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Cisplatin-induced neurotoxicity in cerebellar cortex of male mice involves oxidative stress and histopathology

Azza Attia^{1*}, Cecil Matta¹, Reda ElMazoudy^{2,3*}  and Hanan Khalifa⁴

Abstract

Background: Despite evidence of neurotoxicity, cisplatin is still considered the most potent drug prescribed in human chemotherapy for a broad spectrum of malignancies. The objective was to evaluate the cerebellar cortex damage including oxidative stress biomarkers and histopathology aspects in male mice. One saline control group and two cisplatin groups were intraperitoneally injected with 0, 5, and 10 mg/kg body weight (bw) cisplatin, twice per week for four successive weeks, respectively.

Results: Cisplatin decreased the body weights of treated mice. Serum levels of superoxide dismutase and glutathione peroxidase were significantly reduced in the 5 and 10 mg/kg dose, twice weekly for 4 weeks treatment; in contrast, there was a significant increase of lipid peroxidation. 5 and 10 mg/kg bw of cisplatin caused histopathological damage in the cerebellum tissue characterized by disruption, disorganization, and degeneration with dense pyknotic nuclei of the granular cells. Ultrastructurally, in the cortical region of the cerebellum, the Purkinje cells showed irregular pyknotic nuclei with indistinct nucleoli, cytoplasmic vacuolation, marked indentation of the nuclear membrane, dilatation of the endoplasmic reticulum, and breakdown and disappearance of mitochondrial cristae. Moreover, the molecular layer showed cellular necrosis and an increased number of lysosomal particles. The myelinated nerve fibers showed degenerative areas distinct by splitting, disruption, and loss of the lamellar pattern of the myelin sheath.

Conclusion: These findings provide a confirmed foresight that the in vivo potential treatment of mice with cisplatin induces cerebellum deficits and impairment in neuronal histology. The identified mechanism which evokes neurotoxicity is oxidative stress-dependent status. This mechanism is pharmacologically boosted by great production of free radical reactive oxygen species.

Keywords: Cisplatin, Cerebellum, Purkinje cells, Oxidative stress, Ultrastructure

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Background

Accidentally, Rosenberg in 1965 discovered cisplatin as a cell division inhibitor (Rosenberg, Vancamp, & Krigas, 1965). During 1969, cisplatin was exhibited and explained for its anticancer property in animal patterns (Rosenberg, Vancamp, Trosko, & Mansour, 1969). Afterwards, in chemotherapeutic regimens, cisplatin came to be the mainstay usually used in treating different patterns of malignancies, for instance, esophageal, cervical, testicular, and ovarian cancer and more (Crona et al., 2017). Besides, in the synergistic regimen, cisplatin demonstrated lesser toxicity and a high therapeutic rate (Einhorn, 2002).

An increasing body of clinical evidence indicates that a low dose of cisplatin induces 30% nephrotoxicity in adults and 70% in pediatric states (Un et al., 2020). Unfortunately, the higher accumulated doses of cisplatin also result in nephrotoxicity, neurotoxicity, and cardiotoxicity (Chowdhury, Sinha, Banerjee, & Sil, 2016; Li et al., 2017; Steeghs, de Jongh, Sillevius Smitt, & van den Bent, 2003). Also, in higher cisplatin dose chemotherapy, other severe adverse effects such as myelosuppression, ototoxicity, and nausea were reported (Brock, Knight, & Freyer, 2012; Kim et al., 2015). Likewise, hepatotoxicity is a risk factor concomitant with cisplatin dose in cancer chemotherapy (Pezeshki et al., 2017).

Many reported data of cisplatin-related neurotoxic effects were recorded. Besides, the cerebellum is particularly vulnerable to intoxication and poisoning, especially the cerebellar cortex and Purkinje neurons (Manto, 2012). On administering to experimental animals, cisplatin induces cytotoxicity of the cerebellar cortex through apoptosis of the proliferating granular cells and deterioration of migrating and differentiating Purkinje cells (Pisu et al., 2004). Moreover, it has shown that cisplatin induces *in vitro* death of cerebellar granule cells including the morphological and molecular alterations during the cerebellum development in postnatal rats (Wick et al., 2004). Also, the accumulated cisplatin in the neurites causes axonopathy and degeneration of myelinated fibers along with marked remyelination and layer demyelination (Yoon et al., 2009).

Among the cisplatin-implicated adverse effects are free radical-mediated oxidative stress and subsequent depletion of neural antioxidant enzymes (Yadav, 2019). Aside from an excessive production of reactive oxygen species, apoptosis and mitochondria dysfunction are also involved in the acceleration of DNA damage and trigger caspase activation cisplatin-induced neural toxicity in the peripheral nerve system (Maj, Ma, Krukowski, KN Kavelaars, & Heijnen, 2017). Another cytotoxic mechanism showed that cisplatin or metabolites can interact with a variety of cellular organelles such as the cell membranes, endoplasmic reticulum, nucleus, and lysosomes

resulting in cellular necrosis and apoptosis (Gatti, Cassinelli, Zaffaroni, Lanzi, & Perego, 2015). The peripheral neurotoxicity is a characteristic clinical aspect sign induced by cisplatin in lung tumor (Sharawy, Laila, & Youakim, 2015).

Objective

Concerning the deleterious effects of cisplatin on neural tissue, few studies were focused on the assessment of cisplatin's effect on the cerebellar tissue which is still not fully elucidated. Experimentally, due to the evidence that the cerebellum is one of the highly vulnerable body parts to intoxication and most likely to be influenced in the neural tissue, it was our superior choice for the present study.

Methods

Chemicals

Cisplatin™ (MERCK, Oncoten Pharma Production GmbH, Rodleben, Germany) was purchased from the pharmacy. Cisplatin was freshly prepared by dissolving in 0.9% saline every week.

Experimental animals

An overall number of 45 male albino mice (average weight 30–35 g, age 90–100 days) were utilized in the present study. Male mice were randomly collected from stock kept on the animal house of the Faculty of Agriculture, Alexandria University, Alexandria, Egypt. For 1 week of acclimation, five mice per stainless steel cage were kept at a suitable room temperature of 25 ± 2 °C, with 60–65% humidity, a natural photoperiod of 12 h of light and 12 h of darkness, and free access to food and drinking water. All the present methodological procedures, laboratory animal handling, caring, weighing, dosing, anesthesia, and dissections were executed according to the local committee of Institutional Animal Care and Use Ethics (ACUE), Faculty of Science, Alexandria University, Alexandria (Registration No. AU 04-19-10-21-3).

Experimental design

Male mice were randomly divided into two cisplatin groups and one negative control group (15 mice/each). Mice in the negative control group (GI) were intraperitoneally injected with 0.2 ml/kg of 0.9% saline. Mice in group II (GII) were intraperitoneally injected with 5 mg/kg bw cisplatin dissolved in 0.9% saline. Mice in group III (GIII) were intraperitoneally injected with 10 mg/kg bw cisplatin dissolved in 0.9% saline. The experimental protocol was carried out twice/week, for four consecutive weeks. All mice were administered with an equal volume of cisplatin and/or saline (0.2 ml). This protocol was applied in accordance with the investigated neurotoxic dose (Akman et al., 2015).

Experimental weights

After 24 h of post-saline or post-cisplatin dose (day 29), the body weight of male mice was recorded, and thereafter, the body weight change (%) was estimated.

Collection and preparation of blood, serum, and brain

At the end of the elapsed experimental time, all cisplatin and control mice were weighed for the final weight. Immediately before anesthesia and dissection, blood samples were collected from the orbital venous plexus bleeding of the eye. The animal is stuffed with the thumb and forefinger of the non-dominant hand, and the skin around the eye is pulled taut. A capillary is inserted into the medial canthus of the eye (30° angle to the nose). Slight thumb pressure is enough to puncture the tissue and enter the plexus/sinus. Once the plexus/sinus is punctured, blood will come out through the capillary tube (Parasuraman, Raveendran, & Kesavan, 2010). Thereafter, mice were anesthetized by intraperitoneal injection with a mixture of ketamine hydrochloride (99.0%) and xylazine hydrochloride (99.0%) solution (1 ml/kg body weight of 1:1) marketed for use in laboratory research animals (Cat. No. K-113, Sigma-Aldrich Corporation, USA). Thereafter, the mice were then killed by cervical dislocation and the blood samples were collected; the serum was isolated by clotting for 30 min and centrifuged at 3000 rpm for 15 min at 4 °C and then maintained at -20 °C. On dissection, the brain was collected and washed with normal saline 0.9% for removal of blood, except for 5 mice for DNA analysis from each group.

Serum biochemical analysis

Aliquots of sera were used for measuring the content of superoxide dismutase (SOD) enzyme and glutathione peroxidase (GPx) antioxidative enzymes using colourimetric methodology as advised by commercial kits (Reardon & Allen, 2009; Zelko, Mariani, & Folz, 2002), while the assay of lipid peroxidation content was done through the determination of the level of malondialdehyde (MDA) production following the thiobarbituric acid method (Ermis et al., 2004).

DNA analysis

DNA assay for the brain tissue of 5 mice of both experimental and control groups was performed using the method of Saad, Youssef, and Elshennawy (2009). DNA (G1N70) was isolated from the brain tissue using GenElute™ kits from Sigma-Aldrich, and then the DNA is solubilized in TE buffer (T9285). Two nanograms of the nucleic acid samples was mixed with 3 µL of the loading buffer. The samples were carefully loaded into the wells using pipettes with a suitable marker containing nucleic acid fragments of various sizes. The dye front was tracked using Orange G. After electrophoresis, the gel

was transferred to a UV transilluminator and the image of the gel was acquired. The samples will appear as bright bands. After the run, a fluorescence image of the gel was acquired.

Histopathological methods

On the spot, all brains of mice were cut into small pieces. According to the standard protocol, these small pieces of the brain cerebellum were fixed in neutral buffered formalin (10%) and embedded in paraffin wax. Five-micrometer slices of neutral formalin-fixed and paraffin-embedded tissues of the cerebellum were routinely cut by a microtome. The first step in performing paraffin sections is removing the paraffin wax. After thorough de-waxing, the slide is passed through several changes of alcohol to remove the xylene and then thoroughly rinsed in water. Thereafter, the slides are stained with hematoxylin and eosin (Bancroft & Gamble, 2002). For routine histopathology diagnosis, stained slides were examined using an Olympus B50X microscope.

Ultrastructural methods

Tiny specimens of the cerebellar cortex of both control and cisplatin-treated mice were immediately fixed in 2% 4F1G and rinsed in 0.1 M phosphate buffer at (pH = 7.4, 4 °C) for 1 h. Thereafter, they were post-fixed with 1% buffered osmium tetroxide (OsO₄) for 1–2 h at 4 °C; then, the specimens were washed several times with phosphate buffer for 40 min and dehydrated in ascending concentrations of ethyl alcohol. Tissue specimens were treated with propylene oxide and embedded in a mixture of 1:1 of Epon-Araldite and later in a pre-dried gelatine capsule (dryness at 37 °C oven for 1 h before use). Polymerization was done in the oven at 65 °C for 24 h. Ultrathin sections were cut with a glass knife on LKB ultramicrotome, mounted on 200-mesh naked copper grids, and double-stained with uranyl acetate and lead citrate (Hayat, 2000). Ultrathin sections were inspected under a JEOL 100CX transmission electron microscope (Electron Microscope Unit, EMU, Faculty of Science, Alexandria University, Alexandria, Egypt).

Statistical analysis

Statistical analysis for all data was done using the SPSS software package version 17.0, and the results were expressed as the mean ± standard deviation. The data were analyzed using a one-way analysis of variance (ANOVA). All tests were carried out at a significant level of probability value $P \leq 0.05$. Further, analyses of the data were performed with the least significant difference (LSD) to determine which sample was significantly different from the control.

Results

Cisplatin and body weights

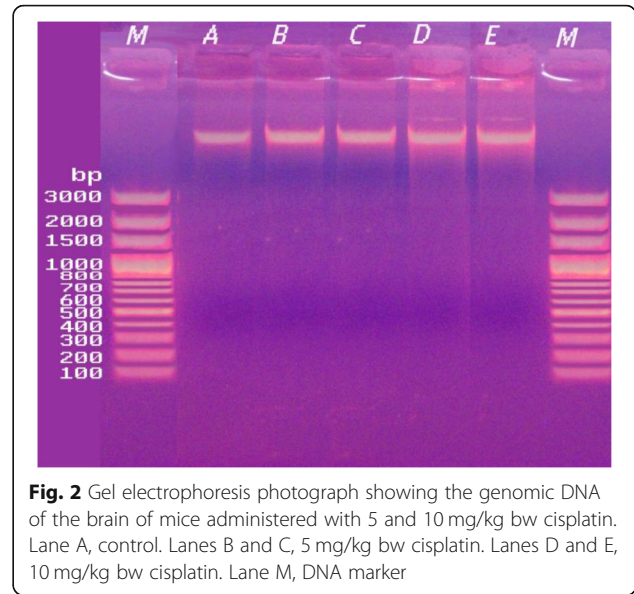
At the end of 4 weeks and depending on dose response, 5 and 10 mg/kg bw cisplatin caused a significant decrease in body weights versus control mice (data not shown).

Cisplatin and oxidative stress

As shown in Fig. 1a, b, cisplatin was capable of influencing the antioxidant enzymes in the blood and also of inducing free radical output, which are in turn the main primary markers of oxidative stress. Both SOD and GPx (Fig. 1a), which are an index of reactive oxygen species generation, were significantly decreased in the sera of 5 mg/kg bw and 10 mg/kg bw cisplatin-treated mice. Also, the MDA, which is an indication of lipid peroxidation (LPO), was significantly increased in the sera of mice that received 5 and 10 mg/kg bw cisplatin (Fig. 1b).

DNA analysis

The gel electrophoresis assay of the genomic DNA extracted from brain tissues of mice treated with the 5 mg/kg bw and 10 mg/kg bw cisplatin (Fig. 2; lanes B, C and

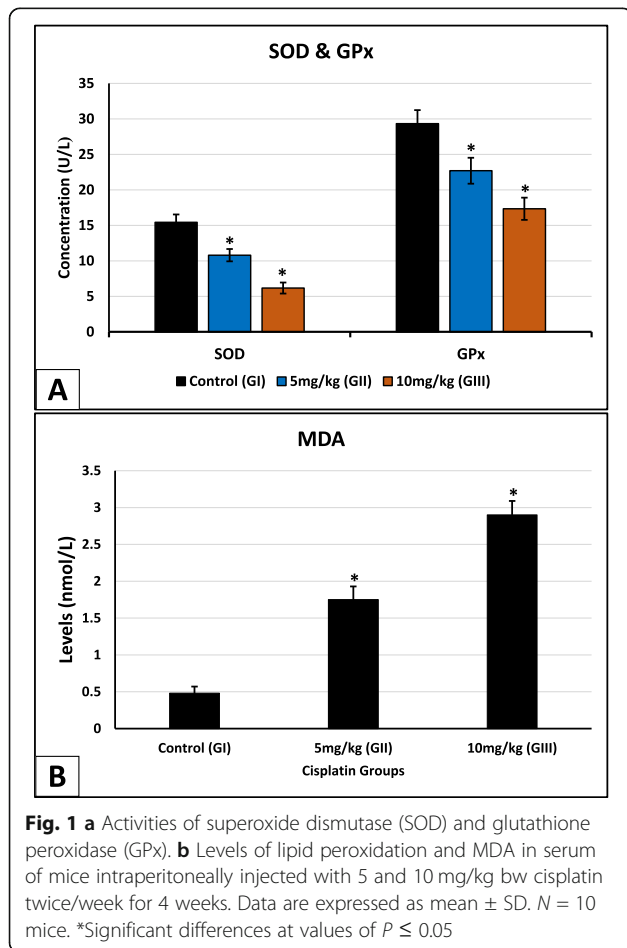


lanes D, E respectively) showed intact bands of genomic DNA (i.e., absence of degradation), compared to the control (Fig. 2, lane A).

Cisplatin and neuro-histopathology

Histopathological alterations of the cerebellar cortex of 5 mg/kg bw cisplatin-treated mice showed an assessment of neural injury as shown in Fig. 3. Cisplatin induced mild disorganization of the monolayer arrangement of Purkinje cells, resulting in loss of the characteristic “flask-shaped” pattern. Most of the Purkinje cells have marked irregular outlines, and their nuclei were hardly identified (Fig. 3c, d) compared with the control cortex (Fig. 3a, b). The granular cells were accumulated in a clumping manner and contained irregular dark nuclei (Fig. 2c, d), while in 10 mg/kg bw cisplatin-treated group, many highly vacuolated cells of the molecular layer of the cerebellar cortex were visualized (Fig. 3e, f). The Purkinje cells were disorganized and irregular in shape, showing severe vacuolation of pyknotic nuclei. Besides, the granular cells showed a multilayer disposition of Purkinje cells, and a halo of empty spaces appeared around most of the Purkinje cells. Their nuclei were hardly identified (Fig. 3e, f).

Mild signs of ultrastructural changes in the cerebellar cortex of mice that received 5 mg/kg bw cisplatin were seen. Large areas of mossy fibers accompanied by a disruption in the mitochondrial cristae were discriminated in the molecular layer (Fig. 4c, d) compared with the control (Fig. 4a, b). Present observations also indicated that various changes were observed which indicate and characterize neurodegeneration after the 5 mg/kg bw cisplatin dose. The most obvious alterations were the shrinkage of Purkinje cells, irregular nuclei with indistinct



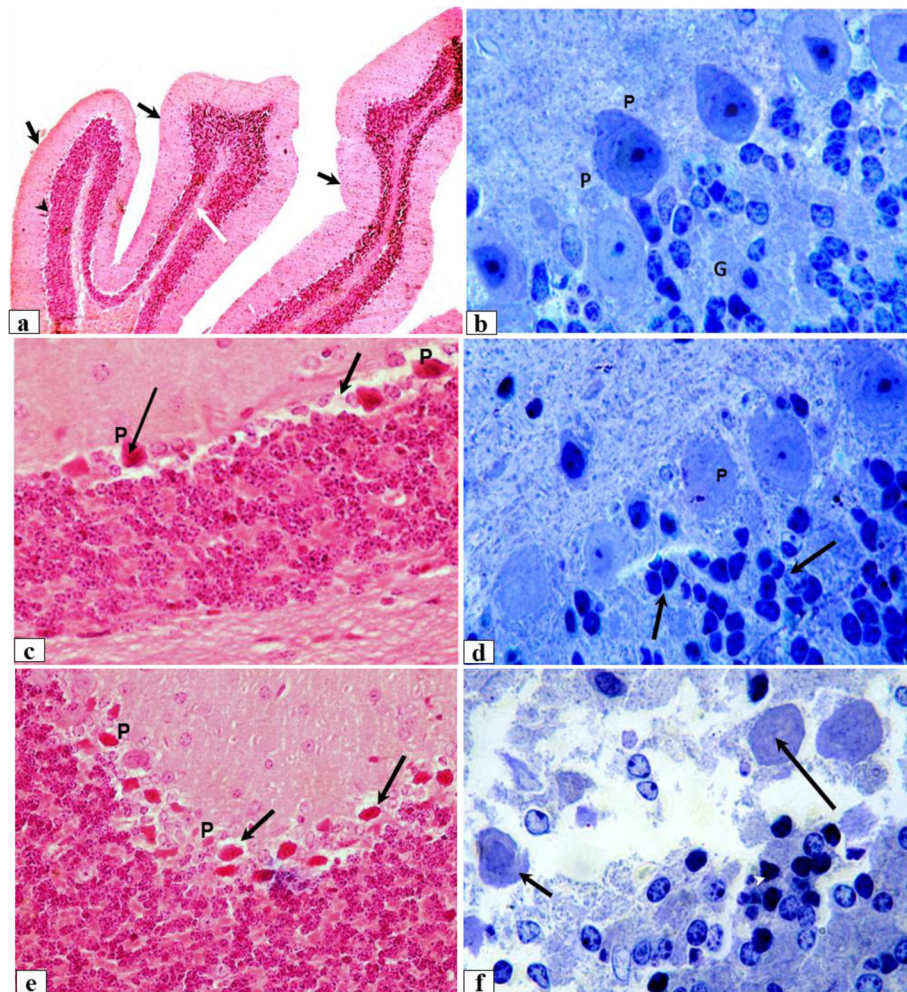


Fig. 3 a–f Light micrograph of sections of the cerebellar cortex of the cerebellum. **a** Control mice showing the folium (arrows) of the gray matter. H&E, $\times 100$ (bar = 200 μm). **b** Semithin section showing the flask-shaped of Purkinje cells (P). Toluidine blue, $\times 1000$ (bar = 20 μm). **c** Mice intraperitoneally injected with 5 mg/kg bw cisplatin showing shrunken Purkinje cells (P) and halo of empty spaces around the Purkinje cells (arrows), $\times 400$ (bar = 50 μm). **d** The nuclei of some Purkinje cells (P) are hardly identified. Note the dense nuclei of the granular cells (arrows). Toluidine blue, $\times 1000$ (bar = 20 μm). **e** Mice treated with 10 mg/kg bw cisplatin showing deposition of the Purkinje cells (P) surrounded by a highly vacuolated cytoplasm (arrow), $\times 400$ (bar = 50 μm). **f** Disorganization of the Purkinje cells with hardly identified nuclei (arrows). Toluidine blue, $\times 1000$ (bar = 20 μm)

nucleoli, dilatation of the endoplasmic reticulum, loss of mitochondrial cristae, fragmentation of the rough endoplasmic reticulum giving small, fragmented rods, and a hypertrophied Golgi zone (Fig. 4c, d).

At the 10 mg/kg bw cisplatin protocol, different events of necrosis among the cells of the molecular layer were observed and exhibited along with an increased number of cytoplasm lysosomal particles. These neurodegenerative changes were associated with disorganization of nuclei of Purkinje cells, leaving a nuclear ghost, destruction of mitochondrial cristae, and marked dilatation of the cisternae of the rough endoplasmic reticulum. Moreover, in the granular neurons, the myelinated axons exhibited numerous areas of degeneration characterized by disruption, splitting, and loss of the lamellar compact structure

of myelin sheath fibers (Fig. 5a, c) compared with the control (Fig. 4a, b). In contrast, the granular cells in the granular layer were still intact.

Discussion

Cisplatin is a global chemotherapeutic drug with definite nephrotoxicity, neurotoxicity, and hepatotoxicity. Because of the prevalence of solid tumor treatment, cisplatin has become extensively used and prescribed on a large scale (Attia, Matta, & Khaliffa, 2015; Paksoy et al., 2011; Üstün, Oğuz, Şeker, Korkaya, & H., 2018). Animal exposure to cisplatin has been confirmed to cause neural injury. However, the detailed investigations behind the neural damage for the cerebellum remain insufficient. Therefore, we conducted this study based on histopathology and oxidative

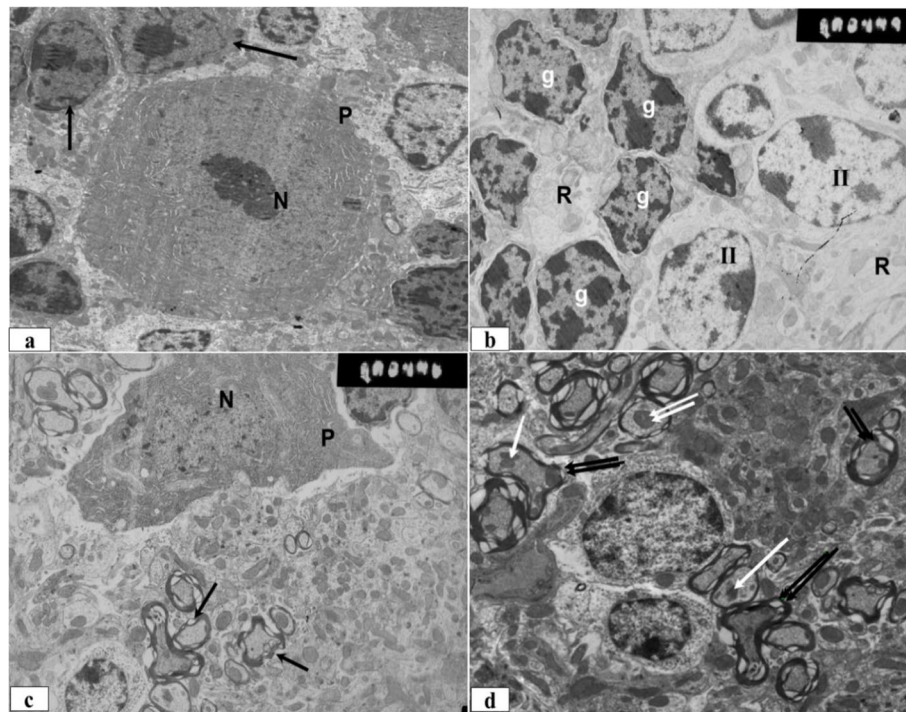


Fig. 4 a–d Electron micrographs of a section of the cerebellar cortex of the cerebellum. **a, b** In control mice showing the nucleus (N) of Purkinje cell (P), the nuclei of the granular (g) layer, and Golgi II cells. **c, d** In mice intraperitoneally injected with 5 mg/kg bw cisplatin showing the nucleus (N) of a shrunken Purkinje cell (P). Notice the splitting of the myelin sheath in some areas of myelinated fibers (arrows) in the molecular layer. $\times 5000$ (bar = 10 μm)

stress assay to further assess the toxicity of cisplatin on mice cerebellum and to explore the potential histopathology and ultrastructure underlying the damage.

Firstly, histopathological findings of mice cerebellum were assessed to confirm the damage to the cerebellar tissues. Consistent with previous studies (Akman et al., 2015), exposure to cisplatin was found to induce cellular hamper and deleterious changes in cerebellar tissues. We then measured the serum antioxidant enzymes of each group, which revealed a decrease of enzymatic activities upon cisplatin treatment, indicating the impairment of biochemical functions of subcellular organelles of neurons (Carozzi, Marmiroli, & Cavaletti, 2010).

The underlying mechanisms were mostly attributed to the high cisplatin toxic effect on body weight loss in chemotherapy treatment which may be due to the acceleration of water elimination by urine associated with continuing dehydration (Tikoo, Bhatt, Gaikawad, Sharma, & Kabra, 2007) or the reduction in food ingestion because of gastrointestinal malaise (Atessahin, Yilmaz, Karahan, Ceribasi, & Karaoglu, 2005). Gastrointestinal malaise may also reflect inhibition of appetite desire and raise the rate of catabolism and disturbance of food digestion and water assimilation (Hassan, Chibber, & Naseem, 2010; Perse & Veceric-Haler, 2018).

Previously, it was concluded that cisplatin induces excessive oxidative stress, enhances apoptosis (Kütük,

Gökçe, Kütük, & Cila, 2019; Mi et al., 2018), and damages the blood–brain barrier in the brain (Blanchette & Fortin, 2011). In the present study, we performed an oxidative stress analysis of mice serum which identified significantly depleted SOD and GPx with an elevation of lipid peroxidation levels in mice brain treated with 5 and 10 mg/kg bw cisplatin. Besides, the deteriorative alterations are induced mostly by oxidative stress exerted by cisplatin, suggesting that cisplatin promotes oxidative stress through the depletion of antioxidant activities (Yilmaz et al., 2004, 2005). Zhu et al. (2016) reported that oxidative stress exerted by cisplatin also enhances the permeability of the mitochondrial membrane liberating pro-apoptotic proteins (Bax, Bak), which in turn stimulate several caspases concerned with apoptosis.

Further enrichment analysis of antioxidant activities showed that multiple biochemical processes were markedly enriched in terms of excessive free radical production, which is highly consistent with the cellular and subcellular changes, indicating the deterioration of neural structures as described in the present results. Several studies have reported the correlation between cisplatin exposure and free radical generation-promoting effects (Santos et al., 2008; Xue et al., 2020).

Other important enriched pathways include cell death and cellular necrosis, which have been proven to be

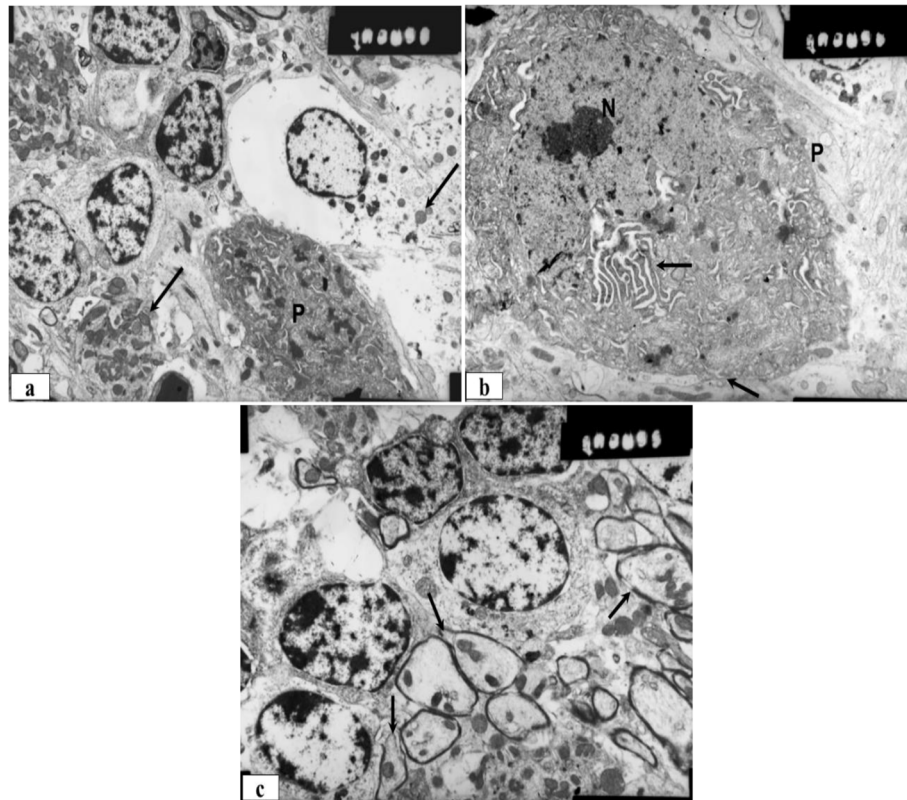


Fig. 5 a-c Electron micrographs of a section of the cerebellar cortex of mice treated with 10 mg/kg bw cisplatin showing a shrunken Purkinje cell (P) with pyknotic nucleus (N). Notice the dilatation in rER and focal areas of splitting myelin sheath of the nerve in **c** (arrows). $\times 5000$ (bar = $10\ \mu\text{m}$)

indispensable for cell injury (Dundar et al., 2016) and may help explain the neuronal degeneration after cisplatin exposure. The apoptosis pathway was found to be significantly enriched as well, which further confirms the present ultrastructural findings of increased apoptosis in the cisplatin-treated brain (Karimi, Amiri, Khalabary, A. R. Mohammadi, & Hosseinimehr, 2019). Apart from oxidative stress and as a cytotoxic agent, cisplatin can trigger cell death during chemotherapy-induced toxicity (Wang, Wu, Fang, Huang, & Zhu, 2019), resulting in activation of signaling of apoptotic pathways which are involved in the pathogenesis of brain injury (Zhang et al., 2015).

The ultrastructure level showed a significant neurodegenerative change in the cisplatin group, suggesting that subcellular apoptosis of neurons may take part in the progression of the histopathological changes described in the present results. Furthermore, in the present findings, the evident cellular death and necrosis could fundamentally be because of the DNA breaking in the neurons following cisplatin exposure and/or arrest of the cell cycle (Attia, Kheirallah, & Khalifa, 2014; Velma, Dasari, & Tchounwou, 2016). The findings of destructive mitochondrial cristae, vacuolization, and dilated cisternae of the rough endoplasmic reticulum along with cell death in terms of splitting and loss of the lamellar

pattern of myelinated axons and disorganization of nuclei of Purkinje cells confirm the apoptotic and cytotoxic effect of cisplatin (Bobilev, Joshi, Barham, Neiss, & Lehmann, 2018; Podratz et al., 2016). On the other hand, accumulation of cisplatin, especially in the neurites, may provide another explanation for neuropathy through nuclear DNA damage and corruption of Schwann cells which are responsible for myelination development (Brouwers, Huitema, Boogerd, W. Beijnen, & Schellens, 2009).

The chemotherapy dose of cisplatin is bio-transformed to active forms, which are the toxic metabolites, by hydrolyzation causing mitochondrial dysfunction (Trombini et al., 2016). Also, the accumulation of cisplatin in the mitochondria exerts toxicity mostly through permanent lysosomal and mitochondrial damage and perturbation of the integrity of the cytoskeleton (Sendao et al., 2006). These active forms are potent that react with DNA forming intra-strand and inter-strand and DNA-protein crosslinks blocking cell division and resulting in DNA damage and apoptotic death in cancer cells (Podratz et al., 2011). Previous studies mentioned that these metabolic bio-transforms can also impair neuronal function and lead to axon loss and nerve corruption (Mong, Petruccio, Kaufman, & Wang, 2008).

Therefore, based on the elevation of lipid peroxidation disintegration-related processes and the depletion of antioxidative activities, combined with the fact that proper antioxidants are critically important for the stabilization and maintenance of brain functions, we suggest that these agents were involved in cisplatin-induced brain injury and that this speculated mechanism is also present in cerebellar cells (Wang et al., 2020).

In summary, these findings provide a confirmed foresight into the potential cisplatin-induced neuropathy in mice cerebellum cells. Cisplatin treatment provokes cisplatin-induced oxidative stress, as well as dysfunction of the cellular ultrastructure. Again, the administration of cisplatin is considered a deleterious chemotherapeutic strategy since cisplatin induces neural injury.

Conclusion

Undoubtedly, the current investigation demonstrated that the common use of cisplatin in chemotherapy results in deleterious neurotoxic effects. Further, cisplatin induces identifiable alterations in neural histology. The principal mechanism of the neurotoxicity might be attributed to the capability of cisplatin to generate free radicals causing oxidative stress.

Abbreviations

CAT: Catalase; GPx: Glutathione peroxidase; H&E: Hematoxylin–eosin; MDA: Malondialdehyde; SOD: Superoxide dismutase; EM: Electron microscope

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Authors' contributions

AA: Supervision, conceptualization, investigation, visualization, methodology, and writing of the original draft. CM: Supervision, thesis administration, and review and editing. RM: Review and editing, data curation, tables, and validation. HK: Funding acquisition, experimental part, data analysis, and software. The authors read and approved the final manuscript.

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Availability of data and materials

Not applicable

Declarations

Ethics approval and consent to participate

The present methodological procedures and laboratory animal care were done under the local committee of Institutional Animal Care and Use Ethics (ACUE) at Alexandria University, Alexandria (Registration No. AU 04-19-10-21-3).

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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References

- Akman, T., Akman, L., Erbas, O., Terek, M. C., Taskiran, D., & Ozsaran, A. (2015). The preventive effect of oxytocin to cisplatin-induced neurotoxicity: An experimental rat model. *BioMed Research International*, 2015, 167235. <https://doi.org/10.1155/2015/167235>.
- Ermiş, B., Ors, R., Yildirim, A., Tastekin, A., Kardas, F., & Akcay, F. (2004). Influence of smoking on maternal and neonatal serum malondialdehyde, superoxide dismutase, and glutathione peroxidase levels. *Annals of Clinical Laboratory Science*, 34, 4–11.
- Atessahin, A., Yilmaz, S., Karahan, I., Ceribasi, A. O., & Karaoglu, A. (2005). Effects of lycopene against cisplatin-induced nephrotoxicity and oxidative stress in rats. *Toxicology*, 212(2-3), 116–123. <https://doi.org/10.1016/j.tox.2005.04.016>.
- Attia, A. A., Kheirallah, N. A., & Khalifa, S. A. (2014). Biochemical and ultra-structural studies of the effect of alprazolam as an anxiolytic drug on the cerebellum of adult male mice. *Journal of Applied Pharmaceutical Science*, 4(01), 074–083. <https://doi.org/10.7324/JAPS.2014.40113>.
- Attia, A. A., Matta, C., & Khaliffa, H. (2015). Genotoxicity and ultrastructural studies of the effect of cisplatin on the cortex of kidney of albino male mice. *International Journal of Biology and Biological Sciences*, 4(1), 1–18.
- Bancroft, J. D., & Gamble, M. (2002). *Theory and practice of histological techniques*, (5th (Ed.) ed., pp. 153–209). Churchill Livingstone.
- Blanchette, M., & Fortin, D. (2011). Blood-brain barrier disruption in the treatment of brain tumors. *Methods in Molecular Biology*, 686, 447–463. https://doi.org/10.1007/978-1-60761-938-3_23.
- Bobylev, I., Joshi, A. R., Barham, M., Neiss, W. F., & Lehmann, H. C. (2018). Depletion of mitofusin-2 causes mitochondrial damage in cisplatin-induced neuropathy. *Molecular Neurobiology*, 55(2), 1227–1235. <https://doi.org/10.1007/s12035-016-0364-7>.
- Brock, P. R., Knight, K. R., & Freyer, D. R. (2012). Platinum-induced ototoxicity in children: A consensus review on mechanisms, predisposition, and protection, including a new International Society of Pediatric Oncology Boston ototoxicity scale. *Journal of Clinical Oncology*, 30(19), 2408–2417. <https://doi.org/10.1200/JCO.2011.39.1110>.
- Brouwers, E. E. M., Huitema, A. D., Boogerd, R., W. Beijnen, J. H., & Schellens, J. H. M. (2009). Persistent neuropathy after treatment with cisplatin and oxaliplatin. *Acta Oncologica*, 48(6), 832–841. <https://doi.org/10.1080/02841860902806609>.
- Carozzi, V. A., Marmiroli, P., & Cavaletti, G. (2010). The role of oxidative stress and antioxidant treatment in platinum-induced peripheral neurotoxicity. *Current Cancer Drug Targets*, 10, 670–682.
- Chowdhury, S., Sinha, K., Banerjee, S., & Sil, P. C. (2016). Taurine protects cisplatin induced cardiotoxicity by modulating inflammatory and endoplasmic reticulum stress responses. *Biofactors*. <https://doi.org/10.1002/biof.1301>.
- Crona, D. J., Faso, A., Nishijima, T. F., McGraw, K. A., Galsky, M. D., & Milowsky, M. I. (2017). A systematic review of strategies to prevent cisplatin induced nephrotoxicity. *Oncologist*, 22, 609–619. <https://doi.org/10.1634/theoncologist.2016-0319>.
- Dundar, H. A., Kiray, M., Kir, M., Kolatan, E., Bagriyanik, A., Altun, Z., ... Olgun, N. (2016). Protective effect of acetyl-L-carnitine against doxorubicin-induced cardiotoxicity in Wistar albino rats. *Archives of Medical Research*, 47, 506e514. <https://doi.org/10.1016/j.jarmed.2016.11.008>.
- Einhorn, L. H. (2002). Curing metastatic testicular cancer. *Proceedings of the National Academy of Sciences of the United States of America*, 99(7), 4592–4595. <https://doi.org/10.1073/pnas.072067999>.
- Gatti, L., Cassinelli, G., Zaffaroni, N., Lanzi, C., & Perego, P. (2015). New mechanisms for old 13 drugs: Insights into DNA-unrelated effects of platinum compounds and drug resistance determinants. *Drug resistance updates: reviews and commentaries in antimicrobial and 15 anticancer chemotherapy*, 20, 1–11. <https://doi.org/10.1016/j.drug.2015.04.001>.
- Hassan, I., Chibber, S., & Naseem, I. (2010). Ameliorative effect of riboflavin on the cisplatin induced nephrotoxicity and hepatotoxicity under photo illumination. *Food and Chemical Toxicology*, 48(8-9), 2052–2058. <https://doi.org/10.1016/j.fct.2010.05.004>.

- Hayat, M. A. (2000). *Principles and techniques of electron microscopy: Biological applications*, (4th ed., pp. 24–96). Cambridge University Press.
- Karimi, S., Amiri, F., Khalatbary, T., A. R. Mohammadi, H. R., & Hosseinimehr, S. J. (2019). Zataria multiflora alleviates cisplatin-induced oxidative stress, apoptosis, and nephrotoxicity in mice. *Jundishapur Journal of Natural Pharmaceutical Products*, 14(3), e6329. <https://doi.org/10.5812/jjnpp.63293>.
- Kim, S. J., Park, C., Lee, J. N., Lim, H., Hong, G. Y., Moon, S. K., ... Park, R. (2015). Erdosteine protects HEI-OC1 auditory cells from cisplatin toxicity through suppression of inflammatory cytokines and induction of Nrf2 target proteins. *Toxicology and Applied Pharmacology*, 288(2), 192–202. <https://doi.org/10.1016/j.taap.2015.07.014>.
- Kütük, S., Gökçe, G., Kütük, G., Cila, M., G. H. E., & Nazıroğlu, M. (2019). Curcumin enhances cisplatin induced human laryngeal squamous cancer cell death through activation of TRPM2 channel and mitochondrial oxidative stress. *Scientific Reports*, 9(1), 17784. <https://doi.org/10.1038/s41598-019-54284-x>.
- Li, H., Tang, Y., Wen, L., Kong, X., Chen, X., Liu, P., ... Xiao, X. (2017). Neferine reduces cisplatin nephrotoxicity by enhancing autophagy via the AMPK/mTOR signaling pathway. *Biochemical and Biophysical Research Communications*, 484(3), 694–701.
- Maj, M., Ma, A., Krukowski, J., KN Kavelaars, A., & Heijnen, C. J. (2017). Inhibition of mitochondrial p53 accumulation by PFT-μ prevents cisplatin-induced peripheral neuropathy. *Frontiers in Molecular Neuroscience*, 10, 108. <https://doi.org/10.3389/fnmol.2017.00108>.
- Manto, M. (2012). Toxic agents causing cerebellar ataxias. *Handbook of Clinical Neurology*, 103, 201–213. <https://doi.org/10.1016/B978-0-444-51892-7.00012-7>.
- Mi, X.-J., Hou, J.-G., Wang, Z., Han, Y., Ren, S., Hu, J.-N., ... Li, W. (2018). The protective effects of maltol on cisplatin-induced nephrotoxicity through the AMPK-mediated PI3K/Akt and p53 signaling pathways. *Scientific Reports*, 8(1), 15922. <https://doi.org/10.1038/s41598-018-34156-6>.
- Mong, P. Y., Petruccio, C., Kaufman, H. L., & Wang, Q. (2008). Activation of rho kinase by TNF-α is required for JNK activation in human pulmonary microvascular endothelial cells. *Journal of Immunology*, 180, 550–558 <https://doi.org/10.4049>.
- Paksoy, M., Ayduran, E., Sanli, A., Eken, M., Aydin, S., & Oktay, Z. A. (2011). The protective effects of intratympanic dexamethasone and vitamin E on cisplatin-induced ototoxicity are demonstrated in rats. *Medical Oncology*, 28(2), 615–621. <https://doi.org/10.1007/s12032-010-9477-4>.
- Parasuraman, S., Raveendran, R., & Kesavan, R. (2010). Blood sample collection in small laboratory animals. *Journal of Pharmacology and Pharmacotherapeutics*, 1(2), 87–93. <https://doi.org/10.4103/0976-500X.215702>.
- Perse, M., & Vecerik-Haler, Z. (2018). Cisplatin-induced rodent model of kidney injury: Characteristics and challenges. *BioMed Research International*, 2018, 1462802. <https://doi.org/10.1155/2018/1462802>.
- Pezeszki, Z., Khosravi, A., Nekuei, M., Khoshnood, S., Zandi, E., Eslamian, M., ... Nematbakhsh, M. (2017). Time course of cisplatin-induced nephrotoxicity and hepatotoxicity. *Journal of Nephropathology*, 6, 163–167. <https://doi.org/10.15171/jnp.2017.28>.
- Pisu, M., Roda, B., Avella, E., & D. and Bernocchi, G. (2004). Developmental plasticity of rat cerebellar cortex after cisplatin injury: Inhibitory synapses and differentiating Purkinje neurons. *Neuroscience*, 129(2004), 655–664. <https://doi.org/10.1016/j.neuroscience.2004.08.023>.
- Podratz, J. L., Knight, A. M., Ta, L. E., Staff, N. P., Gass, J. M., Genelin, K., ... Windebank, A. J. (2011). Cisplatin induced mitochondrial DNA damage in dorsal root ganglion neurons. *Neurobiology of Disease*, 41(3), 661–668. <https://doi.org/10.1016/j.nbd.2010.11.017>.
- Podratz, J. L., Kulkarni, A., Pleticha, J., Kanwar, R., Beutler, A. S., Staff, N. P., & Windebank, A. J. (2016). Neurotoxicity to DRG neurons varies between rodent strains treated with cisplatin and bortezomib. *Journal of the Neurological Sciences*, 362, 131–135. <https://doi.org/10.1016/j.jns.2015.12.038>.
- Reardon, T. F., & Allen, D. G. (2009). Iron injections in mice increase skeletal muscle iron content, induce oxidative stress and reduce exercise performance. *Experimental Physiology*, 94(6), 720–730. <https://doi.org/10.1113/expphysiol.2008.046045>.
- Rosenberg, B., Vancamp, L., & Krigas, T. (1965). Inhibition of cell division in Escherichia coli by electrolysis products from a platinum electrode. *Nature*, 205(4972), 698–699. <https://doi.org/10.1038/205698a0>.
- Rosenberg, B., Vancamp, L., Trosko, J. E., & Mansour, V. H. (1969). Platinum compounds: A new class of potent antitumor agents. *Nature*, 222(5191), 385–386. <https://doi.org/10.1038/222385a0>.
- Saad, A. A., Youssef, M. I., & Elshennawy, L. K. (2009). Cisplatin induced damage in kidney genomic DNA and nephrotoxicity in male rats: The protective effect of grape seed proanthocyanidin extract. *J. Food and Chem. Toxicol.*, 47(7), 1499–1506. <https://doi.org/10.1016/j.fct.2009.03.043>.
- Santos, N. A., Bezerra, C. S., Martins, N. M., Curti, C., Bianchi, M. L., & Santos, A. C. (2008). Hydroxyl radical scavenger ameliorates cisplatin-induced nephrotoxicity by preventing oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. *Cancer Chemotherapy and Pharmacology*, 61(1), 145–155. <https://doi.org/10.1007/s00280-007-0459-y>.
- Sendao, M., Francescato, C., Antunes, H. D., Costa, L. M., R. S., & Bianchi Mde, L. (2006). Comparative study of multiple dosage of quercetin against cisplatin-induced nephrotoxicity and oxidative stress in rat kidneys. *Pharmacological Reports*, 58(4), 526–532.
- Sharawy, N., Laila, R., & Youakim, M. F. (2015). Evaluation of multi-neuroprotective effects of erythropoietin using cisplatin induced peripheral neurotoxicity mode. *Experimental and Toxicologic Pathology*, 67(4), 315–322. <https://doi.org/10.1016/j.etp.2015.02.003>.
- Steeghs, N., de Jongh, F. E., Sillevius Smitt, P. A., & van den Bent, M. J. (2003). Cisplatin-induced encephalopathy and seizures. *Anti-Cancer Drugs*, 14, 443–446.
- Tikoo, K., Bhatt, D. K., Gaikwad, A. B., Sharma, V., & Kabra, D. G. (2007). Differential effects of tannic acid on cisplatin induced nephrotoxicity in rats. *FEBS Letters*, 581(10), 2027–2035. <https://doi.org/10.1016/j.febslet.2007.04.036>.
- Trombini, C., Taina, G., Matilde, M., Thiago, L. R., Julián, B., & Maria, J. B. (2016). Toxic effects of cisplatin cytostatic drug in mussel Mytilus galloprovincialis. *Marine Environmental Research*, 119, 12–21. <https://doi.org/10.1016/j.marenv.2016.05.004>.
- Un, H., Ugan, R., Kose, A., Bayir, D., Cadirci, Y., Selli, E., & J. Halici, Z. (2020). A novel effect of Aprepitant: Protection for cisplatin-induced nephrotoxicity and hepatotoxicity. *European Journal of Pharmacology*, 880(2020), 173168. <https://doi.org/10.1016/j.ejphar.2020.173168>.
- Üstün, R., Oğuz, E., Şeker, K., Korkaya, A., & H. (2018). Thymoquinone prevents cisplatin neurotoxicity in primary DRG neurons. *Neurotoxicology*, 69(2018), 68–76. <https://doi.org/10.1016/j.neuro.2018.09.001>.
- Velma, V., Dasari, S. R., & Tchounwou, P. B. (2016). Low doses of cisplatin induce gene alterations, cell cycle arrest, and apoptosis in human promyelocytic leukemia cells. *Biomarker Insights*, 11, 113–121. <https://doi.org/10.4137/BMI.S39445>.
- Wang, M., Wu, Q., Fang, M., Huang, W., & Zhu, H. (2019). miR-152-3p sensitizes glioblastoma cells towards cisplatin via regulation of SOS1. *Oncology of Targets Therapy*, 12, 9513–9525. <https://doi.org/10.2147/OTT.S210732>.
- Wang, W., Shanmugam, M. K., Xiang, P., Yam, T. Y. A., Kumar, V., Chew, W. S., ... Herr, D. R. (2020). Sphingosine 1-phosphate receptor 2 induces otoprotective responses to cisplatin treatment. *Cancers*, 2020(12), 211. <https://doi.org/10.3390/cancers12010211>.
- Wick, A., Wick, W., Hirrlinger, J., Gerhardt, E., Dringen, R., Dichgans, J., ... Schulz, J. B. (2004). Chemotherapy-induced cell death in primary cerebellar granule neurons but not in astrocytes: In vitro paradigm of differential neurotoxicity. *Journal of Neurochemistry*, 91(5), 1067–1074. <https://doi.org/10.1111/j.1471-4159.2004.02774.x>.
- Xue, D., Pan, S.-T., Zhou, X., Ye, F., Zhou, Q., Shi, F., ... Qiu, J. (2020). Plumbagin enhances the anticancer efficacy of cisplatin by increasing intracellular ROS in human tongue squamous cell carcinoma. *Oxidative Medicine and Cellular Longevity*, 5649174–5649121. <https://doi.org/10.1155/2020/5649174>.
- Yadav, Y. C. (2019). Effect of cisplatin on pancreas and testes in Wistar rats: Biochemical parameters and histology. *Heliyon*, 5(8), e02247. <https://doi.org/10.1016/j.heliyon.2019.e02247>.
- Yilmaz, H. R., Iraz, M., Sogut, S., Ozyurt, Z. Y., Akyol, O., & Gergerlioglu, S. (2004). The effects of erdostiene on the activities of some metabolic enzymes during cisplatin- induced nephrotoxicity in rats. *Pharmacological Research*, 50(3), 287–290. <https://doi.org/10.1016/j.phrs.2004.03.003>.
- Yilmaz, H. R., Sogut, S., Ozyurt, B., Ozugurlu, F., Sahin, S., Isik, B., ... Ozyurt, H. (2005). The activities of liver adenosine deaminase, xanthine oxidase, catalase, superoxide dismutase enzymes and the levels of malondialdehyde and nitric oxide after cisplatin toxicity in rats: Protective effect of caffeic acid phenethyl ester. *Toxicology and Industrial Health*, 21(1–2), 67–73. <https://doi.org/10.1191/0748233705th2160a>.
- Yoon, M. S., Katsarava, Z., Obermann, M., Schafers, M., Liedert, B., Dzagnidze, A., ... Thomale, J. (2009). Erythropoietin overrides the triggering effect of DNA platination products in a mouse model of cisplatin-induced neuropathy. *BMC Neuroscience*, 10(1), 77–86. <https://doi.org/10.1186/1471-2202-10-77>.
- Zelko, I. N., Mariani, T. J., & Folz, R. J. (2002). Superoxide dismutase multigene family: A comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD

(SOD3) gene structures, evolution, and expression. *Free Radical Biological Medicine*, 33(3), 337–349. [https://doi.org/10.1016/s0891-5849\(02\)00905-x](https://doi.org/10.1016/s0891-5849(02)00905-x).

Zhang, L., Yang, X., Li, X., Li, C., Le Zhao, & Hou, H. (2015). Butein sensitizes HeLa cells to cisplatin through the AKT and ERK/p38 MAPK pathways by targeting FoxO3a. *International Journal of Molecular Medicine*, 36(4), 957–966. <https://doi.org/10.3892/ijmm.2015.2324>.

Zhu, K., Jiang, L., Chu, Y., & Zhang, Y. S. (2016). Protective effect of selenium against cisplatin-induced nasopharyngeal cancer in male albino rats. *Oncology Letters*, 12(6), 5068–5074. <https://doi.org/10.3892/ol.2016.5346>.

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