https://doi.org/10.52973/rcfcv-e363960 Revista Científica, FCV-LUZ / Vol. XXXVI

Dose-dependent Cisplatin-induced neurotoxicity: Histopathological and molecular alterations in rat brain

Neurotoxicidad Inducida por Cisplatino dependiente de la dosis: Alteraciones histopatológicas y moleculares en el cerebro de rata

Osman Dağar[1]*, Zeynep Çelik-Kenar[2], Ayşenur Tural–Çifçi[3], Cennet Yaka[2], Muhammed Öner[2], Mehmet Tuzcu[2]

[1]Aksaray University, Eskil Vocational School, Department of Veterinary Medicine. Aksaray, Türkiye.
[2]Selçuk University, Faculty of Veterinary Medicine, Department of Pathology. Konya, Türkiye.
[3]Aksaray University, Faculty of Veterinary Medicine, Department of Pathology. Aksaray, Türkiye.
**Corresponding author: osman.dagar@aksaray.edu.tr*

ABSTRACT

Cisplatin is a widely used chemotherapeutic agent known to cause dose-dependent neurotoxicity, yet the underlying histopathological and molecular mechanisms remain incompletely understood. This study aimed to investigate the neurotoxic effects of different cisplatin dosing regimens on rat brain tissue and to evaluate associated molecular responses, including Tumor necrosis factor-alpha, Heat Shock Protein 70, Brain natriuretic peptide, and Hypoxia-inducible factor 1 alpha expression. Thirty-eight male Wistar albino rats were randomly assigned to five groups: control, single-dose cisplatin (4 mg·kg^{-1} and 8 mg·kg^{-1}), and split-dose cisplatin (2×2 mg·kg^{-1} and 2×4 mg·kg^{-1}). Following administration, brain tissues were collected for histopathological evaluation and quantitative real-time Polymerase Chain Reaction analysis. Histopathological assessments revealed dose-dependent neuronal necrosis, gliosis, perivascular edema, neuronophagia, and congestion, with the most severe alterations observed in the single 8 mg·kg^{-1} dose group. Split-dose regimens elicited milder changes, suggesting that peak plasma concentrations may drive acute neurotoxicity. Molecular analyses demonstrated concurrent upregulation of tumor necrosis factor-alpha and heat shock Protein 70 messenger ribonucleic acid, indicative of an endogenous protective response against cisplatin-induced neuroinflammation. Notably, brain natriuretic peptide and hypoxia-inducible factor 1 alpha levels exhibited increasing trends across all cisplatin – induced groups; these molecules have not previously been investigated in the context of cisplatin-induced neurotoxicity, highlighting a novel aspect of potential adaptive responses. The results indicate that cisplatin neurotoxicity is dose-dependent and more pronounced with high single doses, while split-dose administration may allow partial activation of protective mechanisms, mitigating histopathological damage. Collectively, these findings advance the understanding of cisplatin-induced brain injury, underscore the significance of dosing strategies in minimizing neurotoxic risk, and provide new insights into molecular pathways that may contribute to adaptive responses and neuroprotection.

Key words: Cisplatin; neurotoxicity; histopathology; gene expression; dose-dependent

RESUMEN

La cisplatina es un agente quimioterapéutico ampliamente utilizado, conocido por causar neurotoxicidad dependiente de la dosis, aunque los mecanismos histopatológicos y moleculares subyacentes aún no se comprenden completamente. El objetivo de este estudio fue investigar los efectos neurotóxicos de diferentes regímenes de dosis de cisplatina en el tejido cerebral de ratas y evaluar las respuestas moleculares asociadas, incluyendo la expresión de factor de necrosis tumoral alfa, proteína de choque térmico 70, péptido natriurético cerebral y subunidad alfa del factor inducible por hipoxia–1. Treinta y ocho ratas albinas Wistar macho se asignaron aleatoriamente a cinco grupos: control, cisplatina en dosis única (4 mg·kg^{-1} y 8 mg·kg^{-1}) y cisplatina en dosis dividida (2×2 mg·kg^{-1} y 2×4 mg·kg^{-1}). Tras la administración, se recolectaron tejidos cerebrales para evaluación histopatológica y análisis mediante la reacción en cadena de la polimerasa cuantitativa en tiempo real. Las evaluaciones histopatológicas revelaron necrosis neuronal, gliosis, edema perivascular, neuronofagia y congestión dependientes de la dosis, siendo las alteraciones más graves observadas en el grupo de dosis única de 8 mg·kg^{-1}. Los regímenes de dosis dividida indujeron cambios más leves, sugiriendo que las concentraciones plasmáticas máximas pueden determinar la neurotoxicidad aguda. Los análisis moleculares mostraron una regulación concurrente al alza de factor de necrosis tumoral alfa y proteína de choque térmico 70, indicativa de una respuesta protectora endógena frente a la neuroinflamación inducida por cisplatina. Cabe destacar que los niveles de péptido natriurético cerebral y subunidad alfa del factor inducible por hipoxia–1 mostraron una tendencia al aumento en todos los grupos con neurotoxicidad inducida por cisplatina; estas moléculas no han sido investigadas previamente en el contexto de la neurotoxicidad por cisplatina, lo que resalta un aspecto novedoso de posibles respuestas adaptativas. Los resultados indican que la neurotoxicidad por cisplatina es dependiente de la dosis y más pronunciada con dosis únicas elevadas, mientras que la administración en dosis dividida podría permitir la activación parcial de mecanismos protectores, mitigando el daño histopatológico. En conjunto, estos hallazgos mejoran la comprensión de la lesión cerebral inducida por cisplatina, subrayan la importancia de las estrategias de dosificación clínica para minimizar el riesgo neurotóxico y proporcionan nuevos conocimientos sobre vías moleculares que pueden contribuir a respuestas adaptativas y neuroprotección.

Palabras clave: Cisplatino; dependiente de la dosis; neurotoxicidad; histopatología; expresión génica

Received: 03/04/2026 Accepted: 16/06/2026 Published: 06/07/2026

INTRODUCTION

Cisplatin has markedly enhanced survival in cancer patients; however, its use is frequently accompanied by a range of adverse effects, with neurotoxicity being among the most prominent [1, 2].

Initial investigations concentrated mainly on peripheral nervous system involvement, whereas contemporary studies have progressively highlighted the possible neurotoxic impact on the central nervous system (CNS) [3, 4].

Although cisplatin has limited ability to cross the blood-brain barrier, it has been shown to reach the brain and disrupt hippocampal and cerebellar functions, leading to cognitive impairments in learning, attention, and memory [5, 6].

Previous studies have demonstrated that chemotherapy agents can induce structural alterations in the brain, including reduced grey and white matter in the cortex and corpus callosum and decreased hippocampal volume [7, 8].

Cisplatin exerts its antineoplastic effects primarily by forming adducts with genomic and mitochondrial deoxyribonucleic acid (DNA). These structural alterations induce significant DNA damage and inhibit DNA replication, ultimately leading to programmed cell death through apoptotic or necrotic pathways [9, 10].

Heat shock proteins (HSPs), molecular chaperones generally below 100 kDa, play a critical role in protein folding. The synthesis of HSPs is markedly increased in response to cellular exposure to elevated temperatures ranging from 42 to 44°C [11, 12].

In addition to heat, the expression of HSPs can be induced by various stressors including hypoxia, infection, inflammation, ethanol, heavy metals, trace metals, ultraviolet radiation, and nutrient deprivation, leading them to be referred to as “stress proteins.” HSPs possess cytoprotective properties and have been implicated in neurodegenerative disorders such as multiple sclerosis and Alzheimer’s disease, as well as in a range of brain injuries including ischemia and hemorrhage [13, 14, 15].

Hypoxia-inducible factor 1 alpha (HIF–1α) is a transcription factor that plays a central role in regulating the expression of genes under hypoxic conditions [16, 17]. HIF–1α expression is strongly induced under conditions of decreased oxygen availability in organs [18, 19]. HIF–1α regulates genes primarily involved in oxygen homeostasis and glucose-energy metabolism, and its activation contributes to cellular protection against ischemic injury [20, 21].

Hypoxia-inducible factor 1 alpha is a key regulator of diverse biological processes, encompassing vascular development, blood cell production, epithelial cell proliferation and migration, renal erythropoietin synthesis, glucose metabolism, immune responses, inflammatory pathways, tumorigenesis, autophagy, apoptosis, and epigenetic modifications [17, 22, 23].

Brain natriuretic peptide (BNP), a 32–amino acid hormone initially identified in porcine brain tissue, is primarily produced and secreted by the ventricles of the heart [24]. Plasma or serum concentrations of BNP and its inactive N-terminal pro-B-type natriuretic peptide (NT-proBNP) are widely used as biomarkers for the diagnosis and prognostic assessment of cardiac dysfunction, including heart failure, and studies have shown that BNP expression and release from astrocytes in the brain can increase under hypoxic and stress conditions [25, 26, 27].

In studies examining cisplatin-induced toxicity, variables such as dose, duration of administration, and cisplatin formulation may influence the severity and nature of toxic effects, potentially affecting both the observed efficacy of interventions and the extent of toxicity [28, 29, 30]. Although extensive research has been conducted on the peripheral neurotoxic effects of cisplatin, investigations into its impact on the central nervous system remain relatively limited [31, 32].

Furthermore, only a limited number of studies have investigated the cisplatin doses that induce central neurotoxicity [3, 33].

This study aimed to investigate the dose-dependent effects of cisplatin on the rat cerebral cortex using histopathological and reverse transcription polymerase chain reaction (RT-PCR) analyses, and to assess BNP, HIF-1α, and HSP gene expression. The findings are expected to enhance the understanding of central neurotoxicity mechanisms, facilitate the determination of safe dose ranges, and support more controlled cisplatin treatment planning.

MATERIALS AND METHODS

Experimental design

In this study, 38 male Wistar albino rats (*Rattus norvegicus*), aged 12 weeks and weighing 250–300 g, were used. The animals were obtained from the Selçuk University Experimental Medicine Application and Research Center (Konya, Türkiye) and housed under controlled conditions, with a 12–hour (h) light-dark cycle, a constant temperature of 21°C, and free access to standard chow and water.

All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals to ensure ethical standards and animal welfare. Ethical approval was obtained from the Selçuk University Faculty of Veterinary Medicine Laboratory Animal Production and Research Center Ethics Committee (decision number 2026–29, February 26, 2026).

Healthy male Wistar albino rats were randomLy assigned to one of five experimental groups. The cisplatin doses were selected based on concentrations commonly reported in the literature [34, 35]. The experimental groups were as follows:

- Control group (n = 6): Received a single intraperitoneal (i.p.) injection of 2 mL 0.9 % physiological saline.
- Cisplatin 4 mg·kg^{-1} (n = 8): Received a single i.p. injection of cisplatin at 4 mg·kg^{-1}.
- Cisplatin 2 × 2 mg·kg^{-1} (n = 8): Received two i.p. injections of cisplatin at 2 mg·kg^{-1} each.
- Cisplatin 8 mg·kg^{-1} (n = 8): Received a single i.p. injection of cisplatin at 8 mg·kg^{-1}.

- Cisplatin 2 × 4 mg·kg^{-1} (n = 8): Received two i.p. injections of cisplatin at 4 mg·kg^{-1} each.

General anesthesia was induced with intraperitoneal administration of Ketalar (75 mg·kg^{-1}) and Rompun (10 mg·kg^{-1}). After confirmation of adequate anesthesia, the animals were euthanized by cervical dislocation to prevent suffering, and brain tissues were carefully dissected during the subsequent necropsy procedure.

All injections were administered according to the experimental schedule illustrated in FIG. 1.

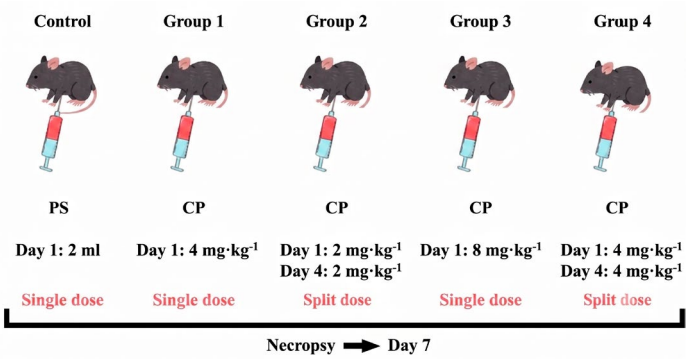

FIGURE 1. Experimental procedure timeline and cisplatin administration representation. PS: Physiological saline, CP: Cisplatin.

Histopathological examination

Brain tissues obtained during necropsy were fixed in 10 % formaldehyde solution for 24 h. Subsequently, the tissues were trimmed to appropriate sizes and placed into tissue processing cassettes. After fixation, the samples were washed under running tap water for 12 h to remove residual fixative. The tissues were then processed using a routine tissue processing system (Leica TP1020, Leica Microsystems, Germany) and embedded in paraffin blocks. Sections of 5 μm thickness were obtained from the paraffin blocks using a microtome (Leica RM2125, Leica Microsystems, Germany) and stained with hematoxylin and eosin (H&E). The prepared brain sections were examined under a light microscope (Olympus BX51, Tokyo, Japan). Histopathological changes including neuronal necrosis, neuronophagia, gliosis, perivascular edema, hyperemia, endothelial cell damage, and mononuclear cell infiltration (MCI) were evaluated and scored as follows: 0 = none, 1 = mild, 2 = moderate, and 3 = severe.

Quantitative Real-Time PCR analysis

Formalin-fixed paraffin-embedded (FFPE) brain tissue sections were first deparaffinized with xylene and subsequently rehydrated through a graded ethanol series. To improve Ribonucleic Acid (RNA) extraction efficiency and reverse protein cross-linking caused by fixation, the samples were treated with proteinase K. Total RNA was then extracted using the SanPrep Column microRNA Miniprep Kit (Cat: SK8811, BIO BASIC) according to the manufacturer's instructions. The concentration and purity of the isolated RNA were determined NanoDrop 2000 spectrophotometrically (Thermo Fisher Scientific, USA), and only samples with acceptable purity ratios were included in subsequent analyses to ensure reliable downstream applications. Complementary deoxyribonucleic acid (cDNA) was synthesized from the extracted RNA using the High-Capacity cDNA Reverse Transcription Kit (Cat: 4368813, Thermo Fisher Scientific) following the manufacturer's protocol.

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to evaluate the mRNA expression levels of BNP, HIF–1α, and HSP70 using a Real-Time PCR system (LightCycler® 96 System, Roche, Switzerland) with the LightCycler 480 SYBR Green Master Mix Kit (Cat: 04707516001, Roche). The primer sequences used for amplification are presented in Table I [36, 37, 38, 39]. The PCR amplification protocol consisted of an initial denaturation step at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 58°C for 10 s, and extension at 72°C for 10 s. Relative gene expression levels were calculated using the 2^−ΔΔCt method after normalization to the housekeeping gene GAPDH.

TABLE I
Primer sequences used for quantitative real-time polymerase chain reaction

Gene	Primer sequence (5'–3')
BNP	F: TGATTCTGCTCCTGCTTTTC R: GTGGATTGTTCTGGAGACTG
HIF–1α	F: GATGGAATGGAGCAGAAGACA R: TAACTGGTCAGCTGTGGTAATC
HSP70	F: GGTCTCAAGGGCAAGATCAG R: TTTCTCAGCCAGCGTGTTAG
TNF–α	F: CCAGGAGAAAGTCAGCCTCCT R: TCATACCAGGGCTTGAGCTCA
GAPDH	F: CACCCTGTTGCTGTAGCCATATTC R: GACATCAAGAAGGTGGTGAAGCAG

F: Forward, R: Reverse, BNP, HIF–1α, HSP70, TNF – α, GAPDH

Statistical analysis

Statistical analyses of the histopathological and Real-Time PCR data were performed using IBM SPSS Statistics 22 and GraphPad Prism software. The normality of the data distribution was evaluated using the Shapiro-Wilk test, while homogeneity of variances was assessed with the Levene test. For datasets showing normal distribution, comparisons among groups were conducted using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test. The results were presented as mean ± standard error (SE), and a P value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Histopathological results

Neuronal necrosis/apoptosis, neuronophagia, gliosis, perivascular edema, congestion, and MCI were evaluated in each case. The severity of histopathological alterations was quantified using a semi-quantitative scoring system, and lesion scores were recorded for each parameter. Statistical analysis results are shown in TABLE II.

TABLE II
Statistical analysis of histopathological scores in the cerebral cortex in an experimental model of cisplatin-induced neurotoxicity

Groups	Neuronophagia	Gliosis	Congestion	Perivascular edema	Neuronal necrosis/ apoptosis	MCI
Control	0,25 ± 0,11^{b}	0,16 ± 0,10^{d}	0,66 ± 0,21^{b}	0,16 ± 0,16^{c}	0,16 ± 0,10^{d}	0,08 ± 0,08^{c}
4 mg·kg^{-1} CP	1,41 ± 0,20^{a}	0,66 ± 0,21^{c}	1,33 ± 0,21^{a}	0,75 ± 0,17^{ab}	0,83 ± 0,16^{bc}	0,83 ± 0,16^{b}
2×2 mg·kg^{-1} CP	1,16 ± 0,27^{a}	0,75 ± 0,17^{bc}	0,83 ± 0,16^{ab}	0,66 ± 0,21^{b}	0,50 ± 0,22^{cd}	0,66 ± 0,21^{b}
8 mg·kg^{-1} CP	1,83 ± 0,27^{a}	1,33 ± 0,16^{a}	1,41 ± 0,20^{a}	1,25 ± 0,17^{a}	1,41 ± 0,20^{a}	1,41 ± 0,20^{a}
2×4 mg·kg^{-1} CP	1,58 ± 0,23^{a}	1,16 ± 0,10^{ab}	1,25 ± 0,17^{ab}	1,16 ± 0,10^{ab}	1,16 ± 0,10^{ab}	1,08 ± 0,10^{ab}

Data are expressed as mean ± standard error (SE). Groups with different superscript letters $^{(a-d)}$ within the same column are significantly different (P<0.05) based on one-way ANOVA followed by Duncan's multiple comparison test. CP: Cisplatin, MCI: Mononuclear cell infiltration

While neuronophagia scores were minimal in the control group, all cisplatin-induced groups showed a significant increase, with the highest scores in the 8 mg·kg^{-1} single-dose group (P<0.05). Glial activation was at basal levels in the control group, while all cisplatin-induced groups showed a significant increase (P<0.05).

The highest scores were observed in the 8 mg·kg^{-1} single-dose group, and the 2×4 mg·kg^{-1} split-dose group showed slightly lower scores than the single-dose group, remaining well above control group levels. Congestion scores were lowest in the control group and increased significantly in the 4 mg·kg^{-1} and 8 mg·kg^{-1} single-dose cisplatin groups (P<0.05).

Split-dose groups (2×2 mg·kg^{-1} and 2×4 mg·kg^{-1}) showed intermediate levels, not significantly different from the control group or single-dose groups. Perivascular edema was minimal in the control group and increased in all cisplatin-induced groups, with the most severe changes in the 8 mg·kg^{-1} cisplatin single-dose group (P<0.05).

Neuronal necrosis/apoptosis was lowest in the control group, elevated in all cisplatin-induced groups, and highest in the 8 mg·kg^{-1} single-dose group (P<0.05).

Mononuclear cell infiltration scores were lowest in the control group. Cisplatin-induced infiltration occurred in all groups, peaking in the 8 mg·kg^{-1} single-dose group (P<0.05).

The 2×4 mg·kg^{-1} split-dose group showed slightly lower scores, but the values were not statistically different from the single-dose 8 mg·kg^{-1} group (P>0.05). Histopathological images of sections obtained from the cerebral cortex of rats, together with the morphological alterations and lesions observed in these sections, are presented in FIG. 2, illustrating the extent and characteristics of the tissue damage across the experimental groups.

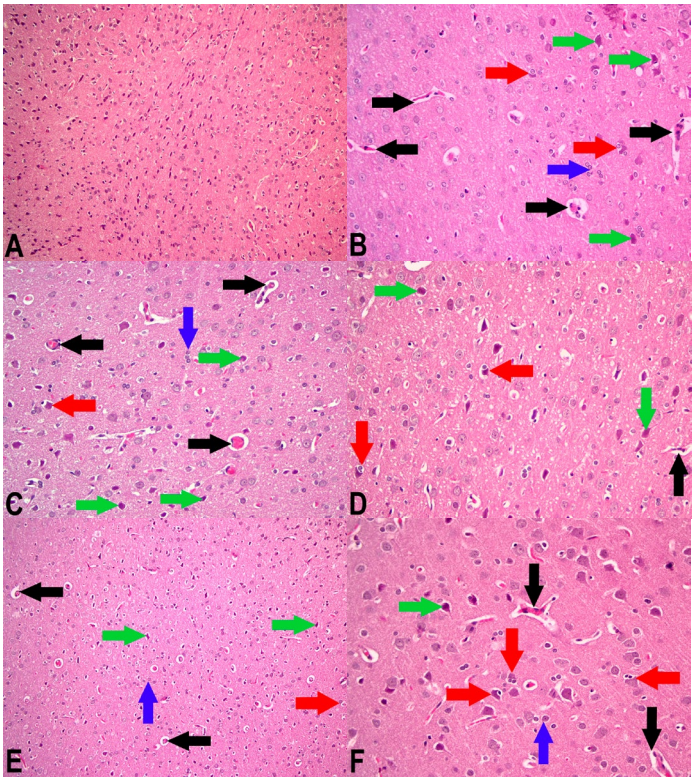

FIGURE 2. Histopathological images of rats cerebral cortex sections stained with H&E. (A) Control group, general architecture of the cerebral cortex (20×). (B) 4 mg·kg^{-1} cisplatin-induced group (40×). (C) 2×2 mg·kg^{-1} cisplatin-induced group (40×). (D) 8 mg·kg^{-1} cisplatin-induced group (40×). (E) 2×4 mg·kg^{-1} cisplatin-induced group (20×). (F) 2×4 mg·kg^{-1} cisplatin-induced group (40×). Vascular congestion and perivascular edema (black arrows), necrotic/apoptotic neurons (green arrows), neuronophagia (red arrows), and gliosis (blue arrows)

Histopathological findings in the present study are consistent with previous reports demonstrating cisplatin-induced neurotoxicity. A study showed that histopathological examinations following the administration of different doses of cisplatin significantly increased neuronal necrosis/apoptosis, gliosis, perivascular edema, neuronophagia, and hyperemia, particularly in the group treated with a single dose of 12 mg·kg^{-1} cisplatin [3].

In addition, cisplatin is known to trigger oxidative stress and DNA damage in brain tissue, leading to microglial activation and subsequent neuronal loss [4]. Cisplatin injection has also been reported to stimulate inflammatory cell migration in neural tissue by increasing lipid peroxidation and nitric oxide levels in brain homogenates [40]. Furthermore, it has been demonstrated that cisplatin disrupts vascular morphology even in vital centers such as the medulla oblongata, activating apoptosis and inflammation pathways and altering gene expression, ultimately leading to tissue edema [41].

In the present study, different dose regimens (4 mg·kg^{-1}, 2×2 mg·kg^{-1}, 8 mg·kg^{-1}, and 2×4 mg·kg^{-1}) were applied to evaluate the dose-dependent neurotoxic effects of cisplatin. The findings, particularly the lack of a significant difference between the 4 mg·kg^{-1} and 2×2 mg·kg^{-1} doses, suggest that the neurotoxic effects of cisplatin become more pronounced beyond a certain dose threshold, which is important for determining optimal dosing in clinical practice.

While these findings are generally consistent with the literature, the detailed histopathological evaluation performed in this study provided a more comprehensive understanding of the process. Specifically, a dose-dependent increase was observed in perivascular edema, neuronal necrosis, neuronophagia, congestion, and gliosis, with the most severe damage detected in the group receiving a single dose of 8 mg·kg^{-1} cisplatin.

Moreover, it was observed that a single high dose (8 mg·kg^{-1}) induced more pronounced histopathological damage compared to the split-dose regimen with the same total dose (2×4 mg·kg^{-1}). It is thought that this difference stems from the higher peak plasma concentrations associated with single-dose administration, which exacerbate acute neurotoxicity; conversely, it is considered that split-dose allows for a more controlled development of cumulative toxicity and the partial activation of cellular defence mechanisms.

Gene expression results

The effects of Cisplatin administration on gene expression levels in brain tissues are presented in FIG. 3. All cisplatin-induced groups showed increased TNF–α mRNA expression compared to the control group. This increase was most pronounced in the 8 mg·kg^{-1} cisplatin group and was statistically significant (P<0.05), whereas differences among the 4 mg·kg^{-1}, 2×2 mg·kg^{-1}, and 2×4 mg·kg^{-1} cisplatin groups were not significant (ns).

Similarly, Heat Shock Protein 70 mRNA expression levels were elevated in all cisplatin-induced groups compared to the control, showing an overall increase ns. The highest expression was observed in the 8 mg·kg^{-1} cisplatin group (P<0.05). BNP mRNA expression levels were also increased in the cisplatin-induced groups compared to the control group. This increase was statistically significant (P<0.05), particularly in the 8 mg·kg^{-1} cisplatin group, while other groups showed moderate elevations. In contrast, HIF–1α mRNA expression levels tended to increase in all cisplatin-induced groups compared to the control group; however, these changes were ns.

Cisplatin-induced neurotoxicity has been associated with a significant increase in pro-inflammatory cytokines, including NF–κB, TNF–α, and IL–6, in brain tissues, indicating the involvement of neuroinflammatory pathways in neuronal damage [5].

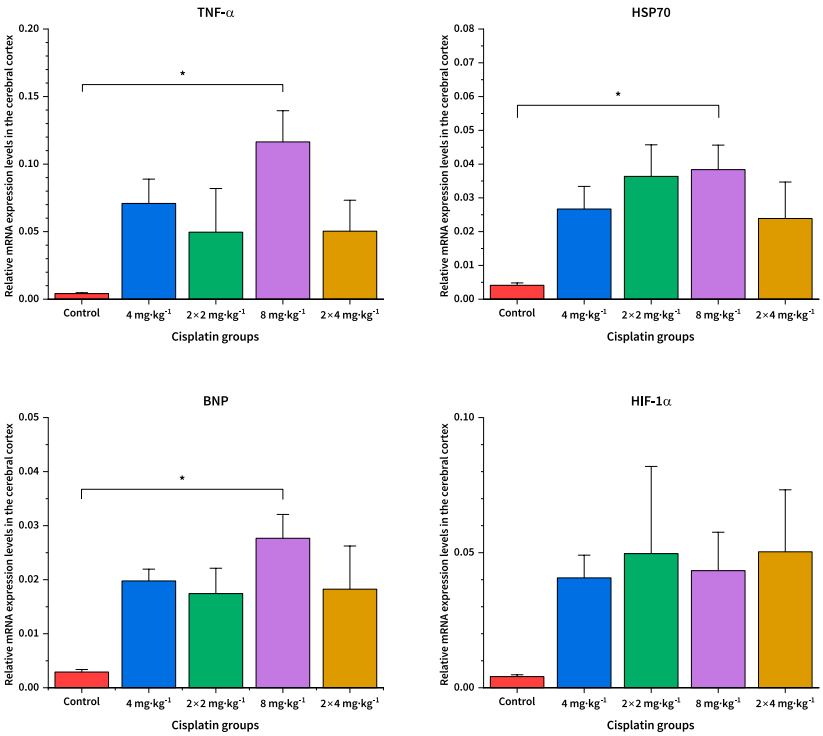

FIGURE 3. TNF–α, HSP70, BNP, and HIF–1α mRNA expression levels in the cerebral cortex. Data are expressed as mean ± standard error (SE). '*' indicates a statistically significant difference compared to the control group, while 'ns' denotes no statistically significant difference relative to the control group

In rat models, cisplatin administration significantly elevated TNF–α levels in the cerebral cortex and induced oxidative stress and apoptotic changes, indicating that TNF–α plays a central role in mediating cisplatin-induced neurotoxicity [42].

Cisplatin has been shown to increase TNF–α release in the dorsal root ganglia, contributing to the development of chemotherapy-induced peripheral neurotoxicity and associated inflammatory responses [43]. Interestingly, the lack of significant differences among the 4 mg·kg^{-1} and split-dose groups (2×2 mg·kg^{-1} and 2×4 mg·kg^{-1}) suggests that the intensity of the neuroinflammatory response in the cerebral tissue may be sensitive to the peak dose concentration (8 mg·kg^{-1}) rather than the total administration frequency, aligning with previous reports on cytokine-driven neurotoxicity.

Cisplatin administration significantly increased BNP and HSP70 mRNA transcript levels in brain tissue. BNP, primarily synthesized by cardiac ventricular cells, has vasodilatory, natriuretic, and diuretic effects [25]. Its levels rise in response to oxidative stress, serving as a protective mechanism against mitochondrial damage in the early stages of cardiac pathology [44, 45].

A significant increase in BNP mRNA expression was observed in cisplatin-induced groups, most prominently in the 8 mg·kg^{-1} group, suggesting an adaptive response to counteract cisplatin-induced oxidative stress and mitochondrial dysfunction.

Heat Shock Protein 70 has been shown to protect cells from oxidative stress by inhibiting lipid peroxidation and apoptosis, enhancing the synthesis of antioxidant enzymes, and preventing tumor necrosis factor-induced cell death through the suppression of interleukin–6 and nitric oxide production [46].

The simultaneous increase in HSP70 and TNF–α mRNA expression observed in this study suggests a strong endogenous defense against cisplatin-induced neuroinflammation. Given that HSP70 has been reported to counteract TNF-induced cytotoxicity by suppressing IL–6 and nitric oxide production, its upregulation in brain tissue likely serves as a critical checkpoint to limit neuronal apoptosis and lipid peroxidation. Thus, the parallel elevation of HSP70 in cisplatin-induced groups can be interpreted as a targeted physiological response to mitigate the pro-oxidant and pro-inflammatory environment.

Hypoxia-inducible factor 1 alpha plays a crucial role in the cellular response to hypoxia, regulating angiogenesis, inflammation, and the expression of various target genes under low-oxygen conditions [19, 23]. In this study, HIF-1α mRNA expression levels tended to increase in all cisplatin-induced groups compared to the control; however, these changes were ns, suggesting that cisplatin-induced oxidative stress did not markedly affect HIF-1α expression in brain tissue.

Further investigations should focus on determining the minimum effective dose of cisplatin that preserves its antitumoral efficacy while minimizing neurotoxic adverse effects. Evaluating low or subclinical cisplatin regimens against the progression of CNS lesions will be critical to clarifying their therapeutic viability. Moreover, long-term longitudinal studies are warranted to comprehensively assess the safety and sustainability of these dose-adjusted cisplatin strategies.

CONCLUSION

Cisplatin administration resulted in marked histopathological and molecular alterations in brain tissue, with neuronal necrosis, gliosis, perivascular edema, neuronophagia, and congestion most pronounced in the single 8 mg·kg^{-1} dose group, although split-dose regimens showed lower absolute scores, these variations did not reach statistical significance. The concurrent increase in TNF–α and HSP70 mRNA expression likely represents an endogenous protective response against cisplatin-induced neuroinflammation. Notably, the observed upward trends in BNP and HIF–1α levels provide novel insights, as the roles of these molecules in cisplatin-induced neurotoxicity have not been previously investigated. Overall, these findings highlight the dose-dependent nature of cisplatin neurotoxicity and the greater severity associated with high single doses, underscoring the importance of optimized clinical dosing strategies to mitigate neurotoxic risk.

Conflict of interest

The authors declared that they have no conflict of interest.

Ethics approval

This study was approved by the Ethics Committee of the Selçuk University Faculty of Veterinary Medicine Laboratory Animal Production and Research Center (Decision No. 2026–29, February 26, 2026).

BIBLIOGRAPHIC REFERENCES

[1] Dos Santos NAG, Ferreira RS, Dos Santos AC. Overview of cisplatin-induced neurotoxicity and ototoxicity, and the protective agents. Food Chem. Toxicol. [Internet]. 2020; 136:111079. doi: https://doi.org/rdm8

[2] Dasari S, Njiki S, Mbemi A, Yedjou CG, Tchounwou PB. Pharmacological effects of cisplatin combination with natural products in cancer chemotherapy. Int. J. Mol. Sci. [Internet]. 2022; 23(3):1532. doi: https://doi.org/gsntwq

[3] Altunkaya M, Ateş MB, Bulut A, Abuşoğlu G, Öztürk B. Cisplatin-induced toxicity in the hippocampus: a dose-dependent mechanism of damage. BMC Pharmacol. Toxicol. [Internet]. 2025; 26(1):215. doi: https://doi.org/rdm9

[4] Durak M, Ozhan O, Yildiz A, Durhan M, Vardi N, Cigremis Y, Parlakpinar H. Protective effect of short-term thymoquinone administration on the central nervous system in cisplatin-induced neurotoxicity. Eur. Rev. Med. Pharmacol. Sci. [Internet]. 2022; 26(19):6935–6943 doi: https://doi.org/rdnb

[5] Alhowail AH. Cisplatin induces hippocampal neurotoxicity and cognitive impairment in rats through neuroinflammation, oxidative stress, and overexpression of glutamatergic receptors mRNA. Front. Pharmacol. [Internet]. 2025; 16:1592511. doi: https://doi.org/g924xv

[6] Owoeye O, Adedara IA, Farombi EO. Pretreatment with taurine prevented brain injury and exploratory behaviour associated with administration of anticancer drug cisplatin in rats. Biomed. Pharmacother. [Internet]. 2018; 102:375–384. doi: https://doi.org/gdnhc9

[7] Sekeres MJ, Bradley-Garcia M, Martinez-Canabal A, Winocur G. Chemotherapy-induced cognitive impairment and hippocampal neurogenesis: a review of physiological mechanisms and interventions. Int. J. Mol. Sci. [Internet]. 2021; 22(23):12697. doi: https://doi.org/grrwpd

[8] George RP, Semendric I, Hutchinson MR, Whittaker AL. Neuroimmune reactivity marker expression in rodent models of chemotherapy-induced cognitive impairment: A systematic scoping review. Brain Behav. Immun. [Internet]. 2021; 94:392–409. doi: https://doi.org/grrwpx

[9] Basu A, Krishnamurthy S. Cellular responses to Cisplatin-induced DNA damage. J. Nucleic Acids [Internet]. 2010; 2010:201367. doi: https://doi.org/c6mnb4

[10] Yang Z, Schumaker LM, Egorin MJ, Zuhowski EG, Guo Z, Cullen KJ. Cisplatin preferentially binds mitochondrial DNA and voltage-dependent anion channel protein in the mitochondrial membrane of head and neck squamous cell carcinoma: possible role in apoptosis. Clin. Cancer Res. [Internet]. 2006; 12(19):5817–5825. doi: https://doi.org/d7dxft

[11] Hagymasi AT, Dempsey JP, Srivastava PK. Heat–shock proteins. Curr. Protoc. [Internet]. 2022; 2(11):e592. doi: https://doi.org/qk6m

[12] Becker J, Craig EA. Heat-shock proteins as molecular chaperones. Eur. J. Biochem. [Internet]. 1994; 219(1–2):11–23. doi: https://doi.org/fxqbzj

[13] Van Noort JM, Bugiani M, Amor S. Heat Shock Proteins: Old and novel roles in neurodegenerative diseases in the Central Nervous System. CNS Neurol. Disord. Drug Targets [Internet]. 2017; 16(3):244–256. doi: https://doi.org/rdnd

[14] Romi F, Helgeland G, Gilhus NE. Heat-shock proteins in clinical neurology. Eur. Neurol. [Internet]. 2011; 66(2):65–69. doi: https://doi.org/bt6c6j

[15] Stetler RA, Gan Y, Zhang W, Liou AK, Gao Y, Cao G, Chen J. Heat shock proteins: cellular and molecular mechanisms in the central nervous system. Prog. Neurobiol. [Internet]. 2010; 92(2):184–211. doi: https://doi.org/cngkw5

[16] Hellwig-Bürgel T, Stiehl DP, Wagner AE, Metzen E, Jelkmann W. Review: hypoxia-inducible factor–1 (HIF–1): a novel transcription factor in immune reactions. J. Interferon Cytokine Res. [Internet]. 2005; 25(6):297–310. doi: https://doi.org/c7rtrk

[17] Basheeruddin M, Qausain S. Hypoxia-Inducible Factor 1–Alpha (HIF–1α): An essential regulator in cellular metabolic control. Cureus [Internet]. 2024; 16(7):e63852. doi: https://doi.org/rdnf

[18] Chu Q, Gu X, Zheng Q, Zhu H. Regulatory mechanism of HIF–1α and its role in liver diseases: a narrative review. Ann. Transl. Med. [Internet]. 2022; 10(2):109. doi: https://doi.org/rdng

[19] Zhang J, Yao M, Xia S, Zeng F, Liu Q. Systematic and comprehensive insights into HIF–1 stabilization under normoxic conditions: implications for cellular adaptation and therapeutic strategies in cancer. Cell. Mol. Biol. Lett. [Internet]. 2025; 30:2. doi: https://doi.org/hbbwkh

[20] Schumacker PT. Hypoxia-inducible factor–1 (HIF–1). Crit. Care Med. [Internet]. 2005; 33(12):423–425. doi: https://doi.org/ffg57v

[21] Howell NJ, Tennant DA. The role of HIFs in ischemia-reperfusion injury. Hypoxia [Internet]. 2014; 2:107–115. doi: https://doi.org/qk6k

[22] Zimna A, Kurpisz M. Hypoxia-Inducible Factor–1 in physiological and pathophysiological angiogenesis: applications and therapies. Biomed. Res. Int. [Internet]. 2015; 2015:549412. doi: https://doi.org/gb57nx

[23] Balamurugan K. HIF–1 at the crossroads of hypoxia, inflammation, and cancer. Int. J. Cancer [Internet]. 2016; 138(5):1058–1066. doi: https://doi.org/ggwwk2

[24] Nakagawa Y, Nishikimi T, Kuwahara K. Atrial and brain natriuretic peptides: Hormones secreted from the heart. Peptides [Internet]. 2019; 111:18–25. doi: https://doi.org/rdnj

[25] Alcidi G, Goffredo G, Correale M, Brunetti ND, Iacoviello M. Brain natriuretic peptide biomarkers in current clinical and therapeutic scenarios of heart failure. J. Clin. Med. [Internet]. 2022; 11(11):3192. doi: https://doi.org/g83qkw

[26] Katoh C, Osanai T, Tomita H, Okumura K. Brain natriuretic peptide is released from human astrocytoma cell line U373MG under hypoxia: a possible role in anti-apoptosis. J. Endocrinol. [Internet]. 2011; 208(1):51–57. doi: https://doi.org/bz44g9

[27] Panagopoulou V, Deftereos S, Kossyvakis C, Raisakis K, Giannopoulos G, Bouras G, Pyrgakis V, Cleman MW. NTproBNP: an important biomarker in cardiac diseases. Curr. Top. Med. Chem. [Internet]. 2013; 13(2):82–94. doi: https://doi.org/f4rsds

[28] Volarevic V, Djokovic B, Jankovic MG, Harrell CR, Fellabaum C, Djonov V, Arsenijevic N. Molecular mechanisms of cisplatin-induced nephrotoxicity: a balance on the knife edge between renoprotection and tumor toxicity. J. Biomed. Sci. [Internet]. 2019; 26(1):25. doi: https://doi.org/gqf7z3

[29] Zavala-Valencia AC, Velasco-Hidalgo L, Martínez-Avalos A, Castillejos-López M, Torres-Espíndola LM. Effect of N-Acetylcysteine on cisplatin toxicity: a review of the literature. Biol.: Targets Ther. [Internet]. 2024; 18:7–19. doi: https://doi.org/hbbpjw

[30] Akşit D, Yazıcı A, Akşit H, Sarı ES, Yay A, Yıldız O, Kılıç A, Ermiş SS, Seyrek K. Selenium protects retinal cells from cisplatin-induced alterations in carbohydrate residues. Balkan Med. J. [Internet]. 2016; 33(4):441–447. doi: https://doi.org/rdnk

[31] Nachnani R, Sepulveda DE, Booth JL, Zhou S, Graziane NM, Raup-Konsavage WM, Vrana KE. Chronic cannabigerol as an effective therapeutic for cisplatin-induced neuropathic pain. Pharmaceuticals [Internet]. 2023; 16(10):1442. doi: https://doi.org/g9853f

[32] Karavelioglu E, Boyaci MG, Simsek N, Sonmez MA, Koc R, Karademir M, Guven M, Eser O. Selenium protects cerebral cells by cisplatin induced neurotoxicity. Acta Cir. Bras. [Internet]. 2015; 30(6):394–400. doi: https://doi.org/rdnn

[33] Kazak F, Akalın PP, Yarım GF, Başpınar N, Özdemir Ö, Ateş MB, Altuğ ME, Deveci MZY. Protective effects of nobiletin on cisplatin induced neurotoxicity in rats. Int. J. Neurosci. [Internet]. 2022; 132(5):531–537. doi: https://doi.org/rdnp

[34] Abdel-Wahab WM, Moussa FI. Neuroprotective effect of N-acetylcysteine against cisplatin-induced toxicity in rat brain by modulation of oxidative stress and inflammation. Drug Des. Dev. Ther. [Internet]. 2019; 13:1155–1162. doi: https://doi.org/rdnq

[35] Gutte H, Oxbøl J, Kristoffersen US, Mortensen J, Kjær A. Gene expression of ANP, BNP and ET–1 in the heart of rats during pulmonary embolism. PLoS One [Internet]. 2010; 5(6):e11111. doi: https://doi.org/dnnmwb

[36] Wang X, Li X, Erhardt JA, Barone FC, Feuerstein GZ. Detection of tumor necrosis factor–α mRNA induction in ischemic brain tolerance by means of real-time polymerase chain reaction. J. Cereb. Blood Flow Metab. [Internet]. 2000; 20(1):15–20. doi: https://doi.org/bhs6nw

[37] Bulut A, Tuzcu M, Ateş MB, Çetin ŞH, Kaya V. Análisis histopatológico y PCR en tiempo real de alteraciones en el cerebro de rata en el síndrome de aplastamiento inducido por glicerol. Rev. Cient. FCV-LUZ [Internet]. 2026; 36(1):e361779. doi: https://doi.org/rdns

[38] Elghamry HA, Mohamed MI, Hassan FM, Abdelfattah DS, Abdelaal AG. Potential use of GAPDH m-RNA in estimating PMI in brain tissue of albino rats at different environmental conditions. Egypt. J. Forensic Sci. [Internet]. 2017; 7(1):24. doi: https://doi.org/ghj4s3

[39] Moneim AEA. *Azadirachta indica* attenuates cisplatin-induced neurotoxicity in rats. Indian J. Pharmacol. [Internet]. 2014; 46(3):316–321. doi: https://doi.org/rdnt

[40] Öztopuz RÖ. Melatonin ameliorates cisplatin-induced neurodegeneration in medulla oblongata through the expressions of Aqp–1,–4, inflammation and apoptosis pathway genes. Turk. J. Biol. [Internet]. 2022 [cited 20 Mar 2026]; 46(2):162–172. Available in: https://goo.su/qASoK

[41] Khadrawy YA, El-Gizawy MM, Sorour SM, Sawie HG, Hosny EN. Effect of curcumin nanoparticles on the cisplatin-induced neurotoxicity in rat. Drug Chem. Toxicol. [Internet]. 2019; 42(2):194–202. doi: https://doi.org/rdnv

[42] Oliveira HR, Coelho MS, Neves FAR, Duarte DB. Cisplatin-induced changes in calcitonin gene-related peptide or TNF–α release in rat dorsal root ganglia *in vitro* model of neurotoxicity are not reverted by rosiglitazone. Neurotoxicology [Internet]. 2022; 93:211–221. doi: https://doi.org/rdnw

[43] Michel L, Rassaf T, Totzeck M. Biomarkers for the detection of apparent and subclinical cancer therapy-related cardiotoxicity. J. Thorac. Dis. [Internet]. 2018; 10(35):4282–4295. doi: https://doi.org/gnb6r7

[44] Chang P, Zhang X, Zhang J, Wang J, Wang X, Li M, Wang R, Yu J, Fu F. BNP protects against diabetic cardiomyopathy by promoting Opa1–mediated mitochondrial fusion via activating the PKG-STAT3 pathway. Redox Biol. [Internet]. 2023; 62:102702. doi: https://doi.org/qk6v

[45] Van Molle W, Wielockx B, Mahieu T, Takada M, Taniguchi T, Sekikawa K, Libert C. HSP70 protects against TNF-induced lethal inflammatory shock. Immunity [Internet]. 2002; 16(5):685–695. doi: https://doi.org/ccq65s

[46] Belenichev IF, Aliyeva OG, Popazova OO, Bukhtiyarova NV. Involvement of heat shock proteins HSP70 in the mechanisms of endogenous neuroprotection: the prospect of using HSP70 modulators. Front. Cell. Neurosci. [Internet]. 2023; 17:1131683. doi: https://doi.org/qk6w