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Reno-Protective Properties of *Azolla pinnata* Extract Against Gentamicin-Induced Kidney Damage Are Attributed to Its Antioxidant Effects

Mohammad Ghanbari Alamoti[1], Farideh Jalali Mashayekhi[2], Naser Hosseini[3], Houshang Najafi[4] Saeed Changizi-Ashtiyani[5*]

[1]Department of Physiology, School of Medicine, Arak University of Medical Sciences, Arak, Iran;
[2]Department of Biochemistry and Genetics, School of Medicine, Arak University of Medical Sciences, Arak, Iran;
[3]Department of Medicinal Plants, Faculty of Agriculture and Natural Resources, Arak University, Arak, Iran;
[4]Department of Physiology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran;
[5]Department of Physiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background: Gentamicin, a powerful aminoglycoside antibiotic, is limited in clinical use due to dose-related kidney toxicity, mainly caused by oxidative stress. *A. pinnata*, an antioxidant-rich aquatic fern, has not been extensively studied for renoprotection against GM-induced kidney damage. This research assessed the protective effects of a hydroalcoholic extract of *A. pinnata* on GM nephrotoxicity.

Methods: Forty male Wistar rats were divided into five groups (n = 8): control, sham, GM (100 mg/kg/day, i.p.), and GM plus *A. pinnata* extract (10 or 20 mg/kg/day, orally). After seven days, renal function markers (serum creatinine and urea), oxidative stress parameters (MDA, FRAP, CAT, and GPX), TNF-α, and renal histopathology were assessed.

Results: GM significantly damaged kidney function and induced oxidative stress, as shown by increased levels of creatinine, urea, and MDA, along with reduced FRAP and CAT activity ($p < 0.05$). Co-treatment with *A. pinnata* extract, especially at 20 mg/kg, significantly lessened these effects by restoring kidney function markers, boosting antioxidant defenses, and lowering lipid peroxidation. The extract did not have a significant impact on either GPX activity or TNF-α levels. Histopathological analysis revealed that GM-induced tubular necrosis and glomerular damage were significantly ameliorated by *A. pinnata* in a dose-dependent manner.

Conclusion: *A. pinnata* extract offers notable protection against kidney damage caused by gentamicin, mainly by enhancing the body's natural antioxidant defenses, decreasing lipid peroxidation, and maintaining the normal structure of kidney tissue. These findings suggest that *A. pinnata* could serve as a valuable complementary treatment to improve the safety of GM use. ***DOI: 10.61186/ibj.5194***

Keywords: Gentamicins, Kidney failure, Oxidative stress

Corresponding Author: Saeed Changizi-Ashtiyani
Department of Physiology, Iran University of Medical Sciences, Tehran, Iran; P.O.Box: 1449614535. E-mail: ashtiyani@yahoo.com;
TEL./FAX number: (+98-21) 86704546; ORCID ID: 0000-0001-9781-5553

List of Abbreviations:
***A. pinnata*:** *Azolla pinnata*; **BW:** body weight; **CAT**: catalase; **DPPH**: 1,1-Diphenyl-2-picrylhydrazyl; **ELISA:** enzyme-linked immunosorbent assay; **FRAP:** ferric reducing antioxidant power; **GM:** gentamicin; **GPX:** glutathione peroxidase; **H&E:** hematoxylin and eosin; **i.p.:** intraperitoneal; **MDA:** malondialdehyde; **SD:** standard deviation; **TNF-α:** tumor necrosis factor-alpha

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INTRODUCTION

Based on recent epidemiological studies, drug-induced nephrotoxicity is a significant clinical concern, responsible for approximately 20% of acute kidney injury cases in hospitalized adults, as demonstrated in a 2024 cohort study of 1,398 patients[1]. Gentamicin (GM), a highly effective aminoglycoside antibiotic against Gram-negative infections, is notoriously limited by its dose-dependent nephrotoxic potential[2,3]. This toxicity primarily manifests as damage to renal tubular cells, involving both necrotic and apoptotic pathways[3,4].

The search for natural compounds to mitigate drug-induced toxicity is a growing field. Natural products have been used in traditional medicine for a long time, with their effectiveness frequently attributed to bioactive components such as polyphenols and flavonoids with strong antioxidant effects[5-7]. *A. pinnata*, a tiny, free-floating water fern, contains high levels of these types of compounds. It is not only nutrient-dense but also contains antioxidants capable of scavenging nitric oxide, superoxide, and DPPH radicals[8-10].

While numerous plants have been investigated for nephroprotection, *A. pinnata* presents a unique profile due to its high biomass yield, nutritional value, and hepatoprotective and antioxidant effects in other models[10-12]. However, its specific influence on antioxidant enzyme activity and histological outcomes in gentamicin-induced nephrotoxicity remains poorly characterized. This study was therefore designed to evaluate the protective effects of a hydroalcoholic extract of *A. pinnata* on renal function, oxidative stress status, and histological damage induced by GM in rats. This study assessed the protective effects of a hydroalcoholic extract from *A. pinnata* on kidney function, oxidative stress levels, and tissue damage caused by GM in rats.

MATERIALS AND METHODS

Plant collection and extract preparation

Fresh aerial parts of *A. pinnata* were gathered from Anzali Lagoon, Iran (GPS coordinates: 37°27'21.07"N, 49°21'48.27"E) during Autumn 2023. A botanist at Shahid Beheshti University verified the plant species, and a voucher specimen (SBUH-2025-901) was archived. The plant material was dried in the shade, ground into powder, and extracted by cold maceration using 70% ethanol (v/v). Specifically, 50 g of the powder was soaked in 300 mL of solvent and stirred at 100 rpm and 37 °C for 72 hours. The residue was then re-extracted with 150 mL of fresh solvent for an additional 24 hours. The combined extracts were filtered, concentrated under reduced pressure, and dried, resulting in a crude solid extract yield of 7.5% (w/w). For administration, the extract was dissolved in DMSO, diluted with normal saline to keep the final DMSO concentration below 1%, stored at 4 °C, and freshly prepared every 72 hours[11].

Experimental design and animal groups

Forty male Wistar rats weighing approximately 200 ± 50 g were randomly assigned into five groups (n = 8 each): (1) control group: received no treatment; (2) sham: received vehicle (DMSO-saline orally, normal saline i.p.); (3) GM: received GM (100 mg/kg BW/day, i.p.) for seven days[4]; (4) GM + AP10: received GM plus *A. pinnata* extract (10 mg/kg BW/day, orally) for seven days[11]; (5) GM + AP20: received GM plus *A. pinnata* extract (20 mg/kg BW/day, orally) for seven days. Male rats were chosen to homogenize the experimental group by eliminating variables associated with the estrous cycle.

Biochemical and histological assessments

After a period of seven days, blood samples and kidney tissues were collected from the anesthetized subjects. Serum levels of creatinine and BUN were measured. Kidney tissues were evaluated for levels of MDA, CAT, GPX, FRAP, and TNF-α. For histological examination, kidney sections were stained with H&E and damage was scored on a scale from 0 to 4 by an observer blinded to the groups.

Statistical analysis

Data were expressed as mean ± SD. Graphs were created using GraphPad Prism software. The assumptions of normality and equal variances were verified using the Shapiro-Wilk and Levene's tests, respectively. Statistical differences were assessed using one-way analysis of variance, followed by Duncan's post hoc test, performed with SPSS version 16. A $p < 0.05$ was considered statistically significant.

RESULTS

Renal function

GM administration significantly increased serum urea and creatinine levels compared to the sham group ($p < 0.05$). Co-treatment with *A. pinnata* extract dose-dependently reduced these elevations, especially at 20 mg/kg, causing a significant reduction compared to the GM-only group ($p < 0.05$), though levels remained above the sham group (Fig. 1).

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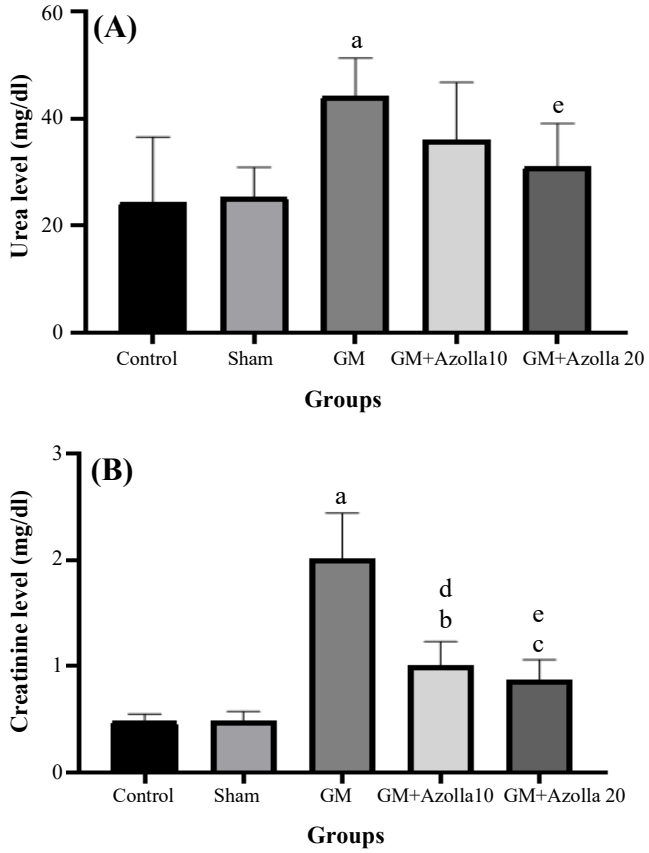

Fig. 1. Serum (A) urea and (B) creatinine levels in the sham, GM, GM+ Azolla 10, and GM + Azolla 20 groups. Values are mean ± SD ($p < 0.05$). [a]GM vs. Sham, [b]GM + Azolla 10 vs. Sham, [c]GM + Azolla 20 vs. Sham, [d]GM vs. GM + Azolla 10, [e]GM vs. GM + Azolla 20.

Oxidative stress indicators

Oxidative stress caused by GM was demonstrated by a notable rise in kidney MDA levels and a reduction in FRAP and CAT activities ($p < 0.05$). Administering *A. pinnata* extract, particularly at a dose of 20 mg/kg BW, significantly counteracted these effects by decreasing MDA levels and restoring FRAP and CAT activities ($p < 0.05$ compared to the GM group). Neither GM treatment nor co-administration with *A. pinnata* significantly affected GPX activity (Fig. 2).

Inflammation

As depicted in Figure 3, there were no significant differences in renal TNF-α levels among the sham, GM, and GM plus *A. pinnata* extract groups ($p > 0.05$).

Renal histopathology

Histological examination revealed normal architecture in the control and sham groups. The GM group exhibited severe damage, including tubular necrosis, epithelial desquamation, intratubular casts, and glomerular damage. Co-treatment with *A. pinnata* extract markedly reduced the severity of these lesions in a dose-dependent manner, with the GM + AP20 group showing only mild, focal damage (Fig. 4).

DISCUSSION

This research offers strong proof that the hydroalcoholic extract of *A. pinnata* offers considerable protection against kidney damage caused by GM, mainly by reducing oxidative stress. Our results support the widely recognized nephrotoxic effects of GM, which include impaired kidney function and severe oxidative injury[4,13,14]. The novelty of our work lies in the detailed demonstration of *A. capacity* to counteract these effects by specifically modulating key components of the renal antioxidant system.

The most striking effect was observed on CAT activity and the non-enzymatic antioxidant capacity FRAP, both of which were depleted by GM and robustly restored by co-treatment with *A. pinnata*. Catalase is an essential primary defense enzyme that breaks down hydrogen peroxide, a major reactive oxygen species produced during GM toxicity[15]. The recovery of catalase activity indicates that *A. pinnata* extract may either directly shield the enzyme from GM-induced damage or enhance its expression, a mechanism that has been supported by research on other flavonoid-rich extracts[16].

The parallel increase in FRAP indicates an enhancement of the overall reducing capacity of the renal tissue, likely due to the direct electron-donating ability of the phenolic and flavonoid constituents of the extracts, such as those previously identified in *A. pinnata*[17-19]. This combined approach effectively reduced lipid peroxidation, as shown by the marked decrease in MDA levels, a key factor in GM-induced cellular damage[3]. Notably, the extract did not affect GPX activity. This selective impact on the antioxidant system is significant, indicating that the renoprotective effect of *A. pinnata* is not a broad, non-specific activation of all antioxidant enzymes but may specifically target pathways related to H_2O_2 metabolism through CAT and direct free radical scavenging via its phytochemicals. The GPX system, which depends on GSH availability, might be less influenced in this particular short-term model or may require a different phytochemical stimulus. This observation is consistent with research on Azolla's response to salinity stress, which also demonstrated varied regulation of antioxidant enzymes[20]. Furthermore, unlike other nephroprotective agents such as saffron extract[21,22], *A. pinnata* did not significantly reduce renal TNF-α levels in our study. This behavior indicates that its primary protective mechanism in this model is antioxidant rather

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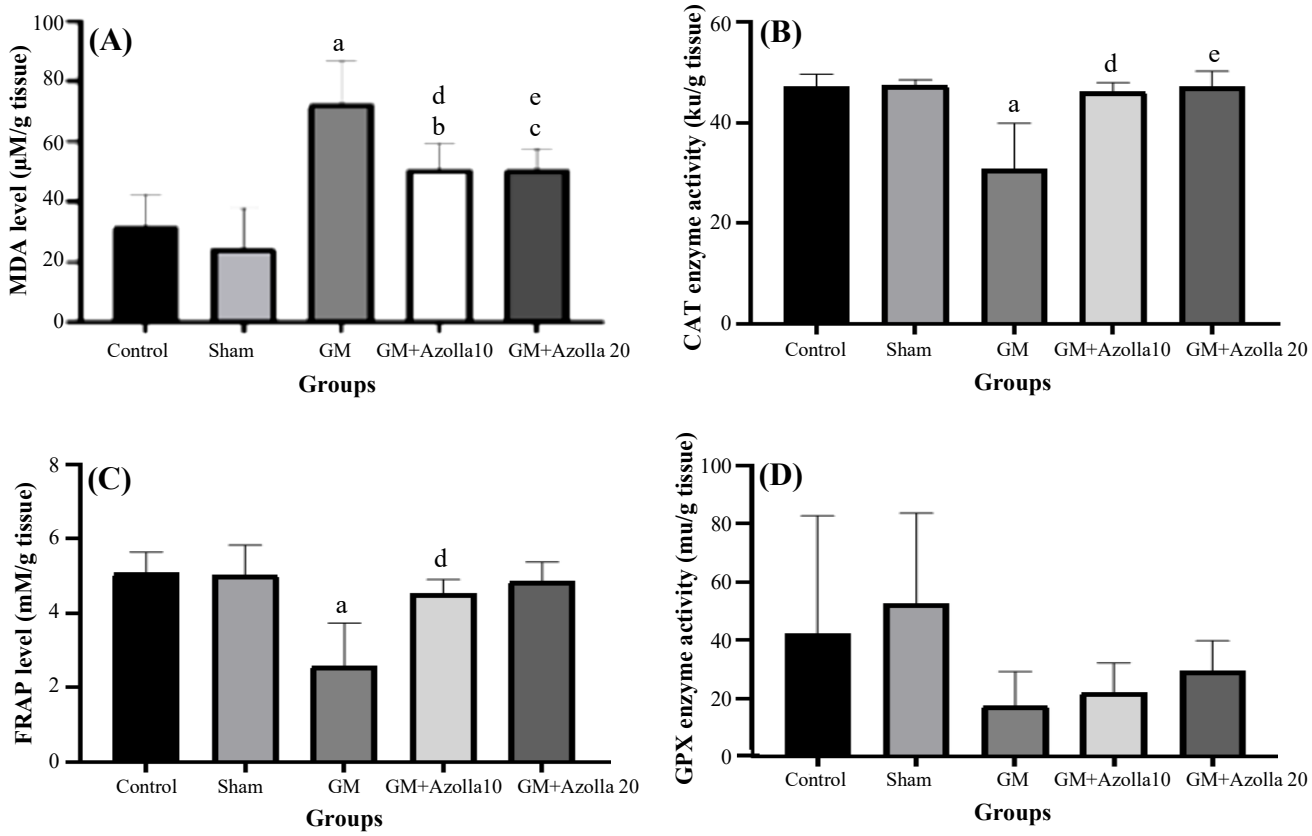

Fig. 2. Oxidative stress parameters in renal tissue. (A) MDA levels, (B) CAT activity, (C) FRAP levels, (D) GPX activity. Values are mean ± SD ($p < 0.05$). [a]GM vs. Sham, [b]GM + Azolla 10 vs. Sham, [c]GM + Azolla 20 vs. Sham, [d]GM vs. GM + Azolla 10, [e]GM vs. GM + Azolla 20.

than anti-inflammatory. The significant histological preservation observed, despite unchanged TNF-α, underscores the paramount role of oxidative stress mitigation in preventing structural damage. The prevention of tubular necrosis and glomerular injury is consistent with the known tissue-protective effects of antioxidants and has been observed with *A. pinnata* in other toxicity models, such as lead-induced hepatotoxicity[10,11,23] and pendimethalin-induced toxicity in fish[10].

The documented phytochemical profile of *A. pinnata* provides a plausible explanation for our results. Research has found that the ethanolic extracts contain strong antioxidants such as quercetin, rutin, and tamarixetin[11,24-27]. Notably, quercetin has been demonstrated to protect against gentamicin-induced kidney toxicity by lowering oxidative stress and cell death[28-29]. Thus, the kidney protection observed in this study is probably due to the combined effects of these compounds, which boost the body's natural defenses and directly neutralize ROS.

There are some limitations to this research. The absence of HPLC analysis prevented the identification of specific bioactive compounds. Using only male rats restricts the applicability of the results to females. Additionally, employing a single sham group instead of separate control groups for each administration method is a design constraint. Future research should include detailed phytochemical profiling, broader cytokine assessments, and evaluation of sex-based differences.

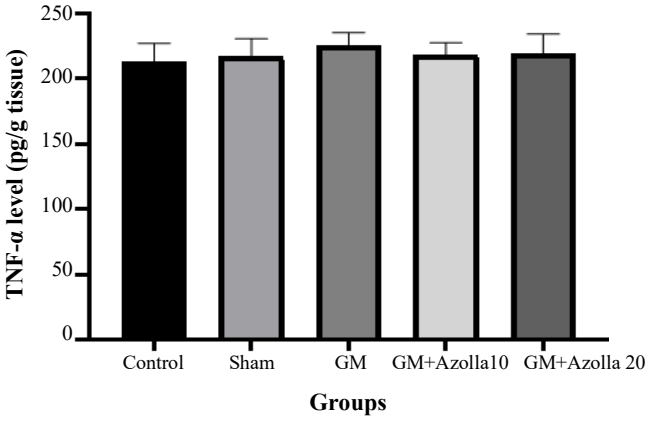

Fig. 3. Renal tissue TNF-α levels across all experimental groups. Values are mean ± SD. No significant differences were found ($p > 0.05$).

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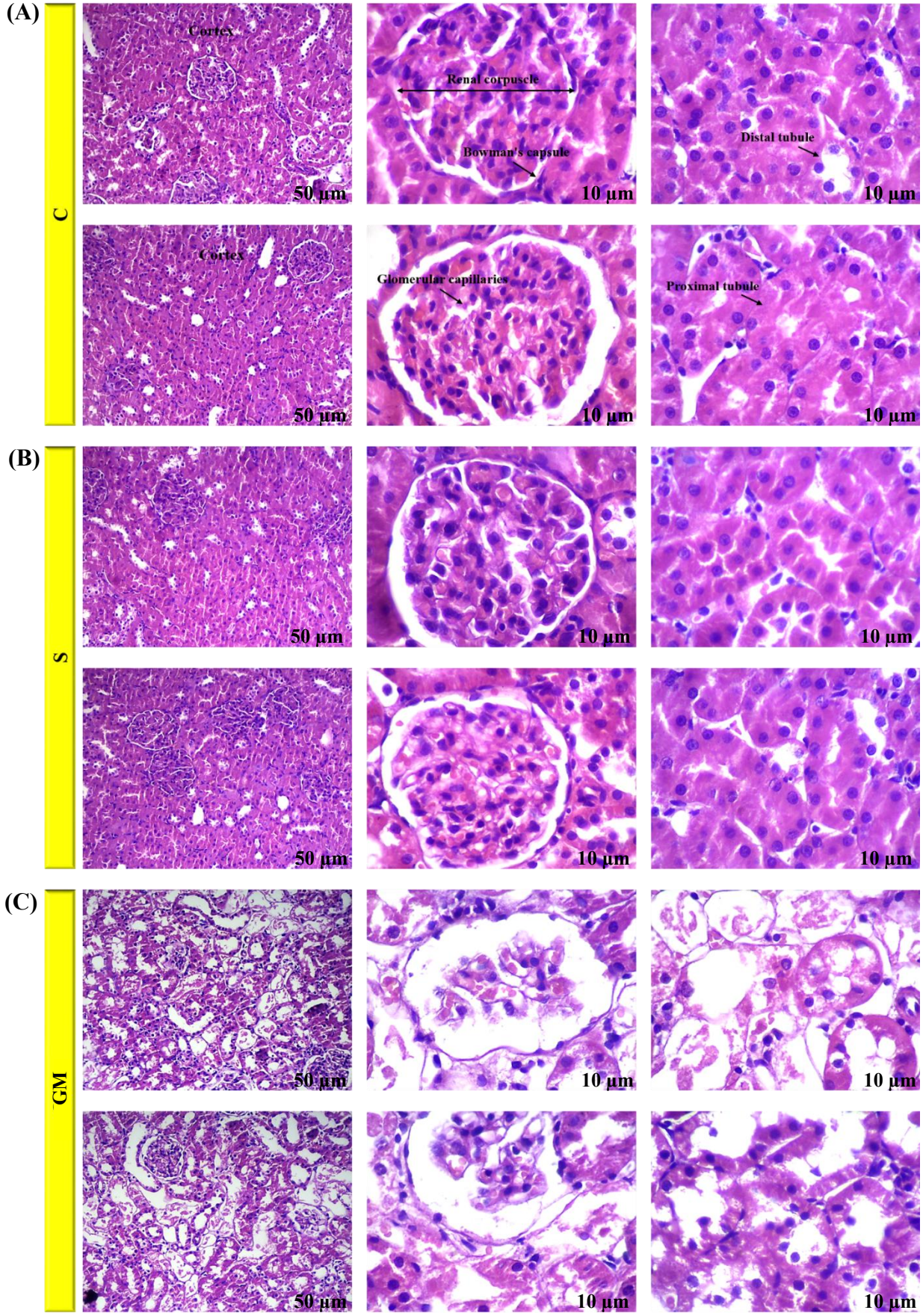

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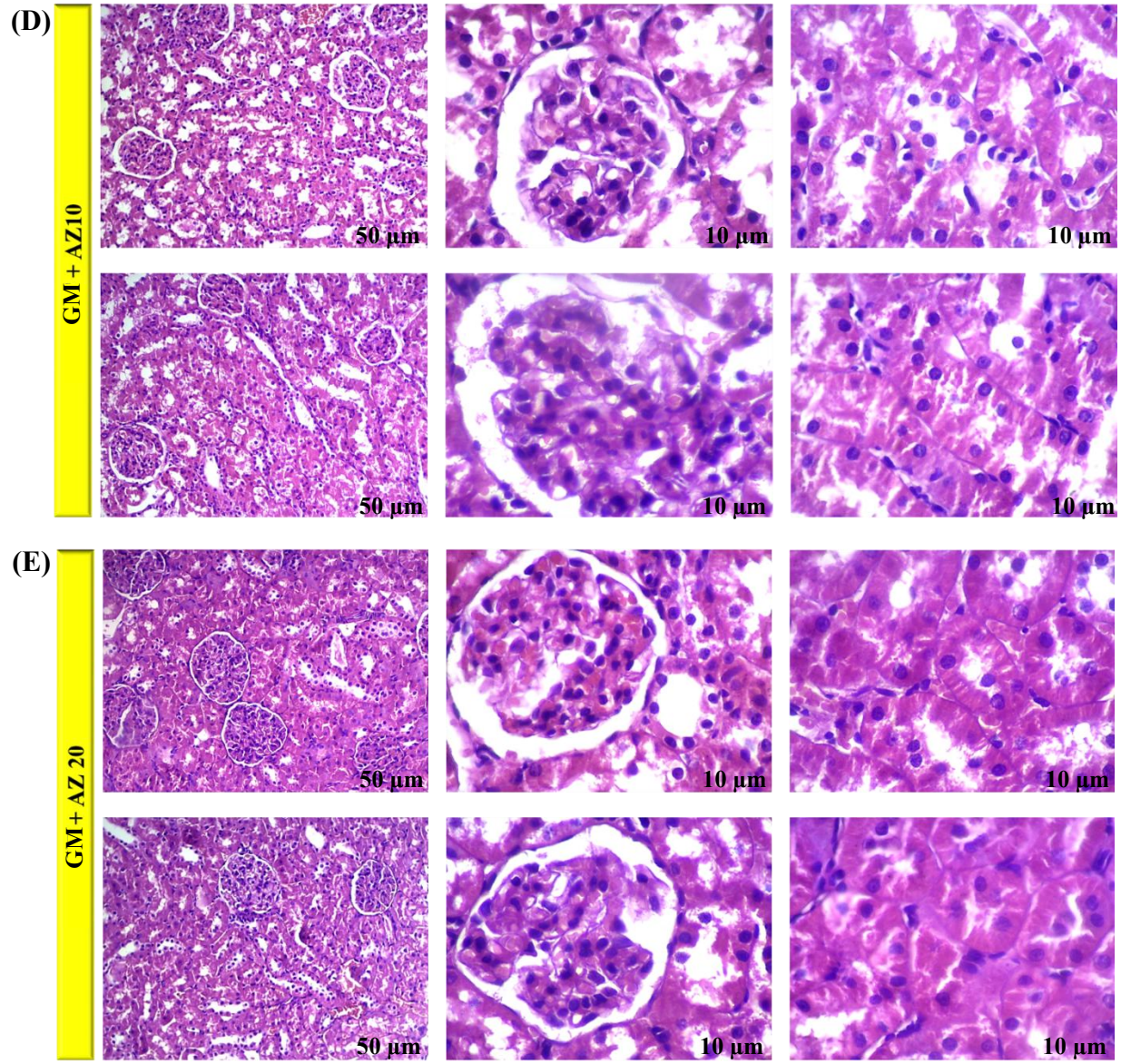

Fig. 4. Representative photomicrographs of kidney sections (H&E staining, 100×). (A) Control group showing normal renal histology; (B) Sham group; (C) GM group; (D) GM + Azolla 10 group; (E) GM+ Azolla 20 group.

CONCLUSION

The hydroalcoholic extract of *A. pinnata* shows strong protective effects against GM-induced kidney damage. Its effectiveness mainly stems from its powerful antioxidant activity, which reduces oxidative stress, maintains antioxidant enzyme function, and prevents structural damage to the kidneys. These findings suggest that *A. pinnata* could be a valuable complementary treatment to improve the safety of GM therapy. Future research should focus on identifying the specific active compounds and elucidating their molecular mechanisms of action.

DECLARATIONS

Acknowledgments

ChatGPT-4.0 (OpenAI) was used solely for preliminary literature identification. Final content validation was performed exclusively by the authors.

Ethical approval

All the experimental procedures in this study were conducted in accordance with the Institutional Animal Care guidelines and approved by the Research Ethics Committee of Arak University of Medical Sciences, Arak, Iran (ethical code IR.ARAKMU.AEC.1401.014).

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Consent to participate

Not applicable.

Consent for publication

All authors reviewed the results and approved the final version of the manuscript.

Authors' contributions

MGA: conceptualization, data curation, formal analysis, investigation, methodology, writing–original draft, and investigation; FJM: conceptualization, writing-review & editing, conceptualization, project administration, resources, supervision, validation, visualization, and writing–review & editing; NH: investigation; HN: methodology; SCA: investigation, data curation, formal analysis, methodology, supervision, validation, writing–original draft, writing–review & editing, conceptualization, project administration, resources, supervision, validation, visualization, and writing–review & editing.

Data availability

All relevant data can be found within the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

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Supplementary information

The online version does not contain supplementary material.

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