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RESEARCH ARTICLE OPEN ACCESS

Therapeutic and preventive effects of pumpkin seed extract on gentamicin-induced nephrotoxicity in rats

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Abstract

Objective: This experiment was designed to demonstrate the therapeutic and preventive effects of pumpkin seed extract against gentamicin-induced nephrotoxicity.

Methods: Forty male albino rats were randomly divided into five groups: control, gentamicin only, pumpkin seed extract only, gentamicin + pumpkin seed extract (treatment group), and pumpkin seed extract + gentamicin (preventive group). The pumpkin seed extract was administered orally at a dose of 500 mg/kg body weight.

Results: Gentamicin administration significantly increased serum levels of urea (~90%), creatinine (~80%), malondialdehyde (MDA, ~70%), and endothelin-1 (ET-1, ~65%), while reducing catalase (CAT) and glutathione (GSH) activities by approximately 45% and 50%, respectively, compared to controls. Co-administration of pumpkin seed extract, both preventively and therapeutically, significantly reversed these changes, restoring biochemical markers close to control levels. Histopathological evaluation confirmed the protective and reparative effects of the extract on kidney tissue.

Conclusion: Pumpkin seed extract (500 mg/kg) demonstrated both preventive and therapeutic potential in mitigating gentamicin-induced nephrotoxicity through antioxidant and anti-inflammatory mechanisms.

Keywords: Renal biomarkers, Nephrotoxicity, Pumpkin seed extract, Endothelin-1, Antioxidant activity

Plain English Summary

This study investigated whether pumpkin seed extract can protect the kidneys from damage caused by gentamicin, a powerful antibiotic known to harm kidney function. Forty rats were divided into five groups, receiving different combinations of pumpkin seed extract and gentamicin. Rats that got gentamicin alone showed kidney damage, with high levels of waste products (urea and creatinine), oxidative stress, and inflammation. Those treated with pumpkin seed extract, either before or after gentamicin, had much healthier kidney function and tissue. The extract significantly reduced oxidative damage and boosted the body's natural antioxidants. Microscopic examination confirmed that kidney tissues were better preserved in treated rats. The results suggest that pumpkin seed extract, rich in antioxidants, can both prevent and repair drug-related kidney damage. The researchers recommend further studies to explore optimal doses and potential human applications.

Introduction

Nephrotoxicity represents a significant medication complication by damage to renal function either acutely or chronically, and both factors depend on

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drug dosage levels and therapeutic period (1). Acute or chronic renal deterioration through toxic metabolite accumulation happens due to agents and drugs in the condition known as nephrotoxicity (2). The drug-induced origin of nephrotoxicity amounts to 20% but grows substantially in older patients because of their longer lifespan and multiple medication use (3). Medical professionals use gentamicin as GENT because it serves as an antibiotic that kills Gram-negative bacteria effectively while treating severe infections (4). The clinical use of gentamicin is limited due to its nephrotoxic potential (5, 6). Reactive oxygen species production in renal tissue is considered the main cause of gentamicin's toxic effects, since it is the most used drug in this antibiotic group (7). ROS triggers vascular muscle cells to constrict and simultaneously decreases the rate at which the kidneys filter blood (GFR) (8). Lipid peroxidation, together with protein modification, results in cellular injury and necrosis when ROS attacks cells (9). The urinary system excretes most of the administered intravenous gentamicin into urine streams, yet some of the administered injection reaches the kidney cortex areas to damage cells there (4).

Alternative healthcare options use herbal drugs that deliver effective and secure treatment methods (10). Similar healing processes occur in various liver diseases due to this treatment method (11). Herbal drugs include numerous antioxidants that aid in safeguarding the human body from dangerous free radicals (12). Pumpkins, under the scientific name *cucurbita pepo L*., belong to the genus *Cucurbita* and the *Cucurbitaceae* family as gourd squashes (13). The plant has various applications across numerous medical treatment areas (14). Unsaturated fatty acids make up the pumpkin seed, the content being linoleic acid, palmitic acid, stearic acid and oleic acid, standing as the main component, while phytosterols join carotenoids, vitamin A and vitamin E as antioxidants (15). Pumpkins also carry proteins together with trace elements, which include zinc, selenium, sodium, calcium, magnesium, potassium and phosphorus (16). These seeds contain phenols and polysaccharides along with triterpenes and possess flavonoids and saponins in addition to sterols and phytosterols, together with tocomonoenols (17). Pumpkin seeds demonstrate therapeutic effects against diabetes, cancer and hypertension, and they also mitigate skin injuries, protect the eyes by minimising cataract development, decelerate ageing effects and halt tumour enlargement based on research findings (18). Antioxidant properties, together with antibacterial and anti-inflammatory effects, characterise pumpkin seeds (19). The lipid components of pumpkins help lower bladder and urethral pressure as well as prevent and treat prostate cancer, according to scientific studies (20). The main purpose of this research was to evaluate how pumpkin seed extract might shield the kidneys and counteract the harmful effects of gentamicin-induced Nephrotoxicity.

Pumpkin seed extract is abundant in bioactive compounds such as flavonoids, phenolic acids, vitamin E, and unsaturated fatty acids. These substances are renowned for their potent antioxidant effects, primarily through neutralising reactive oxygen species and boosting the activity of the body's antioxidant enzymes. Such phytochemicals have demonstrated the ability to reduce oxidative stress and inflammation, two central processes involved in drug-induced kidney damage, which positions pumpkin seed extract as a compelling candidate for renal protection. Although there is increasing evidence supporting the overall health benefits of pumpkin seed extract, its specific potential to safeguard against gentamicin-induced kidney injury remains insufficiently explored. To address this gap, our study aims to assess both the therapeutic and prophylactic effects of pumpkin seed extract in a rat model of gentamicin-related nephrotoxicity. We hypothesise that, owing to its rich antioxidant content, pumpkin seed extract could potentially mitigate renal impairment and oxidative stress caused by gentamicin administration.

Material and methods

Sample

The sample size (n = 8 per group) was determined based on previous similar studies that achieved statistically significant differences using comparable group sizes. Male Wistar rats aged 10–12 weeks and weighing between 260 and 310 g were selected to ensure physiological maturity and homogeneity in metabolic response.

Preparation of pumpkin seed extract

The process involving the harvesting of pumpkin seeds followed the maceration technique detailed by Siddig (21) in his work. Two grams of pumpkin seed powder were macerated in 70% ethanol (1:10 w/v), and the solution was stirred and allowed to sit for 24 hours at room temperature (~25°C) with continuous stirring at 150 rpm. The extract was then filtered and concentrated under reduced pressure using a rotary evaporator. Although phytochemical standardisation (e.g., HPLC fingerprinting or total phenolic content) was not performed in this study, future studies will aim to incorporate quantitative analysis of the active constituents for consistency across batches.

Animals and Experimental Protocol
Research involved male Wistar rats of 10-12 weeks old weighing from 260g to 310g, which were maintained on a standard diet with ad libitum water. The Animal House of the Biology Department at the College of Education for Pure Sciences, Animal Ethical Committee of Anbar University, provided the approval for experimental deployment. Rats were randomly assigned to five groups. The first group was given normal saline solution (3 ml/kg) and was considered a control group, the second group was given pumpkin seed extract (500 mg/kg), the third group: was injected intraperitoneally with gentamicin (80 mg/kg), the fourth group was injected with gentamicin for 12 weeks + was given pumpkin seed extract and was considered a therapeutic group, and the last group, the fifth was given pumpkin seed extract + gentamicin and was considered a preventive group. The experiment lasted 12 weeks. A dose of 500 mg/kg body weight was chosen based on previous studies that demonstrated the antioxidant and nephroprotective potential of pumpkin seed extract in rodent models (36).

Blood collection
We collected blood through heart puncture from all rats by using disposable syringes. After which, test tubes were used to collect the blood samples without anticoagulant. It was then allowed to coagulate before performing biochemical testing. Serum extraction from coagulated blood samples required 5 minutes of runtime using 3,000 rpm centrifugation. The biochemical markers were obtained from the serum after frozen storage at -20°C.

Biochemical Analysis: Determination of Blood Urea and Creatinine
Blood urea level was tested using the Enzymatic Colourimetric Kit (Linear Chemicals, S.L., Spain) based on Fawcett and Scott, 1960 (22) method, while Determination of Serum Creatinine Level, Serum creatinine level was determined using Creatinine Colourimetric Kit (BioMérieux, Urea France) based on Henry, 1974 (23).

Serum lipid peroxidation measurement (MDA)
The levels of MDA, as the marker of lipid peroxidation, were measured by the thio-barbituric acid (TBA) test (24). Spectrophotometric measurement was done at 532 nm to determine the absorbance.

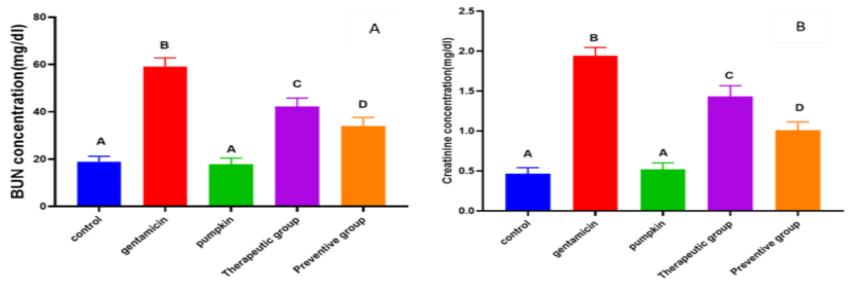
Measuring serum- Catalase (CAT) and Glutathione (GSH)
CAT activities of the serum were assessed according to a previous report (25). For the initiation of the reaction, a sample (20 µL of serum or supernatant) was added to 2 mls of 30 mM hydrogen peroxide (H2O2) in 50 mM potassium phosphate buffer pH. The assay of GSH activity in serum was carried out based on (26).

Determination of Serum End-1
The ELISA kit from BT laboratory, Zhejiang, China, allowed estimation of Serum Endothelin-1 levels following the manufacturer's guidelines to obtain results as ng/L. The study results used ng /L as the measurement unit.

Histopathological Examinations
The rat kidney tissue was fixed in 10% formalin solution, then followed by liquid concentration steps before xylene cleaning and paraffin solution immersion, after which a 5 mm-thick tissue cutting was performed. The researchers cut tissue samples to 5 mm, then stained them with haematoxylin before viewing under the microscope (22).

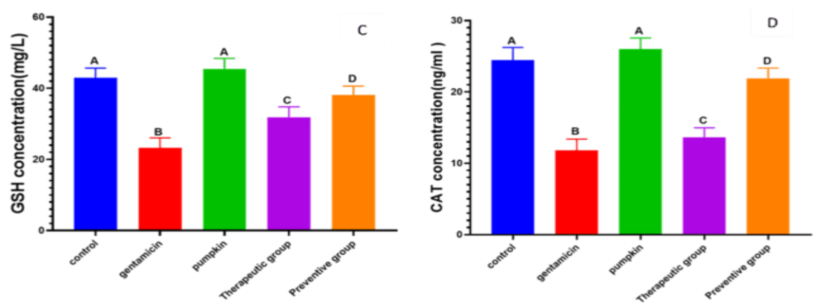
Statistical analysis
The data are presented as mean ± SEM using the Statistical Package for Social Sciences version 20). Data were tested for normality using the Shapiro-Wilk test before applying one-way ANOVA. Tukey's post hoc test was used for pairwise group comparisons. Effect sizes (Cohen's d) were calculated to provide a measure of the magnitude of differences between groups alongside p-values, with significance set at $p \le 0.05$.

Results
The results of our current experiment, shown in Figure (A, B), Illustrates a significant increase in the level of both blood urea and creatinine in the serum of the male rat injected with gentamicin (second group) compared to the healthy control group, while the results in the third group, which was given pumpkin seed extract alone, were similar to the control group. The results of the fourth group (therapeutic) and the fifth group (preventive) showed a significant decrease in the level of the above two indicators compared to the second group.



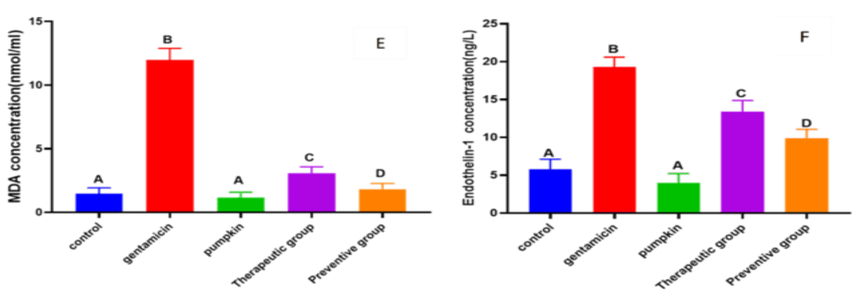
Figures A and B: The effect of pumpkin seed extract on urea and creatinine concentrations in rats treated with gentamicin (The number of rats per group = 8, different capital letters indicate significant difference between the treatment groups, while similar capital letters indicate no significant difference)

The results of this study showed a significant increase in MDA levels and a decrease in the levels of the antioxidants GSH and CAT in the second group compared to the control group. The third group did not show any negative results regarding the above results. The results in the fourth and fifth groups, which were given pumpkin seed extract before or after gentamicin, showed a significant decrease in MDA levels and an increase in the activity of the antioxidants GSH and CAT compared to the second group, as shown in Figures C and D.



Figures C and D: The effect of pumpkin seed extract on GSH and CAT concentrations in rats treated with gentamicin (Number of rats per group = 8, different capital letters indicate significant difference between the treatment groups, while similar capital letters indicate no significant difference)

The results of inflammatory mediators, illustrated by Figure (F), showed an increase in the level of End-1 in the serum of male albino rats in the second group, whose peritoneum was injected with gentamicin alone, compared to the healthy control group. The level of these two indicators was close to the control group in the third group, while the results for the above two indicators showed a reflection in the result in the fourth and fifth groups and a significant decrease in the level of the above measurements after giving pumpkin seeds, whether before or after gentamicin, compared to the second group. It is noteworthy that the results of the fifth preventive group were better than the fourth therapeutic group.



Figures E and F: The effect of pumpkin seed extract on MDA and ET-1 concentrations in rats treated with gentamicin (Number of rats per group = 8, different capital letters indicate significant difference between the treatment groups, while similar capital letters indicate no significant difference)

The histological evaluation showed severe pathological changes in the gentamicin group of kidney tissue, represented by the presence of glomerular degeneration (GD), inflammatory infiltration (LI), cellular degeneration (D), congestion (CON), complete degeneration of some glomeruli (GL), necrosis (N) and sloughing of the lining of the urinary tubules (SL), as in pictures 3, 4, 5 and 6, compared with the control group. The third group, which was given pumpkin seed extract alone, showed a normal pattern almost similar to the control group, as in picture 2. The fourth preventive group showed a clear improvement in the histological structure when compared with the gentamicin group, with the presence of negative effects, including simple deposition of necrotic materials and simple inflammatory infiltration, as in pictures 7 and 8. The fifth treatment group also showed a normal histological pattern, except for the presence of haemorrhage (HE), inflammatory infiltration, cellular degeneration and simple necrosis, as in pictures 9 and 10.

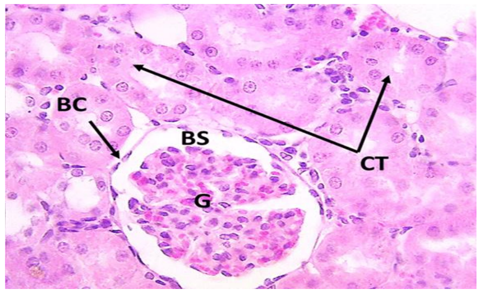


Image 1: A cross section of the kidney of a rat from the control group showing the glomerulus G, Bowman's space BS, Bowman's capsule BC, and the urinary convoluted tubules CT (H and E stain, 400X)

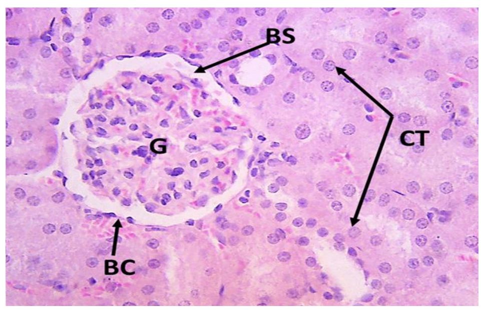


Image 2: A cross section of rat kidney in the pumpkin seed group showing the glomerulus G, Bowman's space BS, Bowman's capsule BC, and urinary convoluted tubules CT (H and E stain, 400X)

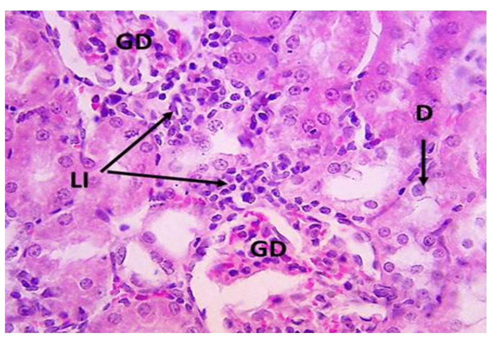


Image 3: A cross section of the kidney of a rat in the gentamicin group showing glomerular degeneration (GD), inflammatory infiltration (LI), and degeneration of D cells (H and E stain, 400X)

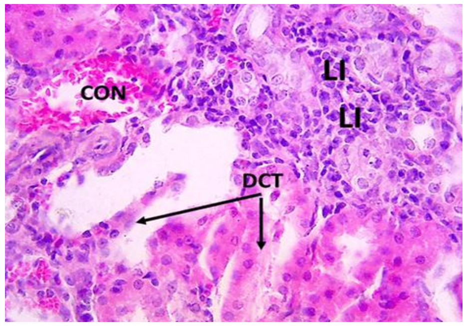


Image 4: A cross section of the kidney of a rat in the gentamicin group showing congestion (CON), severe inflammatory infiltration (LI), and degeneration of convoluted tubule cells (DCT), H and E stain. 400X)

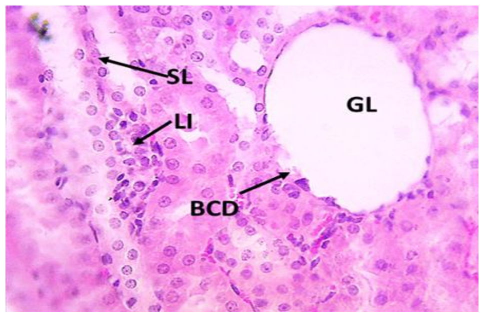


Image 5: A cross section of the kidney of a rat in the gentamicin group showing complete degeneration of the glomerulus (GL), inflammatory infiltration (LI), degeneration of the Bowman's capsule (BCD), and sloughing of the convoluted tubule lining (SL) (H and E stain. 400X)

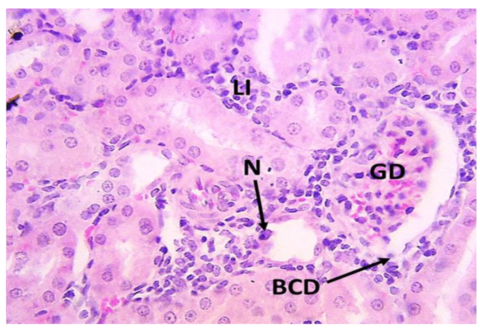


Image 6: A cross section of the kidney of a rat in the gentamicin group showing degeneration of the glomerulus (GD), inflammatory infiltration (LI), degeneration of Bowman's capsule (BCD), and necrosis of some N cells (H and E stain, 400X)

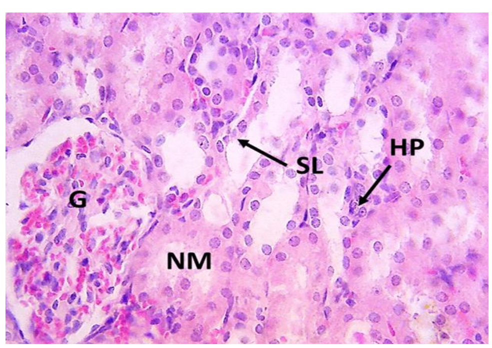


Image 7: A cross section of the kidney of a rat in the pumpkin seeds + gentamicin preventive group showing the normal pattern of the glomerulus G, shedding of some of the lining of the convoluted tubules SL, and simple deposition of necrotic material NM (H and E stain, 400X)

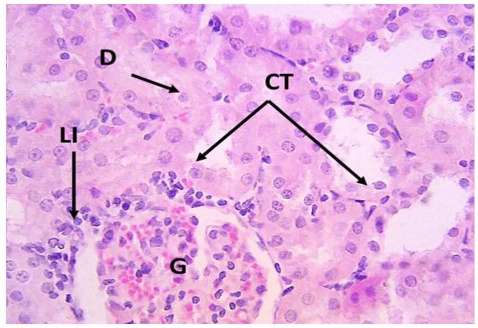


Image 8: Cross section of kidney of a rat in the pumpkin seeds + gentamicin preventive group showing normal glomerular pattern G, convoluted tubules CT, slight inflammatory infiltrate LI, slight degeneration of cells D, (H and E stain, 400X)

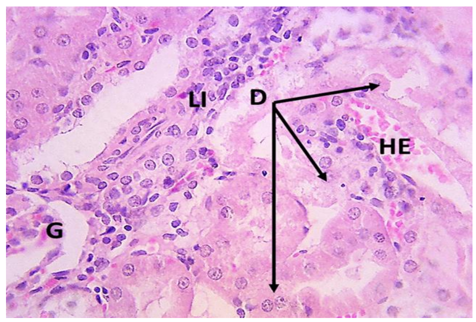


Image 9: Cross section of the kidney of a rat with gentamicin + pumpkin seeds treatment group showing normal glomerular pattern G, haemorrhage HE, slight inflammatory infiltrate LI, degeneration of cells D, (H and E Stain, 400X)

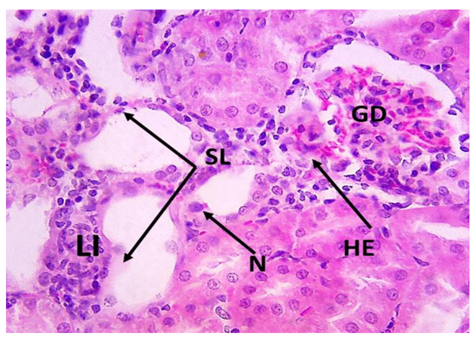


Image 10: A cross section of the kidney of a rat in the gentamicin + pumpkin seeds treatment group showing degeneration of the glomerulus (GD), necrosis of some N cells, sloughing of the lining of the convoluted tubules (SL), inflammatory infiltration (LI), and minor haemorrhage (HE) (H and E stain, 400X)

Discussion

Infections caused by gram-negative bacteria are frequently managed with aminoglycoside antibiotics, such as gentamicin. However, their use is limited because of serious kidney-related side effects (28). The nephrotoxicity associated with aminoglycosides like gentamicin is well recognised (29). Several studies have highlighted the role of oxidative stress, which occurs due to reactive oxygen species (ROS), in causing damage to the kidneys (30). Additionally, these reactive oxygen species contribute to the renal tubular necrosis and acute renal failure triggered by gentamicin (5). Various mechanisms, including superoxide anions and hydroxyl radicals, lead to gentamicin's toxic effects on the kidneys, resulting in cell damage and cell death(8). These mechanisms can include DNA damage, lipid peroxidation, disruption of cellular respiration, interference with the generation of adenosine triphosphate, inhibition of the electron transport chain, and destabilisation of the tubular cell membranes (5).

Recently, there has been an increased emphasis on the use of herbs and herbal supplements for various health issues because they have fewer side effects (31). This study aimed to assess how pumpkin seeds can protect the kidneys from damage caused by gentamicin. The findings of this research indicate that pumpkin seeds can help reduce inflammation in the kidney tubules, oxidative stress, changes in tissue structure, and issues with kidney function caused by gentamicin.

The current research showed that gentamicin caused significant kidney damage in rats by raising levels of blood urea and serum creatinine. Research conducted by (7), along with (32), indicated that the nephrotoxicity linked to gentamicin occurs because these drugs are absorbed by and build up in the proximal tubular epithelial cells, leading to acute tubular necrosis that is dependent on how much and how long the exposure lasts. Furthermore, this accumulation increases the production of reactive oxygen species, which have a crucial role in kidney damage and contribute to the main clinical signs of nephrotoxicity, including elevated urea and plasma creatinine levels (9, 33). explored this nephrotoxicity and found that gentamicin resulted in high serum creatinine levels. This increase in creatinine is a result of gentamicin gathering in the kidneys, causing a reduction in glomerular filtration and, consequently, kidney failure (34). Several studies have shown a strong connection between oxidative stress, particularly reactive oxygen species, and gentamicin-induced nephrotoxicity. Gentamicin causes the mitochondria in the renal cortex to release iron, and the complex of iron and gentamicin boosts the formation of reactive oxygen species (ROS) (8). An excess of ROS leads to kidney function problems, which show as higher levels of serum creatinine and urea (35). We also noticed that when pumpkin seeds are given alongside gentamicin, the serum creatinine and urea levels are lower than in the group treated only with gentamicin. Multiple studies have shown that the antioxidant properties of pumpkin seeds help to lower the increased levels of serum creatinine and urea in rats treated with gentamicin. It is well known that pumpkin seeds provide antioxidant benefits either by directly eliminating free radicals or by enhancing the activity and levels of antioxidant enzymes (36).

The results of serum MDA analysis indicated that the gentamicin-treated group had increased serum MDA intensity compared to untreated control animals. The research findings confirm our study by revealing that gentamicin leads to decreased activity in antioxidant enzymes (GSH and CAT) per other reports. Gentamicin enhances ROS production in cells alongside suppressing renal antioxidant defenses so the drug ultimately lowers antioxidant parameters (GSH, GPX, CAT, SOD) and results in GST activation and MDA lipid peroxidation. Gentamicin treatment makes rat kidneys more vulnerable to ROS damage since the medication induces antioxidant defence enzymes, such as glutathione and catalase, to become deficient (37, 38). The observed reduction in MDA levels and restoration of antioxidant enzyme activities (CAT, GSH) may be attributed to flavonoids, phenolic acids, and vitamin E in pumpkin seeds, which are known to scavenge reactive oxygen species and inhibit lipid peroxidation. These phytochemicals not only enhance the antioxidant defence system but may also modulate signalling pathways involved in oxidative stress and inflammation. Comparable antioxidant and nephroprotective effects have been reported with agents like N-acetylcysteine, which also restores glutathione levels and reduces oxidative stress in gentamicin-induced nephrotoxicity. However, unlike N-acetylcysteine, pumpkin seed extract offers a natural, dietary-based intervention with additional nutritional benefits.

The findings indicate that treatment with pumpkin seeds reduced lipid peroxidation and increased the activity levels of CAT and GSH, leading to improved urinary functions. As mentioned earlier, oxidative stress contributes to renal damage caused by gentamicin. This drug results in quick alterations in the lipid makeup of membranes. These membrane changes may stem from lipid peroxidation triggered by free radicals (39). This notion is backed by higher levels of MDA, an end product of lipid peroxidation, found in the kidneys of rats treated with gentamicin. Our observations noted increased lipid peroxidation in the group receiving gentamicin, aligning with earlier research. The results also showed that levels of CAT and GSH were lower in the rats treated with gentamicin. Pumpkin seeds reversed these biochemical markers of oxidative stress and antioxidant activity (40). We believe that these results align with the antioxidant properties of pumpkin seeds. Moreover, other studies have supported these findings, highlighting how antioxidants can prevent the onset of renal failure in laboratory animals (13).

Elevated levels of ET-1 in serum work as indicators for identifying cardiovascular disease as well as atherosclerosis. Endothelin-1 is produced within the interstitial fluid layer of aorta endothelial cells and other tissues, such as heart tissue and kidney tissue (41, 42) established that elevated serum levels of endothelin-1 occur due to gentamicin-generated oxidative stress in mice, resulting in damaged endothelia that release high concentrations of endothelin-1 together with macrophage and T-cell cellular infiltration as vascular inflammation progresses, ultimately increasing the probability of developing kidney failure (43). Research findings presented in this paper show identical results. Pumpkin seeds demonstrate their antioxidant effects because they contain active compounds such as flavonoids and phenols, and vitamins, resulting in tissue defence against oxidative damage, which enhances the reduction of inflammatory markers, including ET-1 (44).

In this study, we observed that gentamicin leads to problems with kidney function and structure, along with a rise in tissue MDA levels, which aligns with findings from other research. This shows that reactive oxygen species and lipid peroxidation play a part in the kidney damage caused by gentamicin (45). Typically, oxidation involves creating reactive substances, which are caused by hydroxyl radicals and superoxide anions produced from hydrogen peroxide. When aminoglycosides are released from the lysosomes of epithelial cells, reactive oxidative species are generated (46). This release leads to swelling in kidney cells, which disrupts mitochondrial respiration, while gentamicin boosts the generation of ROS within the mitochondria (47). These oxidative agents hinder the respiratory chain and the formation of ATP, encourage the release of cytochrome C and other factors that trigger cell death, cause lipid damage, harm cellular proteins and DNA, induce contractions in mesangial cells, and create stress in the endoplasmic reticulum (48).

Studies have established antioxidant properties in pumpkin seed oil because this plant contains essential fatty acids that support cell membrane fluidity, together with amino acids along with phytosterols, β-β-carotenes, phenolics, flavonoids, and selenium. Pumpkin seed oil serves as a powerful antioxidant since it contains vitamin E (40, 49).

The findings from this study suggest that pumpkin seed extract holds significant translational potential as a natural adjuvant therapy to mitigate drug-induced nephrotoxicity, particularly in clinical scenarios involving aminoglycoside antibiotics like gentamicin. Given its antioxidant and anti-inflammatory properties, the extract may complement conventional treatment strategies to protect renal function, especially in patients at high risk for kidney injury. While these results are promising, clinical trials are necessary to validate efficacy, determine optimal dosing, and evaluate safety in human populations.

Study limitations

This study has some limitations. First, the pharmacokinetic profile and bioavailability of the pumpkin seed extract were not assessed, which limits our understanding of the precise systemic exposure and active constituents. Second, only a single dose (500 mg/kg) was evaluated; dose-response relationships and potential toxicity thresholds remain unexplored. Additionally, the lack of extract standardisation may affect reproducibility. Future studies should aim to quantify active compounds, assess multiple dosing levels, and perform detailed pharmacological profiling.

Conclusion

The intake of 500 mg/kg body weight of pumpkin seed ethanolic extract successfully guarded Wistar rats from developing gentamicin-induced kidney damage. The experimental results showed that pumpkin seed ethanolic extract produces protective effects on the kidneys. Further research needs to be done to identify the compounds present in pumpkin seed, as well as a breakdown of the protection mechanisms involved.

Declarations

Ethics approval and consent to participate

All experimental procedures involving animals were conducted following the ethical standards approved by the Animal Ethical Committee of the College of Education for Pure Sciences, University of Anbar, Iraq (No: 240, 2024/ 02/ 26). The experimental protocol was reviewed and approved under reference number [insert approval/reference number if available. Efforts were made to minimise the number of animals used and to reduce their suffering, following international guidelines for the care and use of laboratory animals.

Consent for Publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution Non-Commercial 4.0 license.

Availability of Data

Data for this work is available from the authors and may be provided upon reasonable request.

Conflicts of Interest

None.

Funding

None.

Authors' contributions

MMS: Conceptualisation, methodology, investigation, data collection, formal analysis, and

writing, original draft preparation.
ALH: Supervision, validation, data interpretation, writing, review and editing, and project administration.
Both authors have read and approved the final manuscript.

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