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Zingiberene attenuates paclitaxel-induced ototoxicity by strengthening cochlear antioxidant defense system in vivo

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ABSTRACT: Paclitaxel is widely used in the treatment of many cancers. Paclitaxel-induced ototoxicity is related to the neurotoxic effects of paclitaxel on auditory peripheral neurons. Zingiberene has significant antitumor and antioxidant properties. This study aimed to determine whether zingiberene protects against the ototoxicity caused by paclitaxel. Twenty-four Wistar Albino rats were divided into four groups. The control group received 1 ml/kg saline on days 1, 7, 14, and 21. The paclitaxel group received 5 mg/kg paclitaxel on days 1, 7, 14, and 21. On days 1, 7, 14, and 21, the zingiberene group received 10mg/kg of zingiberene. Paclitaxel + zingiberene group first 5 mg/kg paclitaxel and 30 minutes later 10mg zingiberene on the 1st, 7th, 14th, and 21st days. A distortion product-evoked otoacoustic emission test (DPOAE) was performed before (day 0) and after (day 22) of the experiment. The pretreatment DPOAE values of the groups were not significantly different. On day 22, the DPOAE results in the paclitaxel group showed a considerable decline. Malondialdehyde levels were substantially higher, and glutathione levels were much lower in the paclitaxel group. The paclitaxel+zingiberene group displayed significantly higher DPOAE levels than the paclitaxel group. Compared to the paclitaxel group paclitaxel+zingiberene, glutathione levels were considerably higher, and malondialdehyde levels were significantly lower. The study findings provide the first evidence in the literature that zingiberene can prevent ototoxicity from paclitaxel-induced hearing loss by lowering the levels of oxidant parameters. It demonstrates that administering zingiberene and paclitaxel together may be a practical clinical approach to alleviate paclitaxel-induced ototoxicity.

KEYWORDS: Antioxidants; ototoxicity; paclitaxel; reactive oxygen species; zingiberene.

1. INTRODUCTION

Ototoxicity is a condition that causes hearing loss permanently or temporarily as a result of exposure to chemotherapeutic or other medications, especially when treating severe systemic illnesses like cancer [1]. More than 150 medicines, including chemotherapeutic drug-induced hearing loss documented more commonly in patients in clinical results, are now recognized as ototoxic. In this scenario, ototoxic hearing loss, which develops after intensive chemotherapeutic treatments due to rising cancer prevalence, is a serious clinical concern that impairs communication and affects the quality of life. Many studies have been published on cisplatin and aminoglycoside antibiotics' ototoxic effects [2]. However, there is limited information on the ototoxic effects of paclitaxel (PCX), which is used to treat various cancers. That is because PCX is commonly used with antineoplastic pharmaceuticals like cisplatin, which are believed to cause hearing loss [3]. However, the recent findings demonstrating that PCX has ototoxic effects particularly call for greater research in this area [4, 5].

PCX, a tetracyclic diterpenoid molecule derived from Pacific yew tree bark, is a taxane family anticancer pharmaceutical with a broad spectrum of activity [6]. PCX, a tubulin stabilizer, inhibits cell proliferation by binding to the β portion of intracellular microtubules, boosting tubulin polymer stability and thereby blocking microtubule depolarization [7]. Like other chemotherapeutic drugs, PCX has many side effects that limit its clinical use. Especially PCX's neurotoxic action causes a decrease in

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sensory nerve conduction velocity over the dorsal root ganglia and adverse effects on peripheral axons. PCX's neurotoxic effects on sensory neurons are projected to be similar to those on neurons in the auditory periphery, implying PCX's ototoxic effects [8-10].

The pathophysiology of chemotherapeutic drug-induced ototoxicity is driven by reactive oxygen species (ROS), which cause oxidative damage to the cochlea. To combat reactive oxygen species (ROS) and keep the oxidative system in balance, the cochlea includes an internal antioxidant mechanism. However, due to the excessive rise in free radicals in the cochlea, this equilibrium is disturbed in favor of oxidants, leading to an increase in lipid peroxidation and oxidative damage [10]. Malondialdehyde (MDA), a biochemical marker utilized as a biomarker of lipid degradation in tissues, is generated as a result of oxidative damage caused by the impacts of ROS [11]. Additionally, it is observed that chemotherapeutic drugs cause ototoxicity with insufficient glutathione (GSH) content and inactivation of the antioxidant system, while they cause excessive free radical generation in the cochlea. As a result of oxidative damage observed in excessive oxidative stress or insufficiency of antioxidant potential, the level of glutathione (GSH), which is an important antioxidant molecule in the structural and functional preservation of the integrity of cell, tissue and organ systems, decreases and pathological conditions occur due to free radical destruction [12]. Oxidative damage can be mitigated by diminishing the generation of ROS or boosting the antioxidant system. As a result, antioxidants are commonly used in contemporary methods to overcome the ototoxic effects of chemotherapeutics [10]. Bioactive secondary metabolites of medicinal plants are frequently included in recent ototoxicity investigations owing to their unique antioxidant content and free radical scavenging capacity [13]. Zingiberene (ZBN), the main component of ginger oil extracted from the medicinally important plant Zingiber officinale, is a monocyclic sesquiterpenoid. Antioxidant, antiviral, antibacterial, antiulcer, and anticancer properties are just some of the pharmacological effects of ZBN [14-16]. Herein, the present research was framed to unveil the anti- ototoxic effects of ZBN by boosting antioxidant levels through scavenging free radical.

2. RESULTS

2.1. Audiological evaluation results

All rats were tested using DPOAE assays pre- and post-dugs treatment to establish baseline hearing sensitivity function. Table 1 shows group means and standard deviations for each experiment for the change in DPOAE threshold shifts between pre- and post-test auditory test sessions. Figure 1 shows the comparison of inter-group analyses. In intra-group and inter-group analyses, baseline DPOAE thresholds (0. day) were similar for all frequencies ($p>0.05$). When the DPOAE values of the PCX group on the 22nd day were compared to the DPOAE values on the first day, the DPOAE values of this group showed a substantial decrease following PCX administration. On the 22nd day, the DPOAE values of the PCX group were considerably lower than the DPOAE values of the CONTROL group. This result demonstrates that PCX induces cochlear damage in rats. There was no significant difference in hearing thresholds between the DPOAE results of the control group on the 22nd day and the DPOAE results on the 0. day. Similarly, the DPOAE values of the ZBN group on the 22nd day did not differ significantly from the DPOAE values of the 0. day. Furthermore, no substantial difference was seen between the 22nd day DPOAE values of the rats given PCX with ZBN and either in the CONTROL group or the 0. day DPOAE values. On the other hand, the DPOAE values of the PCX+ZBN group were considerably greater at all frequencies compared to the DPOAE values of the rats who only received PCX. This demonstrates that PCX's ototoxic action reduces DPOAE values at all frequencies but that ZBN, combined with PCX, prevents this decrease in DPOAE values.

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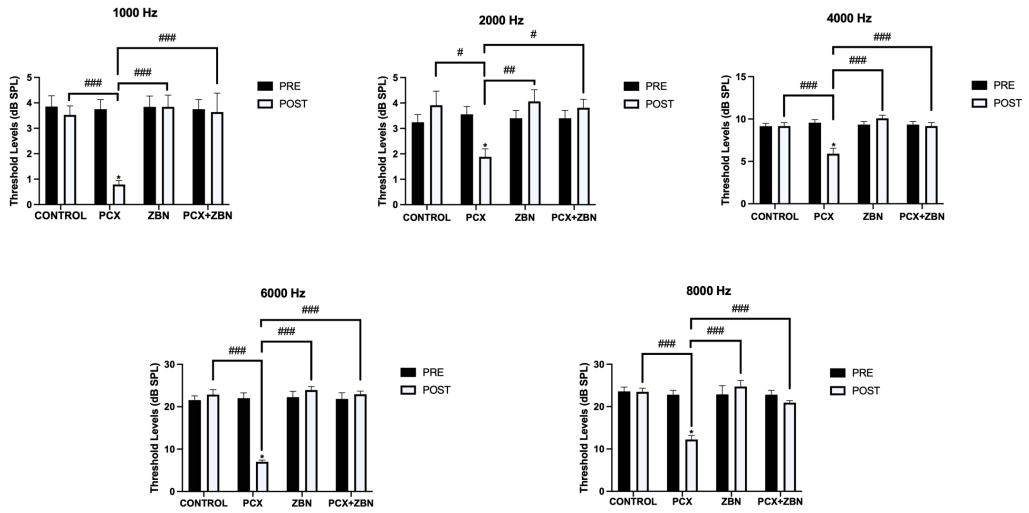

Figure 1. Inter-group comparison of DPOAEs amplitude levels in different frequencies (1000-8000Hz). One-way ANOVA was used to make statistical comparisons, followed by Tukey's Significant Difference test. *p<0.05 denotes that the pre- and post-DPOAE values in the PCX group significantly differed. The #p < 0.05, ##p < 0.01, ###p < 0.001 signs were used for comparison with the PCX group. ZBN: Zingiberene; PCX: Paclitaxel; DPOAE: Distortion product otoacoustic emissions. The values shown in the graphs are the means ± standard deviations.

Table 1. Effect of ZBN treatment on comparison of intra-group the pretreatment (0. day) and post-treatment (22nd day) DPOAE amplitude levels for 1000–8000 Hz frequencies

DPOAE Frequencies	Pre Post	CONTROL	PCX	ZBN	PCX+ZBN
1000	0 day	3,86±0,42	3,75±0,38	3,84±0,43	3,75±0,38
	22nd day	3,53±0,354	0,79±0,15*	3,84±0,46	3,64±0,75
2000	0 day	3,24±0,31	3,56±0,31	3,4±0,31	3,4±0,31
	22nd day	3,91±0,55	1,89±0,31*	4,06±0,46	3,81±0,33
4000	0 day	9,16±0,32	9,57±0,35	9,34±0,37	9,34±0,37
	22nd day	9,17±0,41	5,90±0,66*	10,08±0,37	9,17±0,41
6000	0 day	21,55±1,01	22,47±1,25	22,27±1,36	21,84±1,44
	22nd day	22,87±1,17	6,74±0,41*	23,94±0,83	22,93±0,75
8000	0 day	23,58±1,03	22,8±2,048	22,90±1,031	22,8±1,031
	22nd day	23,49±0,85	12,24±1,47*	24,72±0,96	21,07±0,47

ZBN: Zingiberene; PCX: Paclitaxel; DPOAE: distortion product otoacoustic emissions. The paired-samples T-test was used for intra-group comparisons of DPOAE outcomes. * Post-treatment statistically significant for intra-group comparison. The values shown in the table are the means ± standard deviations.

2. Biochemical evaluation results

As a biochemical evaluation of ototoxicity, the results of GSH and MDA levels are represented in Figures 2 A and B, respectively. MDA levels increased while GSH levels decreased in the PCX group compared to the CONTROL group. This result indicates that PCX triggers oxidative stress in the cochlea, probably contributing to ototoxicity. MDA levels in the PCX+ZBN group were substantially lower than in the PCX group. Also, when the MDA levels of the ZBN and CONTROL groups were examined compared to the PCX group, MDA levels were significantly lower. GSH levels were also

markedly higher in CONTROL, ZBN, and PCX+ZBN groups than in the PCX group. These data revealed that ZBN administration restored MDA and GSH levels that had been depleted by PCX, showing that ZBN treatment was protective against oxidative stress.

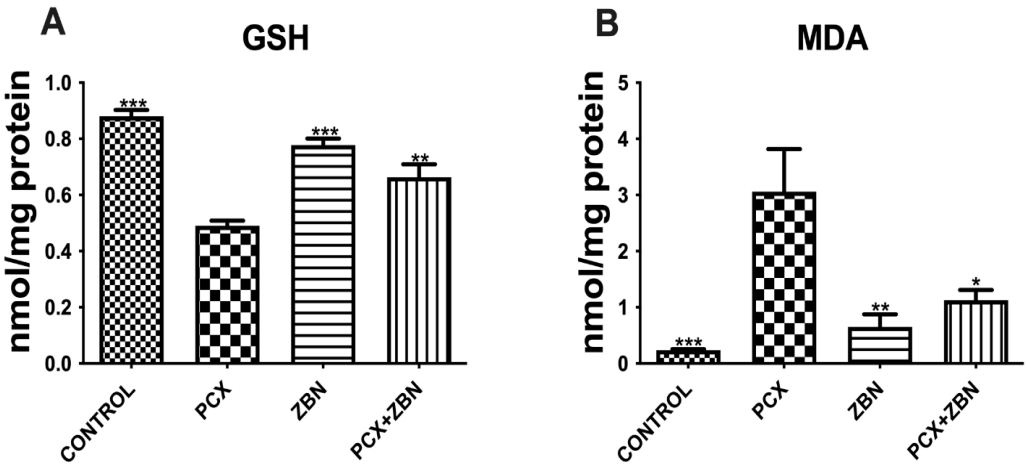

Figure 2. The effect of ZBN on GSH and MDA levels in groups that underwent PCX-induced ototoxicity. One-way ANOVA was used to make statistical comparisons, followed by Tukey's Significant Difference test. The *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ signs were used for comparison with the PCX group. GSH: glutathione; MDA: Malondialdehyde; ZBN: Zingiberene; PCX: Paclitaxel. The values shown in the graphs are the means ± standard deviations.

3. DISCUSSION

ZBN reduced PCX-induced ototoxicity in a rat model. Assessments of DPOAE elevation were used to evaluate the functional impact of ZBN. By lowering the MDA level and raising the GSH level, ZBN demonstrated its antioxidative activity. In conclusion, ZBN's antioxidant properties may suggest that it can be utilized to prevent and treat other illnesses and symptoms, as well as in patients who are worried about ototoxic side effects from anticancer therapy with PCX. We believe that our study is the first to explore the impact of ZBN on ototoxic damage.

Data on the effects of PCX on the inner ear are quite rare, although numerous anticancer drugs have been demonstrated to be ototoxic. The co-administration of PCX with many other antineoplastic drugs, including cisplatin, whose toxic effects have been well-documented, is the main cause of the ototoxic impact of PCX being obscured. As a result, functional abnormalities in the auditory system occurring during or after anticancer therapy were almost always attributed to other ototoxic drugs in combination except for PCX [3, 17]. According to the mechanism of PCX, a microtubule stabilizing agent, it attaches to the β portion of microtubules to prevent depolarization and diminish axonal transmission. The mechanism of PCX results in a common adverse effect of its treatment known as sensorimotor axonal neuropathy, which is dose-related [6, 7]. As β-tubulin isoforms are present in both hair cells and neurons, it is not surprising that considerable damage to both auditory nerve fibers and spiral ganglion neurons occurs, given the known toxic mechanism of PCX [18]. As a result, another plausible cause for why PCX by itself is not thought to be ototoxic is that it may result in "latent hearing loss" by predominantly harming auditory nerve fibers rather than outer hair cells [3, 8, 19]. A few clinical trials on the impact of paclitaxel on sensorineural hearing loss have been documented [20, 21]. Research revealing the link between hearing loss and paclitaxel, particularly in recent experimental studies, is notable and it is observed that paclitaxel reduces DPOAE frequencies [4, 5, 22]. Consistent with these results, a decline in DPOAE results was observed in the PCX group in the current research. Thus, this confirms that PCX damages hearing.

The main cause of ototoxicity brought on by chemotherapeutics is oxidative stress, although the pathogenic mechanism causing the temporary or permanent hearing loss is not entirely known [1, 2, 23, 24]. By boosting the ROS generation and decreasing antioxidant enzyme systems, PCX can start many pathological processes in the cochlea [4]. The cochlea has an effective antioxidant defense mechanism against ototoxic effects, including antioxidants like vitamin C, E, and GSH [25]. However, excessive ROS generation reduces GSH and inhibits antioxidant enzymes, shifting the balance between oxidants and antioxidants in favor of oxidants. Since the ototoxic effect disarms the body's natural defenses, the

increase in lipid peroxidation, which is the primary cause of many pathophysiological conditions, causes cell damage [26, 27]. Consistent with this information, in our study, MDA levels, an indicator of lipid peroxidation, were significantly higher in the cochlea of rats receiving PCX than in the CONTROL group. Also, the GSH level in the cochlear tissues of rats receiving PCX was significantly lower than in the CONTROL group. These findings indicate that PCX impairs the cochlea's endogenous antioxidant defense mechanism and that oxidative stress contributes to cochlear damage.

This gap in the literature will be filled by research and the discovery of substances with potential otoprotective effects, as ototoxicity is the most significant side effect limiting chemotherapeutics in clinical practice and an important factor reducing the quality of life of cancer patients. An ideal otoprotective drug, however, should offer dependable protection with few side effects without diminishing chemotherapeutics' potential to combat tumors [28]. In this context, diminishing the excessive ROS generation that underlies the pathophysiology of ototoxicity or boosting the antioxidant system can reduce the ototoxic effect of chemotherapeutics. Exogenous antioxidants are thus the most remarkable agents and have evolved into the main strategy of ototoxicity treatment in research [5, 22, 29-31].

Our study aimed to determine whether ZBN, a powerful antioxidant, would have an otoprotective effect against PCX-induced ototoxicity. ZBN is a major component of Zingiber officinale and is a monocyclic sesquiterpene with essential medicinal properties including antioxidant [14], antibacterial [32], and antiviral [33], antiulcer [34], and anticancer [16, 35-37]. ZBN effectively decreased angiogenesis and inflammatory response, according to a recent study [38]. To the best of our knowledge, the effects of ZBN on ototoxicity have not been studied. However, several studies have shown that sesquiterpenes have a protective effect on chemotherapeutics-induced ototoxicity. Farnesene, a natural sesquiterpene, has been reported to exert autoprotective effects in PCX-induced ototoxicity by restoring MDA and GSH levels damaged by oxidative damage [22]. Another sesquiterpene, zingerone has been reported to exert otoprotective effects in cisplatin-induced ototoxicity [29]. One study observed that ZBN reduced the levels of increased lipid peroxidation products and normalized the decreased GSH levels in ISO-induced MI rats [38]. In parallel with these results, it was observed in our study that ZBN restored the increased MDA and decreased GSH levels with PCX. These findings might be attributable to ZBN's ability to defend against free radical scavenging activity. In addition, DPOAE frequencies decreasing with PCX returned to values close to the control group with ZBN administration, indicating parallelism between our biochemical results and our DPOAE results. The antioxidative and anti-inflammatory properties of ZBN were observed in the previous study at a dose of 10 mg/kg, and acute toxicity tests chose this experimental dose of ZBN. Therefore, the dose of ZBN (10 mg/kg) used in this study was the same as in the previous study [38]. The data we obtained revealed that this dose was appropriate for ZBN to exert its anti-inflammatory and antioxidant effects in the cochlea. The ZBN-only group's biochemical and DPOAE results also resembled the control group. This outcome shows that ZBN had no adverse effects.

4. CONCLUSION

According to this study, PCX causes ototoxicity in the cochlear tissue, as evidenced by the rise in MDA levels, a fall in GSH levels, and a decline in DPOAE levels. Based on the high-frequency DPOAE results and biochemical evaluation, we can conclude that ZBN may have a protective effect against the ototoxic effects of PCX in rats by preventing oxidative stress on the cochlear and reversing this effect by exhibiting autoprotective activity. Also, ZBN does not cause any adverse effects on the inner ear when used alone. However, more detailed studies on the effects of ZBN are needed to determine the optimal dose required and the use of longer administration times before it can be used in clinical practice. Finally, although this study has a limitation of not evaluating the cochlear tissue histopathologically, the study results might shed light on the future research.

5. MATERIALS AND METHODS

5.1. Ethical approval

The study process was carried out in line with the Declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals. The ethical approval was taken from the Ataturk University Animal Experiments Local Ethics Committee with the Protocol Number:42190979-000-E.1600196671.

5.2. Animals housing and handling

Throughout the experiment, 24 Wistar albino rats weighing approximately 250-300 g were kept in standard plastic cages with ad libitum and standard environmental circumstances such as a room with air conditioning (22 ˚C ± 1 temperature, 55% ± 10% humidity) and a fully automatic lighting system (12 hours light/darkness).

5.3. Study design

Otomicroscopic examinations were performed on the external ear canal, eardrum, and tympanic membranes. Rats with pathologies or impaired hearing were excluded from the study. For performing an otoscopic examination and measuring distortion product otoacoustic emissions (DPOAE), anesthesia was provided intraperitoneally with 40 mg/kg ketamine hydrochloride (Ketalar, Pfizer) and 10 mg/kg xylazine (Xylazinbio; Bioveta). The rats with an otoscopic examination and the first DPOAE measurement (0. day) were randomly allocated into four groups. The groups and the procedure for administering dosing schedules and drug application procedure and measures used in the present study are indicated in Table 2 and Figure 3, respectively. Previous research revealed the dose of ZBN [38] (CAS no. 495-60-3, Guide Chem) and PCX [5, 22] (Sindaxel; Actavis Drug Co.) to be used. One day after the last drug administration (22. day), the second DPOAE readings were measured. The drug treatment protocol and PCX-induced ototoxicity model procedures were described in earlier research [5, 22].

Table 2. Study groups and procedures of drug treatment

Group no (n=6)	Group name	Procedures for drug treatment
Group 1	CONTROL	Received 1ml/kg saline for 1, 7., 14, 21. days (i.p.)
Group 2	PCX	Received 5 mg/kg PCX for 1., 7., 14., 21. days (i.p.)
Group 3	ZBN	Received 10mg/kg ZBN for 1., 7., 14., 21. days (i.p.)
Group 4	PCX+ ZBN	Received first 5 mg/kg PCX and 30 minutes later 10mg/kg ZBN for 1., 7., 14., 21. days (i.p.)

ZBN: Zingiberene; PCX: Paclitaxel; i.p.: Intraperitoneal.

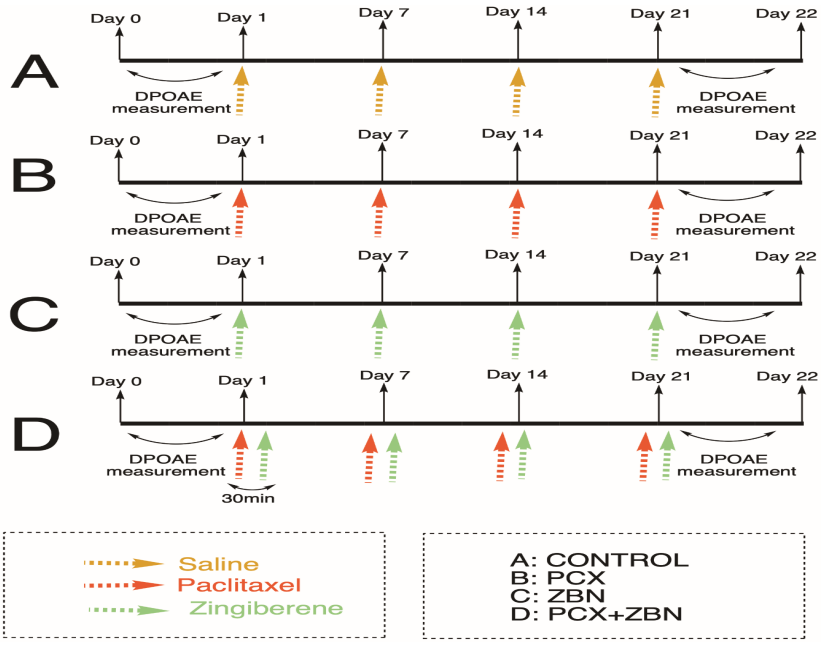

Figure 3. Drug application procedure and measurements used in the present study. ZBN: Zingiberene; PCX: Paclitaxel; DPOAE: Distortion product otoacoustic emissions.

5.4. Audiological evaluation

The MADSEN Capella device was used to take DPOAE measurements. Before each measurement, this equipment was calibrated. All rats were sedated with 40 mg/kg ketamine hydrochloride and 10 mg/kg xylazine before being measured. Rats were otoscopically examined after being anesthetized. In a quiet environment, appropriate probes were placed on the rats' right and left outer ear channels to perform DPOAE measurements. Throughout the experiment, two DPOAE measurements were taken (before and after the last drug administration). Two primary tones consisting of L1=65 dB SPL and L2 =55 dB SPL were presented at L1-L2 difference 10 dB Sound Pressure Level (SPL). Two frequencies ratio f1/f2 = 1.22 was fixed to receive the strongest responses. Measurements consisted of DPgrams. DPgram values were recorded from both ears on days 0 and 22 at 1000, 2000, 4000, 6000, and 8000 Hz. The values shown in the Table1 and Figure 1 are the average DPgram values of the left and right ears for each ear at the same stimulus intensity. Signal-to-noise ratio (SNR) values of 3 dB and higher were considered positive in the DPOAE assessments.

5.5. Biochemical evaluation

The Tissue Lyser II (Qiagen) grinding gear was used to grind cochlear tissue samples out of each rat in liquid nitrogen. The pulverized tissues were then homogenized in 1 mL of phosphate-buffered saline (PBS) homogenate buffer. Malondialdehyde (MDA) and glutathione (GSH) levels, as previously indicated in the literature respectively [5, 17, 22] from the supernatant generated by centrifuging of homogenized tissues, were evaluated in an ELISA reader. The data was depicted in the form of a mean and standard deviation.

5.6. Data analysis

The IBM statistics (version 21.0) program was utilized for statistical analysis. Shapiro-Wilk test, skewness, kurtosis, Q-Q plot, and histograms were used to determine the data's normality. Because the data was normally distributed, parametric tests were utilized. For DPOAE and antioxidants data, Tukey's Significant Difference test was used as a post hoc test of One-way Analysis of Variance (ANOVA). The paired-samples T-test was performed to compare within-group DPOAE data pre and post treatments. Statistical significance was defined at $p<0.05$ values. The data was provided as a mean with a standard deviation.

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