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Hesperidin protects brain and sciatic nerve tissues against cisplatin-induced oxidative, histological and electromyographical side effects in rats

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Abstract

In the present study, the beneficial effect of hesperidin (HP), a *citrus* flavonoid, on cisplatin (CP)-induced neurotoxicity was investigated. A total of 28 rats were equally divided into four groups; the first group was kept as control. In the second and third groups, CP and HP were given at the doses of 7 and 50 mg/kg/day, respectively. In the fourth group, CP and HP were given together at the same doses. The results indicated that although CP caused significant induction of lipid peroxidations and reduction in the antioxidant defense system potency in the brain and sciatic nerve, HP prevented these effects of CP. Besides, CP led to histopathological damage, mainly apoptosis, as well as electromyographical (EMG) changes in sciatic nerve. On the other hand, HP treatment reversed histopathological and EMG effects of CP. In conclusion, CP had severe dose-limiting neurotoxic effects and these effects of CP can be prevented by HP treatment. Thus, it appears that coadministration of HP with CP may be a useful approach to attenuate the negative effects of CP on the nervous system.

Keywords

Cisplatin, hesperidin, neurotoxic effect, oxidative damage, electromyography

Introduction

Cisplatin (*cis*-diamminedichloroplatinum(II); CP) is an effective chemotherapeutic agent used for the treatment of many types of cancers including testis, lung, colorectal, ovarian, breast, and bladder (Carozzi et al., 2010). The adverse effects including reproductive nephrotoxicity and neurotoxicity limit the clinical usage of CP. Neurotoxicity is one of the most important adverse effects of CP and is caused due to dose-limiting CP (Orhan et al., 2004). The neurotoxic mechanism of CP may be due to the fact that platinum accumulates and leads to oxidative damage and apoptotic cell death in the nervous system (Carozzi et al., 2010). Oxidative stress caused by reactive oxygen species can be mainly responsible for peripheral and central neurotoxicity of CP (Rosenberg, 1978). Several *in vivo* and *in vitro* studies (Barabas et al., 2008; Krarup-Hansen et al., 1993) have demonstrated that the side effects of CP on nervous system are characterized by significant changes in the electromyographical (EMG) values (reduced amplitude and induced latency), painful paresthesias of the extremities and numbness, and loss of vibration sense and ataxia. In addition, both short- and long-term administration of CP cause a significant oxidative stress by forming reactive oxygen species or free radicals with a concomitant increase in lipid peroxidation and decline in

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antioxidant enzyme activity in tissues (Beytur et al., 2012; Hartmann et al., 1999; Yüce et al., 2007). Some experimental studies (Olas and Wachowicz, 2004; Orhan et al., 2004; Rodriguez-Menendez et al., 2008; Tuncer et al., 2010) showed that the use of antioxidant therapies such as resveratrol, erythropoietin, melathonin, alpha-lipoic acid, and valproate reduces CP neurotoxicity in animal model. However, there is no study as to whether or not the hesperidin (HP) treatment can prevent CP neurotoxicity.

HP is a bioflavonoid, which is believed to play a role in plant defense, and is found abundantly in *citrus* species such as lemon and orange. Various previous studies (Choi and Ahn, 2008; Menze et al., 2012) revealed that HP exhibits pharmacological activities including antioxidant, anticarcinogenic, antihypertensive, and anti-inflammatory activities but none of which has been confirmed as applicable to humans. Shagirta and Pari (2011) determined that HP treatment protects testicular functions against cadmium toxicity because of antioxidant properties. It is therefore believed that HP is a powerful radical scavenger that promotes cellular antioxidant defense system and can also traverse the brain–blood barrier (Shagirtha and Pari, 2011; Youdim et al., 2003). Thus, HP can prevent neurodegeneration caused by toxic agents and can promote brain functions (Hwang et al., 2012).

Based on this background, we hypothesized that HP may prevent CP-induced neurotoxicity due to its intrinsic biochemical and antioxidant properties. To this end, oxidative status, histopathological, immunohistochemical, and EMG changes in the brain and sciatic nerve of rats were investigated.

Materials and methods

Chemicals

CP (10 mg/10 mL, code 1876A) was obtained from Faulding Pharmaceuticals Plc (Warwickshire, UK). HP was given from Molecula Limited (Gillingham, UK) All other chemicals were purchased from Sigma Chemical Co. (St Louis, Missouri, USA) and were of analytical grade or of the highest grade available.

Animals and treatment

A total of 28 healthy adult male Spraque Dawley rats (aged between 2 and 3 months and weighing 250–300 g) were obtained from the Experimental Animal Institute, Malatya, Turkey, for this experiment. Animals were housed in sterilized polypropylene rat cages, in a 12-h light–dark cycle, at an ambient temperature of 21°C. Diet and water for them were given *ad libitum*. Experiments were performed based on animal ethics guidelines of the Institutional Animals Ethics Committee.

Rats were randomly divided into four equal groups ($n = 7$ in each group). CP was intraperitoneally (i.p.) administered at the dose of 7 mg/kg with a single injection. HP was given at the doses of 50 mg/kg/day for 14 consecutive days by gavages. Group 1 (control) served as negative control and was given isotonic saline (i.p.) and corn oil (orally) as vehicles. In group 2 (CP group), CP was given as a single injection and then corn oil was given for 14 days. Rats in group 3 (HP group) were treated with HP for 14 days without CP. In group 4, the rats were treated with CP and HP (i.e. CP + HP group) together. Tissue samples were collected on day 14 of CP treatment. The animals were killed under ether anesthesia and tissues (brain and sciatic nerve) were immediately removed and dissected over ice-cold glass. Tissue samples were stored at −86°C deep freezer until analysis.

Biochemical assay

The homogenization of tissues was carried out in Teflon glass homogenizer with 150 mM potassium chloride (pH 7.4) to obtain 1:10 (weight in volume) dilution of the whole homogenate. The homogenates were centrifuged at 18,000 *g* (4°C) for 30 min to determine thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) levels, and catalase (CAT) activities and at 25,000 *g* for 50 min to determine glutathione peroxidase (GPx) and copper-/zinc-superoxide dismutase (SOD) activities.

The levels of homogenized tissue TBARS, as an index of lipid peroxidation, were determined by thiobarbituric acid (TBA) reaction using the method of Yagi (1998). The product was evaluated spectrophotometrically at 532 nm and the results are expressed in nanomole per gram tissue. The GSH content of the brain and nerve homogenate was measured at 412 nm using the method of Sedlak and Lindsay (1968). The GSH level was expressed in nanomole per milliliter. SOD activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction due to O_2^- generated by the xanthine/xanthine oxidase system (Sun et al., 1988). One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the NBT reduction rate. The product was evaluated

spectrophotometrically at 560 nm. Results are expressed in international unit per milligram of protein. CAT activity of the tissues was determined according to the method of Aebi (1974). The enzymatic decomposition of hydrogen peroxide (H_2O_2) was followed directly by the decrease in the absorbance at 240 nm. The difference in absorbance per unit time was used as a measure of CAT activity. The enzyme activities are given in k (katalase activity) per milligram of protein. GPx activity was measured by the method of Paglia and Valentina (1967). In the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH), the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to nicotinamide adenine dinucleotide phosphate. The decrease in absorbance at 340 nm was measured. GPx activity is expressed as international unit per milligram of protein. Tissue protein content was determined according to the method developed by Lowry et al. (1951) using bovine serum albumin as standard.

Histological evaluation

For light microscopic evaluation, brain and nerve samples were fixed in 10% formalin and were embedded in paraffin. Paraffin-embedded specimens were cut into 5-μm-thick sections, mounted on slides, and stained with hematoxylin–eosin (H&E) stain. Tissue samples were examined using a Leica DFC280 light microscope and a Leica Q Win Image Analysis system (Leica Micros Imaging Solutions Ltd, Cambridge, UK).

For immunohistochemical analysis, thick sections were mounted on polylysine-coated slides. After rehydrating, samples were transferred to citrate buffer (pH 7.6) and heated in a microwave oven for 20 min. After cooling for 20 min at room temperature, the sections were washed using phosphate-buffered saline (PBS). Then sections were kept in 0.3% H_2O_2 for 7 min and afterward washed with PBS. Sections were incubated with primary rabbit polyclonal caspase-3 antibody (Abcam, Cambridge, MA, USA, Ab4051) for 2 h. They were then rinsed using PBS and incubated with biotinylated goat anti-polyvalent for 10 min and streptavidin peroxidase for 10 min at room temperature. Staining was completed with chromogen + substrate for 15 min, and then the slides were counterstained with Mayer's hematoxylin for 1 min, rinsed in tap water, and dehydrated. The caspase-3 kit was used according to the manufacturer's instructions.

EMG assay

On day 14 of drug administration, the EMG changes were performed under general anesthesia and were carried out on the right sciatic nerve with a Dantec Keypoint electromyography (code number, 9020M0203 and serial number, 1562, Medtronic, Skovlunde, Denmark). Neurophysiological analysis was performed according to the method of Kalender et al. (2009). Briefly, for the stimulations, the sciatic nerve was percutaneously stimulated with supramaximal stimulus for the determination of compound muscle action potentials (CMAPs) with monopolar needle electrodes. Electrical stimulation was square pulse with a frequency of 1 Hz and duration of 0.2 ms. Recorded signals were amplified by an alternating current-coupled preamplifier with filters at 1 Hz and 10 KHz. CMAP records were obtained from distal gastrocnemius muscle with needle electrodes. The common reference (ground electrode) was placed on the tail. The CMAP amplitudes were measured from peak to peak and the latencies were measured from the stimulus artifact to the first deflection from baseline.

Statistical analysis

All values were presented as mean ± SEM. Significant differences ($p < 0.05$) are given in tables. A computer program SPSS 18.0 (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. For the comparison of biochemical and EMG parameters, statistical analyses were performed using one-way analysis of variance and post hoc Duncan's significant difference test.

Results

Biochemical results

The brain SOD, GPx, CAT, GSH, and TBARS values are given in Table 1. The results demonstrated that CP caused a significant increase in TBARS levels and decrease in GSH levels and antioxidant enzyme activities (CAT, SOD, and GPx) in brain tissues compared with other groups. Additionally, HP administration lowered TBARS and elevated GSH levels compared with control values. Additionally, HP treatments together with CP led to significant decline in elevated TBARS levels and induction in antioxidant enzyme activity and GSH levels close to the values of the control group.

The levels of TBARS, SOD, GPx, CAT, and GSH in sciatic nerve tissue are given in Table 2. TBARS levels were significantly increased and SOD, GPx,

Table 1. Changes in SOD, CAT, and GPx activities and GSH and TBARS levels in brain tissue of rats administered with CP and HP (mean ± SEM; $n = 7$).[a]

	TBARS (nmol/g tissue)	GSH (nmol/mL)	SOD (U/mg protein)	CAT (k/mg protein)	GPx (U/mg protein)
Control	8.60 ± 0.28[b]	90.4 ± 1.49[b]	34.2 ± 2.46[b]	0.024 ± 0.001[b]	2.39 ± 0.073[b]
CP	11.88 ± 0.72[c]	71.5 ± 2.25[c]	18.2 ± 1.48[c]	0.014 ± 0.0003[c]	1.23 ± 0.090[c]
HP	6.62 ± 0.43[d]	98.8 ± 3.31[d]	34.4 ± 1.74[b]	0.027 ± 0.0009[b]	2.41 ± 0.081[b]
CP + HP	9.62 ± 0.67[b]	86.3 ± 1.16[b]	26.0 ± 1.04[d]	0.018 ± 0.0005[d]	1.93 ± 0.112[d]

SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; GSH: glutathione; TBARS: thiobarbituric acid reactive substances; CP: cisplatin; HP: hesperidin.
[a]Means bearing different superscripts within same column were significantly different ($p \leq 0.05$).

Table 2. Changes in SOD, CAT, and GPx activities and GSH and TBARS levels in nerve tissue of rats administered with CP and HP (mean ± SEM; $n = 7$).[a]

	TBARS (nmol/g tissue)	GSH (nmol/mL)	SOD (U/mg protein)	CAT (k/mg protein)	GPx (U/mg protein)
Control	4.32 ± 0.33[b]	225.4 ± 11.4[b]	3.86 ± 0.16[b]	0.023 ± 0.0009[b]	1.95 ± 0.121[b]
CP	7.36 ± 0.51[c]	133.3 ± 2.8[c]	1.88 ± 0.27[c]	0.014 ± 0.0003[c]	1.12 ± 0.087[c]
HP	4.03 ± 0.23[b]	229.0 ± 8.5[b]	4.01 ± 0.30[b]	0.024 ± 0.001[b]	2.05 ± 0.130[b]
CP + HP	4.83 ± 0.26[b]	170.4 ± 7.9[d]	3.03 ± 0.23[d]	0.017 ± 0.0007[b]	1.83 ± 0.115[b]

SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; GSH: glutathione; TBARS: thiobarbituric acid reactive substances; CP: cisplatin; HP: hesperidin.
[a]Means bearing different superscripts within same column were significantly different ($p \leq 0.05$).

CAT, and GSH levels were significantly decreased in CP group compared with control and other groups. However, elevated TBARS levels and lowered SOD, GPx, CAT, and GSH levels were significantly reversed to the value of the control group in rats treated with HP + CP (Table 2).

Histopathological and immunohistochemical results

Histological findings indicated that the brain tissue had a normal histological appearance in control and HP groups. On the other hand, in CP (Figure 1(a)) and CP + HP (Figure 1(b)) groups, the cell infiltration in cerebral cortex was observed compared with that of control (Figure 1(c)) and HP (Figure 1(d)) groups. However, in CP + HP group (Figure 1(b)), the cell infiltration was significantly decreased in comparison with CP group (Figure 1(a)). Also, shrinkage of the cytoplasm and extensively dark pyknotic nuclei in neurons of the cerebral cortex tissue were seen in the CP group (Figure 1(e)). These abnormalities were significantly alleviated when HP has been given together with CP (Figure 1(f); H&E; ×40). The cerebellar cortex showed normal histological appearance, and Purkinje cells showed no histological abnormalities in control (Figure 2(a)) and HP (Figure 2(d)) groups. However, CP led to deeply stained, shrunken, and variously shaped Purkinje cells with pyknotic nuclei (Figure 2(b)), and HP prevented these changes when combined with CP (Figure 2(c)).

In immunohistochemical analysis, caspase-3-stained cells were not observed in the cerebral cortex of control (Figure 3(a)) and HP (Figure 3(d)) groups. On the other hand, the percentage of caspase-3-positive cells was significantly higher in the CP group than other groups (Figure 3(b)). Besides, the density of caspase-3-stained cells was minimal (Figure 3(c)) in CP + HP group. The sciatic nerve was evaluated as transverse sections, and the sciatic nerve degeneration (axonal degeneration and demyelination) was determined in CP (Figure 4(b)) and CP + HP groups (Figure 4(c)). However, these findings were decreased in CP + HP groups compared with CP group. Besides, in control (Figure 4(a)) and HP (Figure 4(d)) groups, sciatic nerve showed normal histological appearance.

EMG results

The latency and amplitude values in sciatic nerve of rats exposed to CP and HP are given in Table 3. The latency time significantly prolonged and amplitude value significantly declined with CP treatment. However, HP administration increased the lowered amplitude value and decreased the elevated latency

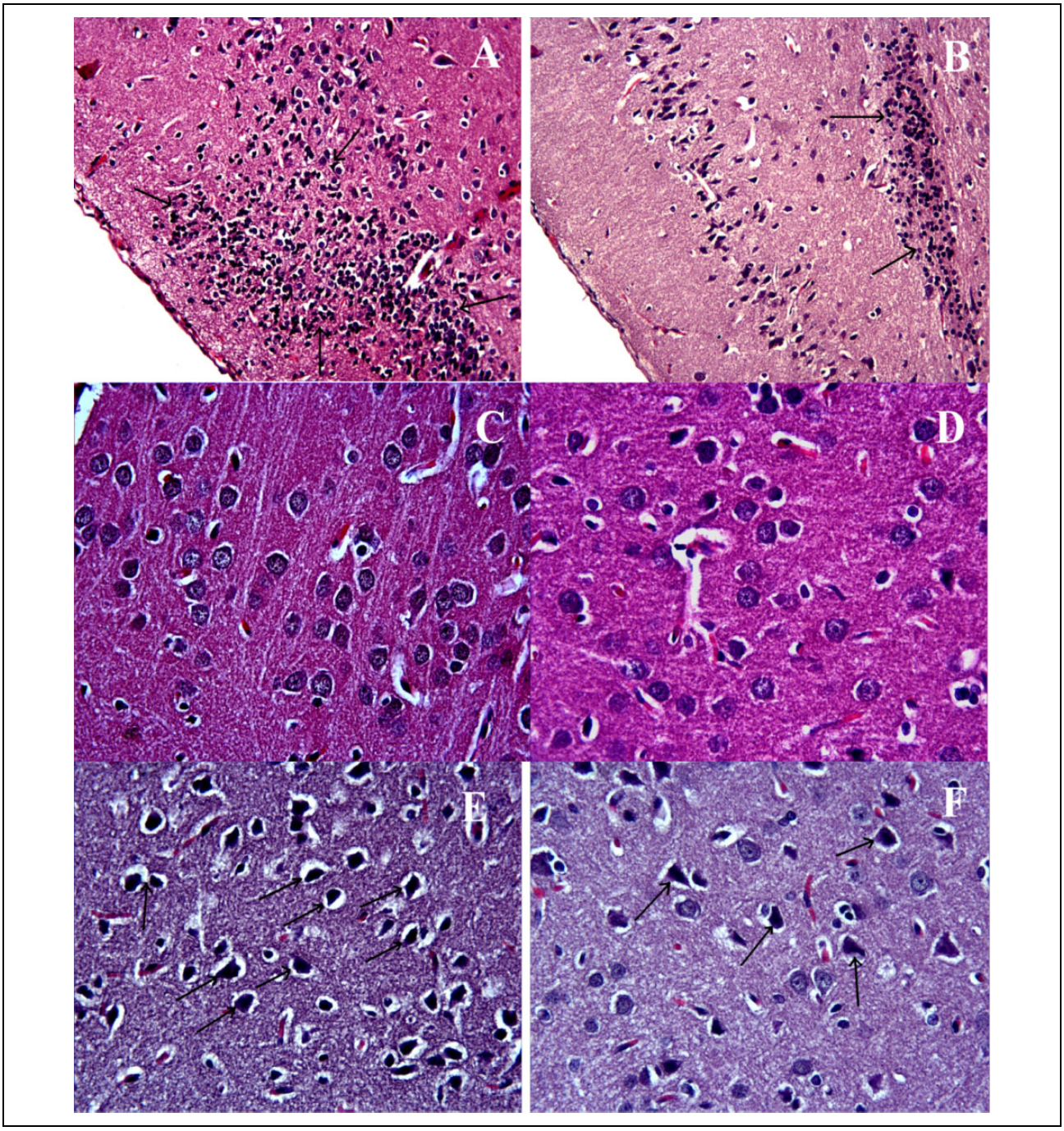

Figure 1. In CP group (a), intensive cell infiltration (arrows) was detected. This infiltration (arrows) was decreased in CP + HP group (b); H&E, ×20. In control (c) and HP (d) groups, neurons of cerebral cortex showed normal histological appearance. In CP group (e), shrinkage cytoplasm and extensively dark pyknotic nuclei in neurons (arrows) of the cerebral cortex tissues were seen. These findings (arrows) were decreased in CP + HP groups (f); H&E, ×40. CP: cisplatin; HP: hesperidin; H&E: hematoxylin–eosin.

back to the control value. These results also indicated that although CP treatment negatively affected CMAPs (decreased amplitude and increased latency), HP treatment completely brought CMAP values closer to control value.

Discussion

CP treatment can cause neurological side effects in cancer patients and this situation can be limiting the clinical dosage of CP. For that, the prevention of side

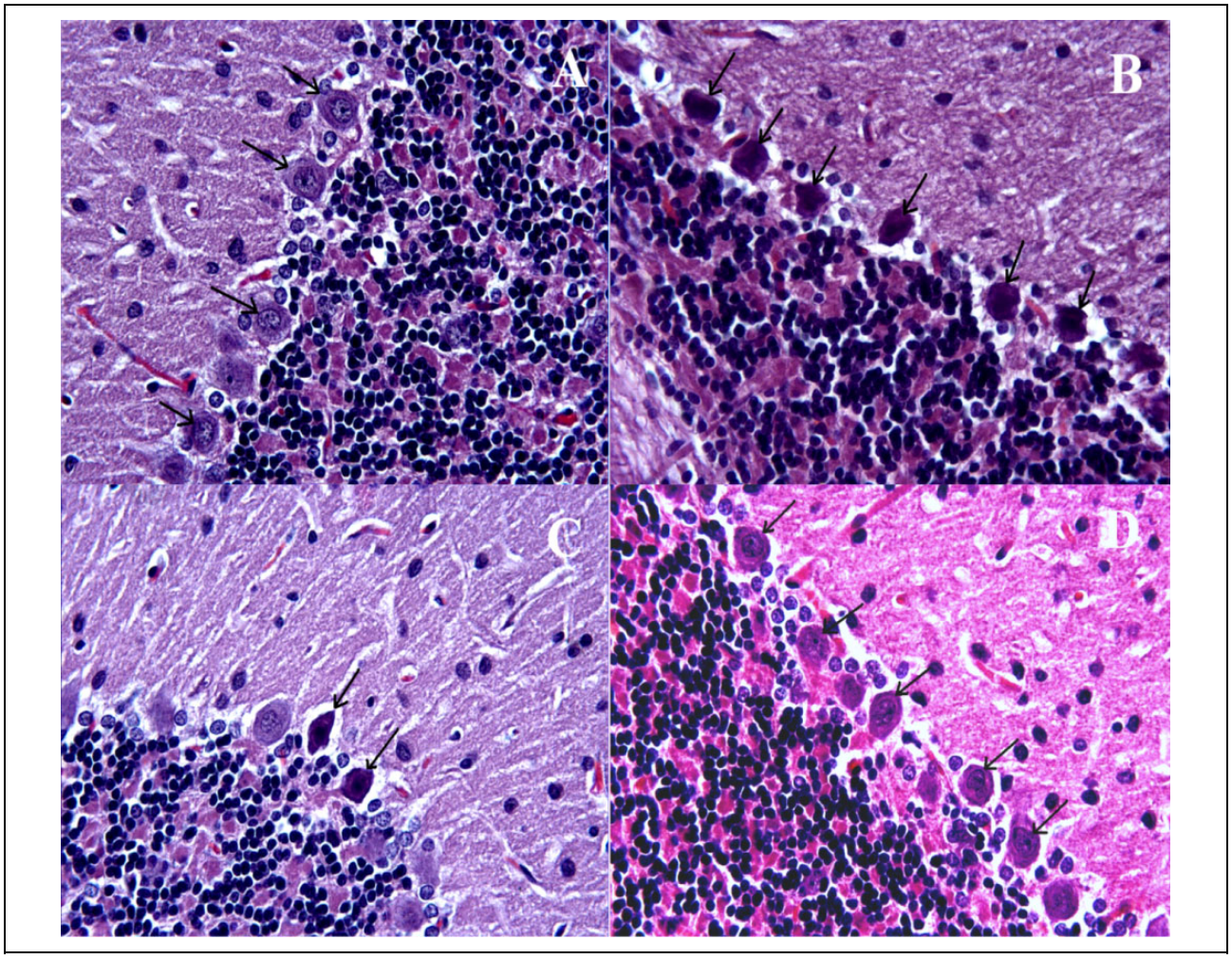

Figure 2. In control (a) and HP (d) groups, cerebellar cortex had showed normal histological appearance. In CP group (b), deeply stained Purkinje cells with pyknotic nuclei (arrows) were seen. In CP + HP group (c) Purkinje cell degeneration (arrows) was decreased; H&E, ×40. CP: cisplatin; HP: hesperidin; H&E: hematoxylin–eosin.

effects of CP is very important in terms of treatment protocol, benefits in life quality, and widening the dose limits. In the present study, it was determined that CP-induced neurotoxic effects via increased oxidative stress, histopathological defects, and EMG changes also decreased antioxidant status in rats. However, HP treatment can reverse toxic effects of CP on the nervous system when used together with CP.

Oxidative evaluations

Oxidative stress, a condition of an imbalance between the free radicals and antioxidant defense system, is an important factor in the pathogenesis of neurological disorders due to the fact that the nervous system has high content of polyunsaturated membrane lipids (Acar et al., 2012). Recent studies (Carozzi et al., 2010; Hwang et al., 2012) indicated that CP led to neurotoxic effects in human and animals via induction of lipid peroxidations. Our study showed that CP produced a significant increase in the TBARS levels; as a result, it induced lipid peroxidations in the brain and sciatic nerve. Also the enzyme activity of antioxidants (SOD, CAT, and GPx) was suppressed and the GSH levels were declined with CP treatment. Therefore, CP clearly led to an imbalance of oxidative system and caused oxidative damage in central and peripheral nervous system. Carozzi et al. (2010) indicated that oxidative stress has a significant role in the neurological toxicity of platinum complex and may be the primary effect mechanism of CP on the nervous system. Rybak et al. (2000) indicated that CP caused 165% elevation in malondialdehyde (MDA) levels, nearly 50% depletion of GSH, and about 50% reduction in the

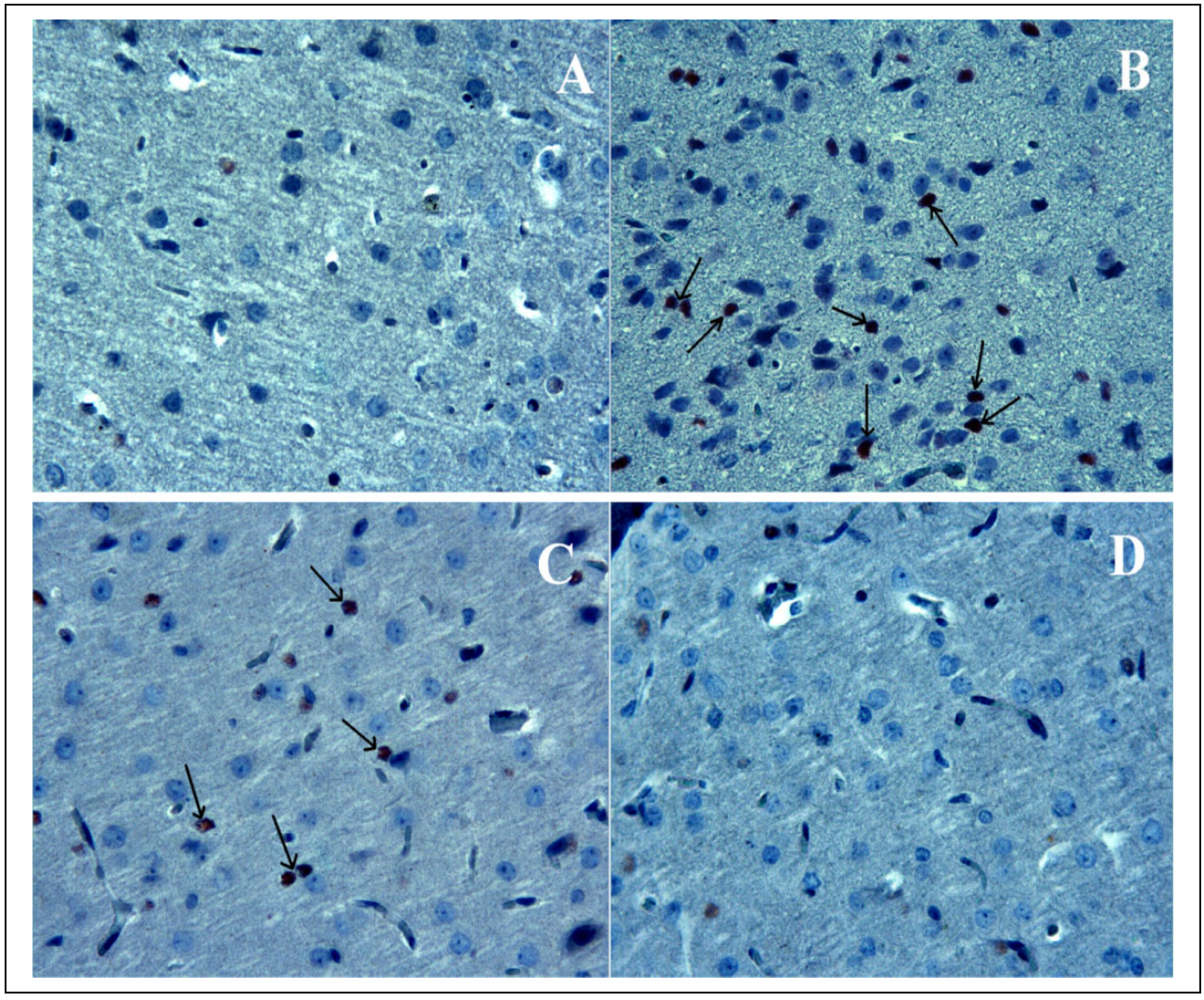

Figure 3. Immunohistochemical expression of caspase-3 in the cortex of control and experimental groups of rats. In control (a) and HP groups (d), there were no caspase-3-stained cells. Caspase-3-stained cells (arrows) increased in CP group (b). The number of positive-stained cells (arrows) decreased in CP +HP group (c); caspase-3, ×40. CP: cisplatin; HP: hesperidin.

activities of SOD, GPx, and GSH reductase, while CAT activity was reduced to 70% of the control values in rats. Also Qu et al. (2012) reported that MDA levels significantly increased; however, antioxidant parameters decreased upon CP treatment in the nervous system. These studies (Rybak et al., 2000; Qu et al., 2012) showed that CP and other platinum complexes produced oxidative changes in the nervous system and these results agree with our findings. Therefore, our results clearly demonstrate that CP induces oxidative damage in the brain and sciatic nerve of the rats.

Several researchers suggested that citrus flavonoids such as HP have strong antioxidant activity and they hypothesized that the antioxidant action of flavonoids may reduce multi-organ toxicity caused by oxidative stress (Choi and Ahn, 2008; Hwang and Yen, 2008; Hwang et al., 2012). Indeed, the present study demonstrated that HP therapy could significantly prevent oxidative stress through returning the elevated TBARS levels and declined antioxidant enzyme activity and GSH levels back to the normal in the brain and sciatic nerve when given together with CP. Choi and Ahn (2008) investigated neuroprotective effects of HP in mice and they suggested that chronic HP treatment has a neuroprotective effect by inhibiting the oxidative damage (decline of TBARS), together with activating the antioxidant defense system. In the same study, TBARS levels were declined by 21.36%, while SOD, GPx, and GSH levels were increased by 20.58%, 15.15%, and 21.83%,

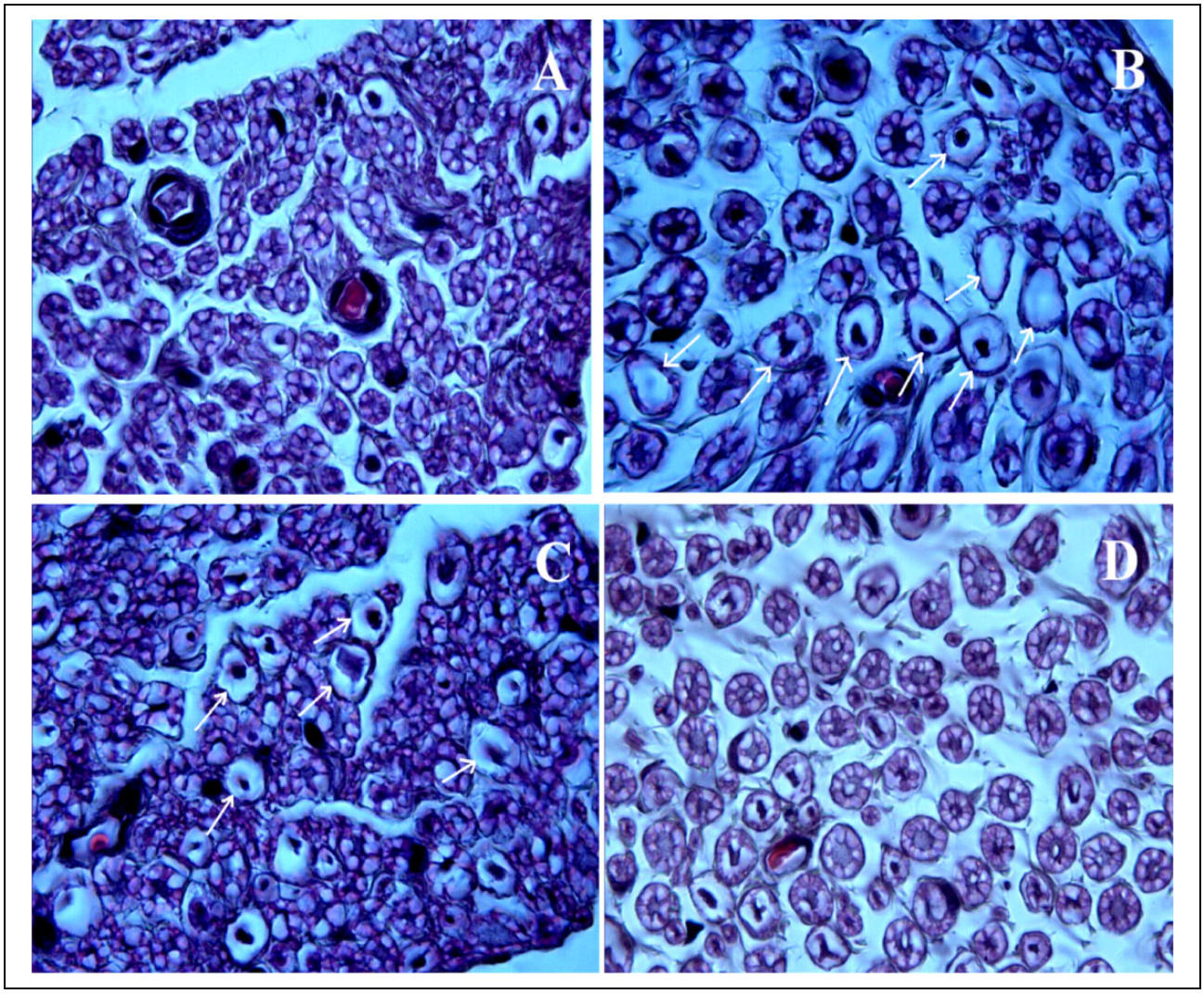

Figure 4. We compared sciatic nerve transverse sections in all the groups. The control (a) and HP (d) groups showed normal histological appearance. In CP group (b), nerve degeneration was prominent. This degeneration was decreased in CP + HP group (c); H&E, ×100. CP: cisplatin; HP: hesperidin; H&E: hematoxylin–eosin.

Table 3. The CMAP values for CP and HP groups ($n = 7$, mean ± SEM).[a]

Group	Latency (ms)	Amplitude (mV)
Control	1.32 ± 0.051[b]	13.11 ± 0.73[b]
CP	2.31 ± 0.112[c]	10.22 ± 0.57[c]
HP	1.20 ± 0.043[b]	14.70 ± 0.61[b]
CP + HP	1.25 ± 0.048[b]	13.34 ± 0.61[b]

CMAP: compound muscle action potential; CP: cisplatin; HP: hesperidin.
[a]Means bearing different superscripts within same column were significantly different ($p \leq 0.05$).

respectively, with HP treatment in mice. Besides, Menze et al. (2012) investigated the potential effects of HP against 3-nitropropionic acid-induced neurotoxicity in rats and indicated that HP significantly prevented neurotoxic effects via its antioxidant properties. All the results from previous studies agree with our results. Finally, HP treatment can be beneficial for the neurotoxic effects of CP when given together, and neuroprotection may be due to its radical scavenging, antioxidant, and anti-inflammatory activities.

Histological changes

In the present study, it was demonstrated that CP significantly produced histopathological alterations in the brain and sciatic nerve tissues. Primarily, hemorrhage in arachnoid layer, cell infiltration in cerebral cortex, shrinkage in the cytoplasm, and extensively dark pyknotic nuclei in neurons of the cerebral cortex

were observed in the brain tissue after CP treatment. Additionally, apoptosis was clearly seen in neurons of the cerebral cortex upon immunohistochemical evaluation, and these data were supported by caspase-3 staining. Demyelination and axonal degeneration occurred in sciatic nerve after CP administration. However, histopathological damages formed were significantly reversed with HP-combined treatment. Results from previous studies (Abou-Elghait et al., 2010; Kirchmair et al., 2005) are in agreement with our findings, and they determined that CP treatment increased histopathological damage in brain and sciatic nerve tissue. Menze et al. (2012) also reported similar findings that HP treatment can be beneficial for the protection of the nervous system against 3-nitropropionic acid-induced neurotoxicity in rats. It was considered that the histopathological effects may be attributed to the imbalance between oxidant and antioxidant status induced by CP. Moreover, this situation may contribute to severe neurological disorders such as peripheral neuropathy. For this reason, a decrease in elevated oxidative stress in the nervous system as a result of HP treatment is of importance in CP neurotoxicity.

EMG alterations

Peripheral neurotoxicity, determined by CMAPs latency and CMAPs amplitude with electromyography, is the main toxic effect of CP. First, we showed that CP administration significantly prolonged the CMAPs latency. Also the mean amplitude of CMAPs, depending on the number of axons that conduct impulses from the stimulus point to the muscle, the number of functioning motor end-plates, and the muscle volume (Korte et al., 2011; Stålberg and Erdem, 2002) were significantly declined with CP treatment. Because CMAPs (compound muscle action potentials) latency depends on the degree of demyelination in tissue, the histological findings about demyelination in the current study support this conclusion. Besides, it was indicated that the fall in the mean amplitude of CMAPs and the elevation in the duration of distal latency implies that a significant number of sciatic nerve fibers were affected by the CP leading to peripheral neuropathy. The previous studies (Carozzi et al., 2009; Tuncer et al., 2010) demonstrated that CP treatment caused peripheral neuropathy that led to a decrease in maximum and minimum upstroke velocity values in rats. These findings were in agreement with our findings. The peripheral neurotoxic effects of CP may result from its oxidative and histopathological properties on the sciatic nerve. In this context, for preventing CP effects on peripheral neuropathy, many antioxidant agents were tested such as melathonin, alpha lipoic acid, and valproate (e.g., Orhan et al., 2004; Rodriguez-Menendez et al., 2008; Tuncer et al., 2010) and some of these were found to be efficient. When given together with CP, HP prevented side effects of CP in terms of peripheral neuropathy via a significant decline in elevated latency value and induction in lower amplitude value. These results suggested that HP treatment can fully reverse peripheral neuropathy induced by CP with changing latencies and amplitude values.

Conclusion

In conclusion, the present study revealed the toxic effects of CP on central and peripheral nervous system with oxidative stress, histopathological damage, and EMG alterations. Also, the use of HP in combination with CP minimized its toxicity, which was evident from decreasing TBARS levels, histological changes in tissue, EMG alterations, and increasing antioxidant enzyme activities (SOD, CAT, and GPx) and GSH levels. The beneficial effects of HP against CP-induced neurological damage may be due to its antioxidant, anti-inflammatory, and free radical-scavenging properties. Therefore, it appears that HP, a citrus flavonoid, can prevent and protect against neurological damage caused by CP in rats.

Conflict of interest

The authors declared no conflict of interest.

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