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Effect of andaliman fruit on the improvement of kidney function and histological structure

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ABSTRACT

Glomerulonephritis is a disease of inflammation on kidney's glomerulus that is marked by the damage of glomerular capillary wall, increase of membrane permeability, and decrease of glomerular filtration rate. Glomerulonephritis needs to be detected and treated early to minimize kidney damage. Chronic glomerulonephritis can slowly be progressive and periodically cause the loss of kidney function. Oxidative stress and inflammation is known to have a role in the pathogenesis of Acute Glomerulonephritis. Andaliman fruit (Zanthoxylum acanthopodium DC) is known to have plenty of benefits, namely as antioxidant and anti-inflammation. To determine the effect of andaliman fruit ethanol extract towards measurement of creatine, urea, and kidney histologic structure on mice models with acute glomerulonephritis. Research design is an experimental study using the pretest posttest with control group design method on Streptokinase induced acute glomerulonephritis Rattus norvegicus rat model. This study compares the effect of andaliman fruit ethanol extract on control group and treatment group receiving each the dose of 100 mg/kg/day, 200 mg/kg/day and 300 mg/kg/day. There was a significant difference ($p<0.05$) creatinine and urea level between groups after given the andaliman fruit ethanol extract on acute glomerulonephritis rat model after 14 days. The most effective change on kidney histologic structure could be seen in a group receiving the dose of 100 mg/kg/day. This study showed the anti-inflammatory activity of andaliman fruit (Zanthoxylum acanthopodium DC) that could be seen from the improvement of kidney function and histologic structure.

1. Introduction

Glomerulonephritis is an inflammatory disease on kidney glomerulus that was marked by the damage of glomerular capillary wall, increasing of glomerular membrane permeability, and decreasing of glomerular filtration rate. Based on the onset of disease, glomerulonephritis was categorized into acute and chronic glomerulonephritis. Acute glomerulonephritis could be caused by the infection of bacteria, viral, fungi,

protozoa or other immunological events [1]. Infection related kidney disease should be detected and treated rapidly to minimize the damage of the kidney.

Streptokinase that was produced by group A *streptococcus β-hemolyticus* has the ability to bind with plasmin or plasminogen. Activation of plasminogen results in increased plasminogen conversion to plasmin. Plasmin is an enzyme that degrades extracellular matrix which could cause inflammation on glomerulus and lead to kidney fibrosis [2], [3]. The histological structure of kidney on rat induced Streptokinase showed damage on glomerular epithelial cells and kidney tubule epithelium as well as the appearance of fibroblast cells on kidney histopathology [4]. Treatment of glomerulonephritis aims to overcome the inflammation and prevent kidney fibrosis. Kidney fibrosis is characterized by excessive accumulation of extracellular matrix, resulting in progressive loss of kidney function; also known as chronic kidney disease.

Chronic kidney disease is one of the world's health issues. In the U.S., the prevalence of chronic kidney disease is 14.4% in 2020, and is predicted to keep on increasing to 16.7% in 2030 [5]. In Indonesia, the prevalence of chronic kidney disease also increased; from 0.18% in 2013 to 0.3% in 2018 [6]. Early stage of chronic kidney disease does not show clinical manifestations, even though there was already a decrease in kidney function. Complaints and clinical symptoms are noticed by the patients in late stages.

Indonesia is well known as a tropical country that is enriched with flora and fauna, the usage of herbs/ plants as part of traditional medicine is common in Indonesia. Andaliman fruit (*Zanthoxylum acanthopodium DC*) grows well in North Sumatera, especially in the mountain region of Simalungun, North Tapanuli, Toba Samosir, and Dairi district. Traditionally, andaliman fruit is often used as spices in Bataknese cuisine [7].

Oxidative stress and inflammation are known to take part in the pathogenesis of acute glomerulonephritis. Oxidative stress is caused by an imbalanced between the production of free radicals and its degradation by the antioxidant system. Andaliman fruit contains secondary metabolites such as alkaloid, glycoside, steroid/triterpenoid, flavonoid, tannin and saponin [8]. Several studies claimed that andaliman fruit had a lot of biological activities, such as antioxidant, antimicrobial, anti-inflammatory, anticancer, antiaging, anti halitosis, and others [9].

Nanoparticles of andaliman fruit shown anti-inflammatory and antioxidant properties in atherosclerotic rats by improving the endothelial dysfunction, confirmed by the increased expression of HSP70 on immunohistochemistry examination [10]. Administration of andaliman fruit methanol extract also improves the appreance of necrotic liver and kidney histology on Benzopyrene-induced rat [11]. Andaliman fruit has an antimicrobial activity against Escherichia coli, Staphylococcus aureus and Salmonella typhimurium that could be potentially used as a food preservatives [12]. Andaliman fruit (Zanthoxylum acanthopodium) has also been reported for its cytotoxic activity. The cell toxicity evaluation was shown against 4T1 breast cancer cells [13] and P388 leukemia cells [14].

Antioxidants have the role to neutralize free radicals, so cellular damage could be restrained. Therefore, the authors are interested in examining andaliman fruit either to prevent or to slow down the progression of kidney disease.

2. MATERIALS AND METHODS

The study was conducted from January to April 2022 in the Pharmacology Laboratory, Faculty of

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Pharmacy, University of Sumatera Utara (USU), Medan. The research design is an experimental study using the pretest posttest with control group design method. Ethical approval of this study was obtained from research ethics committee of Universitas Prima Indonesia, number 006/KEPK/UNPRI/I/2022.

2.1 Experimental Design

Experimental animals were 35 healthy Wistar rats (*Rattus norvegicus*), at the age of 8-11 week old, weighing 200-250 grams, and were given a 7-day period for acclimatization. The experimental animals were randomly divided into five groups of seven rats each. The group divisions are KN as a normal control group, K- (Streptokinase induced) as a negative control group, P1 (Streptokinase induced+treated with a dose of 100 mg/kg/day), P2 (Streptokinase induced+treated with a dose of 200 mg/kg/day) and P3 (Streptokinase induced+treated with a dose of 400 mg/kg) based on the previous study [11]. Streptokinase was administered intravenously through the coccygeal vein with the dose of 6,000 IU per rat on day 1, 6 and 11 of research [4].

2.2 Andaliman Fruit Ethanol Extract

One kilogram of fresh andaliman fruit was harvested from Toba Samosir district, North Sumatra. Andaliman fruit is then washed and dried in a drying cabinet for 7 days. Dried andaliman fruit was milled into simplicia (weighs 650 grams) and macerated with 4.5 litres of 96% ethanol for 5 days, stirred regularly, and filtered using a filter paper. The residue then re-macerated with 1.5 litres of 96% ethanol for another 2 days. The filtrate obtained from both processes were combined and concentrated with a rotary evaporator and water bath until it formed a thick extract.

2.3 Sample Collection

On day 16 of research, two experimental animals in each group were used to measure the creatinine level, urea level and kidney histological structure as a pretest. Whole blood sample was obtained from the cardiac puncture. Both kidneys of each rat were harvested and stored in 10% buffered formalin for histological examination. The other 25 rats would be given the treatment based on the group division for 14 days.

a) Creatinine and Urea Level

The creatinine and urea level was evaluated in the Integrated Laboratory of University of Sumatera Utara (USU) Medan using the photometric determination of serum. The reagents used for the measurement of creatinine and urea level were Glory Diagnostics Kit (Indonesia).

b) Kidney Histological Structure

Rat's kidney preparation was stained with Hematoxylin-Eosin (HE) histological stains. The histology sample preparation and the assessment of preparation was done in the Histology Laboratory, Faculty of Medicine, University of Sumatera Utara (USU) Medan. Kidney histological structure before and after treatment was evaluated using the EGTI scoring system (score 0-4) which quantitatively scores endothelial, glomerular, tubular and interstitial lesions [15].

2.4 Data analysis

The data obtained were analyzed using SPSS software version 25. Statistical analysis that was used to determine whether there were differences between groups was One Way Anova. If there was a significant difference among treatment groups ($p<0.05$), it would be followed by Mean Tukey's HSD test. Wilcoxon test was done to compare the means of creatinine and urea level before and after treatment. Kidney histological structure was analyzed descriptively.

3. RESULT

3.1 Qualitative Phytochemical Screening

The phytochemical screening was carried out at the Biology Laboratory of the Faculty of Pharmacy, University of North Sumatera. The results of phytochemical screening of andaliman fruit ethanol extract can be seen in Table 1.

Table 1. Result of phytochemical screening

No.	Secondary metabolite	Reagent	Result
1.	Alkaloid	Dragendroff Bouchardat Meyer	+
2.	Flavonoid	Mg^+ powder + Amil Alcohol + HCl	+
3.	Glycoside	Molish + H_2SO_4	+
4.	Saponin	Aquadest (shaking)	-
5.	Tanin	$FeCl_3$	+
6.	Triterpen/ Steroid	Lieberman-Bouchart	+

The result showed that the secondary metabolites found in andaliman fruit ethanol extract are alkaloid, flavonoid, glycoside, saponin, tanin, triterpene/ steroid.

3.2 Creatinine and Urea Level

Increase of BUN value and creatinine were part of response of kidney filtration rate overcoming the decrease caused by the inflammation on glomerulus. Results of serum creatinine and urea value could be seen on Table 2 and Table 3.

Table 2. Creatinine level before and after treatment

Group	Creatinine (Pre Test)	Creatinine (Post Test)	Difference	*p-value*
KN	0.81 ± 0.08	0.78 ± 0.04 [a]	(-) 0.03	0.655
K-	0.77 ± 0.12	0.93 ± 0.03 [b]	0.16	0.180
P1	0.78 ± 0.10	0.98 ± 0.06 [b]	0.20	0.180
P2	1.16 ± 0.16	0.98 ± 0.04 [b]	(-) 0.18	0.180
P3	1.24 ± 0.34	1.06 ± 0.05 [b]	(-) 0.18	0.655

Note: Value is expressed as Mean ± SD
Different superscript indicate statistical significance ($p<0.05$) determined by Anova test
p-value was determined by Wilcoxon test

The results obtained from *one-way Anova test* showed that there was a significant difference in creatinine level between groups ($p<0.05$). The *post hoc Tukey HSD test* as seen on Table 2 showed that there is significant difference in creatinine level between negative control group and normal control group ($p<0.05$). However, the creatinine level in negative control group did not differ from P1 ($p>0.05$), P2 ($p>0.05$) and P3 ($p>0.05$) groups that received andaliman fruit ethanol extract. The normal control group has the lowest creatinine level (0.78 ± 0.04 mg/dl), while P3 has the highest creatinine level (1.06 ± 0,].05 mg/dl).

Wilcoxon test was used to compare the measurement of creatinine level before and after treatment as seen on Table 2. There is no significant difference in creatinine level before and after treatment in normal control group ($p=0.655$), negative control group ($p=0.180$), P1 ($p=0.180$), P2 ($p=0.180$) and P3 ($p=0.655$).

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Table 3. Urea level before and after treatment

Group	Urea (Pre Test)	Urea (Post Test)	Difference	*p-value*
KN	49,70 ± 0,14	35,28 ± 5,35 [a]	(-) 14,42	0,180
K-	69,85 ± 15,77	67,38 ± 10,57 [b]	(-) 2,47	0,655
P1	44,85 ± 7,57	32,43 ± 7,77 [a]	(-) 12,42	0,180
P2	40,10 ± 22,49	32,56 ± 3,04 [a]	(-) 7,54	0,655
P3	35,35 ± 1,20	28,80 ± 4,24 [a]	(-) 6,55	0,180

Note: Value is expressed as Mean ± SD
Different superscript indicate statistical significance ($p<0.05$) determined by Anova test
p-value was determined by Wilcoxon test.

The results obtained from *one-way Anova test* showed that there was a significant difference in urea level between groups ($p<0.05$). The *post hoc Tukey HSD test* showed that normal control group, P1, P2 and P3 significantly differ from the negative control group. The highest urea level was 67.38 ± 10.57 mg/dl, followed by the normal control group (35.28 ± 5.35 mg/dl), P2 (32.56 ± 3.04 mg/dl), P1 (32.43 ± 7.77 mg/dl) and P3 (28.80 ± 4.24 mg/dl).

Wilcoxon test was used to compare the measurement of urea level before and after treatment as seen on Table 3. There is no significant difference in urea level before and after treatment in normal control group ($p=0.180$), negative control group ($p=0.655$), P1 ($p=0.180$), P2 ($p=0.655$) and P3 ($p=0.180$).

3.3 Kidney Histological Structure

The kidney structures were examined using a light microscope at the magnification of 100 and 400 times. Kidney tissure were stained with hematoxylin-eosin (HE) and evaluated using the EGTI scoring system. Representative photomicrographs of each group before and after treatment can be seen in Figure 1.

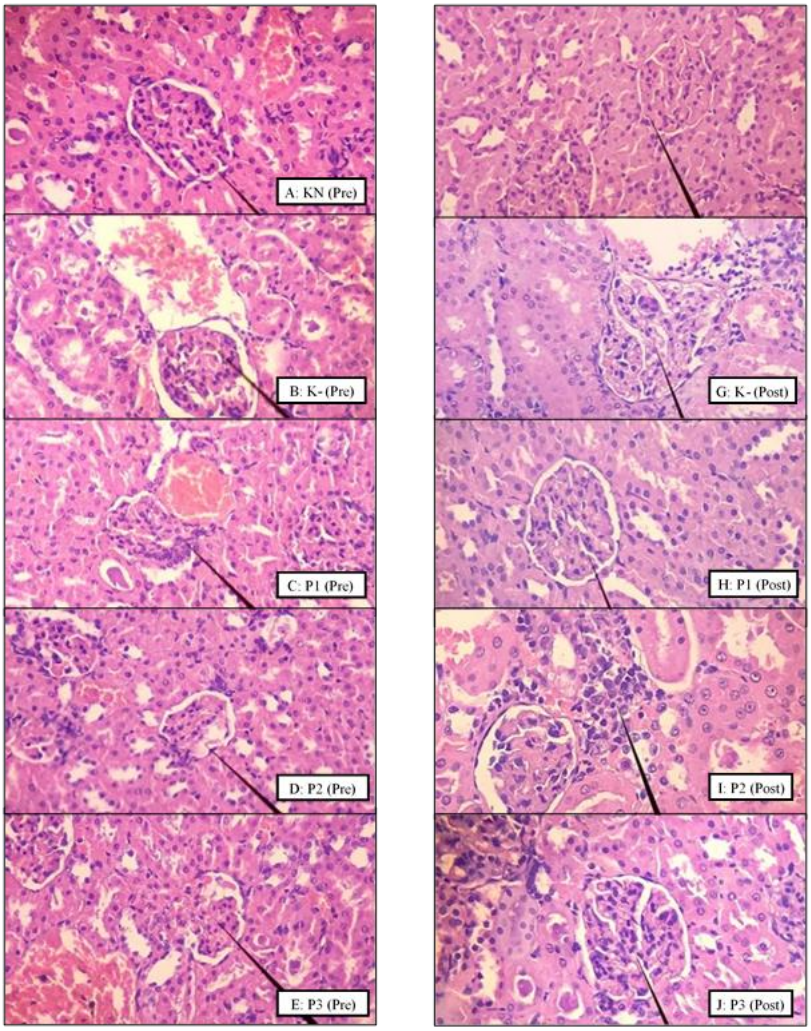

Figure 1. (a-e) Kidney histological structure before treatment. (f-j) Kidney histological structure after treatment

KN: normal control, K-: Streptokinase induced acute glomerulonephritis rat model, P1: Streptokinase induced+treated with 100 mg/kg/day, P2: Streptokinase induced+treated with 200 mg/kg/day, P3: Streptokinase induced+treated with 400 mg/kg/day

Normal appearance of glomerulus, Bowman's capsule, tubular and interstitial space can be seen on Figs. 1a and 1e. The glomerulus appeared as a network of capillaries that are surrounded by Bowman's space. The stratum parietale of Bowman's capsule is lined by simple squamous epithelial cells. Both proximal tubules and distal tubules appeared normal. Cuboidal epithelial cells lining the proximal tubules appeared to be stained more eosinophilic than distal tubules. Proximal tubules also have smaller lumens and are lined with a brush-border, whilst distal tubules have no brush-border [16]. Proximal tubules are more prone to toxic injury and ischemic [17].

The photomicrographs of acute glomerulonephritis rat models that were induced with 3 x 6,000 IU of Streptokinase can be seen on Figs. 1b, 1c, 1d and 1e with varying degrees of damage. The glomerular capillary tuft was retracted and some showed glomerular fibrosis, endothelial cells swelling and disruption, thickened basal membrane of the tubular cells and loss of brush border cells, inflammation and necrosis can be seen within the interstitial compartment

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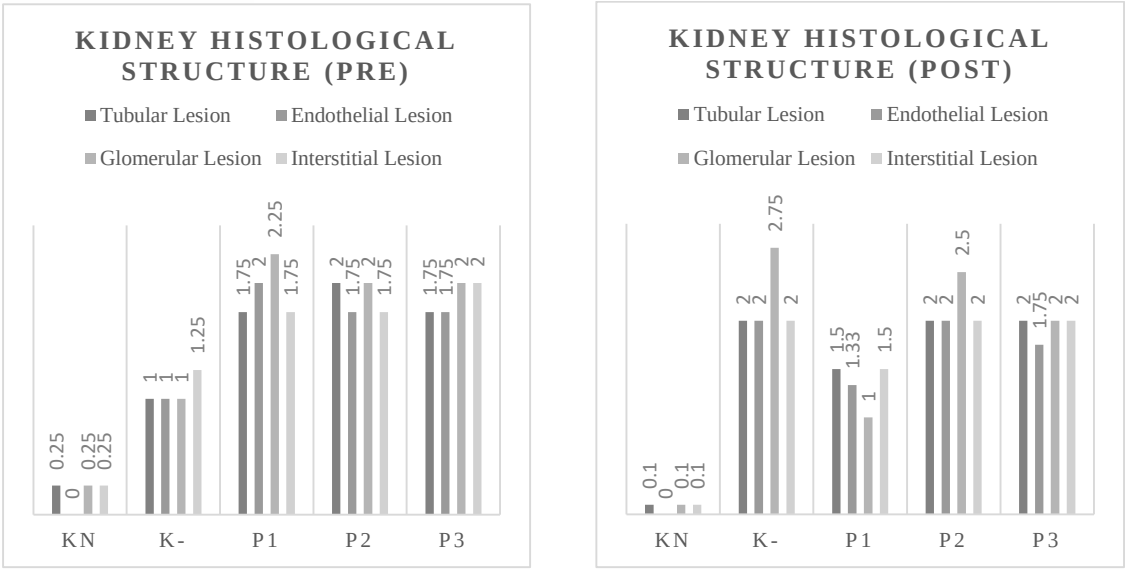

Figure 2. EGTI scoring of kidney histological structure before and after treatment

The severity of lesions in each group was confirmed using the EGTI scoring system (Fig. 2). The scoring showed that group K-, which was induced with Streptokinase and did not receive any treatment revealed a higher score compared to before. The glomerulus progresses to glomerular fibrosis, endothelial cells disruption, thickened basal membrane of the tubular cells and loss of brush border cells. There was inflammation, haemorrhage and necrosis in less than 25% of the tissue within the interstitial compartment.

Group P1 that received treatment of 100 mg/kg/day andaliman fruit ethanol extract for 14 days showed most improvement on kidney histological structure. The other groups that received treatment of andaliman fruit ethanol extract showed a better score compared to the group that did not receive any.

4. DISCUSSION

Andaliman fruit reportedly has various health benefits other than traditionally used as spice and food preservatives. Andaliman fruit contains phytochemical components of alkaloid group, glycoside, steroid/triterpenoid, flavonoid, tannin and saponin [8]. Flavonoid and alkaloid substances in andaliman fruit have the activity of antioxidant and anti-inflammatory. Flavonoid was a polyphenol compound that was synthesized by fruits and plant leaves. In-vitro study showed flavonoid had the ability to decrease the formation of free radicals through scavenging and chelating mechanisms [18].

Antioxidant activity from the ethanol extract and alkaloid fraction of andaliman fruit while examined using free radical *1,1-diphenyl-2-picrylhydrazyl* (DPPH) entrapment method showed the activity of antioxidant in strong category on the pH of 7 and was very strong on the pH of 9 [19]. The study that was done towards Lipopolysaccharide (LPS) induced macrophages showed anti-inflammatory effect by decreasing the production of inflammatory mediators such as TNF-α, IL-6, MMP-9, COX-2 and iNOS [20].

This study used Streptokinase in the induction of acute glomerulonephritis. Streptokinase is a streptococcal protein that is widely used as a thrombolytic agent to lyse fibrin clots in some cases of myocardial infarction. Administration of a single dose of Streptokinase 6,000 IU intravenously via coccygeus vein was able to increase the levels of Nitric Inducible Oxide Synthase (iNOS) and Malondialdehyde (MDA) expression on rat kidneys [21]. Administration of repeated doses of Streptokinase could worsen the glomerular and tubular damage that lead to kidney fibrosis. A recent study reported that administration of 3

x 6,000 IU of Streptokinase decreases the expression of e-cadherin, an adherens junction localized on the surface of epithelial cells that maintains cell to cell integrity [4].

Streptokinase as a plasminogen activator results in increased plasminogen conversion to plasmin. Activation of plasmin could induce glomerular damage by degrading extracellular matrix proteins by metalloproteinase enzymes, accumulating and activating pro-inflammatory cells [2]. This process releases inflammation mediators such as bradykinin, TNF-α, IFN-γ, IL-1 and further releases of Inducible Nitric Oxide Synthase (iNOS). iNOS expression increases the amount of free radicals as Nitric Oxide (NO) in the kidneys that exerts nephrotoxic injury. Nitric Oxide is synthesized endogenously, released by activated macrophages, causing vasodilatation and inhibits thrombocyte aggregation [21].

Kidney has excretory function that removes metabolic waste products and toxins, also maintains fluid and electrolyte balance. In acute or chronic kidney injury, metabolic waste products such as uric acid, urea and creatinine level increases in condition where urinary excretion declines [22]. Creatinine is a product of creatine and creatinine phosphate metabolism in skeletal muscle. Creatinine is metabolized in the body constantly, is filtered through glomerulus and secreted by proximal tubules [23]. Urea is an end product of protein metabolism. Urea level is mainly dependent on protein intake and kidney's function to secrete urea [24]. On special condition such as rhabdomyolysis, usage of medicine such as cephalosporin and sulfa could increase the level of creatinine serum; meanwhile liver cirrhosis, hyperbilirubinemia, fluid overload and loss of muscle mass could decrease the creatinine serum. Serum creatinine as a biomarker to diagnose kidney failure was assessed to be less sensitive and less specific [25].

Proximal tubule cells are more susceptible to damage because of its function to reabsorb glomerular filtrate [17]. The increase of serum creatinine level oftentimes was slower. The other biomarkers that can be used as an early detection to kidney damage such as Cystatin C, NGAL (N-acetyl-b-D-glucosaminidase), KIM-1 (Kidney Injury Molecule-1), α1-microglobulin, β2-microglobulin, lysozyme and others. Biomarkers that are secreted by tubulus are rated to be more sensitive and specific for early detection or mild kidney damage [25], [26].

A high performance liquid chromatographic (HPLC) method with diode array detection revealed the presence of ascorbic acid, phenolic acids and flavonoids in four different solvents extracts of *Zanthoxylum acanthopodium* leaves. The less polar media as in the 80% aq. ethanol extract contained more phenolic acids and flavonoids. The flavonoids identified were catechin, rutin, myricetin, quercetin, apigenin and kaempferol18. Flavonoid-rich extract demonstrated a protective action against Cadmium-mediated kidney injury through the reduction of inflammatory markers IL-1β expression in the kidney, reducing morphological changes of glomerulus and proximal tubules, confirmed by the histopathological examinations [27].

The antioxidant property of flavonoids has been reported as a nephroprotective agent. Pretreatment of oral administration of rutin at a single dose of 200 mg/kg one hour before lipopolysaccharide (LPS) injection significantly protects the kidneys with acute kidney injury. Rutin administration lowered serum creatinine and BUN level compared to control group, restored kidney's level of malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and catalase activity. Pretreatment of rutin also inhibited the release of inflammatory mediators such as TNF-α, IL-6, nuclear factor-kappa B (NF-κB), toll-like receptor 4 (TLR4), cyclooxygenase-2 (COX2) and restored kidney's level of sirtuin-1 (SIRT1) [28]. Rutin improves kidney function on Chronic Kidney Disease rat models, as levels of creatinine, BUN, plasma uric acid, phosphate ion concentrations, and proteinuria were all decreased. Rutin also decreased the expression of HO-1 (heme

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oxygenase 1), an oxidative stress marker; but did not reduce the expression of TGF-β and TNF-α [29]. Oral administration of pectolinarigenin, a natural flavonoid, dose-dependently suppressed myofibroblast activation, extracellular matrix deposition and effectively lessened the kidney's tubulointerstitial fibrosis after injury. Pectolinarigenin inhibited the expression of α-SMA, collagen I, fibronectin in the kidney tissue and blocked the activation of SMAD3 and STAT3 signaling [30].

Andaliman fruit methanol extract was reported to be able to decrease the necrotic area of liver and kidney necrosis caused by the induction of Benzopyrene [11]. Andaliman fruit extract has a dose-dependent effect on kidneys. The highest dosage of andaliman fruit extract (300 mg/kg) significantly improved the kidney function and the appearance of kidney histological structure marked by decreased the width of the necrotic area and increased the number of rescue glomerular structures on diabetic nephropathy rats induced by Streptozotocin [31]. However, the histology examination from this study showed that the lower dosage of andaliman fruit ethanol extract (100 mg/kg) showed better improvement than the other groups that received higher dosage (200 mg/kg and 300 mg/kg) based on appearance of glomerulus and tubules.

The degree of kidney damage induced by Streptokinase before and after treatment was scored using the EGTI scoring system as a reliable method assessing kidney histology. The most effective improvement could be seen in the treatment group receiving the dosage of 100 mg/kg/day for 14 days.

5. CONCLUSION

There was a significant difference ($p<0.05$) of creatinine and urea level between groups after giving the andaliman fruit ethanol extract on acute glomerulonephritis rat models. The research showed the potential of andaliman fruit (*Zanthoxylum acanthopodium DC*) ethanol extract as an anti-inflammatory with the improvement of kidney histological structure on acute glomerulonefritis rat models after given the extract for 14 days.

This study suggested to investigate the optimum dose for its antioxidant potential and long-term safety of andaliman fruit extract. Further research to use a more specific kidney impairment parameter such as Cystatin-C, NGAL, KIM-1 and others are encouraged.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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