The effect of taxifolin on oxidative sciatic nerve damage induced by cobalt chloride in rats: a biochemical and histopathological evaluation

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Cobalt is a trace element that increases lipid peroxidation and malondialdehyde levels and reduces the antioxidant defense mechanisms of nerve cells. High levels of cobalt exposure may cause peripheral neuropathy, but the mechanism behind this has not yet been elucidated. Taxifolin is a flavonoid whose antioxidant and anti-inflammatory properties are well-known. We aimed to investigate the effect of taxifolin on cobalt-induced oxidative sciatic nerve damage. Eighteen albino male Wistar rats were assigned to three groups: Control, Cobalt, and Taxifolin + Cobalt groups. Total oxidant and total antioxidant status and levels of malondialdehyde, total glutathione, and superoxide dismutase were measured to determine the effect of taxifolin on cobalt-induced sciatic nerve injury. The following statistically significant effect of taxifolin was observed: It prevented cobalt-induced oxidative sciatic nerve damage by reducing malondialdehyde levels and total oxidant status and increasing total antioxidant status, total glutathione levels, and superoxide dismutase levels. In a histopathological analysis, we observed similar findings in Control and Taxifolin + Cobalt groups. We determined that taxifolin is effective in preventing cobalt-induced oxidative damage in sciatic nerve injury.

Key words: sciatic neuropathy, antioxidants, superoxide dismutase, malondialdehyde, glutathione, neuroprotective effect

INTRODUCTION

Cobalt (Co) is a trace element involved in the synthesis of cobalamin (vitamin B12) that plays an important role in cellular growth, differentiation, and development (Kikuchi et al., 2018). Daily intake of vitamin B12 for an adult has been calculated as 2.4 μg/day (about 0.1 μg/day as Co) (Battaglia et al., 2009; Kikuchi et al., 2018). Intake of lower amounts of vitamin B12 may lead to anemia (Kikuchi et al., 2018; Zheng et al., 2019); conversely, high doses of cobalt exposure could cause peripheral neuropathy, but the mechanism behind this has not yet been elucidated (Kikuchi et al., 2018). It has also been proven that Co, which could cross the blood-brain barrier, is toxic to the central nervous system (Zheng et al., 2019).

Co is frequently used in skin-whitening creams, in orthopedic and dental implants, and in the hard metal industry, which produces cutting tools that are primarily used for manufacturing industrial products and parts (Denizoğlu and Duymuş, 2006; Andersson et al., 2020; Tanoğlu et al., 2020; Ashraf et al., 2021). Co can enter the body in a nanoparticular form, but the toxic effect of Co nanoparticles has been reported to be attributable mainly to Co ions dissolved from

particulate aggregates (Kikuchi et al., 2018). Elevated levels of Co were reported in blood and urine samples of occupationally exposed workers (Karovic et al., 2007). Additionally, heavy metal debris and corrosion products released from the surface of Co-alloyed prostheses and implants have a detrimental effect on all organs, including the nervous system (Keegan et al., 2008; Clarke et al., 2015; Tanoğlu et al., 2020). Moreover, peripheral neuropathies have been reported in patients with Co-containing orthopedic implants (Clarke et al., 2015).

Cobalt chloride ($CoCl_2$) increases lipid peroxidation and malondialdehyde levels and reduces the antioxidant defense mechanisms of nerve cells (Zheng et al., 2019). These alterations are the main mechanisms of the pathogenesis of Co-associated neurotoxicity (Zheng et al., 2019). It was reported that the neurotoxic effect of $CoCl_2$ in brain tissue may be prevented through the repair of reduced antioxidant enzyme activity and the inhibition of increased lipid peroxidation (Akinrinde and Adebiyi, 2019).

Taxifolin (Tax) (3,5,7,3,4-pentahydroxy flavanone or dihydroquercetin) is a flavonoid which is a bioactive constituent of onion, French maritime pine bark, milk thistle, orange, and grapefruit. It has been revealed in the literature that Tax has various pharmacological effects, including antioxidant, anti-inflammatory, antiviral, antibacterial, hepatoprotective, anti-cancerous, and neuroprotective properties (Zu et al., 2014; Tanaka et al., 2019; Yang et al., 2019). The inhibitor effects of Tax on the production of reactive oxygen species were demonstrated in a study by Cai et al. (2018). The results of various studies suggest that Tax may be useful in mitigating and preventing Co-induced sciatic nerve injury. Therefore, in our current study, we aimed to investigate the effects of Tax on Co-induced oxidative sciatic nerve damage using biochemical and histopathological analyses. Few studies have examined the effects of different flavonoids on peripheral nerve damage (Yaşar et al., 2019; Basu and Basu, 2020; Uddin et al., 2020). To the best of our knowledge, no study has investigated the effects of Tax on Co-induced sciatic nerve damage.

METHODS

Animals

In this study, a total of 18 male, 10 to 12-week-old albino Wistar rats weighing between 275 and 287 grams were obtained from Ataturk University Medical Experimental Application and Research Center. The rats underwent a two-week adaptation period to the new en-

vironment. The rats were caged individually in a controlled environment with a 12-hour light/dark cycle, temperature of 23 ± 2°C, humidity of 40 to 70%, and free access to food and drinking water.

The experimental study protocols were approved by the Ethics Committee of Ataturk University, Erzurum Turkey Animal Care Ethics Committee (Number: 255, Date: 26.12.2019), and the study was performed in accordance with the National Institute of Health's guide for the care of laboratory animals (NIH Publications No. 8023).

Chemicals

In this study, thiopental sodium was supplied from İbrahim Etem – Menarini Pharmaceuticals (İstanbul/TURKEY), cobalt chloride ($CoCl_2 \times 6 H_2O$) from Merck (Darmstadt/Germany), and taxifolin from Evalar (Moscow/Russia, Catalog Number: RU.77.99.88.003.E.012499.12.14).

Experimental procedure

Eighteen male albino Wistar rats were divided into the following three groups: a control group (n=6), Co group rats receiving $CoCl_2$ solution ($CoCl_2 \times 6 H_2O$), and Tax + Co group rats receiving taxifolin and $CoCl_2$ solution. In the control group, only distilled water was applied *via* oral gavage into the stomach. In the Co group, distilled water was initially applied, and one hour after the administration of distilled water, 150 mg/kg $CoCl_2$ was applied *via* oral gavage into the stomach for 7 consecutive days, as described in a study by Akinrinde and Adebiyi (2019). In the Tax + Co group, 50 mg/kg Tax was initially applied, and one hour after Tax administration, 150 mg/kg $CoCl_2$ was applied *via* oral gavage into the stomach for 7 consecutive days, as described in a study by Salcan et al. (2020). At the end of this period, all animals were sacrificed through the intraperitoneal administration of 50 mg/kg thiopental sodium. A linear incision of approximately 4.5 cm was made from the posterolateral region of the left and right thighs. The skin in that area has been separated over the muscles. After the dissection of the gluteal muscle, the sciatic nerve was exposed between the femoral, biceps, and semitendinosus muscles using forceps. The sciatic nerve was isolated from the surrounding tissues up to the branching point in the popliteal area and dissected using scissors. The middle third of sciatic nerves were excised with epineurium bilaterally. The dissection process took four minutes after anesthesia. The middle part of the right sciatic nerve was placed in 10% formalin solution for histopathological examination.

The middle part of the left sciatic nerve was placed in the Eppendorf tube for the evaluation of biochemical parameters.

Total oxidant status (TOS), total antioxidant status (TAS), levels of malondialdehyde (MDA), total glutathione (tGSH), and superoxide dismutase (SOD), and the histopathological alterations of the nerve samples were evaluated. A pairwise comparison of the study groups in terms of biochemical and histopathological results were performed.

Biochemical analysis

Approximately 60 mg of samples of sciatic nerve were thoroughly washed with NaCl (0.9%). Tissues were homogenized with liquid nitrogen. After the homogenization process, 2 ml of Tris–HCl buffer solution (pH 7.4, 0.15 M) was centrifuged at 4000xg at 4°C for 10 min. The obtained supernatants were used for biochemical analysis, including protein quantity, malondialdehyde (MDA), total glutathione (tGSH), superoxide dismutase (SOD) levels, and total oxidant and total antioxidant status.

Protein concentration measurement was performed according to the Bradford method (Kruger, 1994). The principle of the measurement is based on the absorbance at 595 nm of the colored complex formed by the binding of Coomassie Brilliant Blue G-250 dye to proteins.

Malondialdehyde analysis

Malondialdehyde measurements were based on a method employed by Ohkawa et al. (1979) involving the spectrophotometrical measurement of the absorbance of the pink-colored complex created by thiobarbituric acid (TBA) and MDA. A tissue-homogenate sample (0.1 mL) was added to a solution containing 0.2 ml of 80 g/L sodium dodecyl sulfate, 1.5 mL of 200 g/L acetic acid, 1.5 mL of 8 g/L 2-thiobarbiturate, and 0.3 mL of distilled water. The mixture was incubated at 95°C for 1 h. Upon cooling, 5 mL of n-butanol:pyridine (15:1) was added. The mixture was vortexed for 1 min and centrifuged at 2000xg for 30 min. The absorbance of the supernatant was measured at 532 nm. The standard curve was obtained using 1,1,3,3-tetramethoxypropane (Ohkawa et al., 1979).

Total glutathione analysis

Total glutathione analysis was undertaken using a method described by Sedlak and Lindsay (1968). 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) is a chromogenic chemical and is reduced seamlessly via sulfhydryl groups. The yellow color produced during the reduction was measured using 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 glutathione reductase) was prepared. Prior to measurement, 0.1 mL of meta-phosphoric acid was added to 0.1 mL of tissue-homogenate and centrifuged at 2,000xg for 2 min for deproteinization. A 0.15 mL cocktail solution was added to the 50 μL of supernatant. The standard curve was obtained using oxidized glutathione (GSSG) (Sedlak and Lindsay, 1968).

Superoxide dismutase analysis

The measurements were performed according to the method proposed by Sun et al. (1988). SOD is formed through the conversion of xanthine to uric acid by xanthine oxidase. When nitro blue tetrazolium (NBT) is added to this reaction, SOD reacts with NBT, and a purple-colored formazan dye occurs. The sample was weighed and homogenized in 2 ml of 20 mmol/L phosphate buffer containing 10 mmol/L EDTA at pH 7.8. The sample was centrifuged at 4000xg for 10 minutes, and supernatant was then used as the assay sample. The mixture, containing 2,450 μL of measurement mixture (0.3 mmol/L xanthine, 0.6 mmol/L EDTA, 150 μmol/L NBT, 0.4 mol/L Na_2CO_3, 1 g/l bovine serum albumin), 500 μL of supernatant, and 50 μL of xanthine oxidase (167 U/l), was vortexed and incubated for 10 min. At the end of the reaction, formazan formed. The absorbance of the purple-colored formazan was measured at 560 nm.

Measurements of total oxidant status and total antioxidant status

The TOS and TAS of tissue homogenates were determined using a novel automated measurement method and commercially available kits (Rel Assay Diagnostics, Turkey), which were both developed by Erel (2004, 2005). The TAS method is based on the bleaching of the characteristic color of a more stable 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation by antioxidants, and the measurement is performed at 660 nm. The results are expressed as nmol hydrogen peroxide (H_2O_2) equivalent/L. In the TOS method, the oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. Ferric ion produces a colored complex with xylenol orange in an acidic medium. The color intensity, which can be mea-

sured with a spectrophotometer at 530 nm, is compared to the total amount of oxidant molecules present in the sample. The results are expressed as µmol Trolox equivalent/L. In this study, the percentage ratio of TOS to TAS was used as the Oxidative Stress Index (OSI), which was calculated by dividing TOS by 100 × TAS.

Histopathological analysis

Initially, sciatic nerve samples were fixed in a 10% formaldehyde solution for a light microscopic assessment. Following the identification process, the sciatic nerve samples were washed under tap water in cassettes for 24 h. The sciatic nerve samples were then passed through a series of increasing ethanol to dehydrate the tissues and were kept for 10 min in each ethanol solution (70%, 80%, 90%, and 100%) to gradually dehydrate the tissues. Sciatic nerve samples were passed through xylol and were embedded in paraffin. Four-to-five micrometer sections were prepared from the paraffin blocks, and hematoxylin–eosin staining was applied. The photos of the samples were taken following the assessment performed using the Olympus DP2-SAL firmware program (Olympus® Inc. Tokyo, Japan). In the serial slices of each experimental group, including a central and five peripheral slices, six areas in each of six sections at 100x magnification were selected and scored in terms of destruction, perineurium disjunction, Schwann cell degeneration, and congestion. The severity of the histopathological findings in each section were graded as 0-normal, 1-mild damage, 2-moderate damage, and 3-severe damage. The histo-

pathological assessment of the slices was performed by a pathologist blinded to the study groups.

Statistical analysis

The sample size was calculated by accepting the type 1 error level as 0.05 and the power of the study and the effect size as 0.8; the total sample size was calculated to be at least 15 models. A statistical analysis was performed using SPSS version 25 (IBM, NY, USA). All samples in the groups have exhibited a normal distribution, according to the Kolmogorov-Smirnov test and parametric ANOVA test used for the statistical evaluation. The means ± standard deviations of the groups were calculated for the evaluation of the descriptive data. A Bonferroni correction was performed for the pairwise comparisons of the study groups. All histopathological grading scores displayed a normal distribution according to a Kolmogorov-Smirnov test. Therefore, an ANOVA test was used for the comparison of the histopathological results of the groups. The level of statistical significance was accepted as 0.05.

RESULTS

The means ± standard deviations and p-values of the pairwise comparison of the groups in terms of TOS, TAS, and the levels of MDA, tGSH, and SOD were indicated in Table 1. MDA levels and TOS were statistically significantly lower in the Tax + Co group compared to the Co group. In the Tax + Co group, tGSH

Table 1. Means ± standard deviations and the pairwise comparison of the groups.

	Control (C) Mean ± SD	Cobalt (Co) Mean ± SD	Taxifolin + Cobalt (Tax + Co) Mean ± SD	Significant differences between groups	p values for pairwise comparisons
MDA (µmol/gr protein)	4.5 ± 0.14	8.1 ± 0.23	5.0 ± 0.1	Co > C Tax + Co > C Co > Tax + Co	<0.001* <0.001* <0.001*
tGSH (nmol/gr protein)	12.2 ± 0.42	5.5 ± 0.28	10.8 ± 0.38	C > Co C > Tax + Co Tax + Co > Co	<0.001* <0.001* <0.001*
SOD (U/gr protein)	12.7 ± 1.12	4.6 ± 0.4	10.6 ± 0.38	C > Co C > Tax + Co Tax + Co > Co	<0.001* <0.001* <0.001*
TOS (nmol H2O2/mg protein)	6.0 ± 0.62	11.7 ± 0.53	6.5 ± 0.42	Co > C Tax + Co > C Co > Tax + Co	<0.001* 0.39 <0.001*
TAS (µmol Trolox equivalent/mg protein)	8.5 ± 0.47	3.5 ± 0.43	7.8 ± 0.22	C > Co C > Tax + Co Tax + Co > Co	<0.001* 0.008* <0.001*

* Statistically significant; Significance level p<0.05; MDA: malondialdehyde, tGSH: total glutathione, SOD: superoxide dismutase, TOS: total oxidant status, TAS: total antioxidant status.

and SOD levels and TAS were statistically significantly higher than the Co group.

The histopathological analysis results for the control group indicated a normal histological structure of the sciatic nerve samples, including the myelinated nerve fibers together with the axon in the central location, the surrounding Schwann cells, the connective tissue around the bundle of myelinated nerve fibers, and the vessels (Fig. 1A). In contrast to the control group, degenerated and swollen myelinated nerve fiber axons, loss of the central location, and hypertrophic and hyperplasic images of the Schwann cells forming the myelin sheath and the vessel congestions were observed in the Co group. It was observed that peripheral nerve fascicles lost their connection with the perineurium. The perineurium was irregular and discrete (Fig. 1B and 1C). In the Tax + Co group, the myelinated

nerve fibers were observed mostly in a normal structure with the centrally located axons. Additionally, the Schwann cells displayed normal morphology, and the vessel congestion was not observed (Fig. 1D). The quantitative data concerning histopathological changes in sciatic nerves are presented in Table 2.

DISCUSSION

The most important finding of the current study was that cobalt-induced oxidative damage in the sciatic nerve could be reduced with the administration of taxifolin. Lethal dose 50 (LD50) values in Wistar rats have been determined for different Co compounds. Speijer et al. (1982) determined the LD50 dose for cobalt chloride as 418 mg/kg in Wistar rats. Death has
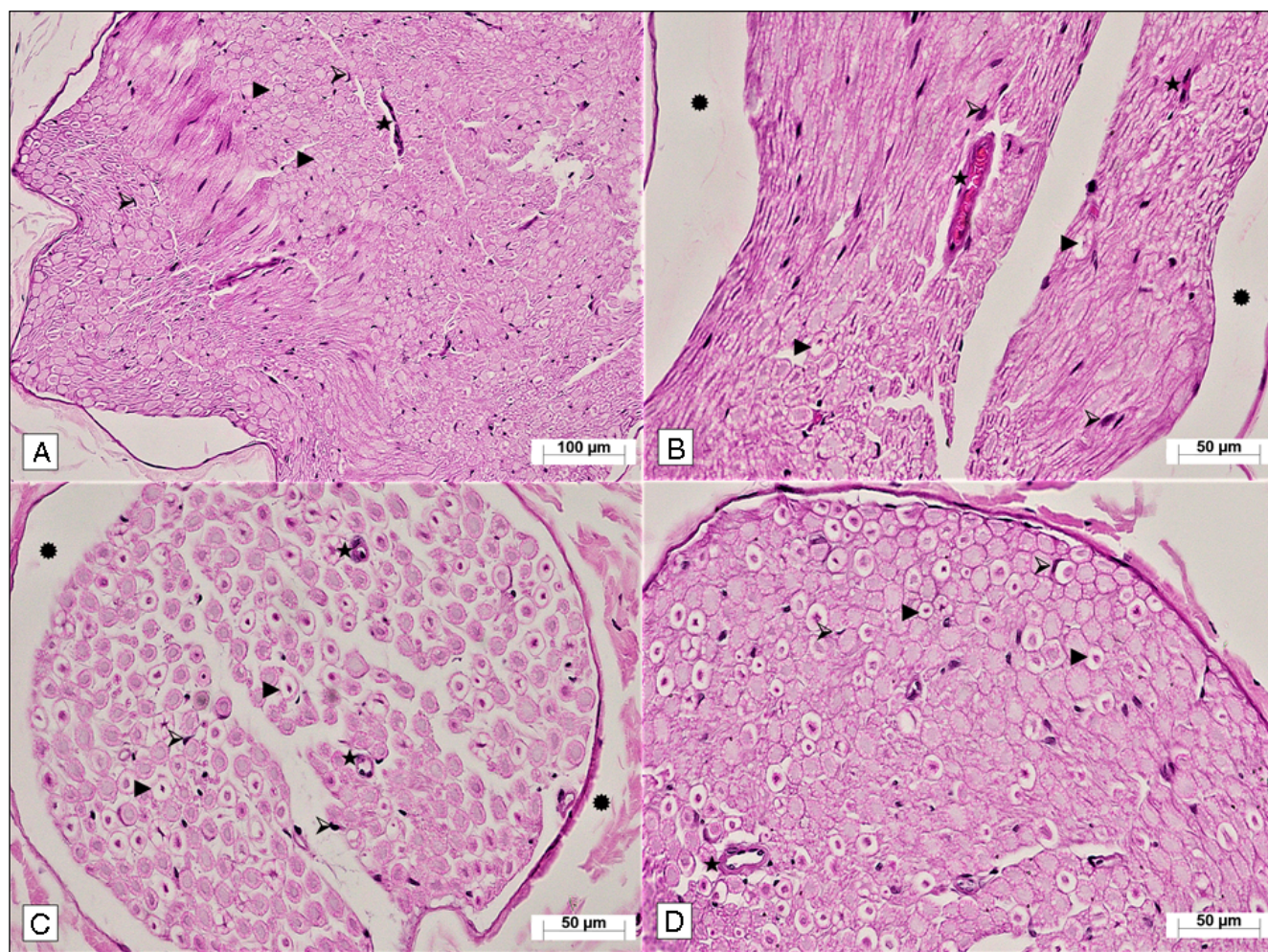

Fig. 1. (A) Hematoxylin-eosin staining of the sciatic nerve section in the control group; myelinated axon (▶), Schwann cell (➤), blood vessel (★), ×200. (B) Hematoxylin-eosin staining of the sciatic nerve section in the cobalt group; degenerated myelinated axon (▶), hypertrophic and hyperplasic Schwann cell (➤), congested blood vessel (★), disjunction in perineurium (✳) ×400. (C) Hematoxylin-eosin staining of the sciatic nerve section in the cobalt group; degenerated myelinated axon (▶), hypertrophic and hyperplasic Schwann cell (➤), congested blood vessel (★), disjunction in perineurium (✳) ×400. (D) Hematoxylin-eosin staining of the sciatic nerve section in the cobalt + taxifolin group; myelinated axon (▶), Schwann cell (➤), blood vessel (★), ×400.

Table 2. Means ± standard deviations and the comparison of the groups according to the histopathological analysis results.

	Control Mean ± SD	Cobalt Mean ± SD	Cobalt + Taxifolin Mean ± SD	P Value
Destruction	0.0 ± 0.0	2.5 ± 0.5	0.5 ± 0.5	<0.001
Perineurium Disjunction	0.0 ± 0.0	2.7 ± 0.4	0.4 ± 0.3	<0.001
Schwann Cell Degeneration	0.0 ± 0.0	2.5 ± 0.6	0.6 ± 0.4	<0.001
Congestion	0.0 ± 0.0	2.5 ± 0.5	0.5 ± 0.5	<0.001

The severity of histopathological findings in each section was graduated as 0-normal, 1-mild damage, 2-moderate damage, and 3-severe damage.

been reported in Sprague-Dawley rats by the gavage administration of 161 mg/kg Co chloride (Domingo et al., 1984; Faroon, 2004). In our study, 150 mg/kg CoCl$_2$ was applied and we did not observe any physical side effects.

In a study of Wang et al. (2009) after a single oral dose of 50 mg/kg Tax, the peak plasma concentration as 1438.8 ± 359.7 ng/mL, time to maximum concentration as 9.5 ± 3.3 min, the half-life value as 48.0 ± 29.0 min were measured in rats. It has been shown that the rapid absorption of Tax from the gastrointestinal tract and rapid distribution to the blood plasma, liver, heart, spleen, brain, skeletal muscles, lungs and kidneys of the rats. Tax is then metabolized and excreted *via* urine, but the amount of pure taxifolin eliminated through urine is quite lower (Wang et al., 2009; Das et al., 2021). Severe side effects due to Tax have not yet been reported (Das et al., 2021). Additionally, animal studies have shown that Tax is nontoxic even at high doses (Bernatova and Liskova, 2021). The highest dose of Tax applied on rats was 1,500 mg/kg, and this dosage was considered to be the "No Observed Adverse Effect Level of Tax." Assuming a factor of 100, the acceptable daily intake dose for humans was calculated to be 15 mg/kg (Das et al., 2021). In our study, a 50 mg/kg/day dosage of taxifolin was administered.

We examined MDA, tGSH, and SOD levels, TOS, and TAS to investigate the oxidative stress. MDA is a marker that indicates the severity of lipid peroxidation, which causes the destruction of structural proteins and cellular structures (Ohkawa et al., 1979).

Glutathione plays an important role in the oxidation-reduction balance of cells by removing toxic metabolites and maintaining their sulfhydryl groups (Sedlak and Lindsay, 1968). The antioxidant system typically responds through enzymes associated with glutathione synthesis to any stress inducer (Kubrak et al., 2012). SOD is a metalloenzyme that catalyzes the conversion of superoxide to hydrogen peroxide. The measurement of TAS and TOS capacities provides more valuable information than the individual measurements of oxidants and antioxidants. Measuring antioxidant and oxidants individually is time-consuming and expensive, as it requires the use of complex techniques. Therefore, TAS and TOS measurements are more commonly adopted methods (Erel, 2004; 2005).

Co is an essential element for the synthesis of vitamin B12, as it leads to human cell differentiation and development. Exposure to high levels of Co is also known to have toxic effects (Apostoli et al., 2013). Co toxicity was introduced for the first time in 1966 with the addition of a foam-stabilizing agent containing Co into beer, called "Beer Drinker's Cardiomyopathies" (Morin and Daniel, 1967; Apostoli et al., 2013; Peters et al., 2017). Co toxicity primarily affects mitochondrial functions (Karovic et al., 2007; Catalani et al., 2012). Exposure to high levels of Co destructs the mitochondrial membrane and increases the release of apoptogenic factors (Karovic et al., 2007; Catalani et al., 2012). Co-related mitochondrial dysfunction mimics hypoxia, which leads to DNA fragmentation, activates caspase, and increases reactive oxygen species (ROS) production (Karovic et al., 2007; Catalani et al., 2012; Lan et al., 2016). When the amount of ROS exceeds the antioxidant capacity of the cell, irreversible cell damage occurs (Lan et al., 2016).

Although Co is believed to be an inert ion *in vitro*, several studies have shown increased serum and remote tissue Co ion levels due to wear, corrosion, stress, friction, and fatigue of implants *in vivo* (Keegan et al., 2008; Apostoli et al., 2013). The toxic effects of Co particles, which are released from Co alloy implants, have been demonstrated in the literature (Keegan et al., 2008; Clarke et al., 2015). Although the mechanism of Co toxicity has not yet been fully elucidated, findings such as blindness, deafness, tinnitus, hand tremor, cognitive impairment, depression, vertigo, heart failure, and peripheral neuropathy related to Co toxicity have been reported (Keegan et al., 2008; Catalani et al., 2012; Apostoli et al., 2013; Clarke et al., 2015). Although the *in vivo* studies about Co-related polyneuropathy are limited, it is known to be a significant factor that negatively affects quality of life

(Oldenburg et al., 2009; Ikeda et al., 2010). The auditory and visual neuropathic effects of Co have been extensively investigated (Apostoli et al., 2013).

In the histopathological examinations of our study, destruction, perineurium disjunction, Schwann cell degeneration, and congestion levels were statistically significantly higher in the Co group compared to the control group. In a study by Apostoli et al. (2013) which investigated the neuropathic effects of Co in rabbits, severe retinal and cochlear ganglion cell depletion, along with optic nerve damage and loss of sensory cochlear hair cells, were observed. However, unlike our study results, no significant ultrastructural changes were observed in the sciatic nerve. The different results may be related to the usage of rabbits and the application of lower amounts of Co in the study by Apostoli et al. (2013).

In our study, we found that MDA levels and TOS were statistically significantly higher in the Co group compared to the control group. In the Co group, tGSH and SOD levels and TAS were statistically significantly lower than in the control group. Kubrak et al. (2012) also reported that high doses of Co ions increased the reactive oxygen products in the heart and gills of goldfish, inducing the antioxidant defense system by activating enzymes, such as SOD.

Taxifolin is a bioactive constituent of onion, French maritime pine bark, milk thistle, orange, and grapefruit (Zu et al., 2014; Tanaka et al., 2019; Yang et al., 2019). Tax is a catechol-type flavonoid that has strong antioxidant and anti-glycation activity (Nam et al., 2015; Tanaka et al., 2019). Tax, which has been widely used in medicine, improves immune system, reduces the formation of cancer cells, exhibits strong antibacterial activity, and prevents neurocognitive disorders (Zu et al., 2014; Tanaka et al., 2019; Yang et al., 2019). Tax acts as an antioxidant that reduces the reactive oxygen species and enzymes inhibition, inducing the chelation of metal ions and generating membrane-bound antioxidants (Leopoldini et al., 2004; Yang et al., 2019). It has been demonstrated that the antioxidant activity of Tax is due to the electrochemical redox potentials, especially that of the ring A hydroxyl groups evaluated by deoxyribose degradation assay (Chobot et al., 2016; Sunil and Xu, 2019).

In our study, we determined the significant antioxidant effects of Tax in preventing Co-induced oxidative nerve damage by reducing MDA levels and TOS and by increasing the tGSH, SOD levels, and TAS. Additionally, the histological analysis results support the protective effect of Tax on Co-induced sciatic nerve injury.

Some studies have revealed the antioxidant effect of Tax on different types of cells, and our results also support these results (Peters et al., 2017; Sunil and Xu, 2019; Yang et al., 2019). Zhang et al. (2019) have proven that Tax increases TAS and SOD activity in cardiomyocytes, decreases MDA levels, and diminishes the oxidative processes triggered by Di-2-ethylhexyl phthalate. Tang et al. (2019) reported that Tax increases SOD activity and reduces MDA levels in ischemic myocardial cells. Nam et al. (2015) found that Tax reduces MDA levels in proteasome inhibitor-induced oxidative processes and promotes glutathione formation.

In our study, Tax induced a statistically significant decrease in MDA levels and TOS, which is an indicator of oxidant activity. We also observed a statistically significant increase in TAS and levels of mean tGSH and SOD, which indicate the antioxidant activity. We detected no statistically significant difference between the Tax + Co and control groups in terms of mean TOS. The absence of a significant difference is evidence that Tax could reverse the increased total oxidant status due to Co toxicity to the mean values measured in the control group.

Our study is the first study, which investigate the protective effects of Tax on Co-induced peripheral neuropathy by measuring the TOS, the TAS, and the levels of MDA, tGSH, and SOD quantitatively. We noticed that the histopathological findings of Co-induced sciatic nerve damage were milder in the Tax + Co group compared to the Co group. We believe that the milder histopathological changes could be a result of the protective effect of Tax on the sciatic nerve.

There are some limitations of our study. This is an experimental study affected by all the biases of animal studies. We did not evaluate the effect of the Co and Tax levels on blood or on sciatic nerve tissue, and we did not analyze the effects of Tax and Co with different doses and durations. We designed our study with a sample size of six per study group, which may be considered relatively small. However, the total sample size prior to the study was calculated to be at least 15 models and the *post hoc* analysis of our study has revealed that the power of the study was 99%.

CONCLUSION

Our experimental data suggests that low doses of Tax, when co-administered with cobalt, may alleviate cobalt-related sciatic nerve injury. Further studies should focus on the *in vivo* antioxidant pathways of Tax to determine the protective mechanism on nerve tissue. Prospectively, well-designed human studies are necessary to determine whether these antioxidants, which have been shown to be effective in preventing nerve damage in rats, are also effective in humans.

REFERENCES

Akinrinde AS, Adebiyi OE (2019) Neuroprotection by luteolin and gallic acid against cobalt chloride-induced behavioural, morphological and neurochemical alterations in Wistar rats. Neurotoxicology 74: 252–263.

Andersson L, Hedbrant A, Bryngelsson I-L, Persson A, Johansson A, Ericsson A, Vihlborg P, Sjögren B, Särndahl E, Stockfelt L, Westberg H (2020) Respiratory health and inflammatory markers – exposure to cobalt in the swedish hard metal industry. J Occup Environ Med 62: 820–829.

Apostoli P, Catalani S, Zaghini A, Mariotti A, Poliani PL, Vielmi V, Semeraro F, Duse S, Porzionato A, Macchi V, Padovani A, Rizzetti MC, et al. (2013) High doses of cobalt induce optic and auditory neuropathy. Exp Toxicol Pathol 65: 719–727.

Ashraf T, Taneez M, Kalsoom S, Irfan T, Shafique MA (2021) Experimental calculations of metals content in skin-whitening creams and theoretical investigation for their biological effect against tyrosinase enzyme. Biol Trace Elem Res 199: 3562–3569.

Basu P, Basu A (2020) In vitro and in vivo effects of flavonoids on peripheral neuropathic pain. Molecules 25: 1171.

Battaglia V, Compagnone A, Bandino A, Bragadin M, Rossi CA, Zanetti F, Colombatto S, Grillo MA, Toninello A (2009) Cobalt induces oxidative stress in isolated liver mitochondria responsible for permeability transition and intrinsic apoptosis in hepatocyte primary cultures. Int J Biochem Cell Biol 41: 586–594.

Bernatova I, Liskova S (2021) Mechanisms modified by (−)-epicatechin and taxifolin relevant for the treatment of hypertension and viral infection: knowledge from preclinical studies. Antioxidants 10: 467.

Cai C, Liu C, Zhao L, Liu H, Li W, Guan H, Zhao L, Xiao J (2018) Effects of taxifolin on osteoclastogenesis in vitro and in vivo. Front Pharmacol 9: 1286.

Catalani S, Rizzetti M, Padovani A, Apostoli P (2012) Neurotoxicity of cobalt. Hum Exp Toxicol 31: 421–437.

Chobot V, Hadacek F, Bachmann G, Weckwerth W, Kubicova L (2016) Pro- and antioxidant activity of three selected flavan type flavonoids: catechin, eriodictyol and taxifolin. Int J Mol Sci 17: 1986.

Clarke A, Pulikottil-Jacob R, Grove A, Freeman K, Mistry H, Tsertsvadze A, Connock M, Court R, Kandala NB, Costa M, Suri G, Metcalfe D, et al. (2015) Total hip replacement and surface replacement for the treatment of pain and disability resulting from end-stage arthritis of the hip (review of technology appraisal guidance 2 and 44): systematic review and economic evaluation. Health Technol Assess 19: 1–668.

Das A, Baidya R, Chakraborty T, Samanta AK, Roy S (2021) Pharmacological basis and new insights of taxifolin: A comprehensive review. Biomed Pharmacother 142: 112004.

Denizoğlu S, Duymuş ZY (2006) Evaluation of cobalt, chromium, and nickel concentrations in plasma and blood of patients with removable partial dentures. Dent Mater J 25: 365–70.

Domingo JL, Llobet JM, Bernat R (1984) A study of the effects of cobalt administered orally to rats. Arch Farmacol Toxicol 10: 13–20.

Erel O (2004) A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 37: 112–119.

Erel O (2005) A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38: 1103–1111.

Faroon O, Keith S (2004) Toxicological profile for cobalt. ID: 136031015.

Ikeda T, Takahashi K, Kabata T, Sakagoshi D, Tomita K, Yamada M (2010) Polyneuropathy caused by cobalt-chromium metallosis after total hip replacement. Muscle Nerve 42: 140–143.

Karovic O, Tonazzini I, Rebola N, Edström E, Lövdahl C, Fredholm BB, Daré E (2007) Toxic effects of cobalt in primary cultures of mouse astrocytes. Biochem Pharmacol 73: 694–708.

Keegan GM, Learmonth ID, Case C (2008) A systematic comparison of the actual, potential, and theoretical health effects of cobalt and chromi-

um exposures from industry and surgical implants. Crit Rev Toxicol 38: 645–674.

Kikuchi S, Ninomiya T, Kohno T, Kojima T, Tatsumi H (2018) Cobalt inhibits motility of axonal mitochondria and induces axonal degeneration in cultured dorsal root ganglion cells of rat. Cell Biol Toxicol 34: 93–107.

Kruger NJ (1994) The Bradford Method for Protein Quantitation. In: Basic Protein and Peptide Protocols. Humana Press, New Jersey, p. 9–16.

Kubrak OI, Rovenko BM, Husak V V, Vasylkiv OY, Storey KB, Storey JM, Lushchak VI (2012) Goldfish exposure to cobalt enhances hemoglobin level and triggers tissue-specific elevation of antioxidant defenses in gills, heart and spleen. Comp Biochem Physiol Part C Toxicol Pharmacol 155: 325–332.

Lan AP, Chen J, Chai ZF, Hu Y (2016) The neurotoxicity of iron, copper and cobalt in Parkinson's disease through ROS-mediated mechanisms. BioMetals 29: 665–678.

Leopoldini M, Pitarch IP, Russo N, Toscano M (2004) Structure, conformation, and electronic properties of apigenin, luteolin, and taxifolin antioxidants. A first principle theoretical study. J Phys Chem A 108: 92–96.

Morin Y, Daniel P (1967) Quebec beer-drinkers' cardiomyopathy: etiological considerations. Can Med Assoc J 97: 926–928.

Nam YJ, Lee DH, Shin YK, Sohn DS, Lee CS (2015) Flavanonol taxifolin attenuates proteasome inhibition-induced apoptosis in differentiated PC12 cells by suppressing cell death process. Neurochem Res 40: 480–491.

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351–358.

Oldenburg M, Wegner R, Baur X (2009) Severe cobalt intoxication due to prosthesis wear in repeated total hip arthroplasty. J Arthroplasty 24: 825.e15-825.e20.

Peters RM, Willemse P, Rijk PC, Hoogendoorn M, Zijlstra WP (2017) Fatal cobalt toxicity after a non-metal-on-metal total hip arthroplasty. Case Rep Orthop 2017: 1–5.

Salcan I, Dilber M, Bayram R, Suleyman E, Erhan E, Karahan Yi S, Naz Yazici G, Coban A, Suleyman H (2020) Effect of taxifolin on cobalt-induced ototoxicity in rats: a biochemical and histopathological study. Int J Pharmacol 16: 522–528.

Sedlak J, Lindsay RH (1968) Estimation of total, protein-bound, and non-protein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 25: 192–205.

Speijers GJA, Krajnc EI, Berkvens JM, Logten MJ van (1982) Acute oral toxicity of inorganic cobalt compounds in rats. Food Chem Toxicol 20: 311–314.

Sun Y, Oberley LW, Li Y (1988) A simple method for clinical assay of superoxide dismutase. Clin Chem 34: 497–500.

Sunil C, Xu B (2019) An insight into the health-promoting effects of taxifolin (dihydroquercetin). Phytochemistry 166: 112066.

Tanaka M, Saito S, Inoue T, Satoh-Asahara N, Ihara M (2019) Novel therapeutic potentials of taxifolin for amyloid-β-associated neurodegenerative diseases and other diseases: recent advances and future perspectives. Int J Mol Sci 20: 2139.

Tang Z, Yang C, Zuo B, Zhang Y, Wu G, Wang Y, Wang Z (2019) Taxifolin protects rat against myocardial ischemia/reperfusion injury by modulating the mitochondrial apoptosis pathway. PeerJ 7: e6383.

Tanoğlu O, Say F, Yücens M, Alemdaroğlu KB, İltar S, Aydoğan NH (2020) Titanium alloy intramedullary nails and plates affect serum metal ion levels within the fracture healing period. Biol Trace Elem Res 196: 60–65.

Uddin MS, Mamun A Al, Rahman MA, Kabir MT, Alkahtani S, Alanazi IS, Perveen A, Ashraf GM, Bin-Jumah MN, Abdel-Daim MM (2020) Exploring the promise of flavonoids to combat neuropathic pain: from molecular mechanisms to therapeutic implications. Front Neurosci 14: 478.

Wang X, Xia H, Xing F, Deng G, Shen Q, Zeng S (2009) A highly sensitive and robust UPLC–MS with electrospray ionization method for quantitation of taxifolin in rat plasma. J Chromatogr B 877: 1778–1786.

Yang CL, Lin YS, Liu KF, Peng WH, Hsu CM (2019) Hepatoprotective mechanisms of taxifolin on carbon tetrachloride-induced acute liver injury in mice. Nutrients 11: 2655.

Yaşar H, Ersoy A, Keskin Cimen F, Mammadov R, Kurt N, Arslan Y (2019) Peripheral neurotoxic effects of cisplatin on rats and treatment with rutin. Adv Clin Exp Med 28: 1537–1543.

Zhang Y, Shi G, Cai J, Yang J, Zheng Y, Yu D, Liu Q, Gong Y, Zhang Z (2019) Taxifolin alleviates apoptotic injury induced by DEHP exposure through cytochrome P450 homeostasis in chicken cardiomyocytes. Ecotoxicol Environ Saf 183: 109582.

Zheng F, Luo Z, Zheng C, Li J, Zeng J, Yang H, Chen J, Jin Y, Aschner M, Wu S, Zhang Q, Li H (2019) Comparison of the neurotoxicity associated with cobalt nanoparticles and cobalt chloride in Wistar rats. Toxicol Appl Pharmacol 369: 90–99.

Zu Y, Wu W, Zhao X, Li Y, Wang W, Zhong C, Zhang Y, Zhao X (2014) Enhancement of solubility, antioxidant ability and bioavailability of taxifolin nanoparticles by liquid antisolvent precipitation technique. Int J Pharm 471: 366–376.