



ORIGINAL RESEARCH

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The effect of ferulic acid against cisplatin-induced ototoxicity

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Abstract

Ototoxicity refers to cellular damage or functional disorder developing in the inner ear in association with any therapeutic agent or chemical substance. Cisplatin, one of these agents capable of causing ototoxicity, is a chemotherapeutic used in several malignancies. Ferulic acid (FA) is a phenolic acid with known anti-oxidative and anti-inflammatory properties. The purpose of this study was to perform a biochemical and histopathological investigation of the protective efficacy of FA against cisplatin-induced ototoxicity in rats. Twenty-four Wistar rats were used in this study. Animals were randomly assigned into four groups of six rats each. Rats in the control group received intraperitoneal injection of 1 ml saline for four consecutive days. Rats in the cisplatin group received a single intraperitoneal administration of 10 mg/kg cisplatin. Rats in the FA group received intraperitoneal FA at 100 mg/kg for four days, and rats in the cisplatin+FA group received intraperitoneal FA at 100 mg/kg 1 h after administration of intraperitoneal cisplatin at 10 mg/kg, this procedure being performed for four consecutive days. Rats were sacrificed 24 h after the final drug administration. Cochlear tissues were removed for biochemical and histopathological analysis. MDA levels were significantly higher in cochlear tissues of the rats receiving cisplatin compared to the control group. MDA levels in rats receiving cisplatin and treated with FA were significantly lower than those in the cisplatin group. Activities of SOD and GPx decreased significantly in the cochleas of rats administered cisplatin compared to the control group. Both SOD and GPx activities were significantly higher in rats administered FA and cisplatin in combination compared to the cisplatin only. Histopathological evaluation of cochleas of the rats revealed significant protection of FA against cisplatin induced ototoxicity. This study, the first in the literature, shows that FA exhibits a protective effect against cisplatin-induced ototoxicity.

Keywords: Cisplatin, ototoxicity, ferulic acid

Introduction

Ototoxicity refers to cellular damage or functional disorder developing in the inner ear in association with any therapeutic agent or chemical substance. Cisplatin, one of these agents capable of causing ototoxicity, is a chemotherapeutic used in several malignancies. The molecular pathology underlying cisplatin-induced ototoxicity has not yet been fully explained. Studies have shown that cisplatin affects several cochlear components, such as outer hair cells, spiral ganglion cells and the stria vascularis [1]. Ototoxicity developing in association with cisplatin may occur as the result of various pathways, such as apoptosis, overproduction of reactive oxygen species (ROS) and decreased endogenous antioxidant expression [2].

Permanent, bilateral and progressive sensorineural hearing loss develops in 60-80% of patients treated with cisplatin [3]. The severity of the loss depends on the frequency of drug administration and the dosage. Although hearing loss is not a life-threatening

condition, it is nevertheless important in terms of impairing quality of life and causing communication problems. Ototoxicity is the main side-effect restricting cisplatin use. The major aim in patients receiving anti-tumor therapy with cisplatin is to minimize this side-effect. Various antioxidant agents such as D-methionine, allopurinol, flunarizine, curcumin and lutein have been used for this purpose in experimental studies [3-7].

Ferulic acid (FA) is a phenolic acid with known anti-oxidative and anti-inflammatory properties. Phenolic compounds are employed as therapeutic agents in neurodegenerative diseases, cancer, diabetes, coronary heart diseases, and inflammatory states. Previous studies have indicated that, due to its antioxidant activities, FA is capable of neutralizing nitric oxide and hydroxyl radical groups responsible for DNA damage [8].

To the best of our knowledge, no previous studies have investigated the protective effect of FA against ototoxicity. The purpose of this study was to perform a biochemical and histopathological investigation of the protective efficacy of FA against cisplatin-induced ototoxicity in rats.

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Material and Method

Animals

Twenty-four Wistar rats weighing 270 ± 20 g were used. All animals were housed in special cages in chambers with fixed temperature ($23 \pm 20^\circ\text{C}$) and humidity ($60\% \pm 5\%$) and with equal day/night cycles. Rats were allowed free access to food and water. All procedures were conducted in line with Care and Use of Laboratory Animals Guide regulations.

Experimental Procedure

FA was obtained from Sigma-Aldrich Chemical Co. and cisplatin from Koçak Farma Co. (Koçak, Istanbul, Turkey). Animals were randomly assigned into four groups of six rats each. Rats in the control group received intraperitoneal injection of 1 ml saline for four consecutive days. Rats in the cisplatin group received a single intraperitoneal administration of 10 mg/kg cisplatin. Rats in the FA group received intraperitoneal FA at 100 mg/kg for four days, and rats in the cisplatin+FA group received intraperitoneal FA at 100 mg/kg 1 h after administration of intraperitoneal cisplatin at 10 mg/kg, this procedure being performed for four consecutive days. Rats were sacrificed using 400 mg/kg intraperitoneal thiopental sodium 24 h after the final drug administration. Cochlear tissues were removed and stored in a deep freeze at -80°C for biochemical analysis. Cochlear tissues were also fixed in 10% formaldehyde for histopathological analysis.

Biochemical Analysis

After sacrifice, all rats' cochlear tissues were stored at -80 until the day of study. Cochlear tissue from each rat was ground in liquid nitrogen using a Tissue Lyser II grinding jar set (Qiagen, Hilden, Germany). All tissue specimens were homogenized using 1.15% KCL buffer solution at 9 ml per gram. Supernatant was then collected by centrifuging the homogenate at 10000 rpm for 30 min. Activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD) and levels of malondialdehyde (MDA) in each supernatant specimen were determined using an ELISA reader [9-11]. Mean absorbance values were calculated, a standard curve was plotted and an equation was obtained from the standard absorbances. This equation was used to compute proportions of GPx, MDA and SOD. SOD and GPx activities, and MDA levels identified in tissues were expressed as U/ml, iU/L, and nmol/ml, respectively. Levels of proteins in samples were measured using the method described by Lowry. Bovine serum albumin was employed as the standard commercial protein (total protein kit-TP0300-1 KT; Sigma Chemical Co., Munich, Germany).

Histopathological Analysis

After the animals had been sacrificed, the bilateral temporal bones were removed and the cochleas isolated. Cochlear tissues were fixed for 48 h in 10% formalin solution for histopathological evaluation. For decalcification, tissues were stored for 96 h in Osteosoft decalcification solution (Merc, HC313331, Germany) and then washed under running tap water for 24 h. Tissue were then passed through 80% alcohol (2 times x 12 h), 90% alcohol (2 times x 12 h), 96% alcohol (2 times x 12 h), 100% alcohol (2 times x 12 h), chloroform (3 times x 5 h), and liquid paraffin (12 h) and embedded in paraffin blocks. Sections 4 μm in thickness were taken from each block and placed onto glass slides. Preparation for histopathological analysis were stained with hematoxylin and eosin and studied under a light microscope. Sections examined under light microscopy were classified in terms of lesions as none (-), mild (+), moderate (++) or severe (+++), and photographs

were taken.

Statistical Analysis

SPSS 17.0 software was used for statistical analyses. Distribution of biochemical data was assessed by means of the Shapiro-Wilks test. Since normal distribution was determined the parametric One-Way ANOVA test was used. The Post-hoc Turkey test was used to determine the source of variation. The non-parametric Kruskal-Wallis test was used to analyze differences between groups among data obtained by semiquantitative means data, while the Mann Whitney U test was used for comparisons between two groups. All results were given as mean \pm standard deviation and $p < 0.05$ was accepted as statistically significant.

Results

Biochemical Findings

Chemical changes taking place in rat cochlear tissues are summarized in Figure 1.

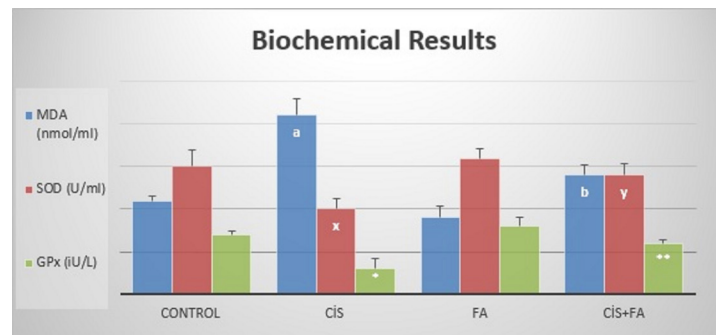


Figure 1. Biochemical results

a: There is statistically significant difference between Cis and Control groups in terms of MDA levels. b: There is statistically significant difference between Cis +FA groups in terms of MDA levels ($p < 0.05$). x: There is statistically significant difference between Cis and Control groups in terms of SOD activity ($p < 0.05$). y: There is statistically significant difference between Cis and Cis+FA groups in terms of SOD activity ($p < 0.05$). *: There is statistically significant difference between Cis and Control groups in terms of GPx activity ($p < 0.05$). **: There is statistically significant difference between Cis +FA groups in terms of GPx activity ($p < 0.05$).

MDA levels were significantly higher in rats receiving cisplatin compared to the control group. MDA levels in rats receiving cisplatin and treated with FA were significantly lower than those in the cisplatin group. The administration of FA exhibited a protective effect against MDA elevation, a marker of lipid peroxidation, caused by cisplatin.

Activities of SOD and GPx, two components of the antioxidant enzyme system, decreased significantly in the cochleas of rats administered cisplatin compared to the control group. Both SOD and GPx activities were significantly higher in rats administered FA and cisplatin in combination compared to the cisplatin only. The administration of FA was determined to protect against a decrease in antioxidant enzymes caused by cisplatin.

Histopathological Findings

The organ of Corti, stria vascularis and spinal ganglion exhibited a normal structure in the control and FA groups (Figure 2, 4). A severe decrease in outer hair cell numbers was determined in the organ of Corti in the cisplatin group, compared to an increase in numbers in the cisplatin+FA group. Desquamation and erosion were observed in vessels in the stria vascularis in the cisplatin group (Figure 3). These lesions were significantly milder in the cisplatin+FA group. Degenerated and eosinophilic necrotic cells were observed in the spinal ganglion in the cisplatin group. In the

cisplatin+FA group, while mildly degenerated cells were observed, no cells with necrotic dilation and congestion were seen (Figure 5). Histopathological cochlear findings are summarized in Table 1.

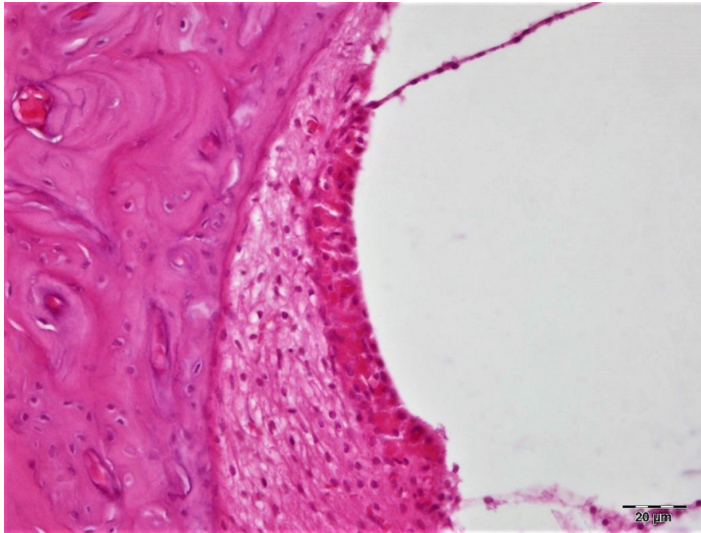


Figure 2. Normal histological structure of the cochlea in the control group, H&E, Bar: 20 μm

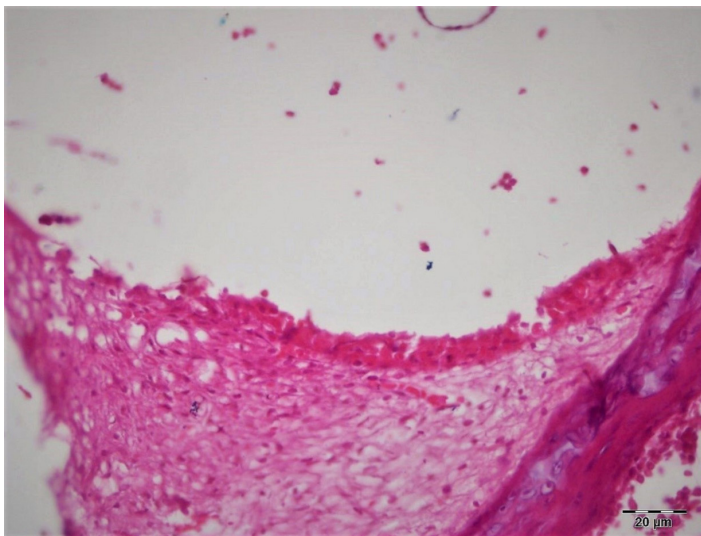


Figure 3. Desquamation and erosion in the stria vascularis and dilation and congestion in vessels in the cisplatin group, H&E, Bar: 20 μm

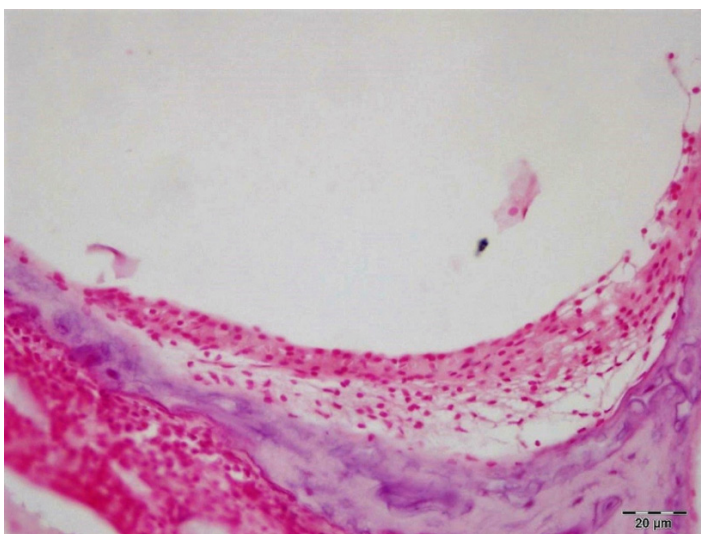


Figure 4. Normal histological structure of the cochlea in the ferulic acid group, H&E, Bar: 20 μm

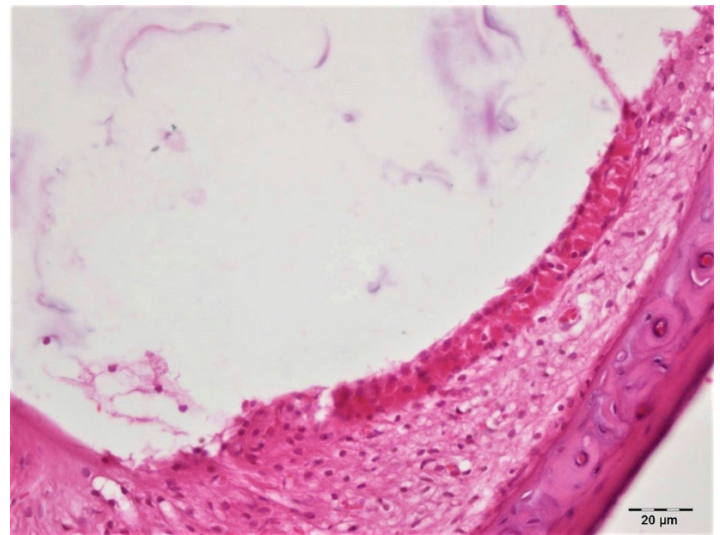


Figure 5. Mild dilation and congestion in the vessels of the stria vascularis and a moderate decrease in outer hair cell numbers in the organ of Corti in the cisplatin+ferulic acid group, H&E, Bar: 20 μm

Table 1. Histopathological features of cochlear tissues

		Control	Cis group	FA Group	Cis + FA group
Organ of Corti	Number of outer hair cells	+++	+	+++	++
Stria vascularis	Desquamation-erosion	-	+++	-	+
	Dilation and congestion in vessels	-	+++	-	++
spinal ganglion	Degenerated cells,	-	+++	-	+
	eosinophilic necrotic cells	-	+++	-	-

Cis: Cisplatin

Discussion

Cisplatin is an antineoplastic agent widely used in the treatment of several forms of cancer. It exhibits its antineoplastic effect by inhibiting DNA synthesis. The most important side-effects limiting its use are nephrotoxicity, neurotoxicity and ototoxicity. Since nephrotoxicity can be controlled with hydration therapy, ototoxicity is the principal side-effect restricting cisplatin therapy⁷. The mechanism by which cisplatin induces ototoxicity has still not yet been fully explained. Studies have shown that cisplatin causes hearing loss by affecting various regions of the cochlea. The histopathological finding receiving the greatest focus is degeneration of outer hair cells. Damage beginning in the tip links of the stereocilia of outer hair cells gradually comes to involve loss of all the outer hair cells. This loss begins in the basal part and progresses to the apex. It also causes collapse in Reissner's membrane and atrophy in supporting cells in the organ of Corti and the stria vascularis [12-13].

The molecules and pathology responsible at the cellular level for this damage are still unclear, although oxidative stress is thought to be the most important factor. Studies have suggested that cisplatin exhibits an ototoxic effect by causing an increase in the production of free oxygen radicals in cellular structures in the cochlea. At the same time, cisplatin has also been shown to reduce cochlear antioxidant enzyme levels. Cisplatin results in the formation of dysfunctional proteins and enzymes by compromising DNA

synthesis by accumulating in cochlear tissues. This will result in an increase in free oxygen radicals and a decrease in the antioxidant enzyme system. The increase in ROS and decrease in the antioxidant system result in oxidative stress, a cytotoxic condition. Oxidative stress causes cochlear cell damage and cell death [14]. In our study, the MDA levels were higher in rats treated with cisplatin while the activities of SOD and GPx were lower. These results showed the effect of oxidative stress on cisplatin-induced ototoxicity.

In the light of these pathophysiological processes, it has been suggested that reducing ROS levels while increasing antioxidant enzyme levels can protect against cisplatin-induced ototoxicity. Various antioxidant agents have been investigated for this purpose in ototoxicity. The purpose of our study was to investigate the protective effect of FA, a powerful antioxidant agent, in cisplatin-induced ototoxicity.

FA (4-hydroxy-3-methoxycinnamic acid) is a phenolic compound widely present in plant species, including many grains, fruits and vegetables, and is easily metabolized [15]. In a study of the antidepressant and antioxidant efficacy of FA, Zeni et al. reported that oxidative stress induced with corticosterone and depression-like behavioral changes in rats improved with the administration of FA [16]. Gu et al. determined that FA increased the differentiation and survival of neural stem cells in vivo in mice with gentamycin-induced neuronal hearing loss. At the same time, they reported that in vivo FA restored ABR threshold shifts and impairments in DPOAE results in neuronal hearing loss [17]. Fetoni et al. determined that FA exhibited a protective effect against noise-related hearing loss in guinea pigs. They reported that FA exhibited this protective effect by reducing oxidative stress and apoptosis and increasing the viability of hair cells in the organ of Corti. At the same time, they reported that FA also exhibited its protective effect by increasing antioxidant enzyme levels in addition to its ROS scavenging activity [18]. Bami et al. investigated the protective effect of FA in cisplatin-induced nephrotoxicity and observed that FA was significantly effective in terms of protecting against oxidative stress, raising antioxidant levels and restoring histopathological changes to normal [8]. We also determined that FA reduced oxidative stress in cisplatin-induced ototoxicity, increased antioxidant enzyme levels and corrected histopathological disturbances in the cochlea in cisplatin-induced ototoxicity in rats. The principal limitation of our study is that clinical tests such as DPOEA and ABR capable of showing functional damage occurring in the rat cochlea were not performed.

Conclusion

This study, the first in the literature, shows that FA exhibits a protective effect against cisplatin-induced ototoxicity. FA exhibits this effect by reducing oxidative stress in the cochlea.

Competing interests

The authors declare that they have no competing interest

Financial Disclosure

The financial support for this study was provided by the investigators themselves.

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