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Ameliorative effects of betaine on cisplatin-induced cardiotoxicity in rats through anti-inflammatory pathways

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Abstract

Cisplatin (CS) is a broad-spectrum chemotherapeutic agent that causes serious adverse effects, such as cardiotoxicity, despite its potent anti-tumor efficacy. This study aimed to evaluate the cardioprotective effects of betaine in rats exposed to repeated low-dose CS administration using histopathological and immunohistochemical methods. Forty female Wistar albino rats were divided into four groups, including control, betaine, CS, and CS + betaine. Betaine (250 mg kg^{-1}) was administered orally on a daily basis for four weeks, while CS (8.00 mg kg^{-1}) was administered intraperitoneally once a week for the same duration. Cardiomyocytes were then examined using histopathological and immunohistochemical methods. The data were analyzed using one-way analysis of variance and Tukey tests. Histopathological analysis revealed cardiomyocyte disorganization, myofibril loss, and increased eosinophilia in the CS group. Betaine treatment partially prevented CS-induced histological damage, contributing to the cardiac muscle structure preservation. Immunohistochemical analyses demonstrated a significant increase in transforming growth factor-beta and interferon gamma expressions in the CS group, whereas betaine administration reduced transforming growth factor-beta levels. Interleukin 6 expression was lower in the CS + betaine group compared to the CS group. No significant differences were observed between groups regarding Interleukin-1β expression. These findings suggest that betaine may have protective effects against CS-induced cardiotoxicity. Its anti-inflammatory properties appear to mitigate cardiomyocyte damage.

Introduction

Cisplatin (CS) is a broad-spectrum alkylating chemotherapeutic agent used against various tumor types, including sarcomas and carcinomas.[1] The CS exerts its anti-tumor effect primarily by interacting with guanines in nuclear DNA, thereby arresting cell division.[2] Unfortunately, the mechanism of action of CS is not specific to cancer cells. While effectively inducing apoptosis in malignant cells, CS is also associated with a range of dose- and time-dependent toxic effects on healthy tissues, including nephrotoxicity, cardiotoxicity, and hepatotoxicity.[3-5] Direct damage to the nuclear and mitochondrial genetic materials, along with the tissue response to the oxidative stress–inflammation–apoptosis cascade induced by CS, ultimately leads to histopathological alterations and functional impairment in the affected tissues.[6]

It is known that homocysteine levels in blood increase in response to CS.[7] Homocysteine plays a role in a biochemical cascade involving oxidative stress, caspase activation, DNA damage, and dysfunction of the endoplasmic reticulum and mitochondria.[8] The formation of methionine from homocysteine can occur through betaine or 5-methyltetrahydrofolate pathways, with betaine playing a crucial role in this mechanism through methylation.[9]

Betaine is a methylated derivative of glycine that is naturally found in various plants and animals.[10,11] Numerous studies have shown that betaine exhibits anti-oxidant properties, providing protective effects in the kidneys, heart, nerves, and liver.[9,12,13]

Due to its ability to activate the anti-oxidant defense system, betaine is also referred to as an anti-inflammatory agent.[14] Betaine transfers a methyl group to toxic metabolites, such as homocysteine, through the betaine-homocysteine methyltransferase enzyme, converting it

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into methionine while itself being transformed into dimethylglycine.[15,16]

This study aimed to investigate the cardioprotective properties of betaine against potential side effects induced by CS in healthy rats subjected to the repeated low-dose CS treatment. In this context, the ability of betaine to protect the heart by exhibiting anti-inflammatory properties was examined using histopathological and immunohistochemical methods.

Materials and Methods

Groups and chemicals. In the present study, a total of forty female Wistar albino rats, aged 8 - 10 weeks and weighing 200 - 250 g, raised at the Experimental Research Application and Research Center of Erciyes University, Türkiye, were utilized. The rats were housed in plastic cages under standard conditions with a regular pellet diet and water, and maintained at a temperature of 22.00 ± 2.00 ˚C and a 12-hr light/12-hr dark cycle. Four groups were formed from randomly selected rats (n = 10), including control, betaine, CS, and CS + betaine. Betaine hydrochloride (Sigma-Aldrich, St. Louis, USA) at a dose of 250 mg kg^{-1} was dissolved in physiological saline solution and administered orally *via* gavage.[17] Cisplatin (Koçak Farma, Istanbul, Türkiye) at a dose of 8.00 mg kg^{-1} was administered intraperitoneally to the corresponding groups.[18] The control group received physiological saline solution for 30 days, while the betaine and CS + betaine groups were treated with betaine dissolved in physiological saline solution *via* gavage. Cisplatin was administered once a week for four weeks (a total of four CS injections) to the CS and CS + betaine groups. Two weeks after the final dose of betaine, the rats were anesthetized by intraperitoneal administration of ketamine (50.00 mg kg^{-1}; Pfizer, Istanbul, Türkiye) and xylazine (10.00 mg kg^{-1}, Bayer, Istanbul, Türkiye), after which they were sacrificed. Ethical approval for conducting the experimental studies was obtained from the Local Ethics Committee for Animal Experiments at Erciyes University, Türkiye (05.12.2024, Approval No: 24/238). All animals were treated humanely in accordance with the Guide for the Care and Use of Laboratory Animals.

Tissue preparation. For histopathological examination, immediately after euthanasia, heart tissues were rapidly excised and immersed in a 10.00% formaldehyde solution and fixed for 72 hr. The tissues were then processed according to the standard histological tissue processing procedures. Briefly, the tissues were washed in running tap water, dehydrated through increasing concentrations of alcohol, cleared with xylene, and embedded in paraffin blocks. Sections of 5.00 µm thickness were cut from the paraffin blocks and mounted onto poly-L-lysine-coated slides.

Hematoxylin and Eosin (H&E) and immunohistochemical stainings. The obtained sections were stained with H&E for histopathological evaluation. In addition, immunohistochemical staining was performed to assess inflammation during the histopathological assessment. For this purpose, primary antibodies were used as follows: Transforming growth factor-beta (TGF-β; 1/100 dilution, Bioss, Woburn, USA), interleukin 6 (IL-6; 1/400 dilution, Affinity Biosciences, Cincinnati, USA), IL-1β (1/100 dilution, Bioss), and interferon gamma (IFN-γ; 1/200 dilution, Bioss). Immunohistochemical staining was performed using the avidin-biotin-peroxidase method (Ultravision Polyvalent (Rabbit-Mouse) Horseradish Peroxidase Kit, 125 mL, ThermoFisher Scientific, Waltham, USA). The stained slides were examined under a microscope (Olympus BX51; Olympus, Tokyo, Japan), and images were captured from four randomly selected areas of each preparation. The immunoreactivity intensities of the obtained images were measured using the ImageJ Software (National Institutes of Health, Bethesda, USA).[19]

Statistical analysis. Statistical analyses of the data in this study were performed using GraphPad Prism (version 7.0; GraphPad Software Inc., San Diego, USA). The normality of the data distribution was assessed using the D'Agostino & Pearson normality test. Since all data showed a normal distribution, one-way analysis of variance was used, followed by Tukey *post-hoc* test for multiple comparisons between groups. A p-value of < 0.05 was considered statistically significant.

Results

Histopathological findings. The histopathological evaluation was performed based on the findings obtained from H&E staining of myocardial tissue sections, which were examined under a light microscope. In the control and betaine groups, the cardiac muscle fibers exhibited a standard histological architecture with centrally located oval nuclei and homogeneous cytoplasm showing standard acidophilic staining. In the CS group, the muscle fibers were widely separated, showing cardiomyocyte disorganization. The cytoplasm exhibited varying degrees of eosinophilia, mostly observed in necrotic areas with or without visible nuclei. In addition, some cardiomyocytes exhibited loss of myofibrils and minimal cytoplasmic vacuolization, leading to a loss of eosinophilia, thus presenting a disrupted architecture of the heart muscle. In the CS + betaine group, the standard architecture of the heart muscle was partially preserved compared to the CS group. While certain muscle fibers displayed typical cytoplasmic features with centrally positioned nuclei, others demonstrated reduced eosinophilia associated with cytoplasm myofibrils loss. Disorganization, characterized by separation of muscle fibers, was also present (Fig. 1).

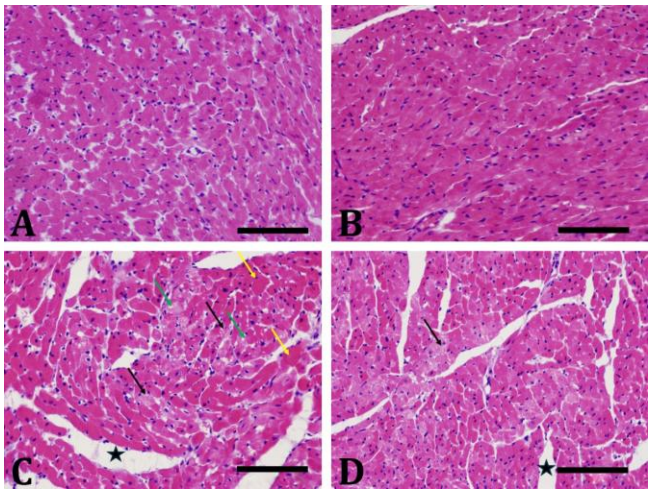

Fig. 1. Photomicrographs of myocardium in **A)** Control, **B)** betaine, **C)** Cisplatin (CS) and **D)** CS + betaine groups. Asterick: Disorganization of cardiomyocytes with widely separated muscle fibers; black arrow: Loss of myofibrils; green arrow: Minimal cytoplasmic vacuolization; yellow arrow: Intensely eosinophilic cytoplasm, (Hematoxylin and Eosin staining; Bars = 100 µm).

Immunohistochemical findings. Following histopathological evaluation, cardiomyocytes were stained with TGF-β, IL-6, IL-1β, and IFN-γ primary antibodies using immunohistochemical methods (Fig. 2). The obtained density measurements results were statistically analyzed (Table 1). In this context, evaluation of TGF-β expression revealed that the CS group exhibited significantly higher expression levels compared to the control and betaine groups, whereas the CS + betaine group showed a marked reduction in expression relative to the CS group. According to the immunoreactivity assessment for IL-6, no significant difference was observed between the control, betaine, and CS groups, whereas the CS + betaine group showed significantly lower expression than the CS group. As for IL-1β, although there was no significant difference between the groups, the CS group exhibited higher expression. When evaluating IFN-γ, it was found that the CS group had significantly higher levels than the control and betaine groups. At the same time, no significant difference was observed between the CS + betaine and CS groups.

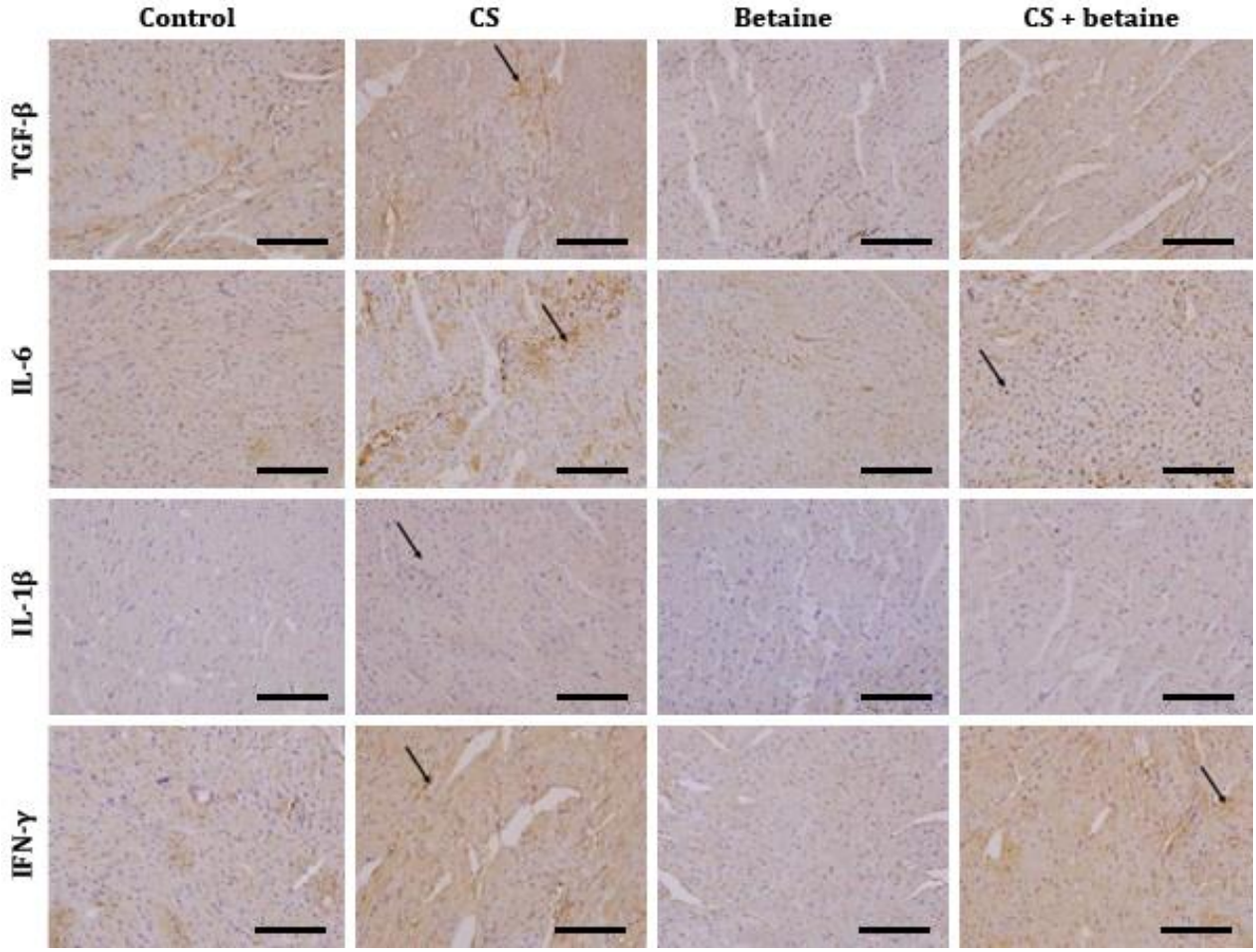

Fig. 2. Transforming growth factor-beta (TGF-β), interleukin 6 (IL-6), IL-1β, and interferon gamma (IFN-γ) immunohistochemical stainings of the heart tissue in different experimental groups, (Bars = 100 µm). The expressions intensities in the groups are indicated by arrows. CS: Cisplatin

Table 1. Immunoreactivity intensities of different parameters in the heart tissue of the experimental groups. Data are expressed as mean ± standard deviation of pixels *per* 305 µm × 446 µm in ImageJ Software.

Parameters	Control	CS	Betaine	CS + Betaine	*p-value*
Transforming growth factor-beta	73.41 ± 2.37[a]	75.64 ± 2.48[b]	71.70 ± 2.14[c]	71.65 ± 1.63[cd]	0.0001
Interleukin-6	74.22 ± 3.18[ab]	75.75 ± 3.60[b]	74.75 ± 2.60[ab]	73.79 ± 2.20[a]	0.0245
Interleukin-1β	72.79 ± 2.05[a]	73.96 ± 2.33[a]	72.84 ± 2.33[a]	72.86 ± 1.71[a]	0.0441
Interferon gamma	73.35 ± 1.72[a]	77.74 ± 2.38[b]	72.60 ± 1.69[a]	77.25 ± 2.18[b]	0.0001

CS: Cisplatin.
[a-d] Different letters indicate the statistical differences between groups ($p < 0.05$).

Discussion

In recent years, betaine, which has been used as a dietary supplement in animal nutrition,[9] has garnered attention in human health research due to the discovery of its anti-oxidant and anti-inflammatory properties.[20] This study presented the first data regarding betaine treatment showing anti-inflammatory properties in CS-induced cardiotoxicity. The obtained data demonstrated that betaine consumption suppressed CS-induced inflammation by reducing TGF-β, IL-6, IL-1β, and IFN-γ levels and promoting histopathological improvement in rat cardiomyocytes.

Cisplatin-induced cardiotoxicity, although less common than ototoxicity or gastrointestinal toxicities, is one of the major limiting factors in cancer treatment, and clinical cases have been increasingly reported in recent years.[21] Clinically, various cardiac events induced by CS have been reported.[22] The cause of these symptoms can be attributed to the endothelial damage and various vascular events.[23] The mechanism underlying CS-induced cardiotoxicity is thought to involve the generation of reactive oxygen species (ROS) and consequent oxidative stress, which in turn triggers apoptosis and inflammatory responses.[24]

As stated, while much of the literature acknowledges that oxidative stress following CS treatment is associated with the resulting damage, there is also evidence indicating that inflammation may be the primary initiator of CS-related toxicity.[25] The immune system primarily regulates the inflammatory response through pattern-recognition receptors, which detect pathogen- or damage-associated molecular patterns.[26] Activation of various receptors subsequently stimulates key transcription factors, such as nuclear factor kappa B (NF-κB).[27] This leads to the release of pro-inflammatory cytokines, such as IL-1β, IL-6, and tumor necrosis factor-alpha (TNF-α), as well as chemokines, like Chemokine (C-X-C motif) ligand 2 and IL-8.[28] These mediators coordinate the recruitment of immune cells to the affected area and promote inflammation; however, if this process persists, it may result in tissue damage.[25]

For this reason, natural or synthetic anti-inflammatory compounds with the potential to significantly alleviate CS-related toxicity have increasingly emerged as promising therapeutic candidates.[25] In addition to more commonly used anti-inflammatory agents, such as resveratrol,[29] honey,[30] and curcumin,[31] betaine has also been included in studies on CS-induced toxicity, and has been shown to reduce CS-induced nephrotoxicity by inhibiting NF-κB, IL-6, IL-1β, and TNF-α.[32,33]

Additionally, it is known that high ROS levels can lead to pathological processes, such as inflammation.[34] It has been shown that CS administration produces a variety of inflammatory factors and chemokines in cardiomyocytes, resulting in damage to the cardiomyocytes.[35]

The formation of ROS and endothelial damage resulting from CS treatment triggers the production of pro-inflammatory mediators.[31] Following CS-induced intracellular damage, the transcription factor NF-κB is activated, leading to the induction of synthesis and expression of cytokines and pro-inflammatory mediators, such as TNF-α, IL-1β, and IL-6, which exacerbate tissue damage.[36] The increase in inflammatory cytokines observed in the present study due to the CS treatment was consistent with the results reported in previous studies.[36]

Therefore, anti-oxidant therapies may provide myocardial protection against CS. For this purpose, many natural anti-oxidants, such as resveratrol, apocynin, and alpha-lipoic acid, can protect cardiomyocytes against ROS.[37] Potential cytoprotective strategies against CS include curcumin,[38] vitamin E,[39] N-acetylcysteine,[40] and Aloe vera.[41] The general mechanism of action of these agents involves the improvement of mitochondrial dysfunction, attenuation of oxidative damage, and a subsequent reduction in inflammation and cell death.[42]

In this context, reducing oxidative stress and inflammation may help alleviate CS-induced cardiotoxicity. Therefore, the present study investigated the effects of betaine, a natural anti-oxidant and anti-inflammatory compound, on inflammatory markers in CS-treated rats. Betaine treatment directly reduces homocysteine levels by inducing the remethylation process, which converts homocysteine into methionine.[12] Hence, the beneficial properties of betaine are promising. Studies have shown that hyperhomocysteinemia ultimately induces oxidative stress and apoptosis.[43,44] Betaine exerts its primary anti-oxidant mechanism by acting as a methyl group donor through betaine-homocysteine methyltransferase, converting homocysteine into methionine.[20] Additionally, it reduces apoptosis and inflammation markers (NF-κB and IL-1β) levels.[17] The NF-κB pathway regulates many

genes involved in inflammation, including pro-inflammatory cytokines, such as TNF-α and IL-1β. Therefore, it is not surprising that chronic NF-κB activation is involved in many inflammatory diseases.[45] As a result, betaine exerts anti-inflammatory effects by inhibiting the NF-κB signaling pathway.[20] In the present study, the anti-inflammatory property of betaine is supported by its ability to reduce specific inflammatory markers. Previously, it has been shown that betaine restores the activity of anti-oxidant enzymes, inhibits TNF-α, NF-κB, and caspase 3, and protects rats from CS-induced nephrotoxicity.[32] The CS-induced oxidative stress triggers a series of inflammatory reactions, including the elevation of TNF-α, IL-1β, and IL-6, leading to tissue damage. This damage is being mitigated using various substances, such as taurine, which is known for its anti-inflammatory properties.[35] The present study demonstrated that betaine significantly decreased the levels of TGF-β and IL-6, indicating its role in the suppression of inflammatory processes. This finding is consistent with a former study.[46] However, IL-1β did not show sufficient expression in any of the groups, resulting in no significant outcome. At the same time, IFN-γ was significantly elevated in response to CS, although this could not be adequately reduced by betaine. It has been shown that betaine provides various benefits, including improving heart health by preventing oxidative stress through its anti-oxidant role and reducing inflammation.[20,47] It has been shown that betaine can reverse cardiovascular complications and myocardial infarction.[47] Therefore, anti-oxidants may offer a promising approach to alleviate cardiac damage in CS chemotherapy patients.[48]

The present study demonstrated significant improvement in cardiac histology at the light microscopic level in the CS + betaine group. Findings, such as partial restoration of the standard histological structure and organization of the heart muscle fibers, were consistent with previous similar studies.[49] This improvement can be attributed to the previously known anti-oxidant and anti-inflammatory potential of betaine.[20] The reduction in inflammatory cytokines observed in the present study following betaine administration is consistent with the findings of earlier studies.[50]

In conclusion, this study provides novel evidence that betaine exerts protective effects against CS-induced cardiotoxicity, primarily through its anti-inflammatory properties. Histopathological analysis revealed that betaine alleviated myocardial structural damage, while immunohistochemical findings demonstrated a significant reduction in TGF-β and IL-6 expressions levels. Although IL-1β and IFN-γ did not show statistically significant improvements, the overall trend supports the regulatory effect of betaine on inflammatory mediators. Considering these results, betaine may represent a promising therapeutic candidate for mitigating CS-related cardiac side effects. Further research is warranted to explore the full therapeutic potential of betaine in cardioprotection.

Conflict of interest

The authors declare no conflict of interest.

References

1. Patanè S. Cardiotoxicity: cisplatin and long-term cancer survivors. Int J Cardiol 2014; 1 75(1): 201-202.
2. Rocha CRR, Silva MM, Quinet A, et al. DNA repair pathways and cisplatin resistance: an intimate relationship. Clinics (Sao Paulo) 2018; 73(suppl 1): e478s. doi: 10.6061/clinics/2018/e478s.
3. de Jongh FE, van Veen RN, Veltman SJ, et al. Weekly high-dose cisplatin is a feasible treatment option: analysis on prognostic factors for toxicity in 400 patients. Br J Cancer 2003; 88(8): 1199-1206.
4. Uehara T, Yamate J, Torii M, et al. Comparative nephrotoxicity of cisplatin and nedaplatin: mechanisms and histopathological characteristics. J Toxicol Pathol 2011; 24(2): 87-94.
5. Xing JJ, Hou JG, Liu Y, et al. Supplementation of saponins from leaves of *Panax quinquefolius* mitigates cisplatin-evoked cardiotoxicity *via* inhibiting oxidative stress-associated inflammation and apoptosis in mice [Portuguese]. Antioxidants (Basel) 2019; 8(9): 347. doi: 10.3390/antiox8090347.
6. Peres LA, da Cunha AD Jr. Acute nephrotoxicity of cisplatin: molecular mechanisms. J Bras Nefrol 2013; 35(4): 332-340.
7. Tanyeli TT, Karadaş H, Akyıldız İ, et al. Effect of folic acid on cisplatin-induced ototoxicity: a functional and morphological study. J Int Adv Otol 2019; 15(2): 237-246.
8. Bleich S, Degner D, Sperling W, et al. Homocysteine as a neurotoxin in chronic alcoholism. Prog Neuropsychopharmacol Biol Psychiatry 2004; 28(3): 453-464.
9. Craig SA. Betaine in human nutrition. Am J Clin Nutr 2004; 80(3): 539-549.
10. Moon K, Guallar E, Navas-Acien A. Arsenic exposure and cardiovascular disease: an updated systematic review. Curr Atheroscler Rep 2012; 14(6): 542-555.
11. Sakamoto A, Nishimura Y, Ono H, et al. Betaine and homocysteine concentrations in foods. Pediatr Int 2002; 44(4): 409-413.
12. Alirezaei M, Jelodar G, Niknam P, et al. Betaine prevents ethanol-induced oxidative stress and reduces total homocysteine in the rat cerebellum. J Physiol Biochem 2011; 67(4): 605-612.
13. al-Hafyan S, Asoodeh A, Baghshani H, et al. Ameliorative potential of betaine against arsenite-induced hepatotoxicity and nephrotoxicity. Com Clin Pathol 2024; 33(1): 155-162.

14. Jung YS, Kim SJ, Kwon DY, et al. Alleviation of alcoholic liver injury by betaine involves an enhancement of antioxidant defense via regulation of sulfur amino acid metabolism. Food Chem Toxicol 2013; 62: 292-298.
15. Day CR, Kempson SA. Betaine chemistry, roles, and potential use in liver disease. Biochim Biophys Acta 2016; 1860(6): 1098-1106.
16. Barak AJ, Beckenhauer HC, Tuma DJ. Methionine synthase. a possible prime site of the ethanolic lesion in liver. Alcohol 2002; 26(2): 65-67.
17. Hussein RM, Al-Dalain SM. Betaine downregulates microRNA 34a expression *via* a p53-dependent manner in cisplatin-induced nephrotoxicity in rats. J Biochem Mol Toxicol 2021; 35(9): e22856. doi: 10.1002/jbt.22856.
18. Fu Y, Cai J, Li F, et al. Chronic effects of repeated low-dose cisplatin treatment in mouse kidneys and renal tubular cells. Am J Physiol Renal Physiol 2019; 317(6): F1582-F1592.
19. Ülger M, Ülger B, Turan IT, et al. Evaluation of the effects of favipiravir (T-705) on the lung tissue of healty rats: an experimental study. Food Chem Toxicol 2025; 196: 115235. doi: 10.1016/j.fct.2025.115235.
20. Zhao G, He F, Wu C, et al. Betaine in inflammation: mechanistic aspects and applications. Front Immunol 2018; 9: 1070. doi: 10.3389/fimmu.2018.01070.
21. Oun R, Rowan E. Cisplatin induced arrhythmia; electrolyte imbalance or disturbance of the SA node? Eur J Pharmacol 2017; 811: 125-128.
22. Talley RW, O'Bryan RM, Gutterman JU, et al. Clinical evaluation of toxic effects of cis-diamminedichloroplatinum (NSC-119875)--phase I clinical study. Cancer Chemother Rep 1973; 57(4): 465-471.
23. Licciardello JT, Moake JL, Rudy CK, et al. Elevated plasma von Willebrand factor levels and arterial occlusive complications associated with cisplatin-based chemotherapy. Oncology 1985; 42(5): 296-300.
24. Xia J, Hu JN, Zhang RB, et al. Icariin exhibits protective effects on cisplatin-induced cardiotoxicity via ROS-mediated oxidative stress injury *in vivo* and *in vitro*. Phytomedicine 2022; 104:1 54331. doi: 10.1016/j.phymed.2022.154331.
25. Domingo IK, Latif A, Bhavsar AP. Pro-inflammatory signalling PRRopels cisplatin-induced toxicity. Int J Mol Sci 2022; 23(13): 7227. doi: 10.3390/ijms23137227.
26. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell 2010; 140(6): 805-820.
27. Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. Clinical Microbiol Rev 2009; 22(2): 240-273.
28. Germolec DR, Shipkowski KA, Frawley RP, et al. Markers of inflammation. Methods Mol Biol 2018; 1803: 57-79.
29. Said RS, Mantawy EM, El-Demerdash E. Mechanistic perspective of protective effects of resveratrol against cisplatin-induced ovarian injury in rats: emphasis on anti-inflammatory and anti-apoptotic effects. Naunyn Schmiedebergs Arch Pharmacol 2019; 392(10): 1225-1238.
30. Hamad R, Jayakumar C, Ranganathan P, et al. Honey feeding protects kidney against cisplatin nephrotoxicity through suppression of inflammation. Clin Exp Pharmacol Physiol 2015; 42(8): 843-848.
31. Kumar P, Sulakhiya K, Barua CC, et al. TNF-α, IL-6 and IL-10 expressions, responsible for disparity in action of curcumin against cisplatin-induced nephrotoxicity in rats. Mol Cell Biochem 2017; 431(1-2): 113-122.
32. Hagar H, Medany AE, Salam R, et al. Betaine supplementation mitigates cisplatin-induced nephrotoxicity by abrogation of oxidative/nitrosative stress and suppression of inflammation and apoptosis in rats. Exp Toxicol Pathol 2015; 67(2): 133-141.
33. Al Za'abi M, Ali H, Al Sabahi M, et al. The salutary action of melatonin and betaine, given singly or concomitantly, on cisplatin-induced nephrotoxicity in mice. Naunyn Schmiedebergs Arch Pharmacol 2021; 394(8): 1693-1701.
34. Freitas I, Boncompagni E, Tarantola E, et al. In situ evaluation of oxidative stress in rat fatty liver induced by a methionine- and choline-deficient diet. Oxid Med Cell Longev 2016; 2016: 9307064. doi: 10.1155/2016/9307064.
35. Chowdhury S, Sinha K, Banerjee S, et al. Taurine protects cisplatin induced cardiotoxicity by modulating inflammatory and endoplasmic reticulum stress responses. Biofactors 2016; 42(6): 647-664.
36. Ma X, Yan L, Zhu Q, et al. Puerarin attenuates cisplatin-induced rat nephrotoxicity: the involvement of TLR4/NF-κB signaling pathway. PLoS One 2017; 12(2): e0171612. doi: 10.1371/journal.pone.0171612.
37. Qi L, Luo Q, Zhang Y, et al. Advances in toxicological research of the anticancer drug cisplatin. Chem Res Toxicol 2019; 32(8): 1469-1486.
38. Bahadır A, Ceyhan A, Öz Gergin Ö, et al. Protective effects of curcumin and beta-carotene on cisplatin-induced cardiotoxicity: an experimental rat model. Anatol J Cardiol 2018; 19(3): 213-221.
39. Ibrahim MA, Bakhaat GA, Tammam HG, et al. Cardioprotective effect of green tea extract and vitamin E on cisplatin-induced cardiotoxicity in mice: toxicological, histological and immunohistochemical studies. Biomed Pharmacother 2019; 113: 108731. doi: 10.1016/j.biopha.2019.108731.
40. Rosic G, Selakovic D, Joksimovic J, et al. The effects of N-acetylcysteine on cisplatin-induced changes of cardiodynamic parameters within coronary autoregulation range in isolated rat hearts. Toxicol Lett 2016; 242: 34-46.
41. Erfani Majd N, Shahraki R, Tabandeh MR, et al.

Protective effects of *Aloe vera* gel on cisplatin-induced oxidative stress, apoptosis and neurons structure in rat hippocampus. Vet Res Forum 2022; 13(1): 111-119.
42. Oliveira CA, Mercês ÉAB, Portela FS, et al. An integrated view of cisplatin-induced nephrotoxicity, hepatotoxicity, and cardiotoxicity: characteristics, common molecular mechanisms, and current clinical management. Clin Exp Nephrol 2024; 28(8): 711-727.
43. Almashhadany A, Shackebaei D, Van der Touw T, et al. Homocysteine exposure impairs myocardial resistance to ischaemia reperfusion and oxidative stress. Cell Physiol Biochem 2015; 37(6): 2265-2274.
44. Baggott JE, Tamura T. Homocysteine, iron and cardiovascular disease: a hypothesis. Nutrients 2015; 7(2): 1108-1118.
45. Schuliga M. NF-kappaB signaling in chronic inflammatory airway disease. Biomolecules 2015; 5(3): 1266-1283.
46. Hagar H, Husain S, Fadda LM, et al. Inhibition of NF-κB and the oxidative stress-dependent caspase-3 apoptotic pathway by betaine supplementation attenuates hepatic injury mediated by cisplatin in rats. Pharmacol Rep 2019; 71(6): 1025-1033.
47. Ashtary-Larky D, Bagheri R, Ghanavati M, et al. Effects of betaine supplementation on cardiovascular markers: a systematic review and meta-analysis. Crit Rev Food Sci Nutr 2022; 62(23): 6516-6533.
48. Mukhopadhyay P, Horváth B, Zsengellér Z, et al. Mitochondrial-targeted antioxidants represent a promising approach for prevention of cisplatin-induced nephropathy. Free Radic Biol Med 2012; 52(2): 497-506.
49. Shariati S, Shirani M, Azadnasab R, et al. Betaine protects mice from cardiotoxicity triggered by sodium arsenite through antioxidative and anti-inflammatory pathways. Cardiovasc Toxicol 2024; 24(6): 539-549.
50. Patel D, Yadav P, Singh SK, et al. Betaine alleviates doxorubicin-induced nephrotoxicity by preventing oxidative insults, inflammation, and fibrosis through the modulation of Nrf2/HO-1/NLRP3 and TGF-β expression. J Biochem Mol Toxicol 2024; 38(1): e23559. doi: 10.1002/jbt.23559.