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ARAŞTIRMA / RESEARCH

Otoprotective effects of farnesene against oxidative damage induced by paclitaxel

Paklitakselin neden olduğu oksidatif hasara karşı farnesenin otoprotektif etkileri

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Abstract

Purpose: This study explores the biochemical and functional effects of farnesene, which has potent free radical scavenging and antioxidant properties, on paclitaxel-induced ototoxicity.

Materials and Methods: Eighteen male Wistar albino rats were allocated into three groups of six rats at random. No paclitaxel or farnesene was given to the control group throughout the research. Paclitaxel was given four times intraperitoneally at a dose of 5 mg/kg (1st, 7th, 14th & 21st days) in the paclitaxel group. In the Farnesene + Paclitaxel group, 5 mg/kg paclitaxel was given first, followed by 4 times 50 mg/kg farnesene intraperitoneally 30 minutes later (1st, 7th, 14th & 21st days). Otoacoustic emission measurement was taken on days 0 and 21 in all rats. After that, the animals were sacrificed, and their cochleas were extracted for biochemical testing.

Results: Paclitaxel caused oxidative stress in the cochlea, which considerably elevated malondialdehyde levels and lowered glutathione levels in cochlear tissues. Furthermore, the paclitaxel group's distortion product otoacoustic emission values were significantly lower than the other groups. Improvements in the damage produced by paclitaxel in various biochemical and functional parameters were observed in the Farnesene+Paclitaxel group.

Conclusion: The study findings imply that farnesene, a natural antioxidant, reduced paclitaxel-induced hearing loss in rats, and a combination of farnesene and paclitaxel therapy may have protected from paclitaxel-induced ototoxicity for future clinical use.

Keywords: Antioxidants, farnesene, otoacoustic emission, ototoxicity, paclitaxel.

Öz

Amaç: Bu çalışmanın amacı, güçlü serbest radikal süpürücü ve antioksidan özelliklere sahip farnesenin paklitaksel kaynaklı ototoksisite üzerindeki etkilerini biyokimyasal ve fonksiyonel yönden araştırmaktır.

Gereç ve Yöntem: On sekiz erkek Wistar albino sıçan, altı sıçandan oluşan üç gruba rastgele ayrıldı. Araştırma boyunca kontrol grubuna paklitaksel veya farnesen verilmedi. Paklitaksel grubuna, 5mg/kg paklitaksel intraperitoneal olarak dört kez (1., 7., 14. ve 21. günlerde) verildi. Farnesen + paklitaksel grubuna, önce 5 mg/kg paklitaksel, 30 dakika sonra 50 mg/kg farnesen intraperitoneal olarak 4 kez (1., 7., 14. ve 21. günlerde) verildi. 0. ve 21. günlerde tüm sıçanların otoakustik emisyon ölçümü yapıldı. Daha sonra hayvanlar sakrifiye edildi ve biyokimyasal testler için kokleaları çıkarıldı.

Bulgular: Paklitaksel, önemli ölçüde malondialdehit seviyelerini yükselterek ve glutatyon seviyelerini düşürerek kokleada oksidatif strese neden oldu. Ayrıca paklitaksel grubunun distorsiyon ürünü otoakustik emisyon değerleri diğer gruplara göre anlamlı derecede düşüktü. Farnesen+paklitaksel grubunda ise paklitakselin çeşitli biyokimyasal ve fonksiyonel parametrelerde oluşturduğu hasarda iyileşmeler gözlendi.

Sonuç: Çalışma sonuçları doğal bir antioksidan olan farnesen'in sıçanlarda paklitaksel kaynaklı işitme kaybını azalttığını, farnesen ve paklitaksel kombinasyonunun gelecekte klinik kullanım için paklitaksel kaynaklı ototoksisiteden koruyabileceğini göstermektedir.

Anahtar kelimeler: Antioksidanlar, farnesen, otoakustik emisyon, ototoksisite, paklitaksel

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INTRODUCTION

Ototoxicity is the destruction of the cochlear and vestibular organs generated by exposure to various therapeutic pharmaceuticals, resulting in permanently or temporarily hearing impairment[1]. Drug-induced hearing loss is widespread, particularly in patients taking chemotherapy treatments. Chemotherapeutic agents are used more frequently as the global rate of cancer incidence continues to rapidly rise[2]. As a consequence of the rising cancer prevalence, hearing loss due to chemotherapeutic drug use is becoming a severe health concern, leading to social and academic reductions in a person's quality of life [3]. Therefore, it is important to develop treatment options against chemotherapeutic-induced ototoxicity.

Paclitaxel (PCX), a taxane plant product, is an effective anti-cancer agent widely utilized in the treatment of ovarian, lung, breast, and cervical cancers, as well as many head and neck malignancies, since its discovery [4-7]. PCX, a tubulin stabilizer, upsets the balance in microtubule stability by binding to the ß portion of microtubules, blocking dynamic depolarization of the microtubule network, thereby revealing its antitumor activity [8]. Despite its demonstrated efficacy, PCX has been linked to neurotoxicity, peripheral neuropathy, and various sensory complaints such as numbness and paresthesia [9, 10]. The neurotoxic effects of PCX, particularly on the dorsal root ganglia (DRG), slow the conduction of sensory nerves, while its neurotoxic effects on satellite cells impair peripheral axons [11]. The sensitivity of neurons to PCX toxicity has guided clinical studies of the ototoxic effect of PCX on peripheral auditory neurons [12 13]. Recent studies on animal models have reported that substances such as curcumin, carvacrol, resveratrol, gallic acid, and eugenol, all of which have potent antioxidant properties, can attenuate chemotherapeutic drugs induced ototoxicity such as cisplatin and PCX[14-18]. Ototoxicity is accompanied by free radicals, resulting in oxidative damage to cochlear cells, which is why antioxidants are used in much research on preventing and restoring ototoxicity. Reactive oxygen species (ROS) build up in the cochlea, depleting intracellular glutathione (GSH) levels and impairing the antioxidant defense mechanism. The oxidative damage in the cochlea is attributed to a decrease in the antioxidant defense system and an increase in lipid peroxidation [19]. Antioxidants have become popular to combat ototoxicity as a result of this [20].

Sesquiterpene lactones, a member of the terpene family, have important roles as pharmaceutical agents due to their many biological and therapeutic potentials such as antioxidant, antinociceptive, anti-cancer, and anti-inflammatory [21-23]. Farnesene (FNS) is a sesquiterpene lactone present in various plants and foods, with a wide range of biological effects. FNS has been proved to have a wide range of essential bioactivities, including neuroprotective effect [24,25], DPPH radical scavenging [26], antibacterial properties [27], and anticarcinogenic effect [28]. FNS, which has neuroprotective effects [24], may protect peripheral auditory neurons from ototoxicity triggered by PCX's neurotoxic impact. Furthermore, FNS's strong antioxidant capacity and chelating activity [26] may protect against ototoxicity caused by the production of free radicals that generate oxidative injury.

Ototoxicity caused by the use of chemotherapy medications is becoming a severe health concern as cancer prevalence rises[2,3]. Even though PCX is efficient chemotherapy that is widely used in the treatment of various cancers[4-7], PCX has many side effects, such as neurotoxicity, which may be the cause of its ototoxicity[12,13]. However, the hypothesis that its neurotoxic effects can be viewed as an ototoxic impact has begun to gain traction, though there is a gap in the literature on PCX's ototoxic effect.

Recent research shows that the use of antioxidant-strong substances in preventing and treating ototoxicity is generally effective [14-18]. There is no research on the impact of FNS, which has high antioxidant and neuroprotective capabilities, on ototoxicity in the literature. As a result, this research hypothesizes that FNS' neuroprotective and antioxidant activities may have an otoprotective effect by reducing PCX's neurotoxic effects and that FNS' anti-cancer effect may aid in PCX's cancer treatment.

Our review of the literature found that the impact of FNS on ototoxicity is unknown, and there have been no previous studies on the role of FNS on PCX-induced ototoxicity. Then, this study aims to conduct a biochemical and functional examination to see if FNS has a potential protective effect against PCX-induced ototoxicity.

MATERIALS AND METHODS

Animals and chemicals

The experiment was carried out in the Experimental Animals Laboratory of the Ataturk University Medical and Experimental Application and Research Center in compliance with the standards of the Declaration of Helsinki and the National Animal Care and Use Guidelines for Laboratory Animals. The animals were received from the Ataturk University Medical Experimental Application and Research Center (Turkish Acronym: ATADEM). Ataturk University Animal Research Local Ethics Committee (Turkish Acronym: HADYEK) accepted the study's ethical procedures and protocols with the approval of all participants, with the decision dated 26.08.2016 and numbered 41190979-000-E.1600196748.

Eighteen male Wistar albino rats weighing 250-300 g, 7-9 weeks old, were used in the study. The animals were kept in polypropylene cages with a 12-hour dark/light cycle, 55 %±10 % humidity, and a temperature-controlled environment (22±1°C). They had free access to food and water. The animals were housed in a room with a

background noise level of about 50 decibels. All rats' outer ears and tympanic membranes were inspected. Any rats with ear issues or no otoscopic examination and distortion of otoacoustic emissions (DPOAE) waves at any of the studied frequencies were ruled out. PCX from Actavis Pharma (Sindaxel; Actavis Drug Co., Istanbul, Turkey); FNS from Sigma–Aldrich Chemical Co. Ltd (CAS No. 18794-84-8), ketamine from Pfizer (Ketalar, 50 mg/mL vial), and xylazine from Bioveta (Xylazinbio; Bioveta, Ankara, Turkey) were among the pharmaceuticals utilized in the study.

Experimental design

DPOAE measurement were performed[15] under anesthesia (ketamine - 40 mg/kg + xylazine - 5 mg/kg) before drug administration on day 0. Afterward, animals with no auditory pathological findings and whose baseline hearing threshold was evaluated were randomly divided into three groups.

1-Control group (n=6): 1 ml of saline was applied 4 times (1st, 7th, 14th & 21st days) (i.p.).

2-Paclitaxel group (n=6) (PCX): 5 mg/kg PCX was administered 4 times (1st, 7th, 14th & 21st days) (i.p.).

3-Farnesene + Paclitaxel group (n=6) (FNS+PCX): The first 5 mg/kg PCX was given and 30 minutes later 50 mg/kg FNS was administered 4 times (1st, 7th, 14th & 21st days) (i.p.).

After the first DPOAE measurements, rats were divided into groups. In the present study, the number of animals in each group was determined as 6, as stated in previous ototoxicity studies[18, 29-31]. The doses of FNS and PCX were also decided based on the previous research (i.e., FNS[32] and PCX [14, 15]). Throughout the trial, the rats in the control group were given 1 ml of saline four times (1st, 7th, 14th, and 21st days). 5 mg/kg PCX (i.p.) was given four times throughout the experiment (1st, 7th, 14th, and 21st days) to construct an ototoxicity model in the PCX and FNS + PCX groups. In the treatment group, FNS + PCX group, 50 mg/kg FNS was applied 4 times (1st, 7th, 14th & 21st days) during the experiment 30 minutes after each PCX administration (i.p.). One day after (22. days) the final drug application, animals were re-anesthetized for a second DPOAE measurement[15], and sacrificed animals' cochleas were removed for biochemical investigations.

Auditory assessment

Under general anesthesia, DPOAE measurements were taken with the MADSEN Capella device by inserting a rat-suitable probe into the external ear canal of constant strength, and frequency changes were used to measure otoacoustic emissions. The frequencies (f1 and f2) were set to f1/f2= 1.22, the stimulus intensity levels were set to L1=65dB, L2=55dB, and the L1-L2 difference was set to 10 dB sound pressure level (SPL) 33. The distortion product gram (DPgram) was used to make the measurements. The measurements were taken at 2000, 4000, 6000, and 8000-hertz frequencies.

Biochemical assessment

Cochlea tissues from each rat were exposed to grind with liquid nitrogen using a Tissue Lyser II (Qiagen) grinding jar set. Approximately 100 mg of tissue from the ground samples was then weighed, homogenized with phosphate-buffered saline (PBS) in an Eppendorf tube, and centrifuged to perform various biochemical treatments 15. GSH 34 and MDA 35 levels were assessed as per the literature. The mean and standard deviation were used to represent the values.

Statistical analysis

IBM SPSS (Version 21.0) was employed for statistical analysis. Shapiro-Wilk test was used to determine the data's normality. As the data were normally distributed, parametric tests were utilized in the analysis. For homogeneity of variances, the Levene test was performed. One-Way Analysis of Variance (ANOVA) tests with Tukey's Significant Difference test for homogeneous variances and Games Howell test for non-homogeneous variances were used to evaluate differences between experimental groups for DPOAE and antioxidant parameter data. A paired T-test was used to compare within-group DPOAE values before and after treatments. A statistically significant difference was outlined at $p<0.05$ values. The data was recorded as a mean with a standard deviation.

RESULTS

Table 1 details the DPOAE test findings of pre (day 0) and post (day 22) in all groups. Pre (day 0) DPOAE measurements did not differ statistically significantly among groups and within groups ($p > 0.05$). DPOAE levels pre- and post- PCX treatment were statistically different (for 2000Hz p=0.001; for 4000 Hz p=0.000; for 6000Hz p=0.000; for 8000Hz p=0.002). The control group's pre-and-post-DPOAE levels did not

differ (for 2000Hz p=0.76; for 4000Hz p=0.17; for 6000Hz p=0.89; for 8000Hz p=0.96) (see Table 1). Intra-group comparisons were made using the paired T-test. The results were presented as means ± standard deviations. * was used to compare pre- and post- DPOAE thresholds within the same group (* *p< 0.05*). PCX: paclitaxel; FNS: farnesene; Hz: hertz; DPOAE: distortion product otoacoustic emissions.

When the DPOAE levels of the PCX and FNS + PCX groups were compared on day 22, the FNS+ PCX group had considerably higher DPOAE levels at all frequencies (for 2000Hz *p*=0.000; for 4000 Hz *p=0.031*; for 6000Hz *p=0.000*; for 8000Hz *p=0.000*) (see Figure 1). There was no significant change in DPOAE levels on day 22 between the Control and FNS+ PCX groups (for 2000Hz *p*=0.270; for 4000 Hz *p=0.998*; for 6000Hz *p=0.912*; for 8000Hz *p=0.390*) (see Figure 1). The results suggest that the treatment with FNS protects against PCX-induced ototoxicity.

Table 1. Pre-test and post-test intra-group comparison of DPOAE measurements

Groups	Test	2000Hz	4000Hz	6000Hz	8000Hz
Control	PRE	4.24±0.39	12.44±0.63	21.26±0.96	23.49±1.71
	POST	4.02±0.70	10.69±0.69	21.57±1.41	23.58±0.95
PCX	PRE	4.02±0.70	12.44±0.63	21.26±0.96	24.06±2.19
	POST	0.97±1.14*	6.67±0.21*	11.30±0.32*	12.44±0.73*
FNS+PCX	PRE	4.00±0.96	12.44±0.63	21.26±0.96	25.43±2.64
	POST	2.43±0.43	11.35±1.27	22.33±1.73	26.00±1.44

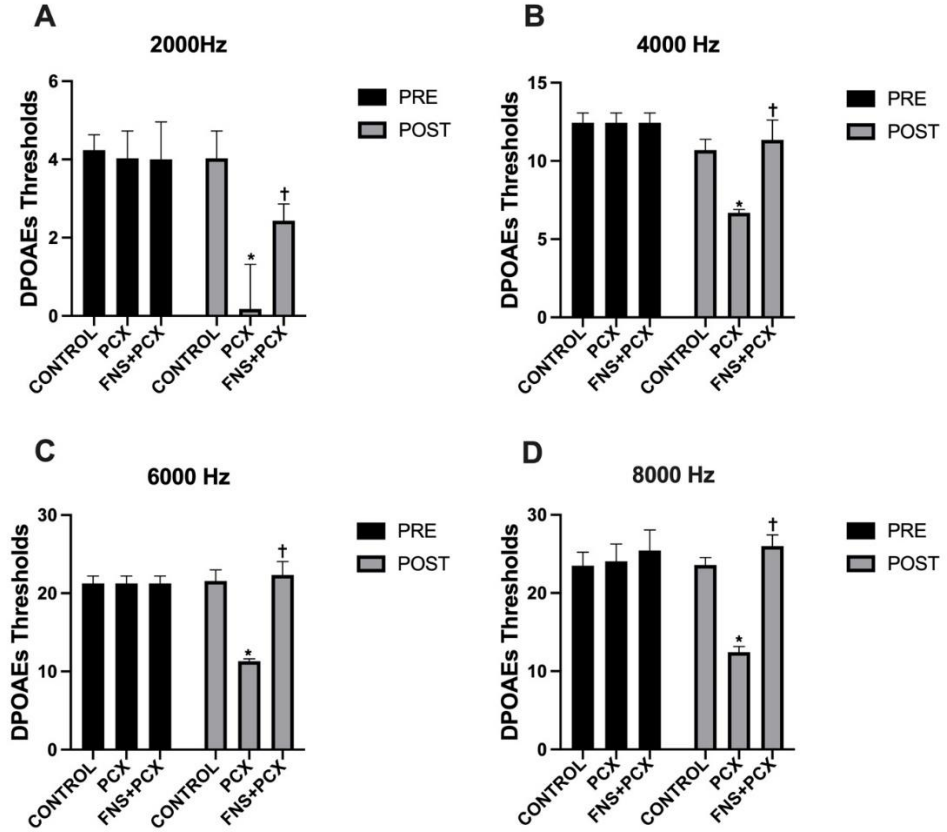

Figure 1. Intergroup comparison of hearing thresholds of DPOAES values at 2000 Hz (A), 4000 Hz (B), 6000 Hz (C), and 8000 Hz (D).

One-way ANOVA was used for the statistical comparisons, followed by the Games Howell test. *$p<0.05$ denotes that the PCX group differed significantly from the other groups; †$p<0.05$ denotes that the FNS+PCX group differed significantly from the PCX group. PCX: paclitaxel; FNS: farnesene; DPOAE: otoscopic examination and distortion product otoacoustic emissions; Hz: hertz.

Figure 2 depicts the results of analyses of the oxidative stress markers GSH and MDA levels. While GSH levels ($p=0.000$) declined, MDA levels ($p=0.000$) increased compared to the control group in the PCX group. When the PCX+FNS group was compared to the control group, there was no significant difference in both MDA ($p=0.123$) and GSH ($p=0.128$) levels. This result shows that FNS mitigates the oxidative damage caused by PCX. The MDA levels in the PCX group were more significant than in the PCX+FNS group ($p=0.000$; Figure 2A). Furthermore, GSH levels in the PCX+FNS group were observed to be considerably greater than in the PCX group ($p=0.000$; Figure 2B).

One-way ANOVA followed by Tukey's post hoc test was used for the analyses. *$p< 0.05$, **$p<0.01$, and ***$p<0.001$ denote that the PCX group differed significantly from the other groups. †$p< 0.05$, ††$p< 0.01$, and †††$p<0.001$ denote that the Control group differed significantly from the other groups. The data were presented as means ± standard deviations. PCX: paclitaxel; FNS: farnesene; MDA: malondialdehyde; GSH: glutathione.

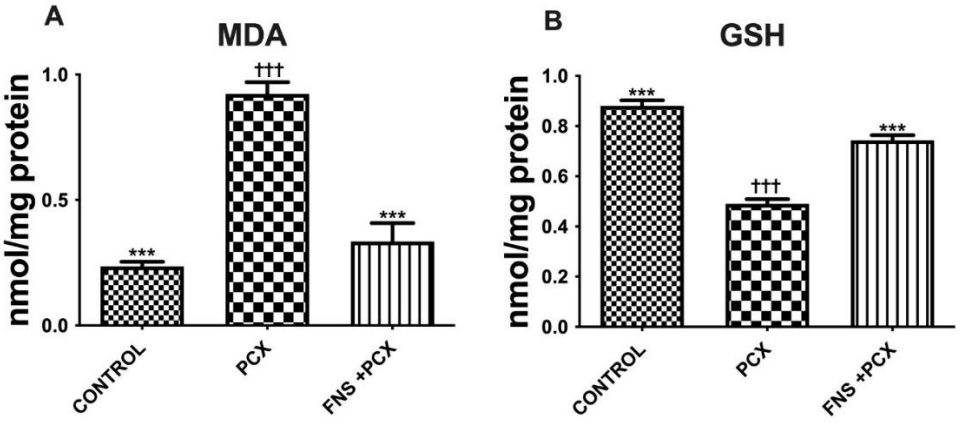

Figure 2. The effect of FNS on MDA (A) and GSH (B) levels in the cochlea tissue after Paclitaxel-induced ototoxicity.

DISCUSSION

The study showed that FNS effectively prevented ototoxicity after exposure to PCX in an experimental rat model. FNS's effects were assessed functionally by taking measurements of DPOAE and biochemically by analyzing oxidative stress markers. The DPOAE results verified PCX-induced ototoxicity, and FNS indicated that it could protect against PCX-induced hearing impairment by lowering auditory threshold shifts. Furthermore, FNS demonstrated its antioxidant function by restoring MDA and GSH levels harmed by PCX-induced oxidative damage.

Ototoxicity, defined as cellular damage or dysfunction in the inner ear, is a frequent complication depending on chemotherapeutics, particularly in cancer patient[1]. Chemotherapy-induced hearing impairment can negatively influence a patient's quality of life, rendering it a life-threatening matter[3]. Consequently, much research has been carried out against the ototoxic effects of chemotherapeutic drugs[14, 36, 37]. However, no study acknowledged that a therapeutic approach exists currently.

Since its introduction into clinical practice, PCX, also known as Taxol, has piqued interest for its anti-cancer properties in cancers such as breast cancer, ovarian cancer, uterine cancer, and head and neck cancers[38]. Despite its widespread usage as one of the most potent and effective antineoplastic drugs to treat advanced and resistant malignancies, there is limited research on PCX-induced ototoxicity[39]. As PCX is combined with antineoplastic drugs like cisplatin, which is known to cause ototoxicity, these antineoplastic drugs are blamed for causing ototoxicity, while PCX is exonerated[11]. PCX suppresses tumor cell proliferation by promoting tubulin polymerization. However, by blocking axonal transport in neurons, this scenario is blamed for developing neuropathy, a restrictive adverse effect of paclitaxel. The neurotoxic effects of PCX on sensory nerve conduction velocity in the DRG suggest that such effects on peripheral auditory neurons will be similar[40]. In this context, recent studies[14, 15] emphasizing a link between hearing loss and PCX treatment suggest that much research on the ototoxic consequences of PCX is needed.

DPOAE test was used in our study, as it is effective even in the early stages of damage since it represents the activity of outer hair cells[41]. DPOAE levels were measured twice in rats on day 0 and day 22 of the study. At all frequencies, 2nd DPOAE measures in PCX-treated rates were considerably lower than both 1st DPOAE measurements and those in the control group. This result demonstrates that PCX generates ototoxicity and hearing loss at all frequencies tested. On day 22, however, DPOAE levels in the FNS+PCX group were considerably higher than in the PCX group. These results show that FNS protects against PCX-induced ototoxicity by acting as a protector. The fact that there was no significant difference between the control group and the FSN+PCX group at all frequencies in the second DPOAE measurement indicates that FSN, through its neuroprotective properties, alleviates the hearing impairment detected in the outer hair cells at an early stage. Furthermore, the correlation of the DPOAE results with the biochemical results suggests that FSN protects against ototoxicity.

Although the exact mechanism of chemotherapeutic-induced ototoxicity is unknown, it is thought to be associated with an excessive formation of free radicals in the cochlea, which triggers the oxidative processes of the significant factor [42]. There is an intrinsic antioxidant defense system in the cochlea that includes glutathione and antioxidant enzymes. When the ototoxic effect overwhelms the endogenous defense system, it can harm cochlear cells by producing excessive ROS production, GSH depletion, and increased lipid peroxidation. Exogenous antioxidants, which both prevent uncontrolled ROS formation and improve the antioxidant defense system, have thus become the key target for counteracting the chemotherapeutics' ototoxic effect [43, 44].

Exogenous antioxidants such as *Nigella sativa* oil [45], pycnogenol[46], pomegranate[47], resveratrol[48], and gallic acid [17] have been utilized as otoprotectors in research. Based on this background, we investigated the effects of FNS, a natural sesquiterpenoid recognized for its antioxidant properties, on paclitaxel-induced ototoxicity. Sesquiterpene lactones, a terpene family member, have been found to include active compounds with various biological effects, including FNS[46]. FNS is shown to have antioxidant[26], antifungal[50], anticarcinogenic[28], and neuroprotective properties[25]. According to Arslan et al.[24], FNS has a neuroprotective function in ß amyloid toxicity by enhancing antioxidant capacity while decreasing oxidative capacity. FNS alleviated oxidative stress in hydrogen peroxide-induced toxicity, according to Turkez et al.[25]. FNS was reported to have a free radical scavenging activity in another study [26]. FNS's free radical scavenging action implies that it may be responsible for its antioxidant properties. As a result, FNS has been studied as an antioxidant in the treatment of various oxidative processes; however, it has never been explored for its putative prevention against PCX-induced ototoxicity. MDA levels were substantially higher, and GSH levels were significantly lower in the cochleas of PCX-treated rats compared to the control group in our research. These data show that oxidative stress plays a role in PCX-induced cochlear damage, which is in line with earlier research [14, 15]. The decrease of MDA levels and the increase of GSH levels with FNS treatment repaired this oxidative imbalance, which PCX had disrupted. FNS enhanced the cochlea's antioxidant defense mechanisms, according to these results. Similarly, carvacrol[15], a terpenoid like FNS, showed similar protective effects against PCX-induced ototoxicity.

Finally, our biochemical findings reveal that FNS can protect against ototoxicity by raising antioxidant enzyme levels and decreasing oxidant parameters for the first time in the literature. The recovery observed in DPOAE results after FNS administration implied

that FNS has a protective effect against the ototoxicity with PCX. However, comprehensive prospective randomized research is required to confirm the beneficial effects of FNS on routine clinical use.

This study has two major limitations. The first limitation is the number of animals in groups. Due to the small number of rats in each group, we could not detect minor changes in physiological studies. Because the small sample size may have influenced the DPOAE data and statistical analysis results, much research is needed to assess the positive effects of FNS on ototoxicity. The second limitation is the lack of histopathological examinations. Much comprehensive research is also needed since histopathological studies indicate that FNS protects against PCX-induced ototoxicity and is not harmful when delivered alone to cells.

Looking at the literature, FNS appears to have various essential bioactivities, including neuroprotective effect[24,25], antioxidant[26], antibacterial[27], and anticarcinogenic[28] properties. The antioxidant qualities of FNS, its protective action against neurons, and the neurotoxic effect of PCX are thought to cause ototoxicity through peripheral auditory neurons, as stated in the previous research[11,15], which drove our research. It is noteworthy that substances with antioxidant properties are generally studied in ototoxicity studies[20,45-48]. Despite this knowledge and outcomes, research on the effects of FNS with antioxidant qualities on ototoxicity is limited in the literature. This study aimed to fill in the gap in the literature by presenting findings on a previously unexplored topic and suggested a novel treatment option for a significant side effect such as ototoxicity, which is becoming more common as cancer cases rise.

Yazar Katkıları: Çalışma konsepti/Tasarımı: BD, FA. AT; Veri toplama: BD, FA. AT; Veri analizi ve yorumlama: BD, FA; Yazı taslağı: BD; İçeriğin eleştirel incelenmesi: BD, FA. AT; Son onay ve sorumluluk: BD, FA. AT; Teknik ve malzeme desteği: FA, AT; Süpervizyon: BD, FA. AT; Fon sağlama (mevcut ise): yok.
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