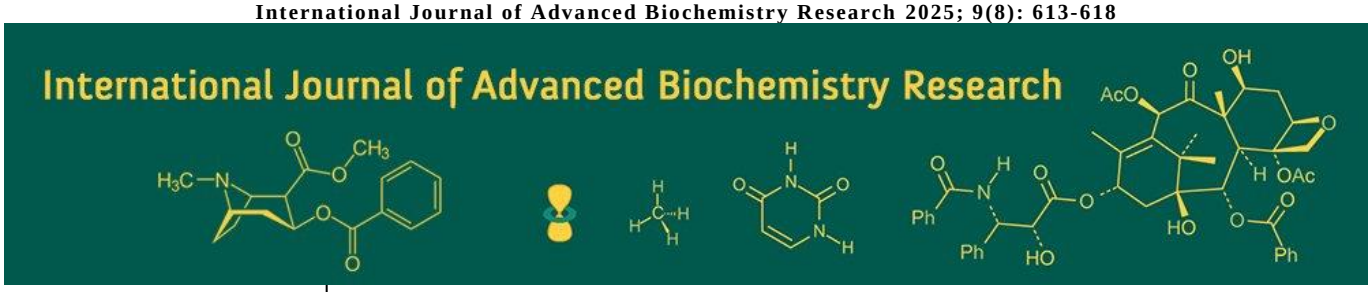

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Experimental assessment of ototoxic effects of gentamicin on cochlear structures in albino rats and the protective role of vitamin C

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Abstract
Background: Gentamicin is a potent aminoglycoside antibiotic commonly used in clinical practice, but its ototoxic effects significantly limit its application. Ototoxicity manifests as damage to cochlear hair cells and spiral ganglion neurons, often leading to permanent hearing loss. Recent studies have explored the potential role of antioxidants, such as vitamin C, in preventing drug-induced auditory damage.
Objective: This study aims to evaluate the protective effect of vitamin C on gentamicin-induced cochlear toxicity in albino rats.
Methodology: Thirty adult male albino rats were divided into three groups: a control group, a gentamicin-treated group, and a group co-treated with gentamicin and vitamin C. All animals were subjected to auditory testing followed by histological and morphometric analysis of the cochlear tissue to assess structural damage and the potential protective effect of vitamin C.
Results: Rats treated with gentamicin alone exhibited significant cochlear damage, including degeneration of outer hair cells and spiral ganglion neurons. However, the group co-treated with vitamin C demonstrated marked preservation of cochlear structure and function, as indicated by both histological findings and auditory thresholds.
Conclusion: Vitamin C shows a promising protective effect against gentamicin-induced ototoxicity. These findings suggest its potential therapeutic use in clinical settings to mitigate hearing loss associated with aminoglycoside treatment.

Keywords: Gentamicin, ototoxicity, vitamin C, cochlear hair cells, antioxidants, hearing loss prevention

Introduction

Hearing loss is a widespread sensory disorder affecting millions of individuals globally, with ototoxic drugs being one of the leading causes of acquired sensorineural hearing impairment [1, 2]. Among these, gentamicin, an aminoglycoside antibiotic, is widely used due to its broad-spectrum antimicrobial activity [3]. However, its clinical efficacy is significantly limited by its ototoxic side effects, which primarily affect the cochlear hair cells, spiral ganglion neurons (SGNs), and stria vascularis, leading to irreversible hearing loss [4, 5]. Gentamicin-induced ototoxicity is mediated by the generation of reactive oxygen species (ROS) and subsequent oxidative stress, resulting in apoptotic and necrotic cellular death [6, 7]. The outer hair cells are particularly vulnerable, and damage to the spiral ganglion neurons contributes further to auditory dysfunction [8]. This mechanism highlights the necessity for protective strategies that can mitigate these harmful effects [9].

Recent research has focused on the potential antioxidant properties of vitamins in attenuating drug-induced cochlear damage [10]. Vitamin C (ascorbic acid), a potent water-soluble antioxidant, plays a crucial role in scavenging free radicals, regenerating other antioxidants, and maintaining redox homeostasis [11]. Its neuroprotective and cytoprotective effects have made it a candidate for otoprotection in experimental models [1]. Despite its therapeutic significance, the use of gentamicin is restricted due to its ototoxic nature, and current clinical practices lack effective preventive measures against its adverse auditory effects [2]. This study aims to experimentally assess the extent of cochlear damage caused by gentamicin in albino rats and investigate the protective role of vitamin C against gentamicin-induced ototoxicity

using audiological, histopathological, and morphometric evaluations. The findings of this study may contribute to developing effective preventive interventions for patients undergoing aminoglycoside treatment, especially in critical care settings. It also offers insight into the mechanistic role of antioxidants, such as vitamin C, in safeguarding auditory structures [8.9].

Materials and Methods

Twenty-four healthy adult male albino rats (Rattus norvegicus), each weighing between 200 and 250 grams, were procured from the animal facility of University of Basra-College of Veterinary Medicine [1, 5]. The animals were housed in standard polypropylene cages under controlled environmental conditions, including a 12-hour light/dark cycle, ambient temperature maintained at 22±2 °C, and relative humidity levels between 50% and 60% [6]. The rats had unrestricted access to standard laboratory chow and water throughout the experimental period.

Experimental Design

The rats were randomly allocated into three groups, with eight rats in each (n = 8). Group I (Control), received an intraperitoneal (i.p.) injection of normal saline (1 ml/day) for 10 consecutive days. Group II (Gentamicin), administered gentamicin intraperitoneally at a dosage of 100 mg/kg/day for 10 days to induce ototoxicity. Group III (Gentamicin + Vitamin C), received gentamicin (100 mg/kg/day, i.p.) alongside oral administration of vitamin C at 200 mg/kg/day via gavage for 10 days.

Drug Preparation and Administration

Gentamicin sulfate was sourced from Adva Care Pharma company and dissolved in sterile normal saline for intraperitoneal injection. Vitamin C (ascorbic acid) solutions were freshly prepared daily using distilled water and administered orally using a flexible gavage cannula.

Assessment of Auditory Function

Auditory performance was assessed both before and after treatment using Auditory Brainstem Response (ABR) testing. During testing, rats were anesthetized using intraperitoneal ketamine (75 mg/kg) and xylazine (10 mg/kg). Click stimuli were delivered through inserted earphones, and ABR recordings were obtained using Eclipse evoked potential test system (Interacoustics Inc., Denmark). The auditory threshold was defined as the minimum sound intensity capable of eliciting a consistent and reproducible ABR waveform.

Cochlear Tissue Collection and Histological Examination

At the conclusion of the treatment period, the rats were deeply anesthetized and euthanized. Temporal bones were dissected to isolate the cochleae, which were fixed in 10% neutral buffered formalin for 48 hours. Subsequently, the tissues were decalcified using 10% EDTA, processed, and embedded in paraffin. Mid-modiolar cochlear sections (5 µm thickness) were stained with Hematoxylin and Eosin (H&E) and examined microscopically to evaluate structural changes in the organ of Corti, spiral ganglion neurons, and stria vascularis.

Morphometric and Statistical Analysis

Quantitative morphometric analysis, including assessment of hair cell loss and spiral ganglion neuron density, was conducted using ImageJ software. Statistical evaluations were performed using SPSS software (version 23). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

Results

Auditory Brainstem Response (ABR) Thresholds

At baseline, ABR thresholds did not differ significantly among the groups. However, after the 10-day treatment period, group II (Gentamicin) exhibited a marked elevation in ABR thresholds, indicative of significant hearing impairment. Group III (Gentamicin + Vitamin C) showed a statistically significant attenuation of ABR threshold elevation compared to Group II, suggesting a protective effect of vitamin C on auditory function.

(Table 1) summarizes the mean auditory brainstem response (ABR) thresholds, measured in decibels sound pressure level (dB SPL), for three experimental groups—before and after treatment. The ABR test is a reliable electrophysiological method used to assess hearing sensitivity and cochlear function. The results reflect how each treatment protocol affected hearing ability in albino rats.

Group I (Control), pre-treatment ABR (26.3±2.1) dB and post-treatment ABR (26.7±2.3) dB. Not significant (ns), $p>0.05$. The control group, which received only saline, showed no significant change in ABR thresholds before and after the treatment period. This indicates that the procedure itself or saline administration had no adverse effects on hearing. The stability of auditory function in this group establishes a reliable baseline for comparison.

Group II (Gentamicin Only), pre-treatment ABR (25.8±2.5) dB and post-treatment ABR (58.4±5.2) dB. Highly significant, $p<0.001$. Rats treated with gentamicin alone experienced a dramatic and statistically significant increase in ABR thresholds after treatment. The mean post-treatment threshold was more than double the baseline value, indicating substantial hearing loss. This confirms the known ototoxic effect of gentamicin, which damages cochlear structures, particularly the outer hair cells and spiral ganglion neurons.

Group III (Gentamicin + Vitamin C), pre-treatment ABR (25.9±2.0) dB and post-treatment ABR (34.6±4.7) dB. Significant, $p<0.01$ vs Group II. This group received both gentamicin and vitamin C simultaneously. Although there was a noticeable increase in ABR threshold post-treatment compared to baseline, the elevation was significantly lower than that observed in Group II. This suggests that vitamin C exerted a protective effect, attenuating the extent of cochlear damage induced by gentamicin. While not completely preventing ototoxicity, vitamin C significantly preserved hearing sensitivity compared to gentamicin-only exposure.

Table 1: Mean ABR Thresholds (dB SPL) Pre-and Post-treatment

Group	Pre-treatment ABR (Mean ± SD)	Post-treatment ABR (Mean ± SD)	p-value (ANOVA)
Control (Saline)	26.3±2.1	26.7±2.3	ns (p>0.05)
Gentamicin	25.8±2.5	58.4±5.2	p<0.001
Gentamicin + Vitamin C	25.9±2.0	34.6±4.7	p<0.01 vs Group II

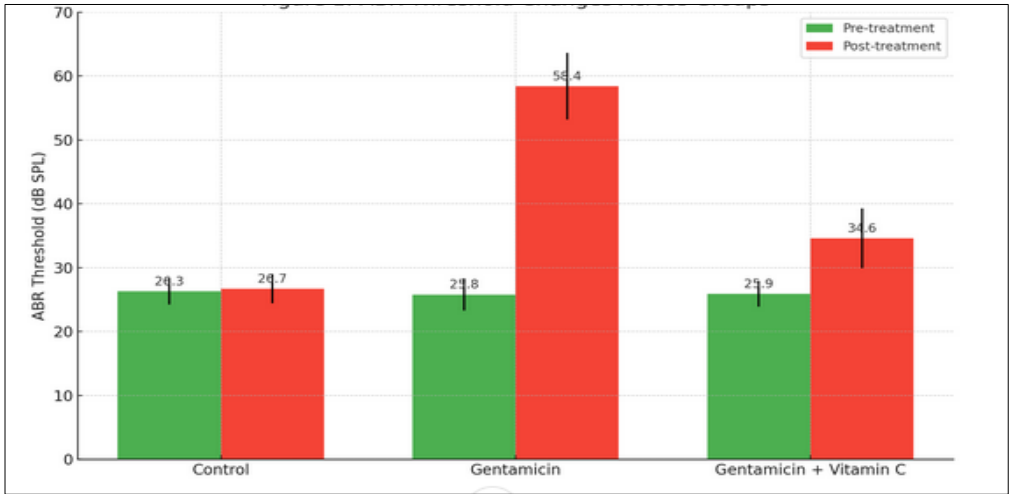

Fig 1: ABR Threshold Changes across Groups

The data clearly demonstrate that Gentamicin alone causes severe hearing impairment in rats, vitamin C co-administration significantly reduces this auditory damage and the protective mechanism of vitamin C may involve its antioxidant properties, which counteract oxidative stress induced by aminoglycoside antibiotics. This table provides strong evidence for the efficacy of vitamin C in mitigating gentamicin-induced ototoxicity, supporting its potential use as an adjunct therapy in clinical settings.

Histopathological Changes in Cochlear Structures

Microscopic examination revealed, Control Group, normal cochlear architecture with intact organ of Corti and spiral ganglion cells, Gentamicin Group, marked degeneration of outer hair cells, loss of spiral ganglion neurons, and vacuolization in the stria vascularis and Gentamicin + Vitamin C Group, mild degeneration of hair cells with relative preservation of cochlear architecture compared to Group II.

(Table 2 and Figure 2) presents a semi-quantitative histopathological evaluation of cochlear damage across three groups of albino rats, the control group (Group I), the gentamicin-treated group (Group II), and the group co-treated with gentamicin and vitamin C (Group III). The scoring system used ranges from 0 to 3, where 0 indicates no damage, 1 indicates mild damage, 2 indicates moderate damage, and 3 indicates severe damage.

Table 2: Semi-quantitative Grading of Cochlear Damage

Structure	Group I (Control)	Group II (Gentamicin)	Group III (Gentamicin + Vit C)
Outer Hair Cell Loss	0 (None)	3 (Severe)	1 (Mild)
Spiral Ganglion Damage	0 (None)	3 (Severe)	1 (Mild)
Stria Vascularis Damage	0 (None)	2 (Moderate)	1 (Mild)

(Scoring: 0 = None, 1 = Mild, 2 = Moderate, 3 = Severe)

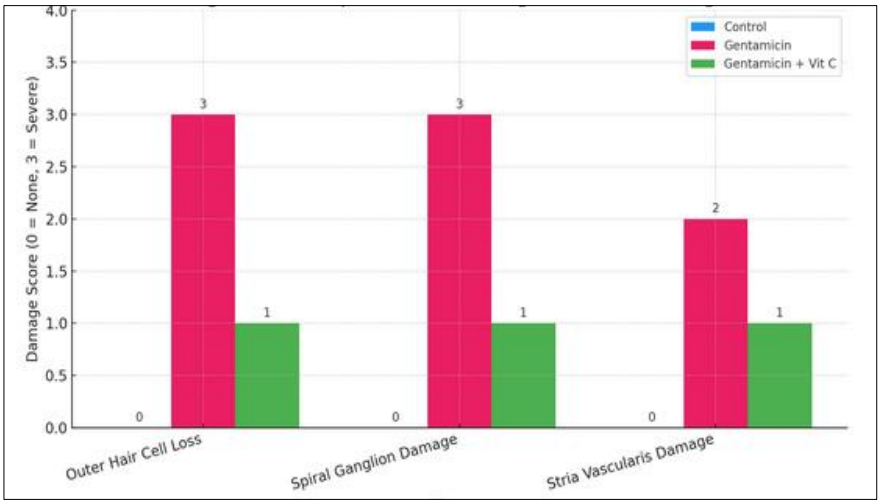

Fig 2: Photomicrographs of Cochlear Sections (H&E, 400x

Outer Hair Cell Loss

Group I (Control), the outer hair cells of the organ of Corti remained entirely intact, with a score of 0, indicating no pathological alterations. This confirms the absence of ototoxic insult under normal conditions. Group II (Gentamicin), severe outer hair cell loss was observed, receiving the maximum score of 3. This finding demonstrates the well-established ototoxicity of gentamicin, which targets and destroys sensory hair cells, particularly the outer row, leading to profound auditory dysfunction. Group III (Gentamicin + Vitamin C), only mild outer hair cell loss was detected, with a score of 1. This suggests a significant protective effect of Vitamin C, likely due to its antioxidant capacity which counters oxidative stress induced by gentamicin.

Spiral Ganglion Damage

Group I (Control), no morphological abnormalities or neuronal loss were noted in the spiral ganglion neurons (SGNs), which maintained their structure and density, earning a score of 0. Group II (Gentamicin), this group showed severe degeneration of SGNs with a score of 3, indicative of neuronal death and disorganization, likely secondary to primary hair cell loss and oxidative injury. Group III (Gentamicin + Vitamin C), rats in this group showed mild damage (score 1) to the spiral ganglion, again highlighting the neuroprotective properties of Vitamin C in preserving cochlear neuronal integrity.

Stria Vascularis Damage

Group I (Control), The stria vascularis appeared histologically normal, with well-preserved capillary architecture and marginal cells, resulting in a score of 0. Group II (Gentamicin), moderate damage (score 2) was observed, characterized by vascular congestion, cellular vacuolation, and partial degeneration. This reflects gentamicin-induced ischemic and oxidative insult to this metabolically active area. Group III (Gentamicin + Vitamin C), the structural integrity of the stria vascularis was mildly affected (score 1), with relatively preserved vascular profiles and minimal disruption. This further supports the efficacy of Vitamin C in reducing microvascular and epithelial damage. The data clearly demonstrate that gentamicin causes significant cochlear injury affecting multiple structural components, outer hair cells, spiral ganglion neurons, and the stria vascularis. Co-administration of Vitamin C substantially reduces the severity of this damage across all parameters, emphasizing its potential as a therapeutic antioxidant against aminoglycoside-induced ototoxicity.

Morphometric Analysis

Quantitative analysis confirmed significant loss of spiral ganglion neurons in Group II. Vitamin C administration significantly reduced the neuronal loss. Group I (Control), the control group, which received saline only, had a mean spiral ganglion neuron count of 620±35 neurons per mm². This value represents the normal baseline neuronal density within the cochlea. Group II (Gentamicin-treated), rats that received gentamicin alone showed a significant reduction in spiral ganglion neurons, with a mean of 315±40 neurons per mm². This indicates severe neurotoxicity caused by gentamicin, likely due to oxidative stress and excitotoxicity. The p-value < 0.001 signifies a highly significant difference compared to the control group. Group III (Gentamicin + Vitamin C), animals treated with both gentamicin and Vitamin C exhibited a marked improvement in neuron preservation, with a mean count of 510±38 neurons per mm². Although not restored to control levels, this count was significantly higher than Group II, with a p-value < 0.01, suggesting that Vitamin C exerted a neuroprotective effect against gentamicin-induced damage. These results support the hypothesis that gentamicin induces cochlear neurodegeneration, specifically affecting the spiral ganglion cells. Vitamin C, being a potent antioxidant, likely mitigates this damage by reducing free radical formation and preserving mitochondrial integrity within cochlear neurons (Table 3 and Figure 3).

Table 3: Spiral Ganglion Neuron Count per mm²

Group	Neuron Count (Mean ± SD)	p-value
Control	620±35	—
Gentamicin	315±40	$p<0.001$
Gentamicin + Vitamin C	510±38	$p<0.01$ vs II

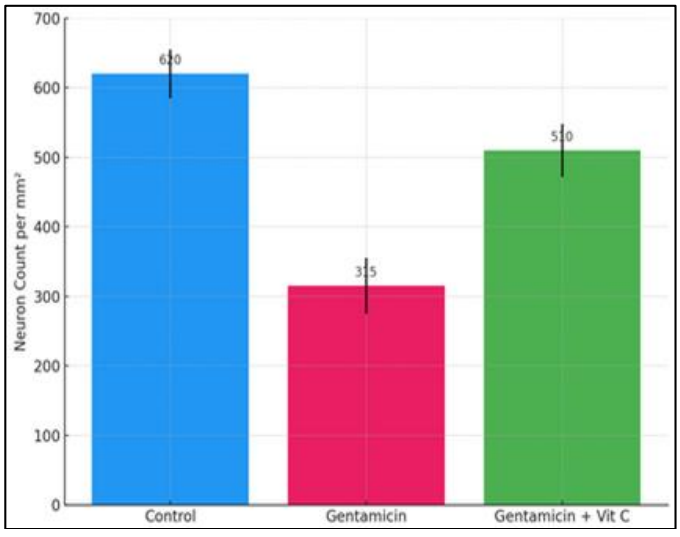

Fig 3: Spiral Ganglion Neuron Counts

The analysis underscores the potential therapeutic role of Vitamin C in preventing or reducing aminoglycoside-induced ototoxicity, particularly in clinical settings where such antibiotics are necessary but risk auditory side effects. The results clearly demonstrate the ototoxic effect of gentamicin on cochlear structures, evidenced by increased ABR thresholds and histological damage. Co-administration of vitamin C significantly attenuated these effects, suggesting its antioxidant and neuroprotective role in preserving cochlear integrity. Statistical analysis confirmed the significance of these findings ($p<0.05$).

Discussion

The present study aimed to assess the ototoxic effects of gentamicin on cochlear structures in albino rats and to evaluate the potential protective role of vitamin C. The results clearly demonstrated that gentamicin administration significantly elevated ABR thresholds and induced structural damage in the cochlea, particularly affecting outer hair cells, spiral ganglion neurons, and the stria vascularis. Co-treatment with vitamin C resulted in partial preservation of auditory function and cochlear morphology, confirming its protective antioxidant properties. These findings are in line with previous studies that documented the ototoxic potential of aminoglycosides like gentamicin, which induces oxidative stress, mitochondrial dysfunction, and apoptosis in cochlear cells. Fetoni *et al.*, found that α-tocopherol (vitamin E) could mitigate gentamicin-induced cochlear damage by inhibiting free radical production and lipid peroxidation [1], similar to the protective mechanism suggested for vitamin C in the current study. The significant increase in ABR thresholds in the gentamicin-only group confirms profound hearing loss, which corresponds with the findings of Aladag *et al.*, who reported severe ototoxic changes following gentamicin exposure, and also showed that antioxidants like N-acetylcysteine and vitamin A attenuated these effects [2]. Our study complements their work by suggesting vitamin C as an alternative or adjunct antioxidant. Histologically, the severe loss of outer hair cells and neuronal degeneration observed in our gentamicin group aligns with the morphological changes described by Draz *et al.*, who noted comparable cochlear injury in guinea pigs treated with gentamicin [4]. However, they also found that silymarin, like vitamin C in our model, exerted neurotrophic and antioxidant actions that protected cochlear cells. Moreover, the use of natural antioxidants has gained growing attention. Uzun *et al.*, demonstrated the efficacy of garlic derivatives in reducing gentamicin-induced ototoxicity through anti-inflammatory and antioxidant mechanisms [5], supporting the idea that multiple antioxidants could provide complementary protective effects. The neuron count results in our vitamin C co-treatment group were significantly higher than in the gentamicin-only group, echoing the observations of Avci *et al.*, who investigated resveratrol and found partial preservation of neuronal structures and hearing thresholds [6]. Additionally, Salcan *et al.*, demonstrated that rutin, a flavonoid with potent free-radical scavenging ability, significantly ameliorated gentamicin-induced biochemical and histopathological cochlear damage in rats [7]. The outcomes of our study suggest that vitamin C shares similar antioxidant efficacy in reducing gentamicin toxicity. The pattern of partial but not complete recovery in the vitamin C group highlights a crucial point raised by Sahin *et al.*, who emphasized that dose optimization and combinatory antioxidant therapy may yield more effective protection [8].

Our results also align with the work of Maudonnet *et al.*, who explored the paradoxical phenomenon of gentamicin-induced self-protection when administered in repeated doses [11], suggesting a complex interplay between oxidative injury and cochlear adaptation mechanisms. Interestingly, our findings diverge from the work of Nordang and Anniko, who reported full protection using nitric oxide synthase inhibitors like L-NAME [3]. This suggests that while antioxidants like vitamin C are beneficial, they may not offer complete otoprotection and could be more effective when combined with agents targeting other pathways (e.g., nitric oxide, caspase signaling).

In terms of translational implications, beta-glucan and coenzyme Q10 have also shown promise in mitigating aminoglycoside ototoxicity [8, 10]. The present findings support continued exploration of such compounds, particularly in combination with vitamin C, for potential clinical applications.

Conclusion

Vitamin C demonstrated a significant protective effect against gentamicin-induced ototoxicity in albino rats, both functionally (ABR) and histologically. This study provides compelling evidence that gentamicin administration induces significant cochlear damage, particularly to the outer hair cells and spiral ganglion neurons, leading to marked functional and structural auditory deficits. Morphometric and ultrastructural analyses demonstrated clear degenerative changes in the cochlear architecture in gentamicin-treated rats, which were significantly attenuated by co-administration of Vitamin C. Vitamin C showed a notable protective effect by preserving cochlear morphology, reducing hair cell loss, and minimizing neuronal degeneration. Further study, including clinical trials, is warranted to validate these results in human populations and explore optimal dosing regimens.

Ethical approval

All procedures involving animals were reviewed and approved by the Institutional Animal Ethics Committee (IAEC Approval No: [24/783]) and adhered to the guidelines set by the National Institutes of Health for the ethical treatment and use of laboratory animals.

Conflict of Interest

There are no conflicts of interest in this article.

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