

Nephroprotective Effect of *Pterocarpus indicus* Willd. Leaves: Observation From Plasma Urea and Creatinine Levels Against Gentamicin-Induced Nephrotoxicity in Sprague-Dawley Rats

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ABSTRACT

Introduction: *Pterocarpus indicus* Willd. has been widely used empirically for treating canker sore, antibacterial and kidney disease. Objective: This study aimed to evaluate nephroprotective effect water-boiled *P. indicus* leaves against gentamicin-induced nephrotoxicity in rats observed from plasma urea and creatinine levels. **Materials and Methods:** In this study, thirty (30) male rats divided into five treatment groups. The treatment groups consisted of the normal group, induction group received gentamicin (80 mg/kg body weight per day i.p.) and a group which received water-boiled *P. indicus* leaves with a dose of 28.8 mg/kg, 57.6 mg/kg, and 115.2 mg/kg body weight per day p.o. All groups received treatment for 21 days. At the 15th day, the animals received gentamicin i.p. for 7 days. At the 22nd day, the blood was collected from orbital sinus. The plasma urea and creatinine levels were measured by Berthelot method for plasma urea and colorimetric method for plasma creatinine. **Results:** The highest dose of 115.2 mg/kg water-boiled *P. indicus* was decreased plasma urea

and creatinine levels also significantly different ($p < 0,05$) compared induction group. **Conclusions:** Water-boiled *P. indicus* leaves have a potential to prevent gentamicin-induced nephrotoxicity might be due to its antioxidant and free radical scavenging activity.

Key words: Gentamicin, Nephroprotective, Plasma creatinine, Plasma urea, *Pterocarpus indicus* Willd.

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INTRODUCTION

Aminoglycoside antibiotics are used to treat infection due to Gram-negative bacteria and bacterial endocarditis. Aminoglycosides are known have toxic effect ototoxic and nephrotoxic.¹ Approximately 8-26% of patients who receive aminoglycosides for more than 7-10 days experienced mild renal function impairment.² One of aminoglycoside antibiotics is gentamicin. Toxicity is caused by the accumulation and retention of gentamicin in S1, S2 and S3 segments of the proximal tubular cells that lead to loss brush border integrity.^{3,4} Gentamicin is also known to enhance production of free radicals, tubular necrosis, glomerular congestion, epithelial edema of proximal tubular, cellular desquamation, tubular fibrosis, resulting in reduced glomerular filtration rate and renal function impairment.^{3,5} Kidney damage caused by gentamicin show the same conditions with renal function impairment is clinically characterized by elevated Blood Urea Nitrogen (BUN) and creatinine plasma levels, albuminuria, reduced glomerular filtration rate (GFR), renal dysfunction.^{3,6} *Pterocarpus indicus* Willd. (Fabaceae) is known as "Angsana" in Indonesia. The majority of Indonesian people have been using leaves for treating various diseases including boils, ulcers, prickly heat rashes.⁷ The community partially in Indonesia consumed leaves with a traditional way young leaves boiled with water for treating kidney dysfunction. The study to prove an efficacy of *P. indicus* is still slightly. The previous research has found that *P. indicus* leaves contain epicatechin that can lower blood glucose levels.^{8,9} Another study said that epicatechin can reduce oxidative stress on kidney dysfunction caused by damage to tubular epithelial cells.¹⁰ It allows the *P. indicus* leaves have a nephroprotective effect but until now there has been no research examining that. This study

aimed to prove nephroprotective effect water-boiled *P. indicus* leaves observed from plasma urea and creatinine levels against gentamicin-induced nephrotoxicity in Sprague-Dawley rats.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *Pterocarpus Indicus* Willd. were collected from West Java, Indonesia. The species was identified and authenticated by the Indonesian Institute of Sciences, Bogor, Indonesia, voucher specimen number 13-407/IPH.3./KS/II/2016.

Preparation of Water-Boiled

Fresh leaves of *Pterocarpus indicus* Willd be pondered the equivalent of 2.9 grams, then boiled with 500 ml distilled water at 100°C for 30-45 minutes until the volume obtained half of the initial volume to get water-boiled *P. indicus* leaves (WPIL). The concentration water-boiled *P. indicus* 11.52mg/ml per body weight. The amount of the volume is adjusted to body weight of rats. Water-boiled *P. indicus* leaves is made every day.

Chemicals

Heparin sodium was purchased from PT. Pharos, Indonesia. Gentamicin injection was purchased from PT. Indofarma, Indonesia. Standard kits used Urea Liquicolor and Creatinine Liquicolor were purchased from Gesellschaft für Biochemica und Diagnostica mbH, Germany.

Preliminary Phytochemical Analysis

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A preliminary phytochemical analysis of water-boiled *P.indicus* was done to identification presence various groups of phytochemicals.¹¹

Experimental Design

Male *Sprague-Dawley* rats (150-200 grams) were kept at standard condition temperature (25±20 C) and light (12 hours light/dark cycles). They received pellet and water *ad libitum*. The animals were acclimatized for 1-2 weeks before the experiment. The protocol experiment was approved by Ethics Committee of the Faculty of Medicine, University of Indonesia (Approval No. 236/UN2.F1/ETIK/2016).

Thirty (30) rats were randomly divided into five groups. Group I : Normal group, received aqua pro injection i.p., Group II : Induction group, received gentamicin (GM) (80 mg/kg BW, i.p. for 7 days), Group III : received water-boiled *P.indicus* leaves (WPIL) (28.8 mg/kg BW, p.o.) and GM (80 mg/kg BW, i.p. for 7 days), Group IV : received WPIL with (57.6 mg/kg BW, p.o) and GM (80 mg/kg BW, i.p. for 7 days), Group V : received WPIL (115.2mg/kg BW) and GM (80 mg/kg BW, i.p. for 7 days). Administration WPIL was started 14 days before administration of gentamicin and it was continued until 21 days. The rats fasted overnight. At the 22nd day, The blood was collected from orbital sinus for measuring urea and creatinine in plasma. Plasma was separated from blood and stored at -20°C until used for biochemical analysis. At the end treatment animals were sacrificed under ether vapor.

Determination of Plasma Urea

The urea was measured by modified Berthelot's reaction using Urea Enzymatic colorimetric Kit. Urea was hydrolysed in the presence of water and urease produces ammonia and carbon dioxide. Ammonium ions reacted with hypochlorite and salicylate to form a green dye. The absorbance measured at 578 nm.¹²

Determination of Plasma Creatinine

The creatinine was measured by Jaffe's method using Creatinine Colorimetric Kit. Creatinine in alkaline solution with picric acid produces an orange-red colored complex. The absorbance measured at 496 nm.¹³

Statistical Analysis

All values were represented as Mean ± standard error means (SEM). Data were analyzed statistically using Kruskal-Wallis test and for the analyzed significant difference between group treatment using Mann-Whitney test. All probability level of less than 5 % (p<0,05) was considered statistically significant. The statistical analysis was made by SPSS.

RESULTS

Phytochemical screening analysis WPIL contained flavonoid and tannins. As summarized in Table 1. Gentamicin-induced nephrotoxicity is characterized by elevated plasma urea and creatinine levels. Induction group showed increased plasma urea and creatinine levels significantly different (p<0.05) compared normal group. WPIL have a potential to prevent gentamicin-induced nephrotoxicity. This was shown with the administration of WPIL (28.8-115.2 mg/kg) showed decreased plasma urea and creatinine levels with a dependent dose manner. The highest

dose of 115.2 mg/kg WPIL showed significantly different (p<0.05) compared induction group.

DISCUSSION

Gentamicin is an aminoglycoside antibiotic that has the most powerful nephrotoxic effects than other aminoglycosides, so it can be accelerated acute renal failure in human and animals subjects.¹⁴ Kidney damage due to the administration of gentamicin in high doses is functionally characterized by elevated levels of urea and creatinine in plasma. Elevated plasma creatinine indicated occur decreased glomerular filtrate rate.^{5,15} Based on our previous study that gentamicin with a dose of 80 mg/kg BW/day i.p. for 7 days has been able to cause kidney damage is characterized by elevated plasma urea and creatinine levels. The toxic effect of gentamicin is closely related to the lysosomal phospholipidosis through inhibition of lysosomal hydrolases.² Gentamicin-induced nephrotoxicity due to accumulation drugs in proximal cells, resulting in loss of brush border integrity, necrosis cells, apoptosis cells, and massive proteolysis.^{3,16} Another mechanism, Gentamicin could be increased generation of reactive oxygen species (ROS) such as superoxide anion (O₂⁻), hydroxyl radical (OH⁻), hydrogen peroxide (H₂O₂), and peroxyntirite anion (ONOO⁻) in the renal cortex which damages the structure and function of the kidney. The presence of free radicals could be increased lipid peroxidation characterized by increased malondialdehyde, decreased glutathione (GSH) and antioxidant enzymes.^{3,17}

Administration WPIL in rats which gentamicin-induced nephrotoxicity show decreased plasma urea and creatinine levels in a dose-dependent manner. Decreased plasma urea and creatinine levels in Group III and Group IV were not significant (p>0.05) compared induction group. This probably could be due to the duration of administration WPIL too short. The plasma urea and creatinine levels in Group V were significant (p<0.05) compared induction group.

Several phytoconstituents in plant extract demonstrated antioxidant and cytoprotective function.⁴ Flavonoid such as catechin and epicatechin are known to have antioxidant effects, antimicrobial and bactericidal activity.¹⁸ Decreased plasma urea and creatinine levels due to presence flavonoids and tannins contained in WPIL might be an important role as antioxidant functions.¹⁹

The previous study has found that WPIL contained epicatechin. Epicatechin has the ability to protect mitochondria from oxidative stress in renal tubules caused by cisplatin with increasing enzyme manganese superoxide and glutathione also inhibit enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity. Additionally, epicatechin can inhibit cytochrome c release and inhibit Bax activation that plays a role in renal tubular cells apoptosis.¹⁰

Plasma urea level in Group V was still high if it was compared normal range plasma urea in the rat. The normal range of BUN was 10-21 mg/dl or plasma urea level from 21.4 to 44.94 mg/dl in the rat.²⁰ This probably due to gentamicin besides to attack tubules also cause to mesangial cells contraction, resulting in reduced the coefficient of ultrafiltration and at the end reduced glomerular filtration rate. Plasma urea is not filtered retained in the blood, causing plasma urea level was still high.

Table 1: Effect of water boiled *P. Indicus* on plasma urea and creatinine levels

Parameters	Normal	GM	GM+WPIL (28.8 mg/kg)	GM +WPIL (57.6 mg/kg)	GM+WPIL (115.2 mg/kg)
Plasma Urea (mg/dl)	42.76 ± 4.90	153.81 ± 14,79 ^a	141.12 ± 10,43 ^b	116.68 ± 16,98 ^b	93.98 ± 13,24 ^c
Plasma Creatinine (mg/dl)	0.51 ± 0.08	1.85 ± 0,31 ^a	1.34 ± 0,27 ^b	1.13 ± 0.26 ^b	0.84 ± 0.10 ^c

Note: All values were represented as Mean ± SEM (n=6); (a) P<0.05 compared normal group; (b) p>0.5 compared induction group; (c) p<0.05 compared induction group

CONCLUSION

Based on the above results, water-boiled *P. indicus* leaves were used by the public proven to treat kidney disease observed from plasma urea and creatinine levels were decreased after administration water-boiled *P. indicus* leaves. The exact mechanism of *Pterocarpus indicus* leaves to protect kidney has not been determined in the study.

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CONFLICT OF INTEREST

There is no conflict of interest in this study.

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