RESEARCH ARTICLE
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Is Gentamicin-Induced Ototoxicity Reversible with Delayed Administration of Nigella Sativa Oil? An Experimental Study

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ABSTRACT

Objective: Gentamicin (GM) is a potent antibiotic but is known to cause irreversible ototoxic effects. Nigella sativa oil (NSO) can offer protective prophylaxis against ototoxicity when administered prior to GM treatment. This study aims to assess the impact of delayed NSO administration on GM-induced ototoxicity using the auditory brainstem response (ABR) test.

Material and Methods: Adult male Sprague-Dawley rats were randomly divided into three groups, each consisting of six animals. All groups received intraperitoneal (i.p.) GM (120 mg/kg i.p.) for a duration of 10 days. The first two groups were given 0.3 ml/kg/day NSO for five days, while the third group received 0.9% saline for the same duration. Group 1 involves the early administration of NSO (NSOE) 10 days after starting GM, Group 2 involves the late administration of NSO (NSOL) 25 days after starting GM, and Group 3 is the saline (control) group. Hearing thresholds were recorded for both ears prior to treatment initiation and again 30 days after the start of treatment.

Results: Two rats expired and were excluded from the study. A total of 64 ABR test results were obtained. No difference was found between the pre- and post-treatment ABR values in Groups 1 and 2. However, a significant increase in ABR thresholds was observed only in Group 3. A significant difference was found in post-treatment hearing thresholds ($p<0.001$) between Group 3 and the other two groups.

Conclusion: The results suggest the delayed administration of NSO within a period of 30 days, whether earlier or later, to be able to effectively reverse the GM-induced ototoxic effects.

Keywords: Gentamicin, nigella sativa oil, ototoxicity, auditory brainstem response test

INTRODUCTION

Aminoglycosides (AG) are highly effective antibiotics utilized in the treatment of serious infections (1, 2). Nonetheless, they are well-established to possess ototoxic properties, with the risk of ototoxicity escalating with higher dosages, more frequent administration, and prolonged treatment durations (3, 4).

Nigella sativa (NS) is an annual flowering plant with a significant historical background in traditional medicine (5). The oil has been traditionally used to support respiratory, stomach, and intestinal health (6). Thymoquinone and other active constituents of NS have recently been found to have numerous potential therapeutic properties. NS is commonly used as a natural remedy for various illnesses and conditions, including asthma, diabetes, hypertension, cough, bronchitis, inflammation, eczema, headache, dizziness, influenza, and fever (7). Previous research has demonstrated that, when used together or as a preventative measure with ototoxic drugs, NSO offers protection against ototoxicity. However, the efficacy of NSO in reversing the ototoxic effects after treatment with GM has not been assessed (3, 8, 9). This study plans to evaluate the effect of administering nigella sativa oil (NSO) up to 30 days after starting gentamicin (GM) treatment on GM-induced ototoxicity using the auditory brainstem response (ABR) test.

MATERIALS and METHODS

The study was carried out using male Sprague-Dawley rats weighing between 300-400 g that had been bred at the Bursa Uludağ University Experimental Animal Breeding and Research Unit. The surgical interventions were approved by the Bursa Uludağ University Ethical Committee on Animal Research (Date: October 5, 2022, No: 2022-10/07). The study was planned taking into account the Guide for the Care and Use of Laboratory Animals. The animals were placed in secure cages

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with unrestricted access to food and water. The study evaluated the ABR thresholds in all rats and included 18 rats with normal hearing thresholds. The rats were divided into three groups, each consisting of six animals.

Group 1: GM+NSOE (Early administration starting on day 10 after the first GM dose)

Group 2: GM+NSOL (Late administration starting on day 25 after the first GM dose)

Group 3: GM+Saline (Control group; saline started on day 25 after the first GM dose)

All three groups were administered GM at a dose of 120 mg/kg intraperitoneally (i.p.) for a duration of 10 days. Groups 1 and 2 received NSO (0.3 ml/kg/day i.p.) for 5 days in the early and late stages, respectively. Group 3 was given saline (0.3 ml/kg/day i.p.) and served as the control group. Adequate and appropriate doses were determined based on the results from the previous study (9).

ABR tests were performed before starting the GM treatment and again 30 days post-start of GM treatment on both ears of the rats while under general anesthesia with sevoflurane using the Interacoustics Eclipse EP15 device. A total of 64 test results were obtained from 16 rats. ABR results were compared statistically among the groups. Clicks were delivered using an in-ear headphone. A total of 2,000 responses to repetitive stimuli were recorded and averaged at a frequency of 31.1 Hz. These responses were then analyzed within a duration of 18 milliseconds. ABR thresholds were determined by positioning the active needle electrode on the vertex of the head, while placing the reference needle electrodes on both sides of the mastoid region. The ground needle electrode was inserted subcutaneously into the sacral region (Figure 1). The minimum level of intensity required to observe Wave V was determined as the threshold for the ABR.

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics (ver. 28.0; IBM, USA). All variables were presented as Mean±SD. The Wilcoxon signed-rank test was used to compare within-group parameters and one-way analysis of variance (ANOVA) test to compare the variables among the groups, where $p<0.05$ is considered statistically significant.

RESULTS

Two rats (one in Group 1, one in Group 3) were excluded from the study due to death before completion of the experiment. The ABR tests were conducted before the initiation of the GM treatment and on day 30 after treatment had commenced. A total of 64 test results were obtained, comprising 32 measurements for both the right and left ears of the rats. Table 1 presents the average values of the hearing thresholds before and after treatment as determined by the ABR results.

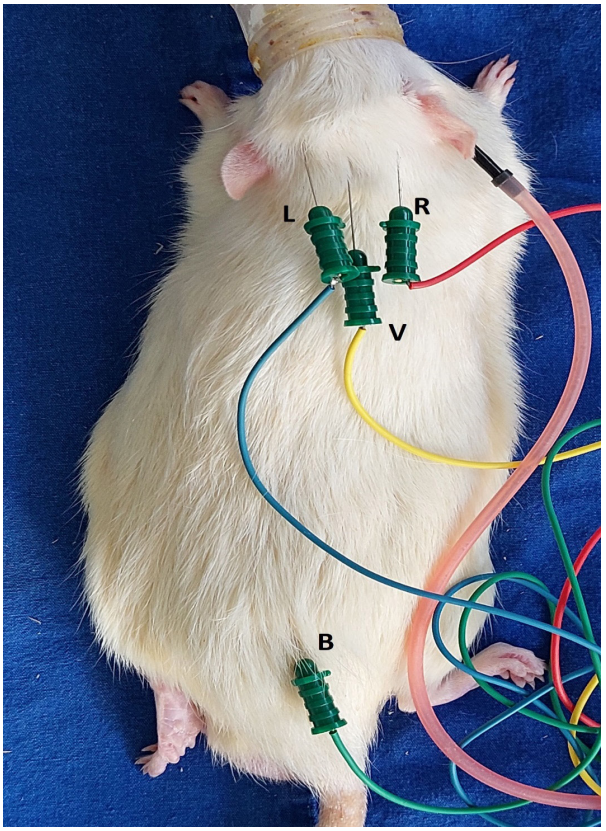

Figure 1: Sevoflurane anesthesia was given in order to perform the ABR test. ABR thresholds were obtained by placing the active needle electrode in the vertex (V), the reference needle electrodes bilaterally in the mastoid region (L and R), and the ground needle electrode in the back region subcutaneously (B).

The mean ABR measurements pre- and post-treatment were compared. No difference was found between the pre- and post-treatment ABR values for Groups 1 and 2. However, a significant increase in ABR threshold was observed only in Group 3 ($p=0.038$). When comparing the pre-treatment measurements alone, no significant difference was observed among the groups ($p=0.316$), while a significant difference became evident in terms of the post-treatment values ($p<0.001$). Figures 2 and 3 illustrate examples from the pre- and post-treatment ABR recordings.

DISCUSSION

Ototoxicity is a frequently reported adverse drug reaction, and its impact on hearing loss greatly affects quality of life (10). In a survey conducted on children, notable developmental differences were present between those with hearing loss and those with normal hearing. Children affected by ototoxicity have also been revealed to experience lower quality of life in terms of communication skills, independence, and emotional development (11).

Drug-induced ototoxicity may occur during or after drug therapy (12). Patients who complain of hearing loss are referred to an

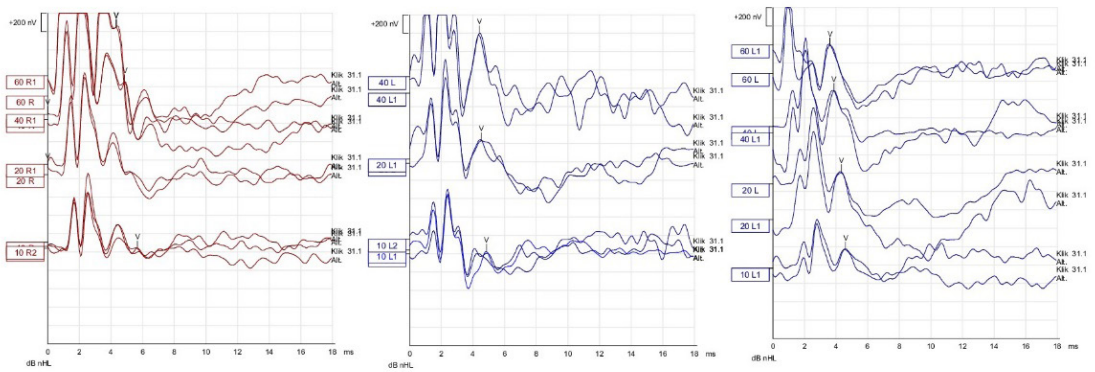

Figure 2: Examples of the pre-treatment ABR test results for the rats from Groups 1, 2, and 3.

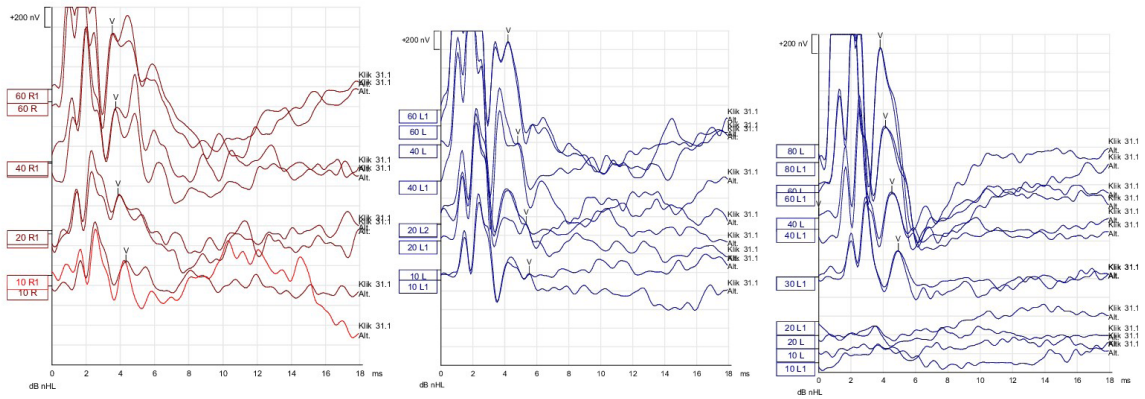

Figure 3: Examples of the post-treatment ABR test results for the rats from Groups 1, 2, and 3.

otorhinolaryngology department. However, the irreversible nature of the toxic effects presents challenges for physicians in terms of providing appropriate treatment. Rizk et al.'s review study reported 194 drugs to be able to cause ototoxicity when administered systemically. According to their findings, the most common class of medication was antimicrobials, followed by psychotropics (13). Ototoxic drugs can affect the inner ear through various mechanisms (14). The ototoxic effects of AG are reported to typically be bilateral and permanent (3, 15). These drugs target the outer hair cells at the basal turn of the cochlea and are hypothesized to enter the hair cells and marginal cells of the stria vascularis, thereby inhibiting mitochondrial protein synthesis via ribosomal binding. This process also induces the formation of ferric cations that produce reactive oxygen species (ROS) (12, 16). The risk of AG-induced ototoxicity has been reported to increase with the mutation of the gene encoding the mitochondrial 12s rRNA (17).

Table 1: Mean hearing levels for all groups (dB) ± SD: Pre and post-treatment results

Group No.	Group Name	Pre-treatment ABR Hearing Threshold (dB, mean)	Post-treatment ABR Hearing Threshold (dB, mean)	p
1	GM+NSOE	10	10	1
2	GM+NSOL	10.83±2.88	10.3±2.88	1
3	GM+Saline	12±4.22	19±7.38	**0.038**

ABR: Auditory brainstem response test; dB: Decibel; GM: Gentamicin; NSO: Nigella sativa oil; NSOE: Early NSO started on day 10 after first GM dose; NSOL: Late NSO started on day 25 after the first GM dose

Currently, no universally recognized agent is found to be utilized in clinical practice for treating GM-induced ototoxicity (3). Antioxidants have protective properties against prooxidant ROS and have been demonstrated to be able to sometimes have prooxidant effects on their own (7). However, *in vitro* tests have shown NSO, which possesses antioxidant properties, to not act as a prooxidant (18, 19). The present study has discovered the ABR test results of the rats that had been given NSO after GM administration to be identical to those obtained before GM administration. One month after GM administration, the hearing thresholds of the rats not administered NSO was observed to be significantly lower than those of rats treated with NSO.

Nigella sativa (NS) possesses a range of biological effects, including anti-inflammatory, anti-hyperlipidemic, anticancer, antioxidant, antidiabetic, and wound healing properties (6, 20, 21). Limited studies have examined the efficacy of NSO in reversing drug-induced hepatotoxicity. Jaswal et al. treated rats with antituberculosis drugs for 8 weeks (3 days/week), followed by NS for 8 weeks (3 days/week) and reported NS to exhibit excellent hepatoprotective abilities and to reverse hepatotoxic effects (22). Similarly, Hassan et al. suggested thymoquinone to be able to reverse the oxidative stress damage produced by atorvastatin in the rat liver, proposing its use in reversing hepatic injury (23).

Previous research has shown NSO to provide protection against ototoxicity when used concomitantly or prophylactically with ototoxic drugs (3, 8, 9). Sagit et al.'s experimental study conducted with rats demonstrated histopathologically that GM-caused ototoxicity was reduced when high doses of thymoquinone were administered simultaneously with GM (8). In another study, Edizer et al. reported the simultaneous administration of intratympanic NSO and GM in rats for 3 weeks to partially alleviate the ototoxic effect caused by GM (3). The previous study utilizing dose titration determined the optimal prophylactic dose of NSO to prevent ototoxicity (9). However, the effectiveness of NSO administration in reversing late-detected ototoxic effects after GM treatment has not been previously evaluated. The objective of the current study has been to examine whether delayed administration of NSO is able to effectively mitigate permanent hearing loss in GM-induced ototoxicity, and the findings have revealed administering 0.3 ml/kg/day NSO for 5 days within 30 days of the start of GM treatment to effectively reverse GM-induced ototoxicity and to completely restore hearing function. The study proposes the short-term administration of appropriate doses of NSO in the later period after starting the use of ototoxic drugs to be able to provide otoprotective benefits.

Recognizing the limitations of the current study is imperative. Firstly, the reversibility of the toxic effects was not confirmed through cochlear histopathological studies. Additionally, the current study only assessed the effectiveness of NSO in reversing ototoxic effects within the first month. Future investigations should evaluate the retrospective benefits of NSO application during later periods.

CONCLUSION

The study has demonstrated the administration of 0.3 ml/kg/day NSO at any time within 30 days of initiating GM treatment to be able to effectively reverse ototoxic effects. Further research is necessary to assess the possibility of reversing ototoxic effects through NSO administration beyond the initial 30-day period. The ability of NSO to reverse ototoxic effects offers hope for the management of late-onset drug-induced ototoxicity.

Ethics Committee Approval: This study was approved by the Ethics Committee of the Bursa Uludağ University Ethical Committee on Animal Research (Approval No. 2022-10/07 dated October 5, 2022).

Peer Review: Externally peer-reviewed.

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

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