European Review for Medical and Pharmacological Sciences 2022; 26: 6935-6943

Protective effect of short-term thymoquinone administration on the central nervous system in cisplatin-induced neurotoxicity

M.A. DURAK[1], O. OZHAN[2], A. YILDIZ[3], M. DURHAN[4], N. VARDI[3], Y. CIGREMIS[4], H. PARLAKPINAR[2]

[1]Department of Neurosurgery, [2]Department of Pharmacology, [3]Department of Histology and Embryology, [4]Department of Medical Biology and Genetic, Medical School, Inonu University, Malatya, Turkey

Abstract. – OBJECTIVE: This study was performed to investigate the potential beneficial effects of thymoquinone (TQ) on brain tissue based on biochemical and histopathological analyses in cisplatin (CIS) treated rats with central nervous system (CNS) neurotoxicity.

MATERIALS AND METHODS: The rats were randomly divided into 4 groups with 8 rats in each group (n:8). Group 1: (Control), saline was administered for 3 days at a volume of 0.5 ml per day intraperitoneal (i.p.). Group 2: (CIS Group), one dose of CIS was administered (7 mg/kg i.p.). Group 3: (TQ Group), TQ was given at a dose of 5 mg/kg per day for 3 days (i.p.). Group 4: (CIS+TQ Group), one dose of 7 mg/kg was initiated half an hour before administration of CIS and one dose of 5 mg/kg per day was administered TQ i.p. for 3 days.

RESULTS: Malondialdehyde levels were found to be statistically significantly higher in the CIS group compared to the control group. Degenerative changes observed in the CIS+TQ group were found to be milder than in the CIS group. In the CIS+TQ group, a statistically significant decrease in the severity of caspase-3 immunoreactivity was found when compared to the CIS group. It was found that the severity of neurofilament immunoreactivity monitored in neuronal extensions was similar in all groups. In the CIS+TQ group, the severity of tau protein's immunoreactivity was similar to that of the CIS-group.

CONCLUSIONS: According to the results obtained in our study, beneficial effects were obtained in reducing neurotoxicity with short-term TQ application in rats treated with CIS treatment.

Key Words:
Thymoquinone, Cisplatin, Neurotoxicity, Oxidative stress, Rat.

Introduction

Cancer is a leading cause of death worldwide and is commonly treated with chemotherapy using platinum complex agents. Cisplatin (CIS) was the first of these agents to be developed for clinical use and is still widely used in cancer treatment[1,2]. Platinum complexes trigger programmed cell death mediated by the formation of intra-strand DNA crosslinks and thus inhibit replication[1,3]. In clinical practice, CIS is used alone or in combination with other chemotherapeutic agents or radiotherapy. It is an active heavy metal compound that can be used to treat many cancer types, including lung, bladder, testicular, breast, pancreatic, endometrial, esophageal, and head and neck cancers, lymphoma, and melanoma[4,5]. The efficacy of CIS increases significantly with increasing doses, but higher doses are associated with higher rates of side effects, such as serious nephrological toxicity, ototoxicity, and neurotoxicity, and therefore their use is limited[4,6,7]. Neurotoxicity is the most common side effect seen during the use of CIS, which causes dose limitation[6,8]. Some previous studies[8,9] reported that antioxidant supportive therapy is beneficial for reducing the neurotoxicity associated with platinum-based chemotherapy, and that it increases the effectiveness of anticancer treatment; many bioflavonoids have been used for this purpose. Studies[10-13] on these effects of flavonoids have showed that thymoquinone (TQ) from black cumin seeds has antioxidant, antiallergenic, antiviral, antidiabetic, anti-inflammatory, and anticarcinogenic activities. Previously, TQ compounds have been reported[11,14,15] to have neuroprotective effects. Overall, those studies focused on neurotoxicity in

Corresponding Author: Mehmet Akif Durak, MD, Ph.D; e-mail: akif.durak@inonu.edu.tr

the peripheral nervous system. However, central nervous system (CNS) neurotoxicity may also occur in patients undergoing treatment with CIS, and there have been very few studies[16,17] on the effect of TQ on these side effects.

Our study was performed to investigate the potential beneficial effects of TQ on brain tissue based on biochemical and histopathological analyses in CIS-treated rats with CNS neurotoxicity.

Materials and Methods

Animals and Groups

This study was conducted with the approval of the Animal Ethics Committee of Inönü University (Reference Number: 2016/A-11). Thirty-two Wistar albino male rats, 8-10 weeks old and weighing 210-270 g, were obtained from the Inönü University Laboratory Animal Research Center. The rats were placed in a controlled environment with a constant humidity of 60±5% and temperature of 21±2°C under a 12 h/12 h light/dark cycle. The rats were divided into four groups of eight animals each by random selection: the Control group received intraperitoneal (i.p.) injection of 0.5 mL saline daily for 3 days; the CIS group received a single dose of 7 mg/kg CIS i.p.; the TQ group received TQ at 5 mg/kg/day (i.p.) for 3 days; the CIS+TQ group received 5 mg/kg TQ (i.p.) 30 min before a single dose of 7 mg/kg CIS (i.p.) and then 5 mg/kg TQ (i.p.) as a single daily dose for 3 days. Cisplatin (DBL)™ injectable solution (Orna Pharmaceuticals, Istanbul, Turkey) was administered i.p. directly, and TQ (CAS number: 490-91-5; Sigma-Aldrich, St. Louis, MO, USA) was dissolved in saline. The doses of the drugs used were selected according to the results of previous dose-response studies[18,19].

On day 4 of the study, the rats were sacrificed under anesthesia, and the brain tissues were dissected out for sampling. Before the surgical procedures, 10 mg/kg xylazine (Bayer, Istanbul, Turkey) and 100 mg/kg ketamine hydrochloride (Parke-Davis, Istanbul, Turkey) were administered i.p. Doses not exceeding 10% of the initially administered doses were repeated intermittently if needed to maintain the anesthesia.

Tissue Preparation and Biochemical Analysis

Fresh brain tissues taken for detailed biochemical examinations were homogenized using a mixer grinder (MM 400; Retsch, Haan, Germany) and then centrifuged to obtain homogenates. The supernatants were used for copper-zinc superoxide dismutase (CuZn-SOD) enzyme analysis and measurement of reduced glutathione (GSH) and protein levels. Homogenized tissues were directly assessed for malondialdehyde (MDA) content, according to the method reported by Mihara and Uchiyama[20], and the results are presented as nmol MDA/g wet tissue. The level of GSH was measured using the method of Ellman, and the results are presented as nmol GSH/g wet tissue[21]. CuZn-SOD activity was measured by the method of Sun et al[22], and the values obtained are presented as U/g protein[22]. After the determination of the protein concentration according to the Biurea method, a calibration curve was created using bovine serum albumin[23].

Histopathological Analysis

Cerebral tissues were stored in 10% formaldehyde solution and embedded in paraffin, and then sections 4-5 μm thick were cut from the paraffin blocks. Sections were stained with hematoxylin and eosin to determine the general morphological structure. Cerebral cortex congestion and neuronal degeneration were evaluated.

Immunohistochemical Analysis

The sections were subjected to deparaffinization and rehydration processes and then placed in a pressure cooker and boiled with 0.01 M citrate (pH 6.0) for 15-20 minutes. To block endogenous peroxidase activity, sections were treated with 3% hydrogen peroxide for 12 min. After washing with phosphate buffered saline (PBS) for 5 min, the sections were treated with Ultra V block (Thermo Scientific, Waltham, MA, USA) to block nonspecific binding and then incubated at 37°C for 60 min with primary antibody (Caspase-3; Thermo Scientific). After washing with PBS at 37°C for 10 min, the tissues were treated with biotin-conjugated secondary antibodies. Subsequently, the sections were incubated with streptavidin peroxidase at 37°C for 10 min, and the chromogen-treated sections were then stained with hematoxylin and covered with water-based sealer.

For immunohistochemical analysis, at least 10 images were randomly obtained from each section at 40× magnification using the Leica DFC-280 research microscope and Leica Q Win Image Analysis System (Leica Microsystems, Wetzlar, Germany). The percentage of immunohistochemical staining in each image was determined using ImageJ (National Institutes of Health, Bethesda,

MD, USA) and calculated as (immunohistochemically stained area/total area) × 100.

Statistical Analysis

To detect even small effects, sample sizes required for a power of 0.80 were calculated using NCSS software (NCSS, Kaysville, UT, USA). The data were analyzed using SPSS for Windows (version 22.0; IBM Corp., Armonk, NY, USA). The normality of the distribution was confirmed by applying the Kolmogorov-Smirnov 's test. Based on the results of the normality test, analysis of variance (ANOVA) was performed followed by multiple comparison testing using Tukey's test for homogeneous variance. The results of the histopathological analyses are expressed as the mean ± standard deviation (SD). Biochemical analysis values that were significant in the Kruskal-Wallis' H test were then subjected to the Conover's test. The values are reported as the median (min-max). In all analyses, $p<0.05$ was taken to indicate statistical significance.

Results

Histopathological Analysis

On histological analysis, the cerebral cortex was normal in appearance in the Control and TQ groups. Neurons in sections from these groups showed round, large, euchromatic nuclei and normal morphological features (Figure 1A and B). On the other hand, degenerative changes were observed in neurons in the CIS group, including shrunken acidophilic cytoplasm and pycnotic nuclei (Figure 1C). In addition to the changes observed in the cerebral cortex, the CIS group showed cerebral cortex congestion. The CIS+TQ group also showed degenerated neurons and congestion but less severe than those in the CIS group (Figure 1D).

Immunohistochemical Analysis

Low levels of caspase-3 immunoreactivity were observed in the neuronal cell bodies in the Control and TQ groups (Figure 2A and B). Caspase-3 immunoreactivity was significantly higher in the CIS group than the Control group (Figure 2C). The CIS+TQ group showed a significant decrease in the level of caspase-3 immunoreactivity compared with the CIS group (Figure 2D). The levels of caspase-3 immunoreactivity in all of the experimental groups are presented in Table I. The Control and TQ groups showed low levels of tau protein immunoreactivity in neurons and neuroglia cells (Figure 3A and B) in contrast to the higher immunoreactivity in the CIS group (Figure 3C), but this difference was not significant (*p*

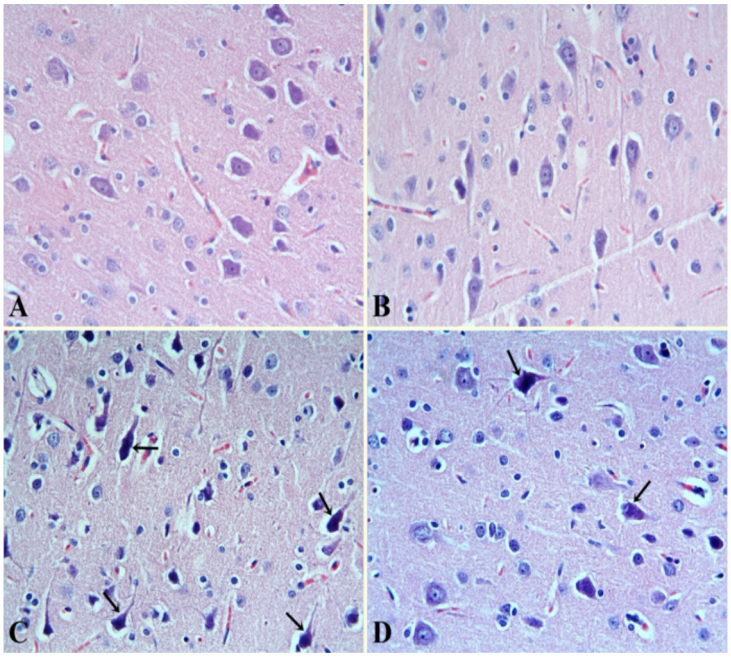

Figure 1. It is observed that the cerebral cortex belonging to the control **(A)** and TQ **(B)** groups has a normal histological structure. The presence of degenerated neurons (arrows) in the cerebral cortex was striking in the CIS-treated group **(C)**. Degenerative changes in neurons were observed to decrease in the CIS+TQ group **(D)**. H-E x40.

Table I. Percentage of caspase-3, tau and neurofilament immunoreactivity of the groups.

	Caspase-3	Tau	Neurofilament
Control	0.14±0.18	1.35±0.79	19.60±5.32
TQ	0.17±0.23	0.70±0.35	24.28±4.48
CIS	1.71±1.07[a]	1.98±0.95	18.51±6.98
CIS+TQ	0.49±0.92[b]	1.82±0.90	17.13±5.03

[a]Significant increase compared to the control group (p=0.002). [b]Significant decrease compared to CIS-treated group (p=0.041).

> 0.05). The intensity of tau protein immunoreactivity was similar between the CIS+TQ and CIS groups ($p < 0.05$) (Figure 3D). Table I shows the tau immunoreactivity levels in all groups. Neurofilament immunoreactivity intensity in neuronal extensions was similar among all groups ($p > 0.05$) (Figure 4, Table I).

Biochemical Findings

The tissue MDA, GSH, and SOD levels were examined in all groups, and the results demonstrated significantly higher MDA levels in the CIS group than the Control group ($p = 0.017$). There were no differences in the MDA level among the other groups or in GSH and SOD levels among any of the groups (Table II).

Discussion

Cancer treatment remains difficult in many cases. The platinum complex CIS is widely used in chemotherapy. The therapeutic effect of CIS can be increased by administering a high dose. However, high doses of the drug are associated with serious side effects, including neurotoxicity, ototoxicity, and nephrotoxicity. These side effects limit the use of high doses and therefore reduce the therapeutic efficacy[4,7,24-27]. Neurotoxicity is the most common side effect of CIS and can be very difficult or impossible for patients to tolerate[8]. It was originally believed that CIS neurotoxicity affects the peripheral sensory nerves, while the brain and spinal cord tissues are protected by the blood-brain barrier, and thus the central nervous system is rarely affected[16]. However, it has been reported that this is not true in cases where blood-brain barrier integrity is impaired, such as brain metastasis[16,26,28]. In addition, it has been reported[29,30] that side effects, such as posterior reversible encephalopathy syndrome, headache, gait instability, hallucinations, cognitive disorders, epileptic seizures, and dysarthria, occur with the use of CIS. Thus, CIS can show side effects that affect both the peripheral and central nervous systems. Neurotoxic complications, such as headache, gait instability, hallucinations, cognitive disorders, encephalopathy, seizures, dysarthria, glove-sock-style paresthesia, numbness, and sensory peripheral neuropathy, occur frequently in patients undergoing chemotherapy with CIS[26,28,29]. In addition, Dietrich et al[31] reported that CNS progenitor cells and oligodendrocytes were strongly affected by administration of CIS both *in vivo* and *in vitro*. In their study, application of CIS induced apoptotic events in the corpus callosum and dentate gyrus of the hippocampus[30]. Some studies[32-34] reported that CNS findings, such as increased anxiety, depression, and behavioral changes in the form of cognitive dysfunction, can be observed due to oxidative damage, inflammation, and apoptosis with the use of platinum compounds. Therefore, the present study was performed to evaluate the beneficial effects of TQ against CIS-induced neurotoxicity in rats due to its powerful antioxidant and anti-inflammatory effects.

Oxidative damage, inflammation, and apoptosis occur with mitochondrial dysfunction. The

Table II. Biochemical parameters.

Cerebrum tissue	MDA (nmol/gwt)	GSH (nmol/gwt)	SOD (U/g protein)
Control	88.05 (84.30-112.20)	248.30 (240.10-264.60)	215.20 (193.20-267.60)
TQ	101.70 (80.90-104.70)	248.30 (223.90-260.50)	214.90 (188.00-274.90)
CIS	106.10 (93.20-119.00)[a]	252.30 (240.10-276.80)	194.05 (166.80-241.10)
CIS+TQ	96.90 (92.50-109.50)	254.35 (223.90-272.70)	187.50 (161.50-216.10)

[a]Significant increase when compared to control group (p=0.017).

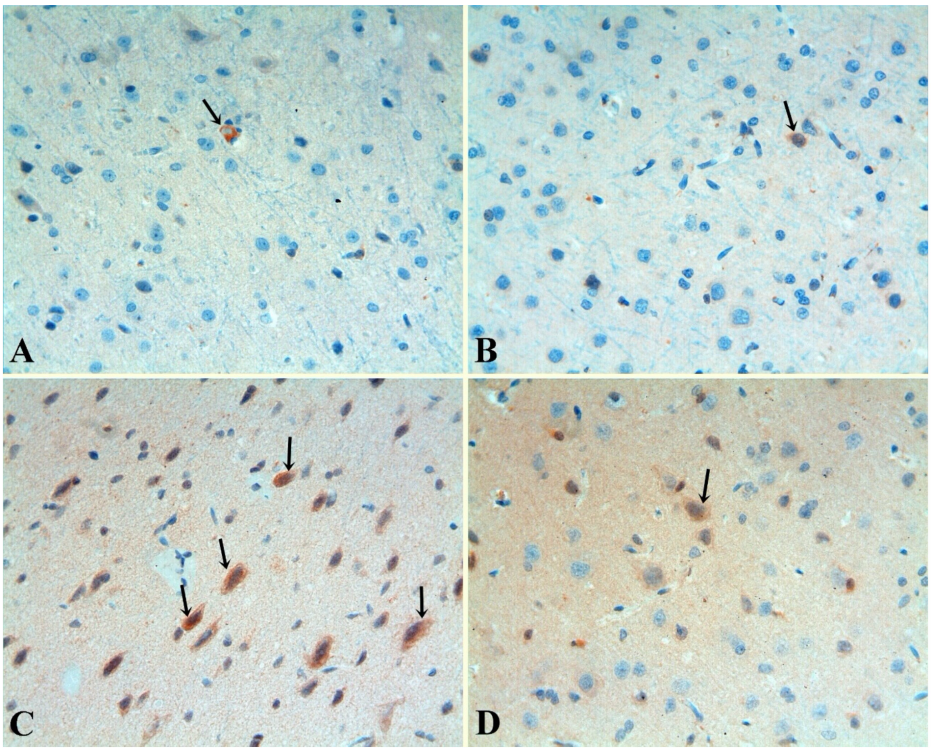

Figure 2. Caspase-3 immunoreactivity intensity. Control **(A)**, TQ **(B)**, CIS **(C)**, CIS+TQ **(D)** groups. Arrows point to caspase-3 positive neurons. Caspase-3 x40.

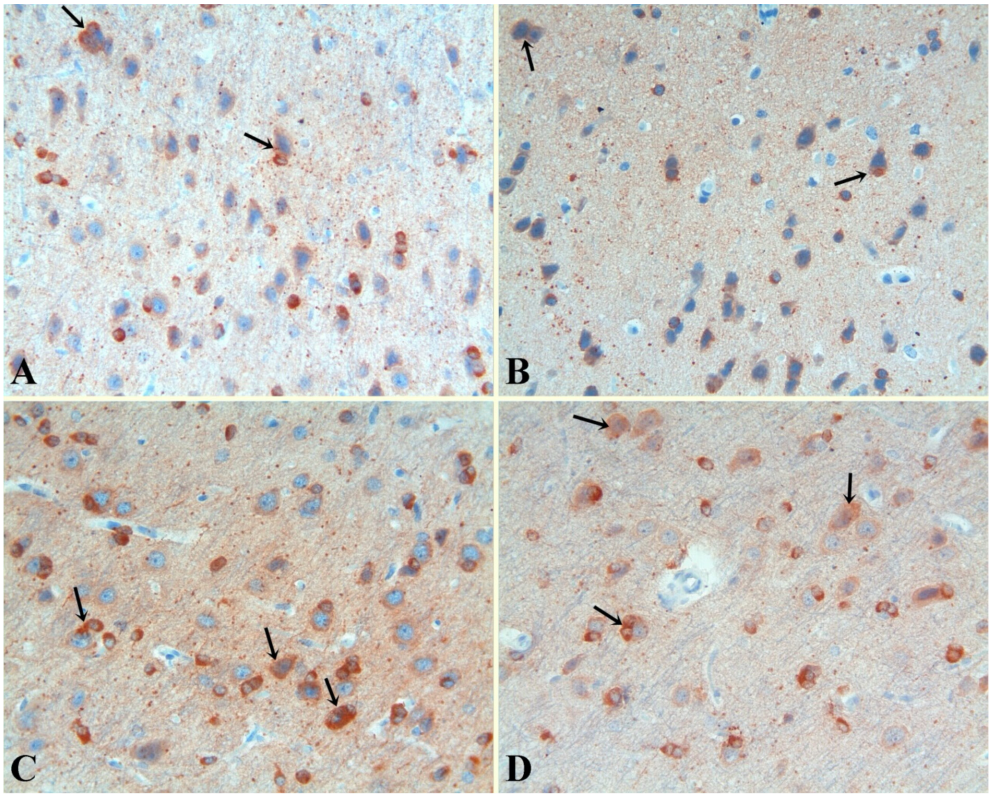

Figure 3. Tau immunoreactivity intensity. Control (**A**), TQ (**B**), CIS (**C**), CIS+TQ (**D**) groups. Arrows point to tau positive neurons. Tau x40.

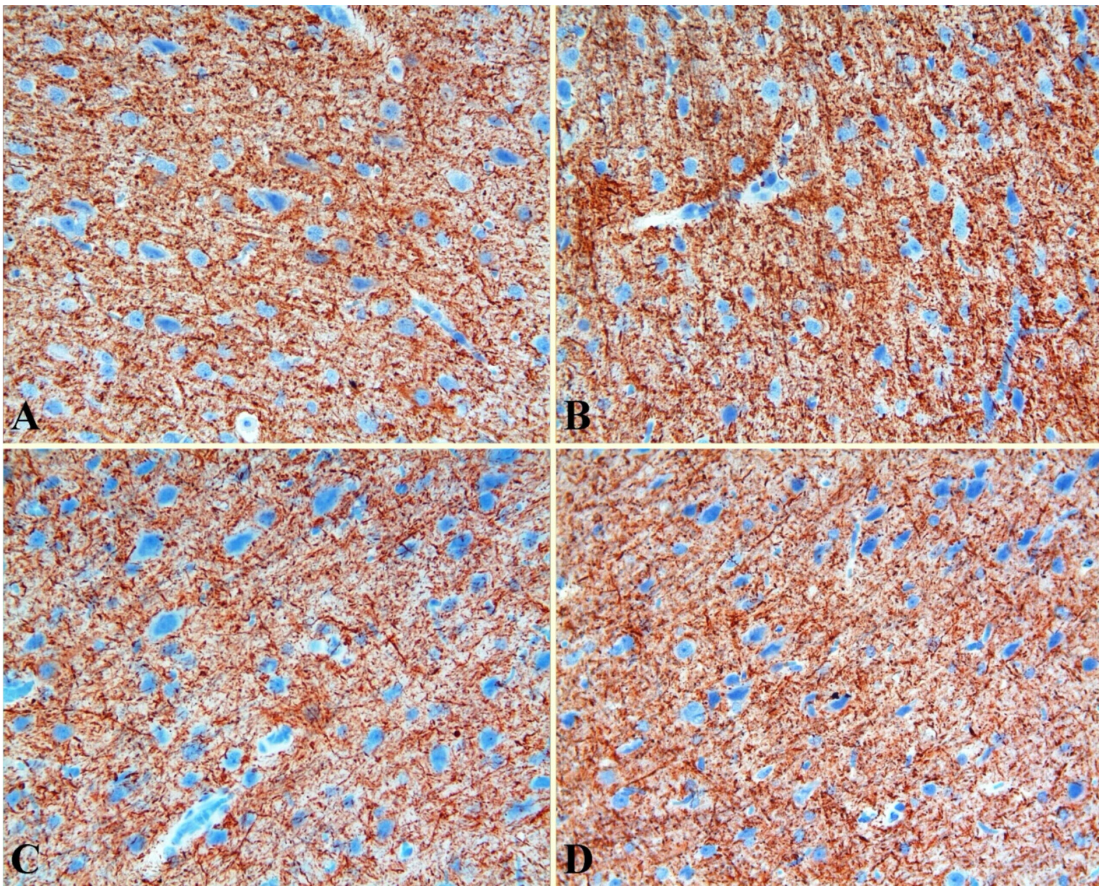

Figure 4. Neurofilament immunoreactivity intensity. Control (**A**), TQ (**B**), CIS (**C**), CIS+TQ (**D**) groups. Neurofilament x40.

increase in production of reactive oxygen species (ROS) as a result of mitochondrial dysfunction in neurons has been identified as a potential mechanism of CIS neurotoxicity. CIS administration has been reported to down-regulate cytochrome B mRNA expression, resulting in mitochondrial DNA damage, thus leading to excessive ROS production[14,35,36]. The histopathological results of the present study showed that degenerative changes occurred in neurons in the treatment groups given CIS. The degenerative neurons exhibited shrunken acidophilic cytoplasm and pycnotic nuclei and cerebral cortex congestion in the neurons in these groups (Figure 1C and D). This degeneration was thought to be due to oxidative damage and inflammation. Excessive ROS production causes lipid peroxidation that damages biological membranes. Under normal conditions, cells have antioxidant mechanisms that protect tissues against ROS-induced damage. The endogenous antioxidants GSH and SOD are the main defense mechanisms against the damage induced by ROS by rescuing potentially damaging free radical groups[37]. Lipid peroxidation in the cell leads to production of aldehydes, hydrocarbon gases, and MDA as end products. The amount of MDA formed by lipid peroxidation is used to determine the degree of oxidative damage in tissues[38]. Therefore, we examined the changes in MDA, GSH, and SOD levels to determine the degree of oxidative damage in the cerebral cortical tissues of the rats in our study. Consistent with the literature, we found a significantly increased MDA level, which is an indicator of oxidative damage due to lipid peroxidation, in the CIS group compared to the Control group. Antioxidant supplementation has been reported[9,11,14,18,39,40] to be effective in reducing ROS levels or attenuating the effects of ROS by reducing tissue damage. In this context, antioxidant supplementation to reduce or prevent the effects of increased ROS levels associated with CIS treatment may have beneficial effects on neurotoxic side effects. Some studies[11,14,18,39] have emphasized that antioxidant supportive therapy is beneficial in reducing neurotoxicity due to platinum-based chemotherapy and increasing the effectiveness of

anticancer therapy. Previous *in vitro* and *in vivo* studies[9,11,39] have showed that TQ has anti-inflammatory and antioxidant effects.

In the present study, application of TQ tissue significantly decreased MDA levels. This observation showed that TQ prevents the decreases in GSH and SOD activities by decreasing the MDA level, thus preventing neuronal lipid peroxidation. This effect is likely due to reductions in neuronal degeneration and therefore apoptosis.

We also performed histopathological analyses to determine the inhibitory effect of TQ on the oxidative mechanisms that lead to neuronal degeneration and death. We observed degenerated neurons and occlusions in the CIS+TQ group, but the extents of these changes were less severe in this group than the CIS group. We detected a low level of tau immunoreactivity, which is an indicator of degeneration in neurons and neuroglial cells, in the Control and TQ groups. However, tau immunoreactivity was increased in the CIS group, albeit not significantly ($p > 0.05$). In addition, we investigated the immunoreactivity of caspase, which inactivates enzymes required for DNA repair and replication and cleaves cytoskeletal proteins, causing cell membrane budding and ultimately apoptosis[41-44]. We detected a low level of caspase-3 immunoreactivity in the neuronal bodies in the Control and TQ groups. On the other hand, caspase-3 immunoreactivity was significantly higher in the CIS group than Control group. The CIS+TQ group also showed a significant decrease in caspase-3 immunoreactivity compared with the CIS group. In our histopathological and biochemical analyses, TQ application significantly decreased the CNS-damaging effects of CIS in rats.

Conclusions

This study showed that short-term TQ application had beneficial effects by reducing neurotoxicity in rats treated with CIS. Further large-scale, randomized, controlled clinical trials are required to validate these neuroprotective effects of CIS chemotherapy.

Conflict of Interest

The authors of this article do not declare any relationship with any company whose products or services may be related to the subject of the article.

Funding

This work was supported by the Research Fund of Inonu University. Project Number: TSA-2020-2149.

Ethics Approval

This study was conducted with the approval of the Animal Ethics Committee of Inönü University (Reference Number: 2016/A-11).

Authors' Contributions

Mehmet Akif Durak, Hakan Parlakpinar and Onural Ozhan are the coordinators of this study and they planned the study protocol design. MAD, HP and OO made the mandatory requirements for the study. OO were responsible for drug administration and data collection. HP and OO performed the surgical procedures. The histopathological evaluations were carried out by Azibe Yildiz, and Nigar Vardi, whereas Merve Durhan and Yilmaz Cigremis performed biochemical experiments. OO and HP were responsible for data and statistical analysis and interpretation of the results. This manuscript was written by MAD, OO and HP. The final manuscript was revised collaboratively by MAD, OO and HP.

ORCID ID

Mehmet Akif Durak: 0000-0003-0827-2708
Hakan Parlakpinar: 0000-0001-9497-3468
Nigar Vardi: 0000-0003-0576-1696

References

1) Cepeda V, Fuertes MA, Castilla J, Alonso C, Quevedo C, Pérez JM. Biochemical mechanisms of cisplatin cytotoxicity. Anticancer Agents Med Chem 2007; 7: 3-18.
2) Kelland, L. The resurgence of platinum-based cancer chemotherapy. Nat Rev Cancer 2007; 7: 573-584.
3) Chiorazzi A, Semperboni S, Marmiroli P. Current View in Platinum Drug Mechanisms of Peripheral Neurotoxicity. Toxics 2015; 3: 304-321.
4) Wang D, Lippard SJ. Cellular processing of platinum anticancer drugs. Nat Rev Drug Discov 2005; 4: 307-320.
5) Hanigan MH, Devarajan P. Cisplatin nephrotoxicity: molecular mechanisms. Cancer Ther 2003; 1: 47-61.
6) Cvitkovic E. Cumulative toxicities from cisplatin therapy and current cytoprotective measures. Cancer Treat Rev 1998; 4: 265-281.
7) dos Santos NA, Carvalho Rodrigues MA, Martins NM, dos Santos AC. Cisplatin-induced nephrotoxicity and targets of nephroprotection: an update. Arch Toxicol 2012; 86: 1233-1250.

8) Bobylev I, Joshi AR, Barham M, Neiss WF, Lehmann HC. Depletion of Mitofusin-2 Causes Mitochondrial Damage in Cisplatin-Induced Neuropathy. Mol Neurobiol 2018; 55: 1227-1235.
9) Ragheb A, Attia A, Eldin WS, Elbarbry F, Gazarin S, Shoker A. The protective effect of thymoquinone, an anti-oxidant and anti-inflammatory agent, against renal injury: a review. Saudi J Kidney Dis Transpl 2009; 20: 741-752.
10) Ahmad N, Ahmad R, Alam MA, Samim M, Iqbal Z, Ahmad FJ. Quantification and evaluation of thymoquinone loaded mucoadhesive nanoemulsion for treatment of cerebral ischemia. Int J Biol Macromol 2016; 88: 320-332.
11) Mendonça LM, da Silva Machado C, Teixeira CC, de Freitas LA, Bianchi Mde L, Antunes LM. Curcumin reduces cisplatin-induced neurotoxicity in NGF-differentiated PC12 cells. Neurotoxicology 2013; 34: 205-211.
12) Darakhshan S, Bidmeshki Pour A, Hosseinzadeh Colagar A, Sisakhtnezhad S. Thymoquinone and its therapeutic potentials. Pharmacol Res 2015; 95: 138-158.
13) Gholamnezhad Z, Havakhah S, Boskabady MH. Preclinical and clinical effects of Nigella sativa and its constituent, thymoquinone: A review. J Ethnopharmacol 2016; 190: 372-386.
14) Kandeil MA, Mahmoud MO, Abdel-Razik AH, Gomaa SB. Thymoquinone and geraniol alleviate cisplatin-induced neurotoxicity in rats through downregulating the p38 MAPK/STAT-1 pathway and oxidative stress. Life Sci 2019; 228: 145-151.
15) Üstün R, Oğuz EK, Şeker A, Korkaya H. Thymoquinone prevents cisplatin neurotoxicity in primary DRG neurons. Neurotoxicology 2018; 69: 68-76.
16) Gregg RW, Molepo JM, Monpetit VJ, Mikael NZ, Redmond D, Gadia M, Stewart DJ. Cisplatin neurotoxicity: the relationship between dosage, time, and platinum concentration in neurologic tissues, and morphologic evidence of toxicity. J Clin Oncol 1992; 10: 795-803.
17) Isaev NK, Chetverikov NS, Stelmashook EV, Genrikhs EE, Khaspekov LG, Illarioshkin SN. Thymoquinone as a Potential Neuroprotector in Acute and Chronic Forms of Cerebral Pathology. Biochemistry (Mosc) 2020; 5: 167-176.
18) Almutairi MM, Alanazi WA, Alshammari MA, Alotaibi MR, Alhoshani AR, Al-Rejaie SS, Hafez MM, Al-Shabanah OA. Neuro-protective effect of rutin against Cisplatin-induced neurotoxic rat model. BMC Complement Altern Med 2017; 17: 472.
19) Hajipour S, Sarkaki A, Dianat M, Rashno M, Khorsandi LS, Farbood Y. The effects of thymoquinone on memory impairment and inflammation in rats with hepatic encephalopathy induced by thioacetamide. Metab Brain Dis 2021; 36: 991-1002.
20) Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978; 86: 271-278.
21) Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82: 70-77.
22) Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34: 497-500.
23) Hiller A, Greif RL, Beckman WW. Determination of protein in urine by the biuret method. J Biol Chem 1948; 176: 1421-1429.
24) Mollman JE. Cisplatin neurotoxicity. N Engl J Med 1990; 322: 126-127.
25) Park SB, Goldstein D, Krishnan AV, Lin CS, Friedlander ML, Cassidy J, Koltzenburg M, Kiernan MC. Chemotherapy-induced peripheral neurotoxicity: a critical analysis. CA Cancer J Clin 2013; 63: 419-437.
26) Decatris MP, Sundar S, O'Byrne KJ. Platinum-based chemotherapy in metastatic breast cancer: current status. Cancer Treat Rev 2004; 1: 53-81.
27) Ju SM, Bae JS, Jeon BH. AMP-activated protein kinase contributes to ROS-mediated p53 activation in cisplatin-induced nephrotoxicity. Eur Rev Med Pharmacol Sci 2021; 25: 6691-6700.
28) Namikawa K, Asakura M, Minami T, Okazaki Y, Kadota E, Hashimoto S. Toxicity of cisplatin to the central nervous system of male rabbits. Biol Trace Elem Res 2000; 74: 223-235.
29) Zahir MN, Masood N, Shabbir-Moosajee M. Cisplatin-induced posterior reversible encephalopathy syndrome and successful re-treatment in a patient with non-seminomatous germ cell tumor: a case report. J Med Case Rep 2012; 29: 409.
30) Ishihara M, Matsutani N, Ota S, Seki N. A Case of Posterior Reversible Encephalopathy Syndrome Induced by Cisplatin/Pemetrexed Chemotherapy for Lung Cancer. Case Rep Oncol 2017; 10: 235-238.
31) Dietrich J, Han R, Yang Y, Mayer-Pröschel M, Noble M. CNS progenitor cells and oligodendrocytes are targets of chemotherapeutic agents in vitro and in vivo. J Biol 2006; 5: 22.
32) Kanat O, Ertas H, Caner B. Platinum-induced neurotoxicity: A review of possible mechanisms. World J Clin Oncol 2017; 8: 329-335.
33) Abdelkader NF, Saad MA, Abdelsalam RM. Neuroprotective effect of nebivolol against cisplatin-associated depressive-like behavior in rats. J Neurochem 2017; 141: 449-460.
34) Lomeli N, Di K, Czerniawski J, Guzowski JF, Bota DA. Cisplatin-induced mitochondrial dysfunction is associated with impaired cognitive function in rats. Free Radic Biol Med 2017; 102: 274-286.
35) Chiu GS, Maj MA, Rizvi S, Dantzer R, Vichaya EG, Laumet G, Kavelaars A, Heijnen CJ. Pifithrin-μ Prevents Cisplatin-Induced Chemobrain by Preserving Neuronal Mitochondrial Function. Cancer Res 2017; 77: 742-752.
36) Kelley MR, Jiang Y, Guo C, Reed A, Meng H, Vasko MR. Role of the DNA base excision repair protein, APE1 in cisplatin, oxaliplatin, or carboplatin induced sensory neuropathy. PLoS One 2014; 9: e106485.
37) Wagner FC Jr, Dohrmann GJ, Bucy PC. Histopathology of transitory traumatic paraplegia in the monkey. J Neurosurg 1971; 35: 272-276.

38) Schwab ME, Bartholdi D. Degeneration and regeneration of axons in the lesioned spinal cord. Physiol Rev 1996; 76: 319-370.

39) Turan MI, Cayir A, Cetin N, Suleyman H, Siltelioglu Turan I, Tan H. An investigation of the effect of thiamine pyrophosphate on cisplatin-induced oxidative stress and DNA damage in rat brain tissue compared with thiamine: thiamine and thiamine pyrophosphate effects on cisplatin neurotoxicity. Hum Exp Toxicol 2014; 33: 14-21.

40) Pietta PG. Flavonoids as antioxidants. J Nat Prod 2000; 63: 1035-1042.

41) Adams JM, Cory S. Life-or-death decisions by the Bcl-2 protein family. Trends Biochem Sci 2001; 26: 61-66.

42) Adrain C, Martin SJ. The mitochondrial apoptosome: a killer unleashed by the cytochrome seas. Trends Biochem Sci 2001; 26: 390-397.

43) Spierings DC, de Vries EG, Vellenga E, van den Heuvel FA, Koornstra JJ, Wesseling J, Hollema H, de Jong S. Tissue distribution of the death ligand TRAIL and its receptors. J Histochem Cytochem 2004; 52: 821-831.