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Comparison of Gambier Extract (Uncaria Gambier Robx) and Angiotensin Receptor Blocker on Proteinuria Reduction and Antioxidants - Enhancement in Nephrotic Rat Models

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ABSTRACT

Background: Proteinuria is a significant clinical manifestation that causes edema in several diseases, including Nephrotic Syndrome (NS). Untreated proteinuria is strongly linked to the progression of kidney failure. One of the adjuvant therapies could be used to reduce proteinuria such as Angiotensin Receptor Blocker (ARB) including Losartan®. Gambier is a traditional medicinal plant widely known for its antioxidant effects. Catechin, a compound contained in Gambier Extract (GE), has been used to reduce microalbuminuria in diabetics. However, its application in NS has not been widely studied. **Objective:** This study compared the effects of GE and ARB in reducing proteinuria and increasing antioxidant activity levels, as well as reported histopathological findings in the nephrotic Wistar rat model. **Methods:** An experimental design study with a control group and a posttest was conducted. The experimental animals were divided into four groups: the control group (K1), the group with puromycin aminonucleoside (PAN) injection (K2), the group with PAN injection + GE (K3), and the group with PAN injection + Losartan® (K4). The standard GE used was Sarie Uncariae® by Toyo Brothers, PT while the ARB (Losartan®) was obtained from Novell, PT. Protein urine, the activity level of total superoxide dismutase (T-SOD), and malondialdehyde (MDA) were assessed using the colorimetric method. Renal histopathology was assessed based on Rollerman's criteria. **Results:** Gambier extract significantly reduced proteinuria, as depicted by a decrease in protein/volume urine ($p = 0.009$), increased antioxidant activity, as illustrated by an elevation in T-SOD activity levels ($p = 0.007$), and tended to decrease MDA levels compared to Losartan®. Based on histopathological findings, GE tended to reduce the percentage of kidney damage in rats induced by puromycin. **Conclusion:** Gambier extract has been shown a higher antioxidant effect by increasing T-SOD activity levels, reducing proteinuria and also exhibiting a tendency to diminish kidney damage.

Keywords: Gambier, Losartan®, SOD, MDA, Proteinuria.

1. BACKGROUND

Proteinuria is a significant clinical manifestation causing edema in nephrotic syndrome (NS). The mechanism of proteinuria in idiopathic NS is associated with immunological dysfunction, leading to the emergence of circulating factors affecting glomerular filtration permeability and podocyte damage (1). The primary treatment goal for NS is the reduction of proteinuria. Untreated proteinuria is strongly associated with the loss of kidney function (2). Various agents, including angiotensin-converting enzyme inhibitors (ACE-i) and angiotensin receptor blockers (ARB), are considered renoprotective agents in reducing proteinuria (3). However, these drugs can pose challenges in patients with advanced kidney disease due to their impact on depleting the glomerulus filtrate rate (GFR) and increasing the risk of hyperkalemia (4,5).

In Indonesia, the use of traditional medicine is common, particularly with gambier (3). Gambier is a traditional medicinal plant widely known for its antioxidant effect. It contains active ingredients, such as catechins, which are

phenolic compounds widely used for their antioxidants, anti-inflammatory, and anti-microbial properties (6). Catechins are also found in fruits, green tea, black tea, and some medicinal plants. Previous studies have shown that catechins extracted from green tea can reduce proteinuria in diabetic nephropathy patients (7). However, the role of Gambier Extract (GE) as an adjuvant therapy to reduce proteinuria in NS has not been widely studied. The antioxidant effect includes increasing primary antioxidant enzymes, such as superoxide dismutase (SOD), and decreasing the end product of the redox reaction, namely malondialdehyde (MDA). A previous study examined the effectiveness of catechins in GE, where administering catechins from GE at a dose of 26 mg/200 g body weight could reduce MDA levels (8). Furthermore, Hase et al,. observed a decrease in albumin excretion in rat models of induced diabetes which consumed tea containing catechins (9).

This study aims to investigate the efficacy of GE in treating NS by observing the reduction in proteinuria and the increase in antioxidants. The primary goal is to quantify the role of GE in curing NS through the reduction in proteinuria and the increase in antioxidant activity. For comparison to GE, Losartan®, previously used as a renoprotective agent in proteinuria Wistar Rats model, is utilized.

2. OBJECTIVE

The aim of the study was to compare the effects of Gambier extract (Uncaria gambir Robx) and an angiotensin receptor blocker (Losartan®) on proteinuria reduction, antioxidant levels, and kidney histopathological changes in a proteinuria Wistar Rats model.

3. MATERIAL AND METHODS

Procedure and Ethical Statement

Male Wistar Rats, aged eight weeks and weighing 150 ± 350 g, were used as the research subjects. The rats were randomly divided into four groups. They were individually housed in cages maintained at an average temperature of 20°C and subjected to a 12-hour light/dark cycle. A one-week acclimatization period was provided for the rats to adjust to the indoor environment, during which they had free access to water and food within their cages. This study was a pure experiment with a posttest control group design. Proteinuria in rat model was induced by subcutaneous injection of Puromycin aminoglycoside (PAN) (Puromycin by InvivoGen, USA) at a dose of 1.5 mg/100 g BW on days 1-5 and repeated on days 30-34 (10).

Male Wistar Rats in different groups received different treatments, such as the control group given aquabidest injection (K1), the group given PAN injection (K2), the group given PAN injection + GE (K3), and the group given PAN injection + Losartan® (K4).

Animal experiments conducted in this study adhered to the ARRIVE guidelines (11). Animals were monitored before and after each injection to ensure no abnormalities in weight (>10%), appearance, or behavior (vocalization, respiration, and movements). Every step taken was made and ensured to minimize animal suffering. The Research Ethics Committee at the Faculty of Medicine, Universitas Sumatera Utara approved the experimental protocols (approval No. 0587/KEPH-FMIPA/2021).

Materials

This study used the Gambier extract brand of Sarie Uncariae® (Toyo Brothers, PT) given at a dose of 26 mg/200 g BW, dissolved in 1 ml of aquabidest, and administered orally via rigid nasogastric tube for 60 days. The angiotensin receptor blocker, brand Losartan®, was obtained from Novel, PT (Reg no: CKL 1033523217A1) administered orally for 60 days at a dose of 10 mg/kg BW dissolved in 1 ml of aquabidest. Male Wistar Rats were purchased from the pharmacology laboratory in the Faculty of Medicine, Universitas Sumatera Utara. Laboratory examination.

The total protein Colorimetric Assay Kit (Coomassie Brilliant Blue Method) from Elabscience® was used to assess proteinuria. A 50 µL sample was mixed with a chromogenic agent and incubated for 10 minutes at room temperature. Optical density (OD) values were measured using spectrophotometry at 595 nm absorbance. Proteinuria levels were expressed in mg/ml. The Total-Superoxide Dismutase (T-SOD) activity assay kit (Hydroxylamine method) from Elabscience® was used to assess T-SOD activity. The sample was mixed with the provided reagent using a vortex mixer and incubated at 37°C for 40 minutes. Afterward, a chromogenic agent was added to the solution and allowed to stand for 10 minutes at room temperature. The OD value was measured at 550 nm for each tube, with results expressed in U/ml. The MDA Colorimetric assay kit (TBA method) from Elabscience® was used to assess the MDA level.

The assessment of serum and urine creatinine levels was carried out using a colorimetric assay kit (Sarcosine Oxidase Method, Elabscience®). Six microliter (µL) of serum was mixed into 180 µL of enzyme solution A and incubated for 5 minutes at 37°C. Then, 60 µL of the enzyme solution B was added and incubated for an additional 5 minutes at 37°C. The examination was performed at 515 nm wavelength. Then, incubated at 37°C for 3 min. Next, we measure the OD 2 values at 515 nm. The calculation of the sample concentration was based on the OD value of the sample, expressed in µmol/L.

Serum urea was assessed by using a colorimetric assay kit (Urease Method) from Elabscience®. Urea was broken down into ammonia and carbon dioxide ions by urease. The reaction between ammonia ions and phenolic chromogenic substances resulted in the formation of blue substances in an alkaline medium. The intensity of the blue substances was proportional to urea content, and the results were calculated using a colorimetric test at 580 nm. A total of 0.02 ml sample and reagents were added to the EP tubes and incubated before the OD value of each tube was measured using a 1 cm optical path cuvette at 580 nm. The calculated results were expressed in mmol/L.

Histopathological Examination

Histopathological examination was performed using Haematoxylin-Eosin (HE) and Trichome- Mansson

Wistar rat demographic data on day 0 and day 60		K1	K2	K3	K4
Body weight, average (SD)	Day 0	239.71 (19.5)	255.20 (30.29)	233.00 (11.80)	254.80 (14.18)
	Day 60	286.43 (25.7)	284.80 (20.41)	274.50 (9.40)	253.80 (26.19)
Body length, average (SD)	Day 0	21.63 (0.66)	21.40 (0.22)	0.80 (0.53)	20.70 (0.35)
	Day 60	22.86 (0.38)	21.90 (0.22)	21.58 (0.49)	22.00 (0.71)
Body circumference	Day 0	13.87 (0.53)	14.18 (0.44)	13.92 (0.34)	14.10 (0.26)
	Day 60	14.57 (0.53)	14.60 (0.55)	13.83 (0.41)	14.20 (0.45)

Table 1. Wistar rat demographic data

Parameter	K1	K2	K3	K4	p
Urine volume D 60, average (SD)	8.09 (5.73)	12.28 (9.53)	24.17 (7.49)	6.76 (1.54)	0.001
Urine total protein, average (SD)	1.02 (0.75)	1.12 (0.87)	0.21 (0.16)	1.06 (0.58)	0.082
Urine protein/urine volume	0.34 (0.45)	0.19 (0.23)	0.01 (0.013)	0.15 (0.09)	0.009
Plasma creatinine, average (SD)	0.53 (0.04)	0.54 (0.06)	0.47 (0.04)	0.53 (0.06)	0.108
Plasma urea, average (SD)	17.28 (5.82)	34.51 (10.90)	15.95 (11.86)	30.31 (9.48)	0.008
Creatinine clearance, average (SD)	0.43(0.33)	0.27(0.35)	0.14(0.12)	0.61(0.37)	0.333
T-SOD level, average (SD)	89.53 (3.37)	76.83 (12.94)	85.54 (5.51)	74.36 (6.37)	0.007
MDA level, average (SD)	4.81 (2.25)	6.45 (1.37)	6.30 (1.19)	6.83 (3.02)	0.352

Table 2. Changes in renal function and oxidative stress

staining to assess the qualitative degree of kidney damage, including the severity of damage to glomeruli and tubules. The staining was carried out immediately after the kidneys were removed. The kidneys from immobilized rats were taken and fixed in 10% buffered formalin, then cut and processed using standard techniques before embedding in paraffin. The assessment of renal histopathology was performed by an anatomical pathologist based on the criteria established by Rollerman et al. The Rollerman criteria range from no histological damage to severe histological damage and are based on the alterations observed in the glomerulus, tubules, and interstitials. In the mild stage, there will be an illustration that is close to a normal overview, in which the glomerular features show inflammatory infiltrates, a high number of glomerular cells, and apoptosis of endothelial cells. While, the tubular pattern shows apoptotic cells, coarse protein staining, and slight dilation of the tubules. In the moderate stage, there is an overview of tubular dilatation and more obvious tubular cell damage, in which the tubule will show more apoptotic cells, more obvious dilation, thickened basement membrane, and a little tubular protein cylinder. In the severe stage, there will be an overview of severe tubular dilation, cell-rich infiltrates, and regenerated tubules, in which the glomerular appearance shows smaller vascular lumina and more optic spaces due to glomerular shrinkage. While the tubular pattern shows a flat and complete loss of epithelium, severe tubular dilation, and peripheral fibrosis (12,13).

Statistical analysis

The data were tested for normality using the Shapiro-Wilk test, and the values of all variables were expressed as mean ± standard deviation (SD). One way ANOVA test were used because the data was normally distributed. A p-value <0.05 was considered statistically significant.

4. RESULTS

The demographic data of the Wistar rats as research subjects are presented in Table 1.

Table 1 demonstrates no significant differences in body weight, body length, and abdominal circumference from day 0 to day 60 among the four groups of rats.

Table 2 presents the changes in renal function parameters and oxidative stress. Urine volume on day 60 of rats in group K2, K3, and K4 increased, where a significant difference was observed in urine volume among all four groups. The analysis result of the 24-hour proteinuria examination revealed no significant difference among all four groups. The injection of PAN (K2) showed a tendency to increase protein excretion. However, the groups receiving GE (K3) and Losartan® (K4) had the tendency to have reduced protein excretion.

All rats from all groups showed similar plasma creatinine levels. There was no significant difference in plasma creatinine levels and creatinine clearance. However, there was a significant difference in urea levels among all four groups. Urea levels of rats given GE (K3) were significantly decreased to the level close to the control group (K1).

MDA level analysis revealed no significant difference among all four groups. Puromycin injection had increased MDA level in K2, K3, and K4 groups, although the increases were not significant. In contrast, there was a significant difference in T-SOD activity among all four groups. It was observed that rats given GE (K3) had the highest T-SOD activity level compared to the other PAN-induced-groups.

Figure 1 shows the average T-SOD enzyme activity and MDA levels among the four groups. Figure 1A displays that the K3 group had the highest average T-SOD activity. Meanwhile, Figure 1B shows that no group was dominant in terms of the average MDA level, although the values in the K3 group and K4 group were lower than the values in the K2 group.

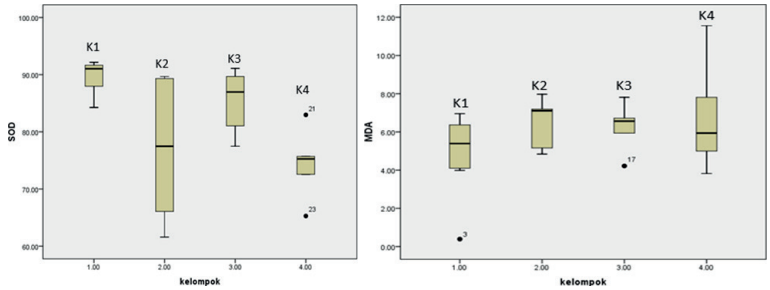


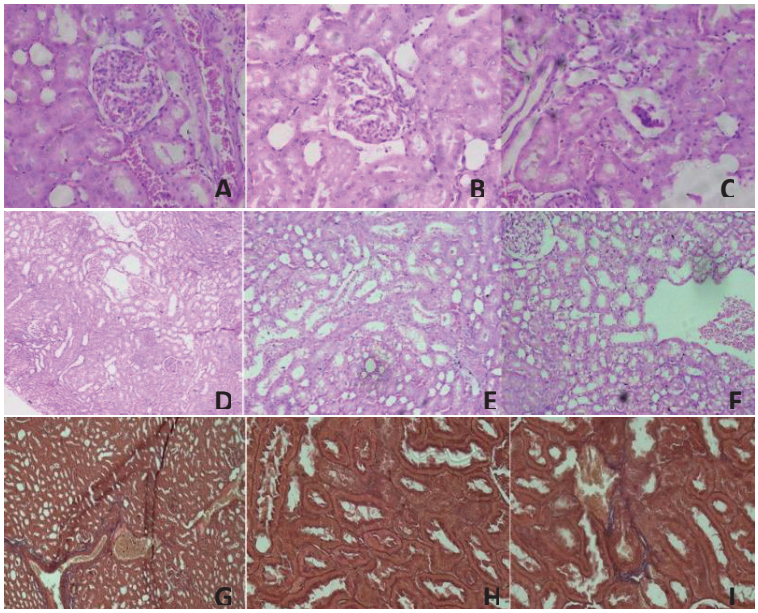
Figure 1: (A) The average activity of the T-SOD enzyme in the four groups (B) Average MDA levels in the four groups

a decrease in protein/volume urine in group with PAN injection + GE (K3). This finding is in line with Siregar's study, which also showed a decreasing effect on proteinuria (16).

Our study found that the PAN-induced rats treated with GE exhibited increased T-SOD activity and the lowest levels of proteinuria, suggesting that the inhibition of free radicals was highly beneficial in reducing proteinuria and minimizing the occur-

Figure 2. Histopathological appearances: (A) Normal glomerulus, (B) Glomerulus with interspace Bowman starting to widen, and (C) Constricting glomerulus with widened interspace Bowman. Description of the degree of tubular damage: (D) Mild tubular dilatation with apoptotic cells, (E) Moderate tubular dilatation with more pronounced apoptotic cells and thinning of the tubular basement membrane, and (F) Severe tubular dilatation with cells mitosis and thickening of the tubular basement membrane. Assessment of fibrosis in rat renal tubules by Manson Trichrome staining: (G) Interstitial fibrosis, (H) Tubular thickening, and (I) Tubular fibrosis.

A histopathological assessment was conducted to evaluate changes and the degree of kidney damage in the glomeruli, tubules, and interstitium (Rollerman's criteria). In group K2, kidney histopathology showed that all rats had severe kidney damage. Meanwhile, there was a decrease in the percentage of rats given GE (K3), where only 60% of rats had criteria for severe kidney damage and 40% had moderate kidney damage histopathologically. This demonstrated that GE can potentially provide histopathologic repair in PAN-induced rats. Meanwhile, 43% of the rats displayed severe damage in the group receiving Losartan® (K4).



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5. DISCUSSION

The antiproteinuric effect of catechins is attributed to the inhibition of podocyte apoptosis mediated by antioxidants (14). On the other hand, Losartan® reduces proteinuria through several mechanisms, such as lowering glomerulus transcapillary pressure, obstructing TGF-beta 1, and preventing podocyte apoptosis (15). In this study, a significant reduction in proteinuria was observed only in subject treated with GE (Table. 2). Hase et al., reported that the administration of catechins in diabetic rats resulted in reduced albumin in the urine after 12 weeks of treatment (9). The administration of GE to PAN-induced rats reduced proteinuria, indicating the role of GE in the glomerulus through its antioxidant properties mediated by catechins. This study demonstrated a significant reduced proteinuria as depicted by rence of renal sclerosis. The antioxidant effect includes increasing primary antioxidant enzymes, such as superoxide dismutase (SOD), and decreasing the end product of the redox reaction, namely malondialdehyde (MDA). T-SOD converts two free radical superoxide anions into oxygen and hydrogen peroxide (non-free radical properties) during free radical formation reactions, while MDA levels reflect the level of lipid peroxidation and cell damage in cells. This aligns with the study by Pane et al., where the administration of GE served as a free radical inhibitor enhancing T-SOD enzyme activity and decreasing proteinuria in diabetic patients (17). Losartan®, however, has no ability to enhance anti-oxidant effect (Table 2).

The creatinine clearance of the rats in this study was not significantly affected by the administration of GE. This result was consistent with several previous studies where there was no significant difference in creatinine clearance after administering GE to rats for 14 days

(16,17,18). Our study monitored the rats for 60 days, and the results still showed no significant difference in creatinine clearance. The plasma urea levels in rats given GE (K3) were close to those of rats in the control group but significantly lower than those from rats given Losartan® (K4). These findings suggested that GE regulated redox processes in the glomerulus in a way similar to the rats in the control group. Therefore, we concluded that the renal function of the rats did not change significantly after PAN injection + GE (K3). This result was consistent with the study by Afolabi et al., who stated that catechin could decrease the levels of creatinine and plasma urea in rats given nephrotoxic agents (19).

Our study found GE provided the highest T-SOD activity level and the lowest MDA level compared to the other groups that received PAN injection (Table 2). This finding aligned with the study by Pane et al., which showed decrease in MDA levels and an increase in T-SOD in diabetic patients who received GE (17). Additionally, Samarghandian, Azimi-Nezhad, and Farkhondeh established that the dose of catechin would dependently lower MDA serum levels and increase T-SOD activity level in the catechin-treated diabetic groups (20). Meanwhile, Simos et al. reported that catechins provided the increase in T-SOD activity levels, but MDA levels remained stable in catechins-administered rats (21). Therefore, we concluded that GE showed a better antioxidant effect than Losartan®. Previously, Ivanov et al., found no statistical differences in the antioxidant activities of superoxide dismutase, glutathione peroxidase, and glutathione reductase among groups of sham-operated rats (SHAM, n=7), rats with acute renal failure (ARF, n=7), and rats that received Losartan® after induction of acute renal failure (ARF + LOS, n=9) (22).

The stable MDA value was hypothesized to be due to the fact that MDA is the end result of a series of 12 redox reaction steps; therefore, many factors affected the MDA value. Meanwhile, T-SOD is an enzyme activated in the early stage of the redox reaction. Thus, this could be the reason why T-SOD activity increased after GE administration (23).

Our study is the first study to describe the effect of GE and ARB on kidney histopathology. The effects of GE on kidney histopathological damage have not been thoroughly researched, which will serve as the foundation for additional research on the related matter. Histopathological findings showed 60% of rats given GE (K3) exhibited severe kidney damage, while the remaining rats showed moderate damage (Figure 2). Meanwhile, histopathological findings showed milder tubular dilatation and milder tubular fibrosis in rats given Losartan®. These findings aligned with the study by Nguyen et al., who proved that the renin-angiotensin-aldosterone (RAA) system was activated by PAN injection. Hence, the administration of ARB (Losartan®) provided a renoprotective effect (24).

6. CONCLUSION

This study demonstrated that GE showed enhancement antioxidant effect than Losartan®, which was proven by a higher T-SOD activity level of PAN-induced rats. In addition, GE also had effect in reducing proteinuria and exhibiting a tendency to diminish kidney damage. These findings supported the role of GE as an adjuvant therapy for treating proteinuria in various renal diseases and further research is needed on the use of GE as an alternative therapy that acts as a renoprotective, especially in patients with nephrotic syndrome.

- **Author's contribution**: RSS gave substantial contributions to the conception, preparation, drafting and design by acquiring, analyzing, and interpreting data for the research. ORR, DH, AL, NKJ, PCE, MR and MMA were involved in the revision of the article and intellectual content. AL, ORR and DH approved the final version for publication and were accountable for all aspects of the work, including ensuring that questions related to the accuracy or integrity of any part of the work are investigated and resolved.
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