ENT Updates

ORIGINAL ARTICLE

ENT UPDATES 11(1):8-13
DOI: 10.5152/entupdates.2021.887158

Effect of Rutin on Gentamicin-Induced Ototoxicity in Rats: A Biochemical and Histopathological Examination

Abstract

Objective: Gentamicin is a broad-spectrum aminoglycoside antibiotic administered parenterally for moderate to severe gram-negative infections. Ototoxicity is an important side effect that limits gentamicin use. The aim of this study is to investigate the effect of rutin on gentamicin-induced ototoxicity in rats biochemically and histopathologically.

Methods: Distilled water was administered by oral gavage to healthy controls (HG) and cobalt administered group (GC). 50 mg/kg rutin was administered by oral gavage to rutin + gentamicin (RGG) group. After one hour, 100 mg/kg gentamicin was injected intraperitoneally (i.p) to the RGG and GC animal groups. This procedure was repeated once a day for 14 days.

Results: Malondialdehyde (MDA), nuclear factor-κB(NF-κB), tumor necrosis factor alpha (TNF-α) and interleukin-1 beta(IL-1β) levels in the cochlear nerve tissue of gentamicin-treated animals were significantly higher compared to healthy controls and rutin + gentamicin treated rats. On the other hand, the amount of total Glutathione (tGSH) was significantly lower compared to the control and rutin group. Histopathological examination revealed degenerated myelinated nerve fibers in the gentamicin group and Schwann cell nuclei were generally not seen. There was a high accumulation of collagen fiber in the tissue and dilated blood capillaries. In the rutin group, myelinated nerve fibers mostly exhibited normal morphology, Schwann cell nuclei were evident and the vessels were normal.

Conclusion: Our experimental results suggest that rutin may be effective in the treatment of gentamicin-associated ototoxicity.

Keywords: Biochemical, gentamicin, histopathological, ototoxicity, rutin

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Cite this article as: Salcan İ, Dilber M, Bayram R, Süleyman E, Yazıcı GN, Çoban A, Süleyman H. Effect of Rutin on Gentamicin-Induced Ototoxicity in Rats: A Biochemical and Histopathological Examination. *ENT-Updates*. 2021; 11(1): 8-13

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Received: *February 26, 2021*
Accepted: *March 5, 2021*

Introduction

Gentamicin is a broad-spectrum aminoglycoside antibiotic that is administered parenterally for moderate to severe gram-negative infections. Gentamycin has broad bacteriocidal activity against many aerobic gram negative and some aerobic gram positive organisms. As other aminoglycosides, gentamicin is thought to act by bounding to bacterial ribosomes and inhibiting protein synthesis. Common side effects of gentamicin are dizziness, headache, confusion, nausea, and skin rash.[1] Important dose-related adverse effects are oto- and nephrotoxicity for all aminoglycosides.[2] High concentrations of aminoglycosides are found mainly in renal tubular cells and inner ear hair cells, further explaining the cause of nephro- and ototoxicity.[1] Aminoglycoside-related hair cell degeneration is one of the main causes of hearing loss in millions of people worldwide. Overproduction of reactive oxygen species (ROS) has been shown to play a role in the pathogenesis of this ototoxicity of aminoglycosides.[3] Furthermore, studies have shown that proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) are the major components on the basis of gentamicin ototoxicity.[4]

Rutin (3,3,4,5,7-pentahydroxyflavone-3-rhamnoglucoside) to be investigated in this study for its protective effect against gentamicin-induced ototoxicity is a vitamin P_1 flavonoid.[5] In addition, rutin has been shown to inhibit adhesion and migration of leukocytes, maintain vascular barrier integrity, and reduce hyperpermeability.[6] Rutin has been found to be

effective against edema that occurs through increased capillary permeability and the leakage of plasma into the tissue.[7] It has been reported that rutin has many properties such as antioxidant, anticancer, antidiabetic, antimicrobial, antiulcer and tissue regenerative properties.[8,9] Further studies have shown that rutin suppresses TNF-α and nuclear factor-κB (NF-κB) production, which are known to be proinflammatory molecules.[6] In the study of Taşlı NG et al., it is stated that the protective effect of rutin on tissue is due to its antioxidant, anti TNF-α and anti-IL-1β properties .[10] All this information suggests that rutin may be effective in the treatment of gentamicin-induced ototoxicity. There are no studies in the literature investigating the effect of rutin against gentamicin-induced ototoxicity. Therefore, the aim of this study is to investigate the effect of rutin against gentamicin-induced ototoxicity in rats using biochemical and histopathological methods.

Methods

Animals

A total of 24 male albino Wistar male rats weighing 290-300 grams were obtained from the medical experimental research center of Ataturk University to be used in this study. The approval for the research has been received from the local animal care committee in the Ataturk University (26.12.2019-256). The animals were divided into three groups prior to the experiment and fed at normal room temperature (22°C).

Chemical Substances

Gentamicin (2 mL ampoule -80 mg) was supplied from KOCAK Farma (Turkey), thiopental sodium was supplied from I.E. Ulagay (Turkey), and rutin was supplied from Solgar (United States).

Experimental Groups

Experimental animals were divided into healthy control (HG), rutin + gentamicin (RGG), and gentamicin control (GC) groups.

Experimental Procedure

50 mg/kg rutin was administered by oral gavage to RGG (n-8) and GC (n-8) animals, and equal volume of distilled water was administered to the HG (n-8) group. One hour after administration of rutin and distilled water, gentamicin was injected intraperitoneally (i.p) into the RGG and GC animals at a dose of 100 mg/kg .[11] This procedure was repeated once a day for 14 days. At the end of this period, animals were killed by high dose anesthesia (50 mg/kg thiopental sodium) and their inner ears were removed. Biochemical and histopathological examinations were performed on the inner ear cochlear nerve tissue. The biochemical and histopathological results obtained from the RGG and HG group were compared with the results from the GC group.

Biochemical Analysis

Prior to dissection, all tissues were rinsed with phosphate buffered saline solution. The tissues were homogenized in ice-cold phosphate buffers (50 mM, pH 7,4) that were appropriate for the variable to be measured. The tissue homogenates were centrifuged at 5,000 rpm for 20 min at 4°C, and the supernatants were extracted to analyze total Glutathione (tGSH) and MDA. All tissue results were expressed by dividing to g protein. All spectrophometric measurements were performed via microplate reader (Bio-Tek, USA).

Malondialdehyde (MDA) Analysis

MDA measurements were based on the method used by Ohkawa et al.,[12] involving spectrophotometrical measurement of absorbance of the pink-colored complex formed by thiobarbituric acid (TBA) and MDA. The tissue-homogenate sample (25 µL) was added to a solution containing 25 µL of 80 g/L sodium dodecyl sulfate and 1 mL mixture solution (200 g/L acetic acid + 1.5 mL of 8 g/L 2-thiobarbiturate . The mixture was incubated at 95 ^{0}C for 1 h. Upon cooling, 1 mL of n-butanol: pyridine (15:1) was added. The mixture was vortexed for 1 min and centrifuged for 10 min at 4000 rpm. The absorbance of the supernatant was measured at 532 nm. The Standard curve was obtained by using 1,1,3,3-tetramethoxypropane.

Total Glutathione (tGSH) Analysis

According to the method defined by Sedlak J and Lindsay RH. DTNB (5,5′-dithiobis [2-nitrobenzoic acid]) disulfate is chromogenic in the medium, and DTNB is reduced easily by sulfhydryl groups .[13] The yellow color produced during the reduction is measured by spectrophotometry at 412 nm. For measurement, a cocktail solution (5.85 mL 100 mM Na-phosphate buffer, 2.8 mL 1 mM DTNB 3.75 mL 1 mM NADPH, and 80 µL 625 U/L Glutathione reductase was prepared. Before measurement, 0.1 mL meta-phosphoric acid was added to 0.1 mL tissue-homogenate and centrifuged for 2 min at 2000 rpm for deproteinization. The 0.15 mL cocktail solution was added to 50 µL of supernatant. The Standard curve was obtained by using GSSG.

NF-ƘB, TNF-α and IL-1β Analysis

Tissue-homogenate NF-ƘB and TNF-α concentrations were measured using rat-specific sandwich enzyme-linked immunosorbent assay. Rat NF-ƘB ELISA immunoassay kits (Cat. No:201-11-0288, SunRed). Rat TNF-α and Rat IL-1β ELISA kits (Cat no: YHB1098Ra, Shanghai LZ). Analyses were performed according to the manufacturers' instructions. Briefly, monoclonal antibody specific for rat NF-ƘB, TNF-α and IL-1β were coated onto the wells of the micro plates. The tissue homogenate, standards and biotinylated monoclonal antibody specific and streptavidin-HRP were pipetted into these wells and then incubated at 37C^{0} for 60 min. After washing, chromogen reagent A and chromogen reagent B were added, acting upon the bound enzyme to produce a color. It was incubated at 37 C^{0} for 10 min. Then stop solution was added. The intensity of this colored product is directly proportional to the concentration of rat NF-ƘB, TNF-α and IL-1β present in the original specimen. At the end of the course, the well plates were read at 450 nm The absorbance of the samples was calculated with formulas using standard graphics.

Histopathological Examination

Tissue samples were taken into 10% formaldehyde solution and identified for 72 hours. After identification, the tissues were taken onto cassette and washed under tap water for 24 hours. Tissues were then dehydrated by being passed through increasing alcohol series (70%, 80%, 90% and 100%). Testicular tissues made transparent in xylol were embedded in paraffin blocks and 4-5 micron thick sections were taken. The sections were stained with Hematoxylin - Eosin double staining and evaluated and photographed using Olympus DP2-SAL firmware program (Olympus® Inc. Tokyo, Japan). Histopathological evaluation was performed

by a histologist blinded to the study groups. The histopathological damage severity in each ovarian tissue section was scored between grade 0-3 (0-normal, 1-mild damage, 2-moderate damage, and 3-severe damage).

Statistical Analysis

For statistical analysis, IBM SPSS 22 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) was used. The results were presented as mean ± standard deviation (SD). The normality assumption of variables were confirmed with the Kolmogorov- Smirnov test. All biochemical measurements were normally distributed and for comparison between groups Anova was used. The assumption of homogeneity of variances were met for all variables. Tukey's HSD tests was used as post-hoc test. For all tests $P < 0.05$ was considered as statistically significant.

Results

I-Biochemical Results:

MDA Analysis Results

The amount of MDA in the cochlear nerve tissue of the animal group administered with gentamicin alone was significantly higher compared to the healthy and rutin group ($P < 0.001$, $P < 0.001$, respectively). Rutin significantly prevented the increase in MDA due to gentamicin. There was no significant difference in the amount of MDA between the healthy and rutin group ($P = 0.060$) (Figure 1).

tGSH Analysis Results

As can be seen in Figure 2, the amount of tGSH in the cochlear nerve tissue of the animal group administered with gentamicin alone was significantly lower compared to the healthy and rutin group ($P < 0.001$, $P < 0.001$, respectively). Rutin significantly prevented the decrease in tGSH induced by gentamicin. There was no significant difference in the amount of tGSH between the healthy and rutin group ($P = 0.058$).

NF-ĸB Analysis Results

The amount of NF-ĸB in the cochlear nerve tissue of the animal group administered with gentamicin alone was significantly higher compared to the healthy and rutin group ($P < 0.001$, $P < 0.001$, respectively). Although rutin decreased NF-ĸB levels that were elevated due to gentamycin, it could not bring it down to the same level with the healthy group ($P < 0.001$) (Figure 3).

TNF-α Analysis Results

The amount of TNF-α in the cochlear nerve tissue of the animal group administered with gentamicin alone was significantly higher compared to the healthy and rutin group ($P < 0.001$, $P < 0.001$, respectively). Although rutin decreased TNF-α levels that were elevated due to gentamycin, it could not bring it down to the same level with the healthy group ($P < 0.001$) (Figure 4).

IL-1β Analysis Results

The amount of IL-1β in the cochlear nerve tissue of the animal group administered with gentamicin alone was significantly higher compared to the healthy and rutin group ($P < 0.001$, $P < 0.001$, respectively). IL-1β levels were similar between the healthy controls and the rutin group ($P = 0.102$) (Figure 5).

II- Histopathological Findings:

When the cochlear nerve tissue sections taken from the healthy control (HG) group were evaluated histopathologically, myelinated nerve fibers displayed a normal histological structure with Schwann cell nuclei and supportive tissue around it (Figure 6). When the sections taken from the gentamicin (GC) group were evaluated, it was observed that myelinated nerve fibers were severely swollen and edematous, axons had generally lost their central localization, and when most nerve fibers were evaluated, it was found that they lost their Schwann cell connections. Collagen accumulation and dilatation of blood capillaries was noteworthy (Figure 7). His-

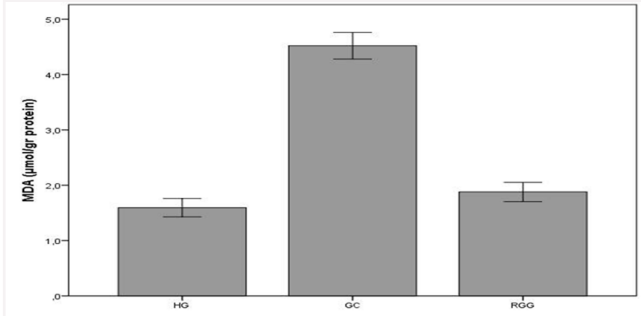

Figure 1. The amount of MDA in the cochlear nerve tissue of the HG, GG, and RGG groups

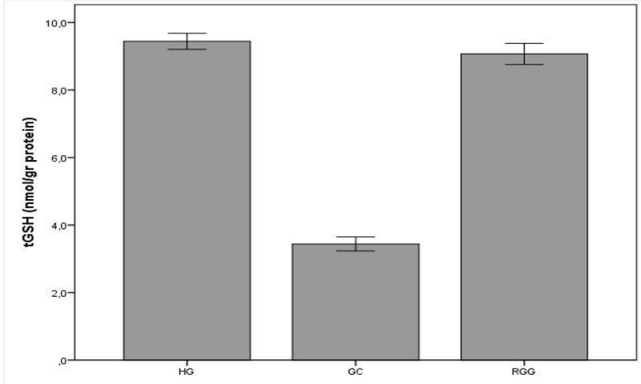

Figure 2. The amount of tGSH in the cochlear nerve tissue of the HG, GG, and RGG groups

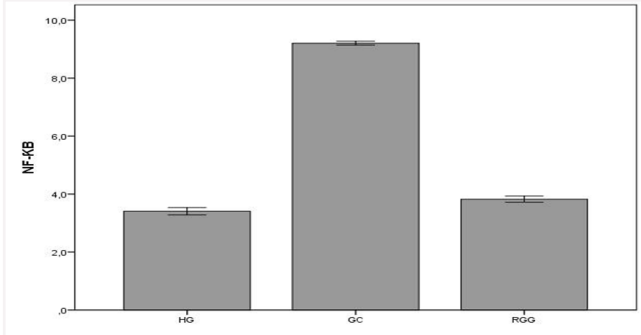

Figure 3. The amount of NF-KB in the cochlear nerve tissue of the HG, GG, and RGG groups

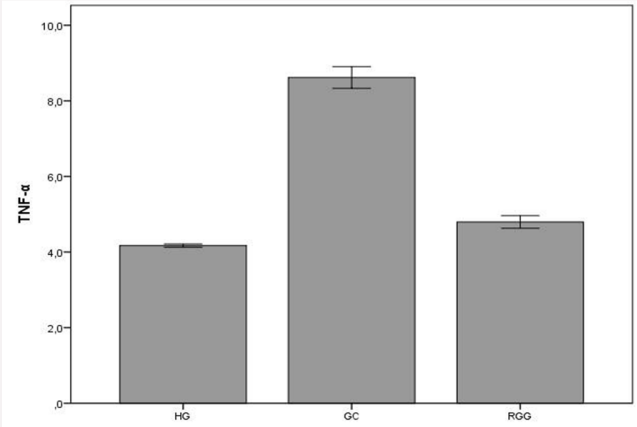

Figure 4. The amount of TNF-α in the cochlear nerve tissue of the HG, GG, and RGG groups

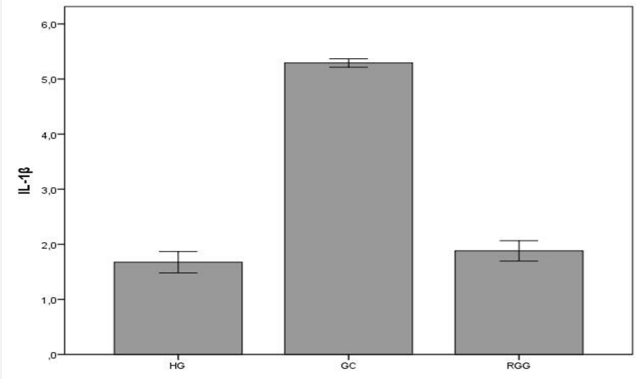

Figure 5. The amount of IL-1β in the cochlear nerve tissue of the HG, GG, and RGG groups

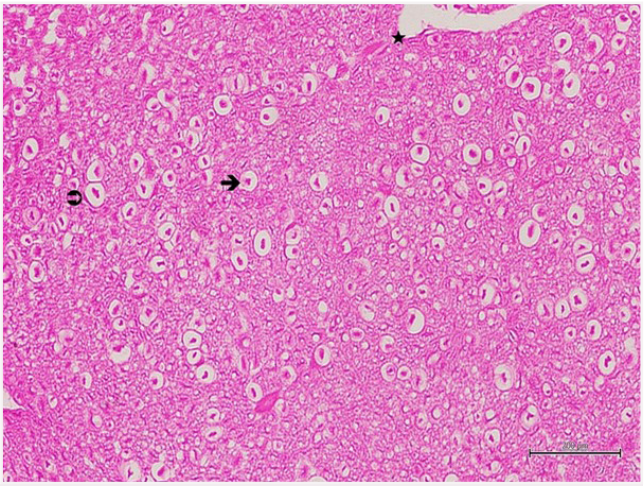

Figure 6. Hematoxylin - Eosin staining in the cochlear nerve tissue of the healthy control (HG) group. ➔: myelinated axon; ➲: Schwann cell; ★: blood vessel (HEx400)

topathological damage was milder in the treatment group, and it was found that the overall appearance of the tissue was similar to the control group (Figure 8).

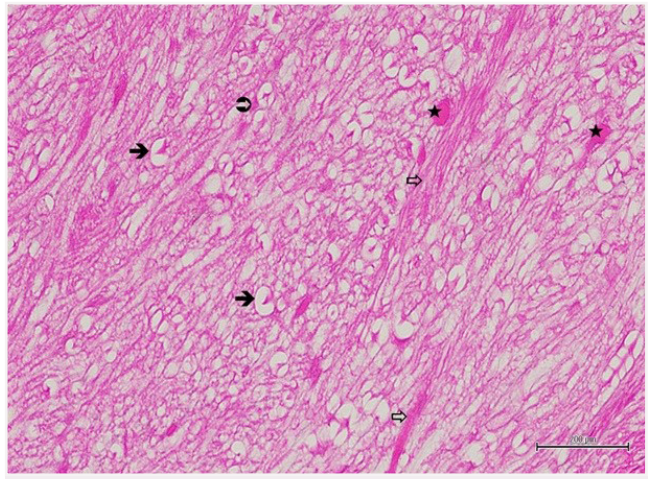

Figure 7. Hematoxylin - Eosin staining in the cochlear nerve tissue of the gentamicin control (GC) group. ➔: degenerate and edematous myelinated axon; ➲ : degenerate Schwann cell; ⇨: collagen deposition; ★: dilated blood vessel (HE x400)

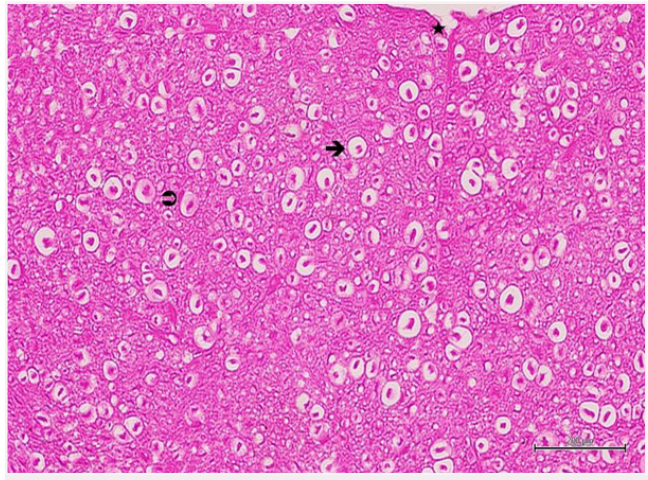

Figure 8. Hematoxylin - Eosin staining in the cochlear nerve tissue of the rutin-treated (RGG) group. ➔: myelinated axon; ➲: Schwann cell; ★: blood vessel (HE x400)

Discussion

The effect of rutin on gentamicin-induced ototoxicity in rats was investigated in this study. To assess the ototoxic effect of gentamicin, the cochlear nerve tissue was examined biochemically and histopathologically. Ototoxicity is also defined as degeneration of the inner ear structures (cochlear and vestibular) for various reasons and disruption of their functions.[14] As known, ototoxicity still continues to be an important cause of sensorineural hearing loss.[15] Excessive ROS formation accumulating in the cochlea and leading to cell death is held responsible for the pathogenesis of ototoxicity.[16] As shown by our biochemical results, an increase in the amount of MDA, which is the final product of ROS related lipid peroxidation (LPO) reaction, was observed in the cochlear nerve tissue of gentamicin treated animals. MDA is an oxidant molecule that causes crosslinking and polymerization of membrane components, maintaining and increasing LPO damage.[17] Wang AM et al. [18] reported

that the LPO event occurring with ROS plays a role in gentamicin-induced cocheotoxicity. Draz El et al. [19] experimentally demonstrated that one of the important factors of gentamicin-induced ototoxicity is MDA. The amount of MDA in the rutin administered group was similar to that of the healthy controls. This indicates that rutin is preventing gentamicin-induced increase in MDA production. There are no studies in the literature investigating the effect of rutin on MDA levels increased by gentamicin in cochlear nerve tissue. However, there are studies showing that it inhibits gentamicin-associated MDA increase in kidney tissue and protects kidney tissue from oxidative damage.[20] This shows that our experimental results are supported by previous studies.

In the present study, there was also a significant decrease in the amount of tGSH in the gentamicin group, which is commonly used to evaluate antioxidant activity in the cochlear nerve tissue. As it is known, GSH is one of the important endogenous antioxidant molecules in protecting tissue integrity against ROS and maintaining tissue functions at normal levels [21]. However, if antioxidants can no longer neutralize oxidants sufficiently, the balance between oxidants and antioxidants is disrupted in favor of oxidants.[22] Koçak I et al. [11] showed that oxidant/antioxidant balance changes in favor of oxidants in gentamicin-induced ototoxicity. Disruption of this balance in favor of oxidants leads to tissue damage, which is expressed as oxidative stress.[23] As can be seen from our experimental results, while the oxidant/antioxidant balance was in favor of oxidants in the gentamicin group, it was shifted in favor of antioxidants in the rutin group. We have shown that rutin suppresses the reduction of tGSH and enzymatic antioxidants by gentamicin, shifting the oxidant/antioxidant balance in favor of antioxidants.[20]

Furthermore, in the present study, it was observed that gentamicin significantly increased proinflammatory cytokine levels such as TNF-α and IL-1β in cochlear nerve tissue compared to healthy and rutin groups. In a previous study, it was stated that proinflammatory cytokines such as tumor TNF-α and IL-1β are the major components that form the basis of gentamicin ototoxicity.[4] There is evidence showing that gentamicin increases TNF-α and IL-1β expression in the cochlea.[4] Bas E et al.'s[24] study shows that gentamicin-induced increase in TNF-α and IL-1β is associated with antioxidant reduction. There is no data in the literature on the effect of rutin on these parameters in cochlear nerve tissue. However, it has been suggested that the protective effect of rutin may have been due to the inhibition of overproduction of antioxidant and proinflammatory cytokines (TNF-α, IL-1β).[11]

As shown by our experimental results, gentamicin group had high levels of MDA, TNF-α and IL-1β and low levels of tGSH, and exhibited histopathological findings such as severe degenerated and edematous myelinated axons, collagen accumulation, and dilated blood vessels in cochlear nerve tissue. On the other hand, there was a marked decrease in the number and severity of such observations in the rutin group. It has been stated in the literature that degeneration of spiral ganglion neurons of hair cells is one of the main causes of hearing loss.[25] It has also been reported that sensorineural hearing loss is often associated with damage to the cochlear hair cells or neurons of the auditory pathway.[26] Furthermore, there is also information in the literature stating that loss of hair cells initiates the gradual degeneration of spiral ganglion neurons, the primary afferent neurons of the cochlea. It has also been reported that these neurons are normally myelinated via Schwann cells, and myelin loss occurs as a precursor to neural degeneration.[27]

Gentamicin administration caused an increase in oxidant and proinflammatory cytokines and a decrease in antioxidants in the cochlear nerve tissue of animals. Gentamicin also resulted in marked histopathological damage in the cochlear nerve tissue. Rutin administration significantly prevented the gentamicin-induced increase in oxidant and cytokine levels, the decrease in antioxidant levels and histopathological damage development. These experimental results suggest that rutin may be useful in the treatment of gentamicin-induced ototoxicity.

Ethics Committee Approval: Ethical committee approval was received from thethe local animal care committee in the Ataturk University (26.12.2019-256).

Peer-review: Externally peer-reviewed.

Informed Consent: N/A.

Author Contributions: Concept - İ.S., H.S., M.D.; Design - İ.S., H.S., M.D.; Supervision - İ.S., A.Ç., E.S.; Resource - İ.S., H.S., GN.Y.; Materials - H.S., R.B., A.Ç.; Data Collection and/or Processing - H.S., G.N.Y., A.Ç., R.B., E.S.; Analysis and/or Interpretation - İ.S., M.D., A.Ç.; Literature Search - İ.S., M.D., R.B., E.S., H.S.; Writing - M.D., İ.S., H.S.; Critical Reviews - A.Ç., R.B., E.S., H.S.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

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