Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2025.202

RESEARCH ARTICLE

Neuroprotective Potential of Betaine in Cisplatin-Treated Rats: A Histopathological and Immunohistochemical Analysis

Menekşe Ülger*[1] and Latife Çakir Bayram[2]

[1]Department of Histology and Embryology, Faculty of Medicine, Erciyes University, 38039 Kayseri, Turkey
[2]Department of Pathology, Faculty of Veterinary Medicine, Erciyes University, 38039 Kayseri, Turkey
*Corresponding author: menekseulger@erciyes.edu.tr

ARTICLE HISTORY (25-163)

Received: February 21, 2025
Revised: May 21, 2025
Accepted: May 23, 2025
Published online: July 14, 2025

Key words:
Betaine
Central nervous system
Cisplatin
Neuroprotection
Neurotoxicity
Rat

ABSTRACT

Cisplatin (CS) is a widely used chemotherapeutic agent, but its side effects, such as neurotoxicity, limit its clinical application. The present study investigated the protective role of betaine, a methyl donor with antioxidant and anti-inflammatory properties, against CS induced neurotoxicity mediated by oxidative stress using immunohistochemical methods. Forty female Wistar albino rats (8-10 weeks old, 200-250g) were randomly divided into four groups: Control, Betaine, CS, and CS+Betaine. Betaine (250mg/kg) was administered orally, while CS (8mg/kg) was administered intraperitoneally once a week for four weeks. Histopathological and immunohistochemical analyses were performed on the cerebral cortex/medulla and hippocampal tissues using HIF-1α, VEGF, EPO, GFAP, and caspase-3 markers. Statistical analyses were performed using GraphPad Prism 10. Histopathological examination revealed that CS administration caused hypereosinophilic degeneration in pyramidal neurons, which was alleviated in the CS+Betaine group. Immunohistochemical analysis showed that CS significantly increased the expression of HIF-1α, VEGF, EPO, and GFAP compared to the control group, while co-administration with betaine tended to reduce these increases; however, the reductions did not reach statistical significance. Caspase-3 expression was also high in the CS group, indicating increased apoptosis, while betaine attenuated this effect. Betaine may exert neuroprotective effects through its antioxidant and anti-apoptotic potential. These findings suggest that betaine may have therapeutic potential in reducing CS-associated neurotoxicity.

To Cite This Article: Ülger M and Bayram LC 2025. Neuroprotective potential of betaine in cisplatin-treated rats: a histopathological and immunohistochemical analysis. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2025.202

INTRODUCTION

Cancer, a leading cause of mortality worldwide, continues to drive efforts aimed at reducing the adverse effects of chemotherapy on healthy tissues and organs, while fostering the development of more effective and targeted treatment options. Cisplatin (CS) is a platinum-based chemotherapeutic used in various malignancies such as ovarian, head, and neck cancers (Minerva *et al.*, 2023). Its antineoplastic activity is achieved by the formation of DNA crosslinks and adducts, in addition to the creation of superoxide radicals (Barabas *et al.,* 2008). The fact that the platinum ions it contains cross-link the purine bases in DNA and interfere with DNA repair mechanisms plays a great role in making CS so effective (Goodsell, 2006). Cancer cells grow and multiply faster compared to normal cells. Therefore, with chemotherapeutics such as CS, cells with these characteristics are aimed to be destroyed. However, healthy normal cells also exhibit these characteristics and are affected by chemotherapy, consequently (Polat *et al.,* 2023). Therefore, CS often causes toxicity in multiple organs, such as the liver, kidneys, gastrointestinal, cardiovascular, hematological, and nervous systems (Dasari *et al.,* 2022). CS causes oxidative damage and toxicity in cells not only through its association with DNA but also through the formation of reactive oxygen and nitrogen species (Gündoğdu *et al.,* 2019). Among the systems known to be affected by Cs toxicity, the nervous system is particularly affected by this oxidative environment due to its vulnerability. (Uttara *et al.,* 2009). Based on this information, it was concluded that the protection of endogenous antioxidant systems, whose function is to eliminate oxidative stress and which are disrupted by CS applied during treatment, is important for maintaining the functionality of the cells.

Betaine is a non-toxic, natural product that is a methyl derivative of glycine and both taken through the diet and synthesized endogenously in the liver and kidneys. (Day & Kempson, 2016). It acts as an osmoprotectant in renal medulla cells and reduces the amount of homocysteine in the liver through methylation of homocysteine (Barak *et al.,* 1996; Kempson *et al.,* 2013). It is also known that the cellular uptake of betaine in the brain is mediated by betaine-γ-aminobutyric acid (GABA) transporter 1 (BGT-1)(Kempson *et al.,* 2014). Thus, the transport of betaine across the membrane in the brain allows it to take its place as a helpful compound against neurodegeneration and oxidative stress in the central nervous system (CNS) (Bhatt *et al.,* 2023). When these beneficial effects of betaine are taken into consideration, it is understood that various mechanisms should be elucidated at the molecular and cellular level concerning CS neurotoxicity. CS-induced oxidative environment occurs in the tissue caused by an increase in free radicals, disruption of the cell oxidant/antioxidant balance, cellular hypoxia, and various specific adaptation mechanisms (Karabulut *et al.,* 2021; Magar *et al.,* 2024). One of these mechanisms is managed by hypoxia-inducible factor-1 alpha (HIF-1α) activation. HIF-1α is a central regulator of cellular responses to hypoxia and plays a role in neurorepair(Amin *et al.,* 2021). Angiogenesis, neurogenesis, and remyelination processes are triggered in the brain by the induction of HIF-1α (Khan *et al.,* 2017). In addition to HIF-1α, hypoxia-induced transcription factors such as vascular endothelial growth factor (VEGF) and erythropoietin (EPO) are also involved in this mechanism (Magar *et al.,* 2024). Induction of angiogenesis is regulated by VEGF (Singh *et al.,* 2012). The expression of EPO in glial cells, neurons, and endothelial cells supports the angiogenic effect of VEGF by providing neuroprotective effects and stimulating endothelial cells (Marti, 2004). Glial fibrillary acidic protein (GFAP) is an intermediate filament that provides cell integrity and strength of the astrocyte cytoskeleton. In addition to being a structural component of the cell, GFAP is also known to be involved in biomechanical and molecular signaling. GFAP is a marker for astrocytes, which are known to be induced and expressed at higher levels in conditions such as brain injury or CNS degeneration (Middeldorp & Hol, 2011).

This study aimed to investigate the potential neuroprotective effect of betaine against CS-induced neurotoxicity via analysis of alterations in a portion of the HIF-1α signaling pathway, including VEGF and EPO. At the same time, evaluation was made on cell death by caspase-3 expression. The results of the study were evaluated by histopathological and immunohistochemical analyses.

MATERIALS AND METHODS

Ethics approval: The experimental procedures were approved by the Erciyes University Animal Experiments Local Ethics Committee (Approval No: 24/236, dated 05.12.2024). All animals were treated humanely and in accordance with the Guide for the Care and Use of Laboratory Animals. Forty female Wistar albino rats, 8–10 weeks old and weighing 200–250g, raised at Erciyes University Experimental Research Application and Research Center (DEKAM), were used in this study. The rats were fed with standard pellet feed and water and housed in plastic cages at 22±237°C and a 12-h light/12-h dark cycle.

Groups and chemicals: Four groups were randomly selected from rats and were as follows (n=10): Control, Betaine, CS, CS+Betaine. Betaine hydrochloride (B3501-100G, Sigma-Aldrich, USA) (250mg/kg) (Hussein & Al-Dalain, 2021) was administered orally by gavage after being dissolved in physiological serum, while CS (CP-Koçak 50mg/100mL Vial Containing Concentrated Solution for Infusion) was administered intraperitoneally at 8mg/kg (Fu *et al.,* 2019). While physiological serum was administered to the control group for 30 days, betaine was administered to the Betaine and CS+Betaine groups. CS administration was applied to the CS and CS+Betaine groups once a week for 4 weeks (4 CS ejections in total). Two weeks after the final dose of betaine, the animals were euthanized by general anesthesia with 50mg/kg ketamine and 10mg/kg xylazine.

Histopathologic analysis: For histopathological examination, immediately after sacrifice, brain tissues were quickly placed in 10% formaldehyde and fixed for 72 hours. Then, a routine histological tissue follow-up procedure was performed. Accordingly, tissues were washed in running tap water, passed through increasing grades of alcohol, clarified with xylene, and embedded in paraffin blocks. 5µM-thick sections were taken from paraffin blocks and placed on polylysine slides. The obtained sections were stained with Hematoxylin and Eosin (H&E) for histopathological evaluation.

Immunohistochemical analysis: Immunohistochemical staining was performed to evaluate inflammation and apoptosis using primary antibodies to HIF-1α (1:100 dilution, bs-0737R, Bioss), VEGF (1:100 dilution, ab-9530, Abcam), EPO (1:100 dilution, ab-226956, Abcam), GFAP (1:100 dilution, NB-12016997, Novus Biologicals) and caspase-3 (1:100 dilution, E-AB-13815, Elebscience). Immunohistochemical staining was performed by the avidin-biotin-peroxidase method (Ultravision Polyvalent (Rabbit-Mouse) Horseradish Peroxidase (HRP) Kit, 125 mL, Thermo Fisher Scientific, Waltham, Massachusetts, USA). For immunohistochemical staining, dilution ranges specified in the data sheet were tested to determine the dilution ranges of primary antibodies. For negative control, PBS was left instead of the primary antibody at the appropriate stage, and other steps were performed in the same way. The stained slides were examined under a microscope, and images were taken from randomly selected areas for each preparation (Olympus BX51; Olympus, Tokyo, Japan). For GFAP expression, three different regions were obtained from the cerebral cortex for each preparation; 30 astrocytes in the medullary area were selected one by one per group, and one dentate gyrus, Cornu Ammonis (CA) Areas: CA1, CA2, CA3 were chosen for the areas in the hippocampus. Immunoreactivity intensities were measured from the obtained images using the ImageJ Software program (Ülger *et al.,* 2025). And the investigators performing these histological assessments were blinded.

Statistical analysis: Statistical analyses for the data in this study were performed using GraphPad Prism 10 software. The normality of data distribution was assessed using the D'Agostino & Pearson normality test. Data were analyzed using a one-way ANOVA test for normal distribution and Tukey's post-hoc test for multiple group comparisons. A p-value of $P<0.05$ was considered statistically significant.

RESULTS

Histopathological findings: In the histopathological examination of brain sections stained with hematoxylin and eosin at the light microscopic level, the control group showed standard cerebral cortex architecture with basophilic cytoplasm and granular cells containing centrally located nuclei and pyramidal neurons containing large and well-defined nuclei. Although there were hyper-eosinophilic degenerated pyramidal neurons with karyolysis in several areas in the CS group, these changes were mild and limited in extent. The histological structure in the tissues of the Betaine group and the CS+Betaine group also reflected standard architecture (Fig. 1). The blood vessels in the brains of all groups had a standard architecture with intact endothelium and uniform wall structure. Granular cells of the dentate gyrus of the hippocampus had a standard histological structure. Other cells, such as basket cells and interneurons, also maintained standard histological architecture. The hippocampus sections of all groups were similar (Fig. 1).

Immunohistochemical findings: In this study, in addition to general histological evaluation, the cortex and hippocampal areas of the brain were evaluated using immunohistochemical methods. In this context, the immunoreactivity of the tissues to HIF-1α, EPO, VEGF, GFAP, and caspase-3 primary antibodies was evaluated. The data obtained from the measurements were evaluated statistically.

It was observed that the results of the Control and Betaine groups were similar in terms of all antibodies evaluated in the cerebral cortex and hippocampus, and there was no statistically significant difference between them. However, when HIF-1α reactivity was evaluated in the cerebral cortex, a significant increase was observed in the CS group compared to the Betaine group ($P<0.05$), while this CS-induced increase tended to decrease in the CS+Betaine group. HIF-1α expression in the hippocampus was found to be significantly higher in the CS group compared to the Control group ($P<0.05$) (Fig. 2, 3) (Table 1).

VEGF expression in the cerebral cortex was found to be significantly higher in the CS group compared to the Betaine group ($P<0.05$). The density in the CS+Betaine group decreased compared to the CS group. No statistically significant difference was found between the groups in terms of VEGF expression in the hippocampus (Fig. 2, 3) (Table 1).

In terms of EPO expression in the cortex, the CS and CS+Betaine groups were found to be significantly higher than the Control group ($P<0.05$). There was no significant difference between the CS and CS+Betaine groups. When the EPO expression in the hippocampus was evaluated, the CS group was found to be significantly higher than the Control group ($P<0.05$). The CS+Betaine group decreased slightly compared to the CS group (Fig. 2, 3) (Table 1).

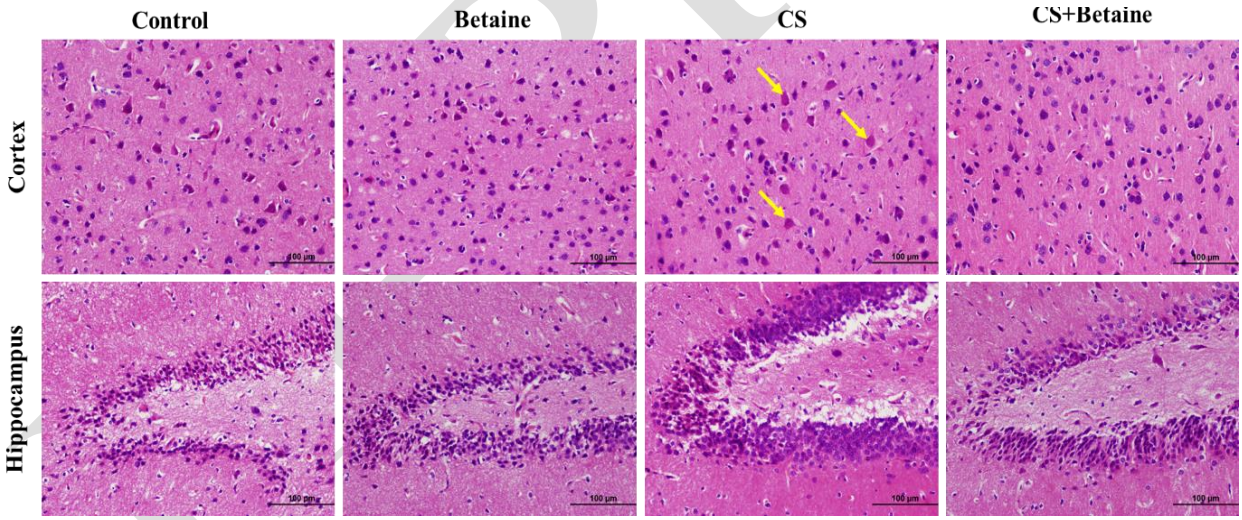

Fig. 1: Photomicrographs of brain sections obtained from the cerebral cortex and hippocampus regions of rats from all groups stained with H&E. (Original magnification = 40X; scale bar = 100μM). Yellow arrow: pyramidal neuron with hyper-eosinophilic degeneration. Abbreviations: CS; Cisplatin, H&E; Hematoxylin and Eosin.

Table 1: Statistical analysis of HIF-1α, VEGF, and EPO immunoreactivity intensities in the cerebral cortex and hippocampus across experimental groups

Markers	Control	Betain	CS	CS+Betain	*p*
HIF-1α (Cortex)	62.25±9.1^{ab}	58.45±12.9^{b}	68.29±8.6^{ac}	67.65±8.5^{a}	*0.0004*
HIF-1α (Hippocampus)	68.75±9.1^{a}	70.10±7.2^{ab}	74.49±8.4^{b}	72.24±7.0^{ab}	*0.0361*
VEGF (Cortex)	60.51±7.92^{ab}	58.7±6.2^{a}	64.03±6.8^{b}	61.59±5.3^{ab}	*0.0213*
VEGF (Hippocampus)	72.84±10.1^{a}	73.43±8.2^{a}	75.66±8.6^{a}	74.01±6.6^{a}	*0.6071*
EPO (cortex)	65.29±4.7^{a}	67.17±9.9^{ab}	72.26±5.7^{bc}	73.73±2.3^{c}	*0.0001*
EPO (Hippocampus)	76.80±6.8^{a}	79.82±8.5^{ac}	84.67±4.0^{b}	83.31±3.3^{bc}	*0.0001*

Data are expressed as mean ± standard deviation. Mean values with different superscripts $^{(a, b, c)}$ within the same row indicate significant differences between groups. $P<0.05$ was considered significant. Density measurements are expressed as pixels in the ImageJ program. Abbreviations: CS; Cisplatin, HIF-1α; hypoxia-inducible factor-1 alpha, VEGF; vascular endothelial growth factor, EPO; erythropoietin.

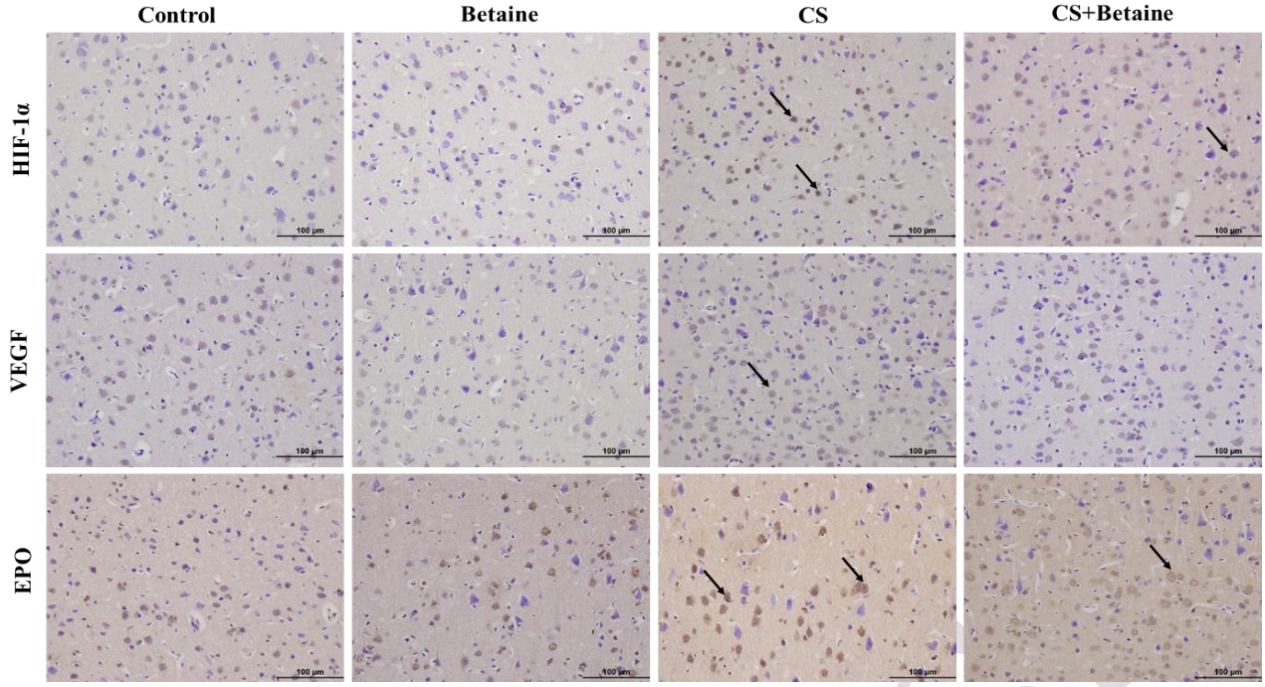

Fig. 2: Photomicrographs of sections were stained with HIF-1α, VEGF and EPO antibodies in the cerebral cortex in all groups. Glia cells showing positive immunoreactivity were stained brown; some are indicated by black arrows. The immunoreactivity intensity for HIF-1α and VEGF appears to be quite low in the Control and Betaine groups. While the CS group was stained more intensely than the CS+Betaine group, both groups exhibited more pronounced immunoreactivity compared to the Control and Betaine groups. For EPO, there appears to be a lower intensity of immunoreactivity in the Control and Betaine groups than CS-treated groups. (Original magnification = 40X, scale bar = 100μM). Abbreviations: CS; Cisplatin, HIF-1α; hypoxia-inducible factor-1 alpha, VEGF; vascular endothelial growth factor, EPO; erythropoietin.

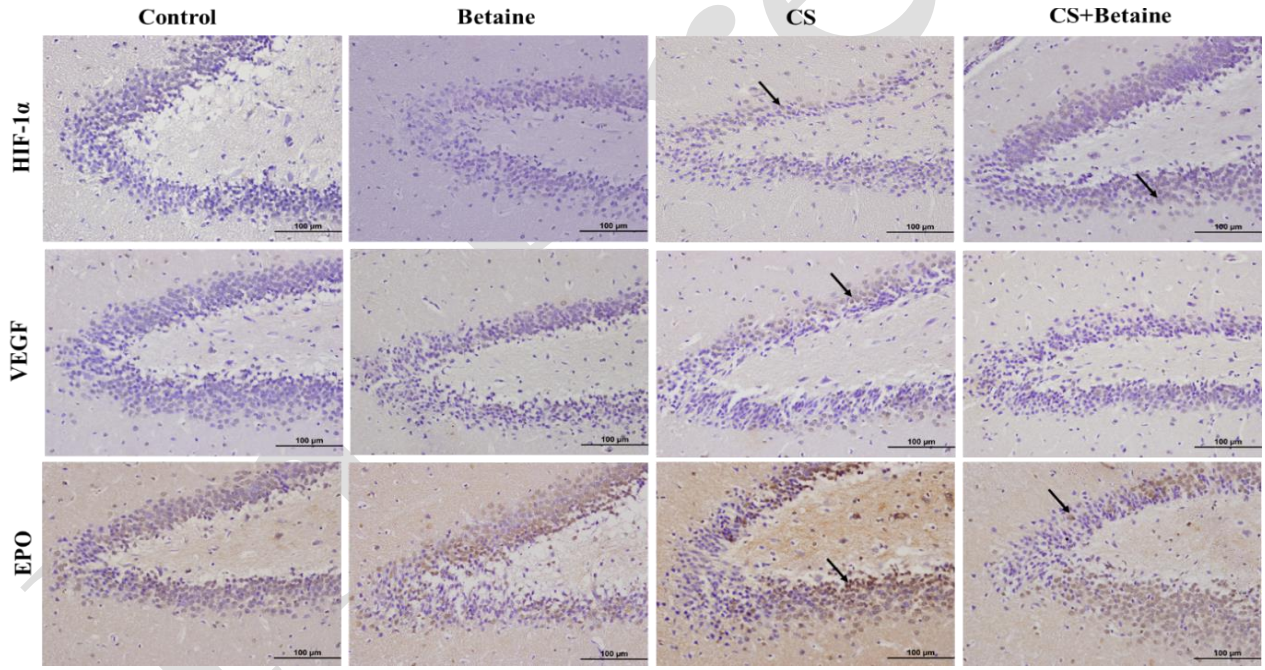

Fig. 3: Cross-sectional photomicrographs were stained with HIF-1α, VEGF and EPO antibodies in all groups in the hippocampus. The cells showing immunoreactivity are stained brown; some of them are indicated by black arrows. There seems to be quite weak immunostaining for HIF-1α and VEGF in the Control and Betaine groups. Although the CS group was stained slightly more intensely than the CS+Betaine group, both these groups showed more pronounced immunoreactivity compared to the Control and Betaine groups. A weaker immunostaining is observed in the Control and Betaine groups for EPO compared to the CS-treated groups. The density in the CS group is also partially higher than in the CS+Betaine group. (Original magnification = 40X, scale bar = 100μM). Abbreviations: CS; Cisplatin, HIF-1α; hypoxia-inducible factor-1 alpha, VEGF; vascular endothelial growth factor, EPO; Erythropoietin.

Since GFAP expression was observed more intensely in the medullary area, measurements were made in this region. According to the statistical evaluation of the measurement results, GFAP expression was significantly higher in the CS group compared to the Control group ($P<0.05$). The results in the CS+Betaine group) were lower than the CS group. In the GFAP results in the hippocampus, the CS group was found to be significantly higher than both the Control and Betaine groups ($P<0.05$). The expression in the CS+Betaine group tended to decrease compared to the CS group (Fig. 4, 5) (Table 2).

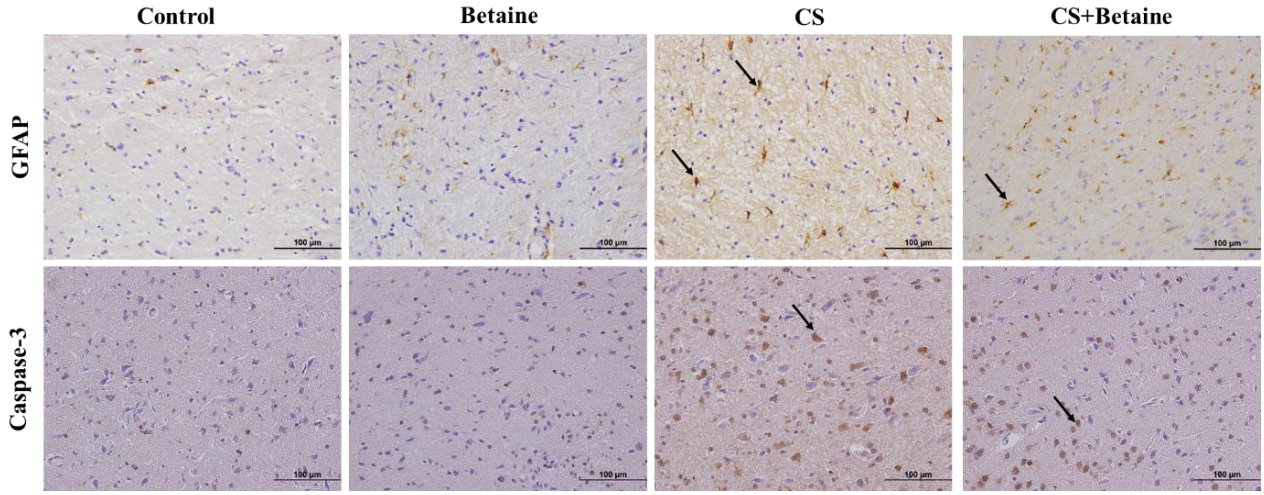

Fig. 4: Neurons in the cerebral medulla showing immunoreactivity with GFAP and caspase-3 antibodies are stained brown; some are indicated with black arrows. In the GFAP immunoassay, while normal expression was observed in the Control and Betaine groups astrocytes showing strong positive reaction in GFAP staining were detected in CS-treated rats. It was found that this density was higher in the CS group than in the CS+Betaine group. Remarkably, in the Control and Betaine groups, normal positive caspase-3 expression was observed without apoptotic cells. In rats treated with CS, however, significantly more apoptotic cells showed up with caspase-3 positivity. Caspase-3 expression seemed to be slightly less in the CS+Betaine group compared to the CS group. (Original magnification = 40X, scale bar = 100µM). Abbreviations: CS; Cisplatin, GFAP; Glial fibrillary Acidic Protein.

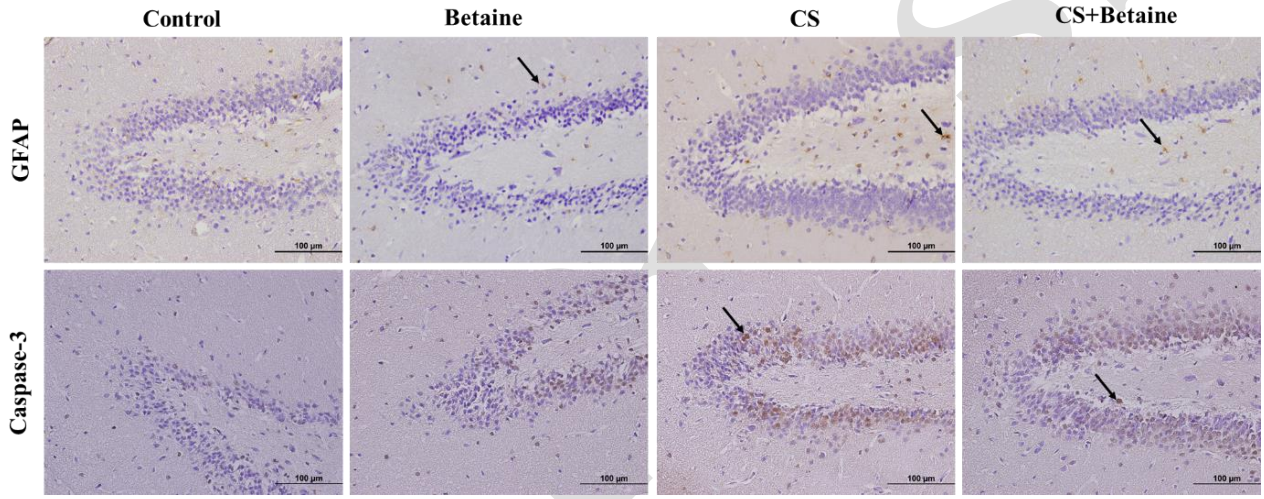

Fig. 5: Neurons in the hippocampus showing immunoreactivity with GFAP and caspase-3 antibodies are stained brown; some are indicated with black arrows. Normal expression was observed in the Control and Betaine groups on immunohistochemically stained hippocampus sections for GFAP. Astrocytes showing a strong positive reaction in the CS group were detected in the hippocampal area. A partially less intense reaction was observed in the CS+Betaine group compared to the CS group. Normal positive caspase-3 expression was observed in the Control and Betaine groups for Caspase-3 immunoreactivity, without apoptotic cells. In rats treated with CS, a dense and large number of cells showing caspase-3 positivity were detected. There was slightly less Caspase-3 expression in the CS+Betaine group compared to the CS group. (Original magnification = 40X, scale bar = 100µM). Abbreviations: CS; Cisplatin, GFAP; Glial fibrillary Acidic Protein.

Table 2: Statistical analysis of GFAP and Caspase-3 immunoreactivity intensities in the medulla and hippocampus across experimental groups

Markers	Control	Betain	CS	CS+Betain	*p*
GFAP (Medulla)	55.27±6,4^{a}	57.02±6.8^{ab}	61.04±7.9^{b}	58.49±7.6^{ab}	*0.0196*
GFAP (Hippocampus)	68.67±8.2^{a}	70.08±7.0^{a}	77.89±9.7^{b}	72.64±5.6^{ab}	*0.0001*
Caspase-3 (Cortex)	55.16±6.3^{a}	56.80±6.9^{ab}	61.80±8.5^{b}	58.79±7.8^{ab}	*0.0059*
Caspase-3 (Hippocampus)	69.29±6.5^{a}	69.57±6.8^{a}	78.88±10.4^{b}	76.87±8.0^{b}	*0.0001*

Data are expressed as mean ± standard deviation. Mean values with different superscripts $^{(a, b, c)}$ within the same row indicate significant differences between groups. P<0.05 was considered significant. Density measurements are expressed as pixels in the ImageJ program. Abbreviations: CS; Cisplatin, GFAP; Glial fibrillary Acidic Protein.

In the results of caspase-3 immunoreactivity applied to evaluate apoptosis, significantly increased expression was detected in the cerebral cortex in the CS group compared to the Control group (P<0.05). Lower expression was observed in the CS+Betaine group compared to the CS group. In the hippocampus, the CS group showed higher expression than the Control and Betaine groups, while it tended to decrease in the CS+Betaine group. As a result of all immunohistochemical evaluations performed in the cerebral cortex/medulla and hippocampus areas, it was observed that there was no statistically significant difference between the CS+Betaine group and the CS group (Fig.4, 5) (Table 2).

DISCUSSION

This study found that HIF-1α expression was upregulated in CS-induced neurotoxicity, and VEGF and

EPO expressions increased accordingly. In addition, it was observed that GFAP expression increased in astrocytes, especially in the medullary regions, due to CS. It is now known that CS reacts with nucleophilic regions in DNA to form strong bonds and thus affects function and structural integrity regardless of tissue (Woźniak & Błasiak, 2002). This study presented new findings showing that betaine therapy mitigates some of the changes caused by cisplatin and may contribute to the maintenance of brain tissue and functional integrity. Neuronal degeneration, which is known to occur due to CS, was observed to disappear with the protection of betaine in the current study (Mert *et al.,* 2024). Cisplatin disrupts tissue oxygenation and extracellular matrix integrity not only in tumors but also in healthy brain tissue. Therefore, it prepares the ground for triggering processes that will provide oxygenation by activating the factors that hypoxia induces as a primary response (Ahmed *et al.,* 2018). The upregulation of HIF-1α in the cerebral cortex and hippocampus in our study results can be considered an indicator of hypoxic signaling. Our results revealed that genes associated with HIF-1α signaling also increased their protein expression. VEGF expression, which increased in parallel with HIF-1α upregulation, is one of these associated genes. VEGF expression increases in response to impaired oxygenation to promote angiogenesis (Sun, 2022). In the study, the fact that VEGF upregulation in the cerebral cortex rather than the hippocampus gave statistically more significant results may suggest that angiogenesis-related oxygenation is experienced more intensely in the cortex. Therefore, it may reveal that CS causes greater neurotoxicity in the cortex compared to the hippocampus. Similarly, it has been shown that VEGF expression may be expressed at different levels in different regions of the brain following chronic cerebral hypoperfusion (Jun *et al.,* 2020). These data must be revealed and supported in more detail with various mechanisms and pathways. It has been shown that protective mechanisms are activated in conditions that will pave the way for ischemia, regardless of the source of neurotoxicity. Increased expression of VEGF and EPO, target genes of HIF-1α, functions as a protective mechanism by triggering tolerance against focal permanent cerebral ischemia (Bernaudin *et al.,* 2002). It has been reported that HIF-1α/EPO hypoxic signaling initiates hippocampal neurogenesis following hypoxia-ischemia, and when returned to normoxia, HIF-1α expression decreases and disappears (Lu *et al.,* 2014). The results of this study also revealed that increased HIF-1α, VEGF, and EPO expression are protective mechanisms in the cortex and hippocampus of the brain against CS neurotoxicity. For this reason, it was aimed to investigate the effects of betaine on CS neurotoxicity via HIF-1α/VEGF/EPO signalization pathway by immunohistochemical methods in this study.Due to the diversity of mechanisms underlying the regulation of neurogenesis, the focus on the use of products that will trigger this is still waiting to be elucidated, and new information is being added to the literature every day. Studies have been conducted investigating the protective effects of natural plants such as curcumin, ginseng, and *Nigella sativa* against cisplatin-induced neurotoxicity (Abdelhiee *et al.,* 2025). In a recent study, it was shown that betaine shows antioxidant activity by reducing serum homocysteine concentration and improves sperm parameters through this effect (Ijab *et al.,* 2022).Additionally, some studies indicate that betaine reduces kidney-specific parameters, inflammation, apoptosis, and cytokine levels in cisplatin-induced nephrotoxicity (Al Za'abi *et al.,* 2021; Hussein & Al-Dalain, 2021). However, to the best of our knowledge, this study provides the first evidence of a relationship between CS-induced neurotoxicity potential neuroprotective effect of betaine. The study aimed to minimize CS neurotoxicity by long-term application of betaine. Although BGT-1 expression is found in the cerebral cortex, cerebellum, brainstem, and hippocampus, betaine concentration has been reported to be lower in the brain than in the liver and kidney (Bhatt *et al.,* 2023). Therefore, it can be said that betaine has effective results in HIF-1α/VEGF/EPO signaling. Despite CS neurotoxicity, a decrease in HIF-1α/VEGF/EPO expressions was detected in the betaine-applied group. There is limited information on how betaine changes HIF-1α signaling or whether it changes or not. We know that there is an upregulation of HIF-1α in the brain due to ischemia or neurotoxicity and an increase in EPO and VEGF, which induce various pathways related to neuroprotection due to this increase (Fan *et al.,* 2009). The study's results support this information with increased HIF-1α/VEGF/EPO expressions due to CS-induced neurotoxicity. Decreased HIF-1α/VEGF/EPO expressions due to the effect of betaine may suggest that betaine is a substance that can be used in neurotoxicity. We also know that excessive HIF-1α expression is involved in the pathogenesis of CNS-related diseases such as stroke, traumatic brain injury, and Alzheimer's disease by facilitating mitochondrial dysfunction, inflammasome formation, and cell death (Choi, 2024). Therefore, HIF-1α/VEGF/EPO signaling can be an important mechanism for developing treatment strategies. Considering the intensive blood supply of the brain due to its structure and function, VEGF is considered one of the main regulators of vascular development and homeostasis (Rattner *et al.,* 2019). VEGF production controlled by HIF provides neuron development and neural dendritic and axon growth. Thus, VEGF affects neuronal development and physiological function (Pan *et al.,* 2017). It is also known that EPO exhibits antioxidant properties by protecting against CS-induced oxidative damage (Rjiba-Touati *et al.,* 2011). HIF-1α/EPO signaling also increases CNS neurogenesis, triggering impaired hippocampal neurogenesis (Lu *et al.,* 2014). The results of our study revealed that CS neurotoxicity occurs through upregulation of HIF-1 target genes VEGF and EPO and that betaine may become a new therapeutic target in this HIF-1α/VEGF/EPO signaling.

Another parameter evaluated in the present study was the expression of GFAP in the hippocampal and medullary areas. It is known that astrocytes, in response to almost all types of CNS pathology, such as trauma, ischemia, and neurodegeneration, show a characteristic change called reactive gliosis, the hypertrophy of their cellular processes. In these reactive astrocytes, the production of intermediate filaments such as vimentin, nestin, and GFAP increases (Pekny & Pekna, 2004). The present study showed strong positive GFAP expression and hypertrophic astrocytes with extended extensions in the CS group. It was shown that the astrocytosis seen with this increased GFAP expression

could be partially reduced with betaine in both hippocampal and medullary areas. It was reported that the increased GFAP expression and apoptotic markers in the cerebral cortex due to CS were reversible with Urchin extracts (Khalil *et al.,* 2023). Additionally, hippocampal GFAP expression was shown to be increased in a Streptozotocin-induced Alzheimer's disease model, and a correlation was demonstrated between neuronal damage and glial cell infiltration (Abbas *et al.,* 2021). In a study examining the neurotoxicity of CS, it was shown that CS triggers oxidative stress and apoptosis in the rat hippocampus, and this effect is reduced by aloe vera gel, a herbal product (Erfani Majd *et al.,* 2022). In addition, studies are showing that betaine gives effective results where neurodegeneration is triggered and apoptosis occurs by reducing the expression of caspase-3 (Kusat Ol *et al.,* 2016; Sogut *et al.,* 2017). This study showed the potential antiapoptotic effect of betaine because the resulting high level of caspase-3 expression due to CS neurotoxicity tends to decrease in animals treated with CS+Betaine. Thus, immunohistochemical techniques have demonstrated that HIF-1α/VEGF/EPO signaling is associated with increased GFAP expression and apoptotic cell death via the caspase-3 pathway in CS neurotoxicity.

Conclusions: Cisplatin-induced neurotoxicity involves complex signaling pathways, including oxidative stress and hypoxia-related responses. The results of the present study show that CS uses HIF-1α/VEGF/EPO signaling while triggering neurotoxicity in the cerebral cortex and hippocampus. It has also been shown that increased GFAP expression and apoptosis due to CS can be reduced with the contributions of betaine. Betaine may have therapeutic value as it exhibits ameliorative effects on this signaling and warrants further investigation as a neuroprotective agent.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest: The authors declare that he has no conflict of interest.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author's contribution: MÜ conceived and designed the study, analyzed the tissue samples and data, wrote the manuscript, LÇB conducted the experiment and reviewed the final version.

REFERENCES

Abbas H, Gad HA, Khattab MA, *et al.,* 2021. The Tragedy of Alzheimer's Disease: Towards Better Management via Resveratrol-Loaded Oral Bilosomes. Pharmaceutics 13:1635.

Abdelhiee EY, Fadl SE, Ashry A, *et al.,* 2025. Harnessing Nature: The Role of Medicinal Plants in Alleviating Cisplatin Toxicity. Pak Vet J 45:73-83.

Ahmed EM, Bandopadhyay G, Coyle B, *et al.,* 2018. A HIF-independent, CD133-mediated mechanism of cisplatin resistance in glioblastoma cells. Cell Oncol (Dordr) 41:319-328.

Al Za'abi M, Ali H, Al Sabahi M, *et al.,* 2021. The salutary action of melatonin and betaine, given singly or concomitantly, on cisplatin-induced nephrotoxicity in mice. Naunyn Schmiedebergs Arch Pharmacol 394:1693-1701.

Amin N, Chen S, Ren Q, *et al.,* 2021. Hypoxia Inducible Factor-1α Attenuates Ischemic Brain Damage by Modulating Inflammatory Response and Glial Activity. Cells 10:1359.

Barabas K, Milner R, Lurie D, *et al.,* 2008. Cisplatin: a review of toxicities and therapeutic applications. Vet Comp Oncol 6:1-18.

Barak AJ, Beckenhauer HC and Tuma DJ, 1996. Betaine, ethanol, and the liver: a review. Alcohol 13:395-398.

Bernaudin M, Nedelec AS, Divoux D, *et al.,* 2002. Normobaric hypoxia induces tolerance to focal permanent cerebral ischemia in association with an increased expression of hypoxia-inducible factor-1 and its target genes, erythropoietin and VEGF, in the adult mouse brain. J Cereb Blood Flow Metab 22:393-403.

Bhatt M, Di Iacovo A, Romanazzi T, *et al.,* 2023. Betaine-The dark knight of the brain. Basic Clin Pharmacol Toxicol 133:485-495.

Choi YK, 2024. Detrimental Roles of Hypoxia-Inducible Factor-1α in Severe Hypoxic Brain Diseases. Int J Mol Sci 25:4465.

Dasari S, Njiki S, Mbemi A, *et al.,* 2022. Pharmacological Effects of Cisplatin Combination with Natural Products in Cancer Chemotherapy. Int J Mol Sci 23:1532.

Day CR and Kempson SA, 2016. Betaine chemistry, roles, and potential use in liver disease. Biochim Biophys Acta 1860:1098-1106.

Erfani Majd N, Shahraki R, Tabandeh MR, *et al.,* 2022. Protective effects of Aloe vera gel on cisplatin-induced oxidative stress, apoptosis and neurons structure in rat hippocampus. Vet Res Forum 13:111-119.

Fan X, Heijnen CJ, van der Kooij MA, *et al.,* 2009. The role and regulation of hypoxia-inducible factor-1alpha expression in brain development and neonatal hypoxic-ischemic brain injury. Brain Res Rev 62:99-108.

Fu Y, Cai J, Li F, *et al.,* 2019. Chronic effects of repeated low-dose cisplatin treatment in mouse kidneys and renal tubular cells. Am J Physiol Renal Physiol 317:F1582-f1592.

Goodsell DS, 2006. The molecular perspective: Cisplatin. Stem Cells 24:514-515.

Gündoğdu R, Erkan M, Aydın M, 2019. Chronic effects of repeated low-dose cisplatin treatment in mouse kidneys and renal tubular cells. Assessment of the Effectiveness of Quercetin on Cisplatin-Induced Ototoxicity in Rats. J Int Adv Otol 15:229-236.

Hussein RM and Al-Dalain SM, 2021. Betaine downregulates microRNA 34a expression via a p53-dependent manner in cisplatin-induced nephrotoxicity in rats. J Biochem Mol Toxicol 35:e22856.

Ijab R, Ayen E, Khaki A, *et al.,* 2022. Evaluation of dietary betaine on post-thawed semen quality in mature bulls during summer heat stress. Vet Res Forum 13:61-70.

Jun YH, Ju GS, Chung YY, *et al.,* 2020. Differential Expression of Vascular Endothelial Growth Factor in the Cortex and Hippocampus upon Cerebral Hypoperfusion. In Vivo 34:191-197.

Karabulut D, Akin AT, Unsal M, *et al.,* 2021. L-Carnitine ameliorates the liver by regulating alpha-SMA, iNOS, HSP90, HIF-1alpha, and RIP1 expressions of CCL4-toxic rats. Iran J Basic Med Sci 24:184-190.

Kempson SA, Vovor-Dassu K and Day C, 2013. Betaine transport in kidney and liver: use of betaine in liver injury. Cell Physiol Biochem 32, 32-40.

Kempson SA, Zhou Y and Danbolt NC, 2014. The betaine/GABA transporter and betaine: roles in brain, kidney, and liver. Front Physiol 5:159.

Khalil EA, Swelim H, El-Tantawi H, *et al.,* 2023. Sea urchin (Diadema savignyi) extract as a novel protective agent against cisplatin induced neurotoxicity in rats. BMC Pharmacol Toxicol 24:11.

Khan M, Khan H, Singh I, *et al.,* 2017. Hypoxia inducible factor-1 alpha stabilization for regenerative therapy in traumatic brain injury. Neural Regen Res 12:696-701.

Kusat Ol K, Kanbak G, Oğlakcı Ilhan A, *et al.,* 2016. The investigation of the prenatal and postnatal alcohol exposure-induced neurodegeneration in rat brain: protection by betaine and/or omega-3. Childs Nerv Syst 32:467-74.

Lu J, Jiang L, Zhu H, *et al.,* 2014. Hypoxia-inducible factor-1α and erythropoietin expression in the hippocampus of neonatal rats following hypoxia-ischemia. J Nanosci Nanotechnol 14: 5614-5619.

Magar AG, Morya VK, Kwak MK, *et al.,* 2024. A Molecular Perspective on HIF-1α and Angiogenic Stimulator Networks and Their Role in Solid Tumors: An Update. Int J Mol Sci 25:3313.

Marti HH, 2004. Erythropoietin and the hypoxic brain. J Exp Biol 207:3233-3242.

Mert H, Kerem Ö, Mıs L, *et al.,* 2024. Effects of protocatechuic acid against cisplatin-induced neurotoxicity in rat brains: an experimental study. Int J Neurosci 134:725-734.

Middeldorp J and Hol EM, 2011. GFAP in health and disease. Prog Neurobiol 93:421-443.

Minerva, Bhat A, Verma S, *et al.,* 2023. Cisplatin-based combination therapy for cancer. J Cancer Res Ther 19:530-536.

Pan ZG, Mao Y and Sun FY, 2017. [VEGF enhances reconstruction of neurovascular units in the brain after injury]. Sheng Li Xue Bao 69:96-108.

Pekny M and Pekna M, 2004. Astrocyte intermediate filaments in CNS pathologies and regeneration. J Pathol 204:428-437.

Polat OA, Karabulut D, Akkul Z, *et al.,* 2023. Evaluation of histologic, antiapoptotic and antioxidant effects of melatonin against the acute ocular toxicity of Cisplatin. Tissue Cell 85:102226.

Rattner A, Williams J and Nathans J, 2019. Roles of HIFs and VEGF in angiogenesis in the retina and brain. J Clin Invest 129: 3807-3820.

Rjiba-Touati K, Boussema IA, Belarbia A, *et al.,* 2011. Protective effect of recombinant human erythropoietin against cisplatin-induced oxidative stress and nephrotoxicity in rat kidney. Int J Toxicol 30:510-517.

Singh N, Sharma G and Mishra V, 2012. Hypoxia inducible factor-1: its potential role in cerebral ischemia. Cell Mol Neurobiol 32: 491-507.

Sogut I, Uysal O, Oglakci A, *et al.,* 2017. Prenatal alcohol-induced neuroapoptosis in rat brain cerebral cortex: protective effect of folic acid and betaine. Childs Nerv Syst 33:407-417.

Sun L, 2022. F-box and WD repeat domain-containing 7 (FBXW7) mediates the hypoxia inducible factor-1α (HIF-1α)/vascular endothelial growth factor (VEGF) signaling pathway to affect hypoxic-ischemic brain damage in neonatal rats. Bioengineered 13:560-572.

Uttara B, Singh AV, Zamboni P, *et al.,* 2009. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol 7:65-74.

Ülger M, Ülger B, Turan IT, *et al.,* 2025. Evaluation of the effects of favipiravir (T-705) on the lung tissue of healty rats: An experimental study. Food Chem Toxicol 196:115235.

Woźniak K and Błasiak J, 2002. Recognition and repair of DNA-cisplatin adducts. Acta Biochim Pol 49:583-596.