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Original Article

Effect of Pycnogenol® on Noise-Induced Hearing Loss in Rats

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BACKGROUND: This study aims to elucidate the potential protective effects of Pycnogenol® against noise-induced hearing (NIHL) loss in a rat model.

METHODS: This study employed a randomized controlled design to investigate the potential protective effects of Pycnogenol® against NIHL in a rat model. Twenty-five male Wistar albino rats were randomly assigned to 5 groups (n = 5 per group): a control group receiving saline administration, a noise exposure group, a noise+saline receiving group, only Pycnogenol® receiving group, and finally, a Pycnogenol® treatment group receiving daily oral administration of Pycnogenol® at 40 mg/kg/day via gavage for 7 days following noise exposure. All groups were subjected to auditory brainstem response assessments at 4 time points: pre-exposure (baseline), post-exposure day 1, day 7, and day 21. Both noise exposure and the Pycnogenol® treatment groups were exposed to 4 kHz narrowband noise at 120 dB SPL for 4 hours. Following sacrifice, histological and immunohistochemical evaluations were conducted on cochlear tissues. Statistical analyses were performed using SPSS software version 25 to determine significant differences between groups and across time points.

RESULTS: Outcome of this research shows that the auditory brainstem response thresholds and cochlear morphology between the experimental and control groups are significantly different from each other, suggesting that Pycnogenol® may have the potential to prevent NIHL loss in rats.

CONCLUSION: Pycnogenol® shows potential in protecting against NIHL. However, further research, particularly at the molecular level, is necessary to better understand its therapeutic mechanisms and its specific impact on auditory metabolic processes.

KEYWORDS: Acoustic trauma, animal models, antioxidants, pycnogenol, noise-induced hearing loss

INTRODUCTION

Noise-induced hearing loss (NIHL) is a widespread public health issue, affecting an estimated 5-16% of the global population, according to the World Health Organization. Loud noise exposure is a leading cause of this preventable condition.[1] The development of NIHL is attributed to a combination of mechanical and metabolic stress on the inner ear, primarily driven by excessive free radical production.[2] This damage can impact various parts of the auditory system, including hair cells, the spiral ganglion, and auditory nerve fibers, potentially leading to sensorineural hearing loss.[3,4] Preventing NIHL involves minimizing noise exposure through measures like hearing protection and reducing sound levels. Additionally, various antioxidants and other pharmacological agents hold promise as preventative or treatment options.[5]

Pycnogenol®, a natural antioxidant derived from the bark of the French maritime pine, has attracted considerable attention for its various therapeutic potentials.[6,7] Numerous studies have confirmed its potent antioxidant and anti-inflammatory properties due to its

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unique chemical composition.[8] In particular, Pycnogenol® has shown efficacy in alleviating a number of conditions associated with oxidative stress, including nephrotoxicity, tinnitus, hepatotoxicity, and diabetes mellitus.[9–11]

Despite the potential of Pycnogenol® in mitigating oxidative stress-related conditions, no research to date has investigated its effects on NIHL. Further investigation is warranted to assess the efficacy and safety of Pycnogenol® in mitigating NIHL.

MATERIALS AND METHODS

Animals

This investigation used 25 male Wistar albino rats (Rattus norvegicus), approximately 3.5 months old, obtained from the Ethical Committee of Experimental Studies, İstanbul Medipol University (approval number: E-38828770-772.02-2865, date: 28.03.2023). Sample size was determined using G-Power.[12,13] Rats weighed between 250 and 350 g. Rats exhibiting signs of ear infection or abnormalities of the outer ear canals or eardrum were not included in the study.

All rats were housed in the Experimental Animal Laboratories for the duration of the study, maintained on a 12-hour light/dark cycle at room temperature (22-24°C) with ad libitum access to food. Animal care and handling procedures adhered to the National Institutes of Health guidelines. Ambient noise levels in the acoustic measurement area, where animal cages were located, were maintained below 50 dBA. Following stimulated otoacoustic emission testing (to check for pathologies of peripheral hearing), 25 rats were randomly assigned to 5 groups (n = 5 per group).

Five distinct experimental groups were established as follows:

Group I: Rats in this group received daily oral administration of saline solution (40 mg/kg/day) via gavage for a duration of 7 days. This group served as the control group.

Group II: Rats in this group were exposed to noise, serving as the noise exposure group without any treatment.

MAIN POINTS

- Pycnogenol is widely recognized for its strong antioxidant and anti-inflammatory effects, showing promising therapeutic potential in recent research. This study is the first to investigate the impact of pycnogenol on noise-induced hearing loss (NIHL) within the current body of literature.
- Pycnogenol can be conveniently taken orally, making it easy to administer and likely to improve patient compliance.
- In this research, the impact of pycnogenol on NIHL was evaluated through objective electrophysiological tests, which offer more reliable and precise measurements than subjective evaluations in comparable animal studies.
- The partially protective effect of pycnogenol observed in NIHL suggests the need for additional studies at different doses to clarify its full therapeutic potential.
- If further research supports these outcomes, pycnogenol could emerge as a practical pharmacological approach for preventing NIHL.

Group III (Noise + Saline): Rats in this group were exposed to noise and received daily oral administration of saline solution (40 mg/kg/day) via gavage for 7 days, serving as a control for the effects of noise exposure.

Group IV: Rats in this group received daily oral administration of Pycnogenol® (40 mg/kg/day) dissolved in distilled water via gavage for 7 days. Pycnogenol® was supplied by Solgar (Turkey). Administration commenced after the noise exposed.[14–16]

Group V (Pycnogenol® + Noise): Rats in this group were exposed to noise, followed by daily oral administration of Pycnogenol® (40 mg/kg/day) dissolved in distilled water via gavage for 7 days. This group allowed for the evaluation of the potential protective effects of Pycnogenol® against NIHL.

Anesthesia Procedure

To induce sedation in rats, a combination of ketamine (40 mg/kg; Ketasol vial, Richter Pharma AG, Wels, Austria) and xylazine (10 mg/kg; Rompun vial, Bayer, Istanbul) was administered intraperitoneally.[17]

Noise Exposure

Noise exposure was exclusively applied to Groups II, III, and V, excluding Groups I and IV. These groups were subjected to 4 kHz centered narrow-band noise (which is selected for its ability to imitate human noise exposures, its targeted impact on the rat cochlea's sensitivity, its established use in research for consistent comparisons, and its ability to isolate NIHL without affecting other frequencies) at 120 dB SPL intensity for 4 hours.[4,18,19] The noise, generated by an interacoustics AC-40 audiometer, was amplified using a Konig PRO-20008s amplifier. Hourly monitoring of noise levels was conducted using a PCE-430 sound-level meter. During noise exposure, each rat was individually housed in a cage measuring 15 x 15 x 15 cm.[20]

The Auditory Brainstem Response

Hearing thresholds at various frequencies were determined using auditory brainstem response (ABR) testing. Needle electrodes (0.40 mm diameter, 12 mm length; Ambu, Malaysia) with impedances between 0 kOhms and 3 kOhms were used. Stimulation and recordings were performed using an Intelligent Hearing Systems device calibrated to ANSI standards prior to experimentation.

The stimuli used in the ABR tests consisted of a 0.1 ms click stimulus and 4 ms tone burst stimuli (2 ms rise/fall, 0 ms plateau) at frequencies of 4, 8, 12, 16, and 32 kHz. Tone bursts utilized a Blackman envelope. All tests were conducted using alternating polarity.

Stimulus presentation varied based on frequency: insert earphones (ER-3A) were used for 4 and 8 kHz frequencies, while high-frequency transducers (high-frequency animal speaker) were employed for 12, 16, and 32 kHz frequencies.

A neonatal probe was used for all ABR recordings. Stimuli were presented at a repetition rate of 19.3 Hz, with 750 recordings averaged per waveform. Averaged waveforms underwent band-pass filtering (100-3000 Hz).

Threshold detection began at 80 dB SPL and decreased in 20 dB steps. As wave amplitudes decreased and approached the hearing

threshold, 5 dB steps were employed for intensity reduction. The hearing threshold was defined as the lowest intensity at which wave II was detectable. İn rats, ABR wave II is often preferred for threshold determination due to its robust and reliable nature, being less susceptible to noise and artifact compared to other waves. This makes it a clearer and more consistent measure of auditory function, especially when assessing hearing thresholds.[21] The recording window spanned 14 ms, encompassing 2 ms pre-stimulus and 12 ms post-stimulus. The estimated duration of the ABR test for each animal was approximately 1.5 hours. The threshold detection procedure involved monitoring wave II across all tested frequencies, commencing at 80 dB SPL. The threshold was established as the lowest intensity level at which wave II was discernible.

ABR tests for threshold determination were conducted at 4 distinct time points: before noise exposure (pretest), 1 day after noise exposure (day 1), 7 days after noise exposure (day 7), and 21 days after noise exposure (day 21).

Histological Sample Preparation

Following the completion of tests on day 21, the rats were euthanized under anesthesia, and their temporal bones were promptly dissected to extract the otic capsules. The extracted otic capsules were then immersed in a 10% formalin solution for fixation. Subsequently, the samples underwent decalcification with formic acid. Decalcification was carried out over a 1-month period in a formic acid solution that was refreshed twice weekly. Post-decalcification, the samples were processed through an alcohol series and embedded in paraffin.

Following stabilization of ear tissue specimens in a 10% neutral formaldehyde solution (pH 7.0-7.4) at +4°C, tissue tracing procedures were carried out. The specimens were then embedded in paraffin blocks, and 5-µm-thick sections were cut. These sections were analyzed using a Leica DM 6000 B microscope with the Leica Application Suite software.[22,23]

Immunohistochemical Assessment of Apoptosis

About 5 µm ear sections were prepared and stained using the TUNEL technique. Analysis was performed using Stereo Investigator version 11.0 image analysis software. To maintain objectivity, a defined area within each frame was selected for cell counting. The apoptotic index (%) was calculated (for each corti, spiral ganglion, stria vascularis, spiral ligament, and hair cells) by dividing the number of apoptotic cells by the total number of cells within the designated area and multiplying by 100. (Hair cells counted for each corti: 3 outer hair cells, 1 inner hair cell.)

Statistical Analysis

Data analysis involved several statistical tests. Initially, the Shapiro-Wilk test was employed to assess the normality of data distribution at each time point (pretest, day 1, day 7, and day 21). As the Shapiro-Wilk test indicated a non-normal distribution ($P < .05$), subsequent analyses utilized non-parametric methods. The Kruskal-Wallis test was chosen for overall group comparisons, examining differences in ABR threshold at each time point and cell apoptosis index results between the control and treatment groups. Pairwise comparisons between groups were conducted using the Mann-Whitney U-test.

All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) version 25 (IBM SPSS Corp.; Armonk, NY, USA), and results are presented as mean ± SD (mean ± SD). Statistical significance was established at $P < .05$.

RESULTS

Auditory Brainstem Response

In this study, a series of experiments was conducted on rats divided into different groups to assess their effects on hearing. Data obtained from the 5 groups were compared. The groups were determined as follows: Saline, Noise, Noise+Saline, Pycnogenol® and Noise+Pycnogenol® groups.

Baseline auditory brainstem response thresholds, assessed on day 0 prior to any interventions, exhibited no statistically significant differences across all experimental groups. This homogeneity at baseline underscores the comparable hearing status of the subjects at the study's outset. Furthermore, ABR assessments conducted on day 1, following the noise exposure protocol, revealed no statistically significant differences between the groups subjected to noise exposure and those in the control condition. This finding suggests that the noise exposure paradigm resulted in a consistent and evenly distributed impact on cochlear function across the noise-exposed animals.

Analysis of repeated ABR threshold measurements revealed a statistically significant difference between day 7 and day 21 ($P < .05$), indicating a temporal effect on hearing recovery. Among the noise-exposed groups, the Noise+Pycnogenol® group demonstrated significant improvements in ABR thresholds at specific frequencies. On day 7, significant improvements were observed at 4 kHz ($P = .002$) and 8 kHz ($P = .016$). By day 21, these improvements extended to 12 kHz ($P = .049$) and 16 kHz ($P = .036$), with sustained significance at 4 kHz ($P = .002$) and 8 kHz ($P = .015$) (see Tables 1-5). These findings suggest that Pycnogenol® treatment may promote hearing recovery following noise exposure. Notably, no significant differences were observed among the groups not subjected to noise exposure.

Comparing ABR thresholds between day 1 and day 21 revealed statistically significant improvements in the noise-exposed groups at frequencies of 4 kHz ($P = .004$), 8 kHz ($P = .008$), 12 kHz ($P = .046$), and 16 kHz ($P = .009$). This suggests a time-dependent recovery of auditory

Table 1. Mean and Standard Deviation Values of 4 kHz ABR Thresholds for All Groups on Days 0, 1, 7, and 21

	Day 0	Day 1	Day 7	Day 21
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Group I	18.50 ± 3.37	19.5 ±2.84	17.50 ± 3.54	17.50 ± 4.25
Group II	13.00 ± 6.32	70.50 ± 5.50	51.00 ± 11.01	53.50 ± 9.14
Group III	17.00 ± 3.50	67.50 ± 8.25	65.00 ± 8.16	58.00 ± 5.37
Group IV	15.50 ± 4.97	17.00 ± 2.58	17.00 ± 3.50	17.50 ± 3.54
Group V	14.17 ± 4.17	67.50 ± 7.94	50.00 ± 8.79	44.58 ± 7.53
$P^{\text{Group total}}$	.052	**.000***	**.000***	**.000***
$P^{\text{Groups II,III,V}}$	.105	.549	**.002***	**.002***
$P^{\text{Groups I,IV}}$	.143	.105	.971	.912

Asterisks (*) indicate significant differences between groups. *$P < .05$.

Table 2. Mean and Standard Deviation Values of 8 kHz ABR Tthresholds for All Groups on Days 0, 1, 7, and 21

	Day 0	Day 1	Day 7	Day 21
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Group I	12.00 ± 2.58	15.00 ± 4.71	11.00 ± 3.16	12.50 ± 3.54
Group II	11.00 ± 5.16	70.00 ± 6.77	51.00 ± 13.90	49.50 ± 12.35
Group III	11.50 ± 4.74	71.00 ± 8.10	62.00 ± 7.53	58.00 ± 5.87
Group IV	12.00 ± 5.87	12.00 ± 3.50	12.50 ± 2.64	12.00 ± 3.50
Group V	10.42 ± 3.34	73.75 ± 6.78	49.58 ± 9.16	46.25 ± 7.72
$P^{\text{Group total}}$	.893	**.000***	**.000***	**.000***
$P^{\text{Groups II, III, V}}$	.956	.388	**.016***	**.015***
$P^{\text{Groups I,IV}}$	1.000	.218	.353	.971

Asterisks (*) indicate significant differences between groups. *P < .05.

Table 4. Mean and Standard Deviation Values of 16 kHz ABR Thresholds for All Groups on Days 0, 1, 7, and 21

	Day 0	Day 1	Day 7	Day 21
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Group I	21.50 ± 8.18	22.00 ± 6.32	21.50 ± 6.26	23.00 ± 6.75
Group II	22.50 ± 4.25	75.50 ± 8.96	58.00 ± 11.83	64.00 ± 9.07
Group III	21.50 ± 4.12	76.50 ± 12.48	64.00 ± 10.22	58.50 ± 11.32
Group IV	18.00 ± 6.32	22.00 ± 4.22	22.00 ± 4.83	22.50 ± 5.84
Group V	19.58 ± 3.96	75.00 ± 7.07	57.50 ± 10.98	51.67 ± 11.15
$P^{\text{Group total}}$	.226	**.000***	**.000***	**.000***
$P^{\text{Groups II, III, V}}$	.266	.885	.365	**.036***
$P^{\text{Groups I, IV}}$	.218	.971	.853	.796

Asterisks (*) indicate significant differences between groups. *P < .05.

function following noise exposure. However, consistent with previous findings, no significant differences were observed among the groups that did not undergo noise exposure, further supporting the specificity of these improvements to NIHL (Figure 1).

HISTOLOGICAL IMAGING RESULTS

Histological examinations involved staining tissue samples with TUNEL staining, which enabled the quantification of apoptosis rates in the spiral ganglion, stria vascularis, spiral ligament, and hair cells. Cellular assessments were performed in 3 distinct regions: basal, middle, and apical. All apoptotic index measurements are reported as percentages to facilitate comparisons.

No statistically significant difference was detected between the control group and the Pycnogenol® group, nor among the noise group, noise+saline group, and noise+ Pycnogenol® group for examining hair cells.

However, significant differences were observed in the Noise+ Pycnogenol® group compared to the control groups when examining apoptosis rates in the spiral ganglion ($P = .000$), stria vascularis ($P = .000$), and spiral ligament ($P = 0.000$) (see Supplementary Figure 1). These findings suggest that Pycnogenol® treatment may exert protective effects against noise-induced apoptosis in these cochlear structures (see Table 6). The observed histological improvements across all investigated parameters further support the potential therapeutic benefits of Pycnogenol® in mitigating NIHL.

DISCUSSION

Antioxidant agents represent a prominent area of investigation for NIHL prevention and treatment, with numerous preclinical and clinical studies exploring their potential to mitigate oxidative stress within the cochlea.[24]

Several studies have demonstrated the protective effects of various antioxidants against NIHL. Kilic et al. found berberine to be effective in reducing oxidative stress associated with NIHL, while Tziridis et al. showed similar protective effects with Ginkgo biloba. Bahaloo et al. further demonstrated the efficacy of myricetin in preventing NIHL. However, to date, no research has investigated the potential of Pycnogenol® as a protective agent against NIHL.[25–27]

The role of free radicals in the development of NIHL is widely recognized. High-intensity noise exposure disrupts the delicate balance within the cochlea, leading to an overproduction of reactive oxygen species.[18] This, in turn, triggers a cascade of events including oxidative stress, cellular damage, and ultimately, cell death. These free radicals damage cell membranes, proteins, and DNA, impairing cochlear function and contributing to hearing loss.[28] The protective effects of antioxidants against NIHL are largely attributed to their ability

Table 3. Mean and Standard Deviation Values of 12 kHz ABR Thresholds for All Groups on Days 0, 1, 7, and 21

	Day 0	Day 1	Day 7	Day 21
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Group I	19.00 ± 2.11	18.50 ± 2.42	18.00 ± 2.58	20.00 ± 3.33
Group II	18.00 ± 5.37	79.50 ± 9.85	59.00 ± 8.10	63.00 ± 11.35
Group III	18.00 ± 2.58	82.00 ± 9.49	67.50 ± 7.17	66.00 ± 6.58
Group IV	15.00 ± 5.27	16.00 ± 3.16	15.00 ± 3.33	17.00 ± 5.37
Group V	14.58 ± 4.50	79.17 ± 9.49	64.58 ± 9.16	55.42 ± 10.54
$P^{\text{Group total}}$	**.064**	**.000***	**.000***	**.000***
$P^{\text{Groups II, III, V}}$	.126	.918	.099	**.049***
$P^{\text{Groups 1,IV}}$	.063	.105	.075	.190

Asterisks (*) indicate significant differences between groups. *P < .05.

Table 5. Mean and Standard Deviation Values of 32 kHz ABR Thresholds for All Groups on Days 0, 1, 7, and 21

	Day 0	Day 1	Day 7	Day 21
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Group I	23.50 ± 4.12	23.50 ± 6.26	22.00 ± 4.22	21.50 ± 5.30
Group II	22.00 ± 3.50	79.50 ± 10.39	54.50 ± 16.41	60.00 ± 18.41
Group III	17.50 ± 7.17	80.50 ± 10.39	66.50 ± 12.70	59.00 ± 15.06
Group IV	19.00 ± 9.66	20 .00 ± 6.67	22.00 ± 4.83	24.50 ± 4.38
Group V	18.33 ± 4.44	81.25 ± 7.72	57.92 ± 13.73	49.17 ± 15.79
$P^{\text{Group total}}$	.098	**.000***	**.000***	**.000***
$P^{\text{Groups II, III, V}}$	.071	.805	.110	.124
$P^{\text{Groups I, IV}}$	.481	.247	.971	.247

Asterisks (*) indicate significant differences between groups. *P < .05.

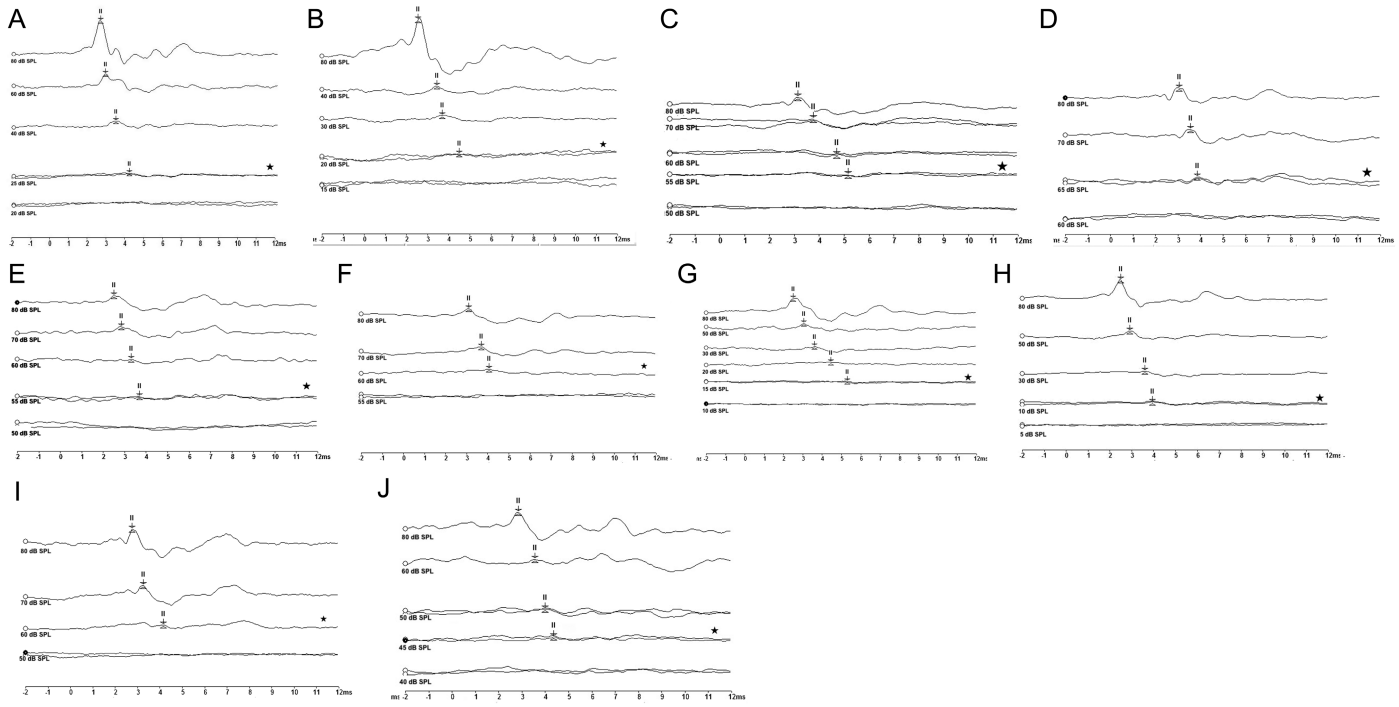

Figure 1. (A-J). Wave morphology of auditory evoked brainstem potentials obtained at 4 kHz on day 1 and day 21. (A) Wave morphology of auditory evoked brainstem potentials obtained on day 1 at a frequency of 4 kHz in saline group. (B) Wave morphology of auditory evoked brainstem potentials obtained on day 21 at a frequency of 4 kHz in saline group. (B) Wave morphology of auditory evoked brainstem potentials obtained on day 1 at a frequency of 4 kHz in noise group. (D) Wave morphology of auditory evoked brainstem potentials obtained on day 21 at a frequency of 4 kHz in noise group. (E) Wave morphology of auditory evoked brainstem potentials obtained on day 1 at a frequency of 4 kHz in noise+saline group. (F) Wave morphology of auditory evoked brainstem potentials obtained on day 21 at a frequency of 4 kHz in noise+saline group. (G) Wave morphology of auditory evoked brainstem potentials obtained on day 1 at a frequency of 4 kHz in Pycnogenol® group. (H) Wave morphology of auditory evoked brainstem potentials obtained on day 21 at a frequency of 4 kHz in Pycnogenol® group. (I) Wave morphology of auditory evoked brainstem potentials obtained on day 1 at a frequency of 4 kHz in Pycnogenol® +noise group. (J) Wave morphology of auditory evoked brainstem potentials obtained on day 21 at a frequency of 4 kHz in Pycnogenol® +noise group.

to mitigate this oxidative stress cascade. Pycnogenol is a complex mixture of diverse polyphenolic compounds, including proanthocyanidins, catechins, and taxifolins, which exhibit potent antioxidant properties. These compounds not only directly scavenge free radicals but also mitigate inflammation by inhibiting the NF-κB pathway and bolster endogenous antioxidant defense mechanisms by activating the Nrf2 pathway. Furthermore, evidence suggests that Pycnogenol may reduce oxidative stress at the cellular level by improving mitochondrial function.[6,29–31]

Eryılmaz et al. demonstrated the protective effect of pine bark extract against metabolic damage in a cisplatin-induced ototoxicity model. Given that cisplatin ototoxicity shares similarities with NIHL in terms of oxidative stress and cell death pathways, this finding further supports the potential of pine bark extract as a therapeutic agent for NIHL.[32]

In our study, significant differences were observed in terms of the efficacy of Pycnogenol® when examining the apoptotic results of the spiral ganglion, stria vascularis, and spiral ligament in the Noise+Pycnogenol® group compared to the control groups. Specifically, the Noise+ Pycnogenol® group exhibited significantly reduced apoptosis in all 3 structures compared to the noise-exposed control group, suggesting a protective effect of Pycnogenol® against noise-induced cellular damage. In addition to histological studies, investigating the intracellular metabolism of NIHL using non-invasive tests is important for guiding studies in humans. Therefore, in our study, the ABR test was also performed.

All groups exhibited similar ABR thresholds at the pretest, confirming comparable hearing levels before noise exposure. Following noise exposure, the Pycnogenol® treatment group demonstrated significantly smaller ABR threshold shifts compared to the noise-only and other control groups. This protective effect was observed at frequencies of 4, 8, 12, and 16 kHz, and was particularly pronounced on days 7 and 21 post-exposure. These findings strongly suggest that Pycnogenol® administration effectively mitigated NIHL.

We believe that the lack of significant differences in hair cell counts between groups may be attributed to the limitations of analyzing modiolar sections, which are not ideal for examining hair cells oriented along the horizontal axis. Therefore, we suggest that a more objective analysis would require a larger sample size of imaged sections.

Pycnogenol® presents as a promising antioxidant with numerous favorable properties. Its accessibility, established human dosage guidelines, and diverse health benefits make it a strong candidate for further research. Notably, Pycnogenol® has demonstrated a favorable safety profile in toxicity studies. These factors support the need for further investigation into Pycnogenol®'s therapeutic potential in humans.

Table 6. Apoptosis and Normal Cell Averages and Apoptotic Indexes (%) of the Spiral Ganglion, Stria Vascularis, Spiral Ligament, and Hair Cells with TUNEL Staining in All Groups Are Shown. (Hair Cells Are Counted for Each Corti: 3 Outer Hair Cells, 1 Inner Hair Cells.)

		Basal					Middle					Apical				
Groups		I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
SG	**A**	3.25	15.88	14.88	3.64	8.00	3.63	14.25	15.25	4.00	7.00	3.13	13.50	16.13	4.91	3.00
	N	210.13	177.88	189.38	205.73	197.50	208.25	167.13	174.38	206.09	181.75	195.63	167.50	167.00	187.00	173.63
	I	1%	8%	8%	2%	4%	2%	8%	8%	2%	4%	2%	7%	9%	3%	2%
$p^{\text{Groups I, IV}}$				.473					.571					.016		
$p^{\text{Groups II, III, V}}$				**.000*****					**.000*****					**.000*****		
SL	**A**	4.75	16.63	18.88	5.73	7.63	4.00	19.38	17.50	4.18	6.13	4.13	16.50	18.25	5.18	7.13
	N	86.63	58.00	57.25	82.64	67.38	91.63	56.00	47.88	84.91	69.63	92.13	65.50	62.13	86.27	72.50
	I	5%	22%	25%	6%	10%	4%	26%	27%	5%	8%	4%	20%	23%	5%	9%
$p^{\text{Groups I, IV}}$				.047					.521					.157		
$p^{\text{Groups II, III, V}}$				**.000*****					**.000*****					**.000*****		
SV	**A**	2,13	14,25	14,25	3,73	5,63	3,75	18,25	17,75	5,27	6,63	2,13	15,88	16,13	3,45	6,00
	N	54,75	35,50	43,75	54,64	45,63	55,88	41,63	42,13	56,73	51,00	48,00	36,38	36,63	48,91	46,25
	I	4%	29%	25%	6%	11%	6%	30%	30%	8%	12%	4%	31%	31%	7%	12%
$p^{\text{Groups I, IV}}$				.012					.069					.012		
$p^{\text{Groups II, III, V}}$				**.000*****					**.000*****					**.000*****		
OHC	**A**	0.27	1.74	1.65	0.43	2.00	0.21	1.28	1.23	0.25	1.01	0.36	1.58	1.33	0.11	0.68
	N	2.73	1.26	1.35	2.57	1.00	2.79	1.72	1.77	2.75	1.99	2.64	1.42	1.67	2.89	2.32
	I	90.90%	42.16%	45.00%	85.71%	33.33%	93.07%	57.26%	59.03%	91.67%	66.20%	87.86%	47.22%	55.56%	96.30%	77.27%
$p^{\text{Groups I,IV}}$				.536					.837					.730		
$p^{\text{Groups II, III, V}}$				.749					.268					.064		
IHC	**A**	0.02	0.20	0.20	0.04	0.02	0.00	0.14	0.13	0.00	0.09	0.00	0.31	0.22	0.00	0.00
	N	0.98	0.80	0.80	0.96	0.98	1.00	0.86	0.88	1.00	0.91	1.00	0.69	0.78	1.00	1.00
	I	97.78%	80.09%	80.00%	95.83%	97.84%	100.00%	85.59%	87.50%	100.00%	90.73%	100.00%	69.4.4%	77.78%	100.00%	100.00%
$p^{\text{Groups I, IV}}$				.963					1.000					1.000		
$p^{\text{Groups II, III, V}}$				.427					.062					.063		

A, apoptosis; I, apoptotic Index; IHC, inner hair cells; N, normal; OHC, outer hair cells; SG, spiral ganglion; SL, spiral ligament; SV, Stria Vascularis.
Asterisks (***) indicate significant differences between groups. *P < .05.

CONCLUSION

In conclusion, while the preliminary findings presented in this study suggest a potential protective role for Pycnogenol® against NIHL, further in vivo research is warranted to elucidate its precise therapeutic mechanisms, particularly its impact on auditory metabolic pathways at the molecular level.

Availability of Data and Materials: All relevant data are included in the article and its supplementary information files.

Ethics Committee Approval: This study was approved by the Ethics Committee İstanbul Medipol University (approval no: E-38828770-772.02-2865; date: March 28, 2023).

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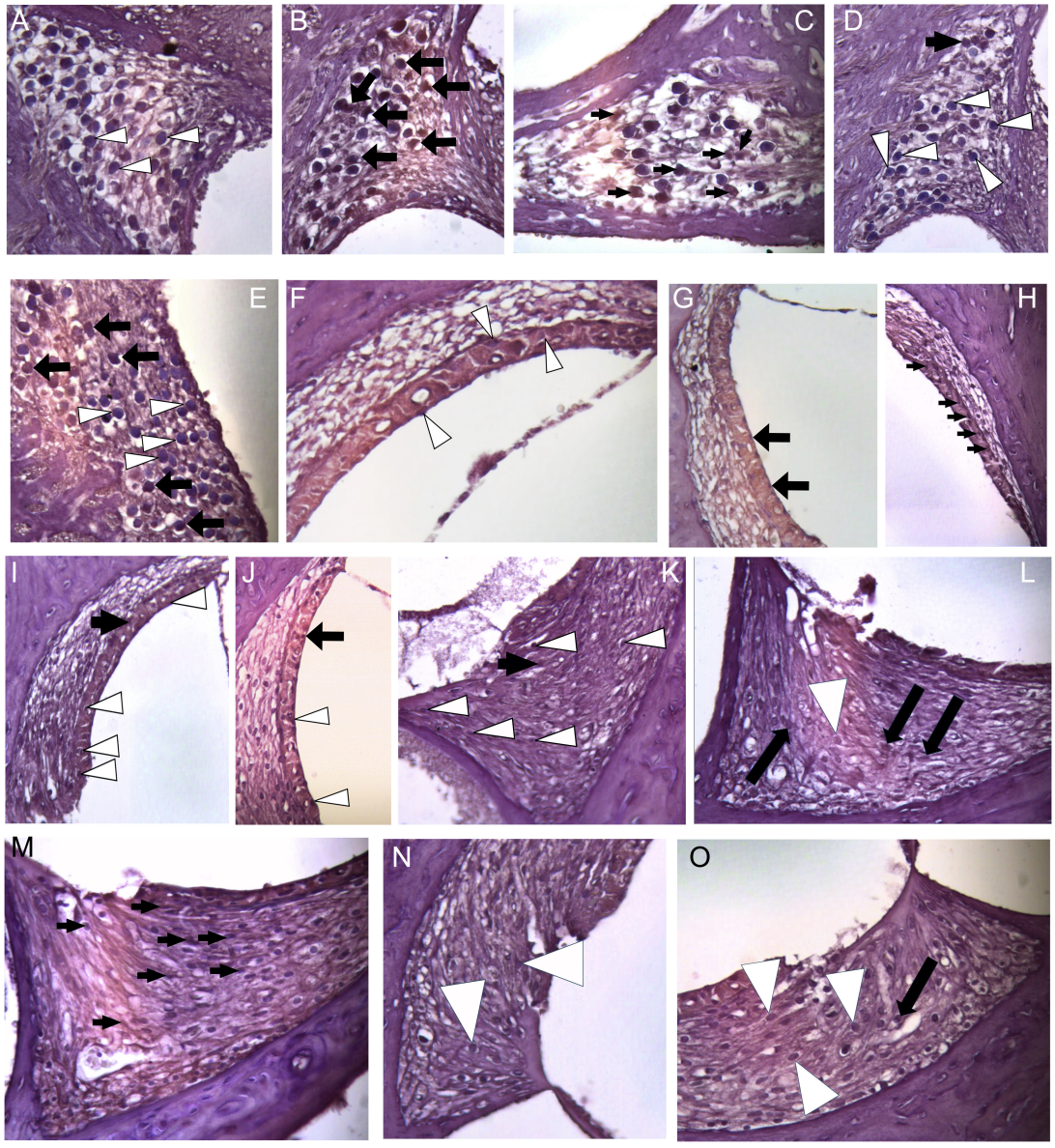

Supplementary Figure 1. a-o. TUNEL staining of spiral ganglion and stria vascularis cross-section of all groups. (a) Spiral ganglion imaging of the saline group 40× (demonstrated white triangle are alive cells). At basal turn, the apoptotic cell index is 1%, at middle turn, the apoptotic cell index is 2%, and at apical turn, the apoptotic cell index is 2%. (b) Spiral ganglion imaging of the noise group 40× (apoptotic cells are indicated by a black arrow.). At basal turn apoptotic cell index is %8, At middle turn apoptotic cell index is %8, At apical turn apoptotic cell index is %7. (c) Spiral ganglion imaging of the noise+saline group 40× (apoptotic cells are indicated by a black arrow.). At basal turn apoptotic cell index is %8, At middle turn apoptotic cell index is %8, At apical turn apoptotic cell index is %9. (d) Spiral ganglion imaging of the Pycnogenol® group 40× (apoptotic cells are indicated by a black arrow and alive cells pointed by a white triangle). At basal turn, the apoptotic cell index is 2%, at middle turn, the apoptotic cell index is 2%, and at apical turn, the apoptotic cell index is 3%. (e) Spiral ganglion imaging of the Pycnogenol®+noise group 40× (apoptotic cells are indicated by a black arrow and alive cells pointed by a white triangle). At basal turn, the apoptotic cell index is 4%, at middle turn, the apoptotic cell index is 4%, and at apical turn, the apoptotic cell index is 2%. (f) Stria vascularis imaging of the saline group 40× (demonstrated white triangle are alive cells). At basal turn, the apoptotic cell index is 4%, at middle turn, the apoptotic cell index is 6%, and at apical turn, the apoptotic cell index is 4%. (g) Stria vascularis imaging of the noise group S40× (Apoptotic cells are indicated by a black arrow.). At basal turn apoptotic cell index is %29, At middle turn apoptotic cell index is %30, At apical turn apoptotic cell index is %31. (h) Stria vascularis imaging of the noise+saline group 40× (apoptotic cells are indicated by a black arrow.). At basal turn apoptotic cell index is %25, At middle turn apoptotic cell index is %30, At apical turn apoptotic cell index is %31. (i) Stria vascularis imaging of the Pycnogenol® group 40× (apoptotic cells are indicated by a black arrow and alive cells pointed by a white triangle). At basal turn, the apoptotic cell index is 6%, at middle turn, the apoptotic cell index is 8%, and at apical turn, the apoptotic cell index is 7%. (j) Stria vascularis imaging of the Pycnogenol®+noise group 40× (apoptotic cells are indicated by a black arrow and alive cells pointed by a white triangle). At basal turn, the apoptotic cell index is 11%, at middle turn, the apoptotic cell index is 12%, and at apical turn, the apoptotic cell index is 12%. (k) Spiral ligament imaging of the saline group 40× (apoptotic cells are indicated by a black arrow and alive cells pointed by a white triangle). At basal turn, the apoptotic cell index is 5%, at middle turn, the apoptotic cell index is 4%, and at apical turn, the apoptotic cell index is 4%. (l) Spiral ligament imaging of the noise group 40× (apoptotic cells are indicated by a black arrow and alive cells pointed by a white triangle). At basal turn, the apoptotic cell index is 22%, at middle turn, the apoptotic cell index is 26%, and at apical turn, the apoptotic cell index is 20%. (m) Spiral ligament imaging of the noise+saline group 40× (apoptotic cells are indicated by a black arrow.). At basal turn apoptotic cell index is %25, At middle turn apoptotic cell index is %27, At apical turn apoptotic cell index is %23. (n) Spiral ligament imaging of the Pycnogenol® group 40× (apoptotic cells are indicated by a black arrow and alive cells pointed by a white triangle). At basal turn apoptotic cell index is %6, At middle turn apoptotic cell index is %5, At apical turn apoptotic cell index is %5. (o) Spiral ligament imaging of the Pycnogenol®+noise group 40× (apoptotic cells are indicated by a black arrow and alive cells pointed by a white triangle). At basal turn apoptotic cell index is %10, At middle turn apoptotic cell index is %8, At apical turn apoptotic cell index is %9.