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Research Article
Hesperidin Alleviates Gentamicin Ototoxicity in Rats by Inhibiting Oxidative Stress, Inflammation and Apoptosis

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Abstract

Background and Objective: Ototoxicity induced by gentamicin (GTN) mediated mainly by oxidative stress, inflammation and apoptosis. Hesperidin (HPN) possesses antioxidant, anti-inflammatory and antiapoptotic activities. This study investigated the possible protective effect of HPN against GTN-induced ototoxicity in rats. **Materials and Methods:** Rats received GTN (100 mg/kg/day, i.p.) for 14 days. The HPN treatment (200 mg/kg/day, p.o.) started on the same day of GTN administration and continued for 14 days. Malondialdehyde, total antioxidant capacity, nitric oxide, calcium ion, tumor necrosis factor-α, interleukin-6, inducible nitric oxide synthase, nitric oxide, nuclear factor-κB p65, Bax/Bcl-2 ratio and cleaved caspase-3 measured in cochlear homogenates. Data analysed by one-way ANOVA test using GraphPad Prism Software Program (version 6.01). **Results:** The HPN significantly decreased malondialdehyde, inducible nitric oxide synthase, nitric oxide and calcium ion and significantly increased total antioxidant capacity in the ear cochleae of GTN-challenged rats. In addition, HPN significantly reduced cochlear tumor necrosis factor-α, interleukin-6, nuclear factor-κB p65, Bax/Bcl-2 ratio and cleaved caspase-3 in rats received GTN. **Conclusion:** The HPN protected against GTN-induced ototoxicity in rats by inhibiting oxidative/nitrative stress, inflammation and apoptosis.

Key words: Hesperidin, gentamicin, cochlea, oxidative stress, inflammation, apoptosis, rats

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The aminoglycoside antibiotic, gentamicin (GTN), is used to eradicate serious infections caused by Gram-negative bacilli. However, aminoglycoside-induced ototoxicity is an adverse effect that limits the usefulness of this antibiotic group. It estimated that 36.7% of aminoglycoside treated patients developed ototoxicity[1]. Both the cochlear and vestibular divisions of the eighth cranial nerve are affected leading to hearing impairment and loss of equilibrium. Aminoglycosides increase Ca^{+2} uptake by the cochlear hair cell mitochondria resulting in enhanced secretion of oxidative enzymes and generation of reactive oxidative species (ROS)[2]. Subsequent exhaustion of endogenous antioxidants, augmented lipid biomembrane peroxidation with increased production of malondialdehyde (MDA) issued[3]. Nitrosative stress and increased production of reactive nitrogen species (RNS), such as nitric oxide (NO), are also involved in aminoglycoside ototoxicity[4]. Additionally, activation of inflammatory cascades and increased production of inflammatory cytokines, such as Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6), are implicated in aminoglycoside ototoxicity[5]. Moreover, increased Ca^{+2} level causes disruption of mitochondrial membrane permeability, activation of the intrinsic apoptotic pathway and induction of caspase-3-dependent execution phase apoptosis[6].

Hesperidin (HPN) is a bioflavonoid obtained mainly from citrus fruits. It possesses antioxidant, antinitrative, anti-inflammatory and antiapoptotic activities. The HPN showed cardioprotective, hepatoprotective, nephroprotective, antidiabetic and anticancer effects[7]. In addition, a previous study revealed that HPN had a protective effect against ototoxicity induced by cisplatin in rats[8]. Therefore, HPN potentially can protect against aminoglycoside ototoxicity and to the best of our knowledge, the possible protective effect of HPN in rat models of GNT-induced ototoxicity not yet been investigated.

MATERIALS AND METHODS

Study area: The study was conducted at the Department of Pharmacology, Faculty of Medicine, Minia University, Egypt, from July, 2023 to March, 2024.

Drugs: The HPN and GTN purchased from Sigma-Aldrich, USA. The HPN was prepared in 0.5% carboxymethylcellulose solution (CMC) and GTN dissolved in physiological saline. Doses used in the present study were selected based on previous investigations[9,10].

Laboratory animals: Sprague-Dawley male rats (weight 220-250 g, each) obtained from the National Research Centre, Giza, Egypt. They housed at 24°C, 45% humidity and 12 hrs light/dark cycle. Rats fed ordinary chew, supplied with tap water *ad libitum* and adapted for one week.

Ethical approval: The study protocol was approved by the Research Ethics Committee, Faculty of Medicine, Al-Baha University, Saudi Arabia (approval number: REC/PHA/BU-FM/2024/20).

Experimental design: Rats were randomly divided into 4 groups (n = 10, each) as follows:

- Group 1 (control) received daily normal saline, i.p., for 14 days and CMC, p.o., for 14 days starting the same day of normal saline administration
- Group 2 received GTN (100 mg/kg/day i.p.) for 14 days and CMC p.o., daily for 14 days starting the same day of GTN administration
- Group 3 GTN (100 mg/kg/day i.p.) for 14 days and HPN (200 mg/kg/day, p.o.) for 14 days starting the same day of GTN administration
- Group 4 received only HPN (200 mg/kg/day p.o.) for 14 days

Sampling and biochemical examinations: Rats were euthanized 24 hrs after administration of drugs by i.p., thiopental (70 mg/kg). The cochleae were dissected out and their dry weights were determined. Cochleae homogenized for 15 min at 5000 rpm in cold potassium phosphate buffer (pH 7.3, 0.05 M). Colorimetric kits were used to assess the levels of MDA, NO, Ca^{+2}, total antioxidant capacity (TAC) (Biodiagnostic, Egypt) and cleaved caspase-3 (R&D Systems, USA). In addition, ELISA kits used to determine TNF-α and IL-6 (R&D Systems, USA), inducible nitric oxide synthase (iNOS) (Cusabio, USA), NF-κB p65 (Lifespan Biosciences, USA), Bax and Bcl-2 (LS Biologicals, USA) in cochlear homogenates.

Statistical analysis: Data analysis by one-way ANOVA test followed by Tukey's test for *post hoc* comparisons done using GraphPad Prism Software Program (version 6.01). Results showed a Mean±SEM and $p<0.05$ was the significance level.

RESULTS

Regarding the oxidative/nitrative stress biomarkers, rats received GNT (100 mg/kg/day, i.p., for 14 days) showed significant increases ($p<0.05$) of MDA, iNOS, NO and Ca^{+2} and a significant decrease ($p<0.05$) of TAC in cochlear homogenates as compared to the control values (Fig. 1a-c).

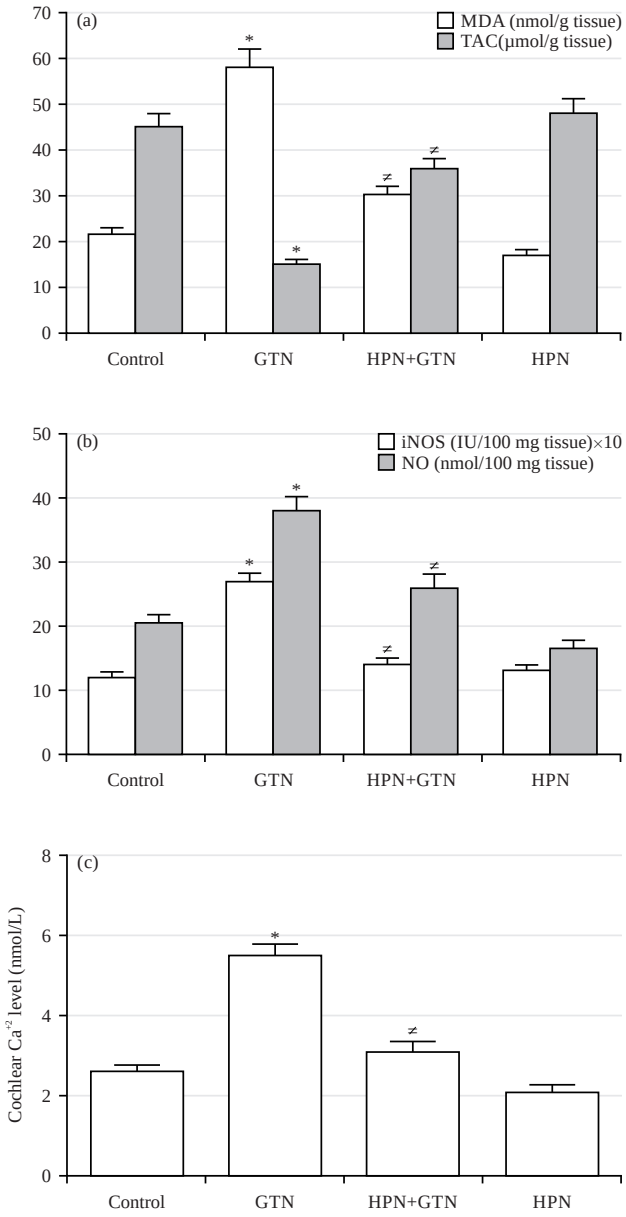

Fig. 1(a-c): Effects of hesperidin (HPN) treatment on (a) Malondialdehyde (MDA) and total antioxidant capacity (TAC), (b) Inducible nitric oxide synthase (iNOS) and nitric oxide (NO) and (c) Ca^{+2} level in the cochleae of gentamicin (GTN)-challenged rats

*p<0.05 vs control, ≠p<0.05 vs GTN group, results are Mean±SEM and n = 10 in each group

Contrarily, HPN (200 mg/kg/day, p.o., for 14 days) starting the same day of GTN administration caused significant reductions ($p<0.05$) of cochlear MDA, iNOS, NO and Ca^{+2} and a significant elevation ($p<0.05$) of TAC in rats challenged with GTN (Fig. 1a-c).

Regarding the inflammatory biomarkers, GNT insult resulted in significant increments ($p<0.05$) of TNF-α, IL-6 and NF-κB p65 in rat cochleae in comparison with the control results (Fig. 2a-b). On the other hand, significant decrements ($p<0.05$) of TNF-α, IL-6 and NF-κB p65 were detected in cochlear homogenates of GTN-challenged rats treated with HPN (Fig. 2a-b).

As regards the apoptotic biomarkers, significant elevations ($p<0.05$) of Bax/Bcl-2 ratio and cleaved caspase-3 were observed in the cochleae of rats that received GNT versus the control rats (Fig. 3a-b). Again, HPN significantly decreased ($p<0.05$) cochlear Bax/Bcl-2 ratio and cleaved caspase-3 in rats that received GTN (Fig. 3a-b).

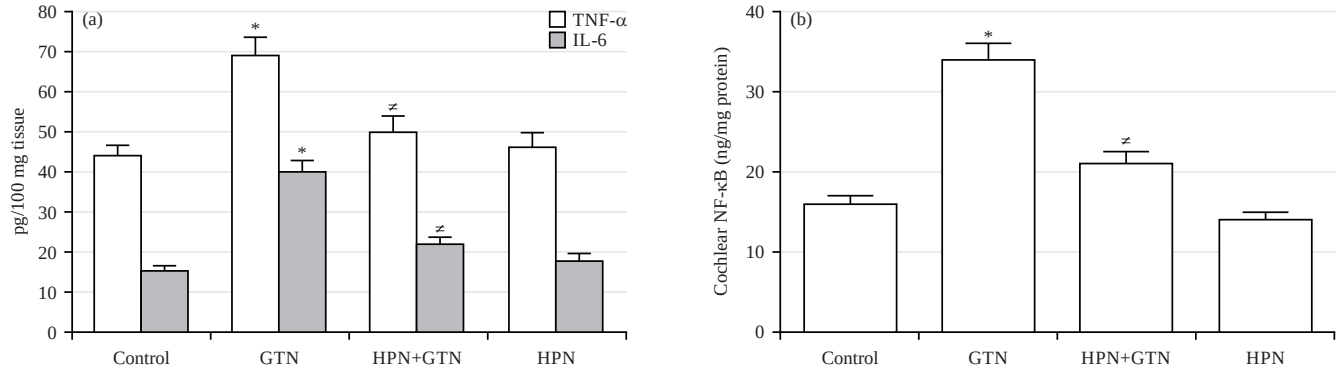

Fig. 2(a-b): Effects of hesperidin (HPN) treatment on (a) Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6) and (b) Nuclear Factor-κB p65 (NF-κB p65) in the cochleae of gentamicin (GTN)-challenged rats

*p<0.05 vs control, ≠p<0.05 vs GTN group, results are Mean±SEM and n = 10 in each group

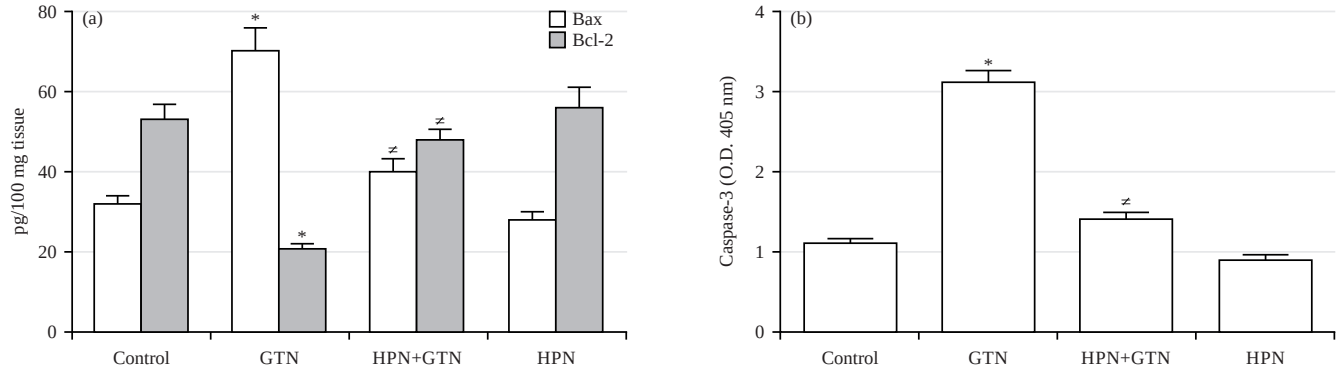

Fig. 3(a-b): Effects of hesperidin (HPN) treatment on (a) Bax and Bcl-2 and (b) Cleaved caspase-3 in the cochleae of gentamicin (GTN)-challenged rats

*p<0.05 vs control, ≠p<0.05 vs GTN group, results are Mean±SEM and n = 10 in each group

DISCUSSION

Prior investigations, similar to the present one, showed that cochlear injury induced by aminoglycosides mediated through oxidative stress, increased ROS generation, depletion of natural antioxidants and enhanced peroxidation of lipid biomembranes. Moreover, activation of iNOS and enhanced production of RNS, as NO, responsible for nitration of macromolecules are involved in GTN-induced cochlear damage[4].

The current study showed that HPN inhibited oxidative/nitrative stress evidenced by reduced MDA, preserved TAC and reduced iNOS activity and NO production in the cochleae of GTN-challenged rats. This is attributed to the ability of HPN to suppress NADPH oxidase-4, the main source of ROS generation during oxidative stress[11]. In addition, HPN provides antinitrative effect by inhibiting iNOS activity and RNS generation[12].

Upregulation of inflammatory cascades is also implicated in the pathogenesis of aminoglycoside ototoxicity. Augmented production of inflammatory cytokines, TNF-α and IL-6, releases the active cytoplasmic NF-κB p65 from its inhibitory subunit IκB. Translocation of NF-κB p65 to the nucleus activates transcription of various inflammatory genes and fosters inflammatory responses[5,13].

Previous studies, similar to the current one, showed that HPN inhibited NF-κB pathway and reduced the generation of NF-κB p65 and inflammatory cytokines[14,15]. This was explained by the capability of HPN to inhibit phosphorylation and degradation of IκB and thereby prevent the release of the active NF-κB p65 unit in the cytoplasm[16].

Moreover, activation of the mitochondrial-dependent apoptotic pathway incriminated in GNT-induced ototoxicity[17]. Increased ROS and inflammatory cytokines disrupt the balance between Bax, the main proapoptotic factor and Bcl-2, the main antiapoptotic factor. The elevation of the Bax/Bcl-2 ratio

increases mitochondrial membrane permeability and enhances the release of mitochondrial apoptogenic factors in the cytoplasm. Eventually, caspase proteases are upregulated and caspase-3-dependent execution phase apoptosis occurs[4,17]. Similar to the present findings, former studies revealed that HPN provided antiapoptotic effect via the reduction of intracellular Ca^{+2} overload responsible for opening the mitochondrial permeability transition pores, restoration of the balance of Bax/Bcl-2 ratio and downregulation of caspase-3 activity[18,19].

The current results indicate that HPN could be a feasible candidate to guard against ototoxicity in patients treated with aminoglycoside antibiotics. However, the limitations of this study include that HPN was used in only a single dose and the safety of HPN was not investigated. Therefore, it is recommended that further studies needed to explore the efficacy of HPN in different doses and also to explore the safety of HPN.

CONCLUSION

The HPN significantly mitigated GTN-induced ototoxicity in rats. This was evidenced by significant decrements of MDA, iNOS, NO, Ca^{+2}, TNF-α, IL-6, NF-κB p65, Bax/Bcl-2 ratio, cleaved caspase-3 and significant increment of TAC in the cochleae of GTN-challenged rats treated with HPN. The otoprotective effect of HPN in this model was conducted via its antioxidant, anti-inflammatory and antiapoptotic activities. Modulation of Ca^{+2} level, iNOS activity and NF-κB pathway seem to be greatly responsible for the protective effect of HPN against the ototoxicity caused by GTN.

SIGNIFICANCE STATEMENT

The HSP significantly reduced MDA, iNOS, NO, Ca^{+2}, TNF-α, IL-6, NF-κB p65, Bax/Bcl-2 ratio, cleaved caspase-3 and significantly increased TAC in the cochleae of GTN-challenged rats. The current study highlights that HPN is a feasible candidate to minimize the risk of ototoxicity in patients treated with aminoglycoside antibiotics. However, further investigations are required to explore the efficacy and safety of HPN in these cases.

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