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Nephroprotective effect of ethanol extract *Abelmoschus manihot* l. Leaves in gentamicin-induced mice

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ABSTRACT

Gentamicin, an aminoglycoside antibiotic, is known for causing nephrotoxicity as its most common side effect, which is mediated by oxidative stress mechanisms, which the present study investigated in focusing on the potential nephroprotective effect of *Abelmoschus manihot* L. leaves (AML) due to their antioxidant activity and flavonoid content. The experiment involved seven groups of mice: untreated, solvent, negative control, and four test groups. The test groups were administered orally with ethanolic extract of AML at doses of 50, 100, 200, and 400 mg/kgBW, respectively, for seven days. On the 8th day, gentamicin was intraperitoneally induced at 112 mg/kgBW. On the 11th day, all mice were euthanized, and blood serum and renal organs were collected for further examination. Despite gentamicin-induced renal damage being evidenced in the euthanized mice through increased creatinine and blood urea nitrogen (BUN) levels, in the negative control group histopathology analysis revealed renal necrosis. Nevertheless, treatment with the ethanolic extract of AML demonstrated a dose-dependent nephroprotective effect on creatinine levels, but not on BUN and histopathology, yet, when considering all the results together, the study showed that the ethanol extract of AML does have a nephroprotective effect in gentamicin-induced nephrotoxicity.

Keywords: *Abelmoschus manihot* L., gentamicin, nephroprotection, renal histopathology

INTRODUCTION

Aminoglycoside has fast bactericidal effect, chemically stable, synergistic with beta-lactam antibiotics, low incidence of resistance, and affordable. However, the nephrotoxic effects reached 10%–25% despite careful monitoring (Kim and Moon, 2012; Hubert and Simmonds, 2015). Gentamicin induced renal damage, with one of the aminoglycoside drugs, was partially reabsorbed by proximal tubular cells (Lopez-Novoa *et al.*, 2011; Randjelovic *et al.*, 2017), generating necrosis of tubular epithelial cells in the proximal part, activating intrinsic apoptotic pathways, breaking the respiratory chain, reducing ATP synthesis, and inducing oxidative stress (Chatterjee, Mukherjee and Nandy, 2012; Otunctemur *et al.*, 2013). However, it has no standard therapy for preventing or curing adverse events on nephrotoxic drugs use.

Besides various approaches, *Abelmoschus manihot* L. leaves (AML) reported for high flavonoid constituent with antioxidant activity (Padmalochana and Dhana Rajan, 2015), with these approaches having been studied to ameliorate gentamicin-induced nephrotoxicity by using natural antioxidant resources such as *Cinnamomun zeylanicum* (Lauraceae), *Thymus vulgaris, Moringa oleifera* (Atsamo *et al.,* 2021*;* Abdel-Azeem *et al.,* 2017; Nafiu *et al.,* 2019).

Instead of modulating the glomerular filtration rate (GFR), urea, and creatinine, the flavonoid has antioxidant effect and prevents lipid peroxidation (Todarwal, Jain and Bari, 2011; Mandey *et al.*, 2014; Luan *et al.*, 2020). AML also

contains micronutrients, hyperin, quercetin-3-o-robinobiosid, isoquercetin, gossipetin-8-o-glucuronide, and myricetin (Taroreh *et al.*, 2016; Kwon *et al.*, 2022).

Hyperin has been proven in inhibiting oxidative response in cisplatin-induced nephrotoxicity by upregulating expression nuclear factor E2-related factor-2 (Nrf2) and Heme oxygenase-1 (HO-1) (Chao *et al.,* 2016; Zhou *et al.*, 2022). In addition to quercetin and isoquercetin having been reported in diverse mechanisms as antioxidants (Ozgen, Kilinc, and Selamoglu, 2016; Aghababaei and Hadidi, 2023), gossipetin, a bioflavonoid, was reported as having antioxidant enzymatic activity in *Rattus norvegicus* (Ijaz *et al.,* 2023). In accordance with other flavonoids, myricetin too has antioxidant activity against cisplatin-induced nephrotoxicity in mice (Hassan *et al.,* 2017; Zhou *et al.,* 2022). In conclusion, although, taken together, studies of AML as a nephroprotector are limited, all known active constituents of AML showed promising antioxidant activity. Therefore, the present study aims to determine nephroprotection activity of AML based on serum creatinine, serum albumin, Blood Urea Nitrogen (BUN), and histopathology examination.

MATERIALS AND METHODS

Materials

Abelmoschus manihot L. leaves were obtained from the Indonesian Spice and Medicinal Research Institute (BALITRO, Bogor, Indonesia); in addition, gentamicin (PT Indofarma Tbk., Jakarta, Indonesia); male white mice (*Mus muculus* L.) with DDY strain; reagent kit and creatinine standard; reagent kit and standard BUN (Blood Urea Nitrogen); (ReiGed Diagnostics, Geziemir, Turkey); high purity grade of ethanol 70%; aqua distillate; sodium methylse-cellulose (Na CMC); xylol; Hematoxylin-Eosin; physiological NaCl 0.9%, saturated picric acid, formaldehyde 40%; glacial acetic acid; chloroform; sodium dihydrogen phosphate monohydrate; disodium hydrogen phosphate anhydrous; etc. used in this study were obtained from Brataco Chemical, Indonesia. Equipment: Spectrophotometry (Microlab 300 & 300 LX EliTech Clinical Systems).

Plant determination.

Determination of *Abelmoschus manihot L*. leaves was carried out at Herbarium Depokensis (DEP), Biota Collection Room, University of Indonesia.

Extract preparation

The dried *Abelmoschus manihot* L. leaves (AML) were powdered and extracted using maceration in ethanol 70% solvent. The maceration was carried out in a shaker at room temperature and followed by evaporate using rotary evaporation. Obtained yield extracted AML (2 gram) was dissolved in CMC Na 0.5% (100 mL) homogenously. The volume of administration was determined based on dose and mice body weight.

Experimental design for animal models

The nephroprotective effect of AML was conducted in Pharmacology Laboratory, Faculty of Pharmacy, Pancasila University, Jakarta, Indonesia after obtaining ethical approval from the Health Research Ethics Committee University of Pembangunan Nasional Veterans Jakarta (101/I/2021/KEPK). All actions were taken by minimizing pain and suffering in experimental animals. Acclimatization was done for one week. Twenty eight mice were divided into seven groups: (I) untreated group without any treatment; (II) The solvent control group was given a 0.5% CMC-Na suspension solution for 7 days orally; (III) negative control was given standard food and drink for 7 days and on day 8th gentamicin (GEN) was given 112 mg/kgBW intraperitoneally; with (IV), (V), (VI), and (VII) group given various doses of AML extract and on the 8th gentamicin (GEN) 112 mg/kgBW intraperitoneally. Sequentially, the test groups were given by doses of 50; 100; 200; and 400 mg/kgBW for 7 days orally. At the end of the study (on day 11), all rats were euthanized. Blood and kidneys were collected for further experiments.

Serum Creatinine and BUN

A total of 1 ml of blood sample was put into a centrifuge tube, left for 20 minutes, and centrifuged at 4000 rpm for 20 minutes. At the same time, serum was taken, put into a microtube, and stored in the freezer at 2°C–5°C.

Serum creatinine and BUN were determined by Spectrophotometry (Microlab 300 & 300 LX EliTech *Clinical Systems*). Serum creatinine was obtained by mixed 5 µL serum, 15 mL of reagent 1 (hydroxide sodium) and 1.25 mL of reagent 2 (picric acid). This mixed solution was homogenized and measured on λ 510 nm. BUN were measured in homogenized solution of 5 µL serum added into 7.5 mL reagent 1 (urease, salicylate, and nitroprusside) and 7.5 mL reagent 2 (sodium hypochlorite and sodium hydroxide) on λ 340 nm.

Renal histopathology

Samples of renal organ were washed with saline solution then dried with filter paper and weighed. Histopathology analysis was prepared by renal fixation in 10% Neutral Buffer Formalin Solution, which was then cut and put into a specimen container. Renal tissue preparations that have been processed are then stained with Hematoxylin-Eosin staining. The preparations were observed under a microscope (Leica DM500) at the appropriate magnification. Histopathological analysis was carried out by an anatomy pathologist. Renal histopathology was carried out descriptively by observing glomerular and renal tubular morphology. The data were obtained in the form of descriptive and were compared regarding the degree of damage based on tubules affected using the Mitchell method (Amarasiri *et al.*, 2020; Ali *et al.*, 2022).

Statistics analysis

The data were analyzed using SPSS version 26 for One Way ANOVA test and followed by Duncan's post-hoc analysis test. The difference is considered significant if the p value <0.05.

RESULTS AND DISCUSSION

Renal Macroscopic Observation

Assessed by observing their color, shape, and consistency (Amang *et al.*, 2020), this macroscopic observation was aimed at observing the visible renal damage after gentamicin (Table I). While negative control and lowest-dose treatment group have blackish red kidney, instead of these two groups, renal macroscopis appears healthy in brownish-red color, good shape and chewy consistency. This indication in negative control might be due to severe hemorrhage, renal vein blood clots, and renal necrotic cells (Sari *et al.*, 2021).

Nephroprotective activity of *Abelmoschus manihot* L. leaves ethanol extract: serum creatinine and BUN

Serum creatinine level and BUN were measured 72-h after gentamicin-induced nephrotoxicity (Figure 1). Gentamicin induced the increase of both parameters significantly compared to the normal and solvent group. On the contrary, where all doses of AML showed similar nephroprotective effects, AML ethanol extract for seven days before gentamicin-induced clearly suppressed the serum creatinine level compared to the untreated group (negative control), despite not being dose-dependent.

Furthermore, the impact of AML treatment before gentamicin-induced was evident in attenuating BUN levels. It was a dose-responsive manner, with higher dose leading to more pronounced effects. However, two higher doses, group 200 and 400 mg/kgBW AML extract, have comparable effects.

Renal histopathology

Normal cells have clear nuclei and round shapes. Glomerular atrophy and enlargement of the capsule space + (25% cell damage) were marked as mild renal damage. Moderate damage is characterized by glomerular atrophy and capsular space enlargement++ (50% cell damage). Renal severe damage noticeably by glomerular atrophy and capsular space enlargement+++ (75% cell damage) (Mitchell and Cotran, 2007; Miller and Zachary, 2017).

The present study showed that gentamicin intraperitonially induced severe renal damage (Table II and Figure 2). Tubular dilatation occurred noticably in the negative control group, and favorable dose-dependent effects were observed in *Abelmoschus manihot* L. leaves ethanol extract treatment group, with low doses of 50 mg/kgBW and 100 mg/kgBW giving the slight improvement counted by 50%.

The primary goal of this study was to investigate the nephroprotective effects of AML after inducing kidney damage with gentamicin 112 mg/kgBW intraperitoneally. Severe damage was subsequently confirmed through histopathological analysis (Figure 2 and Table II). Urea serum and blood ureum nitrogen (BUN) supports histopathological findings (Figure 1).

Gentamicin accumulates in renal proximal tubules and enhances hydrogen peroxide generation by the mitochondria, with this hydrogen peroxide generated during the gentamicin induced oxidative stress in mitochondrial membranes releasing iron from the mitochondria and being mostly derived from the dismutation of superoxide. The released iron makes a complex with gentamicin and accelerates the oxidative stress (Tavafi *et al*., 2012).

Nephrotoxicity is an undesired side effect of gentamicin. Several studies reported the reactive oxygen species (ROS) in gentamicin induced kidney (Sarwar *et al.*, 2022). ROS induce tubular necrosis, decreased GFR and inflammation.

Table I. Renal macroscopic observation

Group	Structure			Photographs
	Colour	**shapes**	**Consistency**	
(i). Normal	Brownish red	Red bean shape and small size	Springy	
(II). Solvent control	Brownish red	Red bean shape and small size	Springy	
(III). Negative controls	Blackish red	Red bean shape and small size	Springy	
(IV). GEN+extract dose of 50 mg/ kgBW	Blackish red	Red bean shape and small size	Springy	
(V). GEN+extract dose of 100 mg/ kgBW	Slightly blackish red	Red bean shape and small size	Springy	
(VI). GEN+ extract dose of 200 mg/ kgBW	Brownish red	Red bean shape and small size	Springy	
(VII). GEN+extract dose of 400 mg/ kgBW	Brownish red	Red bean shape and small size	Springy	

Dependent upon their affinity to kidneys and on drug trapping process, nephrotoxicity of drugs is associated with their accumulation in the renal cortex, (Chatterjee *et al.,* 2012). Excessive production of reactive oxygen species (ROS) cannot be controlled by antioxidants present, which cause the reactive oxygen species (ROS) to react with other molecules. This in turn causes cell damage, which is caused by toxic drugs. One of these drugs is gentamicin (Zulfi *et al.,* 2013).

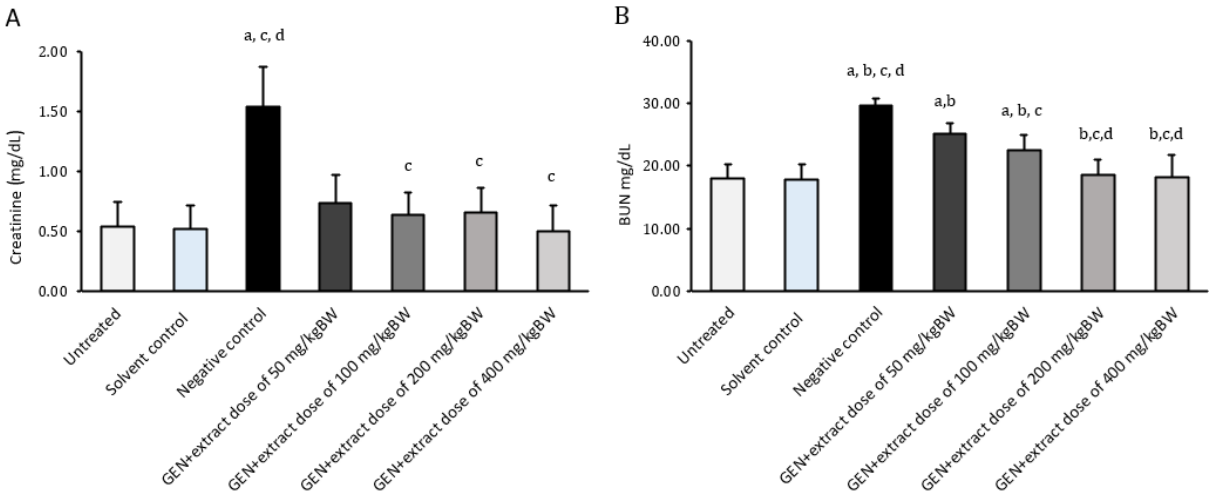

Figure 1. *Abelmoschus Manihot* L. attenuated gentamicin-induced nephrotoxicity by suppressing creatinine serum (mg/dL) and BUN (mg/dL).
creatinine serum and (B) blood urea nitrogen (BUN) in different groups. There was no difference in creatinine and BUN in the normal and solvent control groups. Gentamicin induced elevation of creatinine serum and BUN. AML extract attenuated creatinine and BUN. Normal group (untreated); solvent control group; negative control group (given standard food and drink for 7 days and on day 8th gentamicin (GEN) 112 mg/ kgBW intraperitoneally); Gen+extract group are given by AML 50 mg/kgBW, 100 mg/kgBW, 200 mg/kgBW, or 400 mg/kgBW for 7 days orally and on the 8th gentamicin (GEN) 112 mg/ kgBW intraperitoneally. Data presents in mean±SD and was obtained from 7 separate groups (n = 4). **a)** p values < 0.05 compared with normal group; **b)** p value <0.05 compared with negative group, **c)** p value <0.05 compared with group GEN+extract dose of 50 mg/ kgBW, **d)** compared to p <0.05 with group GEN+ extract dose of 100 mg/ kgBW from one way ANOVA test and followed by Post Hoc Duncan's test

Table II. Scoring Renal Damage

Group	Type Damage		Scoring (%)
	Glomerular Atrophy	Dilation tubules	
(i). Normal	-	-	0
(II). Solvent control	-	-	0
(III). Negative controls	+++	+++	75
(IV). GEN+extract dose of 50 mg/kgBW	++	++	50
(V). GEN+extract dose of 100 mg/ kgBW	++	++	50
(VI). GEN+extract dose of 200 mg/ kgBW	+	+	25
(VII). GEN+extract dose of 400 mg/ kgBW	+	+	25

Gentamicin, as a nephrotoxicity agent, could activate the Extracellular Calcium-Sensing Receptor (CaSR) in the basolateral loop of Henle. CaSR is a specific receptor for divalent and trivalent cations. Gentamicin in the form of polycation becomes a strong ligand for CaSR. Activation of CaSR by gentamicin causes two things: decreased intracellular production of cyclic adenosine monophosphate (cAMP) and increased formation of arachidonic acid. Both cause inhibitions of the reabsorption of Na^+, K^+, Cl^-, $Ca2^+$, $Mg2^+$. Gentamicin-induced cell death causes renal tubular lesions and necrosis. This is related to high levels of urea and creatinine in the blood which are toxic to the body (McLarnon *et al.*, 2002; Magno *et al.*, 2011). This present study demonstrated that gentamicin caused an increase in serum creatinine and BUN level prominently compared to negative controls group (Figure 1).

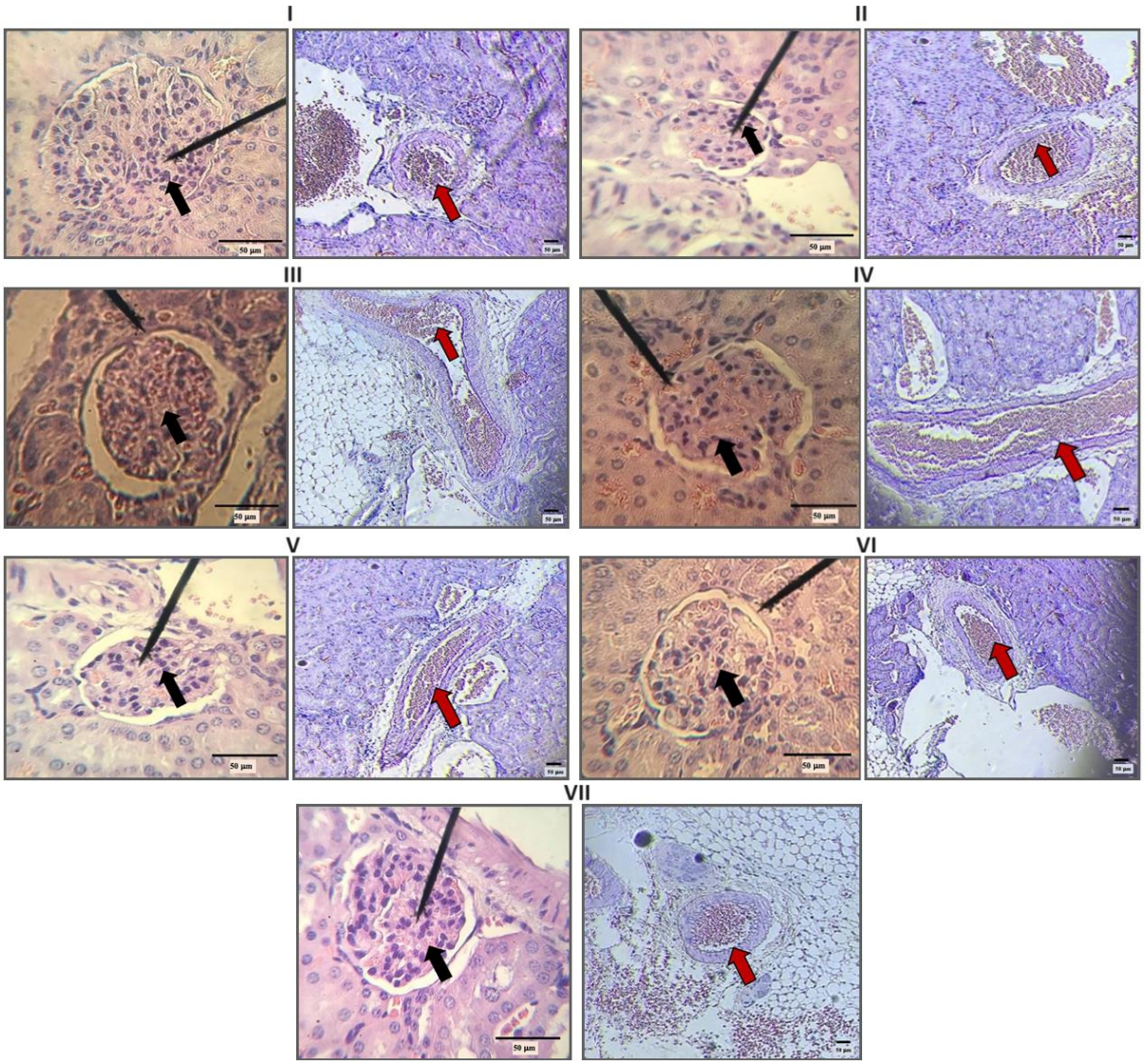

Figure 2. *Abelmoschus Manihot* L. improved gentamicin-induced renal damage.
Normal glomerular and tubular cell morphology can be observed in groups I and II. However, cells that undergo necrosis, glomerular atrophy and tubular dilatation in group III and attenuated in group IV-VII which received different doses AML orally for 7 days advanced. I) Untreated group; II) solvent control; III) gentamicin induced nephrotoxicity but untreated; IV) gentamicin induced nephrotoxicity and treated by AML 50 mg/kgBW; V) gentamicin induced nephrotoxicity and treated by AML 100 mg/kgBW; VI) gentamicin induced nephrotoxicity and treated by AML 200 mg/kgBW; VII) gentamicin induced nephrotoxicity and treated by AML 200 mg/kgBW. AML extract orally administered for 7 days and on day 8th gentamicin 112 mg/kgBW intraperitoneally. Data obtained from sample of 7 separate groups (n = 4). Hematoxylin-Eosin stained. Magnification 40X (left) and 10X (right). Scale bar 50 μm. Glomerular cells (black arrow). Tubular cells (red arrow).

Some natural antioxidant agents possibly overcome gentamicin induced nephrotoxicity. These antioxidants may offer alternatives besides synthetic antioxidants such as butylated hydroxytoluene, octyl gallate, and N-acetylcysteine (Govindappa *et al.*, 2019). The present study used AML ethanol extract to determine its activity. The concomitant use of AML ethanol extract, shown by the decrease in serum creatinine and BUN levels (Figure 1) in the AML treatment group, ameliorated renal damage due to gentamicin induced.

Serum creatinine is well known as a specific renal function marker. If the glomerular filtration rate (GFR) is reduced by 50%, serum creatinine may increase significantly. Dehydration, excessive fatigue, renal infection, and uncontrolled hypertension are the inducer for creatinine serum level (Dahal and Mulukuri, 2015; Wang *et al.*, 2019). This present study measured BUN level from serum. At the same time, urea nitrogen can be an indicator of kidney function where, if there is renal impairment, urea nitrogen will accumulate in the blood (Mitruka, Rawnsley and others, 1977; Wang *et al.*, 2019).

The histological damage in AML treated group was minimal in contrast to the negative control group, where doses 200 mg/kgBW and 400 mg/kgBW of AML ethanol extract for 7 days orally significantly demonstrated lower serum creatinine level when compared with the negative control. This result was in accordance with BUN level (Figure 1) and histopathology examination (Table II & Figure 2).

The nephroprotective effect of AML may be attributed to its ability to scavenge free radical as act as an antioxidant. It is suggested by the presence of flavonoid in AML (Padmalochana and Dhana Rajan, 2015). In accordance with that, flavonoids constituent in spinach and red grapes positively protect renal function due to antioxidant activity (Sarwar *et al.*, 2022). In addition, *Sonchus arvensis L* containing flavonoid showed nephroprotective effect by reducing serum urea and creatinine levels in gentamicin-induced nephrotoxicity (Suliska *et al.*, 2021). Moreover, having high flavonoids, *Kigelia africana* fruits extract was reported to attenuate nephrotoxicity due to gentamicin in rats (Josiah *et al.*, 2020). In a related study, Asparagus *africanus* Lam root extract overcame gentamicin-induced kidney damage by supressed serum creatinine, BUN, and uric acid levels, because of its presences of bioactive secondary metabolites such as flavonoids, tannins, and phenols (Meka Kedir, Dukassa Dubiwak and Tofik Ahmed, 2022).

Flavonoids which are found as active constituents in AML extract also inhibit xanthine oxidase and protein kinase C. Both are enzymes responsible for producing superoxide anion radicals ($O_{2\text{-}}$). Flavonoids also inhibit the enzymes cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase and NADH oxidase. They contribute to the formation of radical oxygen species (ROS). Additionally, flavonoids prevent oxidative stress in the kidney through inducing the synthesis of glutathione S-transferase (GSH). GSH is one of the endogenous antioxidants. Hydroxyl group (-OH) from flavonoids can trap free radical substances (Huang *et al.,* 2011; Zhang *et al.,* 2017; Zhang *et al.,* 2018). In this study, the assessment of endogenous antioxidant enzyme activity was not conducted. In the followed-up research, it was mandatory to perform antioxidant enzymes activity to confirm the mechanism of action from AML ethanol extract.

This study also performed a histopathological examination. It was supported by serum creatinine, and BUN levels. Our histological and microscopic examinations revealed that gentamicin-induced histological damage occurred. Administration of ethanol extract of AML data ameliorated the renal damage, and the amelioration was dose-dependent. The nephroprotective was better at dose of 200 mg/kgBW and 400 mg/kgBW compared to the other group. Parenchymal changes in the glomerulus and tubules were found in this examination (Figure 2 & Table 2). Glomerular atrophy was shown by the size decreasing. Tubular changes were described by widening of the tubular lumen (tubular dilatation). This widening is due to the binding of toxic substances to cell organelles, damaging cell membranes, reducing the ability to produce ATP (Sodimbaku *et al.*, 2016; Sahu *et al.*, 2014; Edeogu *et al.*, 2020).

AML ethanol extract provides protective activity against renal damage through its antioxidant activity, which was possibly caused by the active constituent, flavonoid. Nevertheless, further studies in molecular mechanism and drugs formulation need to be explored, with this as a reminder. AML could constitute a lead to the discovery of a novel drug which will be useful in treatment of drug-induced nephrotoxicity.

CONCLUSION

The AML ethanol extract shows a nephroprotective activity in gentamicin-induced mice by decreasing blood creatinine and BUN levels and can restore histopathology features due to gentamicin nephrotoxicity.

AUTHOR CONTRIBUTION

Ni Made Dwi Sandhiutami designing the experiments, conducted the experiment, analysing and visualized data, writing, editing, and finishing the manuscript. Bantari Wisynu Kusuma Wardhani conducted the experiment, statistical analysis, writing and editing manuscript. Rila Nurefrialia

Nisa conducted experiments and statistical analysis. All authors have read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Ali, A. ., Khan, H. ., Kahrizi, D. ., Pervez, S. ., Alotaibi, G. ., & Rastrelli, L. . (2022). Nephroprotective effects of Helianthus annuus seeds extract in gentamicin induced nephrotoxic male mice. *Cellular and Molecular Biology*, 68(1), pp. 1–7. http://dx.doi.org/10.14715/cmb/2022.68.1.1

Abdel-Azeem, A. S., Hegazy, A. M., Zeidan, H. M., Ibrahim, K. S., & El-Sayed, E. M. (2017). Potential Renoprotective Effects of Rosemary and Thyme Against Gentamicin Toxicity in Rats, *Journal of Dietary Supplements*, 14(4), pp.380-394, DOI: 10.1080/19390211.2016.1253632.

Amang, A.P., Baponwa, O., Mezui, C., Siwe, G.T., Vandi, L.V., Njuh, A., Mbida, H., Kodji E., Vernyuy Tan P.V. (2020). Effects of aqueous extract of root barks of Swartzia madagascariensis (Caesalpiniaceae) on acute kidney failure induced with gentamicin in Wistar rats. *Journal of Medicinal Plants Studies*, 8(4), pp. 183–197. http://www.plantsjournal.com

Amarasiri, S. S., Attanayake, A. P., Arawwawala, L. D. A. M., Jayatilaka, K. A. P. W., & Mudduwa, L. K. B. (2020). Acute and 28-day repeated-dose oral toxicity assessment of Abelmoschus moschatus Medik. in healthy Wistar rats. *Evidence-Based Complementary and Alternative Medicine*, 2020. https://doi.org/10.1155/2020/1359050

Aghababaei, F and Hadidi,M. (2023). Recent advanced in potential health benefits of quercetin. *Pharmaceuticals*, 16, pp 1020. https://doi.org/10.3390/ph16071020

Atsamo, A. D., Lontsie Songmene, A., Metchi Donfack, M. F., Ngouateu, O. B., Nguelefack, T. B., & Dimo, T. (2021). Aqueous Extract from Cinnamomum zeylanicum (Lauraceae) Stem Bark Ameliorates Gentamicin-Induced Nephrotoxicity in Rats by Modulating Oxidative Stress and Inflammatory Markers. *Evid Based Complement Alternat Med*, 2021, pp5543889. doi: 10.1155/2021/5543889.

Chatterjee, P., Mukherjee, A. and Nandy, S. (2012). Protective effects of the aqueous leaf extract of Aloe barbadensis on gentamicin and cisplatin--induced nephrotoxic rats. *Asian Pacific journal of tropical Biomedicine*, 2(3), pp. S1754--S1763. https://doi.org/10.1016/S2221-1691(12)60490-0

Chao, C.S. *et al.* (2016). Hyperin inhibits nuclear factor kappa B and activates nuclear factor E2-related factor-2 signaling pathways in cisplatin-induced acute kidney injury in mice. *Int Immunopharmacology*, 40, pp. 517–523. https://doi.org/10.1016/j.intimp.2016.09.020

Dahal, A. and Mulukuri, S. (2015). Flavonoids in kidney protection. *World J. Pharm. Pharmaceut. Sci*, 4(3), pp. 362–382.

Edeogu, C. O., Kalu, M. E., Famurewa, A. C., Asogwa, N. T., Onyeji, G. N., & Ikpemo, K. O. (2020). Nephroprotective effect of Moringa oleifera seed oil on gentamicin-induced nephrotoxicity in rats: biochemical evaluation of antioxidant, anti-inflammatory, and antiapoptotic pathways. *Journal of the American college of nutrition*, 39(4), pp. 307–315.https://doi.org/10.1080/07315724.2019.1649218

Govindappa, P. K. Gautam V., Tripathi,S.M., Sahni, Y.P., Raghavendra, H.L.S., (2019). Effect of Withania somnifera on gentamicin induced renal lesions in rats. *Revista Brasileira de Farmacognosia*, 29(2), pp. 234–240. https://doi.org/10.1016/j.bjp.2018.12.005

Hassan, S. M., Khalaf, M. M., Sadek, S. A., & Abo-Youssef, A. M. (2017). Protective effects of apigenin and myricetin against cisplatin induced nephrotoxicity in mice, *Pharmaceutical Biology*, 55:1, 766-774. https://doi.org/10.1080/13880209.2016.1275704

Huang, J., Wang, S., Zhu, M., Chen, J., & Zhu, X. (2011). Effects of Genistein, Apigenin, Quercetin, Rutin and Astilbin on serum uric acid levels and xanthine oxidase activities in

normal and hyperuricemic mice. *Food and Chemical Toxicology*, 49(9), pp 1943-1947. https://doi.org/10.1016/j.fct.2011.04.029

Hubert, D. and Simmonds, N. (2015). Living longer with cystic fibrosis. *European Cystic Fibrosis Society*, 390.

Josiah, S.S., Crown, O.O., Akinmoladun, A.C., & Olaleye, M.T. (2020). Renoprotective property of the flavonoid-rich extract of Kigelia africana fruits on gentamicin-induced nephrotoxicity in rats. *Comparative Clinical Pathology, 29*, 815 - 828. https://doi.org/10.1007/s00580-020-03140-w

Kim, S. Y. and Moon, A. (2012). Drug-induced nephrotoxicity and its biomarkers. *Biomolecules & therapeutics*, 20(3), p. 268. https://doi.org/10.4062/biomolther.2012.20.3.268

Kwon H.J., Beom S.H., Hyun, J.A., Kang, E.B., Park, H.E., Han D.G., Choi E.Y., Bong-Jeon A. (2022). Analysis of antioxidant activity, total phenol content, and flavonoid content of Abelmoschus manihot flower extracts. *Korean Journal of Food Preservation*, 29(1), pp. 157–165. https://doi.org/10.11002/kjfp.2022.29.1.157

Lopez-Novoa, J. M., Quiros, Y., Vicente, L., Morales, A. I., & Lopez-Hernandez, F. J. (2011). New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. *Kidney international*, 79(1), pp. 33–45. https://doi.org/10.1038/ki.2010.337

Luan, F., Wu, Q., Yang, Y., Lv, H., Liu, D., Gan, Z., & Zeng, N. (2020). Traditional uses, chemical constituents, biological properties, clinical settings, and toxicities of Abelmoschus manihot L.: a comprehensive review. *Frontiers in pharmacology*, 11, p. 1068. https://doi.org/10.3389/fphar.2020.01068

Magno, A. L., Ward, B. K. and Ratajczak, T. (2011). The calcium-sensing receptor: a molecular perspective. *Endocrine reviews*, 32(1), pp. 3–30. https://doi.org/10.1210/er.2009-0043

Mandey, J. S. Soetanto, H., Sjofjan, O., Tulung, B. (2014). Genetics characterization, nutritional and phytochemicals potential of gedi leaves (Abelmoschus manihot (L.) Medik) growing in the North Sulawesi of Indonesia as a candidate of poultry feed. *Journal of Research in Biology*, 4(2), pp. 1276–1286.

McLarnon, S., Holden, D., Ward, D., Jones, M., Elliott, A., & Riccardi, D. (2002). Aminoglycoside antibiotics induce pH-sensitive activation of the calcium-sensing receptor. *Biochemical and biophysical research communications*, 297(1), pp. 71–77. https://doi.org/10.1016/S0006-291X(02)02133-2

Meka Kedir, W., Dukassa Dubiwak, A. and Tofik Ahmed, E. (2022). Nephroprotective effect of asparagus africanus lam. root extract against gentamicin-induced nephrotoxicity in swiss albino mice. *Journal of Toxicology*, 2022. https://doi.org/10.1155/2022/8440019

Miller, M. A. and Zachary, J. F. (2017). Mechanisms and morphology of cellular injury, adaptation, and death. *Pathologic basis of veterinary disease*, p. 2. doi: 10.1016/B978-0-323-35775-3.00001-1

Mitchell, R. N. and Cotran, R. S. (2007). Cell injury, cell death, and adaptations. Basic Pathology. 8th ed*. Kumar V, Cotran R, Robbins SL (Eds). Elsevier Science, Philadelphia*, pp. 19–22.

Mitruka, B. M., Rawnsley, H. M. and others (1977) *Clinical biochemical and hematological reference values in normal experimental animals.* Masson Publishing USA Inc.

Nafiu, A. O., Akomolafe, R. O., Alabi, Q. K., Idowu, C. O., & Odujoko, O. O. (2019). (2019). Effect of fatty acids from ethanol extract of Moringa oleifera seeds on kidney function impairment and oxidative stress induced by gentamicin in rats. *Biomedicine & Pharmacotherapy*, 117, pp. 109154. https://doi.org/10.1016/j.biopha.2019.109154.

Otunctemur, A., Ozbek, E., Cekmen, M., Cakir, S. S., Dursun, M., Polat, E. C., Somay, A., & Ozbay, N. (2013). Protective effect of montelukast which is cysteinyl-leukotriene receptor antagonist on gentamicin-induced nephrotoxicity and oxidative damage in rat kidney. *Renal failure*, 35(3), pp. 403–410. https://doi.org/10.3109/0886022X.2012.761040

Ozgen, S., Klininc, OK., and Selamoglu, Z. (2016). Antioxidant activity of quercetin: a mechanistic review. *Turk J Agric.Food Sci. Technol,* 4, pp. 1134-1138. https://doi.org/10.24925/turjaf.v4i12.1134-1138.1069

Padmalochana, K. and Dhana Rajan, K. S. (2015). In

vivo nephroprotective potential from leaves extracts of Andrographis paniculata. *World Journal of Pharmacy and Pharmaceutical Sciences*, 4, pp. 1416–1425.

Randjelovic, P., Veljkovic, S., Stojiljkovic, N., Sokolovic, D., & Ilic, I. (2017). Gentamicin nephrotoxicity in animals: Current knowledge and future perspectives. *EXCLI journal*, 16, p. 388. https://doi.org/10.17179/excli2017-165

Sahu, B.D. Tatireddy, S., Koneru, M., Roshan M. Borkar, R.M., Kumar, J.M., Madhusudana Kuncha, M., Srinivas R., Sunder S.R., Sistla R. (2014). Naringin ameliorates gentamicin-induced nephrotoxicity and associated mitochondrial dysfunction, apoptosis and inflammation in rats: possible mechanism of nephroprotection. *Toxicology and applied pharmacology*, 277(1), pp. 8–20. https://doi.org/10.1016/j.taap.2014.02.022

Sari, D.C.R., Budiharjo, S., Afifah, H., Jasmin, D., Kokasih, O., Putri, T.G., Arifiani, K., Setyaningsih, W.A.W., Arfian, N. (2021). Centella asiatica extract attenuates kidney fibrosis through reducing mesenchymal transition and inflammation in ureteral ligation model in mice. *Frontiers in Pharmacology*, 12, p. 621894. https://doi.org/10.3389/fphar.2021.621894

Sarwar, S., Hossain, M.J., Irfan, N.M., Ahsan T., Arefin M.S., Rahman, A., Alsubaie A., Alharthi, B,, Khandaker, M.U., Bradley, D.A. (2022). Renoprotection of selected antioxidant-rich foods (water spinach and red grape) and probiotics in gentamicin-induced nephrotoxicity and oxidative stress in rats. *Life*, 12(1), p. 60. https://doi.org/10.3390/life12010060

Sodimbaku, V., Pujari, L., Mullangi, R. and Marri, S., (2016). Carrot (Daucus carota L.): Nephroprotective against gentamicin-induced nephrotoxicity in rats. *Indian Journal of Pharmacology*, 48(2), p.122. doi: 10.4103/0253-7613.178822

Suliska, N., Praviska, M., kurniati, N.F., Sukandar, E.Y. (2021). Nephroprotective effect of ethanol extract of Sonchus arvensis L. leaves in gentamicin-piroxicam induced rat renal failure. *Journal of Research in Pharmacy*, 25(4). doi**:** 10.29228/jrp.35

Taroreh, M., Raharjo, S., Hastuti, P., Murdiati, A. (2016). Antioxidative activities of various fractions of gedi's leaf extracts (Abelmoschus manihot L. medik). *Agriculture and Agricultural Science Procedia*, 9, pp. 271–278. https://doi.org/10.1016/j.aaspro.2016.02.112

Tavafi, M., Ahmadvand, H. and Toolabi, P. (2012). Inhibitory effect of olive leaf extract on gentamicin-induced nephrotoxicity in rats. *Iran J Kidney Dis*, 6(1), pp. 25-32.

Todarwal, A., Jain, P. and Bari, S. (2011). Abelmoschus manihot Linn: ethnobotany, phytochemistry and pharmacology. *Asian Journal of Traditional Medicines*, 6(1), pp. 1–7.

Wang, R., Hassan, W., Ahmad, F.ud.D.A., Jabeen, Q., Ahmed, H., Iqbal, O. (2019). Citrus aurantium ameliorates cisplatin-induced nephrotoxicity. *BioMed research international*, 2019, pp. 3960908. doi:10.1155/2019/3960908.

Zhang, C, Zhang, G, Liao, Y., Gong, D. (2017). Myricetin inhibits the generation of superoxide anion by reduced form of xanthine oxidase. *Food Chemistry*, 221, pp. 1569-1577. https://doi.org/10.1016/j.foodchem.2016.10.136

Zhang, C., Wang, R., Zhang, G., & Gong, D. (2018). Mechanistic insights into the inhibition of quercetin on xanthine oxidase. *International journal of biological macromolecules*, *112*, 405–412. https://doi.org/10.1016/j.ijbiomac.2018.01.190

Zulfiani, Z., Ilyas, S. and Hutahaean, S. (2013). The influence of vitamin C and E administration on the histological appearance of mouse (Mus musculus L.) kidneys exposed to monosodium glutamate (MSG). *Saintia Biologi*, 1(3), pp. 1–6.

Zhou, J., Nie, R. C., Yin, Y. X., Cai, X. X., Xie, D., & Cai, M. Y. (2022). Protective Effect of Natural Antioxidants on Reducing Cisplatin-Induced Nephrotoxicity. Disease markers, 2022, 1612348. https://doi.org/10.1155/2022/1612348