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# The effectiveness of berberine on noise-induced hearing loss: a rat model

Korhan Kilic[1*], Muhammed Sedat Sakat[1], Abdulkadir Sahin[1], Serkan Yildirim[2], Muhammet Bahaeddin Dortbudak[2]

## SUMMARY

**OBJECTIVE:** Noise-induced hearing loss is a preventable form of hearing loss that has serious social and economic impacts. This study aimed to investigate the protective effect of berberine, a potent antioxidant and anti-inflammatory agent, against Noise-induced hearing loss.

**METHODS:** After applying distortion product otoacoustic emission, 28 female Sprague-Dawley rats were randomly divided into four groups. Group 1 was designated as acoustic trauma group, and rats in this group were exposed to white noise for 12 h at an intensity of 4 kHz 110 dB sound pressure level. Group 2 was the control group. Group 3 was designated as the berberine group, and 100 mg/kg of berberine was administered to rats in this group by intragastric lavage for five consecutive days. Group 4 was designated as the acoustic trauma+berberine group. distortion product otoacoustic emission was repeated on the 6$^{th}$ day of the study and cochlear tissues of rats were dissected for histopathological and immunohistochemical analyses after sacrificing rats.

**RESULTS:** The distortion product otoacoustic emission results showed a significant decrease in signal-noise ratio values at higher frequencies in rats of the trauma group compared to those in other groups. Acoustic trauma caused severe histopathological impairment at cochlear structures together with severe 8-hydroxy-2-deoxyguanosine expression. Rats in the acoustic trauma+berberine group showed mild histopathological changes with mild 8-hydroxy-2-deoxyguanosine expression and better signal-noise ratio values.

**CONCLUSION:** The histopathological and audiological findings of this experimental study showed that berberine provides protection in Noise-induced hearing loss and may have the potential for use in acoustic trauma-related hearing losses.

**KEYWORDS:** Acoustic trauma. Berberine. Noise-induced hearing loss.

## INTRODUCTION

Noise can be defined as unwanted and uncomfortable sound, causing various psychological and physiological effects in humans and adversely affecting the quality of life. Noise is an agent to which individuals are exposed during much of modern life, and that can cause various pathological effects throughout the body. The most important of these effects is seen in the auditory system, the first site impacted by noise[1].

The mechanism underlying noise-related hearing losses is not yet fully understood. However, a combination of apoptosis, oxidative stress, and genetic, physical, and environmental factors is thought to be involved. Oxidative stress is characterized by the presence of DNA damage and lipid peroxidation, generally resulting from the development of free radicals. Noise-induced damage to the cochlea results from degeneration in support cells, afferent nerve fibers, and particularly outer hair cells[2].

The importance of oxidative stress in the pathogenesis of this damage led to the idea of employing antioxidants to prevent it. Various agents have been used for this purpose in the literature[3-7]. Berberine is an alkaloid with a broad pharmacological spectrum and particularly with antioxidant and anti-inflammatory effects[8].

Distortion product otoacoustic emissions (DPOAE) were used to demonstrate noise-related hearing damage. DPOAE are highly sensitive in showing noise-related damage in the inner ear in rats and can yield an earlier response than the auditory brain stem response test[9].

The purpose of this study was to investigate the protective efficacy of the powerful antioxidant and anti-inflammatory agent berberine on noise-related hearing losses using histopathological and immunohistochemical methods and DPOAE.

[1]Ataturk University, Faculty of Medicine, Department of Otorhinolaryngology – Erzurum, Turkey.
[2]Ataturk University, Faculty of Veterinary, Department of Pathology – Erzurum, Turkey.
*Corresponding author: Korhankilic@gmail.com

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## METHODS

This study was approved by the Ataturk University animal experiments ethical committee with number 2021;1:4. The study was conducted at the Ataturk University experimental research laboratory.

### Animals

The study protocol was established with 28 female Sprague-Dawley rats weighing 220–260 g. The Care and Use of Laboratory Animals guideline was implemented throughout the study. Rats were housed in separate cages in a 12:12 h light:dark system. The cages were designed to allow ad libitum access to food and water. The room temperature was set to 22°±2° and the humidity to 50±5%. Environmental noise was kept below 50 dB SPL.

### Study design

Before the study, general anesthesia was applied to all rats using 40 mg/kg ketamine hydrochloride + 10 mg/kg xylazine hydrochloride via the intraperitoneal (i.p.) route. The tympanic membranes of both ears of all rats were subjected to otoscopic examination under general anesthesia. DPOAE was then applied to all rats. Rats with tympanic membrane perforation, findings of otitis media, or failing the DPOAE test were excluded from the study.

The saline solution and berberine used in the study were administered in a single dose by intragastric lavage once daily for 5 days. Saline was administered at a dosage of 8 mg/kg and berberine (Santa Cruz Biotechnology, Inc., Texas, USA) at a dosage of 100 mg/kg. Rats were exposed to acoustic trauma on day 3 of the study. DPOAE measurements were repeated on day 6, and all rats were sacrificed under general anesthesia. The rats' cochlear tissues were excised and set aside for histopathological and immunohistochemical examination.

### Experimental Protocol

Rats were randomized into four groups of seven animals each.

**Group 1** was designated as the acoustic trauma group. Rats in this group received 8 mg/kg of saline solution by orogastric lavage together with acoustic trauma.

**Group 2** was designated as the control group. No drug or acoustic trauma was applied to this group.

**Group 3** was designated as the berberine group. The rats in this group received 100 mg/kg berberine dissolved in saline solution in a single dose once daily for 5 days by intragastric lavage.

**Group 4** was designated as the acoustic trauma+berberine group. Together with the acoustic trauma, the rats in this group received 100 mg/kg of berberine in a single dose once daily for 5 days via intragastric lavage.

### Acoustic Trauma Model

The acoustic trauma was applied to rats in groups 1 and 4 on the 3rd day of the study. For this, the rats were placed into a silent booth inside their cages, equidistant from two speakers. The rats were then exposed to white noise for 12 h at an intensity of 4 kHz 110 dB SPL in a free environment using a GSI Audiostar Pro audiometer (Grason-Stadler, Eden Prairie, Minnesota, USA).

### Distortion product otoacoustic emissions

DPOAE measurements were performed on all experimental animals under general anesthesia before (on day 1) and after (on day 6) noise exposure. The test was conducted following an otoscopic examination in a silent environment using a Madsen Capella 2 (GN Otometrics, Denmark) measurement device, and an appropriate probe was applied to the outer ear canals. DPgram measurements were carried out at 10 frequencies between 2002 and 10000 Hz. Signal-noise ratios (SNR) were compared in the measurement results for days 1 and 6.

### Histopathological examination

Following fixation in 10% formalin solution for 48 h, tissues were allowed to soften for 96–120 h in the Osteosoft decalcification solution (Merck, HC313331, Germany). After softening, the tissue was washed under running water for 24 h. Tissues were then passed through routine processes and then were embedded in paraffin blocks. Sections of 4 µm in thickness were taken and placed onto glass slides. Preparates made ready for histopathological examination were stained with hematoxylin-eosin and examined under a light microscope. These sections were classified based on the presence of lesions as none (–), mild (+), moderate (++), and severe (+++), and they were photographed. Hyperemia in stria vascularis was determined by the diameter of the vessels (<1 µm is defined as none, 1–2 µm as mild, 3–5 µm as moderate, and >5 µm as severe). The degeneration of spiral ganglia was determined by the degenerated cell number (0 is defined as none, 3–5 cells as mild, 6–10 cells as moderate, and >10 cells severe). Structural impairment in outer hair cells was determined by the impaired cell number (0 as none, 3–5 cells as mild, 6–10 cells as moderate, and >10 cells as severe).

### Immunohistochemical examination

All sections placed onto adhesive-containing slides (poly-L-lysine) for immunoperoxidase examination were passed through xylol and alcohol series. After washing with phosphate-buffered saline, endogenous peroxidase inactivation was established by keeping the sections in 3% $H_2O_2$ for 10 min. Following

treatment in a microwave with an antigen retrieval solution for 2×5 min at 500 watts for antigen detection, tissues were left to cool and treated in a microwave. Tissues were then incubated with 8-hydroxy-2-deoxyguanosine (8-OHdG) (catalog no. sc-66036, Santa Cruz, USA) at 37°C for 60 min. Procedures were carried out in compliance with the immunohistochemistry kit instructions (Abcam HRP/DAB Detection IHC kit). 3-3′ Diaminobenzidine was used as the chromogen. Background staining was applied with hematoxylin. To determine the intensity of positive staining from the obtained images, five random areas were selected from each image. As a result of the antibody staining used for the evaluation process, the positive/total area was measured using the ZEISS Zen Imaging Software program.

### Statistical Analysis

Statistical analysis was conducted using the SPSS 20.0 software. Data distribution was checked using the Shapiro-Wilk test. One-way ANOVA and the post-hoc Tukey's test were employed for comparisons of DPOAE results, immunoreactive cells, and immunopositive stained areas of positive antibodies in immunochemical analyses in the case of normal distribution. The nonparametric Kruskal-Wallis test was applied in the analysis of intergroup differences among semi-quantitatively obtained non-normally distributed data at histopathological examination, while the Mann-Whitney U-test was employed for two-way examination. For all tests, a p-value of <0.05 was considered significant.

## RESULTS

### Distortion product otoacoustic emissions results

No difference was observed in SNR values of the control, berberine, and berberine+trauma groups on the 6$^{th}$ day compared to those on the 1$^{st}$ day. No significant difference was observed in SNR values of the trauma group at 2002 and 2383 Hz frequencies on day 6 compared to day 1 values, but significant decreases were determined in SNR values on day 6 at all higher frequencies. This shows that acoustic trauma leads to cochlear damage, particularly at high frequencies. Comparison of post-exposure DPOAE results between groups showed higher SNR values in the berberine+trauma group compared to those in the trauma group, while no significant difference was observed between the berberine+trauma group and the control group. This shows that berberine exhibits effective protection against acoustic trauma in the cochlea. The results of DPOAE are given in Figure 1.

### Histopathological and immunochemical findings

**Acoustic Trauma Group**: Cochlear tissues exhibited degeneration and necrosis in the spinal ganglia, severe erosion in outer hair

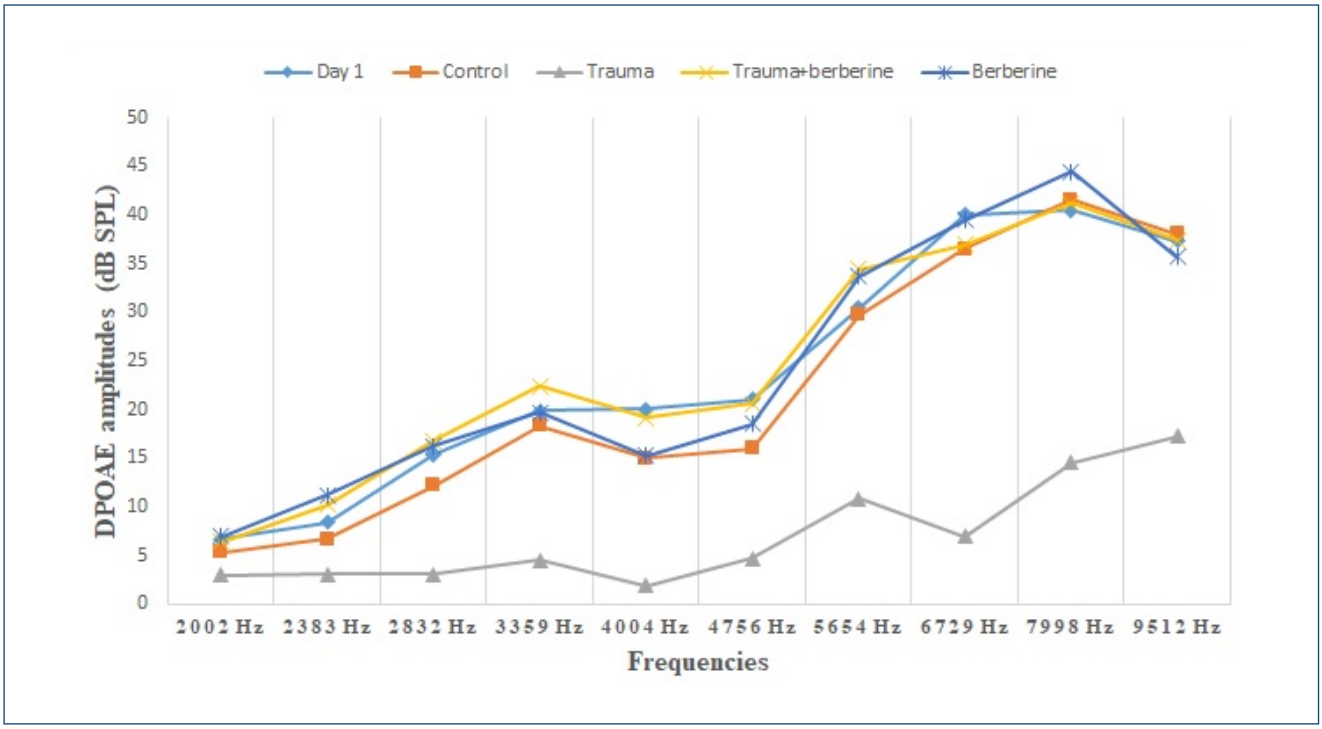


**Figure 1.** Results of distortion product otoacoustic emissions tests before (day 1) and after (day 6) noise exposure. Control, trauma, trauma+berberine, and berberine groups mean the results of distortion product otoacoustic emissions of these groups, performed on day 6.

cells and decreased numbers due to desquamation, and hyperemia in the stria vascularis (Figure 2-A). Immunohistochemical examination of cochlear tissues revealed severe 8-OHdG expression in the spinal ganglia and outer hair cells (Figure 2-E).

**Control and Berberine Groups**: Cochlear tissues exhibited a normal histological appearance in the stria vascularis, spinal ganglia, and outer hair cells (Figure 2-B, C). Immunohistochemical examination revealed negative 8-OHdG expression (Figure 2-F, G).

**Acoustic Trauma+Berberine Group**: Cochlear tissues exhibited mild degeneration in spinal ganglia, mild erosion in outer hair cells, desquamation, and an associated mild decrease in numbers, and moderate hyperemia in the stria vascularis (Figure 2-D). Immunohistochemical examination of cochlear tissues revealed mild cytoplasmic 8-OHdG expression in the spinal ganglia and outer hair cells (Figure 2-H). A statistically significant difference was observed compared with the acoustic trauma group in terms of histopathological and immunochemical results ($p<0.05$). The histopathological and immunohistochemical findings are summarized in Table 1.

## DISCUSSION

Exposure to noise is one of the most common causes of hearing loss. Noise-induced hearing loss (NIHL) is a preventable form of hearing loss involving both genetic and environmental factors. Research has shown that measures taken in the prevention of NIHL are most effective and economical than treatment. Sufficient understanding of the pathophysiology of the disease is important for NIHL to be prevented and even treated. Studies to date have shown that oxidative stress is the most important mechanism in the pathophysiology of NIHL[10].

Under normal physiological conditions, oxidants and antioxidants in the body are maintained in balance. However, increased reactive oxygen species (ROS) following the exposure to noise results in that balance being impaired in favor of oxidants. Increased ROS production also causes apoptotic and necrotic cell death in the cochlea, associated with collapse in support cells and stria edema, dendrite breakdown, and stereocilia defects[11]. The outer hair cells are the first structures affected by exposure to noise in the cochlea. Due to their motility, the outer hair cells are highly energy-dependent. On account of their energy dependency, greater oxygen is used in the mitochondria in case of noise exposure, and more ROS is produced as a side product[12]. Increasing ROS production triggers cell death as a cause of DNA damage and the breakdown of lipid and protein molecules[13]. 8-OHdG is a predominant form that occurs as a result of free radical-induced oxidative lesion of nuclear and mitochondrial DNA. Numerous studies have revealed that 8-OHdG is an important marker of oxidative DNA damage. Semenova et al. stated that 8-OHdG is an important indicator of oxidative DNA damage and an important marker in aging and sleep-wake cycle[14]. Chen et al. also found that 8-OHdG levels increased in the cochlear tissue after noise exposure[15]. In this study, increased expression of 8-OHdG was demonstrated in the cochlear tissue after acoustic trauma.

Several molecules have been employed in research for the purpose of halting or neutralizing this physiological cascade at any stage. This study employed histopathological and audiological methods to determine the impact of berberine in an acoustic trauma model. The effect mechanisms of berberine in diseases derive from its antioxidant and anti-inflammatory properties[16]. Berberine shows its antioxidant and anti-inflammatory effects in three main ways:

1. the inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase,
2. the activation of the Nrf2 pathway, and
3. the inhibition of the NF-kB pathway. With all these effects, it shows antioxidant and anti-inflammatory activity[17].

It has also been shown that berberine inhibits apoptosis caused by oxidative stress by modulating $Ca^{2+}$ deregulation[18].

NADPH stimulation via cytokines, the mitochondrial electron transport chain, and xanthine oxidase generally result in oxidative stress as a cause of ROS overproduction[15]. Berberine reduces this overproduction by inhibiting the NADPH pathway. Jang et al. showed that berberine exhibits a high hydroxyl radical scavenging effect[19]. In this study, the administration of berberine decreased the expression of 8-OHdG in cochlear tissue.

The immunohistochemical and audiological findings of the present experimental study showed that, thanks to its excellent antioxidant and apoptotic effects, berberine exhibited a protective effect on the inner ear in an acoustic trauma model. Studies in the literature have also shown that berberine is used in humans. Jiang et al. reported that berberine is a safe and effective drug in patients with colorectal cancer and is beneficial in the treatment of these patients[20]. Similarly, human studies have shown that berberine is an effective agent in neurodegenerative disorders such as the Parkinson's and Alzheimer's diseases due to its potent antioxidant effect[21]. As oxidative stress is one of the most important steps in the underlying mechanism of NIHL, we predict that berberine can also be used for NIHL in humans with its strong antioxidant activity.

The principal limitation of this study is that biochemical investigation showing oxidative and antioxidative values was not

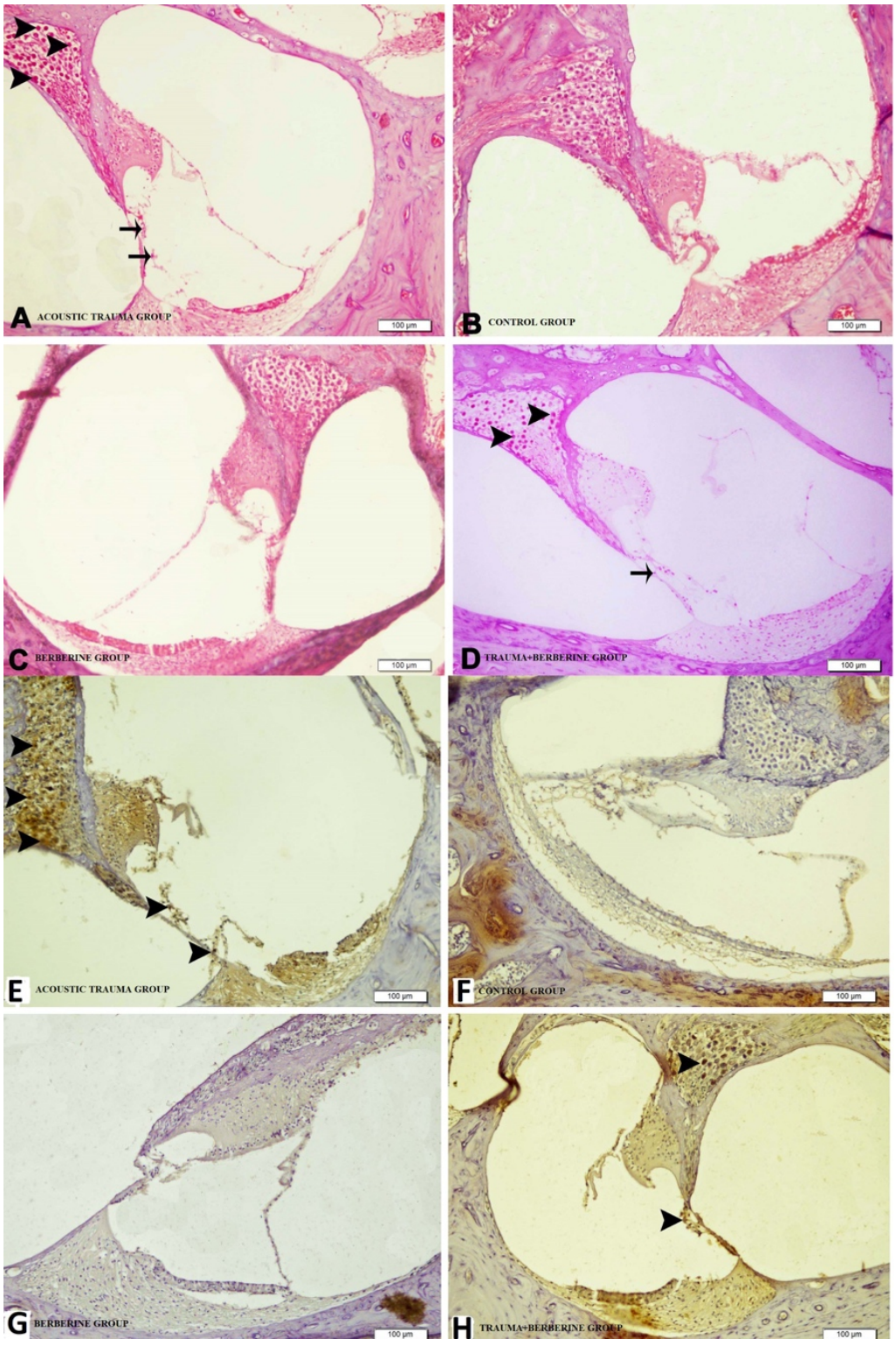


**Figure 2.** Histopathological appearance of cochlear tissues. (A) Acoustic Trauma Group. Degeneration and necrosis in ganglia (arrow heads), desquamation, and severe decrease in number of outer hair cells (arrows). (B) Control Group. Normal histological appearance. (C) Berberine Group. Normal histological appearance. (D) Acoustic Trauma+Berberine Group. Degeneration in ganglia (arrow heads), mild desquamation, and decrease in number of outer hair cells (arrows). Hematoxilen & eosine, Bar: 100 µm. Immunohistochemical appearance of cochlear tissues. (E) Acoustic Trauma Group. Severe cytoplasmic 8-hydroxy-2-deoxyguanosine expression in ganglia and outer hair cells (arrow heads). (F) Control Group. Negative 8-hydroxy-2-deoxyguanosine expression. (G) Berberine Group. Negative 8-hydroxy-2-deoxyguanosine expression. (H) Acoustic Trauma+Berberine Group. Mild 8-hydroxy-2-deoxyguanosine expression in ganglia and outer hair cells (arrow heads). Immunohistochemistry-peroxidase, Bar: 100 µm.

**Table 1.** Histopathological and immunochemical results of cochlear tissues

| | Acoustic Trauma | Control | Berberine | Acoustic Trauma+ Berberine |
|---|---|---|---|---|
| Hyperemia in the stria vascularis | +++ | – | – | ++ |
| Decreased outer hair cells | +++ | – | – | + |
| Degeneration and necrosis in spinal ganglion cells | +++ | – | – | + |
| 8-OHdG expression (mean±SD) | 63.21±3.23[a] | 20.51±6.42[b] | 21.47±5.96[b] | 41.53±5.81[c] |

[a, b, c]different letters show statistically significance ($p<0.05$). Presence of lesions as none (–), mild (+), moderate (++), and severe (+++).

performed. However, 8-OHdG, an important marker of oxidative stress, reduces the scale of that limitation in immunohistochemical terms. As this is an experimental study, there should be difficulties in methodological design and transposing data to human beings.

## CONCLUSIONS

To the best of our knowledge, this is the first study in the literature to show the protective efficacy of berberine in an acoustic trauma model. The histopathological and audiological findings of this experimental study showed that berberine provides protection in acoustic trauma. Berberine may therefore have the potential for use in acoustic trauma-related hearing losses. However, as this is an experimental study, further research is needed on the use of berberine in humans.

## AUTHORS' CONTRIBUTIONS

**KK:** Conceptualization, Data curation, Formal Analysis, Writing – original draft. **MSS**: Conceptualization, Data curation, Formal Analysis, Writing – original draft. **AS**: Data curation, Formal Analysis, Writing – original draft. **SY:** Data curation, Formal Analysis, Writing – original draft. **MBD:** Data curation, Formal Analysis, Writing – original draft.

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