

Protective effect of *Nigella sativa* oil on acoustic trauma induced hearing loss in rats

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Abstract

Acoustic trauma is a common reason for hearing loss. Different agents are used to prevent the harmful effect of acoustic trauma on hearing. The aim of this study was to evaluate the potential preventive effect of *Nigella sativa* (black cumin) oil in acoustic trauma. Our experimental study was conducted with 20 Sprague Downey female rats (mean age, 12 months; mean weight 250 g). All of the procedures were held under general anesthesia. Following otoscopic examinations, baseline-hearing thresholds were obtained using auditory brainstem responses (ABR). To create acoustic trauma, the rats were then exposed to white band noise of 4 kHz with an intensity level of 107 dB in a soundproof testing room. On Day 1 following acoustic trauma, hearing threshold measurements were repeated. The rats were divided into two groups as the study group (n: 10) and the controls (n: 10). 2 mL/kg/day of *Nigella sativa* oil was given to the rats in the study group orally. On Day 4 following acoustic trauma, ABR measurements were repeated again. There was no difference between the baseline hearing thresholds of the rats before acoustic trauma ($P>0.005$). After the acoustic trauma, hearing thresholds were increased and there was no significant sta-

tistically difference between the hearing thresholds of the study and control groups ($P=0.979$). At the 4th day following acoustic trauma, hearing thresholds of the rats in control group were found to be higher than those in the study group ($P=0.03$). Our results suggest that *Nigella sativa* oil has a protective effect against acoustic trauma in early period. This finding should be supported with additional experimental and clinical studies, especially to determine the optimal dose, duration and frequency of potential *Nigella sativa* oil therapy.

Introduction

Exposure to excessive noise is one of the major causes of hearing disorders. It has been estimated that as many as 500 million individuals might be at risk of developing noise-induced hearing loss (NIHL) worldwide.¹ NIHL still remains a problem in developed countries, despite reduced occupational noise exposure, strict standards for hearing protection and extensive public health awareness campaigns. Prolonged exposure to noise at high intensity is associated with permanent hearing threshold shift, as well as poor speech in noise intelligibility.²

Pathogenesis of NIHL has not been completely elucidated yet.³ Numerous studies have shown that high intensity noise exposure causes mechanical and metabolic changes in the cochlea, which in turn lead to apoptosis in the organ of Corti and inner ear damage.^{3,4} Type of damage due to noise exposure can be classified in sublevels as mechanical, neural and metabolic. Mechanical damage can result from the disruption of Reissner's membrane and basilar membrane, loss of stereocilia bundles, disruption of subcellular organelles, injury of the inner and outer hair cells, injury of stria vascularis and spiral ganglion cells, and destruction of the lateral walls of outer hair cells. Neural degeneration is a result of the degeneration of cochlear nerve peripheral terminals on the inner hair cells, spiral ganglion cells and the cell bodies of cochlear nerve afferents, and synaptic degeneration in the central auditory system, especially in the dorsal cochlear nucleus.⁵ Metabolic changes are caused mainly by an increase in free radical species (FRS) that seem to play a primary role in NIHL.⁶ Harmful effects of FRS are generally prevented by endogenous antioxidants; but since endogenous antioxidant capacity is limited, administration of exogenous antioxidants may be beneficial in treating NIHL.^{7,8} Although further research is needed to illuminate the exact molecular mechanisms that eventually result in hearing loss, different agents are used in an attempt to prevent the harmful effect of acoustic trauma on hearing.⁹ Several drugs or supplements including antioxidants, adenosine receptor antagonists, calcium-channel blockers, NMDA receptor antagonists and inhibitors of apoptotic signaling have been used experimentally for prevention and treatment of noise induced hearing loss.

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Antioxidant such as N-acetylcysteine, ACEMg, SPI-1005, D-Methionine are used for treatment in the noise induced hearing loss animal models. In the literature N-acetylcysteine, is the most evaluated for reducing the effect of noise trauma. The results regarding the therapeutic effect of NAC are encouraging.⁵ Additionally, there is a strong synergistic effect when each antioxidant is used in combination with other antioxidants. The studies demonstrate that successful use of antioxidant drugs depends on the optimal timing of treatment and the duration of treatment, which may be highly associated with the time window of free radical formation induced by noise exposure.^{5,10}

Nigella sativa oil is a remarkable herb with its rich historical and religious background. The seeds of the plant commonly known as black seed are the source of the active ingredients. The best seeds come from Egypt where they grow under almost perfect conditions and are used in alternative and complementary medicine all over the world. *Nigella sativa* seeds mainly contain thymoquinone (TQ), dithymoquinone (DTQ), thymohydroquinone (THQ) and thymol (THY). The seeds and the oil of the seeds were shown to have beneficial anti-oxidant, anti-inflammatory, anti-cancer, antimicrobial and immunomodulatory effects.^{11,12} *In vitro*, thymohydroquinone (THQ) was reported to act as a compound that is useful in reactive oxygen species scavenging and have prospective antioxidative properties.¹³ The medical treatment of acoustic trauma is principally based on the anti-oxidant and anti-inflammatory effects of therapeutic agents. Thus, the aim of this study is to determine the potential preventive effect of *Nigella sativa* oil in an animal model of noise-induced hearing loss.

Materials and Methods

This study was approved by the Baskent University Ethical

Committee for Experimental Research on Animals (Project no: DA15/36). All of the procedures were held under general anesthesia in the laboratory for experimental research on animals. The care and handling of the animals were in accordance with the US National Institute of Health (NIH) guidelines.

The study was conducted with 20 Sprague Downey female rats (mean age, 12 months; mean weight, 250 g). Animals were housed in cages in a room with an ambient noise level <50 dB, a 12-h light/dark cycle, and a room temperature of 20-22°C. Animals had free access to water and food during the day. General anesthesia was achieved using ketamin HCL (Ketalar, Pfizer, İstanbul) 60mg/kg and xylazine HCL (Rompun, Bayer, İstanbul) 6 mg/kg.

Following otoscopic examinations, baseline-hearing thresholds were obtained for all rats, using auditory brainstem responses (ABR) and the animals with a hearing threshold of ≤ 20 dB were included in the study. The rats were then exposed to acoustic trauma, using white band noise of 4 kHz with an intensity level of 107 dB in a sound-proof testing room. On the 1st day after noise exposure, ABR measurements were repeated, and the rats were randomized into two groups as the study group (n=10) and the controls (n=10). 2 mL/kg/day of *Nigella sativa* oil was given to the rats in the study group, orally, for three days. On the 4th day following acoustic trauma, ABR measurements were repeated again and hearing thresholds of the animals in study group were compared with those of the controls (Figure 1).

ABR measurements

Baseline hearing status, the effect of noise exposure and potential effect of *Nigella sativa* oil on hearing were screened using ABR. We used an OtoPhyLab (RT Conception, Ferrand, France) instrument and silver transcutaneous needle electrode *electrode aiguille 3/10 connecteur L5CM UU* (Medical Equipment International, Saint Georges, France) for the measurements. The active electrode was placed at the vertex, the reference electrodes

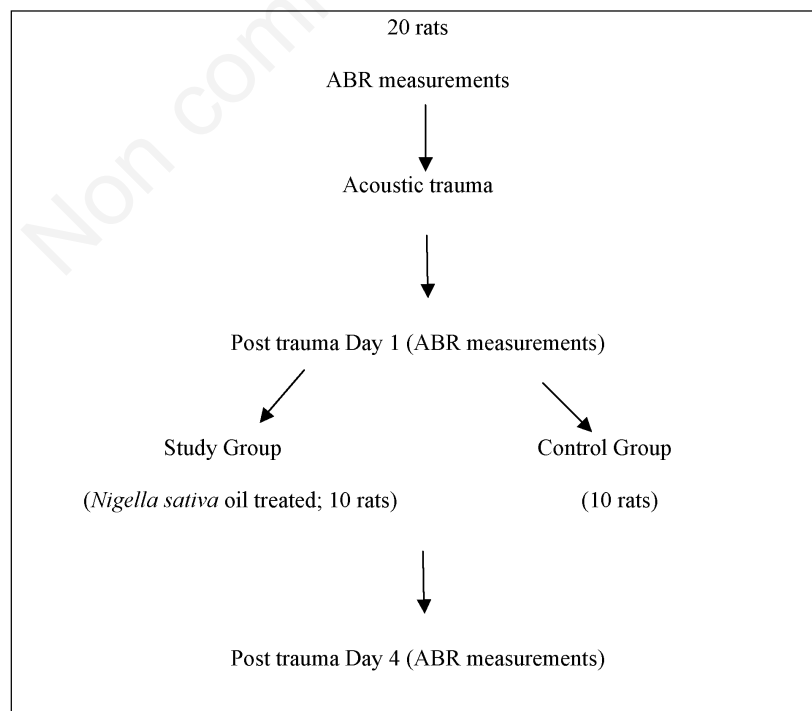


Figure 1. Study design.

were placed in the right and left mastoid regions and the ground electrode was placed on forehead. Click stimuli were used for ABR measurements. The recording was conducted only with impedance $<1.5 \text{ k}\Omega$. Click stimuli had the frequency spectrum of 8-32 kHz and duration of 100 msec. A band-pass filter of 0.1-3 kHz was used during the recordings, and the equipment constantly estimated the residual noise. Stimuli were presented in alternate polarity with a repetition rate of 11.1 per second and 30 msec time interval. The stimulus intensity level began at 90 dBnHL and was decreased in 10 dB steps. The recording stopped after 500 stimuli. Responses were recorded for all researched intensities. The amplitude and latency of recordings were determined by visual identification of the wave V by an experienced evaluator. The smallest level of stimulation intensity that induced a clearly identified Vth wave on the recordings was accepted as the hearing threshold.

Statistical analysis

Statistical analysis was performed using SPSS software (Version 20.0; SSPS Inc., Chicago, IL). Data were expressed as mean \pm standard deviation. Mann Whitney-U test was used for the comparison of variables. A value of $P < 0.05$ was considered statistically significant.

Results

Data from 40 ears of 20 animals were assessed. ABR waves were obtained in all rats before acoustic trauma (Table 1). Baseline hearing thresholds were $\leq 20 \text{ dB}$ in all rats.

Acoustic trauma caused deterioration of hearing thresholds in all 20 rats ($P=0.979$). Hearing thresholds of the rats receiving *Nigella sativa* oil were found to be better than those in the control group, on Day 4 following acoustic trauma ($P=0.03$) (Table 2).

Discussion

Medications developed for the protection against and the treatment of acoustic trauma would certainly reduce the medical costs and result in substantial positive effects on quality of life.¹⁴ In this study, we aimed to assess the effect of *Nigella sativa* oil on acoustic trauma induced hearing loss in an animal model. It was observed that *Nigella sativa* oil reduced the deterioration of the hearing thresholds following noise exposure and has a potential protective effect in acoustic trauma.

Acoustic trauma models in the literature show great variations in terms of methodology and there is no consensus on an ideal trauma model yet. Due to the differences in the duration and severity of the acoustic energy employed, trauma models cannot be compared. The acoustic trauma model used in the present study involved exposure to 107 dB white band noise at 4 kHz for 12 h.

The differences in the acoustic trauma models are also reflected in the hearing assessment methods. Transient evoked otoacoustic emissions (TEOAE), distortion product otoacoustic emissions (DPOAE) and ABR have been used to assess the effects of acoustic trauma quantitatively. TEOAE and DPOAE are the tools that principally reflect the function of the cochlea,¹⁵ while ABR gives a more integrated representation of the function of the whole auditory system. In this study, we attempted to screen the entire auditory pathway and used click-evoked ABR test in the range of 8-32 kHz for hearing threshold measurements. ABR waves were obtained in all rats before the acoustic trauma. The ABR also provided a clear demonstration of the negative effect of acoustic trauma on hearing thresholds in all rats.

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Steroids play an essential role in the management of NIHL. However, the use of steroids may lead to serious complications. There has been an ongoing interest to find a safe and effective therapeutic alternative for the treatment of NIHL. Recently, herbal remedies have gained popularity in the treatment of many health problems. Studies investigating *Nigella sativa* oil (black cumin oil), one of the popular herbal remedies, show that *Nigella sativa* oil and its components have anticancer, antihistaminic, antimicrobial, anti-inflammatory, analgesic, antioxidant, hypoglycemic, and immuno-supportive features.¹⁶

Ustun *et al.* experimentally studied the radio-protective effects of *Nigella sativa* oil on oxidative stress in the tongue tissue of rats, and found that *Nigella sativa* oil had positive effects on tissue damage caused by radiotherapy.¹⁷ Sagit *et al.* reported that timocinon, one of the components of *Nigella sativa* oil, has a protective feature against ototoxicity.¹⁸ Aksoy *et al.* reported that thymoquinone was demonstrated to be a reparative treatment that could be used to relieve inner-ear acoustic trauma in rats.³ Najmi *et al.* found that *Nigella sativa* oil is effective in the treatment of metabolic syndrome as a supplementary treatment.¹⁹ To our knowledge, there is no study in the English literature on the use of *Nigella sativa* oil following acoustic trauma, yet. In the present study, 2 ml/kg *Nigella sativa* oil was used as described before by Hamed *et al.* in a research investigating the effect of *Nigella sativa* oil on hepatorenal toxicity.²⁰ On the other hand, any comment on the duration of the therapy could not be found in the literature. Since the best therapeutic results could be obtained up to 3 days after the acoustic trauma,¹⁰ we aimed to evaluate the early effect of *Nigella sativa* oil on acoustic trauma.

Conclusions

In the present study, we investigated, the effects of the oral use of *Nigella sativa* oil in 20 rats subjected to acoustic trauma, and found that *Nigella sativa* oil has ameliorating effects on hearing thresholds following acoustic trauma. However, our work has limitations. First, the evaluation of the inner ear functions was limited, because only ABR was used. Second, no histopathological evaluation was performed to observe the effectiveness of *Nigella sativa*

Table 1. Latency and amplitude values of ABR waves before acoustic trauma. The values represent mean \pm standard deviation (* msec, **mV).

Waves	Latency*	Amplitude**
I.	1.20 \pm 0.05	4.32 \pm 2.44
II.	2.06 \pm 0.11	3.95 \pm 2.09
III.	2.83 \pm 0.18	1.20 \pm 1.34
IV.	3.75 \pm 0.25	2.98 \pm 1.34
V.	4.63 \pm 0.43	1.01 \pm 0.77

Table 2. Hearing thresholds (mean \pm standard deviation [dB]) of the rats on Day 1 and Day 4 following acoustic trauma.

Parameters	Control Group	Study Group	z value	P
Day 1	41 \pm 7.88	41 \pm 4.47	-0.026	0.979
Day 4	49 \pm 10.2	42 \pm 8.9	-2.171	0.03

oil in the inner ear and the auditory pathway. Although *Nigella sativa* oil reduced the deterioration of the hearing thresholds following noise exposure, the difference was limited. On the other hand, our results are for further research on the role of *Nigella sativa* in acoustic trauma management. Similar studies addressing the treatment regimen in terms of dose and duration should be conducted.

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