The European Research Journal 2026;12(1):119-128 Original Article

DOI: https://doi.org/10.18621/eurj.1803088 *Otorhinolaryngology, Head and Neck Surgery*

In Vivo Evaluation of [Gly14]-Humanin's Protective Effect Against Cisplatin-Induced Ototoxicity

Murat Yaşar[1], Sedat Gökmen[2], Fatma Atalay[1], İrfan Çınar[3], Yusuf Aydin[1], Musa Tatar[4]

[1]Department of Otorhinolaryngology, Head and Neck Surgery, Faculty of Medicine, Kastamonu University, Kastamonu, Turkiye; [2]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Kastamonu University, Kastamonu, Türkiye; [3]Department of Pharmacology, Faculty of Medicine, Kastamonu University, Kastamonu, Türkiye; [4]Department of Histology and Embryology, Faculty of Veterinary Medicine, Kastamonu University, Kastamonu, Türkiye

ABSTRACT

Objectives: This study aimed to evaluate in vivo the potential protective effects of [Gly14]-humanin against cisplatin-induced ototoxicity (CIO) and its modulatory effects against oxidative stress, inflammatory, and apoptotic markers

Methods: Thirty-five male Balb/c mice were randomly divided into five groups: control, cisplatin, cisplatin + [Gly14]-humanin (3 mg/kg), cisplatin + (6 mg/kg), and vitamin E. Cisplatin (10 mg/kg, b.w.) was administered as a single dose. [Gly14]-humanin was administered intraperitoneally for 14 days. Biochemical analyses (Superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA)), gene expression levels (TNF-α, IL-1β, IL-6, CASP-3, CASP-9, Bcl-2/Bax), and histopathological examinations (PARP1, PARP2 immunoreactivity) were performed in cochlear tissue samples to evaluate oxidative stress, inflammation, and apoptosis.

Results: Cisplatin decreased SOD and GSH levels and increased MDA levels in the cochlear tissue ($P<0.05$). With the application of [Gly14]-humanin, these parameters approached their normal levels. Cisplatin increased the values of pro-inflammatory and pro-apoptotic markers (TNF-α, IL-1β, IL-6, Caspase-3 (CAS-3), Caspase-9 (CAS-9)) significantly while reducing the Bcl-2/Bax ratio ($P<0.05$). [Gly14]-humanin administration reversed these effects in a dose-dependent manner. Histological analysis also revealed decreased PARP1 and PARP2 immunoreactivity in [Gly14]-humanin treated groups, comparable to the positive control (vitamin E).

Conclusions: The findings of this study show that [Gly14]-humanin exhibits important protective effects against CIO by attenuating oxidative stress, inflammation, and apoptosis in cochlear tissues. Further studies evaluating the clinical efficacy and reliability of [Gly14]-humanin are now needed.

Keywords: [Gly14]-Humanin, Cisplatin, Ototoxicity, Oxidative Stress, Cochlear Protection

Cisplatin is a widely used anticancer drug, particularly in the treatment of solid tumors such as cancers of the testis, lung, ovary, and head and neck [1, 2]. However, it also causes severe side effects, such as ototoxicity, nephrotoxicity, neurotoxicity, and myelosuppression. Permanent

Received: October 15, 2025 **Accepted:** November 24, 2025 **Available Online:** December 9, 2025 **Published:** January 4, 2026

How to cite this article: Yaşar M, Gökmen S, Atalay F, Çınar İ, Aydin Y, Tatar M.In Vivo Evaluation of [Gly14]-Humanin's Protective Effect Against Cisplatin-Induced Ototoxicity. Eur Res J. 2026;12(1):119-128. doi: 10.18621/eurj.1803088

Corresponding author: Yusuf Aydın, MD., Assist. Prof., Phone: +90 366 280 10 00, E-mail: yusufaydin@kastamonu.edu.tr

Available Online at https://dergipark.org.tr/en/pub/eurj

hearing loss develops in a significant proportion of patients treated with cisplatin (40-80% of adults and at least 40% of children). Hearing loss in cisplatin-induced ototoxicity (CIO) is typically bilateral, sensorineural, progressive, irreversible, and frequently accompanied by tinnitus and vertigo [1, 3, 4].

There are several mechanisms under the CIO. Studies have suggested that CIO may derive from several mechanisms, including oxidative stress, DNA damage, inflammation, apoptosis, and ferroptosis [5-8]. Another important mechanism is thought to be the accumulation of reactive oxygen species (ROS) induced by cisplatin [1, 2, 5, 9]. Excessive intracellular ROS accumulation can trigger cell death by damaging the cellular structure [10, 11]. Cisplatin's inhibition of antioxidant enzyme activity can result in an increase in ROS inside the cochlea. This causes apoptosis of hair cells and spiral ganglion neurons, thus resulting in sensorineural hearing loss [12]. Cisplatin is reported to exhibit an ototoxic effect not only by damaging hair cells in the organ of Corti, but also spiral ganglion neurons, support cells, and the stria vascularis [13].

Humanin is a 24-amino acid peptide synthesized in association with mitochondrial translocation. It is endogenous in origin and biologically active. It was first identified for its protective effects against neurodegenerative processes associated with Alzheimer's disease [14-17]. Humanin and its synthetic derivatives exhibit antiapoptotic, anti-inflammatory, and antioxidant activities against oxidative stress and hypoxic conditions [18-21]. [Gly14]-humanin is one humanin derivative. While the serine amino acid is present in position 14 in the humanin peptide chain, the amino acid glycine is present in [Gly14]-humanin. Research has identified [Gly14]-humanin as a potent humanin analog, particularly noteworthy for its neuroprotective effects [22, 23].

This study aimed to evaluate in vivo the potential protective effects of [Gly14]-humanin against CIO and its modulatory effects against oxidative stress, inflammatory, and apoptotic markers.

METHODS

Chemicals and Reagents

[Gly14]-humanin (99.86% purity), cisplatin (CAS number: 15663-27-1), and all other reagents were purchased from MedChemExpress. Superoxide dismutase (SOD) (Sunred, Cat: 201-02-0291), glutathione (GSH) (Sunred, Cat; 201-02-180), and malondialdehyde (MDA) (Sunred, Cat; 201-02-0626) were purchased from SunRed Biotechnology Company (SRB) Ltd. (Shanghai, China).

Animals

This study was conducted with 35 male Balb/c mice (6–8 weeks old, weighing 20–25 g) obtained from the Kastamonu University Experimental Animals Application and Research Center, Kastamonu. These were housed in standard cages under well-regulated conditions (relative humidity range: 45±5%, temperature: 24±1°C, in a 12-h light/12-h dark cycle). The animals were maintained on a standard pellet diet and allowed ad libitum access to water during the experimental period. All animal procedures described in this study were approved by the Animal Care and Use Committee at Kastamonu University, and all the methods used during the animal experiments were in accordance with the guidelines approved by the Animal Ethics Committee (25.10.2024/11/39).

Experimental Design

The animals were randomly divided into the following five groups:

I. Control group: The healthy control group did not receive any drug administration (n=7).

II. Cisplatin group: A single dose of cisplatin (10 mg/kg, b.w.) was injected intraperitoneally into the mice on the 10th day [24] (n=7).

III. Cisplatin + [Gly14]-humanin group: A single dose of cisplatin (10 mg/kg, b.w.) on the 10th day of the treatment schedule, along with daily administration of [Gly14]-humanin (3 mg/kg b.w./day) for 14 days (n=7).

IV. Cisplatin + [Gly14]-humanin group: A single dose of cisplatin (10 mg/kg, b.w.) on the 10th day of the treatment schedule, along with daily administration of [Gly14]-humanin (6 mg/kg b.w./day) for 14 days (n=7).

V. Vitamin E group: Vitamin E (100 mg/kg b.w./day) was administered by gavage to the mice orally for 14 days (n=7). A table (1) showing the dose schedule of the experiment is presented as a timeline.

TABLE 1. The Dose Schedule of the Experiment is Presented as a Timeline

Experimental day	Control group (0.5 mL)	Cisplatin group (10 mg/kg, b.w.)	[Gly-14] humanin group (3 mg/kg)	[Gly-14] humanin group (6 mg/kg)	Vitamin E group (100 mg/kg)
Day 1-9	Saline only	Saline only	Daily [Gly-14] humanin	Daily [Gly-14] humanin	Daily Vitamin E
Day 10	Saline only	Cisplatin injection	Daily [Gly-14] humanin +Cisplatin injection	Daily [Gly-14] humanin +Cisplatin injection	Daily Vitamin E +Cisplatin injection
Day 11-14	Saline only	Observation	Daily [Gly-14] humanin	Daily [Gly-14] humanin	Daily Vitamin E
Day 15	All animals in all groups were sacrificed under high-dose anesthesia				

In order to evaluate the preventive effects of [Gly14]-humanin against CIO, doses of 3 and 6 mg/kg were selected in the present investigation based on previous research. Vitamin E (100 mg/kg) was also used as a positive control [25].

At the end of the experimental period, 24 h after receiving the last treatment, the cochlea and vestibular system were collected from all the experimental group mice under deep anesthesia. All mice were sacrificed by removing blood from the left ventricle under deep anesthesia with ketamine (100 mg/kg) + xylazine (10 mg/kg). Animals under deep anesthesia were euthanized by cervical dislocation. The cochlea and vestibular system were used for biochemical, molecular, and histopathological analysis.

Biochemical Analysis

The ELISA method was employed for measuring SOD activity and GSH and MDA levels in cochlear tissue samples, using a previously described method. Tissues were homogenized by adding nine times their weight in iced phosphate-buffered saline (PBS; 0.01 M, pH=7.4) solution for the analysis of tissue samples. Centrifugation was performed at 5000 g for 5 minutes, and the resulting supernatant was used for analysis. Commercial kits (Elabscience, Wuhan, China) were used for the measurement of GSH, SOD, and MDA levels. GSH, MDA, and SOD levels were measured using a microtiter plate ELISA reader (Epoch Microplate Spectrophotometer, BioTek, USA).

Gene Expression Analysis TNF-α, IL-1β, IL-6, Caspase-3 (CAS-3), Caspase-9 (CAS-9), Bax, and Bcl-2 mRNA expression levels in tissue samples were determined by the real-time PCR method.

RNA Extraction

The tissue samples were homogenized in Tissue Lyser II (Qiagen) in the presence of liquid nitrogen. Total RNA was isolated from the cochlea and vestibular system of the experimental and control groups with a QIAcube Connect Qiagen kit for RNA isolation according to the manufacturer's instructions, and the recovery was estimated.

Reverse Transcriptase Reaction and cDNA Synthesis: cDNA was reverse transcribed from total RNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems), following the manufacturer's protocol. The recovery of cDNA was then determined using nanodrop spectrophotometry (EPOCH Take3 Plate, BioTek) before storage at -20 °C.

Real-Time Quantitative PCR

Total RNA extraction and cDNA synthesis were performed according to the methods described in our previous studies [26, 27].

According to the manufacturer's instructions, and TNF-α (Mm00443258_m1), IL-1β (Mm00434228_m1), IL-6 (Mm00446190_m1), Bcl (Mm00477631_m1), Bax

(Mm00432051_m1), Caspase-3 (Mm01195085_m1), Caspase-9 (Mm00516563_m1), and β-actin (Mm02619580_g1) mRNA expression as a housekeeping gene were analyzed. Compared with the control group, all data were expressed as fold change in expression compared with the cell groups using the 2-ΔΔCt method. Differences between the groups in terms of oxidative stress, inflammation, and apoptosis were evaluated.

Immunohistochemistry Procedure

Serial sections 5-µm in thickness from the prepared paraffin blocks were placed onto adhesive slides. The streptavidin-biotin complex (sABC) staining method was then applied to determine PARP1 and PARP2 reactivity in the sections. Antigen retrieval was performed once the sections had been deparaffinized in xylol and dehydrated by passage through alcohol series. For that purpose, the slides were placed into a 10-fold diluted citrate buffer (citrate buffer heat-induced epitope retrieval pH: 6) solution and heated in a microwave oven at 800 Watts for 20 min. Following this process, the sections were left to cool for 30 min. After washing in phosphate-buffered saline (PBS) solution for 3×5 min, they were next incubated in 3% hydrogen peroxide (hydrogen peroxide 30% Merck: 108597) solution in the dark for 20 min for the purpose of blocking endogenous peroxidase activity [28]. At the end of the incubation procedure, the sections were rewashed with PBS solution for 3×5 min. In order to prevent nonspecific antibody binding, Ultra V Block (TP-125-HL, Thermo Fisher Scientific) solution was dropped onto the tissues, left for 10 min. Then, they were incubated at +4 °C overnight at appropriate concentrations of primary antibodies [anti-PARP-1 (1:200; catalogue no. 13371–1-AP, Proteintech) and anti-PARP-2 (1:200; catalogue no. 20555-1-AP, Proteintech)] without washing. Following incubation, the sections were washed with PBS for 3×5 min. Secondary antibody kits (TP-125-HL, Thermo Fisher Scientific) were then applied in line with the procedure order and duration, after which AEC chromogen (TA-060-HA, Thermo Fisher Scientific) was applied to show the reaction and render the immunoreactivity visible. A water-compatible covering medium (TA-125-UG, Thermo Fisher Scientific) was applied to the sections counterstained with Gill's hematoxylin were and examined under a microscope. In the negative controls, PBS replaced the primary antibody after protein blocking, and the tissues were incubated with that solution overnight [29].

Statistical Analysis

Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by the Tukey test and with GraphPad Prism 9 software (GraphPad Software, Inc.). P values <0.05 were considered statistically significant [30].

RESULTS

Compared to the control group, SOD activity

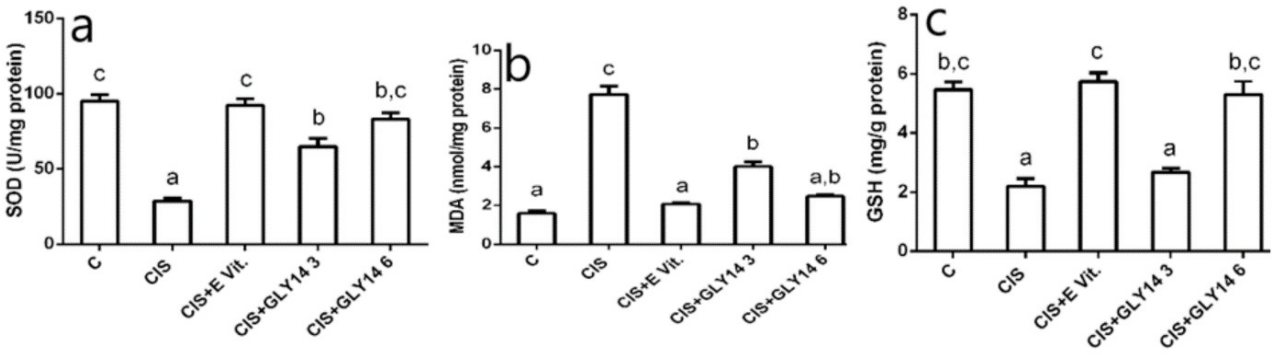

FIGURE 1. Figure 1. Effect of [Gly14]-humanin on antioxidant marker levels in CIS-induced ototoxicity. C, Control; CIS, Cisplatin; GLY 3, [Gly14]-humanin 3 mg/kg; GLY 6, [Gly14]-humanin 6 mg/kg; E Vit, Vitamin E.
Different letters (a, b, c, d, e) in the same column represent a statistically significant difference (P<0.05).

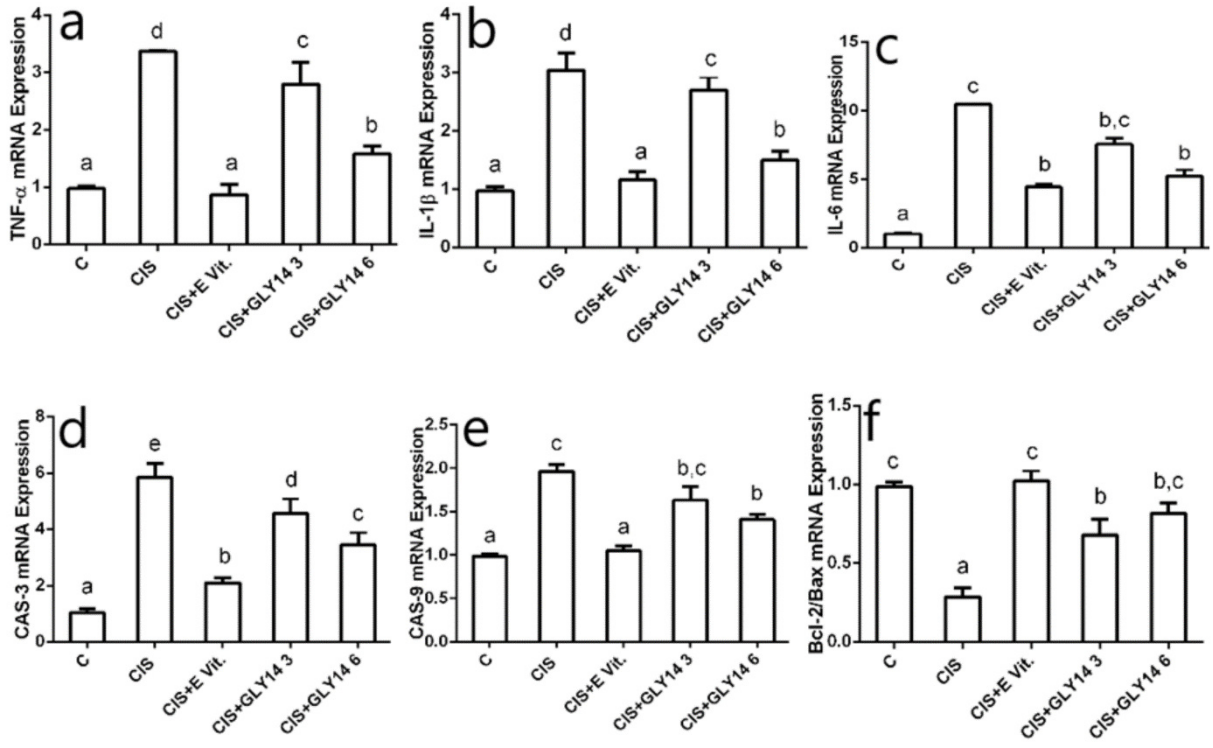

FIGURE 2. The effect of [Gly14]-humanin on inflammation and apoptotic markers mRNA level in CIS-induced ototoxicity. C, Control; CIS, Cisplatin; GLY 3, [Gly14]-humanin 3 mg/kg; GLY 6, [Gly14]-humanin 6 mg/kg; E Vit, Vitamin E. Different letters (a, b, c, d) in the same column represent a statistically significant difference (P<0.05).

(28.2±9.4) and GSH (28.2±9.4) levels were decreased (P<0.001), while MDA (7.64±1.1) levels were increased (P<0.001) with CIS application in the cochlear tissue. With the application of [Gly14]-humanin, these parameters approached their normal levels (Figure 1). Similar findings were found in brain tissue analyses of mice. Decreased SOD activity and GSH levels, and increased MDA levels with CIS application were regulated by [Gly14]-humanin application.

In comparison to the control group, we found significant increases (P<0.001) in pro-apoptotic markers, CAS-3 (5.81±0.7) (P<0.001), inflammatory markers TNF-α (3.3±0.2) (P<0.05), IL-1β (3.2±0.3) (P=0.035), and IL-6 (10.3±0.8) (P<0.001) after cisplatin challenge. In addition, significant reductions (P<0.05) in pro-apoptotic markers, CAS-9 (1.9±0.1) (P=0.035) gene expression and Bcl-2/Bax ratio (0.2±0.1) (P<0.001) after cisplatin application were observed. Moreover, our results revealed that [Gly14]-humanin expecially high dose treatment significantly decreased CAS-3 (3.4±0.7) (P<0.002) gene expression, CAS-9 (1.3±0.5) (P<0.04), TNF-α (1.5±0.3) (P<0.001), IL-1β (1.5±0.3) (P<0.001), and IL-6 (5.2±0.4) (P<0.001), and increased Bcl-2/Bax ratio (0.8±0.1) (P<0.04) gene expression (Figure 2).

TNF-α, IL-1β, IL-6, CAS-3, and CAS-9 values increased significantly with CIS application to mice, while Bcl-2/BAX values were significantly decreased compared to control. These values were normalized depending on the dose (especially [Gly14]-humanin 6 mg/kg) with the administration of [Gly14]-humanin (Figure 2).

In cis groups, both PARP1 and PARP2 exhibited intense immunoreactivity. It was determined that PARP1 immunoreactivity decreased at a similar rate in the [Gly14]-humanin 6 mg/kg and Vitamin E groups. It was determined that PARP2 immunoreactivity decreased similarly in the [Gly14]-humanin 6 mg/kg and Vitamin E. (Figure 3).

Both PARP1 and PARP2 exhibited intense immunoreactivity in the cisplatin group compared to the control groups. Immunohistochemical analysis showed that increased expression of PARP1 in tissue in the cisplatin group tended to decrease with the application of [Gly14]-humanin and Vitamin E (Figure

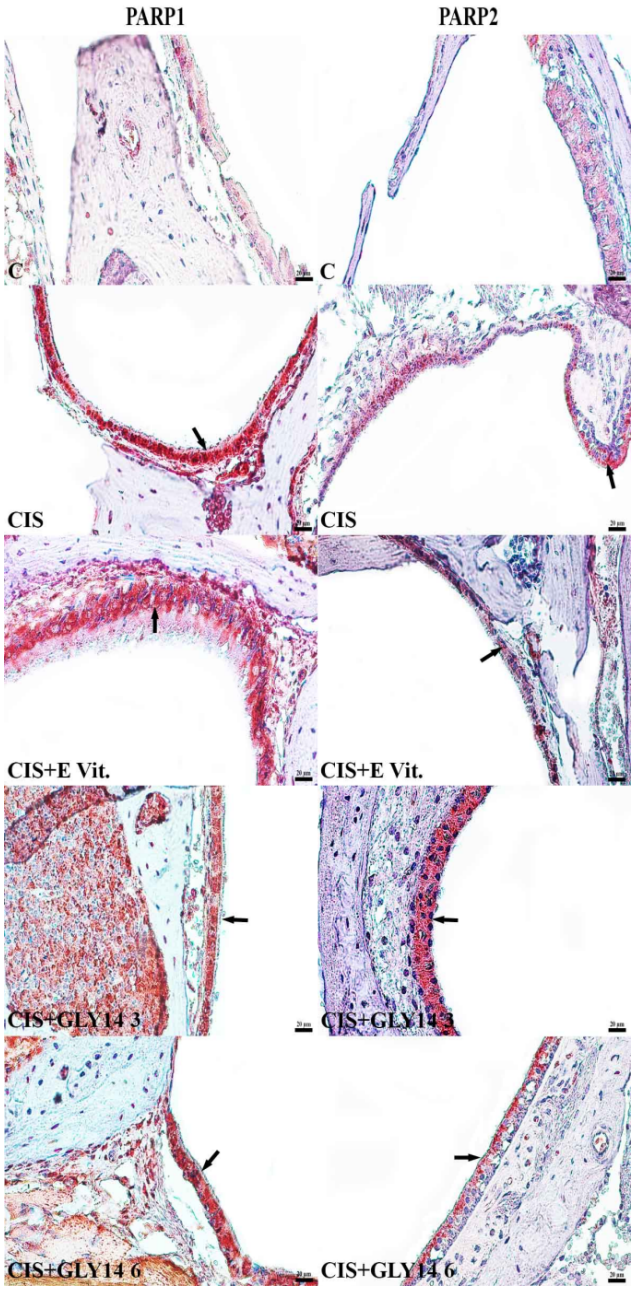

FIGURE 3. Immunohistochemical staining of PARP1 and PARP2 in all groups. Positive-stained cells are shown by arrows. Strept-ABC-IHC, AEC, paraffin section. Scale bar: 20 µm for all panels (C, Control; CIS, Cisplatin; GLY 3, [Gly14]-humanin 3 mg/kg; GLY 6, [Gly14]-humanin 6 mg/kg; E Vit, Vitamin E; PARP, Poly (ADP-ribose) polymerase).

4A). Additionally, the [Gly14]-humanin 6 mg/kg group and Vitamin E group were similar in terms of healing, and less immunoreactivity was observed compared to [Gly14]-humanin 3 mg/kg. Immunohistochemically, the increased expression of PARP1 in the cisplatin group tended to decrease with the application of [Gly14]-humanin and vitamin E (Figure 4B). The increased PARP2 expression with cisplatin administration also decreased with the application of [Gly14]-humanin and vitamin E. In addition, while [Gly14]-humanin 6 mg/kg and vitamin E applications were statistically similar, the vitamin E group was similar to the control group.

DISCUSSION

Cisplatin can produce an ototoxic effect through complex pathophysiological mechanisms. This ototoxic effect largely involves cellular processes such as excessive ROS production in cochlear tissue, oxidative stress, inflammatory responses, and apoptosis [1, 2, 8, 9]. The purpose of this study was to investigate the potential protective effects of [Gly14]-humanin against CIO. The findings showed that [Gly14]-humanin significantly reduces oxidative stress, inflammation, and apoptosis induced by cisplatin, and thus exhibits a protective effect in cochlear tissue. These results suggest that [Gly14]-humanin may exhibit a potential therapeutic effect against CIO.

Cisplatin induces mitochondrial dysfunction in cochlear hair cells and supporting cells, and leads to ROS accumulation by inhibiting antioxidant enzymatic systems. This causes a severe oxidative stress response characterized by a decrease in SOD activity, depletion of GSH levels, and an increase in lipid peroxidation products such as MDA [10-12]. In agreement with the current literature, cisplatin administration in the present study increased MDA levels while decreasing those of SOD and GSH. This confirms that cisplatin triggers a potent oxidative stress response [2, 5, 9]. In this study, we observed that these parameters (SOD, GSH, and MDA) approached their normal levels with [Gly14]-humanin application. The fact that [Gly14]-humanin application restored these oxidative stress markers to close to normal levels shows that this peptide possesses powerful antioxidant properties. This is consistent with previous studies showing that humanin and derivatives thereof exhibit the ability to lower oxidative stress [23, 31, 32]. In particular, a recent study by Liu *et al*. showed that [Gly14]-humanin improved oligoasthenozospermia by

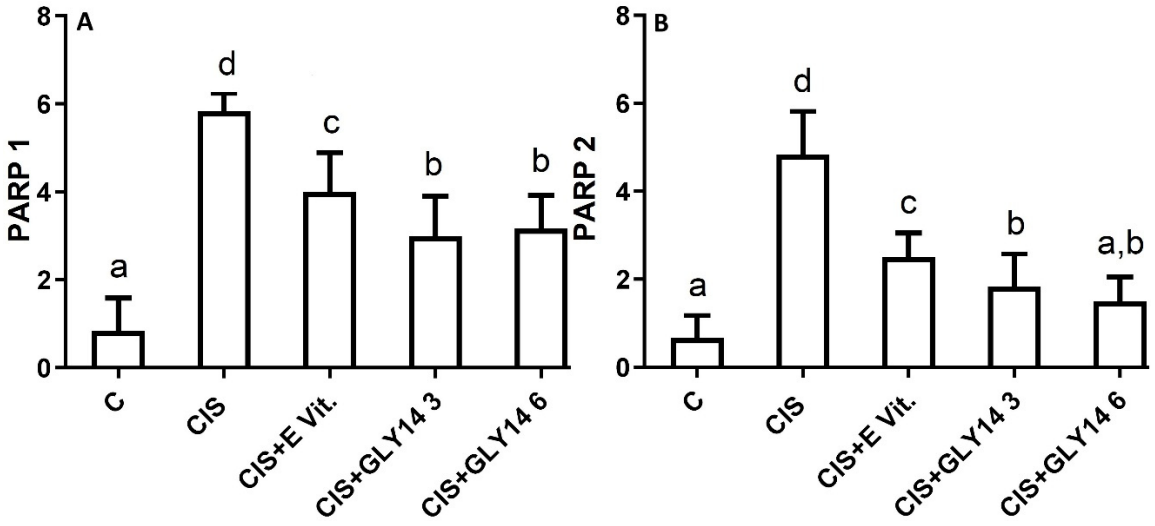

FIGURE 4. Statistical graphical representation of PARP1 (A) and PARP2 (B) IHC scores of inner ear slices. C, Control; CIS, Cisplatin; GLY 3, [Gly14]-humanin 3 mg/kg; GLY 6, [Gly14]-humanin 6 mg/kg; E Vit, Vitamin E; IHC, Immunohistochemistry. Different letters (a, b, c, d, e) in the same column represent a statistically significant difference (P<0.05).

reducing oxidative stress [23]. This suggests that [Gly14]-humanin possesses antioxidant properties at differing doses and in different toxicity models.

Oxidative stress triggers the release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, and initiates an inflammatory cascade by activating transcription factors, including nuclear factor kappa B. In addition, cisplatin-induced oxidative stress and inflammation activate caspase-dependent and –independent apoptotic pathways, thus leading to the programmed death of cochlear hair cells [2, 8].

In our study, significant increases were found in pro-apoptotic markers CAS-3 (P<0.05), inflammatory markers TNF-α (P<0.05), IL-1β (P<0.05) and IL-6 (P<0.05) after cisplatin administration compared to the control group. In addition, significant decreases (P<0.05) were observed following cisplatin administration in anti-apoptotic markers, in CAS-9 (P<0.05) gene expression, and in the Bcl-2/Bax ratio (P<0.05). These values normalized with the application of [Gly14]-humanin, particularly [Gly14]-humanin 6 mg/kg, in a dose-dependent manner. These findings clearly show that cisplatin triggers programmed cell death and an inflammatory response in cochlear cells. The fact that [Gly14]-humanin treatment significantly lowered these inflammatory and apoptotic markers and increased the Bcl-2/Bax ratio shows that it exhibits anti-inflammatory and anti-apoptotic effects. Previous studies have also reported that humanin and its analogs possess anti-apoptotic and anti-inflammatory characteristics [19, 21]. In particular. Jin *et al*. reported that [Gly14]-humanin prevented cell death by obstructing mitochondrial dysfunction [20]. This suggests that GLY14 may exhibit a protective effect by directly targeting cellular damage derived from cisplatin treatment. Apart from its anti-inflammatory and antioxidant properties, [Gly14]-humanin may also have cytoprotective effects by regulating mitochondrial homeostasis and triggering pro-survival pathways like PI3K/Akt signaling and STAT3, which are recognized downstream targets of humanin analogs. The observed decrease in oxidative stress and cochlear apoptosis in our study could be attributed to these pathways.

PARP is an important enzyme in the DNA damage response and cell death. Its activation is known to play an important role in cisplatin-induced cell damage [24]. Histopathological investigation in the present study revealed intense PARP1 and PARP2 immunoreactivity in the cisplatin group, while the application of GLY-14 and vitamin E reduced PARP1 and PARP2 expression. This suggests that [Gly14]-humanin and vitamin E may have the potential to reduce DNA damage and resulting cell death. In particular, the fact that the GLY-14 6 group exhibited

similar healing outcomes to the group in which vitamin E, a known antioxidant and otoprotective agent, was applied suggests that [Gly14]-humanin may be as effective as vitamin E [25].

To the best of our knowledge, this is the first experimental animal study investigating the effect of GLY14 on ototoxicity. Further clinical research evaluating hearing functions and involving long-term follow-up is now needed to confirm these effects in humans.

Strengths and Limitations

One strength of this study is that it compared the effects of two distinct [Gly14]-humanin dosages, 3 mg/kg and 6 mg/kg. The 6 mg/kg dose normalized oxidative stress and inflammatory parameters more effectively than the 3 mg/kg dose. This dose-dependent effect supports the idea of a potential therapeutic role of [Gly14]-humanin. Another strength of this study is the inclusion of vitamin E, with known antioxidant and otoprotective effects, as a positive control. This allowed us to compare the effectiveness of [Gly14]-humanin and vitamin E. That comparison may yield important data regarding the efficacy of [Gly14]-humanin. Further studies including molecular level analyses and different routes of administration (such as intratympanic injection) are now needed to fully elucidate the effect mechanism of [Gly14]-humanin.

However, there are also a number of limitations to this study. The study used waste materials from the project investigating the neuroprotective effect of [Gly14]-humanin on cisplatin-induced neurotoxicity in mice. Therefore, auditory function testing (ABR/DPOAE) could not be performed, and thus, the hearing functions of the animals before and after the experiment could not be compared. In addition, due to the brief follow-up period, this study does not provide information concerning the long-term effects of CIO or the protective potential of [Gly14]-humanin against those long-term effects.

CONCLUSION

The findings of this study show that [Gly14]-humanin exhibits important protective effects against CIO. It may protect cochlear tissue against damage by reducing oxidative stress, inflammation, and apoptosis induced by cisplatin. [Gly14]-humanin may exhibit more pronounced healing at 6 mg/kg. Our findings show that [Gly14]-humanin exhibits similar effects to vitamin E, a known antioxidant. Further studies evaluating the clinical efficacy and reliability of [Gly14]-humanin are now needed.

Ethics Approval and Consent to Participate

This study was approved by the the Kastamonu University Animal Experiments Local Ethics Committee (Decision No: 2024/11-39; date: 25.10.2024). All experimental procedures involving animals were conducted in accordance with the ethical standards of the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: MY, SG, İÇ, MT; Study Design: MY, YA; Supervision: MY, FA, SG; Funding: MY; Materials: MY, SG, İÇ, MT; Data Collection and/or Processing: SG, İÇ, MT; Statistical Analysis and/or Data Interpretation: MY, SG, İÇ; Literature Review: MY, SG, İÇ, FA, YA, MT; Manuscript Preparation: MY, FA, YA; and Critical Review: MY, FA, YA.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author(s) disclosed that they did not receive any grant during the conduction or writing of this study.

Acknowledgments

The authors have no acknowledgments to declare.

Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

Editor's Note

All statements made in this article are solely those of the authors and do not represent the views of their affiliates or the publisher, editors, or reviewers. Any claims made by any product or manufacturer that may be evaluated in this article are not guaranteed or endorsed by the publisher.

REFERENCES

1. Tang Q, Wang X, Jin H, et al. Cisplatin-induced ototoxicity: Updates on molecular mechanisms and otoprotective strategies. Eur J Pharm Biopharm. 2021;163:60-71. doi: 10.1016/j.ejpb.2021.03.008.
2. Rybak LP, Whitworth CA, Mukherjea D, Ramkumar V. Mechanisms of cisplatin-induced ototoxicity and prevention. Hear Res. 2007;226(1-2):157-67. doi: 10.1016/j.heares.2006.09.015.
3. Nagy JL, Adelstein DJ, Newman CW, Rybicki LA, Rice TW, Lavertu P. Cisplatin ototoxicity: the importance of baseline audiometry. Am J Clin Oncol. 1999;22(3):305-308. doi: 10.1097/00000421-199906000-00020.
4. Dai D, Chen C, Lu C, Guo Y, Li Q, Sun C. Apoptosis, autophagy, ferroptosis, and pyroptosis in cisplatin-induced ototoxicity and protective agents. Front Pharmacol. 2024;15:1430469. doi: 10.3389/fphar.2024.1430469.
5. Sheth S, Mukherjea D, Rybak LP, Ramkumar V. Mechanisms of Cisplatin-Induced Ototoxicity and Otoprotection. Front Cell Neurosci. 2017;11:338. doi: 10.3389/fncel.2017.00338.
6. Liu Z, Zhang H, Hong G, et al. Inhibition of Gpx4-mediated ferroptosis alleviates cisplatin-induced hearing loss in C57BL/6 mice. Mol Ther. 2024;32(5):1387-1406. doi: 10.1016/j.ymthe.2024.02.029.
7. Xu B, Li J, Chen X, Kou M. Puerarin attenuates cisplatin-induced apoptosis of hair cells through the mitochondrial apoptotic pathway. Biochim Biophys Acta Mol Cell Res. 2022;1869(4):119208. doi: 10.1016/j.bbamcr.2021.119208.
8. Lu X, Deng T, Dong H, et al. Novel Application of Eupatilin for Effectively Attenuating Cisplatin-Induced Auditory Hair Cell Death via Mitochondrial Apoptosis Pathway. Oxid Med Cell Longev. 2022;2022:1090034. doi: 10.1155/2022/1090034.
9. Ramkumar V, Mukherjea D, Dhukhwa A, Rybak LP. Oxidative Stress and Inflammation Caused by Cisplatin Ototoxicity. Antioxidants (Basel). 2021;10(12):1919. doi: 10.3390/antiox10121919.
10. Finkel T. Signal transduction by reactive oxygen species. J Cell Biol. 2011;194(1):7-15. doi: 10.1083/jcb.201102095.
11. Sies H, Belousov VV, Chandel NS, et al. Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. Nat Rev Mol Cell Biol. 2022;23(7):499-515. doi: 10.1038/s41580-022-00456-z.
12. Gentilin E, Simoni E, Candito M, Cazzador D, Astolfi L. Cisplatin-Induced Ototoxicity: Updates on Molecular Targets. Trends Mol Med. 2019;25(12):1123-1132. doi: 10.1016/j.molmed.2019.08.002.
13. Wang X, Zhou Y, Wang D, et al. Cisplatin-induced ototoxicity: From signaling network to therapeutic targets. Biomed Pharmacother. 2023;157:114045. doi: 10.1016/j.biopha.2022.114045.
14. Kim KH. Intranasal delivery of mitochondrial protein humanin rescues cell death and promotes mitochondrial function in Parkinson's disease. Theranostics. 2023;13(10):3330-3345. doi: 10.7150/thno.84165.
15. Alqahtani SM, Al-Kuraishy HM, Al-Gareeb AI, et al. The neuroprotective role of Humanin in Alzheimer's disease: The molecular effects. Eur J Pharmacol. 2025;998:177510. doi: 10.1016/j.ejphar.2025.177510.
16. Thiankhaw K, Chattipakorn K, Chattipakorn SC, Chattipakorn N. Roles of humanin and derivatives on the pathology of neurodegenerative diseases and cognition. Biochim Biophys Acta Gen Subj. 2022;1866(4):130097. doi: 10.1016/j.bbagen.2022.130097.
17. Karachaliou CE, Livaniou E. Neuroprotective Action of Humanin and Humanin Analogues: Research Findings and Perspectives. Biology (Basel). 2023;12(12):1534. doi: 10.3390/biology12121534.
18. Zhu S, Hu X, Bennett S, Xu J, Mai Y. The Molecular Structure and Role of Humanin in Neural and Skeletal Diseases, and in Tissue Regeneration. Front Cell Dev Biol. 2022;10:823354. doi: 10.3389/fcell.2022.823354.
19. Zapała B, Kaczyński Ł, Kieć-Wilk B, et al. Humanins, the neuroprotective and cytoprotective peptides with antiapoptotic and anti-inflammatory properties. Pharmacol Rep. 2010;62(5):767-777. doi: 10.1016/s1734-1140(10)70337-6.
20. Jin H, Liu T, Wang WX, et al. Protective effects of [Gly14]-Humanin on beta-amyloid-induced PC12 cell death by preventing mitochondrial dysfunction. Neurochem Int. 2010;56(3):417-423. doi: 10.1016/j.neuint.2009.11.015.
21. Hazafa A, Batool A, Ahmad S, et al. Humanin: A mitochondrial-derived peptide in the treatment of apoptosis-related diseases. Life Sci. 2021;264:118679. doi: 10.1016/j.lfs.2020.118679.
22. Li M, Liu Z, Cao X, et al. [Gly14]-Humanin ameliorates high glucose-induced endothelial senescence via SIRT6. Sci Rep. 2024;14(1):30924. doi: 10.1038/s41598-024-81878-x.
23. Liu Y, Feng Q, Zou L, Zhu C, Xia W. Oligoasthenozoospermia is alleviated in a mouse model by [Gly14]-humanin-mediated attenuation of oxidative stress and ferroptosis. Andrology. 2025;13(6):1541-1555. doi:

10.1111/andr.13786.
24. Wang XL, Lin FL, Xu W, Wang C, Wang QQ, Jiang RW. Silybin B exerts protective effect on cisplatin-induced neurotoxicity by alleviating DNA damage and apoptosis. J Ethnopharmacol. 2022;288:114938. doi: 10.1016/j.jep.2021.114938.
25. Aissous I, Benrebai M, Ameddah S, et al. The preventive effects of Centaurea maroccanaBall. extract against oxidative stress induced by cisplatin in mice brains: in vitro and in vivostudies. Drug Chem Toxicol. 2023;46(6):1162-1175. doi: 10.1080/01480545.2022.2139841.
26. Gelen V, Sengul E, Yildirim S, Cinar İ. The role of GRP78/ATF6/IRE1 and caspase-3/Bax/Bcl2 signaling pathways in the protective effects of gallic acid against cadmium-induced liver damage in rats. Iran J Basic Med Sci. 2023;26(11):1326-1333. doi: 10.22038/IJBMS.2023.71343.15525.
27. Çınar İ, Gıdık B, Dirican E. Determination of anti-cancer effects of Nigella sativa seed oil on MCF7 breast and AGS gastric cancer cells. Mol Biol Rep. 2024;51(1):491. doi: 10.1007/s11033-024-09453-1.
28. Tatar M, Tüfekci KK. An investigation of the distributions of ferroptosis and necroptosis mediators in the maternal-fetal interface at different days of rat pregnancy. Anat Histol Embryol. 2024;53(1):e12991. doi: 10.1111/ahe.12991.
29. Tatar M, Tüfekci KK, Uslu S. A determination of the main regulators of necroptosis in testicular tissue under different heat stresses. J Mol Histol. 2025;56(1):74. doi: 10.1007/s10735-024-10350-x.
30. Cinar I, Sirin B, Aydin P, Toktay E, Cadirci E, Halici I, Halici Z. Ameliorative effect of gossypin against acute lung injury in experimental sepsis model of rats. Life Sci. 2019;221:327-334. doi: 10.1016/j.lfs.2019.02.039.
31. Cai H, Liu Y, Men H, Zheng Y. Protective Mechanism of Humanin Against Oxidative Stress in Aging-Related Cardiovascular Diseases. Front Endocrinol (Lausanne). 2021;12:683151. doi: 10.3389/fendo.2021.683151.
32. Klein LE, Cui L, Gong Z, Su K, Muzumdar R. A humanin analog decreases oxidative stress and preserves mitochondrial integrity in cardiac myoblasts. Biochem Biophys Res Commun. 2013;440(2):197-203. doi: 10.1016/j.bbrc.2013.08.055.