Original Article

Nephroprotective effect of sugarcane (*Saccharum officinarum* L.) leaves ethanol extract on gentamicin-induced nephrotoxicity in rats

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Abstract

Kidney damage is commonly attributed to using certain drugs, such as gentamicin, which causes elevated kidney parameters in blood and damage to renal tissue. This damage is often a result of oxidative stress, but it can be mitigated by using antioxidants. Several studies proved the potential of sugarcane (*Saccharum officinarum* L.) leaves as an antioxidant. Therefore, this experiment aimed to examine the nephroprotective action of sugarcane leaves. Twenty-five Wistar rats were separated into the normal, negative, and sugarcane leaf extract (SLE) (200, 400, and 600 mg/kg BW) groups. The animals were handled for 8 days, and then, the blood and tissue were collected 24 h later. The results revealed that SLE prevents increased creatinine, blood urea nitrogen, uric acid, and malondialdehyde levels. The histology analysis indicated that the extract improved kidney morphology and histopathology. Sugarcane leaves have the potential to be a nephroprotective agent.

Key words: Gentamicin, kidney, sugarcane leaves, toxicity

INTRODUCTION

The kidney is essential in eliminating xenobiotics, making it susceptible to toxic effects, including drugs.[1,2] Gentamicin, an aminoglycoside antibiotic, is known to have a nephrotoxic effect.[3] The nephrotoxic effect can be detected through increased renal parameter functions such as creatinine, uric acid, blood urea nitrogen (BUN), and kidney histopathological alteration.[4,5] The histopathological alteration is characterized by tubular necrosis, widening of the Bowman's space, tubular dilation, and glomerular atrophy.[6,7] The mechanism by which gentamicin induces tubular injury is through tubular epithelial cell necrosis and the alteration of the compound participating in transporting water and solutes.[8]

Gentamicin induces nephrotoxicity, possibly by oxidative stress generation such as reactive nitrogen species, hydroxyl radicals, superoxide anions, and hydrogen peroxide in the kidney, and by impairment of intracellular organelles.[9] Gentamicin induces renal damage associated with protein and lipid peroxidation, which can be measured by malondialdehyde (MDA) level.[8,10] Several studies reported that natural antioxidants were essential in nephroprotective activity in gentamicin-induced kidney damage.[5,11]

Sugarcane (*Saccharum officinarum* L.) has been shown to have antioxidant activity in its juice and leaves.[12-14] This plant is primarily cultivated for sucrose production,

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and its leaves are widely used by the community for animal feed and industrial material;[15-17] sugarcane leaves contain high secondary metabolites with vigorous antioxidant activity, such as phenolic compounds and flavonoids.[14] Sugarcane leaves have been proven to contain flavonoids, triterpenoids/steroids, alkaloids, polyphenols, and tannins.[18] Some flavonoids have appeared in a nephroprotective action against different nephrotoxic specialists, leading to intense or persistent kidney damage.[19] Sugarcane juice also exhibited nephroprotective activity by improving antioxidant defense and tissue integrity.[20] Luteolin, a flavonoid in sugarcane leaves, has been shown to have nephroprotective effects.[21] Based on the activity of sugarcane juice and its phytochemical content, sugarcane leaves have potential nephroprotective properties. Studies on the nephroprotective activity of sugarcane leaves on gentamicin toxicity have never been carried out.

SUBJECTS AND METHODS

Subjects

This study included 25 male Wistar rats (8–12 weeks old) weighing 150–200 g, placed into five equal groups of five rats each. The ethical committee approved the experiment's protocol (1195/UN25.8/KEPK/DL/2021).

Materials

Sugarcane leaves were taken in Banyuwangi, Indonesia. The chemicals used were ethanol, hydrochloric acid (HCl), xylol (Merck, German); h and e (Himedia, India); serum creatinine, BUN, and uric acid kits (Elitech, France); and gentamicin sulfate and normal saline from local supplier.

The plant extraction

Sugarcane leaf powder was macerated with 96% ethanol for 48 h; then, the extract was filtered to obtain the first filtrate. The residue was re-macerated for 24 h with the same solvent, yielding the second filtrate. The first and second filtrates were mixed using a rotary evaporator (40°C) to create a condensed sugarcane leaf extract (SLE).

Experimental design

The Wistar rats were separated into five groups (n = 5). Group I (normal group) received saline (1 mL/kg intraperitoneal). Group II (negative group) was given intraperitoneal (i. p) injections of gentamicin (80 mg/kg). Groups III, IV, and V were treated with 80 mg/kg intraperitoneal (i. p) injections of gentamicin and SLE orally at doses of 200, 400, and 600 mg/kg BW. The procedure was continued for 8 days, and on the 9th day, the rats were euthanized. Blood samples and kidneys from each group were taken for biochemical and histological analysis.

Kidney function parameters measurement

The creatinine, BUN, and uric acid serum levels were determined using blood samples and performed using a spectrophotometer per the manufacturer's specifications.

Malondialdehyde measurement

Blood serum and kidney homogenate were added with 1 N HCl and 1% Na-TBA (Sodium Thiobarbituric Acid), vortexed, then incubated for 30 min at 100°C. The precipitate was detached by centrifugation after cooling, and the absorbance was measured using an ultraviolet–visible spectrophotometer (532 nm).[22]

Macroscopic examination and measurement of kidney relative weight

The macroscopic examination was carried out by observing the color and surface texture of the kidneys. The relative kidney weight was computed using (absolute organ weight [g]) ÷ (final body weight of rat) ×100.[23]

Histopathological examination

The kidneys were preserved in 10% neutral-buffered formalin. Furthermore, the fixed tissues were dehydrated with alcohol series and cleared with xylol. Then, kidneys were embedded in paraffin, followed by sectioning and staining. The histology slides were observed with a microscope (Olympus DP21).

Analysis of statistic

Analysis of statistics was conducted using SPSS 20 (IBM, New York, USA) to differentiate between groups through one-way ANOVA, then analyzed with the least significant differences *post hoc*.

RESULTS

Kidney functional parameters

The rats from the negative groups showed high BUN, creatinine, and uric acid levels. Meanwhile, the animals treated with SLE showed lower levels of BUN [Figure 1], creatinine [Figure 2], and uric acid [Figure 3]. The result indicated that the extract had a protective effect on preventing kidney damage.

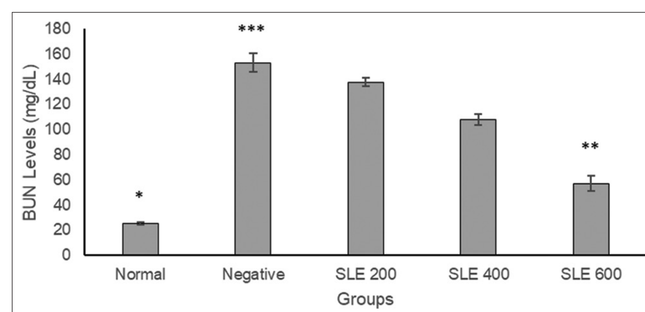

Figure 1: Sugarcane leaf extract's effect on blood urea nitrogen levels. SLE: Sugarcane leaf extract

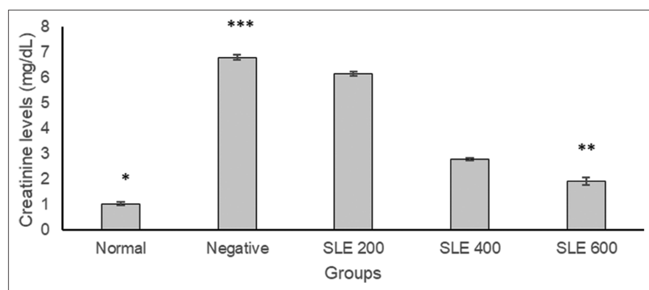

Figure 2: Sugarcane leaf extract's effect on creatinine levels. SLE: Sugarcane leaf extract

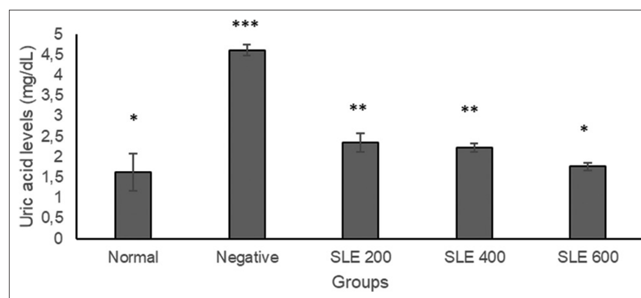

Figure 3: Sugarcane leaf extract's effect on uric acid levels. SLE: Sugarcane leaf extract

Malondialdehyde levels

MDA has been found to increase in patients with renal failure.[10,24,25] Based on the results, the group with the highest MDA levels in serum and kidney was the negative group [Table 1]. The group treated with sugarcane leaves ethanol extract doses of 400 and 600 mg/kg BW showed lower MDA levels, indicating extract activity as an antioxidant [Table 1].

Table 1: The malondialdehyde levels

Group	Serum MDA (nmol/mL) ± SD	Kidney MDA (nmol/mL) ± SD
Negative	15.31±0.19[a]	18.22±0.45[p]
Normal	3.73±0.41[b]	6.92±0.14[q]
SLE 200 mg/kg BW	14.71±0.40[c]	18.16±0.28[p]
SLE 400 mg/kg BW	8.32±1.05[d]	10.50±0.89[r]
SLE 600 mg/kg BW	4.60±0.14[e]	9.06±0.13[s]

The same superscript letter showed that the information differed between groups. MDA: Malondialdehyde, SLE: Sugarcane leaf extract, SD: Standard deviation

Morphology and histology analysis

Kidney weight

The absolute and relative kidney weight data correlated with the intensity of damage and histopathological alteration. Absolute kidney weight is a good indicator of kidney damage regardless of toxic substances' effect on body weight.[26] The results [Table 2] showed no significant difference in all groups ($P > 0.05$), suggesting that the gentamicin-induced renal changes were reversible on its discontinuation.[8]

Table 2: Kidney weight

Group	Kidney absolute weight (g) ± SD	Kidney relative weight (%) ± SD
Negative	1.32±0.11	0.85±0.06
Normal	1.15±0.09	0.71±0.08
SLE 200 mg/kg BW	1.29±0.27	0.81±0.13
SLE 400 mg/kg BW	1.25±0.10	0.78±0.09
SLE 200 mg/kg BW	1.15±0.22	0.77±0.06

SLE: Sugarcane leaf extract, SD: Standard deviation

Kidney morphology

The macroscopic observation [Figure 4] showed the kidney of the negative group appearing paler than the other groups, with dark spots on the surface indicating necrosis. The 600 mg/kg BB SLE dose showed red, almost resembling the normal group.

Kidney histology

Figure 5 shows the treatment effect on rats' kidney histology. The negative and sugarcane leaves extract at 200 mg/kg BW demonstrated tubular dilation and necrosis, widening the distance between the glomerulus, Bowman's capsule, and glomerular atrophy. The 600 mg/kg BW dose was the most effective in improving kidney histopathology by not experiencing tubular dilation, but some tubules still had necrosis.

DISCUSSION

The nephrotoxicity gentamicin mechanism is presumably through the formation of oxidative and nitrosative stress.[11,27] Gentamicin promotes reactive oxygen species (ROS) in mitochondria, thus initiating the intrinsic apoptosis pathway, disrupting the respiration chain, and lowering ATP synthesis, causing cell death.[8] This mechanism induces oxidative stress in tubular cells by reducing antioxidant enzymes (superoxide dismutase and catalase).[8,28] These pathways generate tubular damage and dysfunction by altering the function of the major cellular components involved in water and solute transportation, resulting in epithelial cell necrosis.[8] This nephrotoxicity mechanism is characterized by increased lipid peroxidation, protein denaturation, and DNA damage involving the iNOS/NFκB/p38MAPK pathway.[25,28] Nephrotoxicity due to gentamicin also involves an inflammatory response through increased cell infiltration, causing functional and structural kidney damage.[8] These damages are marked by elevated serum creatinine and BUN, reduced creatinine clearance rate, and histological changes.[29]

In this study, gentamicin induced nephrotoxicity by increasing BUN, serum creatinine, uric acid, and MDA levels, indicating kidney damage caused by oxidative stress. The pathophysiology of chronic renal failure has been discovered to coexist with free radical-induced lipid peroxidative tissue damage.[10] The result showed that

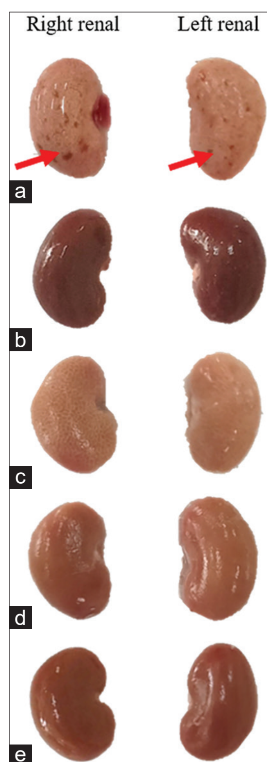

Figure 4: Morphology of rats' kidneys after gentamicin induction. (a) Negative group, necrosis (red arrow), (b) Normal group, (c) Sugarcane leaf extract (SLE) 200 mg/kg, (d) SLE 400 mg/kg, (e) SLE 600 mg/kg

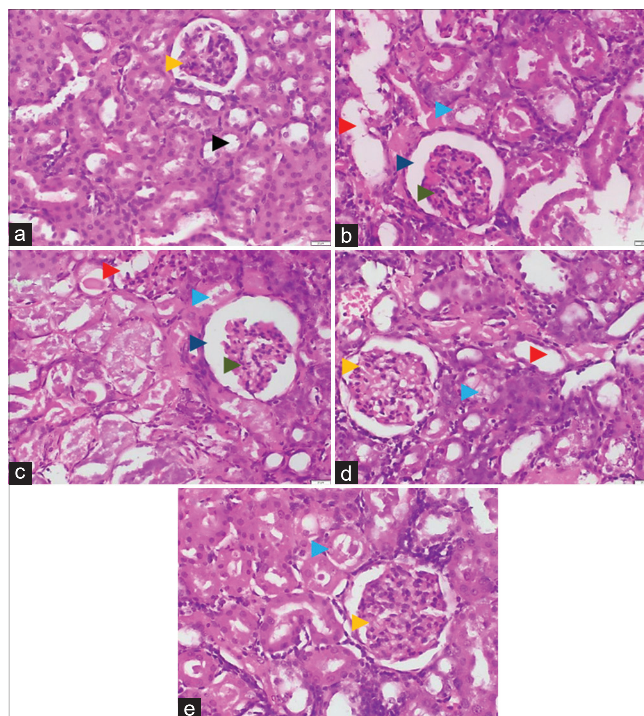

Figure 5: The rat's kidney histology. (a) Normal group, (b) Negative group, (c) Sugarcane leaf extract (SLE) 200 mg/kg, (d) SLE 400 mg/kg, (e) SLE 600 mg/kg, ►: Normal proximal tubule, ►: Normal glomerulus, ►: Tubular dilatation, ►: Tubular necrosis, ►: Widening of Bowman's space, ►: Glomerular atrophy

SLE had lower renal parameters and MDA levels than the negative group. The activity was possibly caused by the phytoconstituents in the extract that acted as antioxidants. According to a previous study, antioxidants and reactive oxygen scavenger agents protect gentamicin-induced nephrotoxicity.[25]

Sugarcane leaf phytochemicals include policosanol and phenolic-polyphenols compounds such as flavonoids.[30] The common mechanism of action for flavonoids as a nephroprotective agent is that they work as an antioxidant and anti-inflammation.[19] The antioxidant of flavonoids work by stabilizing reactive species such as ROS and directly scavenging free radicals including OH, $O2^-$, $^1O_2$, which leads to lipid peroxidation through hydrogen atoms or electron donation transfer in R, RO, and ROO.[5,19,31] Flavonoids inhibit the inflammatory mediators and prevent oxidative stress. Flavonoids also increased glutathione S-transferase antioxidant activity and ROS trapping in mitochondrial succinate oxidase, microsomal monooxygenase, Nicotinamide adenine dinucleotide (NADH) oxidase, and glutathione S-transferase.[32,33]

Luteolin is an essential compound with radical scavenging activity among the flavonoids present in sugarcane leaves.[34,35] Luteolin improved renal dysfunction and reduced oxidative stress, renal tubular cell damage, and apoptosis in nephrotoxic rats by regulating p53-dependent renal tubular apoptosis.[21,36] The nephroprotective of luteolin works by normalizing serum creatinine and BUN, improving oxidative stress by elevating the levels of glutathione, decreasing the levels of 3-nitrotyrosine and 4-hydroxynonenal in cisplatin-induced nephrotoxicity rats.[37] Luteolin also suppresses cytochrome P450-2E1 and reverses inflammation through nuclear factor-kappa B, tumor necrosis factor-alpha, and cyclooxygenase-2 downregulation.[38] Luteolin also normalized uric acid serum, BUN, and urine creatinine and improved histopathological status in albino rats with renal toxicity induced by gentamicin.[21] This compound exhibited a protective effect on the kidney through its anti-oxidative, anti-inflammatory properties, and interaction with the Nrf2/ARE/HO-1 pathway.[39] The results align with previous studies that linked the extract's hepatoprotective activity to its antioxidant activity.[40] This study has limitations, primarily since it was conducted over a short period, and there are no studies on chronic renal failure.

CONCLUSION

SLE had potential activity as a nephroprotective agent by normalizing BUN, creatinine, uric acid, and MDA levels and improving histopathological status, presumably due to its phytoconstituents that had antioxidant and anti-inflammatory effects.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. George B, You D, Joy MS, Aleksunes LM. Xenobiotic transporters and kidney injury. Adv Drug Deliv Rev 2017;116:73-91.
2. Al-Naimi MS, Rasheed HA, Hussien NR, Al-Kuraishy HM, Al-Gareeb AI. Nephrotoxicity: Role and significance of renal biomarkers in the early detection of acute renal injury. J Adv Pharm Technol Res 2019;10:95-9.
3. Morales-Alvarez MC. Nephrotoxicity of antimicrobials and antibiotics. Adv Chronic Kidney Dis 2020;27:31-7.
4. Abdelsameea AA, Mohamed AM, Amer MG, Attia SM. Cilostazol attenuates gentamicin-induced nephrotoxicity in rats. Exp Toxicol Pathol 2016;68:247-53.
5. Aiswarya N, Rashmi RR, Preethi SJ, Chandran V, Teerthanath S, Sunil P, *et al.* Nephroprotective effect of aqueous extract of pimpinella anisum in gentamicin induced nephrotoxicity in wistar rats. Pharmacogn J 2018;10:403-7.
6. Fauzi A, Titisari N, Sutarso, Mellisa V. Gentamicin nephrotoxicity in animal model: Study of kidney histopathology and physiological functions. IOP Conf Ser Earth Environ Sci 2020;465:1-8. [doi:10.18 8/1755-1315/465/1/012005].
7. Yarijani ZM, Najafi H, Hamid Madani S. Protective effect of crocin on gentamicin-induced nephrotoxicity in rats. Iran J Basic Med Sci 2016;19:337-43.
8. Randjelovic P, Veljkovic S, Stojiljkovic N, Sokolovic D, Ilic I. Gentamicin nephrotoxicity in animals: Current knowledge and future perspectives. EXCLI J 2017;16:388-99.
9. Jamshidzadeh A, Heidari R, Mohammadi-Samani S, Azarpira N, Najbi A, Jahani P, *et al*. A comparison between the nephrotoxic profile of gentamicin and gentamicin nanoparticles in mice. J Biochem Mol Toxicol 2015;29:57-62.
10. Sreenivasulu U, Shyam Prasad BR, Durga T. Study of serum malondialdehyde levels in chronic renal failure patients: A hospital based study in Govt. General hospital, Anantapuramu, Andhra Pradesh. Int J Clin Biochem Res 2020;7:138-41.
11. Elgebaly H, Mosa NM, Germoush M, Brahim AC. The nephroprotective effects of olive oil and bee honey against gentamicin-induced nephrotoxicity in rabbits. Aljouf Univ Med J 2016;3:7-13.
12. Abbas SR, Sabir SM, Ahmad SD, Boligon AA, Athayde ML. Phenolic profile, antioxidant potential and DNA damage protecting activity of sugarcane (*Saccharum officinarum*). Food Chem 2014;147:10-6.
13. Ali SE, El Gedaily RA, Mocan A, Farag MA, El-Seedi HR. Profiling metabolites and biological activities of sugarcane (*Saccharum officinarum* Linn.) Juice and its product molasses via a multiplex metabolomics approach. Molecules 2019;24:934.
14. Rao MJ, Ahmed U, Ahmed MH, Duan M, Wang J, Wang Y, *et al*. Comparison and quantification of metabolites and their antioxidant activities in young and mature leaves of sugarcane. ACS Food Sci Technol 2021;1:362-73.
15. Dewanti P, Widuri LI, Alfian FN, Addy HS, Okviandari P, Sugiharto B. Rapid propagation of virus-free sugarcane (*Saccharum officinarum*) by somatic embryogenesis. Agric Agric Sci Procedia 2016;9:456-61.
16. Anur RM, Mufithah N, Sawitri WD, Sakakibara H, Sugiharto B. Overexpression of sucrose phosphate synthase enhanced sucrose content and biomass production in transgenic sugarcane. Plants (Basel) 2020;9:200.
17. Sahu O. Assessment of sugarcane industry: Suitability for production, consumption, and utilization. Ann Agrar Sci 2018;16:389-95.
18. Dewi IP, Viadina ZA, Aldiana M, Fajrin, FA. Phytochemical screening, total flavonoid and lipid peroxidation inhibition of red sugar leaf ethanol extract. J Med Sci 2022;7:797-808.
19. Vargas F, Romecín P, García-Guillén AI, Wangesteen R, Vargas-Tendero P, Paredes MD, *et al.* Flavonoids in kidney health and disease. Front Physiol 2018;9:394.
20. Hussein AY, El-Shafey RS. The possible protective effects of *Saccharum officinarum* l. (Sugar cane) juice co – Supplementation on gentamicin induced acute renal toxicity in adult albino rats. Int J Pharmacol Toxicol 2019;7:29-34.
21. Kashyap S, Sao M, Sharma H, Yadav AP, Kori ML. Nephroprotective effect of luteolin against gentamicin-induced nephrotoxicity in albino rats. Res J Pharm Dos Forms Technol 2019;11:253.
22. Grotto D, Santa Maria LD, Boeira S, Valentini J, Charão MF, Moro AM, *et al.* Rapid quantification of malondialdehyde in plasma by high performance liquid chromatography-visible detection. J Pharm Biomed Anal 2007;43:619-24.
23. Famurewa AC, Akunna GG, Nwafor J, Chukwu OC, Ekeleme-Egedigwe CA, Oluniran JN. Nephroprotective activity of virgin coconut oil on diclofenac-induced oxidative nephrotoxicity is associated with antioxidant and anti-inflammatory effects in rats. Avicenna J Phytomed 2020;10:316-24.
24. Rusu CC, Racasan S, Kacso IM, Moldovan D, Potra A, Patiu IM, *et al.* Malondialdehyde can predict survival in hemodialysis patients. Clujul Med 2016;89:250-6.
25. Ozbek E. Induction of oxidative stress in kidney. Int J Nephrol 2012;2012:465897.
26. Craig EA, Yan Z, Zhao QJ. The relationship between chemical-induced kidney weight increases and kidney histopathology in rats. J Appl Toxicol 2015;35:729-36.
27. Bhide S, Bhojne S, Kanase H, Raut S, Dethe P. Nephroprotective effect of ethanolic extract of flax seed. J Clin Diagn Res 2019;13: FC01-FC05. [doi: 10.7860/jcdr/2019/41506.13025].
28. Mehan S, Dudi R, Kalra S. Renoprotective Effect of Corosolic Acid in Gentamicin-Induced Nephrotoxicity and Renal Dysfunction in Experimental Rats. Ann Pharmacol Pharm 2017; 2:1065.
29. Jaikumkao K, Pongchaidecha A, Thongnak LO, Wanchai K, Arjinajarn P, Chatsudthipong V, *et al.* Amelioration of renal inflammation, endoplasmic reticulum stress and apoptosis underlies the protective effect of low dosage of atorvastatin in gentamicin-induced nephrotoxicity. PLoS One 2016;11:e0164528.
30. Singh A, Lal UR, Mukhtar HM, Singh PS, Shah G, Dhawan RK. Phytochemical profile of sugarcane and its potential health aspects. Pharmacogn Rev 2015;9:45-54.
31. Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. J Nutr Sci 2016;5:e47.
32. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. ScientificWorldJournal 2013;2013:162750.
33. Dahal A, Mulukuri S. Flavonoids in kidney protection. World J Pharm Pharm Sci 2015;4:362-82.
34. Kumar V. Structural characterization of luteolin glycosides flavonoids from Indian plantation white sugar. Orient J Chem 2020;36:773-9.
35. Basist P, Parveen B, Zahiruddin S, Gautam G, Parveen R, Khan MA, *et al.* Potential nephroprotective phytochemicals: Mechanism and future prospects. J Ethnopharmacol 2022;283:114743.
36. Kang HG, Lee HK, Cho KB, Park SI. A review of natural products

for prevention of acute kidney injury. Medicina (Kaunas) 2021;57:1266.

37. Domitrović R, Cvijanović O, Pugel EP, Zagorac GB, Mahmutefendić H, Škoda M. Luteolin ameliorates cisplatin-induced nephrotoxicity in mice through inhibition of platinum accumulation, inflammation and apoptosis in the kidney. Toxicology 2013;310:115-23.
38. Domitrović R, Potočnjak I. A comprehensive overview of hepatoprotective natural compounds: Mechanism of action and clinical perspectives. Arch Toxicol 2016;90:39-79.
39. Alekhya Sita GJ, Gowthami M, Srikanth G, Krishna MM, Rama Sireesha K, Sajjarao M, *et al.* Protective role of luteolin against bisphenol a-induced renal toxicity through suppressing oxidative stress, inflammation, and upregulating Nrf2/ARE/HO-1 pathway. IUBMB Life 2019;71:1041-7.
40. Dewi IP, Kwintana RB, Ulinnuha JU, Rachman F, Christianty FM, Holidah D. Hepatoprotective effect of ethanolic extract of sugarcane (*Saccharum officinarum* Linn.) leaves. J Basic Clin Physiol Pharmacol 2021;32:533-40.