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Research Article
Effect of Taxifolin on Cobalt-induced Ototoxicity in Rats: A Biochemical and Histopathological Study

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Abstract

Background and Objective: Cobalt is a metal with a significant hazard profile, used widely in many industries. The reported hazards of Cobalt include effects on the heart, thyroid, immune system, vision and hearing organs as a result of exposure to high doses. This study aimed to evaluate the biochemical and histopathological effect of taxifolin on Co-induced ototoxicity in rats. **Materials and Methods:** Distilled water was administered to the Healthy Group (HG) and cobalt-only (Co) groups of animals and a third group was orally given 50 mg kg^{-1} Taxifolin+Cobalt (TCo) by gavage. After 1 hr, 150 mg kg^{-1} $CoCl_2$ was administered orally to the stomach by gavage to the animals in the TCo and the Co groups. This procedure was repeated once a day for seven days. **Results:** Malondialdehyde (MDA), Nuclear Factor-Kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) levels were significantly higher than the HG and TCo groups and the cochlear nerve tissue was damaged by significant histopathological damage in the Co group with low total glutathione (tGSH) levels. In the TCo group, biochemical and histopathological findings were found to be close to the HG group. **Conclusion:** This study revealed the role of oxidative stress and proinflammatory cytokines in the pathogenesis of Co ototoxicity. Our results suggest that taxifolin may be useful in the treatment of Co-related ototoxicity.

Key words: Cobalt, cochlear nerve tissue, taxifolin, MDA, NF-κB, TNF-α, IL-1β, tGSH

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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

Cobalt (Co) is a divalent metal used in the production of metal alloys, batteries and pigments[1]. In a previous study, it was stated that the Co element had a significant hazard profile among the metal used widely in many industries[2]. People are typically exposed to Co through their diet, inhalation, or contact with soil and water. The harmful consequences of chronic exposure include heart, liver, kidney, thyroid and immune system disorders and vision and hearing impairment[3]. Metallosis, a putative medical condition involving metal particles caused by the wearing of metal-on-metal hip prostheses containing Co and chrome (Cr) may lead to severe weight loss, heart and thyroid dysfunctions, blindness and sensorial neuropathy-associated hearing loss[1,4]. The effects of Co on sensory hair cells, neurons and supporting cells in the cochlea have been investigated and its toxicity for these structures had been demonstrated[1]. In the basic mechanism of ototoxicity induced by Co, the accumulation of Reactive Oxygen Species (ROS) and the role of proinflammatory cytokine expression has been shown[5]. There are other studies showed the relationship of Co toxicity with ROS[6]. It has been reported that this event may cause mitochondrial swelling, decreased electrical membrane potential and damage that begins with mitochondrial dysfunction[7].

Taxifolin (3,5,7,3,4-pentahydroxy flavanone or dihydroquercetin), which we investigated in terms of its protective effect against Co ototoxicity in our study, is an antioxidant flavonoid[8]. Flavonoids are a group of secondary metabolic compounds widely found in plants. They are key ingredients of the human diet and have become very popular due to their many health-promoting and disease-preventive effects[9]. Research has revealed that taxifolin has various pharmacological effects, including antioxidant, anti-inflammatory, antiviral, antibacterial, anticancer and neuroprotective activities. Besides, taxifolin has been documented to inhibit ROS production[10]. All this information from the literature suggests that taxifolin may be useful in reducing and preventing Co ototoxicity. Therefore, the aim of the study was to biochemically and histopathologically investigate the effect of taxifolin on Co-induced ototoxicity in rats.

MATERIALS AND METHODS

Study area: The study was carried out at Medical Experimental Practice and Research Laboratory, Ataturk University, Turkey, from January-June, 2020.

Sample collection: In this study, a total of 24 albino Wistar male rats weighing between 280 and 295 g were used. All the animals were obtained from the Medical Experimental Application and Research Center of Ataturk University. The approval for the research has been received from the local animal care committee the Ataturk University (26.12.2019-258). The animals were housed and fed at normal room temperature (22°C) in groups before the experiment.

Chemical substances: Taxifolin was obtained from Evalar-Russia, cobalt ($CoCl_2X6H_2O$) from Merck-Germany and thiopental sodium from I.E. Ulagay-Turkey.

Experiment groups: The experimental animals were divided into HG (control group), $CoCl_2$ only (Co) and Taxifolin+$CoCl_2$ (TCo) groups.

Experimentation: Taxifolin was orally administered to the TCo group (n = 8) at a dose of 50 mg kg^{-1} by gavage. Distilled water was administered as a solvent for the Co (n = 8) and HG (n = 8) groups in the same way. About 1 h after the administration of taxifolin and distilled water, 150 mg kg^{-1} $CoCl_2.6H_2O$ was orally administered to the stomach by gavage to the animals in the TCo and the Co groups. This procedure was repeated once a day for 7 days. At the end of this period, all the animals were killed by high-dose anesthesia (50 mg kg^{-1} thiopental sodium) and their inner ears were removed. Biochemical and histopathological examinations were performed on the cochlear nerve tissue of the removed inner ear. The biochemical and histopathological results obtained from the TCo and HG groups were compared with the Co group.

Biochemical analysis: Before dissection, all tissue was rinsed with phosphate-buffered saline solution. The tissues were homogenized in ice-cold phosphate buffers (50 mM, pH 7.4) appropriate for the variable to be measured. The tissue homogenates were centrifuged at 5,000 rpm for 20 min at 4°C and the supernatants were extracted to analyze total glutathione (tGSH) and malondialdehyde (MDA). The results of all tissues were expressed by dividing by G protein. All spectrophotometric measurements were performed using a microplate reader (Bio-Tek, USA).

MDA analysis: Malondialdehyde (MDA) measurements were based on the method used by Ohkawa *et al.*[11], involving the spectrophotometrical measurement of the absorbance of the pink-colored complex formed by thiobarbituric acid (TBA) and MDA. The tissue-homogenate sample (25 µL) was added to a solution containing 25 µL of 80 g L^{-1} sodium dodecyl sulfate and 1 mL mixture solution (200 g L^{-1} acetic acid+1.5 mL of 8 g L^{-1} 2-thiobarbiturate)[11]. The mixture was incubated at 95°C for 1 h. Upon cooling, 1 mL of n-butanol: Pyridine (15:1) was added. The mixture was vortexed for 1min and centrifuged for 10 min at 4,000 rpm. The absorbance of the supernatant was measured at 532 nm. The standard curve was obtained using 1,1,3,3-tetra methoxy propane.

tGSH analysis: According to the method defined by Sedlak and Lindsay, DTNB (5,5'-dithiobis [2-nitrobenzoic acid]) disulfide was used as a chromogenic in the medium and DTNB (Ellman's reagent) was easily reduced by sulfhydryl groups[12]. The yellow color produced during the reduction was measured by spectrophotometry at 412 nm. For the measurement, a cocktail solution (5.85 mL 100 mM Na-phosphate buffer, 2.8 mL 1 mM DTNB, 3.75 mL 1 mM NADPH and 80 µL 625 U L^{-1} glutathione reductase was prepared. Before measurement, 0.1 mL meta-phosphoric acid was added to 0.1 mL tissue-homogenate and centrifuged for 2 min at 2,000 rpm for deproteinization. The 0.15 mL cocktail solution was added to 50 µL of supernatant. The standard curve was obtained using GSSG.

Nuclear factor-kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) analysis: Tissue-homogenate NF-κB and TNF-α concentration were measured using the rat-specific sandwich enzyme-linked immunosorbent assay with dedicated kits (Cat. No: 201-11-0288, SunRed for NF-κB and Cat no: YHB1098Ra, Shanghai LZ for TNF-α and Rat IL-1β). Analyses were performed according to the manufacturers' instructions. Briefly, a monoclonal antibody specific for rat NF-κB, TNF-α and IL-1β were coated onto the wells of the microplates. The tissue homogenate, standards and biotinylated specific monoclonal antibody and streptavidin-HRP were pipetted into these wells and then incubated at 37°C for 60 min. After washing, chromogen reagent A and chromogen reagent B were added, which were acted upon by the bound enzyme to produce a color. The sample was incubated at 37°C for 10 min, after which the stop solution was added. The intensity of this colored product is directly proportional to the concentration of rat NF-κB, TNF-α and IL-1β present in the original specimen. At the end of the course, the well plates were read at 450 nm. The absorbance of the samples was calculated with formulas including standard graphics.

Histopathological examination: The tissues of the subjects were taken into a 10% formaldehyde solution and fixed for 72 hrs. After the fixation process, the tissues were taken into a cassette and washed in running water for 24 hrs and then dehydrated by increased alcohol series (70, 80, 90 and 100%). These sections were stained by hematoxylin-eosin double staining and evaluated and photographed in the Olympus DP2-SAL firmware program (Olympus® Inc. Tokyo, Japan). The histopathological evaluation was performed by a histologist blinded to the study groups.

Statistical analysis: All statistical analyses were performed using IBM SPSS v. 22 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The results were presented as mean±standard deviation (SD). The normality assumption of the variables was confirmed using the Shapiro-Wilk test. All biochemical measurements were normally distributed and for the comparison between the groups, analysis of variance (ANOVA) was used. According to the homogeneity of variances, Tukey's HSD or Games-Howell test was used as a post-doc test. A $p<0.05$ was considered significant for all tests.

RESULTS

Biochemical findings

MDA and tGSH analysis results: As can be seen from Fig. 1, the amount of MDA in the cochlear nerve tissue of the Co group was significantly higher than the TCo group ($p<0.001$ and $p<0.001$, respectively). In the HG and TCo groups, the difference in the MDA amount was calculated to be statistically insignificant ($p = 0.113$). It was also determined that the amount of tGSH in the cochlear nerve tissue of the Co group significantly decreased compared to the HG and TCo groups ($p<0.001$ and $p<0.001$, respectively). In the taxifolin group, tGSH approached the healthy group, but the difference was still statistically significant ($p = 0.018$).

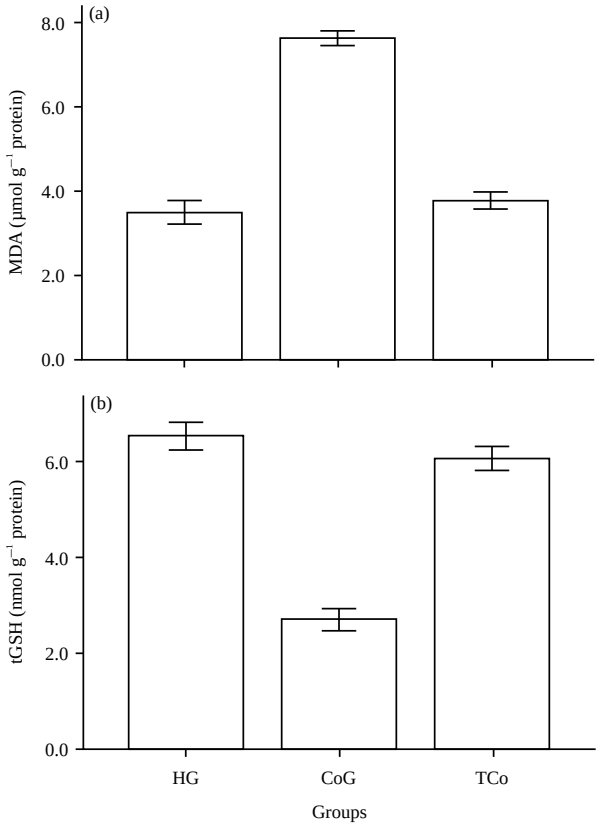

Fig. 1(a-b): Amount of (a) MDA and (b) tGSH in the cochlear nerve tissue of HG, Co and TCo groups

Results of NF-κB, TNF-α and IL-1β analysis: As seen in Fig. 2, the NF-κB ($p<0.001$ and $p<0.001$, respectively), TNF-α ($p<0.001$, $p<0.001$, respectively) and IL-1β ($p<0.001$ and $p<0.001$, respectively) levels were significantly increased in the cochlear nerve tissue of the Co group compared to the HG and TCo groups. In the TCo group, while the IL-1β levels were similar to those of the HG group ($p = 0.156$), the NF-κB and TNF-α levels approached those of the HG group, but the difference was still statistically significant ($p<0.001$ and $p = 0.005$, respectively).

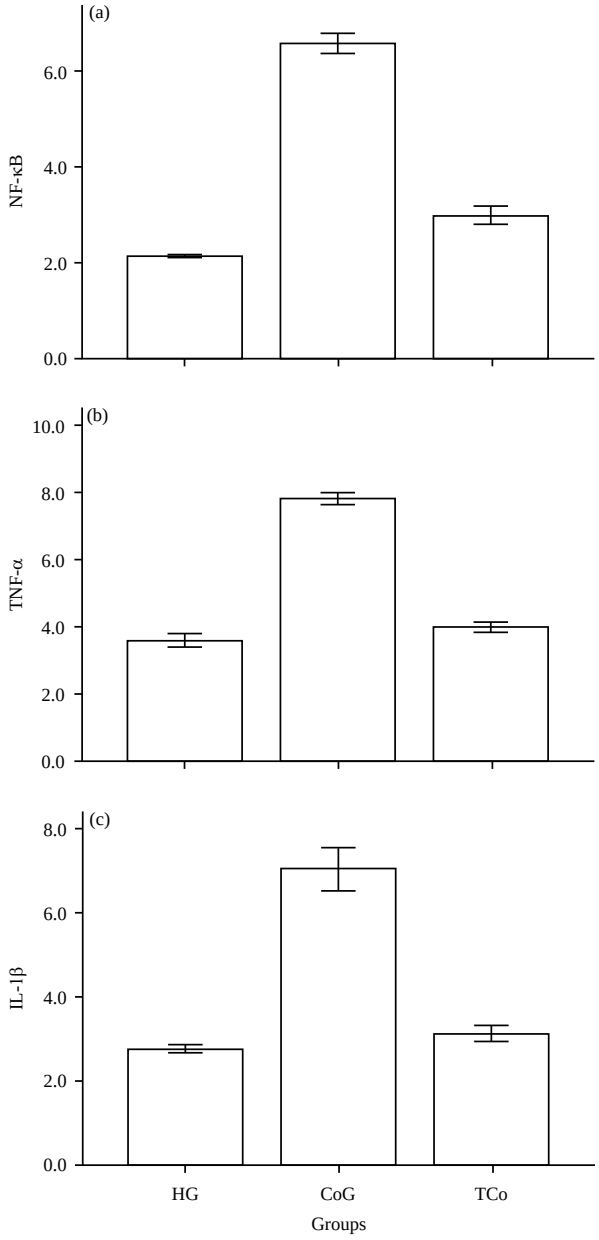

Fig. 2(a-c): (a) NF-κB, (b) TNF-α and (c) IL-1β levels in the cochlear nerve tissue of HG, Co and TCo groups

Histopathological findings: In the histopathological evaluation of the cochlear nerve tissue of the HG group, the tissue was observed to have a normal histological architecture with myelinated nerve fibers with centrally located axons, adjacent Schwann cell nuclei and blood vessels (Fig. 3). However, it was noted that in the Co group of animals, the axons of the myelinated nerve fibers were swollen, edematous and degenerated often losing their central location. Schwann cell nuclei were generally not seen around the myelinated nerve fibers and the interaction between the axon and Schwann cells was lost. Also, it was determined that there was a high accumulation of collagen fiber in the tissue and the blood capillaries were dilated (Fig. 4). Examining the sections of the TCo group, it was observed that the myelinated nerve fibers mostly exhibited normal morphology, Schwann cell nuclei were evident around the nerve fiber and the vessels were normal (Fig. 5).

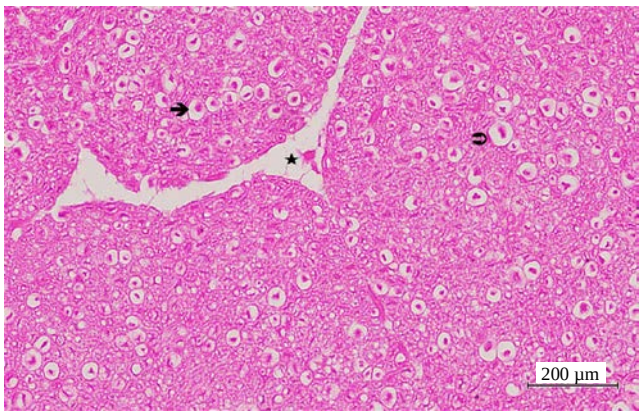

Fig. 3: Hematoxylin-eosin staining in the cochlear nerve tissue of the healthy group
➔: Myelinated axons, ⊃: Schwann cells, ★: Blood vessels (HE ×400)

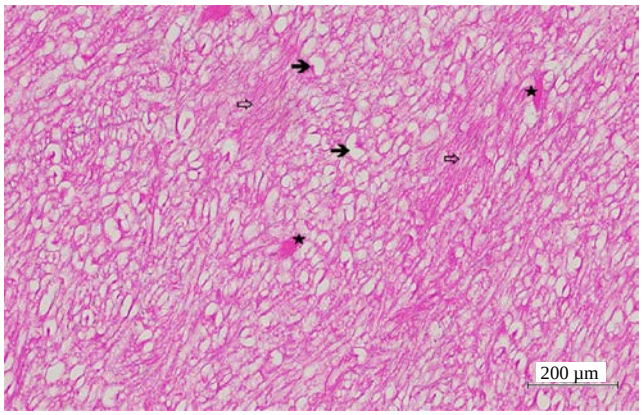

Fig. 4: Hematoxylin-eosin staining of the cochlear nerve tissue of the Co group
➔: Degenerated myelinated axons, ⇨: Accumulation of collagen, ★: Dilated blood vessels (HE ×400)

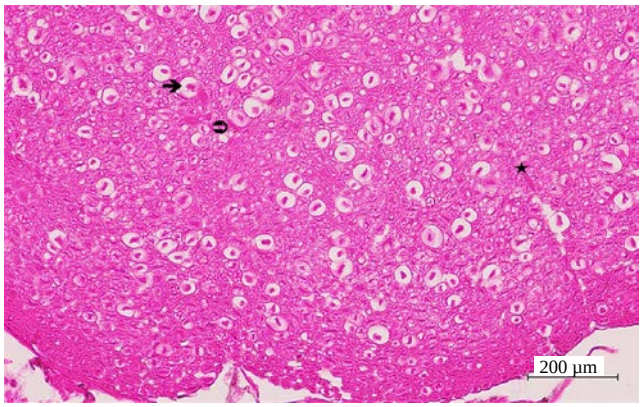

Fig. 5: Hematoxylin-eosin staining of the cochlear nerve tissue of the group treated with taxifolin
➔: Myelinated axons, ⊃: Schwann cells, ★: Blood vessels (HE ×400)

DISCUSSION

In this study, the effect of taxifolin on Co-induced ototoxicity in rats was investigated biochemically and histopathologically. Our biochemical experimental results showed that MDA production in the cochlear nerve tissue of the Co group animals increased compared to the HG and TCo groups and whereas tGSH production was decreased. As stated above, the basic mechanism implicated in ototoxicity induced by Co is the overproduction of ROS[5]. As known, ROS affects cell membrane fatty acids and initiates lipid peroxidation (LPO), a process that continues as an auto-catalytic chain reaction of poly-unsaturated fatty acids and causes tissue damage[13]. The best known of various aldehydes resulting from LPO is MDA, which can cause serious damage by leading to the cross-linking and polymerization of membrane components[14]. In a study by Akinrinde *et al.*[15], it was argued that oxidative stress mediators, such as ROS, hydrogen peroxide (H_2O_2) and MDA play a role in the pathogenesis of Co toxicity. In a study by Li *et al.*[1], it was documented that the increase of the superoxide radical production was responsible for the harmful effects of $CoCl_2$ on peripheral auditory nerve fibers and spiral ganglion neurons. The results of our experiment showed that taxifolin suppressed the overproduction of Co-associated MDA in the cochlear nerve tissue. In a study that supports our experimental results, the data revealed that taxifolin inhibited ROS overproduction[10]. No studies were found showing the effect of taxifolin on the amount of MDA in cochlear nerve tissue but it has been experimentally demonstrated that taxifolin reduces oxidative retinal damage due to the increase in the MDA amount[16].

In this study, it was also found that tGSH was decreased in the cochlear nerve tissue of the Co group which had higher MDA compared to the HG and TCo groups. In previous studies, it has been reported that $CoCl_2$ causes neurotoxicity by decreasing the amount of GSH and increasing the amount of MDA[17]. Also, it has been reported that Co causes a decrease in antioxidant defense systems and that maintaining normal levels of these antioxidant defense systems plays an important role in reducing Co toxicity[15]. In the cochlear nerve tissue of the animals in the TCo group, the amount of MDA was lower and that of tGSH was higher compared to the Co group. No study investigating the effect of taxifolin on the level of tGSH in the cochlear nerve tissue was found in the literature. However, there is research showing that taxifolin inhibits tGSH consumption[16].

In our study, it was observed that the levels of NF-κB, TNF-α and IL-1β were increased in the cochlear nerve tissue of the Co group compared to the SC and TCo groups. It has been reported in the literature that Co-related cell damage is also associated with the overproduction of the proinflammatory cytokines, as well as ROS[18]. As a result of studies, it has been clarified that Co increases the nuclear translocation of NF-κB in auditory cells[5]. It is also reported that NF-κB induces the expression of TNF-α, IL-1β and other pro-inflammatory cytokines[19]. Lee *et al.*[5] stated that the ototoxic effects of Co were associated with the production of ROS and proinflammatory cytokines in the auditory system. The taxifolin used in our experiment for Co-induced ototoxicity prevented the increase of MDA, NF-κB, TNF-α and IL-1β and the decrease of tGSH by Co. Liu *et al.*[20] also reported that taxifolin suppressed the production of ROS, IL-1 β and TNF-α and NF-κB. In our study, the levels of oxidant and proinflammatory cytokines in being close to those of the HG group indicate that our experimental results are in agreement with the literature.

In our study, it was seen that all the biochemical experimental results were consistent with the histopathological findings. In the Co group, the axons of the myelinated nerve fibers were swollen, edematous and degenerated and lost their central position; surrounding Schwann cell nuclei were not seen and the axon-Schwann cell interaction was lost. Besides, it was determined that there was a high accumulation of collagen fiber in the tissue and the blood capillaries were dilated. However, the histopathological findings in the TCo group were observed to be closer to those of the HG group.

CONCLUSION

Co-administration caused an increase in the oxidative and proinflammatory markers and a decrease in the antioxidant markers in the cochlear nerve tissue of the animals. Significant histopathological damage was detected in the Co group, in which the oxidant and proinflammatory markers were higher and antioxidants were lower. Taxifolin was shown to protect the cochlear nerve tissue against Co toxicity by inhibiting the increase of oxidant and proinflammatory parameters and the decrease of the antioxidants by Co. This indicates the role of oxidative stress and proinflammatory cytokines in the pathogenesis of Co ototoxicity. Our experimental results suggest that taxifolin may be useful in the treatment of Co-related ototoxicity.

SIGNIFICANCE STATEMENT

This study discovers the Co-administration caused an increase in oxidative and proinflammatory markers and a decrease in antioxidants in the cochlear nerve tissue of animals. Significant histopathological damage was detected in the Co group with high oxidant and proinflammatory markers and low antioxidants. It has been observed that taxifolin protects cochlear nerve tissue against Co toxicity by inhibiting the increase of oxidant and proinflammatory parameters with Co and the decrease of antioxidants. This information reveals the role of oxidative stress and proinflammatory cytokines in the pathogenesis of Co ototoxicity. That can be beneficial for taxifolin may be useful in the treatment of Co-related ototoxicity. This study will help the researchers to uncover the critical areas of Co is ototoxic and that its ototoxicity is associated with oxidant and proinflammatory cytokine overproduction that many researchers were not able to explore. Thus a new theory on the benefit of applying natural antioxidant and anti-inflammatory products externally in the treatment of hearing loss that may occur with Co and other agents may be arrived at.

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