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Effect of *Hippophae rhamnoides* extract on Cisplatin-induced ototoxicity in Guinea Pigs

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ABSTRACT

No previous studies have examined the effect of *Hippophae rhamnoides* fruit extract (HRE), which contains both water and fat soluble vitamins, on cisplatin-induced ototoxicity. Therefore, the aim of the study was to investigate the effects of HRE on cisplatin-induced ototoxicity in Guinea pigs and to evaluate the biochemical, gene expression and histopathological changes associated with cisplatin use. Experimental animals were divided into three groups: healthy (HG), HRE+cisplatin (HRC) and cisplatin control (CCG) groups. Biochemical, gene expression and histopathological examinations were carried on the removed inner ear tissues. In the HRC group, the oxidant parameter was lower and the anti-oxidant parameter was higher than in the CCG. These results are supported by gene expression levels and histopathological results. The use of HRE against cisplatin-induced oxidative ototoxicity may be easier, more cost effective and more beneficial than the use of vitamins alone or in combination with each other.

Key words: Cisplatin, Ear, Gene expression, *Hippophae rhamnoides*, Guinea pigs.

INTRODUCTION

Cisplatin is a platinum derivative anticancer compound commonly used for the treatment of many malignant diseases (Sakamoto *et al.* 2000). However, serious toxic effects on the ears have been observed secondary to cisplatin use. These side effects have decreased its popularity. Hearing loss is observed in 15% of patients administered cisplatin (Blakley *et al.* 1994).This indicates that cisplatin is rather cochleotoxic. Kuduban *et al.*(2013) argued that this toxic effect created by cisplatin in the inner ear was the result of oxidative stress that develops through the increase of malondialdehyde (MDA), an oxidant parameter. They further suggested that the oxidative stress may also be caused by decreased concentrations of antioxidants, such as reduced glutathione (tGSH), glutathione reductase (GR) and superoxide dismutase (SOD) (Kuduban *et al.* 2013). Cisplatin has been reported to increase the cellular production of cytokines, including proinflammatory interleukin 1 beta (IL-1β) and tumour necrosis factor alpha (TNF α) (Kim *et al*. 2015). In the current study, *Hippophae rhamnoides* fruit extract (HRE), which we will test for its effects against cisplatin toxicity, contains carotenoids, vitamins and fatty acids, which protect the tissue against inflammation and oxidative stress damage (Kwon *et al.* 2011; Yilmaz *et al.* 2014). Tocopherol is also an important antioxidant (Maurya *et al.* 2015).This suggests that the use of HRE may be more beneficial than the use of either vitamins or antioxidants to treat cisplatin ototoxicity. Therefore, the aim of the study was to investigate the effects of HRE on cisplatin-induced ototoxicity in ats and to evaluate the biochemical, gene expression and histopathological aspects of this form of ototoxicity.

MATERIALS AND METHODS

Animals: For this study, we used 18 male Guinea pigs weighing 750–1000 g that were obtained from the Atatürk University Medical Experimental Application and Research Centre. Each animal was assigned to one of the three groups, and all animals were fed and housed at normal room temperature (22°C) throughout the study. All experiments were carried out in accordance with the National Guidelines for the Use and Care of Laboratory Animals. The study was conducted under the approval of the Local Animal Ethics Committee of Atatürk University (Ethics Committee Number: 2015/181).

Chemical agents: Cisplatin (50 mg/100 ml) was supplied from Liba, Turkey, thiopental sodium was provided by I.E. Ulagay, Turkey and *H. rhamnoides* extract was supplied from Karen Bilim, Turkey.

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Experimental groups: Guinea pigs were divided into three groups: healthy (HG), HRE+cisplatin (HRC) and cisplatin control (CCG) groups.

Experimental procedure: The HRC group was administered HRE (50 mg/kg; *n* = 6) and the CCG (*n* = 6) and HG (*n* = 6) groups were given saline by gavage as a solvent. One hour after the administration of HRE and distilled water, the HRC and CCG groups were intraperitoneally injected with a dose of 5 mg/kg cisplatin. HRE and cisplatin administration was repeated once a day. This procedure was performed for 7 days. After, all Guinea pigs were sacrificed with 50 mg/kg dose of thiopental sodium, and their inner ears were removed. Biochemical, gene expression and histopathological examinations were carried out in the removed inner ear tissues. The results of the HRC group were compared with the results of the CCG and HG groups.

Biochemical analysis

Malondialdehyde analysis: The amount of MDA was measured with the method used by Ohkawa *et al* (1979) .

Total Glutathione analysis: Analysis of GSH was performed according to the method of Sedlak and Lindsay (1968).

IL-1β and TNF-α gene expression analysis: Tissue extracts were obtained by tissue fragmentation. RNA isolation was performed from the extract using a MagNA Pure Compact automatic RNA isolation device and kit. cDNA synthesis was performed from the RNA using the Transcriptor First Strand cDNA Synthesis Kit and the reverse transcription PCR instrument, in accordance with the instruction manual.

Gene expression was performed using cDNA with real-time PCR reactions according to the method described by Yapca *et al* (2015).

Histopathological examination : The tympanic cavity was fixed with 10% buffered formaldehyde for 24 hours at +4°C. After fixation, the preparate was decalcified with 10% EDTA (ethylenediamine tetra-acetic acid) solution for 7 days. It was rinsed with tap water for 3 hours to eliminate decalcification solution and fixed with 10% buffered formaldehyde again for 1 more day. Following a routine tissue monitoring process, 5 µm sections were obtained for histopathological evaluation and stained with hematoxylin-eosin (H&E). The Corti organ, stria vascularis, spinal ganglia, Reissner's membrane, epithelial intermediary layer and lamina propria were evaluated with light microscopy (Olympus BX 51, Japan) by a pathologist who did not know the treatment protocol.

Statistical analysis: The differences between the groups were determined using a one-way ANOVA test followed by Tukey's multiple comparison test using SPSS 18.0 software. Results were expressed as mean ± standard error of the mean (x ± SEM) and significance was declared at $p<0.05$.

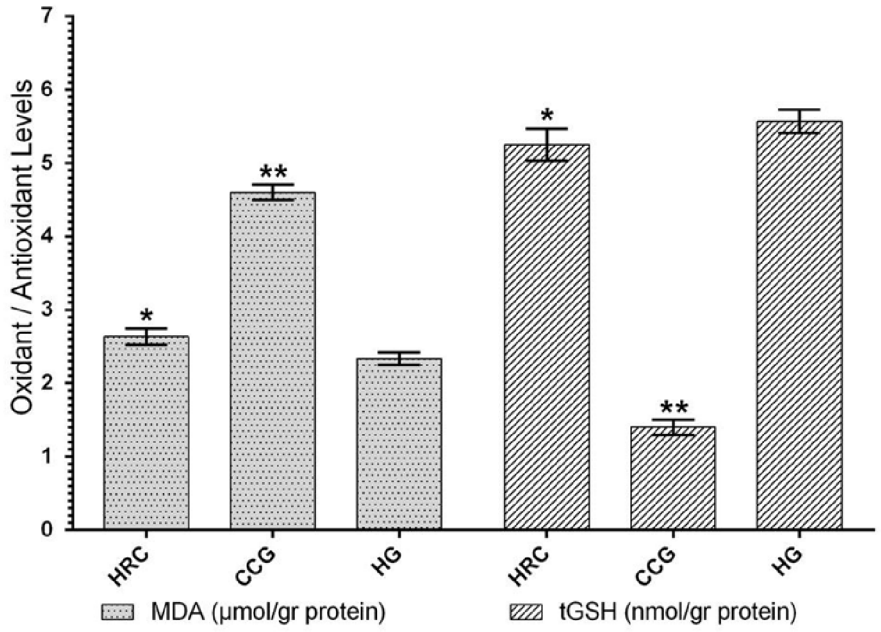

Fig 1: The MDA and tGSH levels in the cochlear tissue of the experimental rat groups.
* $p < 0.001$ according to the CCG group; $p > 0.05$ according to the HG group; **$p < 0.001$ according to the HG group.

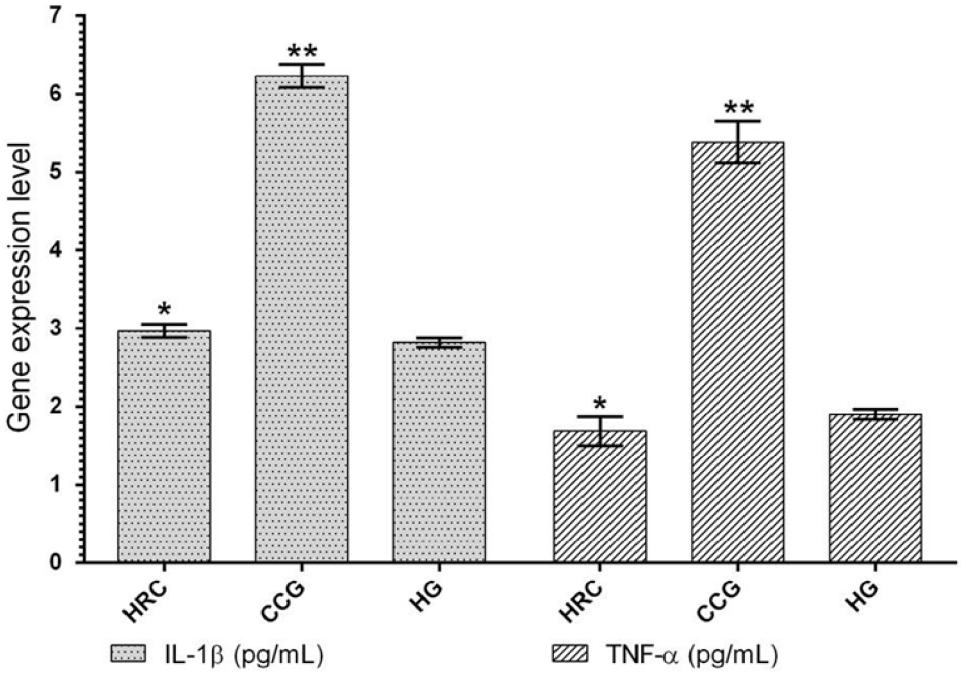

Fig 2: The IL-1β and TNF-α gene expression levels in the cochlear tissue of the experimental rat groups.
* $p < 0.001$ according to the HRC group; $p > 0.05$ according to the HG group; ** $p < 0.001$ according to the HG group.

RESULTS AND DISCUSSION

Biochemical results

MDA and tGSH levels: The MDA amount was 4.6 ± 0.3 nmol/ml ($p < 0.001$) in the cochlear tissue of the CCG group; however, these values were only 2.3 ± 0.2 nmol/ml and 2.6 ± 0.3 nmol/ml in the HG and HRC groups, respectively (Fig 1). Additionally, tGSH levels were significantly lower (1.4 ± 0.3 mg/l, $p < 0.001$) in the group that received cisplatin. However, no significant difference was observed in tGSH amounts between the HG (5.7 ± 0.4 mg/l) and HRC (5.2 ± 0.5 mg/l) groups (Fig 1).

IL-1β and TNF-α gene expression: Cisplatin increased IL-1β gene expression in the cochlear tissue by 6.2 ± 0.4, whereas this value was 3.0 ± 0.2 ($p < 0.001$) in animals given HRE. This indicates that the level of IL-1α gene expression was almost identical between the HRC and HG (2.8 ± 0.1) groups. Whereas TNF-α gene expression was elevated with cisplatin (5.4 ± 0.7, $p < 0.001$), this value was 1.9 ± 0.3 and 1.7 ± 0.5 in the HG and HRC groups, respectively (Fig 2).

Histopathological results: Histopathological appearance was normal in the organ of Corti (Fig 3a), the intermediary layer and the lamina propria of the stria vascularis and lamina propria (Fig 3b), Reissner's membrane (Fig 3c) and the spiral ganglion (Fig 3d) in the inner ear tissue of the HG.

Marked destruction (arrow) was observed in the inner and outer hair cells in the organ of Corti of the CCG group (Fig 4a). There was also damage to the intermediary epithelial layer of the stria vascularis (long arrow) and advanced destruction in the lamina propria (short arrow) of this group (Fig 4b). Additionally, degeneration characterized by swelling (arrow) was observed in the Reissner's membrane of the CCG group (Fig 4c). Cisplatin was also found to cause vacuolization in the ganglion cells (arrow) (Fig 4d).

As seen in Fig 5a, the organ of Corti had a normal appearance (arrow) in the HRC group. Additionally, normal stria vascularis (arrow) and lamina propria (arrow) were observed in the HRC group (Fig 5b). However, a mild degeneration was observed in the cells of Reissner's membrane (arrow) (Fig 5c), whereas the spiral ganglion was found to be healthy (Fig 5d).

In this study, the effect of HRE on cisplatin-induced ototoxicity in Guinea pigs was investigated according to biochemical, gene expression and histopathological aspects. The biochemical results of this experiment demonstrate that MDA levels were higher and GSH levels were lower in the cochlear tissue of animals administered cisplatin than in the HG and HRC groups. MDA is known to be an oxidant andtGSH an antioxidant parameter (Kotb *et al*. 2016; Sangha and Kalra 2016).

The oxidant/antioxidant balance is maintained with the superiority of antioxidants under physiological conditions. Cisplatin is known to increase the production of oxidants in tissues and inhibit the production of antioxidants

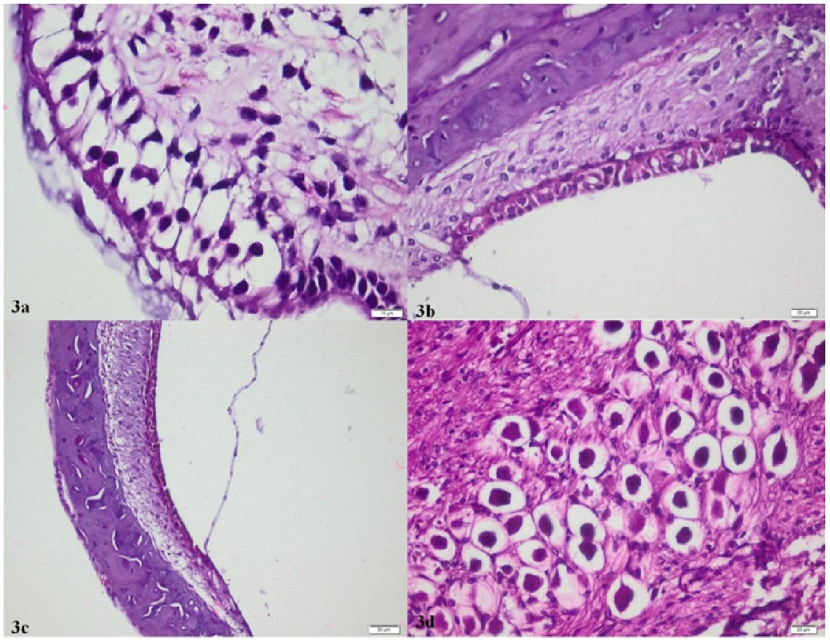

Fig 3; 3a: Normal histopathological view of the organ of Corti containing inner and outer hair cells in the healthy group (HG) (H&Ex100). **3b:** Intermediary epithelial layer of normal stria vascularis and lamina propria in the HG group (H&Ex40). **3c:** Normal histopathological view of Reissner's membrane in the HG group (H&Ex20). **3d:** Normal histopathological view of spiral ganglion in the HG group (H&Ex40).

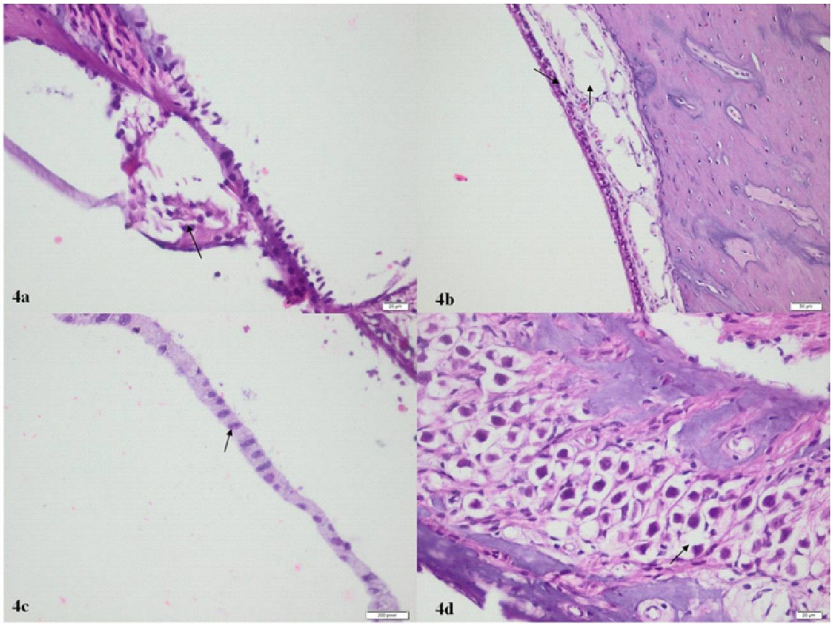

Fig 4; 4a: Destruction in the inner and outer hair cells of the organ of Corti in the cisplatin control group (CCG) (H&Ex40). **4b:** Damage in the intermediary epithelial layer of the stria vascularis and advanced destruction in the lamina propria in the CCG group (H&Ex40). **4c:** Degeneration characterized by swelling in Reissner's membrane in the CCG group (H&Ex40). **4d:** Vacuolization in the cells of the spiral ganglion in the CCG group (H&Ex40).

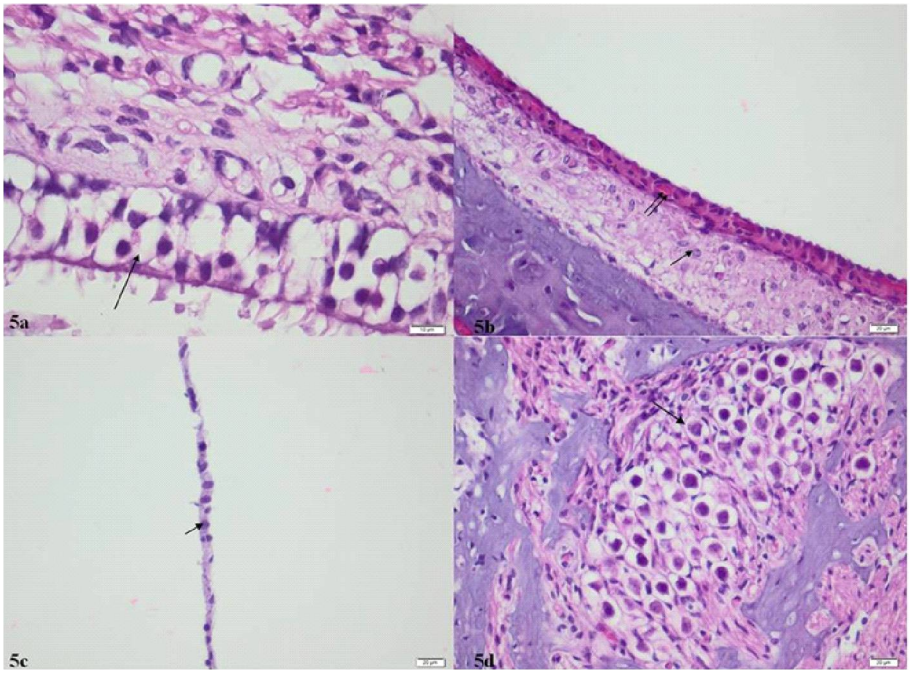

Fig 5; 5a: Normal histopathological view of the organ of Corti containing inner and outer hair cells in the HRC group (H&Ex10). **5b:** Normal intermediary epithelial layer of stria vascularis and lamina propria at the lower part in the HRC group (H&Ex40). **5c:** Mild degeneration in the cells of Reissner's membrane in the HRC group (H&Ex40). **5d:** Normal histopathological view of the spiral ganglion in the HRC group (black arrow) (H&Ex40).

(Teranishi and Nakashima 2003) . In a study by Rybak *et al.* (1995) with Guinea pigs, cisplatin increased the amount of MDA and decreased the amount of tGSH in the cochlea (Rybak *et al.* 1995) .These findings support the results of our study. Again, in the present study, the gene expression of proinflammatory cytokines, such as IL-1β and TNF-α, was also higher in the CCG group, with high MDA and low tGSH amounts observed. In the literature, expression of IL-1β and TNF-α has been shown to play a role in the ototoxicity of cisplatin (So *et al.* 2007). In our study, increased levels of IL-1β and TNF α gene expression in the CCG group are consistent with the findings of the literature.

HRE, which was administered against cisplatin-induced ototoxicity, prevented the increase in MDA and the decrease of tGSH in the cochlear tissue of Guinea pigs. No study has been found regarding the effects of HRE on oxidants/antioxidants and cytokines in cochlear tissue. However, HRE contains carotenoids (α, β, γ) riboflavins, vitamin C, tocopherol, tocotrienol, folic acid, tannin and fatty acids, which feature antioxidant, anti-inflammatory, antiulcer and proinflammatory cytokine antagonist properties (Kwon *et al.* 2011; Yilmaz *et al.* 2014) . Liu *et al.* (2015) demonstrated that *H. rhamnoides* extract both produces antioxidant effects and decreases the production of IL-1β and TNF-α (Liu *et al.* 2015). Numerous studies have suggested that palmitic, oleic, stearic and myristic acid produce anti-inflammatory effects by the inhibition of IL-1β and TNF- α (Hua *et al.* 2006).

Histopathologically, the HRC group had lower MDA, IL-1β and TNF-α levels and higher tGSH levels. However, a mild degeneration was observed in cells of the Reissner's membrane in this group. Cisplatin is known for causing damage in the organ of Corti and inner and outer cells of the organ of Corti (Laurell and Bagger-Sjöbäck 1991). Studies concerning cisplatin have also reported that this drug causes damage in the stria vascularis, Reissner's membrane and spiral ganglion cells (Laurell and Bagger-Sjöbäck 1991; van Ruijven *et al.* 2004; van Ruijven *et al.* 2005).

CONCLUSION

In the present study, we performed biochemical, gene expression and histopathological examinations to demonstrate the damage caused by cisplatin within the inner ear tissue. Additionally, HRE prevented this cisplatin-induced oxidative damage. The beneficial effects of HRE against cisplatin toxicity might be the result of its antioxidant vitamins, fatty acids and other chemical contents. This information suggests that the use of HRE may be beneficial against cisplatin-induced oxidative ototoxicity.

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