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Research Paper

Protective Effects of Premna serratifolia L. Leaf Extract Against Gentamicin-Induced Nephrotoxicity in ICR Mice

Josel D. Cruz[1], Glen S. Nolasco[1], John Dave A. Dicuangco[2*], Lourdes Fatima S. David[1]

[1]Mabalacat City College, Philippines
[2]Don Honorio Ventura State University, Philippines

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Abstract

Many therapeutic drugs, including aminoglycosides, are known to cause nephrotoxicity and impair kidney function. Gentamicin is a type of aminoglycoside commonly used to treat infection caused by gram-negative bacteria, but it can induce nephrotoxicity by accumulation in the proximal convoluted tubules, resulting in stress, degeneration, and injury to the kidney. Although traditional treatments are limited, natural products, such as in Premna serratifolia, have potential for ameliorative bioactivities. This study examined the nephroprotective effects of P. serratifolia ethanolic leaf extract (PSELE) against gentamicin-induced nephrotoxicity in male ICR mice. Twelve male ICR mice were divided into four groups: normal (T0), negative (T-), 0.3 ml/20 g b.w. PSELE-treated (T1), and 0.5 ml/20 g b.w. PSELE-treated (T2). The kidneys were then extracted, weighed, and subjected to histopathological examination. Results showed that PSELE counteracts gentamicin-induced nephrotoxicity by mitigating its effect on weight and kidney tissue alterations in PSELE-treated mice (T1 and T2), with comparable results to normal (T0). The protective effects of the extract were attributed to the phytochemicals that mitigated the oxidative stress of gentamicin. In conclusion, PSELE is a potential therapeutic agent for nephrotoxicity mitigation.

Keywords: *Gentamicin, Histopathological analysis, Kidney, Nephroprotection, Premna serratifolia*

INTRODUCTION

The kidneys are the main organs that filter all the waste materials inside the body. In addition to filtering waste products, it also plays a crucial role in maintaining homeostasis by regulating blood pressure, electrolyte balance, and the production of hormones and red blood cells (Raghavendra & Vidya, 2013). With its physiologic nature, the kidneys are prone to diseases leading to renal failure. When renal failure becomes severe and no longer treatable, the only ways to extend and prolong the person's life are via dialysis or transplantation (Strohmaier et al., 2022).

Medications are a frequent cause of kidney injury due to direct tubular injury, intratubular obstruction, and inflammation (Perazella & Rosner, 2022). Various therapeutic drugs can lead to nephrotoxicity, including aminoglycosides and NSAIDs, which rank first and second to the common cause of kidney toxicity, respectively (Ejaz et al., 2004). Gentamicin is a type of aminoglycoside antibiotic used to treat infections caused by gram-negative bacteria. However, it also induces nephrotoxicity because of the accumulation and retention of aminoglycosides in the proximal convoluted renal tubules (Acharya et al., 2013).

Herbal and natural remedies have gained popularity among the public and have sparked an interest in discovering natural products that can mitigate diseases. Therefore, various studies on plants have been conducted to assess nephroprotection in toxicity-induced by gentamicin (Al-Qarawi et al., 2008; Aiswarya et al., 2018). One potential plant is the *Premna serratifolia*, a common plant traditionally used to treat a variety of ailments. Simamora et al. (2020) demonstrated that

Corresponding author's email: jdadicuangco@dhvsu.edu.ph

water and ethanol extracts *of P. serratifolia contain* phenolic and flavonoid contents. In addition to its presence of phytochemicals, it possesses inhibitory potential in enzymes and antioxidant properties. Other studies have also shown its other bioactivities including antimicrobial, (Rajendran & Basha, 2010), tumor cell suppresion (Selvam et al., 2012), cytotoxic, and hepatoprotective (Vadivu et al., 2009). However, to date, no studies have been conducted to evaluate the protective effects of antidiabetic drugs against drug-induced kidney injury.

The current study primarily aimed to investigate the nephroprotective effects of *P. serratifolia* ethanolic leaf extract (PSELE) against gentamicin-induced renal toxicity in albino mice, which could potentially offer a therapeutic approach to alleviating the renal toxicity associated with gentamicin.

LITERATURE REVIEW

Botanical Description and Secondary Metabolites of *Premna serratifolia* L.

Premna serratifolia L (syn. *P. integrifolia*) belongs to the family Verbenaceae (Reddy et al., 2018), are widely distributed in tropical and subtropical regions. It is a multi-branched shrub or small tree that can grow up to 10 meters tall. The young pubescent branches become smooth when they reach maturity. Leaves are slightly hairy and have an oblong-shaped and crenated-margin with opposite arrangements. *P. serratofoilia* has inflorescent green or yellowish white flowers arranged in conical panicles with green calyx and yellowish corolla (Karmakar et al., 2011). *P. serratifolia* is also known as Agnimantha, and it is widely used in the medicine systems of Ayurveda, Siddha, and Unani (Mali, 2020). In the Philippines, it is also called Alagau-gubat or Mulawin-aso. Its leaves are also cooked or used for different dishes and as natural remedies.

The bioactivities of *P. serratifolia* have been proven to be significant, including antioxidant (Muthukumaran et al., 2013), antimicrobial (Rajendran & Basha, 2010), tumor cell suppresion (Selvam et al., 2012), antiarthritic (Rajendran & Suseela, 2008), cytotoxic, and hepatoprotective (Vadivu et al., 2009). These bioactivities can be accounted for in the presence of bioactive compounds. Phytochemical studies on *P. serratifolia* roots have revealed a similar diversity of secondary metabolites, including alkaloids, carbohydrates, amino acids, steroids, flavonoids, glycosides, tannins, and phenolic compounds, with methanol, chloroform, and aqueous extracts used as solvents (Uppin & Naik, 2017; Mali & Bhadane, 2010). Further sophisticated analysis was conducted by Rency et al. (2015), in which gas chromatography-mass spectrometry (GC-MS) revealed presence of twenty-two (22) bioactive compounds in the ethanolic extract. Singh et al. (2011) reported a total of twenty-nine (29) bioactive compounds in the leaves and root extracts of *P serratifolia*. These findings suggest that *P. serratifolia* is a potential source of pharmacologically active compounds.

Gentamicin as an inducer of nephrotoxicity

Aminoglycoside antibiotics are widely used to treat Gram-negative bacterial infections (Eljaaly et al., 2019; Gad et al., 2011; Thy et al., 2023). However, their clinical efficacy is often limited by nephrotoxic and ototoxic side effects. Aminoglycosides act by binding to the bacterial 30S ribosomal subunit (Długosz & Trylska, 2009; Kaul & Barbieri, 2005), causing misreading of tRNA and inhibiting protein synthesis, which is essential for bacterial growth (Pai et al., 2012).

Gentamicin, a well-known aminoglycoside antibiotic produced by Micromonospura purpurea, has broad-spectrum activity and is effective against various susceptible infections (Azab et al., 2014). This antibiotic is heat-stable and remains active even after autoclaving, making it suitable for microbiological media preparation (Qadir et al., 2011). However, numerous studies have reported that gentamicin can induce nephrotoxicity, affecting approximately 15-30% of treated patients. Clinically, gentamicin-induced nephrotoxicity presents as non-oliguric renal failure, with gradual increases in serum creatinine levels and hypoosmolar urine output emerging after several

days of treatment (Kore et al., 2011).

Gentamicin nephrotoxicity is associated with the generation of reactive oxygen species (ROS) and the formation of iron-drug complexes, which contribute to renal damage. In cases of gentamicin-induced nephrotoxicity, blood urea nitrogen (BUN) and serum creatinine levels are reported to rise significantly (Qadir et al., 2011). Gentamicin, filtered through the glomeruli into tubular urine, binds to anionic phospholipids (e.g., phosphatidylinositol, phosphatidylserine) in the brush border membrane of proximal tubular cells. It is then actively reabsorbed via pinocytosis and processed by lysosomes, where it induces phospholipidosis. The drug enters tubular cells through absorptive-mediated endocytosis after binding to acidic phospholipids and megalin, a large endocytic receptor on the apical membrane of proximal tubules essential for aminoglycoside uptake (Moestrup & Verroust, 2001). This mechanism concentrates gentamicin in lysosomes (Kore et al., 2011), where it disrupts lysosomal and other organellar membranes, including the mitochondria and Golgi apparatus. The subsequent lysosomal rupture releases high concentrations of gentamicin and hydrolytic enzymes into the cytoplasm, impairing intracellular processes such as mitochondrial respiration, electron transport, and microsomal protein synthesis (Qadir et al., 2011).

Histopathological alterations of the kidney

Ozbek (2012) noted that oxidative stress plays a critical role in kidney pathophysiology, induced by various stressors such as drugs, alcohol, and diseases. High oxygen levels can disrupt the balance between reactive oxygen species (ROS) and antioxidant defenses, leading to oxidative stress. ROS can cause direct injury to proteins, lipids, and amino acids through multiple pathways, ultimately resulting in kidney cell damage and death (Wang & Xu, 2003).

According to Qadir et al. (2011), the accumulation of gentamicin in the renal cortex due to its reabsorption in the proximal convoluted tubules results in epithelial cell degeneration and necrosis. This damage is largely attributed to lipid peroxidation and oxidative injury (Qadir et al., 2011; Virani et al., 2016; Pai et al., 2012). Gentamicin enters proximal tubular cells by interacting with anionic phospholipids in cell membranes. This interaction releases iron from the renal cortical mitochondria, forming iron-drug complexes that catalyze free radical formation. These ROS attack DNA and induce renal damage by causing mesangial cell contraction and reducing the glomerular filtration surface area, leading to a decreased glomerular filtration rate (GFR). Gentamicin treatment also leads to significant oxidative accumulation in the kidney and inhibits protein synthesis and DNA replication.

Histopathological analyses by Virani et al. (2016) revealed that gentamicin treatment results in extensive tubular necrosis, a hallmark of gentamicin-induced nephrotoxicity. Reddy et al. (2012) observed pronounced proximal tubular necrosis and epithelial loss in gentamicin-treated samples. Microscopic examination of the affected kidney sections revealed hyaline casts, glomerular congestion, mononuclear cell infiltration, tubular necrosis and degeneration, and intertubular hemorrhage. These findings highlight the severe renal damage associated with gentamicin, emphasizing the role of oxidative injury in its nephrotoxic effects.

RESEARCH METHOD

This study used a Complete Randomized Design (CRD) to evaluate the protective effects of *P. serratifolia* ethanolic leaf extract (PSELE) against gentamicin-induced nephrotoxicity in male albino mice. Key outcomes included kidney weight and histological changes resulting from gentamicin toxicity. A mixed-methods approach was used to comprehensively validate the effectiveness of PSELE as a nephroprotective agent.

Procurement and Authentication of the Plant

The mature leaves of *P. serratifolia* were collected at 12979 Duhat Street, Dau Homesite, Mabalacat City, Pampanga, Philippines. Plant samples were sent to Jose Vera Santos Memorial Herbarium, Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City, Philippines, for authentication. Plant sample was identified and classified as *Premna serratifolia.* Certification and herbarium specimens were kept at the Biology Laboratory, Mabalacat City College, Mabalacat City, Pampanga, Philippines, for further use and documentation (Nolasco et al., 2024).

Experimental Animals

This study used a total of twelve (12) male ICR mice were used to evaluate the protective effects of PSELE against gentamicin-induced nephrotoxicity in male albino mice. The animal subjects were obtained from the University of the Philippines, Diliman, Quezon City, Philippines with ages ranging to 8-12 weeks old. Animals were subjected to acclimatization prior to conduction. Animals were kept at the Biology Laboratory, Mabalacat City College, Mabalacat City, Pampanga, Philippines until the last day of administration. The protocols and ethical considerations were adhered from the Handling Methods of Laboratory Mice and Rats, 1st edition, and Methods of Philippine Association for Laboratory Animal Sciences (PALAS) (Nolasco et al., 2023; Yamauchi et al., 2023).

Preparation of Ethanol Leaf Extract

A total of two (2) kilos of mature leaves were used in this study. The leaves were air-dried for 7 days at room temperature and homogenized by electric mixing. Homogenized samples were soaked in 90% technical grade ethanol for 48 h. The solution was filtered to separate the liquid using filter paper. The solution was then sent to the Angeles University Foundation, Angeles City, Pampanga, Philippines for rotary evaporation. Semisolid sample was lyophilized until the end of the administration period (Nolasco et al., 2023; Yamauchi et al., 2023). A 100% concentration with varying dosages of 0.3ml/20g b.w. and 0.5ml/20g b.w. of mice were used in this study to validate the protective effects of PSELE.

Preparation of chemicals

Gentamicin was purchased from Gonzales Medical Network Enterprises, Angeles City, Pampanga, Philippines. A dose of 80 mg/kg/day was diluted in 1 mL of distilled water and administered intraperitoneally, following the protocol outlined by Prior et al. (2005). This treatment was applied for eighteen (18) days to induce nephrotoxicity, as previously reported (Elewa, 2016).

Treatment Groups

The administration of chemicals was conducted in the morning for 18 days to induce kidney toxicity. All mice were weighed before administration to obtain an accurate dosage of the chemicals. A total of four (4) groups with three (3) replications were established: T0 (normal control), T- (negative control), T1 (0.3 ml/20g PSELE), and T2 (0.5 ml/20g PSELE). The description for each of the treatments is given below.

Table 1. Description of treatment groups.

Treatment groups		Description
Treatment symbol	**Treatment name**	
T0	Normal control	Male ICR mice were treated with distilled water alone (1 ml/20 g per body weight) via intragavage.
T-	Negative control	Male ICR mice were treated with gentamicin along (0.04 ml 20 g per body weight)
T1	0.3 ml/20 g PSELE	Male ICR mice received leaf extract (0.3 ml/20 g per body weight) via intragavage, after which 1-h gentamicin was introduced via intraperitoneal technique (0.04 ml/20 g per body weight)
T2	0.5 ml/20 g PSELE	Male ICR mice received leaf extract (0.5 ml/20 g per body weight) via intragavage, after which 1-h gentamicin was introduced via intraperitoneal technique (0.04 ml/20 g per body weight)

Right kidney extraction

On the 19th day, kidney extraction was performed at Biology Laboratory Mabalacat City College, Mabalacat City, Pampanga, Philippines. Mice were euthanized via cervical dislocation. All specimens, including the left and right kidneys, were weighed before fixation. The right kidneys were extracted and immediately fixed using 10% formaldehyde. Fixed specimens were sent to the High-Precision Diagnostic Center, Angeles City, Pampanga, Philippines for histopathological preparation. Specimens were stained with H&E. A longitudinal section was created to increase the surface area of the observable alterations in the specimen.

Histopathological Analysis

Twelve (12) right kidney slides were examined under LPO (100x) and HPO (400x). Aberrations, such as hyaline cast, glomerular congestion, mononuclear cell infiltration, tubular necrosis and degeneration, and intertubular hemorrhage, were noted. Kidney tissues in the study were classified into levels: no damage, minimal damage, mild damage, moderate damage, and severe damage. Each category corresponds to varying degrees of cellular and tissue injury.

Statistical Analysis

Quantitative data obtained from the study are expressed as mean±standard deviation (SD). Data were analyzed using One-Way Analysis of Variance (ANOVA) followed by post hoc test of Tukey`s multiple comparison test having <0.05 significant level. Statistical analyses were conducted using the IBM Statistical Package for the Social Sciences (SPSS) v22.

FINDINGS AND DISCUSSION

Kidney Weight

Figure 1 shows a comparison of the kidney weights of mice treated with different treatments. Treatment under T2 had the highest mean kidney weight of 0.2270g, whereas T- showed the lowest mean kidney weight of 0.1392 g. The One-Way ANOVA generated a P-value of 0.0039, indicating a statistically significant effect of the treatments on kidney weight variance in male ICR mice. The post hoc test showed that comparisons between T0 and T+, as well as between T+ and T1, were statistically significant (*). Furthermore, the comparison between T+ and T2 was highly statistically significant (**). These findings suggest that PSELE helps maintain kidney weight during the

induction of toxicity.

These findings support the adverse effects of prolonged gentamicin exposure in mice kidneys. According to Aldahmash et al. (2016) and Hayward et al. (2018), gentamicin treatment promotes renal degeneration through the upregulation of oxidants in renal tissue, leading to cell death. This finding is consistent with Udupa and Prakash (2019), which reported significant reductions in kidney weight in mice treated with gentamicin. In contrast, mice treated with both high and low doses of PSELE showed maintained kidney weights, which were congruent to the normal control group (T0), highlighting PSELE's protective effects against gentamicin-induced oxidative stress. Secondary metabolites, such as flavonoids, in PSELE are likely responsible for its nephroprotective activity because they downregulate the oxidative agents generated by gentamicin.

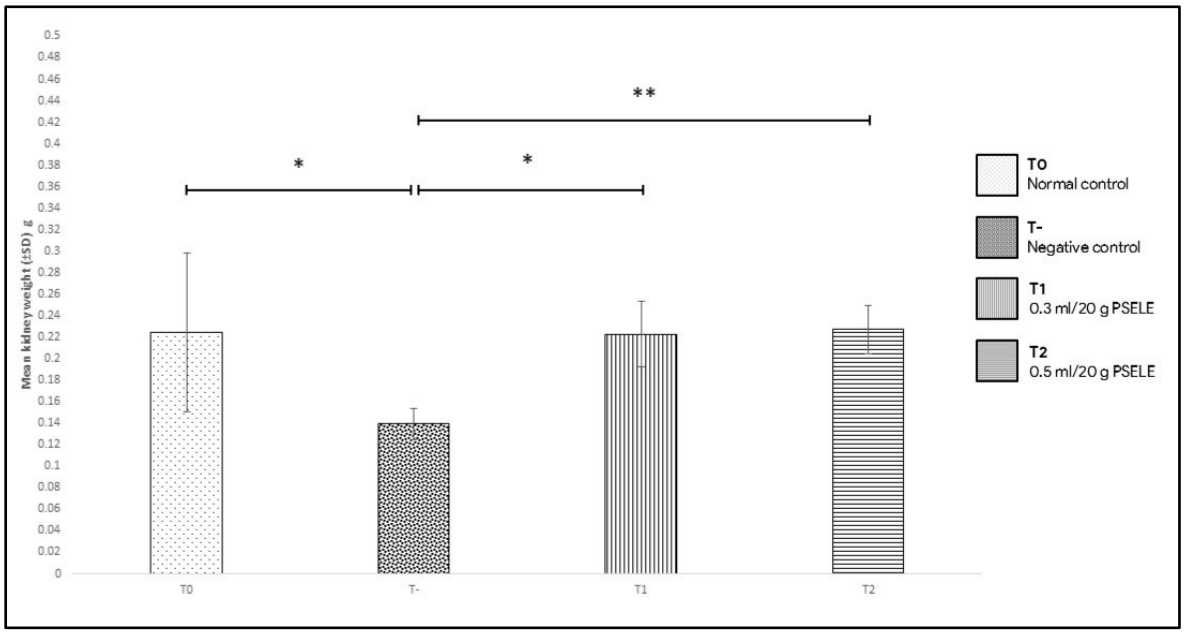

Figure 1. Bar graph of mean kidney weight (±SD) showing significant differences among the treatments.

** - significant (p<0.05); ** - high significant (p<0.01)*

Histopathological examination of the kidneys

Figure 2 presents the histopathological examination results of the right kidneys of selected ICR rats, which were assigned to various treatment groups. The examination highlights the microscopic structural changes in renal tissues. Figure 2A-2B illustrates the preserved histology of the T0 group, showing an intact glomerulus and well-structured renal tubules with no observable alterations. In contrast, mice treated with gentamicin exhibited notable histological changes in kidney structure. Microscopic examination revealed degeneration in both the glomerular and tubular regions, with evident necrosis in gentamicin-treated specimens. Additionally, congestion was observed in the T- group, which likely exacerbated kidney damage. Gentamicin-induced oxidative stress activates inflammatory cells within the kidney, promoting leukocyte infiltration, as shown in Figure 2C-2D (Aghadavod et al., 2016). This finding is consistent with those of Yarijani et al. (2016) and Aldahmash et al. (2016), who reported glomerular atrophy, congestion, necrosis, leukocyte infiltration, and hemorrhage in gentamicin-treated mice. These pathological changes are attributed to free radicals generated by gentamicin, including superoxide, hydroxide, and hydrogen peroxide. Upon gentamicin exposure, the upregulation of these oxidant triggers lipid peroxidation and other oxidative activities, leading to significant kidney tissue damage.

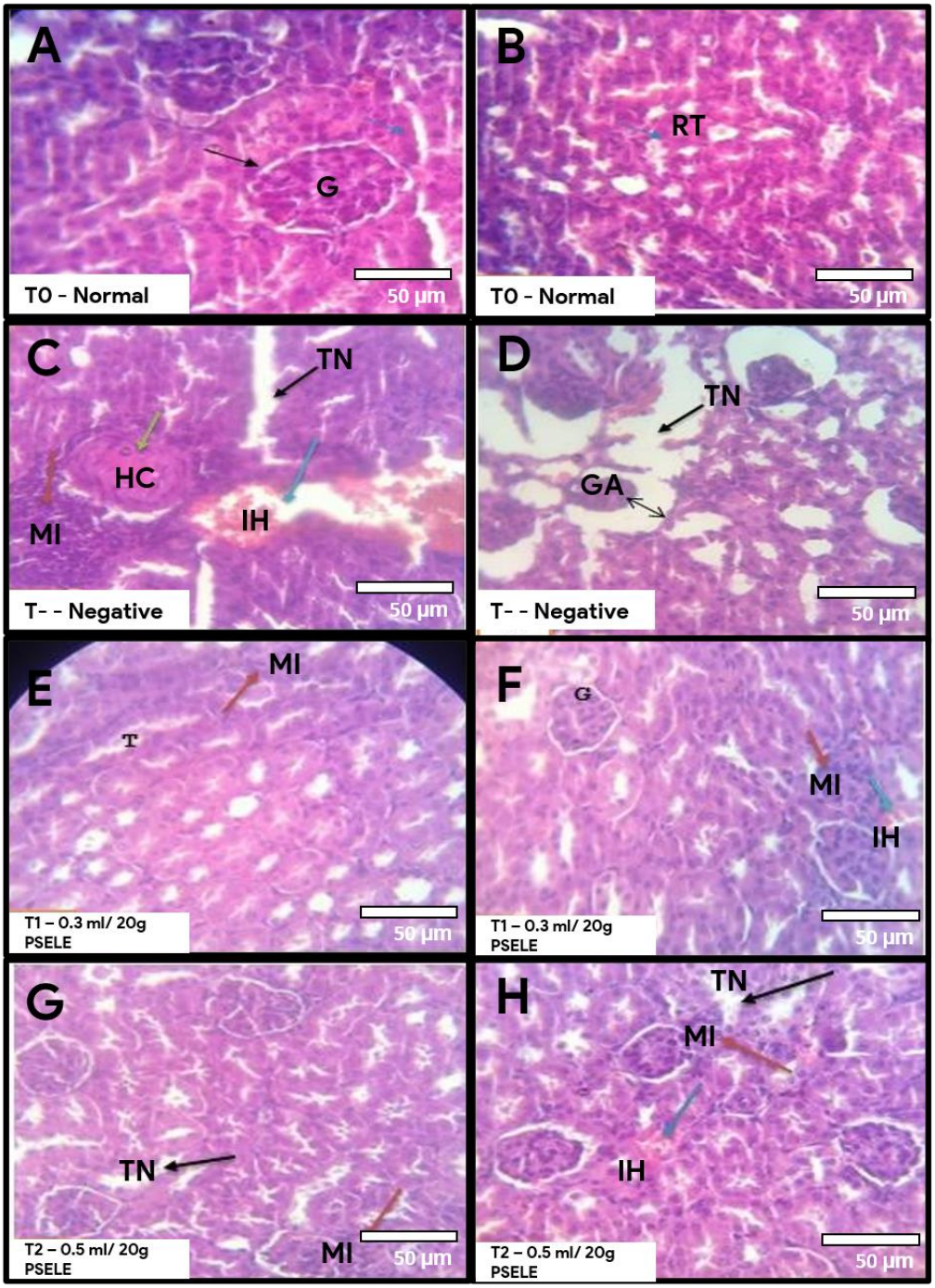

Figure 2. Histopathological observations of kidneys in male ICR mice across all treatments (A-B) T0; (C-D) T-; (E-F) T1; (G-H) T2 under 400x magnification.
G, glomerulus; RT, renal tubules; HC, hyaline cast; GC, glomerular congestion; MI, mononuclear infiltration; TN, tubular hemorrhage; GA, glomerular atrophy or degeneration; IH, intertubular hemorrhage

In mice treated with 0.3 mL/20g b.w. of PSELE, histological examination revealed well-preserved glomeruli, renal tubules, and intact renal cells (Fig. 2E & 2F). In contrast, mice treated with a higher dosage of 0.5 mL/20g b.w. of PSELE exhibited mild degeneration in both the glomerular and tubular linings. A comparison of the histological observations between the two

treatment groups (T1 and T2) showed that T1 exhibited a significant improvement in histoarchitecture compared with T2. Specifically, T1 maintained normal glomeruli and renal tubules, while T2 displayed mild structural alterations. Overall, the kidney tissues from the PSELE-treated mice groups (both T1 and T2) exhibited minimal damage, resembling the condition of the control group (T0), which displayed no damage to minimal damage. These differences can be attributed to the cytotoxic effects associated with higher plant extract dosages, as noted by Biradi and Hullatti (2015). Moreover, the increased effectiveness of lower doses may be due to the complex pharmacodynamics of plant-based extracts, where higher concentrations potentially lead to saturation of receptors or induce mild toxicity. Thus, the potency of the extract was most pronounced in the T1 group.

Vadivu et al. (2009) also demonstrated that PSELE has hepatoprotective potential, suggesting its histoprotective ability. The nephroprotective effects of PSELE may be linked to its secondary metabolites, which include saponins and flavonoids (Lubaina et al., 2016; Yee & Sotanaphun, 2022). Research by Ma et al. (2017) and Vargas et al. (2018) highlighted the crucial role of these metabolites in preserving kidney function and neutralizing the adverse effects of gentamicin. Specifically, Ma et al. (2017) demonstrated that these secondary metabolites exert nephroprotective effects against oxidative stress induced by foreign agents. Therefore, the presence of saponins and flavonoids in PSELE may contribute to its protective effect against gentamicin-induced nephrotoxicity.

CONCLUSION

PSELE possesses nephroprotective effects against gentamicin-induced kidney damage in mice, as supported by gross anatomical weight and histopathological results. Notably, significant differences in kidney weight and comparable results between PSELE-treated mice (T1 and T2) and normal mice (T0) suggest that PSELE had a significant impact on maintaining kidney health and preventing weight loss associated with gentamicin exposure.

Histopathological examination further supported these findings. Kidney tissue from the T1 and T2 groups exhibited minimal to no damage, with structures largely preserved, similar to those in the T0 group. In contrast, gentamicin-treated (T-) displayed moderate to severe renal injury, confirming the toxic effects of gentamicin on kidney tissue. These protective effects of PSELE can be accounted for in the presence of secondary metabolites, such as flavonoids and saponins, which neutralize the oxidative stress and cellular damage caused by gentamicin. Interestingly, the lower dose (T1), at 0.3 ml/20g b.w., also exhibited more pronounced nephroprotection than the higher dose (T2), at 0.5 ml/20 g b.w. These findings imply that PSELE can be used as a therapeutic agent for nephrotoxicity but requires optimal dosing to maximize nephroprotection ability.

LIMITATION & FURTHER RESEARCH

This study will include several key objectives to thoroughly investigate *P. serratifolia*. First, molecular identification will be conducted to verify accurate systematics of the plant species. Second, various solvents, such as butanol, methanol, ether, acetone, chloroform, and water, will be used for the extraction of phytochemicals to obtain a substantial quantity of secondary metabolites. Additionally, other morphological structures of *Premna serratifolia*, including bark, stem, roots, flowers, and fruits, will be assessed for phytochemical extraction. The phytochemicals harvested in the Philippines will be screened in relation to light intensity, followed by the determination and isolation of active phytochemicals to yield pure extracts. Different cell-line assays will be used to obtain comprehensive results regarding the extract efficacy. The effects of pure phytochemical extracts on other mouse tissues will be assessed, along with the use of alternative vehicles as treatments. Blood tests will measure enzymes such as serum creatinine and blood urea nitrogen

(BUN) to assess kidney damage, while grading protocols will be applied to evaluate the changes in kidney tissue accurately. A mitochondrial oxidative stress analysis will be performed on mice treated with pure extracts to investigate the molecular effects on mitochondria. Finally, following extensive studies, clinical trials will be conducted to evaluate the effectiveness of *Premna serratifolia* leaf extract against nephrotoxicity in humans.

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