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RESEARCH ARTICLE

Protective effects of *Piper crocatum* ethanol extract on Gentamicin-induced nephrotoxicity in rats

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Abstract

Background: The kidney is one of the main target organs of toxic substances, including drugs. Gentamicin-induced nephrotoxicity is thought to be caused by the formation of oxidative stress and can be prevented by antioxidants. *Piper crocatum* has potent antioxidant activity. **Objective**: This study aims to determine the nephroprotective activity of *Piper crocatum*. **Method**: The study used Wistar rats in five groups (normal, negative, and ethanol extract of *Piper crocatum* with doses of 250, 500, and 750 mg/kg BW). The nephrotoxicity was induced by gentamicin for eight days. On the ninth day, the kidney function parameters (blood, urea, nitrogen, creatinine and uric acid) and malondialdehyde levels were measured, and kidney histology analysis was performed. **Results**: The results demonstrated that the extracts show lower kidney function parameters and malondialdehyde levels than the negative control. **Conclusion**: *Piper crocatum* is a potential nephroprotective agent.

Introduction

The kidney is an organ with vital functions in the human body (Fristiohady *et al.*, 2020; Gheith & El-Mahmoudy, 2018). It is responsible for regulating fluid, and acid-base balancing; cleansing toxins produced by the body, such as urea and creatinine, and those originating from outside the body, such as drugs. It is also functional in the reabsorption of specific molecules such as glucose and amino acids; regulating blood pressure; and producing several hormones such as erythropoietin and calciferol activation (Gheith & El-Mahmoudy, 2018; Hosohata, 2016). These functions indicate that the kidney is one of the main target organs for toxic substances. Clearance of xenobiotics, such as drugs, by the kidney may cause damage to these organs through acute or chronic injuries, depending on the degree and time of exposure (Gheith & El-Mahmoudy, 2018).

Drug-induced nephrotoxicity is a major drug-related problem caused by acute kidney injury, a sudden decrease in kidney function that occurs within 48 hours and results in nitrogen retention (creatinine and urea). Kidney injuries are one of the leading causes of morbidity and mortality (Chawla & Kimmel, 2012). Deaths from acute kidney injury are estimated to reach two million per year worldwide (Gyurászová *et al.*, 2020; Chawla & Kimmel, 2012). One of the AKI cases is caused by aminoglycoside antibiotics, affecting 10-20% of patients (Morales-Alvarez, 2020). Gentamicin is an aminoglycoside antibiotic most well-known to cause nephrotoxicity. At high doses, gentamicin causes toxic effects in the form of ototoxicity and nephrotoxicity (Hayward *et al.*, 2018). Nephrotoxicity occurs since gentamicin causes tubular damage through tubular epithelial cell necrosis, and reduced adenosine triphosphate (ATP) synthesis, resulting in cell death due to oxidative stress. The nephrotoxicity of gentamicin can be improved with antioxidants based on their ability to scavenge free radicals (Balakumar *et al.*, 2010).

One plant that shows antioxidant activity is the red betel (*Piper crocatum*) (Lister *et al.*, 2020). Red betel belongs

to the *Piperaceae* family with pharmacological effects such as antioxidant and nephroprotective characteristics (Suri *et al*., 2021). Red betel leaves contain bioactive compounds such as flavonoids, tannins, and alkaloids. Flavonoids display strong antioxidant activity in inhibiting lipid peroxidation. These compounds can interact with biomembranes to protect against free radicals. *Piper crocatum* has the potential as a natural antioxidant compound that may reduce free radicals and displays several pharmacological activities such as anti-inflammatory, antimicrobial, antifungal, antihyperglycemic, antiproliferative, and antioxidant (Lister *et al*., 2020; Suri *et al*., 2021). This study aimed to determine the nephroprotective activity of *Piper crocatum* leaves in gentamicin-induced rats.

Methods

Extraction

The red betel leaves (*Piper crocatum*) were obtained from and identified by UPT Materia Medica Batu, Malang, Indonesia (No.074/041/102.7-A/2022). *Piper crocatum* leaf constituents were extracted using the maceration method with 96% ethanol as a solvent for 24 hours with a ratio of 1:10. The extract was subsequently filtered, and the residue was soaked for another 24 hours with the same solvent. The extract was then evaporated in a rotary evaporator to obtain a thick extract of red betel leaves.

Experimental animal

The animals used in this study were male Wistar rats weighing 150-200 mg. The animals were adapted to the laboratory environment for one week with adequate feeding, drinking and regular changing of the husks in a controlled environmental condition (25^{o}C and a 12-hour light/dark cycle). The study was approved by the Ethical Committee of Medical Research Faculty of Dentistry Universitas Jember (No.1287/UN25.8/KEPK/DL/2021).

Experimental design

A total of 25 rats were divided into five groups: normal control (rats were given CMC-Na 0.5% orally and 1 mL/kg BW of normal saline via intraperitoneal (i.p.) injections), negative control (rats were given CMC Na 0.5% orally and 80 mg/kg BW of gentamicin via i.p. injections), and the treatment groups (rats were given 250 mg/kg BW, 500 mg/kg BW, 750 mg/kg BW of ethanol extract of red betel leaf orally and 80 mg/kg BW gentamicin via i.p. injections). The treatment was carried out for eight consecutive days. Rats were sacrificed 24 hours after the final treatment, and blood samples were taken intracardially. Rats' kidneys were removed and fixed in ten percent neutral buffered formalin (NBF) for histopathological studies. The blood samples were used to analyse renal function parameters (serum creatinine, blood urea nitrogen (BUN), and uric acid) and antioxidant activity (plasma and renal malondialdehyde (MDA) levels).

Statistical analysis

The experiment data were statistically analysed using one-way analysis of variance (ANOVA) to determine significant differences between groups in each parameter, followed by post hoc least significant differences (LSD).

Results

Effects of Piper crocatum ethanol extract on renal function parameters

The induction of 80 mg/kg BW of gentamicin for eight consecutive days significantly increased serum creatinine, BUN, and uric acid levels (Table I) when compared to the normal control group ($p < 0.001$) indicating the presence of nephrotoxicity. The administration of *Piper crocatum* ethanol extract at a dose of 500 mg/kg BW showed the prevention of a significant increase in serum creatinine and BUN levels. The administration at doses of 250 mg/kg BW, 500 mg/kg BW, and 750 mg/kg BW was not significantly different from the normal control group ($p = 0.132$; $p = 0.687$; and $p = 0.824$). All of the ethanol extracts affect the uric acid levels as in normal conditions.

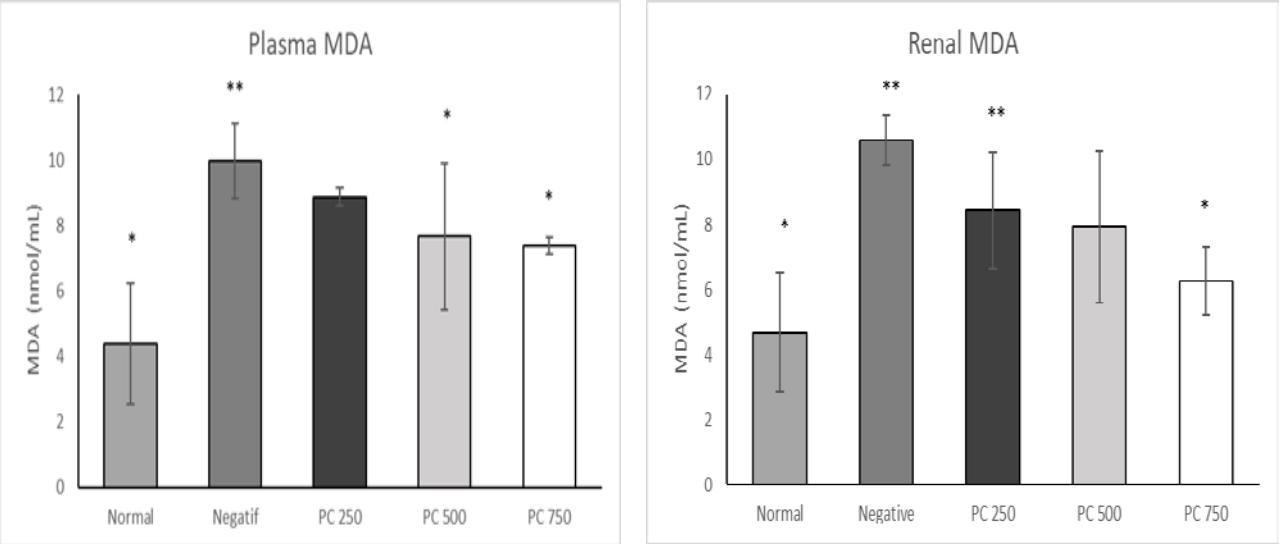
Table I: Effect of *Piper crocatum* ethanol extract on serum creatinine, BUN, and uric acid

Groups	Means ± standard deviation		
	Creatinine (mg/dL)	BUN (mg/dL)	Uric acid (mg/dL)
Normal control	$0.46^{a} \pm 0.02$	$32.14^{a} \pm 0.93$	$1.95^{a} \pm 0.29$
Negative control	$1.47^{b} \pm 0.26$	$90.71^{b} \pm 6.18$	$3.64^{b} \pm 0.17$
Piper crocatum extract 250 mg/kgBB	$0.75^{c} \pm 0.11$	$49.41^{c} \pm 3.16$	$2.63^{ac} \pm 0.59$
Piper crocatum extract 500 mg/kgBB	$0.54^{ad} \pm 0.06$	$41.26^{d} \pm 2.75$	$2.13^{ac} \pm 0.79$
Piper crocatum extract 750 mg/kgBB	$0.67^{acd} \pm 0.10$	$45.28^{cd} \pm 6.25$	$1.85^{ac} \pm 0.66$

The data were statistically analysed using One-way ANOVA, followed by the posthoc LSD test ($p < 0.05$). The means of the columns that share the same letter (a, b, c, or d) are not statistically different.

Effects of Piper crocatum ethanol extract on MDA levels

MDA is a product of lipid peroxidation used to determine tissue damage. The measurement of MDA level plays an essential role in the pathophysiology of kidney disorders. The presence of tissue damage is generally caused by increased MDA levels (Ayala, Muñoz & Argüelles, 2014). As shown in Figure 1, the administration of 80 mg/kg BW of gentamicin caused an increase in plasma and renal MDA levels, suggesting the occurrence of lipid peroxidation or oxidative stress. All *Piper crocatum* leaf ethanol extracts demonstrated the effect of antioxidant activity by preventing the increase in plasma MDA and kidney MDA levels caused by gentamicin.



The data were statistically analysed using One-way ANOVA, followed by the posthoc LSD test $p < 0.05$. The same asterisk symbols (* or **) indicate that the data is not significantly different between groups

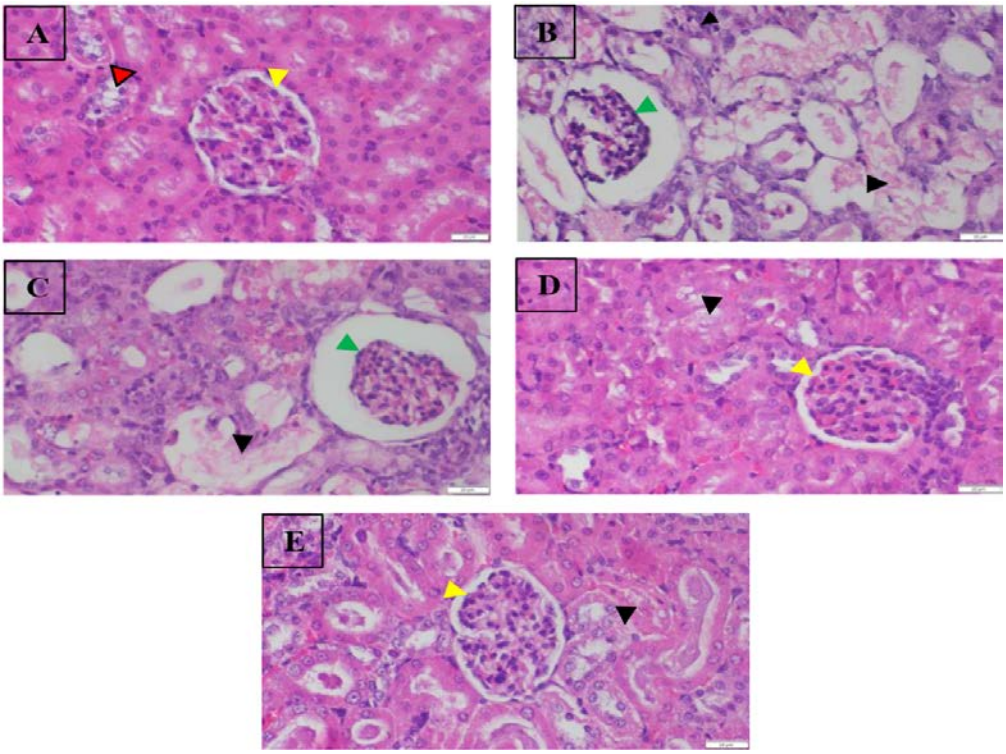
Figure 1: Effect of *Piper crocatum* ethanol extract on plasma MDA and renal MDA

Effects of Piper crocatum ethanol extract on rat kidney histopathology

The characteristics of a normal kidney include a glomerulus with a spherical shape composed of capillaries of simple endothelial cells and proximal convoluted tubules with simple cuboidal cells with microvilli. Gentamicin-induced animals' kidneys showed kidney damage characterised by tubular necrosis. Gentamicin accumulates in the tubules and causes various effects, such as loss of brush border and tubular necrosis (Sharma *et al*., 2021). Gentamicin reduces the number of endothelial cells, causing glomerular atrophy or glomerular shrinkage (Yarijani, Najafi & Madani, 2016). The histology results of the rats' kidneys in the study are presented in Figure 2. The ethanol extract of red betel leaf at doses of 500 and 750 mg/kg BW showed an improvement in the histopathological picture of the kidney.

Discussion

Decreased kidney function due to gentamicin induction occurs due to the accumulation of gentamicin in proximal tubular cells, which causes an increase in reactive oxygen species (ROS). Increasing levels of uric acid can elevate superoxide radicals and hydrogen peroxide, which form reactive oxygen species (ROS). The activity of the *Piper crocatum* ethanol extract, which contains flavonoid compounds, tannins, and alkaloids, can prevent the increase in uric acid levels through its activity as an antioxidant by inhibiting the xanthine oxidase (XO) mechanism. The inhibitory activity of XO by flavonoids is due to the double bonds contained in flavonoids and allows additional reactions by oxidation. The presence of the C_2 and C_3 double bonds will result in the co-planar position of ring B to ring A, making it easier to interact with the xanthine oxidase enzyme and the presence of hydroxyl groups in flavonoids, which introduce an inhibitory effect (Lin *et al*., 2015),). Tannin inhibits xanthine oxidoreductase (XOR), which catalyses hypoxanthine to xanthine to uric acid and produces ROS (Liu *et al*., 2021).



Description: (A) normal control group; (B) negative control group; (C) *Piper crocatum* extract dose 250 mg/kgBW; (D)*Piper crocatum* extract dose 500 mg/kgBW; (E) *Piper crocatum* extract dose 750 mg/kgBW**.**
▶ normal glomerulus**;** ▶ normal proximal tubule; ▶tubular necrosis; ▶glomerular atrophy.

Figure 2: Histology of rat kidney by longitudinal section, hematoxylin-eosin staining, and 40x magnification

Gentamicin causes elevated production of reactive oxygen species (ROS), such as superoxide anion, increased lipid peroxidation, depletion of antioxidant defences, and increased production of inflammatory cytokines (Elgebaly *et al*., 2016). Renal toxicity by gentamicin elevates plasma creatinine and urea levels and causes proximal renal tubular necrosis (Aiswarya *et al*., 2018). ROS regeneration due to gentamicin nephrotoxicity can be prevented by flavonoids through electron transfer and inhibiting lipid peroxidation reactions so that MDA compounds are not formed. Inhibition of the XO and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is another flavonoid mechanism in preventing redox reactions that produce free radicals (Lin *et al*., 2015). The flavonoid contained in red betel leaf is quercetin, which also has a role as a free radical scavenger, preventing damage to cell components caused by free radicals (Suri *et al*., 2021). In addition, tannin works by inhibiting the activity of superoxide and hydroxyl radicals (Namuleme *et al*., 2017). Gentamicin-induced nephrotoxicity also increases the levels of iNOS (inducible Nitric oxide synthase) and NF-κB, which activate proinflammatory cytokines (Mehan *et al*., 2017). Laksmitawati *et al*. (2017) showed that *Piper crocatum* leaves display anti-inflammatory activity by decreasing the TNF-α, IL-1β, IL-6, and NO levels. An increase in TNF-α causes a decrease in glomerular filtration rate (GFR), hence its inhibition elevates GFR and kidney function and prevents renal function parameters) (Mahmoudzadeh *et al*., 2017). Decreasing serum creatinine, BUN, uric acid, plasma MDA, kidney MDA, and improvements in the histopathology of rats' kidneys showed a nephroprotective activity of the ethanolic extract of *Piper crocatum* leaves due to the activity of its bioactive compounds, which have the potential as an antioxidant and anti-inflammatory agent.

Conclusion

Ethanol extract of red betel leaves (*Piper crocatum*) has a nephroprotective activity, which has the potential as an antioxidant and anti-inflammatory agent that may prevent the increase in BUN, creatinine, uric acid, kidney MDA, plasma MDA and may improve the histopathology of rats' kidneys in gentamicin-induced rats.

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Conflicts of Interest

The authors declare no conflict of interest.

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