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Nephroprotective effect of extract *Etlingera elatior* (Jack) R.M. Smith on CCl_4-induced nephrotoxicity in rats

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ABSTRACT

Reactive oxidative stress (ROS) can lead to cell damage, and one of them is the kidney's cell. *Etlingera elatior* (Jack) R.M. Smith can be utilized as an agent that can protect the cell from ROS. This study aimed to investigate the protective effect of *E. elatior* fruit on the kidney's cell. We used experimental animals which were treated with Na-CMC (Group I), Na-CMC (Group II), the extracts of *E. elatior* fruit 200, 300, 400 mg/kg BW for Group III, IV, and V, respectively, for seven days. The blood was collected after treatment. At day 8, group I, III, IV, and V were induced by CCl_4. At day 9, blood was collected and the kidneys were harvested for histopathology analysis. Blood collected were measured for albumin, total protein, urea and creatinine levels. After treatment, albumin and total protein showed no increased levels; urea decreased at doses of 200, 300, and 400 mg/kg BW, respectively, and creatinine levels only decreased at the dose of 400 mg/kg BW ($p<0.05$). The dose of 200 and 300 mg/kg BW showed protecting effects in the tubular cells of renal. Therefore, the ethanol extract of *E. elatior* showed a nephroprotective effect by normalizing the urea and creatinine levels of rats and protecting tubular cells of renal.

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1. Introduction

The kidney plays a vital role in the human body performs the clearance of endogenous waste products and metabolisms, maintaining the acid-base balance, and control of the body's fluid volume. The kidney filters the wastes and reabsorbs the filtered wastes thus the ions are not excreted via urine. Therefore, the renal tubular epithelial cell is at a higher risk of injury (Hasohata, 2016).

Certain types of drug, including aminoglycoside-antibiotic (gentamycin, kanamycin), platinum-based chemotherapy drugs (cisplatin, carboplatin), immunosuppressive drugs (cyclosporine A, tacrolimus), and NSAIDs (nonsteroidal anti-inflammatory drugs) can cause renal damage. The mechanism of drug-induced renal damage mainly involves oxidative stress. Reactive oxidative stress (ROS) increases the activity of free radical producing enzymes lead to oxidative damage, thus exacerbate the renal condition (Hasohata, 2016).

Natural resources as plants that have high antioxidant activity could have the potency to protect cells from free radical exposure. *Etlingera elatior* plant or in local as Wualae can be utilized as a source of antioxidant that beneficial for its protective effect to renal cells. The previous studies showed parts of Wualae as leaves, rhizome, and flower have activity against oxidative agents (Chan et al., 2007; Habsah et al., 2004; Lachumy et al., 2010; Maimulyanti and Prihadi, 2015). Meanwhile, the fruit of Wualae has not been explored yet. Thus, this study aims to investigate the protective effect of Wualae fruit towards the CCl_4-induced kidney of rats.

2. Materials and methods

2.1. Plant collection

Wualae (*Etlingera elatior* (Jack) R.M. Smith) fruit was collected from Abuki district, Konawe Regency, Southeast Sulawesi. The plant was determined at Research Center for Biology, Lembaga Ilmu Pengetahuan Indonesia/ Indonesian Institute of Sciences (LIPI), Cibinong (No. 355/IPH.1.01/If.07/II/2017).

2.2. Fruits extraction

E. elatior fruit (19.04 kg) was wetly sorted then washed under running water. After that, fruits were chopped into small pieces and dried with an oven. Dried fruit was then powdered and obtained 1870 g powder of *E. elatior* fruit. The powdered sample was macerated by using 96% ethanol (3x24 hours) and concentrated with rotary vacuum evaporator (50°C). Total 96.2 g of concentrated extract was obtained (5.15%).

2.3. Animals

Experimental animals used in this study were Wistar male rats. Total of 45 rats (+/- 23 months; 20-30 g) were acclimatized for seven days. All animals involved in this study were according to the Animal Ethics Committee of Universitas Halu Oleo. Animals were divided into five groups (n= 6), then were treated for seven days as follow: Na-CMC (Group I, as negative control); Na-CMC (Group II, as normal control); extract dose of 200 mg/kg BW (Group III); extract dose of 300 mg/kg BW (Group IV); extract dose of 400 mg/kg BW (Group V). At day 7, blood was collected for measurement of albumin, total protein, urea, and creatinine levels.

On day 8, each group was treated with CCl_4(0.4 ml/kg BW) except group II. On day 9, blood was collected by vein tail sampling methods for measurement of albumin, total protein, urea, and creatinine levels. Following by sacrificing the animals and the renal was harvested for histopathology assessment. Removed renal was put in 10% neutral buffer formalin.

2.4. Measurement of albumin level

Serum (10 µl) was reacted toward 1000 µl of albumin reagent, homogenized with vortex and incubated for 10 min (20-25°C). Absorbance was measured under the spectrophotometer (λ 546 nm) for 30 min. The same procedure was conducted to standard.

2.5. Measurement of total protein

Serum (20 µl) was added to 1000 µl of total protein reagent and incubated for 10 min (25°C). Absorbance was measured under the spectrophotometer (λ 546 nm) for 30 min. The same procedure was conducted to the standard solution.

2.6. Measurement of urea level

Serum (10 µl) was reacted toward 1000 µl of urea reagent, homogenized with the vortex. Absorbance was measured under the spectrophotometer (λ 492 nm) for 30 min. The same procedure was conducted to a blank and standard solution.

2.7. Measurement of creatinine level

Standard solution (100 µl), made from a mixture of R1 (picric acid) and R2 (sodium hydroxide) (1:1), was mixed with working reagent (1000 µl) for 30 s, and measured under spectrophotometer (λ 492 nm) as A1. Then, after 2 min, 200 µl of the standard was mixed with working reagent (2000 µl) and measured under spectrophotometer (λ 492 nm). The same procedure was conducted to sample.

2.8. Assessment of histopathology

Renal was fixated by using 10% neutral buffer formalin and dehydrated with increasing dose of alcohol (70%; 80%, 90%, absolute alcohol I; and absolute alcohol II, 2 h each). The clearing was conducted by xylol and infiltrated with paraffin wax. Blocks of paraffin were then cut at 5-6 µm with a microtome. The sections put in warm water (60°C) for 24 h and stained by haematoxylin and eosin (HE), continued examined and photographed by using a light microscope (magnification 400x).

2.9. Data analysis

Data were presented as mean ± standard deviation (SD). Data were analysed with SPSS with difference significance considered at $p<0.05$

3. Results

3.1. Effect of CCl4 on albumin, total protein, creatinine, and blood urea levels

Albumin (Fig. 1) and total protein levels (Fig. 2) were decreasing along with the increasing dose of ethanol extract of *E. elatior* whereas dose of 400 mg/kg BW (Group V) provided a higher effect in reducing albumin levels, followed by treatment of 300 (group IV) and 200 mg/kg BW (group III) respectively. Both levels in various doses pre- and post-treatment showed significant difference ($p<0.05$). Group I (negative group) at both levels showed increased levels, although they were not significant post-treatment with CCl_4.

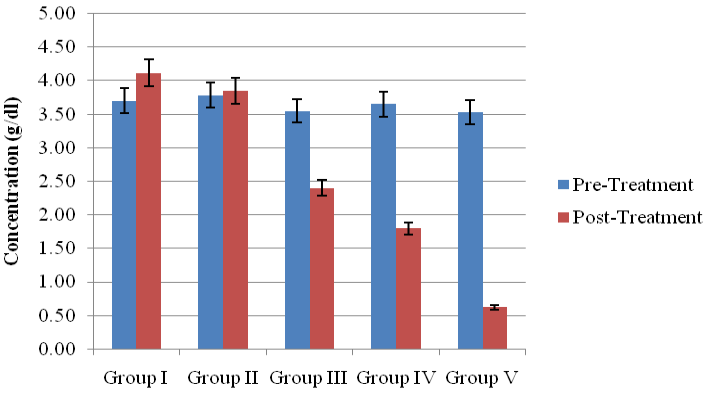

Fig. 1. Albumin levels of rats, pre- and post-treatment with CCl_4 as renal damage inducer. Data presented as mean ± SD (n=6).

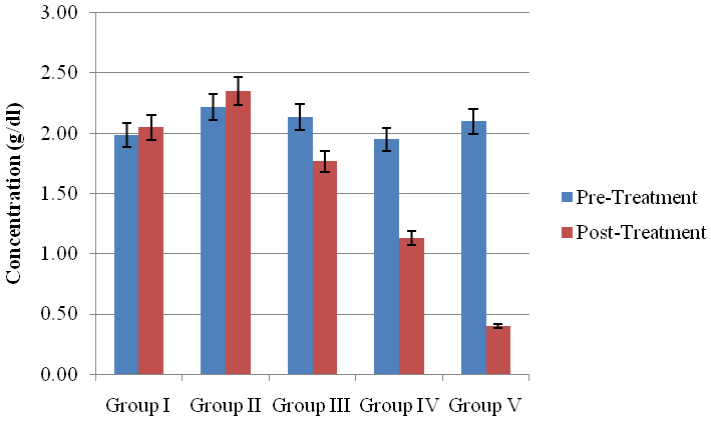

Fig. 2. Total protein levels of rats, pre- and post-treatment with CCl_4 as renal damage inducer. Data presented as mean ± SD (n=6).

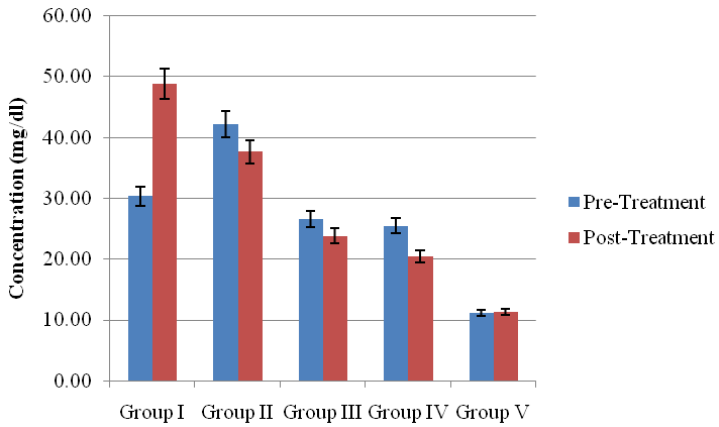

Fig. 3. Urea levels of rats, pre- and post-treatment with CCl_4 as renal damage inducer. Data presented as mean ± SD (n=6).

Based on data obtained, the dose of group III-V provided an effect in decreased urea levels of rats post-induced by CCl_4($p<0.05$) compared to group I, meanwhile only group III and IV post-treatment were showing a significant difference in decreasing urea levels (Fig. 3) compared to pre-treatment ($p<0.05$).

Group III and IV in the measurement of creatinine levels showed no significant difference to the negative group in post-treatment measurement ($p>0.05$), except group V showed a significant difference ($p<0.05$) to the negative control (Group I), although still showed no significant difference ($p>0.05$) compared to pre-treatment. Data presented in Fig. 4.

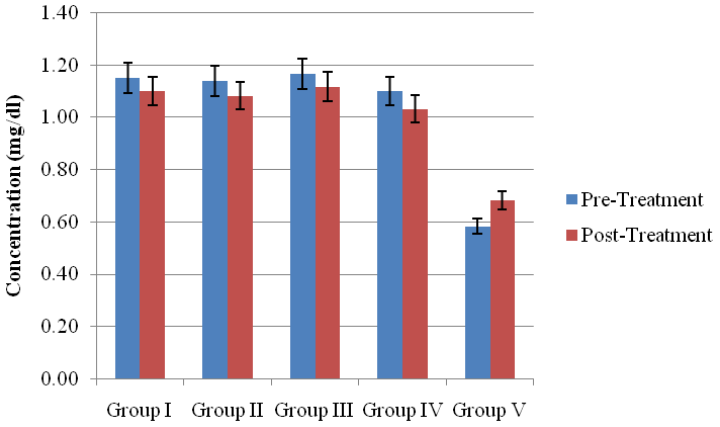

Fig. 4. Creatinine levels of rats, pre- and post-treatment with CCl_4 as renal damage inducer. Data presented as mean ± SD (n=6).

3.2. Histopathology of renal

Histopathology of renal observed if necrosis in the tubular cell occurs. In group II as the normal group which untreated, group III and IV which were treated groups with ethanol extract of fruit *E. elatior* at dose of 200 and 300 mg/kg BW respectively, showed no signs of necrosis at renal tubular cell (Fig. 5c; Fig. 5d), as well as to normal group/ group II (Fig. 5b). However, group V with extract dose of 400 mg/kg BW some signs of necrosis at the tubular cell. Necrosis was characterized by the destruction of proximal tubular epithelial cells (Fig. 5e).

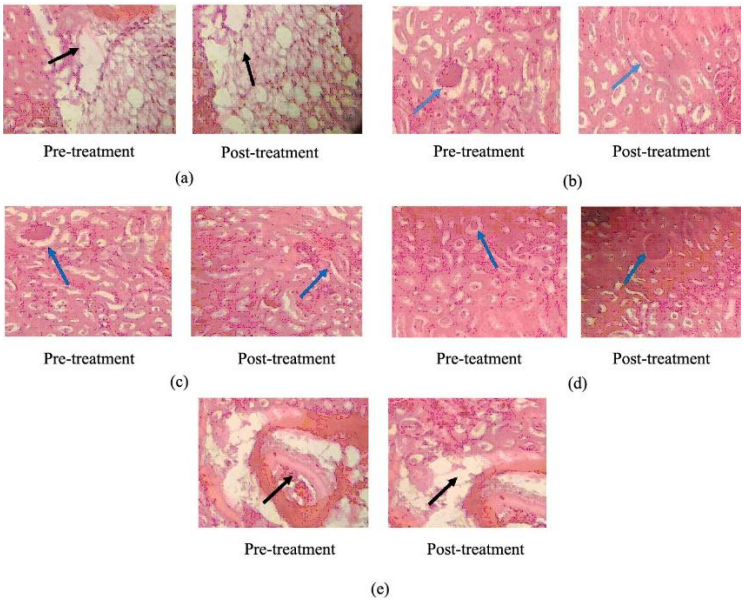

Fig. 5. Renal organs of rats with magnification 400x. (a) Negative group (group I); (b) normal group (group II); (c) *E. elatior* extract 200 mg/kg BW (group III); (d) *E. elatior* extract 300 mg/kg BW; and (e) *E. elatior* extract 400 mg/kg BW.

Negative group (Group I) provided the effect of necrosis due to damage to the proximal tubular epithelial cells. Histopathologically, the necrosis is not describing the presence of a homogeneous and eosinophilic nucleus and cytoplasm with fixed form. Substance actively secreted from blood to urine or the substance reabsorbed from urine resulting in accumulation dose of substances, leads to renal damage (Fig. 5a).

4. Discussion

The nephroprotective activity of ethanol extract of *E. elatior* fruit was conducted on renal damage induced by carbon tetrachloride (CCl_4). Carbon tetrachloride (CCl_4) in various studies induced renal toxicity alongside hepatic toxicity (Khan et al., 2010; Khan and Siddique, 2012). Possible mechanism of CCl_4 in causing renal damage involves renal oxidative stress. Renal damage induced by CCl_4 is most likely due to highly reactive trichloromethyl (CCl_3^-) formed for reductive dehalogenation of CCl_4 by P450 enzyme system thus initiates lipid peroxidation in renal. CCl_4 also affects renal antioxidant enzymes defences such as glutathione peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD), glutathione s-transferase (GST), glutathione-disulfide reductase (GSR), and quinone reductase (QR) lead reactive oxidative stress (ROS) in renal. This ROS leads to tubular necrosis (Aly et al., 2017; Khan et al., 2010; Wu et al., 2012).

In our study, decreased levels of albumin and total protein, and increased levels of creatinine and urea become biomarkers for renal damage induced by CCl_4. Decreased and increased levels of these biomarkers also showed in the previous study conducted by Jedage and Manjunath (2016).

Our findings found that albumin and total protein level of rats treated by ethanol extract at various doses do not provide nephroprotective activity by stabilizing the levels of albumin and total protein pre- and post-treatment. It was unable to normalize the albumin and overall protein levels post-treatment with CCl_4. Decreasing levels of albumin (hypoalbuminemia) and total protein in blood demonstrate a vital sign of impaired renal function (Putri et al, 2016). Therefore, *E. elatior* fruit extract does not normalize both levels.

Creatinine and urea are the metabolic waste product which mostly filtered out of the blood from renal and excreted in urine (Chinnappan et al., 2019; Ezejiofor and Orisakwe, 2019; Vaidya et al., 2008). Our finding suggests that creatinine levels were not affected by the treatment of ethanol extract of fruit *E. elatior* at group III and IV. The post-treatment creatinine levels of the group 400 mg/kg BW slightly increased although not significant compared to pre-treatment but still significantly different from the negative control. Otherwise, blood urea levels were reduced by ethanol extract of fruit *E. elatior* at the dose of 400, 300, and 200 mg/kg BW respectively, conclude ethanol extract was able to normalize the blood urea levels in plasma by excreting via urine.

Histopathology of renal organ showed the potential nephroprotective activity of ethanol extract of *E. elatior* fruit at the dose of 200 and 300 mg/kg BW, although treatment of 400 mg/kg BW showed some necrosis instead. The flavonoid, notably quercetin and kaempferol, are contained in the *E. elatior* plant, which can be found on rhizome, leaves, flower, and stems. Thus allowing *E. elatior* fruit to have the same content which is suspected provides the nephroprotective activity. The flavonoid acts as an antioxidant, inhibits renal impairment by bonding to free radicals (Jackie et al., 2011; Lachumy et al., 2010; Maimulyanti and Prihadi, 2015).

5. Conclusion

The nephroprotective activity of *Etlingera elatior* (Jack) R.M. Smith fruit was proven by its ability in normalizing urea levels of CCl_4-induced rats at concentration 200; 300; and 400 mg/kg BW respectively and creatinine level at a concentration of 400 mg/kg

BW. Also, *E. elatior* at a concentration of 200 and 300 mg/kg BW have the activity to protect tubular cells, while at a concentration of 400 mg/kg BW showed some necrosis.

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Conflict of Interest

The authors declare there is no conflict of interest in this study.

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