RESEARCH ARTICLE

The Histological Impact of Amikacin on Renal Tissue of Rats: The Role of Vitamin D3

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ABSTRACT

Background: Amikacin is a strong aminoglycoside antibiotic which mainly targets Gram-negative bacteria that are resistant to multiple drugs. Its effectiveness remains restricted because of its potential to cause kidney damage. The primary reason for renal injury after amikacin use is excessive oxidative stress causing damage to tubular epithelial cells through enhancing both mitochondrial dysfunction and inflammatory responses. Vitamin D_3 has an antioxidant and anti-inflammatory properties that help protect against tissue damage.

Objective: The research investigated the renal protective effect of vitamin D_3 after amikacin toxicity through histopathological and morphometric assessments of rat kidney tissue.

Methods: Twenty-four adult albino rats were randomly divided into four groups: control, amikacin (300 mg/kg, i.p.), vitamin D_3 (500 IU/kg, orally) + amikacin (500 IU/kg, i.p.), and vitamin D_3 (500 IU/kg, orally) alone. The treatment spanned two weeks for vitamin D_3 followed by seven days of amikacin. Hematoxylin and eosin staining was used to study kidney tissues.

Results: The administration of amikacin alone lead to major kidney tissue damage causing both tubular and glomerular injuries with thinning of epithelial cells and enlarged Bowman's space. The combination of vitamin D_3 with amikacin successfully prevented these lesions, maintained normal glomerular dimensions and protected tubular tissue from damage. The research established a strong negative connection ($r=-0.82$, $p<0.001$) between glomerular diameter and necrotic tubules which confirmed the protection of structural elements.

Conclusion: Vitamin D_3 showed effective protection for kidney health when used to treat amikacin-induced kidney damage. The combination of histological and morphometric analyses confirmed kidney protection through vitamin D_3 which demonstrates its protective effects against aminoglycoside toxicity in kidneys.

Keywords: Amikacin, nephrotoxicity, Vitamin D_3, Morphometric analysis, Rat model.

INTRODUCTION

Aminoglycosides are among the earliest discovered antimicrobial agents. They remain essential for treating severe drug-resistant Gram-negative infections [1]. They are widely used against different pathogens such as Pseudomonas aeruginosa, Klebsiella, Acinetobacter, and Enterobacter [2, 3]. Their bactericidal effect results from irreversible binding to the 30S ribosomal subunit causing disrupted protein synthesis and leading to cell death [4]. However, their clinical use is limited by its dose-dependent nephrotoxicity [5-7].

The multiple pathways of aminoglycoside-induced nephrotoxicity create a complex mechanism that occurs through various overlapping processes [8]. The drug builds up inside proximal tubular epithelial cells through a progressive process which leads to cellular damage [9]. As the intracellular concentrations increase, lysosomal membranes rupture, mitochondrial function declines, and reactive oxygen species (ROS) production exceeds normal limits [10]. The combination of these processes results in tubular necrosis, glomerular shrinkage and inflammation of the interstitial tissue [11]. Structural changes in kidney tissue can be measured through morphometric indicators, including tubular diameter measurements, glomerular size assessments, and the extent of Bowman's space dilation [12, 13]. Morphometric analysis provides an essential complement to traditional histopathological evaluation to measure kidney recovery from medication through exact dimensional assessments.

Research studies from the past few years have investigated methods to reduce kidney damage from aminoglycoside antibiotics by using antioxidants and anti-inflammatory medications as additional treatments. Studies have shown that β-caryophyllene, vitamin E, misoprostol, and vitamin C protect kidney function by decreasing oxidative stress and lipid peroxidation [14, 15, 16, 17]. Vitamin D_3 maintains its original role in calcium–phosphorus homeostasis but it also functions as an immunomodulator and antioxidant through its active form 1,25-dihydroxyvitamin D_3 (calcitriol) which interacts with the vitamin D receptor (VDR) in renal tubular cells [18, 19]. Activation of VDR enables cells to

enhance their internal defense mechanisms through turning on antioxidant enzyme genes such as superoxide dismutase (SOD) and catalase (CAT) [20]. Treatment with vitamin D_3 decreases TNF-α and IL-6 production which results in decreased oxidative and inflammatory stress in renal tissue [21-23].

Research have also showed that vitamin D_3 supplements protect kidney tissue from heavy metal and nephrotoxic substance damage [24], and antibiotics such as gentamicin and vancomycin [25, 26]. This protective effect is mainly mediated through a dual mechanism of redox level normalization and prevention of programmed cell death pathways in tubular cells [27]. However, despite having promising results, it remains unclear how vitamin D_3 affects the structural and morphometric changes caused by amikacin-induced nephrotoxicity. The present work was undertaken to examine the protective roles of vitamin D_3 in a rat model of amikacin-induced renal toxicity. The research combined qualitative histopathological evaluation with quantitative morphometric analysis to develop an unbiased connection between tissue architecture and kidney organ function.

MATERIALS AND METHODS

Experimental Design and Animal Handlin:

Twenty-four healthy adult albino rats (Rattus norvegicus), between 100 and 200 grams were included in the study. The animals were obtained from the College of Veterinary Medicine animal house, University of Mosul, Iraq. The study protocol was reviewed and approved by the Institutional Ethics Committee of the College of Medicine, University of Mosul, and procedures were conducted in full compliance with the NIH Guide for the Care and Use of Laboratory Animals [28]. During the entire study duration, the animals had unrestricted access to a balanced commercial pellet diet and a clean distilled water. We established stable physiological conditions through environmental hygiene and animal welfare monitoring before starting experimental treatments. A one-week acclimatization period was allowed before the animals were randomly divided into four experimental groups (n=6 per group):

- Group I (Control): Received no drugs or vitamin for two weeks.
- Group II (Amikacin-treated): Received amikacin sulfate intraperitoneally (Ajanta Pharma Ltd., India) at a dose of 300 mg/kg body weight daily for seven consecutive days [26] to induce renal toxicity.
- Group III (Vitamin D_3 + Amikacin): Received oral vitamin D_3 (cholecalciferol 1000 IU/mL; MidaPharma, Jordan) at a dose of 500 IU/kg/day for two weeks, followed by amikacin administration in the same dose and regimen as Group II.
- Group IV (Vitamin D_3 alone): Received oral vitamin D_3 only at the same dose and duration as Group III.

Twenty-four hours after the final treatment dose, the animals were anesthetized and sacrificed. Kidneys were excised, rinsed in cold saline, and processed for histological and morphometric assessment.

Histological Preparation and Examination

Kidney tissues were processed according to standard histological methods and 4–5 µm thick serial sections were prepared for analysis. The sections then stained by hematoxylin and eosin (H&E) to evaluate their microscopic tissue structure with high precision. All histopathological scoring was performed by an examiner blinded to eliminate observer bias.

The following histopathological parameters were evaluated qualitatively: Tubular degeneration and necrosis, Glomerular atrophy and Bowman's space dilation, Vascular congestion and interstitial hemorrhage and Mononuclear cellular infiltration. Each alteration was graded semi-quantitatively as: 0 = none; 1 = mild (<25% field involvement); 2 = moderate (25–50%); 3 = severe (>50%) [29].

Morphometric Analysis

Quantitative morphometric assessment of the H&E-stained renal sections was carried out using Image J software (National Institutes of Health, USA), following the procedure outlined by Farris and Colvin [30], with several modifications tailored to the present study. Ten distinct cortical fields were selected from each animal for examination at 400X magnification to achieve proper representation of renal microarchitecture. The study analyzed various structural components including:

- The glomerular diameter (µm) measurement results from averaging two perpendicular measurements that span the glomerular tuft.
- Bowman's space area (µm²): obtained by subtracting the glomerular tuft area from the total area circumscribed by Bowman's capsule.
- The measurement of proximal tubular diameter (µm) takes place between the luminal and epithelial boundaries of proximal convoluted tubules.
- The measurement of tubular epithelial height (µm) represents the vertical distance which runs from the basement membrane to the highest point of the apical surface.
- We determined % by counting necrotic profiles in ten random microscopic fields.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software, version 10.0 (GraphPad Software Inc., San Diego, CA, USA). All quantitative values were expressed as the mean ± standard deviation (SD). The Shapiro–Wilk test results showed that the data followed a normal distribution which allowed us to perform parametric tests for validation. Differences among

experimental groups were examined by one-way analysis of variance (ANOVA), followed by Tukey's post hoc comparison to detect pairwise significance. The analysis used $p < 0.05$ as the significance level to confirm that all measured differences reached statistical significance.

The study used Pearson's correlation coefficient to investigate the relationship between morphometric parameters and histopathological scores and to determine how vitamin D_3 influences renal structure recovery. The research team created bar charts with error bars (± SD) to show the experimental means and their corresponding variation through ± SD.

The laboratory animal research followed internationally accepted ethical standards which protected animal welfare through all experimental procedures. The research team performed experiments with the smallest number of subjects needed to achieve reliable scientific results while keeping animals suffering to a minimum.

RESULTS

Qualitative Histopathological Observations

Normal Renal Structure (Control Group)

The histopathological examination of kidney sections of control group showed normal architectural of kidney which composed of renal tubules, glomeruli and interstitial tissue, no evidence of vascular congestion, interstitial inflammation, or cellular degeneration was observed (Fig. 1).

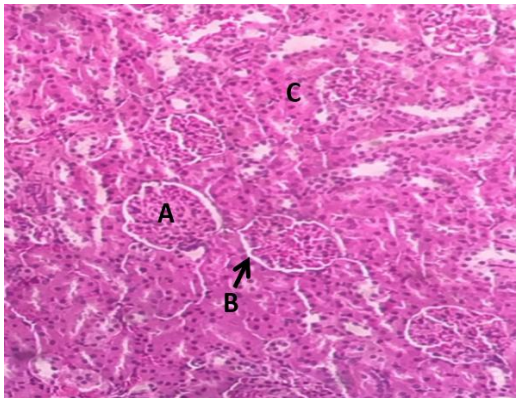

Fig. 1. Photomicrograph of control group showed normal architecture of renal corpuscle (A), bowman's capsule (B) and renal tubules (C) (HE X 200).

Amikacin-Treated Group (Group II)

Sections from rats administered amikacin alone demonstrated marked degenerative and necrotic alterations in renal tissue. The cortical regions displayed lobulation and atrophy of glomerular tuft with expansion of Bowman's space (Fig. 2A), vacuolated and dilated proximal tubules with intraluminal hyaline cast deposition (Fig. 2B), Furthermore, multifocal mononuclear cell infiltration and interstitial hemorrhage were evident (Fig. 2C), together with vascular congestion in the interlobular capillaries (Fig. 2D). These lesions were graded as severe (score=3) in more than 60 % of the examined fields.

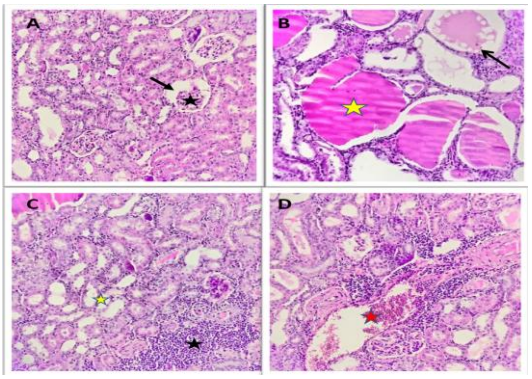

Fig. 2. Photomicrograph of Amikacine treated group showing (A) atrophy of glomerular tuft (black star) with Bowman's space expansion (yellow arrow), (B) marked tubular dilatation and degeneration (black arrow) with hyaline cast deposition in tubular lumen (yellow star) (C) mononuclear cells infiltration (black star) with vacuolar degeneration of epithelial renal tubule (yellow star) (D) blood vessels dilatation and congestion (red star). (HE X 200).

Vitamin D_3 + Amikacin Group (Group III)

Co-administration of vitamin D_3 prior to and during amikacin treatment resulted in remarkable histological preservation compared with the amikacin group. Most renal corpuscles appeared nearly normal, with partial restoration of glomerular size and reduction in Bowman's space widening and mild mononuclear infiltration persisted (Fig. 3A). The majority of proximal tubules exhibited intact epithelial lining, although few scattered hyaline casts (Fig. 3B). Vascular structures showed minimal congestion, indicating reduction in the intensity of vascular injury. Lesion grading in this group declined significantly to mild (score=1) with $p<0.05$ compared with Group II.

Vitamin D_3 Alone Group (Group IV)

Tissue sections obtained from rats treated with vitamin D_3 alone displayed normal renal histological configuration, closely resembling the control group. The glomeruli and tubules retained their structural integrity without evidence of necrosis or inflammation (Fig. 4).

Semi-quantitative scoring demonstrated that amikacin-treated rats exhibited severe structural damage across all assessed parameters, whereas vitamin D_3 pre-treatment significantly reduced lesion severity to mild levels (Table 1).

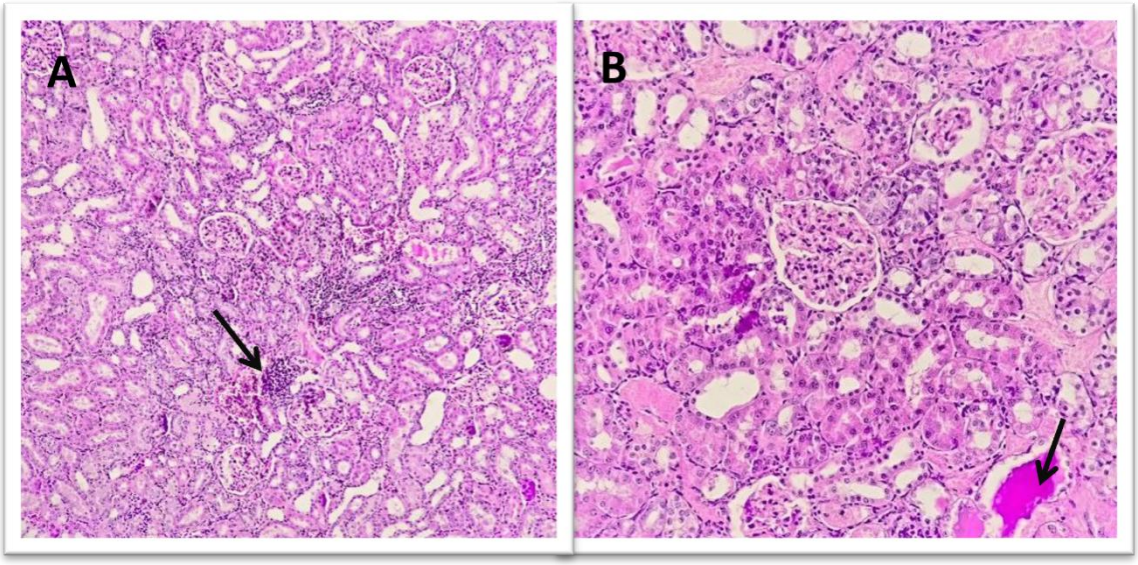

Fig. 3. Photomicrograph of Amikacine + Vit.D_3 treated group showing (A) focal small area of mononuclear cells infiltration (black arrow), (B) focal hyaline cast deposition (black arrow). (HE X 200).

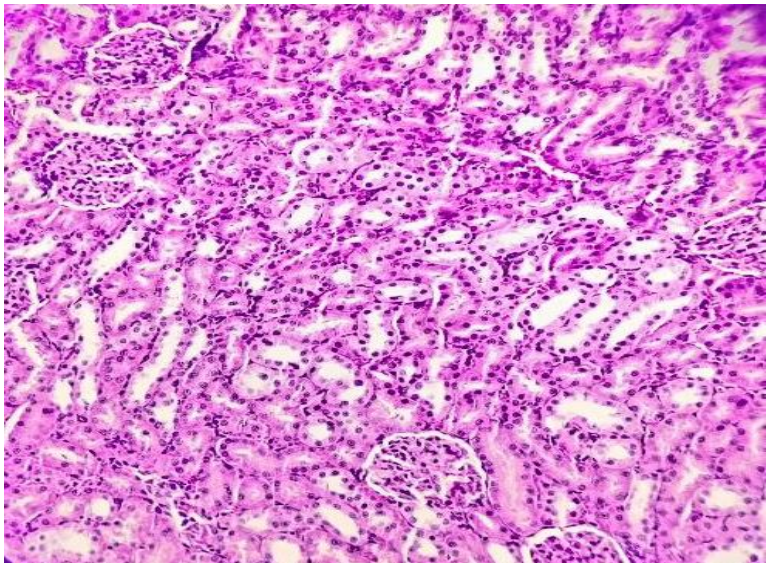

Fig. 4. Photomicrograph of Vit.D3 treated group showed normal histological structure of kidney (HE X 200).

Table 1
Semi-quantitative histopathological scores of renal lesions across experimental groups (mean ± SD).

Histopathological Parameter	Control	Amikacin	Vit D_3 + Amikacin	Vit D_3 alone	p-value
Glomerular atrophy	0.0 ± 0.0	3.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	< 0.001
Bowman's space dilation	0.0 ± 0.0	3.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	< 0.001
Tubular degeneration	0.0 ± 0.0	3.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	< 0.001
Tubular necrosis	0.0 ± 0.0	3.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	< 0.001
Interstitial inflammation	0.0 ± 0.0	3.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	< 0.001
Vascular congestion	0.0 ± 0.0	3.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	< 0.001

Scoring system: 0 = none; 1 = mild (<25%); 2 = moderate (25–50%); 3 = severe (>50% of examined fields).

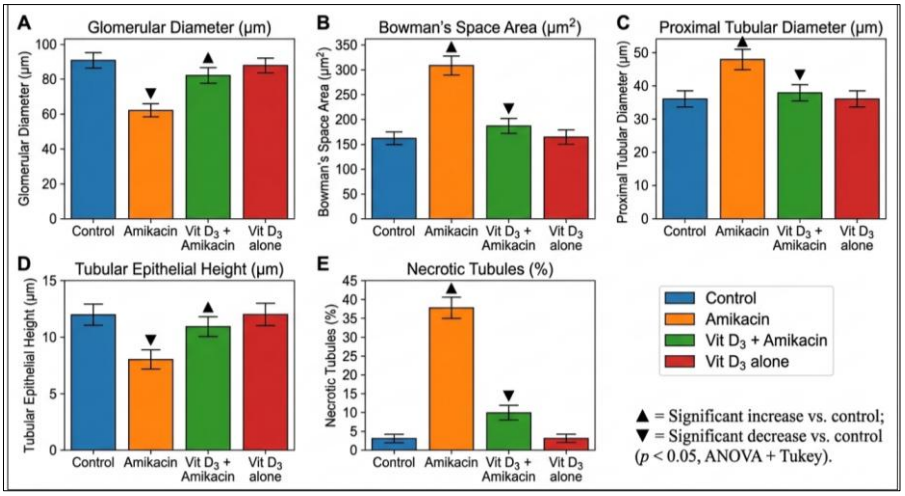

Fig. 5. Morphometric parameters of the renal cortex across different experimental groups. Data are presented as mean ± SD. The bar charts represent (A) Glomerular diameter, (B) Bowman's space area, (C) Proximal tubular diameter, (D) Tubular epithelial height, and (E) Percentage of necrotic tubules. (▲) indicates a significant increase compared to the control group, while (▼) indicates a significant decrease compared to the control group ($p < 0.05$, one-way ANOVA followed by Tukey's post hoc test).

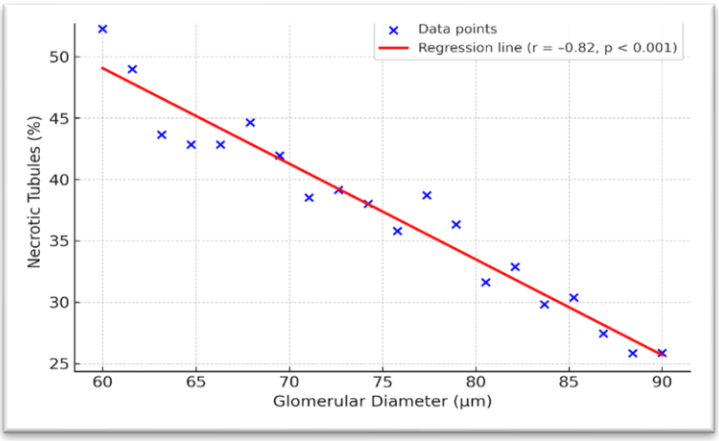

Fig. 6. Scatter plot showing a significant negative correlation between glomerular diameter and the number of necrotic tubules.

Quantitative Morphometric Findings

The quantitative morphometric assessment showed that different experimental groups had distinct and statistically significant differences in their measurements (Fig. 5). Amikacin caused a significant reduction in glomerular diameter (62.3 ± 3.9 µm) compared to control (89.6 ± 4.8 µm), while vitamin D_3 co-treatment increased it (82.4 ± 5.2 µm).

Bowman's space markedly increased in the amikacin group (308.1 ± 21 µm²) versus control (162.4 ± 15.7 µm²) and was reduced with vitamin D_3 + amikacin (186.9 ± 17.3 µm²). While Proximal tubular diameter increased with amikacin (47.9 ± 3.2 µm vs 35.7 ± 2.1 µm in control) and was reduced by vitamin D_3 (37.6 ± 2.4 µm). Tubular epithelial height decreased significantly in the amikacin group (8.1 ± 0.6 µm vs 12.3 ± 0.8 µm) and improved with vitamin D_3 co-treatment (11.2 ± 0.9 µm). Necrotic tubules were significantly increased in the amikacin group (38.4 ± 4.6%) compared to control (2.8 ± 0.5%), while vitamin D_3 co-treatment reduced this value (9.6 ± 1.3%) near control levels (3.1 ± 0.7%).

The combined administration of vitamin D_3 with amikacin attenuated the nephrotoxic effects and improved renal histological architecture. Glomerular dimensions and tubular epithelial thickness showed marked improvement compared with the amikacin group, while the number of necrotic tubules was significantly reduced ($p < 0.05$ vs. amikacin group). The vitamin D_3-only group demonstrated no significant differences from the control group ($p > 0.05$), indicating preservation of normal renal structure.

Correlation Between Morphometric and Histopathological Scores

The research established that glomerular diameter measurements negatively correlated with necrotic tubule percentages at a statistically significant level ($r = –0.82$, $p < 0.001$). indicating that smaller glomeruli existed with more extensive tissue damage. The histopathological lesion scores showed a strong positive correlation with Bowman's space area measurements ($r = 0.79$, $p < 0.01$), (Fig. 6).

DISCUSSION

Many drug-related adverse effects of different pharmacological compounds involve nephrotoxicity. This is especially evident on using aminoglycoside antibiotics including amikacin, gentamicin and tobramycin [31]. The bacterial infection elimination power of these agents remains limited because they tend to build up inside kidney proximal tubular epithelial cells [32]. This accumulation process leads to multiple oxidative, inflammatory and apoptotic reactions which cause acute tubular necrosis and glomerular damage [33].

Previous studies have shown that amikacin causes marked histological damage to the kidneys, including vacuolar degeneration and epithelial desquamation of tubular cells [34] and suggested that tubular necrosis is the primary cause of functional toxicity [35]. Others reported similar histological changes induced by gentamicin in the kidneys [36, 37]. These structural alterations are consistent with the findings of the present study and are explained by oxidative stress mechanisms, in which aminoglycosides disrupt lysosomes, generate reactive oxygen species, and induce apoptosis and further oxidative damage [16].

The morphometric outcomes of the present study provided a quantitative support to the histological observations. The destructive effect of amikacin on renal microarchitecture becomes evident through the combination of decreased glomerular diameter, decreased tubular epithelial height, the expansion of Bowman's space area and the number of necrotic tubules. The experimental results confirmed the histopathological results by showing direct tissue damage effect which proves that morphometric parameters work effectively for nephrotoxicity assessment.

The combination of vitamin D_3 with treatment resulted in decreased degenerative changes severity. The glomeruli returned to their typical structure while the tubular epithelium showed better survival rates and the amount of necrotic tissue decreased. Research studies have proven that vitamin D_3 protects kidneys through its antioxidant and anti-inflammatory properties when used in models of kidney damage caused by gentamicin, cadmium and vancomycin [24, 25, 26]. Mechanistic analyses indicates that this protective effect is mediated via activation of the VDR in renal tissue, initiating signaling pathways that enhance the production of endogenous antioxidant enzymes such as superoxide dismutase and catalase while reducing the production of pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6 [21, 22].

This combined antioxidant and anti-inflammatory response likely accounts for the morphometric recovery observed in the current study, as evidenced by the near-normal glomerular dimensions and epithelial height in vitamin D_3-treated rats. The present findings also align with other studies [38] who showed that vitamin D_3 protects kidneys through the activation of various cytoprotective signaling pathways and the suppression of inflammatory mediators, which prevents cell death. The results of this study suggest that these protective mechanisms operate during amikacin exposure to decrease oxidative DNA damage and lipid peroxidation in renal tissue.

The research achieves its benefits through the combination of quantitative morphometric evaluation with traditional histopathological examination methods. The morphometric method was used to measure glomerular and tubular dimensions with precision, which generated exact data about tissue preservation following vitamin D_3 administration. The study found a significant negative relationship between glomerular diameter measurements and tubular necrosis percentages ($r = -0.82$, $p < 0.001$), which demonstrates that greater structural integrity is strongly linked to a reduction in the extent of renal injury. The quantitative data confirmed the microscopic findings of architectural maintenance in the vitamin D_3–protected group, showing that vitamin D_3 antioxidants produce quantifiable nephroprotection in renal tissue.

The current findings support vitamin D_3's protective role against amikacin-induced renal injury when used as adjunct therapy. Proper supplementation during long-term aminoglycoside treatment may preserve kidney structure through VDR-mediated signaling that enhances the antioxidant and anti-inflammatory responses and minimize tissue damage.

Study Strengths and Limitations

The research provides a solid foundation through its morphological and biochemical results which will help future studies evaluate vitamin D_3 as a protective agent for kidney health. It combined histopathology with quantitative morphometric analysis to provide both descriptive and numerical data on vitamin D_3's protective effects on the kidneys. However, it had methodological limitations, including the absence of biochemical tests (serum urea, creatinine, antioxidant enzymes) and the lack of verification of VDR and Nrf2 pathways via immunohistochemistry, limiting confirmation of molecular mechanisms.

CONCLUSIONS

The study demonstrates that vitamin D_3 effectively attenuates amikacin-induced kidney damage by minimizing tubular necrosis and maintaining glomerular and tubular morphometric dimensions. The findings indicate that vitamin D_3 provides significant

nephroprotection, likely by counteracting the oxidative stress and inflammation associated with amikacin exposure, thereby preserving renal structural integrity. By combining quantitative morphometric analysis with traditional histopathology, the research provides an objective assessment of renal protection and suggests that vitamin D_3 may serve as a supportive option to minimize kidney injury during long-term aminoglycoside treatment.

Acknowledgments

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Conflict of Interest

None.

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Ethical Considerations

The study was approved by Medical Research Ethics Committee, College of Medicine, University of Mosul. Ref No. UOM/COM/MREC/23-24/JUL6.

Author Contribution

The author confirms sole responsibility for the study conception and design, experimental work, data collection, histopathological and morphometric analysis, and manuscript preparation.

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