Clinical and Experimental Health Sciences

# Otoprotective Mechanisms of Carvone As An Antioxidant Agent Against Ototoxic Damage Caused By Paclitaxel

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**ABSTRACT**

**Objective:** Ototoxicity is cellular damage caused by the use of solid treatments as chemotherapeutics in critical illnesses like cancer. The generation of free radicals is linked to fluctuating hearing loss caused by chemotherapeutics. Antioxidants can help to prevent ototoxicity-related oxidative damage. Carvone (CVN) is a monoterpene with excellent antioxidant properties that protect against oxidative damage. This study investigates the biochemical and functional aspects of CVN's putative otoprotective mechanisms against paclitaxel (PCX)-induced ototoxicity.

**Methods:** 24 Wistar albino rats were assigned into four different groups: Control, CVN, PCX, and PCX+CVN. Once a week, the control group received saline. The PCX group received 5 mg/kg PCX intraperitoneally once a week (4 times). Once a week, the CVN group received 50 mg/kg intraperitoneally. The PCX+ CVN group received 5 mg/kg PCX followed by 5 mg/kg CVN once a week. All animals were subjected to deterioration product otoacoustic emission testing before (day 0) and after drug administration (day 23).

**Results:** PCX showed an ototoxic effect by weakening otoacoustic emission values. PCX leads to significant otoacoustic emission value shifts ameliorated by CVN co-treatment (for 2000Hz $p < .001$, for 4000 levels $p < .01$, for 6000Hz $p < .001$, and for 8000 Hz $p < .01$ in PCX+CVN group). Furthermore, the PCX group had significantly greater malondialdehyde levels and significantly lower glutathione levels in the cochlear tissues, compared to the other groups. Co-administered CVN with PCX reversed these effects, making oxidative stress parameters close to those of the control group (for GSH levels $p < .001$, for MDA levels $p < .01$ in the PCX+CVN group).

**Conclusion:** According to the findings, CVN appears to preserve cochlear function in rats against the disruptive effects of PCX.

**Keywords:** Antioxidant, carvone, ototoxicity, oxidative stress, paclitaxel.

## 1. INTRODUCTION

Damage to the inner ear by noise, ototoxic pharmaceuticals, aging, and several disorders leads to hearing loss. Some anticancer drugs, such as cisplatin and paclitaxel (PCX), have been linked to ototoxicity (1). Today, the increase in the usage of chemotherapeutic drugs as the prevalence of cancer rises is increasing the incidence of ototoxic hearing loss. The persistence of hearing loss caused by chemotherapeutic drugs leads to multiple decreases in the quality of life of cancer survivors, and this situation appears as a severe health problem. As a result, there is still a clinical need for treatments to avoid chemotherapeutic-induced ototoxicity.

PCX, a microtubule-stabilizing drug, is among the most effective broad-spectrum chemotherapy (2, 3). PCX alters tubulin polymer balance by increasing microtubule stability, preventing depolarization of the microtubule network, and inhibiting the G2/M phase, demonstrating its anticancer effect (4). It is among the most extensively used anticancer drugs, showing activity in various cancers, including breast, endometrial, lung, and cervical carcinoma (5). PCX has a variety of side effects, the most serious of which is peripheral neuropathy, which has significant dose-limiting toxicity. The impacts of PCX on microtubule polymerase impede axonal transport, causing sensory neurons in the dorsal root ganglia to be damaged and sensory nerve conduction velocity to be reduced, resulting in peripheral neuropathy (6). Even though many chemotherapeutic agents have been demonstrated to have ototoxic effects in considerable detail, there needs to be more research on the ototoxic effects of PCX in the literature (2). However, given the harm that PCX does to sensory neurons, it is assumed that it has a similar effect on neurons in the cochlear, as evidenced by recent investigations (3, 7). In one study, researchers found that 71% of patients developed neuropathic symptoms after paclitaxel administration and that paclitaxel produced early sensory dysfunction and led to permanent neuropathy (8). In another study, the incidence



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of audiogram-confirmed hearing loss in patient groups given platinum only, taxane only, and platinum and taxane ranged from 52.3% to 71.4%. There was no difference between the three chemotherapy groups regarding hearing loss incidence or effects (9).

The accumulation of reactive oxygen species (ROS), which cause damage in cochlear cells and can lead to cell death via apoptosis, is presently thought to be the mechanism of cochlear toxicity caused by chemotherapeutic drugs. Minimizing ROS generation or boosting the antioxidant system is essential to prevent ototoxic damage from cochlear oxidative stress induced by the excessive free radical formation in the inner ear (1). Exogenous antioxidants were shown in studies to protect against ototoxic damage by increasing the ROS scavenger system and reinforcing the deficient endogenous antioxidant synthesis (3, 10-13).

Carvone (CVN) is derived from a variety of pharmaceutical and aromatic plants (cumin, dill, and mint). CVN is a monoterpene with unique pharmacological effects, including anti-inflammatory, anti-tumor, anti-diabetic, antibacterial, fungicidal, and antioxidant capabilities (12). CVN had protective effects against free radical-induced tissue damage reducing consumption of glutathione (GSH) and to decreased elevated malondialdehyde (MDA) levels in a range of experimental models (15-17). However, no studies were conducted to determine whether CVN protects against ototoxicity. Based on the background, this study aimed to assess the preventive efficacy of CVN against ototoxicity induced by PCX in a rat model.

## 2. METHODS

### 2.1. Ethics and Animal Handling

The experiments in this study, authorized by the Animal Experiments Ethics Committee at Ataturk University (ATADEM) (Number: 42190979-000-E.160.011.96726), were undertaken by the principles of the Guide for the Care and Use of Laboratory Animals. Twenty-four male Wistar albino rats (250-300 g) were purchased from the ATADEM at the University of Ataturk. Animals were kept under conventional room and environmental settings (12 hours light/12 hours dark, 22°± 3°C temperature, 55%-10% moisture, background noise level less than 50 dB). Throughout the trial, rats were fed ad libitum.

### 2.2. Drugs and Anesthesia

The dosages of CVN (Sigma-Aldrich Chemical Company, Darmstadt, Germany) and PCX (Sindaxel; Actavis Drug Co., Istanbul, Turkey) utilized were 50 and 5 mg/kg for the rat per weight, respectively, based on previous research (3, 7, 18, 19), and both were given intraperitoneally. Before each recording for otoscopic examination, 50 mg/kg ketamine HCL (Ketalar; Pfizer, Istanbul, Turkey) and 10 mg/kg xylazine (Xylazinbio; Bioveta, Ankara, Turkey) anesthetic mixture was given intraperitoneally.

### 2.3. Experimental Procedures

To perform an otoscopic examination and evaluate distortion product otoacoustic emissions (DPOAE, The MADSEN Capella device, Natus Medical Denmark), all rats were anesthetized intraperitoneally (50 mg/kg ketamine hydrochloride-10 mg/kg xylazine) before the start of the study. The rats were assigned into four groups of six each: Control, CVN, PCX, and PCX+CVN. The size of the experimental groups' samples was established based on prior similar studies (11, 20). Animals in the control group received intraperitoneal 1ml/kg normal saline once a week (1., 8., 15., 22. days). Animals in the PCX group received 5 mg/kg of PCX intraperitoneally once a week (1., 8., 15., 22. days). Animals in the CVN group were given 50 mg/kg intraperitoneally once a week (1., 8., 15., 22. days). Animals in the PCX+ CVN group received 5 mg/kg PCX, followed by 5 mg/kg CVN 30 minutes later once a week (4 consecutive weeks). The process for giving the drug is outlined in Table 1 and Figure 1. The second DPOAE measurements were taken one day following the last drug delivery (23. Day), and the experiment was ended by giving the rats high-dose anesthesia. The rats' cochleas were extracted and preserved in appropriate conditions for biochemical experiments.

***Table 1.*** *Experimental groups, dosing schedules and procedures*

| Groups | Dose | Procedures |
|---|---|---|
| **CONTROL** | 1ml/kg | Saline for 1., 8., 15., 22. days (i.p.) |
| **PCX** | 5 mg/kg | PCX for 1., 8., 15., 22. days (i.p.) |
| **CVN** | 50 mg/kg | CVN for 1., 8., 15., 22. days (i.p.) |
| **PCX+ CVN** | 5 mg/kg- | First PCX and 30 min later CVN for 1., 8., 15., 22. days (i.p.) |

*PCX:Paclitaxel, CVN:Carvone, i.p.:Intraperitoneal*

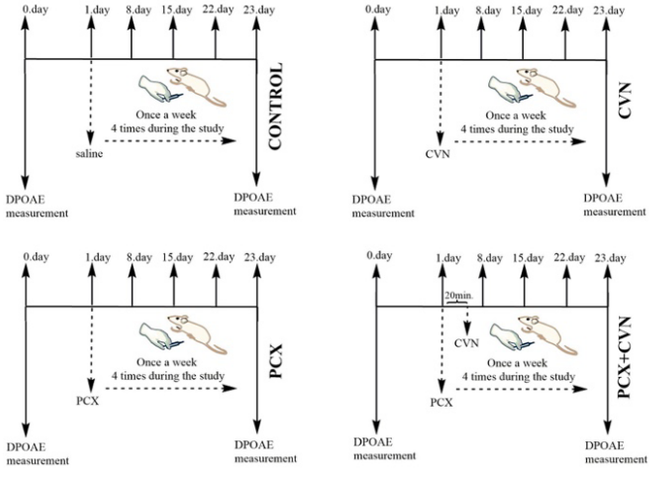


***Figure 1.*** *Drug application protocol. Procedure for administering carvone and paclitaxel. PCX: Paclitaxel, CVN: Carvone.*

### 2.4. Test of Audiological Function

DPOAE dimensions were determined by inserting a fitting probe into the external ear canal under general anesthesia in a quiet setting, following the rats who had already received an otoscopic examination. The difference between the levels of L1 and L2 was kept at 10 dB SPL (sound pressure level) (L1 = 65 dB SPL, L2 = 55 dB SPL). The test was set to two separate frequencies, ratio f1/f2 = 1.22, to obtain the most powerful response. Measurements were recorded in the distortion product gram (DPgram) form (3). DPgram measurements were carried out at 4 frequencies between 2000 and 8000 Hz. Signal Noise Ratio (SNR) values of 3 dB or higher were assumed to indicate positive.

### 2.5. Test of Biochemical Parameters

The harvested cochlea tissues were pulverized using liquid nitrogen in a tissue grinder (The Tissue Lyser II – Qiagen, Hilden, Germany) and then homogenized with the appropriate buffer (PBS) as previously stated (21). An ELISA reader was used to quantify GSH (Sigma-Aldrich Chemical Company, Darmstadt, Germany, CS0260-1KT)(22) and MDA (Sigma-Aldrich Chemical Company, Darmstadt, Germany, MAK085-1KT) (23) levels in the supernatant from each sample. The mean ± standard deviation (SD) was used to express the data.

### 2.6. Statistics

IBM Corp.'s SPSS 21.0 (Armonk, NY, USA) application was used for data analysis. The mean and standard deviation were used to present all the data. Shapiro-Wilk test, skewness, kurtosis, QQ plot, and histograms were used to determine the data's normality. Parametric tests were utilized since the values were normally distributed. For homogeneity of variances, the Levene test was performed. Tukey's Significant Difference test was used for homogenous variances, and the Games Howell test was utilized for non-homogeneous variances. The variations in DPOAE amplitudes within the group were compared using a paired T-test before and after medication delivery (days 0 and 23). In all tests, a p-value less than .05 was considered statistically.

## 3. RESULTS

### 3.1. Results of Audiological Function Test

Table 2 summarizes the DPOAE measurements of the groups on day 0, which is shortly before any drug administration, and on day 23, which is one day after the last administering drugs. For all frequencies tested, DPOAE values at day 0 were not meaningfully different ($p > .05$). DPOAE thresholds alter statistically significantly before and after PCX treatment. Following PCX treatment, DPOAE values (at day 23) declined at all frequencies. At all frequencies of DPOAE measurements on days 0 and 23, no significant change in DPgram values was detected in all groups, excluding the PCX group. On the 23rd day, DPOAE values were significantly lower in the PCX group than in the Control group. On the 23rd day, no significant difference was found between the DPOAE measurements of the CVN and PCX+CVN groups compared to the control group. When the DPOAE measurements of the PCX and PCX+CVN groups were compared on the 23rd day, it was observed that the DPOAE values that had dropped with PCX had dramatically increased with the CVN application (Fig 2).

***Table 2.*** *Intra-group comparison of pre – and post-treatment degradation product otoacoustic emission (DPOAE) thresholds.*

| GROUPS | PRE/ POST | 2000 | 4000 | 6000 | 8000 |
|---|---|---|---|---|---|
| **CONTROL** | PRE | 3.00±0.35 | 8.70±0.63 | 21.7±0.64 | 23.6±1.1 |
| | POST | 2.85±0.16 | 8.20±0.68 | 22.06±0.75 | 23.49±1.02 |
| **PCX** | PRE | 3.00±0.35 | 8.71±0.53 | 21.77±0.59 | 21.56±1.64 |
| | POST | -2.6±0.16 [a] | 6.14±0.30 [a] | 11.11±0.34 [a] | 12.23±0.85 [a] |
| **CVN** | PRE | 3.18±0.20 | 9.48±0.45 | 22.56±1.18 | 22.31±1.33 |
| | POST | 3.31±0.50 | 10.24±0.68 | 19.74±1.79 | 22.56±0.91 |
| **PCX+CVN** | PRE | 3.00±0.35 | 8.71±0.53 | 22.14±1.20 | 22.31±1.33 |
| | POST | 2.70±0.20 | 9.15±0.69 | 16.88±0.20 | 20.75±1.76 |

[a] *(p-values < .05); Within the PCX group was compared PRE and POST medications statistically significant. Paired T-test was utilized for intra-group comparison. PCX:Paclitaxel, CVN:Carvone, i.p.:Intraperitoneal. The values are represented as mean ± SD.*

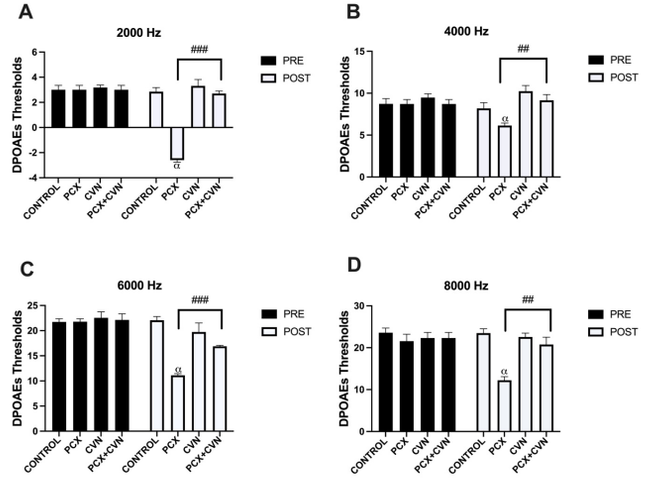


***Figure 2.*** *The intergroup comparison of pre and post-treatment hearing threshold values (A:2000Hz; B:4000Hz; C:6000Hz; D:8000Hz). Degradation product otoacoustic emission (DPOAE) thresholds on day 0 (pretreatment) and day 23 (post-treatment). The DPOAE thresholds differed among the three groups ($^{\alpha}p < .05$ for the control vs. PCX by repeated-measures ANOVA with Games Howell posthoc test). The DPOAE thresholds in the PCX + CVN group on day 23 were attenuated compared with those in the PCX group ($^{\#\#}p < .01$, $^{\#\#\#}p < .001$ for the PCX vs. PCX + CVN groups by repeated-measures ANOVA with Games Howell posthoc test). The values are represented as mean ± SD.*

### *3.2. Results of Biochemical Parameters Test*

Figures 3A and B present the results of the GSH and MDA levels, respectively. There was a substantial increase in MDA levels compared to the control group, while significant reductions in GSH levels were noted in rats given just PCX. When the CVN group was compared to the control group, there was no significant difference in MDA and GSH levels. However, when comparing the PCX+CVN group to the PCX group, there was a substantial rise in MDA levels and a significant decline in GSH levels. Additionally, the PCX+CVN group's MDA and GSH levels were identical to the control group.

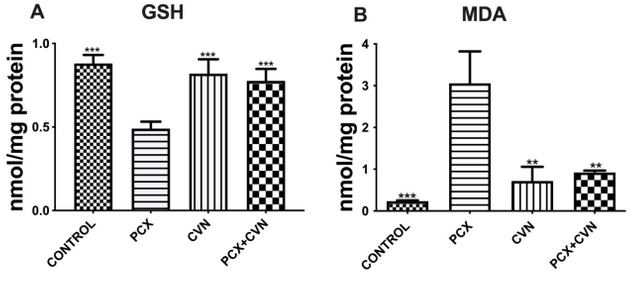


***Figure 3.*** *The effect of carvone on oxidative stress parameters (A: GSH; B: MDA) in groups that underwent paclitaxel-induced ototoxicity. Statistical comparisons were run using one-way ANOVA followed by Tukey's test. The PCX group was compared with the other groups; *p < .05, **p < .01, and ***p < .001 marks were used. The values are represented as mean ± SD.*

## 4. DISCUSSION

This study investigated whether CVN may protect against ototoxicity after exposure to PCX. CVN's preventive effects on oxidative damage in the cochlea following PCX treatment were evaluated biochemically. DPOAE results confirmed PCX-induced ototoxicity. DPOAE results also demonstrated that CVN had a protective effect against PCX ototoxicity, which aligned with our biochemical findings.

In the related literature, various exogenous otoprotector substances, such as thymoquinone (24), carvacrol (3), curcumin (25), and resveratrol (26), have been utilized to reduce ototoxicity caused by various chemotherapeutics by "scavenging" free radicals at a preliminary phase and mitigating oxidative injury via their antioxidant properties.

The pathophysiological processes of generation of ROS, reduction in the antioxidant system, DNA damage, oxidative alterations in proteins, and enhanced lipid peroxidation all contribute to the development of ototoxic consequences associated with chemotherapeutics. Considering these physiological processes, the reason for focusing on antioxidant therapy in ROS-related hearing loss is that administering exogenous antioxidants can prevent inner ear damage via up-regulation of endogenous antioxidant production and the ROS scavenger system (1, 27).

CVN has been demonstrated to be a biologically active molecule in numerous in vitro and in vivo experiments, suggesting that it could be a promising therapeutic candidate (14). Indeed, CVN's potent antioxidant properties make it an attractive candidate for therapeutic development in various oxidative stress-related disorders. Many working groups have examined and documented the antioxidant effects of CVN (14, 16). Compared to α-tocopherol (28), employed as a reference antioxidant in a study, the total antioxidant activity test revealed that CVN exhibited a robust antioxidant activity. Since alpha-tocopherol has been shown to protect against cisplatin-induced ototoxicity in previous research, CVN, which has shown to have more potent antioxidant activities, may have a more significant protective impact than alpha-tocopherol in ototoxicity. Various in vitro techniques, such as lipid peroxidation, 2,2-dipenyl-1-picrylhydrazil (DPPH), and the phosphomolybdenum assay, have been used to examine CVN's antioxidant capacity. As a result, CVN was discovered to have inhibitory activity against thiobarbituric acid reactive species (TBARS), causing the DPPH radical to be scavenged and the reduction of molybdenum, Mo(VI), to Mo(III) (V)(29). So, the potential protective effects of CVN, which is renowned for its powerful antioxidant and anticarcinogenic capabilities, against PCX-induced ototoxicity were explored in this work. To our knowledge, this is the first report to demonstrate a protective impact of CVN against ototoxic damage in the literature.

Some exogenous antioxidant agents used to prevent chemotherapeutic-induced ototoxicity must provide reliable protection without reducing the anticancerous impact potential of chemotherapeutics to be considered an optimal otoprotector. According to various in vitro studies based on cell culture assays, CVN shows antiproliferative effects against a range of cancer cell lines. In an in vivo study of 7,12-dimethylbenz(a)anthracene-induced skin carcinogenesis, CVN had a chemo-preventive effect (14). In another study, CVN showed anticancer activity by inhibiting the proliferation of myeloma KMS-5 cells (28). CVN reduced the migration of breast cancer cell lines and triggered apoptosis in a study testing its antiproliferative and apoptotic activities on breast cancer cells (31). CVN has also been found to protect the retina and optic nerve against PCX-induced cytotoxicity (32). Given this background, it is reasonable to believe that CVN will contribute to the efficacy of the chemotherapeutic treatment in combination and protect against the chemotherapeutic drug's harmful aspects.

Although the cellular mechanisms by which chemotherapeutics such as cisplatin cause the loss of outer hair cells and subsequent degeneration of the organ of corti have been described, the cellular mechanisms by which PCX causes the loss of outer hair cells and subsequent degeneration of the organ of corti are not well understood (2, 7). Even though several anticancer drugs have been demonstrated to be ototoxic, there is very little information on the effects of PCX on the inner ear (7, 33). Because all the adverse effects found with other anti-neoplastic medications like cisplatin in combination use have been traced to drugs

other than PCX, this is the case (34-37). However, some studies demonstrate that PCX can cause sensorineural hearing loss in mice and some histological alterations (38). The pathways that cause cell death in non-proliferating hair cells and inner ear neurons may differ dramatically from the anticancer therapies' traditional modes of action (2). PCX inhibits cell growth and other cellular processes by stabilizing microtubules and preventing microtubule depolymerization from delivering its anti-neoplastic impact (4). Although PCX, like other anti-neoplastic agents, has various adverse effects, peripheral neuropathy is the most prevalent. PCX-induced tubulin polymerization in neurons limits axonal transport, which may cause peripheral neuropathy (39). Sensory abnormalities and decreased sensory nerve conduction velocity are caused by PCX's neurotoxic effects on the dorsal root ganglia. The neurotoxic effects of PCX on peripheral glial cells are assumed to be the cause of its adverse effects on peripheral axons (40, 41). Because β-tubulin isoforms are found in both hair cells and neurons, it is not surprising that considerable damage occurs in both auditory nerve fibers and spiral ganglion neurons, given the recognized destructive mechanism of PCX (42, 43). Due to PCX's possible ototoxic mechanism, it is expected to exacerbate ototoxicity when used with other anti-neoplastic drugs known to induce hearing loss (2). Even though the molecular and cellular causes of PCX ototoxicity are unknown, it is thought that the drug's recognized neurotoxic effects on peripheral glial cells are also responsible for its adverse effects on auditory nerve fibers and spiral ganglion neurons. In this situation, PCX stimulates the generation of free oxygen radicals in both auditory nerve fibers and spiral ganglion neurons, causing oxidative damage to the cochlea and eventually death, according to the recognized damaging mechanism of PCX. PCX was found to have an ototoxic effect in the cochlea by triggering caspase 3 activation, a hallmark of intrinsic apoptosis (7). The triggering of the generation of free oxygen radicals that cause oxidative damage is required for the induction of apoptosis by activating the caspase pathway (7). Excessive ROS generation depletes GSH, causing antioxidant enzymes in the cochlea to be inhibited. The antioxidant defense mechanism is then depleted, resulting in an increase in lipid peroxidation and also cellular damage. High MDA levels are a hallmark of this condition. MDA, a biochemical marker reflected the level of oxygen-free radical and lipid degradation in tissues, is generated as a result of oxidative damage caused by the impacts of ROS. MDA causes changes in ion transport, enzyme activities, structural damage to biological macromolecules such as lipids, proteins, and DNA and disruption of cell membranes. Since it has oxidative stress and inflammatory properties, not only one of the metabolites of cellular damage but also one of the substances that cause cell damage, it plays a role in determining the level of cellular damage (44, 45).

Chemotherapeutic agents that cause ototoxicity have been shown in research to raise MDA levels while decreasing GSH, an essential non-enzymatic endogenous antioxidant (10, 11). PCX raised MDA levels while lowering GSH levels in our study, consistent with previous findings (3, 8, 9). These data indicate that oxidative stress plays a role in the cochlear damage caused by PCX-induced ototoxicity. At the same time, MDA levels were reduced, whereas GSH levels increased due to CVN therapy in this study. According to Mengyuan et al. (15), CVN reversed PCX-induced ototoxicity, reducing oxidative stress and exhibiting otoprotective action due to these findings. CVN dose-dependently decreases MDA levels in rats induced neuronal injury by cerebral I/R. Also, Asle-Rousta et al. (16) showed that CVN reduced MDA levels and enhanced GSH levels in the livers of immobilized rats. Zhao and Du (46) confirmed that CVN increases GSH content and decreases MDA levels on lipopolysaccharide (LPS)-induced acute lung injury in mice. In light of these studies, based on the data in our study, CVN functions as an antioxidant by activating the PCX-mediated reduced antioxidant enzyme system and reducing ROS production.

In the early stages of chemotherapeutic-induced ototoxicity, clinical consequences can be seen. Ototoxicity manifests clinically as gradual, permanent, and dose-dependent sensorineural hearing loss. DPOAE is a simple and inexpensive test frequently used in research to demonstrate hearing loss in the cochlea (47). The DPOAE test was utilized in our research to demonstrate functional impairment in the cochlea. The test was given to all rats twice: once before the first drug was given and again after the last drug was given. According to our DPOAE responses, the DPOAE values of rats receiving PCX were considerably lower than both day 0 and day 23 of all the other groups' DPOAE values at all frequencies. This outcome also demonstrated the ototoxic impact of PCX. Furthermore, compared to the PCX group, rats given CVN with PCX showed considerably greater DPOAE readings. Based on this finding, CVN appears to have a functional protective effect against cisplatin-induced ototoxicity.

It needs to be noted that this experimental research is limited by the need for histopathological examinations. Therefore, further in-depth investigations are necessary to elucidate the protective properties of CVN against PCX-induced ototoxicity in the cochlear tissue, specifically from a histopathological perspective.

## 5. CONCLUSION

PCX successfully induced ototoxicity, evident from the reductions in DPOAE results and biochemical findings. CVN shows distinct signals of protection against PCX ototoxicity after administration. In conclusion, the findings of this study indicate that CVN effectively mitigated oxidative stress parameters by elevating MDA levels ($p < .01$) and significantly reducing GSH levels ($p < .001$). Additionally, CVN demonstrated a beneficial effect on the levels of DPOAE ($p < .01$ at 8000Hz), thereby providing protection against PCX-induced damage. However, more detailed research is needed to determine the optimal dose of CVN before it can be used in clinical practice. In addition, CVN can be used with chemotherapeutic drugs due to its significant anticancer

effect and may be a suitable drug option against ototoxicity, a side effect of chemotherapeutic drugs.

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*Acquisition of data for the study: BD, FA*
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## REFERENCES

[1] Pak JH, Kim Y, Yi J, Chung JW. Antioxidant therapy against oxidative damage of the inner ear: Protection and preconditioning. Antioxidants 2020; 9(11):2-21. DOI: 10.3390/antiox9111076

[2] Dong Y, Ding D, Jiang H, Shi J-r, Salvi R, Roth JA. Ototoxicity of paclitaxel in rat cochlear organotypic cultures. Toxicol Appl Pharmacol. 2014; 280(3):526-533. DOI: 10.1016/j.taap.2014.08.022

[3] Atalay F, Tatar A, Dincer B, Gündoğdu B, Köyceğiz S. Protective effect of carvacrol against paclitaxel-induced ototoxicity in rat model. Turk Arch Otorhinolaryngol 2020; 58(4):241-248. DOI: 10.5152/tao.2020.5714

[4] Yan-Hua Y, Jia-Wang M, Xiao-Li T. Research progress on the source, production, and anti-cancer mechanisms of paclitaxel. Chin J Nat Med. 2020; 18(12):890-897. DOI: 10.1016/S1875-5364(20)60032-2

[5] Alqahtani FY, Aleanizy FS, El Tahir E, Alkahtani HM, AlQuadeib BT, Paclitaxel, in Profiles of drug substances, excipients and related methodology. 2019, Elsevier. p. 205-238. DOI: 10.1016/bs.podrm.2018.11.001

[6] Marupudi NI, Han JE, Li KW, Renard VM, Tyler BM, Brem H. Paclitaxel: A review of adverse toxicities and novel delivery strategies. Expert Opin Drug Saf. 2007; 6(5):609-621. DOI: 10.1517/14740338.6.5.609

[7] Bucak A, Ozdemir C, Ulu S, Gonul Y, Aycicek A, Uysal M, Cangal A. Investigation of protective role of curcumin against paclitaxel-induced inner ear damage in rats. The Laryngoscope 2015; 125(5):1175-1182. DOI: 10.1002/lary.25031

[8] Park SB, Lin CSY, Krishnan AV, Friedlander ML, Lewis CR, Kiernan MC. Early, progressive, and sustained dysfunction of sensory axons underlies paclitaxel-induced neuropathy. Muscle & Nerve 2011; 43(3): 367-374. DOI: 10.1002/mus.21874

[9] Cheung, S, Henderson-Sabes J, Mastick J, Abrams G, Snowberg K, Alfaro E, Quinn M, Paul S, Cooper B, Wallhagen M, Conley Y, Levine J, Miaskowski C. Cancer survivors and neurotoxic chemotherapy: hearing loss and tinnitus. BMJ Support. Palliat. 2022; bmjspcare-2022-003684. DOI: 10.1136/spcare-2022-003684

[10] Kilic K, Sakat MS, Akdemir FNE, Yildirim S, Saglam YS, Askin S. Protective effect of gallic acid against cisplatin-induced ototoxicity in rats. Braz. J. Otorhinolaryngol 2019;85(3):267-274. DOI: 10.1016/j.bjorl.2018.03.001

[11] Sakat MS, Kilic K, Akdemir FNE, Yildirim S, Eser G, Kiziltunc A. The effectiveness of eugenol against cisplatin-induced ototoxicity. Braz. J. Otorhinolaryngol 2019;85(6):766-773. DOI: 10.1016/j.bjorl.2018.07.007

[12] Im GJ, Chang J, Lee S, Choi J, Jung HH, Lee HM, Ryu SH, Park SK, Kim JH, Kim H-J. Protective role of edaravone against cisplatin-induced ototoxicity in an auditory cell line. Hear Res. 2015; 330:113-118. DOI: 10.1016/j.heares.2015.08.004

[13] Özdemir D, Özgür A, Kalkan Y, Terzi S, Tümkaya L, Yılmaz A, Çeliker M, Dursun E. The protective effects of whortleberry extract against cisplatin-induced ototoxicity in rats. Braz. J. Otorhinolaryngol 2019; 85(1):55-62. DOI: 10.1016/j.bjorl.2017.10.009

[14] Bouyahya A, Mechchate H, Benali T, Ghchime R, Charfi S, Balahbib A, Burkov P, Shariati MA, Lorenzo JM, Omari NE. Health benefits and pharmacological properties of carvone. Biomolecules 2021; 11(12):1-26. DOI: 10.3390/biom11121803

[15] Dai M, Wu L, Yu K, Xu R, Wei Y, Chinnathambi A, Alahmadi TA, Zhou M. D-Carvone inhibit cerebral ischemia/reperfusion induced inflammatory response TLR4/NLRP3 signaling pathway. Biomed Pharmacother. 2020; 132:110870. DOI: 10.1016/j.biopha.2020.110870

[16] Asle-Rousta M, Amini R, Aghazadeh S. Carvone suppresses oxidative stress and inflammation in the liver of immobilised rats. Arch Physiol Biochem. 2023; 129(3):597-602. DOI: 10.1080/13813.455.2020.1851726

[17] Vinothkumar R, Sudha M, Viswanathan P, Kabalimoorthy J, Balasubramanian T, Nalini N. Modulating effect of d-carvone on 1, 2-dimethylhydrazine-induced pre-neoplastic lesions, oxidative stress and biotransforming enzymes, in an experimental model of rat colon carcinogenesis. Cell Prolif. 2013; 46(6):705-720. DOI: 10.1111/cpr.12062

[18] Muruganathan U, Srinivasan S, Indumathi D. Antihyperglycemic effect of carvone: Effect on the levels of glycoprotein components in streptozotocin-induced diabetic rats. J Acute Dis. 2013; 2(4):310-315. DOI: 10.1016/S2221-6189(13)60150-X

[19] Muruganathan U, Srinivasan S. Beneficial effect of carvone, a dietary monoterpene ameliorates hyperglycemia by regulating the key enzymes activities of carbohydrate metabolism in streptozotocin-induced diabetic rats. Biomed Pharmacother. 2016; 84: 1558-1567. DOI: 10.1016/j.biopha.2016.11.025

[20] Bekmez Bilmez ZE, Aydin S, Şanli A, Altintoprak N, Demir MG, Atalay Erdoğan B, Kösemihal E. Oxytocin as a protective agent in cisplatin-induced ototoxicity. Cancer Chemother Pharmacol. 2016; 77(4):875-879. DOI: 10.1007/s00280.016.2978-x

[21] Cadirci E, Ugan RA, Dincer B, Gundogdu B, Cinar I, Akpinar E, Halici Z. Urotensin receptors as a new target for CLP induced septic lung injury in mice. Naunyn Schmiedebergs Arch Pharmacol. 2019; 392(2):135-145. DOI: 10.1007/s00210.018.1571-8

[22] Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968; 25:192-205. DOI: 10.1016/0003-2697(68)90092-4

[23] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95(2):351-358. DOI: 10.1016/0003-2697(79)90738-3

[24] Kökten N, Eğilmez OK, Erinç M, Ekici AID, Şerifler S, Yeşilada E, Kalcıoğlu MT. The protective effect of nigella sativa oil against experimentally induced cisplatin ototoxicity: An animal study. J Int Adv Otol. 2020; 16(3):346-352. DOI: 10.5152/iao.2020.7761

[25] Salehi P, Akinpelu OV, Waissbluth S, Peleva E, Meehan B, Rak J, Daniel SJ. Attenuation of cisplatin ototoxicity by otoprotective effects of nanoencapsulated curcumin and dexamethasone in a guinea pig model. Otol Neurotol. 2014; 35(7):1131-1139. DOI: 10.1097/MAO.000.000.0000000403

[26] Avci D, Erkan M, Sönmez MF, Kökoglu K, Günes MS, Gündogdu R, Güleç S, Karabulut D. A prospective experimental study on the protective effect of resveratrol against amikacin-induced ototoxicity in rats. J Int Adv Otol. 2016; 12(3):290-297. DOI: 10.5152/iao.2016.2617

[27] Sergi B, Fetoni A, Ferraresi A, Troiani D, Azzena G, Paludetti G, Maurizi M. The role of antioxidants in protection from ototoxic drugs. Acta Otolaryngol. (Stockh.) 2004; 124(sup552):42-45. DOI: 10.1080/036.552.30410017111

[28] Fetoni A, Sergi B, Ferraresi A, Paludetti G, Troiani D. Protective effects of α-tocopherol and tiopronin against cisplatin-induced ototoxicity. Acta Otolaryngol. (Stockh.) 2004; 124(4):421-426. DOI: 10.1080/000.164.80410016559

[29] Sabir S, Singh D, Rocha J. In Vitro antioxidant activity of S-carvone isolated from Zanthoxylum alatum. Pharm Chem J. 2015; 49(3):187-191. DOI: 10.1007/s11094.015.1251-7

[30] Ding X, Chen H. Anticancer effects of Carvone in myeloma cells is mediated through the inhibition of p38 MAPK signalling pathway, apoptosis induction and inhibition of cell invasion. J BUON 2018; 23(3):747-751.

[31] Patel PB, Thakkar VR. L-carvone induces p53, caspase 3 mediated apoptosis and inhibits the migration of breast cancer cell lines. Nutr Cancer 2014; 66(3):453-462. DOI:10.1080/01635.581.2014.884230

[32] Cinici E, Dilekmen N, Kutlu Z, Dincer B, Cinici O, Balta H, Calık I. Carvone protects against paclitaxel-induced retinal and optic nerve cytotoxicity: a histopathological study. Cutan Ocul Toxicol. 2019; 38(3):290-293. DOI: 10.1080/15569.527.2019.1608229

[33] Sarafraz M, Ahmadi K. Paraclinical evaluation of side-effects of Taxanes on auditory system. Acta Otorhinolaryngol Ital. 2008; 28(5):239-242.

[34] Ridwelski K, Gebauer T, Fahlke J, Kröning H, Kettner E, Meyer F, Eichelmann K, Lippert H. Combination chemotherapy with docetaxel and cisplatin for locally advanced and metastatic gastric cancer. Ann Oncol. 2001; 12(1):47-51. DOI: 10.1023/a:100.832.8501128

[35] Georgoulias V, Ardavanis A, Tsiafaki X, Agelidou A, Mixalopoulou P, Anagnostopoulou O, Ziotopoulos P, Toubis M, Syrigos K, Samaras N. Vinorelbine plus cisplatin versus docetaxel plus gemcitabine in advanced non-small-cell lung cancer: A phase III randomized trial. J Clin Oncol. 2005; 23(13):2937-2945. DOI: 10.1200/JCO.2005.04.016

[36] Ding D, He J, Allman BL, Yu D, Jiang H, Seigel GM, Salvi RJ. Cisplatin ototoxicity in rat cochlear organotypic cultures. Hear Res. 2011; 282(1-2):196-203. DOI: 10.1016/j.heares.2011.08.002

[37] Jamesdaniel S, Coling D, Hinduja S, Ding D, Li J, Cassidy L, Seigel GM, Qu J, Salvi R. Cisplatin-induced ototoxicity is mediated by nitroxidative modification of cochlear proteins characterized by nitration of Lmo4. J Biol Chem. 2012; 287(22):18674-18686. DOI: 10.1074/jbc.M111.297960

[38] Atas A, Agca O, Sarac S, Poyraz A, Akyol MU. Investigation of ototoxic effects of Taxol on a mice model. Int. J. Pediatr. Otorhinolaryngol. 2006; 70(5):779-784. DOI: 10.1016/j.ijporl.2005.11.011

[39] Pace A, Nisticò C, Cuppone F, Bria E, Galiè E, Graziano G, Natoli G, Sperduti I, Jandolo B, Calabretta F. Peripheral neurotoxicity of weekly paclitaxel chemotherapy: A schedule or a dose issue? Clin. Breast Cancer 2007; 7(7):550-554. DOI: 10.3816/CBC.2007.n.010

[40] Hu L-Y, Mi W-L, Wu G-C, Wang Y-Q, Mao-Ying Q-L. Prevention and treatment for chemotherapy-induced peripheral neuropathy: therapies based on CIPN mechanisms. Curr. Neuropharmacol. 2019; 17(2):184-196. DOI: 10.2174/1570159X156.661.70915143217

[41] Cavaletti G, Cavalletti E, Oggioni N, Sottani C, Minoia C, D'incalci M, Zucchetti M, Marmiroli P, Tredici G. Distribution of paclitaxel within the nervous system of the rat after repeated intravenous administration. Neurotoxicology 2000; 21(3):389-393.

[42] Hallworth R, Ludueña RF. Differential expression of β tubulin isotypes in the adult gerbil cochlea. Hear Res. 2000; 148(1-2):161-172. DOI: 10.1016/s0378-5955(00)00149-0

[43] Jensen-Smith HC, Eley J, Steyger PS, Ludueña RF, Hallworth R. Cell type-specific reduction of β tubulin isotypes synthesized in the developing gerbil organ of Corti. J Neurocytol. 2003; 32(2):185-197. DOI: 10.1023/b:neur.000.000.5602.18713.02

[44] Barrera G, Pizzimenti S, Daga M, Dianzani C, Arcaro A, Cetrangolo GP, Gentile F. Lipid peroxidation-derived aldehydes, 4-hydroxynonenal and malondialdehyde in aging-related disorders. Antioxidants 2018; 7(8): 102. DOI: 10.3390/antiox7080102

[45] Papac-Milicevic N, Busch CL, Binder CJ. Malondialdehyde epitopes as targets of immunity and the implications for atherosclerosis. Adv Immunol. 2016; 131: 1-59. DOI: 10.1016/bs.ai.2016.02.001

[46] Zhao M, Du J. Anti-inflammatory and protective effects of D-carvone on lipopolysaccharide (LPS)-induced acute lung injury in mice. J King Saud Univ Sci. 2020; 32(2):1592-1596. DOI: 10.1016/j.jksus.2019.12.016

[47] Abdala C, Visser-Dumont L. Distortion product otoacoustic emissions: A tool for hearing assessment and scientific study. The Volta Review 2001; 103(4):281-302.

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