preventing tubular injury on mice with UUO model. This study was a quasi-experimental research with post-test only with control group design. Subjects were 25 male adult Swiss Webster mice (2-3 months old, 20-30 g weight). Subjects were divided into five groups: SO (Sham Operation), U7 (UUO day-7), U14 (UUO day-14), UC7 (UUO+CGA day-7) and UC14 (UUO+CGA day-14). CGA 14 mg/kg body weight/day was induced intraperitoneally. Tubular injury scores were examined with Periodic Acid Schiff staining. The data obtained from this study were analyzed using SPSS. UUO group with CGA showed the tubular injury score was lower than the UUO group without CGA with p-value < 0,005. The application of chlorogenic acid could prevent tubular injury in mice model of UUO.

1 INTRODUCTION

Chronic renal disease was a global public health problem with increased prevalence and incidence, poor prognosis and high costs. According to the Global Burden of Disease (2015), chronic renal disease is the 18th leading cause of death in 2010 and increased to 12th in 2015 (Neuen *et al*., 2017). The global prevalence of chronic renal kidney is 13.4% and tends to increased as the elderly population increased, and the incidence of diabetes mellitus (DM) and hypertension (Kemenkes, 2017). Central Sulawesi Province was the region with the highest prevalence of chronic kidney disease in Indonesia, which was 0.5%, followed by Aceh, Gorontalo and North Sulawesi (Kemenkes, 2013).

Chronic kidney disease results from an injury such as an obstruction. This injury causes renal structure changes such as renal fibrosis, tubular atrophy, apoptosis and inflammation (Ucero *et al.*, 2013). Fibrosis was an excessive deposition of extracellular matrix proteins in tissues that caused scar formation. The existence of structural changes due to fibrosis in the kidneys results in renal physiological function loss to become *End-Stage Renal Disease* (ESRD) (Neuen *et al*., 2017).

Unilateral Ureteral Obstruction (UUO) was the most representative experimental method for obtaining renal fibrosis inflammation models (Ucero

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et al., 2013). The UUO method was performed by binding or unilateral ureteric ligation in experimental animals. That could be subacute kidney injury characterized by tubular cell injury, interstitial inflammation, and increased intratubular pressure and renal fibrosis inflammation (Ucero *et al.*, 2013). Tubular epithelial cells have multicellular structures that bind together and acting as a *barrier* and absorption/secretion. Injuries at tubular epithelial cells were the beginning of renal fibrosis (Guarino *et al.*, 2009). A tubular injury would make a decrease in renal blood flow.

Chlorogenic acid was a compound *polyphenol* in coffee which had contain a high antioxidant. Chlorogenic acid effectively decreased the metabolites *of arachidonic acid*, *nitric oxide* and the production of proinflammatory cytokines compared to ibuprofen (Chauhan *et al.*, 2012). This active ingredient improved kidney dysfunction and kidney injury induced by cisplatin that suppressed oxidative stress, apoptosis, and autophagy by increasing kidney regeneration (Domitrović *et al.*, 2014). Research on the role and utilization of chlorogenic acid to prevent chronic renal disease was still not widely carried out. The existing studies have not explained the various pathways for preventing fibrosis in the incidence of Chronic renal kidney, so this study needs to be done to assess chlorogenic acid's role on the degree of renal tubular injury used the UUO mice model so that it could be considered as a new therapeutic agent.

Based on the above background, this study's formulation problem was as follows: How is the effect of chlorogenic acid on tubular injury scores at the UUO mice model? General-purpose of this research is to assess the role of administration chlorogenic acid in preventing tubular injury in the UUO mice model.

2 METHODS

This research was true experimental research used post-test only controlled group design. Consists of control and treatment groups. The subjects were 25 mature male Swiss Webster mice which were divided into 5 groups, SO (Sham Operation/Control) group, U7 (day-7 UUO + aquadest), U14 (day-14 UUO + aquadest), UC7 (day-7 UUO + chlorogenic acid), and UC14 (day-14 UUO + chlorogenic acid).

This study has obtained permission from the Medical and Health Research Ethics Committee Faculty of Medicine, Universitas Tadulako based on the ethics feasibility certificate number B.0941/UN28.1.30/KL/2018 on February 26, 2018.

2.1 Animal Model of Unilateral Ureteral Obstruction (UUO)

Mice were anaesthetized using *pentobarbital* with a dose of 0.1ml/10gBW/ times. After the anaesthesia effect was achieved, the mice were placed on the operating table with a pronation position. Mice hair on the right-back was shaved then disinfected used *povidone-iodine*, clamped with tweezers, then cut the skin for ± 1.5 cm parallel to the body axis lumbar region to the right lateral *vertebrae* region (region *flank*). Cutting was done until the peritoneum visible. Pinch the peritoneum with tweezers and then scissors ± 1 cm until the organs were *visceral* visible. Identify the kidneys first, then used tweezers twisted over the inferior pole and then pull the ureter up. Perform a double ligation of the ureter in the inferior polus using 0.4 silk thread in the proximal area. The peritoneum and skin were seen layer by layer using silk 0.4, then gave *povidone-iodine* as an antiseptic.

2.2 Chlorogenic Acid Administration

Chlorogenic acid (Sigma-Aldrich C3878-1G) was injected in mice with a dose of 14 mg/kg BW/day intraperitoneally. A total of 25 mice of swiss webster strains weighing 20-30 grams, 2-3 months old were used in this study. Mice were divided into 5 groups. The distribution for each group as follows: sham operation (SO) group, intraperitoneal distilled water injection for 14 days as a control; mice with UUO injected with distilled water intraperitoneally for 7 days called group U7; mice with UUO injected with chlorogenic acid for 7 days called UC7 group; mice with UUO injected with distilled water intraperitoneally for 14 days called group U14; mice with UUO injected with chlorogenic acid for 14 days group UC14.

2.3 Histopathological Examination with Staining of Periodic Acid Schiff (PAS)

Mice kidney would make by paraffin blocks and cut to 4μm thickness. The tissue slides were halarized and drained with flow water, followed by oxidation with *periodic acid* 0.5%for 5 minutes. The slides were washed with flow water. The slides were soaked in the reagent *Schiff* for 15 minutes and washed with flow water. Counterstained with haematoxylin for 1 minute and then washed with flow water. The slide was then dehydrated and mounted.

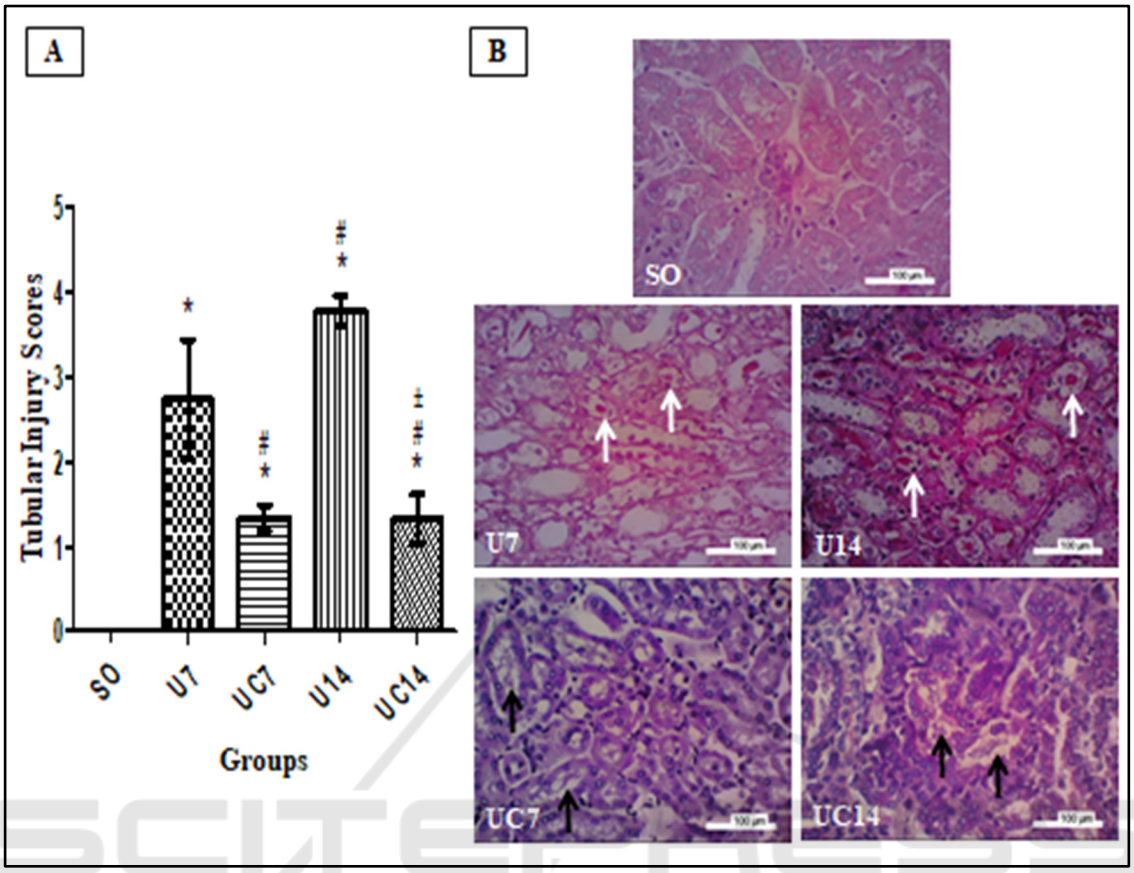

Figure 1: (A) *Kruskal Wallis test* p=0,000. *=p<0,05 vs SO, #=p<0,05 vs U7, ±=p<0,05 vs U14. (B) Microscopic findings of the kidney with PAS staining. Description: White arrows show intraluminal cas*t* in renal tubules that had atrophy. The black arrow shows the brush border.

2.4 Statistical Analysis

Tubular injury scores classified from 0 to 4 (0 = normal, 1 = tubular injury < 25% visual field, 2 = tubular injury involving 25% -50% visual field, 3 = tubular injury involving more than 51% -75% field view, 4 = tubular injury involving > 75% visual field). The assessment used a magnification of 400 times and counted as many as 15 fields of view randomly and did not overlap. This research variable has a ratio scale.

Data were analyzed using the statistical programme. Normality test used Shapiro-Wilk and homogeneity test used the Levene Test. The normality test for tubular injury scores was not normally distributed, then used the Kruskal Wallis test.

3 RESULTS

Histopathological examination of this study found that there was good tubular appearance in the SO group, with cuboidal epithelial cells in the proximal tubule and distal, and the tubule's lumen still wide. Besides that, the brush border was still intact, and no protein was found *cast* in the lumen of the tubules. While in the U7 and U14 groups tubular appearance was found with various signs of injury such as the presence of inflammatory areas, atrophic tubules, loss of brush border in the proximal renal tubules, and tubular dilatation with formation cast intraluminal. Protein cast could be formed from podocyte damage, resulting in protein escaping from glomerular filtration, tissue hypoxia causes epithelial cell apoptosis. Finally, together with the brush border, it is released into the lumen. Compared to the UC7 and UC14 groups that received chlorogenic acid, this group clearly saw improvements in injury, namely the

brush border still attached to the epithelium with intact epithelial cells and an inflammatory cell also appear to be reduced. Tubular dilation in some regions was still found with protein cast in the lumen, but in small amounts when compared to the U7 and U14 groups.

The tubular injury score results in the five sample groups found that the U7 and U14 groups had a higher tubular injury score compared to tubular injury scores in the SO group. Simultaneously, tubular injury scores in the UC7 and UC14 groups who received chlorogenic acid were lower than those in the tubular injury scores in the U7 and U14 groups who did not get chlorogenic acid. This result shows that the UUO model could cause a tubular injury that ends with renal fibrosis. Kidney fibrosis occurs due to obstruction, which causes an increased in intratubular pressure and vasoconstriction in the kidneys, thus ending with tissue ischemia. Also, in this study, it was found that chlorogenic acid administration in the UUO model can inhibit tubular injury, thereby preventing renal fibrosis.

Statistical analysis results showed that there were significant differences in tubular injury scores in the U7 group (2,73±0,7) and U14 (3,77±0,18) compared to the tubular injury scores in the SO (0) group. This result shows that UUO was able to induce tubular injury, which could end with renal fibrosis. Comparison of tubular injury scores between the UC7 groups (1,33±0,15) and UC14 (1,32±0,29) showed a significant difference compared to tubular injury scores in the U7 and U14 groups. Tubular injury scores indicated this result in the UC7 and UC14 groups given lower chlorogenic acid than tubular injury scores in the U7 and U14 groups who were not given chlorogenic acid.

4 DISCUSSION

In this study, it was found that chlorogenic acid had roles in preventing tubular injury in laboratory UUO mice to prevent renal fibrosis. This phenomenon could be seen because the tubular injury score in laboratory mice given chlorogenic acid was lower than the tubular injury score in laboratory mice that were not given chlorogenic acid.

According to the result, it was found that unilateral ureter ligation in experimental animals could cause kidney tubular injury, which resulted in renal fibrosis. UUO method created a higher degree of obstruction because ligation of one ureter caused a total obstruction, increasing intratubular pressure and causing secondary vasocontriction to the kidney, leading to decreased GFR and tissue ischemia. This condition caused further damage to kidney tubules so that most tubular epithelial cells would undergo apoptosis, resulting in atrophy (Ucero *et al.*, 2013).

In this study, the tubular injury score was higher in U7 and U14 groups which received UUO than in SO group. An increased tubular injury score was observed in exeperimental animals with longer UUO (Tateishi *et al.*, 2015). This result showed that UUO was a representative model to create renal fibrosis (Ucero *et al.*, 2013). Other studies also explained tubular injury caused by UUO by providing an overview of tubular damage, tubular epithelial cells apoptosis, fibrosis, and interstitial inflammation. Increasing cell death could be seen in day-3 to day-14 after UUO, and specific histologic marker of chronic kidney disease could be obtained after 1-2 weeks of UUO. Cell death was started by an increased hydrostatic tubular pressure accompanied by increased free radical reactions and oxidative injuries in proximal tubules (Xu *et al.*, 2013). Moreover, the tubular injury score was significantly higher in UC7 and UC14 groups which underwent UUO than in SO group. Therefore, further study about the roles of chlorogenic acid to tubular injury should be conducted.

In this study, the tubular injury score was lower in UUO groups, UC7 and UC14, which were given chlorogenic acid than in UUO groups, U7 and U14, which were not given chlorogenic acid. This result is consistent with the previous study, which stated that there were decreased tubular necrosis, intraluminal cast, and tubular dilatation in laboratory mice given chlorogenic acid (Domitrović *et al.*, 2014). The decrease in tubular injury score in groups given chlorogenic acid was due to antioxidant, anti-inflammatory, antibacterial, and anticarcinogenic properties of chlorogenic acid (Naveed *et al.*, 2018; Tajik *et al.*, 2017).

The limitation in this study is that it has not investigated the role of chlorogenic acid in renal fibrosis on epithelial cell markers and mesenchymal cell markers that affect the occurrence of tubular injury, therefore further research is needed in this regard.

5 CONCLUSION

Tubular injury score in the UUO mice model given chlorogenic acid was lower than the UUO mice model without chlorogenic acid. Further research can be sharpened by researching the role of chlorogenic acid in the tubular injury and the analysis of the role

of chlorogenic acid in renal fibrosis by examining other epithelial cell markers and other mesenchymal cell markers.

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