ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Betaine Mitigates Cyclophosphamide-Induced Hepatotoxicity via Antioxidant Restoration and Modulation of Wnt3A/Lrp5 Signaling Pathways in Rats

Sıçanlarda Betain Antioksidan ve Wnt3A/Lrp5 Sinyal Yolaklarının Modülasyonu Yoluyla Siklofosfamid Kaynaklı Hepatotoksisiteyi Azaltır

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ABSTRACT

Aim: *Cyclophosphamide (CP) is an effective alkylating agent used in the treatment of malignancies and autoimmune diseases. However, toxic metabolites generated during hepatic metabolism induce oxidative stress, inflammation, and apoptosis, leading to hepatotoxicity that limits its clinical use. Betaine (BTN), a natural methyl donor found in foods such as beets, spinach, and wheat, has antioxidant, anti-inflammatory, and anti-apoptotic properties. This study investigated the protective effects of BTN against CP-induced liver injury in rats.*

Materials and methods: *Thirty-five Wistar albino rats were randomly assigned to five groups: Control, BTN, CP, CP+BTN125, and CP+BTN250. Betaine (125 or 250 mg/kg) was administered orally for seven days, while CP (200 mg/kg) was injected intraperitoneally as a single dose on day 7. Blood and liver samples were collected for biochemical, molecular, and histopathological analyses. Serum AST and ALT activities; tissue MDA, SOD, CAT, GPx, and GSH levels; gene expression by RT-PCR; and histopathological changes were evaluated.*

Results: *Cyclophosphamide significantly increased ALT, AST, and MDA levels while decreasing SOD, CAT, GPx, and GSH levels (p<0, 05). Betaine improved these parameters in a dose-dependent manner, with 250 mg/kg showing greater efficacy (p<0, 05). TNF-α and NF-κB expression increased in the CP group but were significantly suppressed by BTN (p<0, 05). Betaine also reduced apoptosis by decreasing Bax and caspase-3 expression and increasing Bcl-2 expression (p<0, 05). Moreover, Wnt3A and Lrp5 expression, reduced by CP, was significantly restored following BTN treatment (p<0, 05). Histopathological examination confirmed that BTN markedly attenuated CP-induced liver damage.*

Conclusion: *BTN exerted significant hepatoprotective effects against CP-induced liver injury through antioxidant, anti-inflammatory, and anti-apoptotic mechanisms, suggesting its potential as a supportive therapeutic strategy for reducing CP-associated hepatotoxicity*

Key words: *cyclophosphamide; betaine; apoptosis; inflammation; hepatotoxicity*

ÖZET

Amaç: *Siklofosfamid (CP), yüksek dozlarda kanser tedavisinde, düşük dozlarda ise bazı otoimmün hastalıkların tedavisinde yaygın olarak kullanılan etkili bir ajandır. Ancak CP'nin karaciğerde metabolize edilmesi sırasında oluşan toksik ara metabolitler hepatotoksisiteye yol açmakta ve bu durum ilacın klinik kullanımını sınırlandırmaktadır. Betain (BTN), pancar, ıspanak ve buğday gibi çeşitli besinlerde doğal olarak bulunan bir bileşiktir. BTN'nin oksidatif stres, enflamasyon ve hücre ölümü gibi hasar mekanizmaları üzerinde koruyucu etkilere sahip olduğu bilinmektedir. Bu çalışmada, BTN'nin CP kaynaklı karaciğer hasarına karşı potansiyel koruyucu etkilerinin değerlendirilmesi amaçlandı.*

Gereç ve Yöntem: *Çalışmada toplam 35 Wistar albino sıçan rastgele beş gruba ayrıldı: kontrol, BTN, CP, CP+BTN125 ve CP+BTN250 mg/kg. Betain (125 mg/kg ve 250 mg/kg) yedi gün boyunca oral yolla uygulanırken, CP (200 mg/kg) deneyin yedinci gününde tek doz intraperitoneal olarak verildi. Deney sonunda kan örnekleri ve karaciğer dokuları toplandı. Elde edilen örneklerde biyokimyasal, moleküler ve histopatolojik analizler gerçekleştirildi. Serum AST ve ALT düzeyleri ile doku MDA, SOD, CAT, GPx ve GSH düzeyleri ölçüldü. Gen ekspresyonları RT-PCR yöntemiyle analiz edildi ve karaciğer dokuları histopatolojik olarak değerlendirildi.*

Bulgular: *Siklofosfamid uygulaması ALT, AST ve MDA düzeylerinde anlamlı artışa, SOD, CAT, GPx ve GSH düzeylerinde ise anlamlı azalmaya neden oldu (p<0,05). Betain tedavisi bu parametrelerde doza bağımlı iyileşme sağladı ve özellikle 250 mg/kg dozunun daha etkili olduğu belirlendi (p<0,05). TNF-α ve NF-κB gibi enflamatuvar genlerin ekspresyonu CP grubunda artarken, BTN tedavisi bu artışı anlamlı olarak baskıladı (p<0,05). Ayrıca BTN, Bax ve kaspaz-3 düzeylerini azaltıp Bcl-2 ekspresyonunu artırarak apoptozu hafifletti (p<0,05). Cyclophosphamide grubunda azalan Wnt3A ve Lrp5 gen ekspresyonları BTN tedavisiyle anlamlı düzeyde arttı (p<0,05). Histopatolojik incelemede CP'nin belirgin karaciğer hasarı oluşturduğu, BTN uygulamasının ise bu hasarı önemli ölçüde azalttığı gözlendi.*

Sonuç: *Betain, CP kaynaklı hepatotoksisiteyi azaltarak karaciğer dokusu üzerinde belirgin koruyucu etkiler göstermiştir.*

Anahtar kelimeler: *siklofosfamid; betain; apoptozis; inflamasyon; hepatotoksisite*

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Introduction

Cyclophosphamide (CP) is an alkylating agent widely used in oncology for the treatment of breast, prostate, and ovarian cancers at high doses, and in the management of autoimmune conditions such as Wegener's granulomatosis at lower doses (1). Following oral or intravenous administration, CP undergoes hepatic bioactivation by the cytochrome P450 enzyme system, yielding 4-hydroxycyclophosphamide (4-HC) as an initial intermediate (2). Due to its chemical instability, 4-HC spontaneously decomposes intracellularly into phosphoramide mustard, which forms covalent bonds with guanine residues and induces DNA cross-linking (3). This structural damage halts replication and transcription, selectively driving apoptosis in rapidly proliferating tumor cells (4). Despite its clinical utility, CP is associated with a range of adverse effects—including myelosuppression, hemorrhagic cystitis, alopecia, and gonadal toxicity—and its hepatotoxic potential frequently limits both dosage and treatment duration (3,5,6). These constraints have driven interest in identifying adjunctive agents that reduce organ toxicity without compromising the drug's antineoplastic activity.

Betaine (BTN) is a trimethylglycine derivative present in beets, wheat germ, spinach, and various marine invertebrates (7,8). It enters systemic circulation through dietary consumption and the oxidation of choline (8). Beyond its role in one-carbon metabolism, BTN has shown therapeutic benefit in several hepatic disease models, including drug-induced liver injury and viral hepatitis (9–12). Its hepatoprotective capacity has been particularly well documented in CCl_4-induced hepatotoxicity models, where it attenuates oxidative damage, inflammation, and cell death (8). Protective effects of BTN have also been reported in experimental renal injury models (13).

This study aimed to determine whether BTN could protect against CP-induced hepatotoxicity in rats by assessing its effects on liver function markers, oxidative stress parameters, inflammatory cytokine expression, and apoptotic signaling.

Material and Methods

Research and publication ethics

Animal studies were approved by the Aksaray University School of Medicine Animal Experiments Local Ethics Committee (2024–072, 16.08.2024).

Drug and chemicals

Cyclophosphamide (Eczacıbaşı Pharmaceutical Marketing Co., Istanbul, Türkiye) and BTN (Sigma, U. S. A., 97% purity) were used at the doses reported in the literature (6,15).

Groups And Experiment Protocol

In this study, 35 Wistar albino rats were randomly allocated to five groups, with seven animals in each group (n=7). All experimental procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) and national regulations for the care and use of laboratory animals. Animals were housed in standard polypropylene cages (3–4 rats per cage) under controlled environmental conditions (temperature: 22±2°C, humidity: 50–60%, 12-hour light/dark cycle) and given free access to standard chow and water.

Groups:

1. Control: rats were given physiological saline both orally and intraperitoneally for 7 days.
2. Betaine: 250 mg/kg orally given BTN for 7 days.
3. Cyclophosphamide: On Day 7, a single dose of 200 mg/kg CP i.p. was administered.
4. Cyclophosphamide + Betaine 125:125 mg/kg of betaine was administered orally for 7 days, and a single dose of 200 mg/kg cyclophosphamide was administered i.p. on Day 7.
5. Cyclophosphamide + Betaine 250:250 mg/kg of betaine was given orally for 7 days and 7. A single dose of 200 mg/kg cyclophosphamide was administered i. p. on the day.

Selection Criteria

We specified that only healthy, adult (8–10 weeks old), weight-matched (220–250 g) Wistar rats obtained from an accredited institutional animal facility be included in the study. Animals were verified to be free of infectious diseases and congenital anomalies by veterinary examination before the study. Only animals with normal feeding behavior and baseline activity levels were selected. However, rats were excluded from the study if they exhibited 1) any signs of systemic disease during loss (more than 10%) before treatment, 3) aggression or injury from handling, or 4) adverse reactions that could com-promise data integrity during experimental procedures.

Tissue Collection

Twenty-four hours post-final treatment (day 8), subjects underwent light sevoflurane anesthesia followed by decapitation. Blood was collected into additive-free vacuum tubes, centrifuged (3.000×g, 10 min, 4°C), and the isolated serum was aliquoted for cryopreservation at -20°C pending biochemical assays. Liver tissues were immediately bisected: one aliquot snap-frozen at -20°C for biochemical analysis, while the contralateral portion was immersion-fixed in 10% neutral buffered formaldehyde for subsequent histological processing.

Liver Function Markers

Serum AST (Aspartate Aminotransferase) and ALT (Alanine Aminotransferase) levels were measured using a commercial kit in accordance with the manufacturer's procedures to assess liver function.

Lipid Peroxidation Analysis

Lipid peroxidation was quantified spectrophotometrically at 532 nm by measuring the chromophore formed from the reaction between thiobarbituric acid (TBA) and malondialdehyde (MDA) extracted from liver samples. Tissues were homogenized in ice-cold 1.15% KCl solution. Following centrifugation (1,000×g, 15 min, 4°C), the supernatant was assayed for MDA content using the established method of Placer et al. (15).

Analysis of Antioxidant Markers

Hepatic antioxidant status was assessed by quantifying SOD, CAT, and GPx enzymatic activities using commercial ELISA kits. GSH concentrations were determined following Sedlak and Lindsay's protocol (16). Total tissue protein content, required for normalizing enzyme activities, was measured according to Lowry et al. (17).

RT-PCR Analysis

Hepatic mRNA levels for Table 1 targets were quantified via reverse transcription PCR (RT-PCR). Total RNA was extracted using QIAzol Lysis Reagent (Qiagen, 79306), with subsequent cDNA synthesis performed with the OneScript Plus cDNA Synthesis Kit (ABM, G236, Canada). Quantitative PCR amplification reactions contained BlaSTaq 2X qPCR MasterMix (ABM, G891) and gene-specific primers and were performed in a Rotor-Gene Q thermocycler (Qiagen) under manufacturer-specified thermal cycling conditions. Relative transcript levels were normalized to β-Actin and calculated via the 2-ΔΔCT algorithm (18).

Table 1. *Primer sequences*

Gene	Sequences (5'-3')	Length (bp)	Accession No
NF-kB	F: AGTCCCGCCCCTTCTAAAAC R: CAATGGCCTCTGTGTAGCCC	106	NM_001276711.1
TNF-a	F: CTCGAGTGACAAGCCCGTAG R: ATCTGCTGGTACCACCAGTT	139	NM_012675.3
Caspase-3	F: ACTGGAATGTCAGCTCGCAA R: GCAGTAGTCGCCTCTGAAGA	270	NM_012922.2
Bax	F: TTTCATCCAGGATCGAGCAG R: AATCATCCTCTGCAGCTCCA	154	NM_017059.2
Bcl-2	F: GACTTTGCAGAGATGTCCAG R: TCAGGTACTCAGTCATCCAC	214	NM_016993.2
Wnt3A	F: GTCACAACAATGAGGCTGGG R: AGGTCTTCACTTCGCAACTG	96	NM_001107705
Lrp5	F: CTTCATCCACCGTGCCAACC R: CCAGTAGAGTGTGTCCCCAG	106	NM_001106321
b-Actin	F:CAGCCTTCCTTCTTGGGTATG R: AGCTCAGTAACAGTCCGCCT	360	NM_031144.3

Histopathological analysis

Liver tissue samples were immersed in 10% formalin fixative for 72 hours. Following fixation, tissues underwent sequential dehydration in progressively concentrated ethanol solutions (70%, 80%, 90%, and 100%), followed by xylene clearing to remove residual alcohols. The processed tissues were then embedded in paraffin blocks .Using a microtome, we obtained 5-μm thin sections, which were subsequently stained with Hematoxylin and Eosin (H&E) for structural evaluation. All histological assessments were performed using an Olympus CX43 light microscope (Japan). Histopathological analysis was performed in a blinded manner by two independent observers who were unaware of the experimental group assignments. Histopathological damage was semi-quantitatively assessed in randomly selected fields per animal. The criteria for the evaluation were based on sinusoidal dilatation, inflammatory cell infiltration, congestion and degeneration of hepatocytes. According to histopathological findings, 0: no damage; 1: mild damage; 2: moderate damage; 3: severe damage.

Statistical analysis

Statistical analyses were performed using IBM Statistical Package for Social Sciences (SPSS) program. Results are expressed as mean ± SD. Since the number of samples in the study was fewer than 50, the Shapiro-Wilk test was used to assess whether the data were normally distributed. As a result of the test, $p>0.05$ was

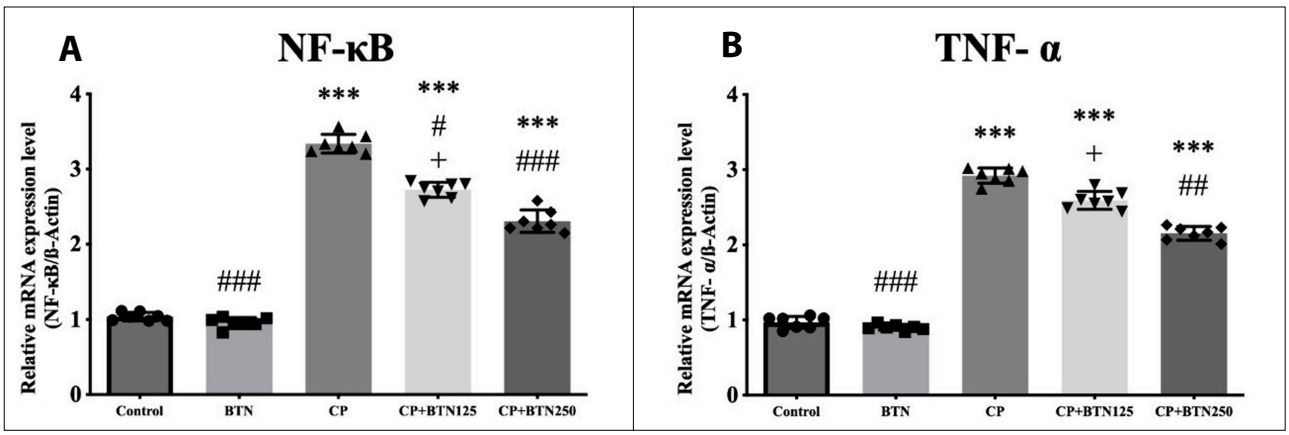

Figure 1. *Effect of CP and BTN on NF-κB and TNF-α mRNA transcription levels in rat liver tissues (n=7). Values are given as mean ± SD. Statistical significance (Control and others: *p<0.05, **p<0.01, ***p<0.001; CP and others: #p<0.05, ##p<0.01, ###p<0.001; CP+BTN125 vs. CP+BTN250: +p<0.05, ++p<0.01, +++p<0.001.) was analyzed using One-Way ANOVA.*

Table 2. *Effects of CP and BTN on serum liver functions parameters and oxidant/antioxidants parameters in rat liver tissues (n=7)*

	Control	BTN	CP	CP+BTN 125	CP+BTN 250
MDA	33.55±2.39^{a}	32,74±2.26^{a}	54.02±2.99^{d}	45.34±1.57^{c}	37.41±1.29^{b}
GSH	5.71±0.47^{d}	6.29±0.67^{d}	2.53±0.38^{a}	3.44±0.21^{b}	4.61±0.15^{c}
GPx	37.42±1.88^{d}	38.44±2.15^{d}	15.84±1.61^{a}	23.38±1.94^{b}	30.75±1.67^{c}
SOD	23.30±2.22^{d}	23.84±1.92^{d}	9.34±1.28^{a}	14.36±1.11^{b}	18.58±1.19^{c}
CAT	51.52±2.13^{d}	52.25±2.00^{d}	32.97±1.91^{a}	40.00±1.53^{b}	45.56±1.57^{c}
AST	65.28±2.97^{a}	65.07±3.17^{a}	107.99±7.37^{d}	91.52±3.18^{c}	80.99±2.89^{b}
ALT	33.39±2.79^{a}	34.51±3.20^{a}	64.28±3.32^{d}	54.57±2.52^{c}	45.65±3.29^{b}

Values are given as mean ± SD. Statistical significance (Different letters indicate statistical difference: ^{a,b,c,d}p<0.05) was analyzed using One-Way ANOVA.

obtained for the markers. After this value, ANOVA and Tukey tests were performed to statistically evaluate the data. Statistical significance was determined at p<0.05, p<0.01, p<0.001.

Power analysis was performed for sample size, with 7 animals per group, using an alpha level of 0.05 and a power of 0.86. The study of Dhouibi et al. (19) was taken as a reference for power analysis.

Results

Liver Function Findings

Cyclophosphamide treatment significantly elevated ALT and AST levels versus controls (p<0.001). Coadministration of BTN with CP markedly attenuated these elevations relative to CP-only treatment (p<0.001; Fig. 1). Betaine exhibited dose-dependent efficacy, with higher doses demonstrating superior hepatoprotective effects (p<0.001; Table 2).

Oxidative Stress Findings

The CP group exhibited significantly suppressed SOD, CAT and GPx activities and GSH concentrations (p<0.001), but elevated MDA (p<0.001) compared with controls. Betaine supplementation, conversely, increased antioxidant parameters and reduced MDA in a dose-responsive manner, demonstrating significantly greater restoration at 250 mg/kg than at 125 mg/kg (p<0.001; Table 2).

Inflammatory Cytokines Findings

Cyclophosphamide administration significantly upregulated hepatic mRNA expression of proinflammatory cytokines NF-κB and TNF-α versus controls (p<0.001; Fig. 1). Betaine co-treatment dose-dependently suppressed these transcripts, with 250 mg/kg demonstrating significantly greater inhibition than 125 mg/kg (p<0.05; Fig. 1).

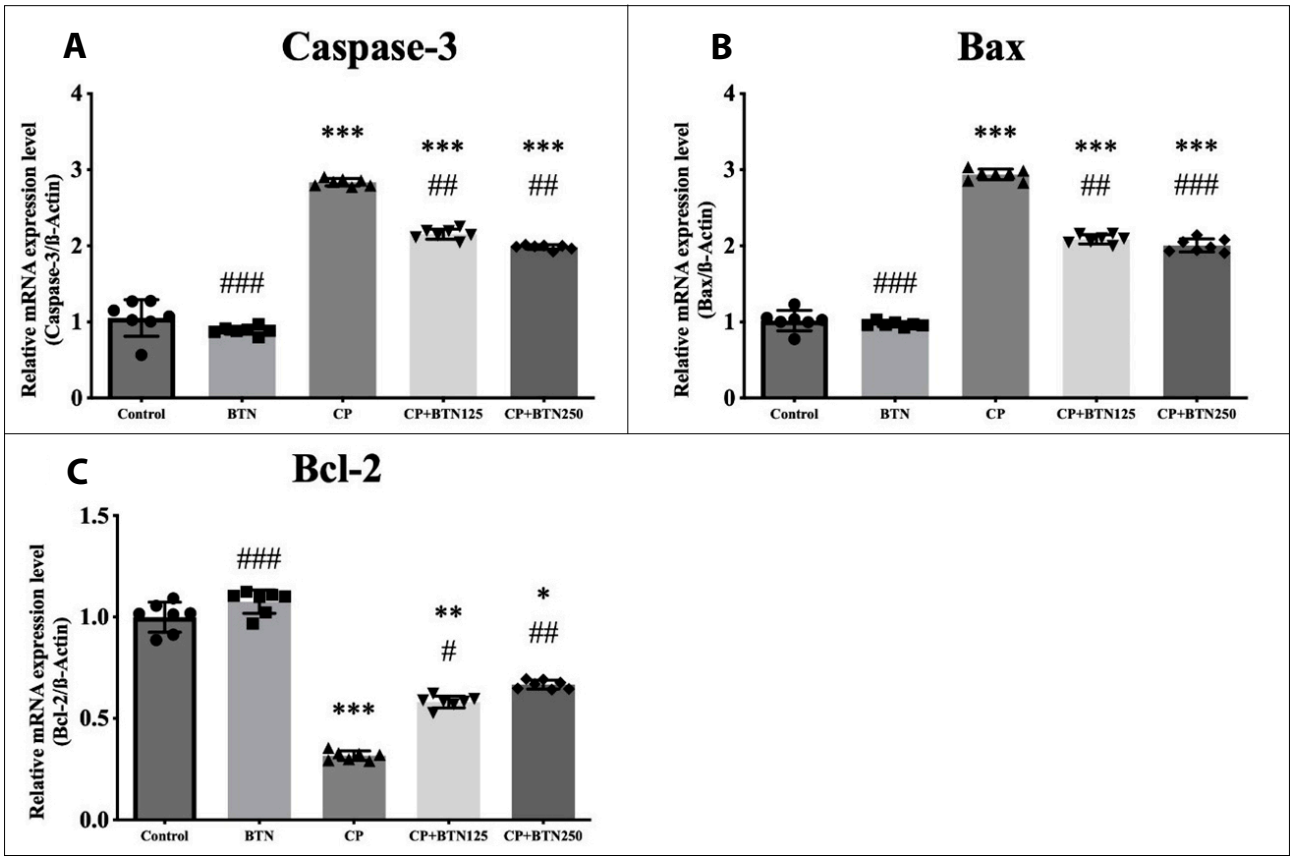

Figure 2. *Effect of CP and BTN on Caspase-3, Bax and Bcl-2 mRNA transcription levels in rat liver tissues (n=7). Values are given as mean ± SD. Statistical significance (Control and others: *p<0.05, **p<0.01, ***p<0.001; CP and others: #p<0.05, ##p<0.01, ###p<0.001; CP+BTN125 vs. CP+BTN250: +p<0.05, ++p<0.01, +++p<0.001.) was analyzed using One-Way ANOVA.*

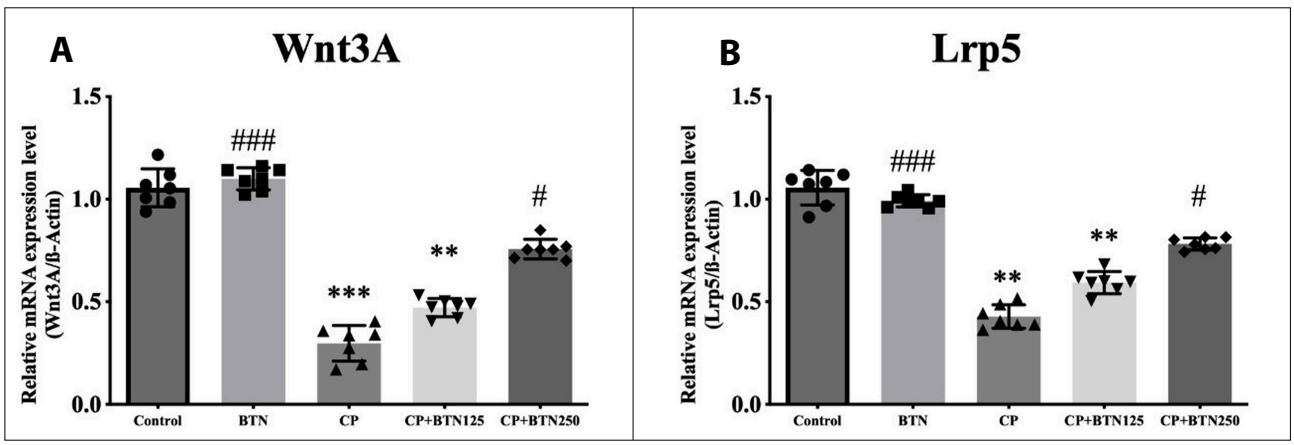

Figure 3. *Effect of CP and BTN on Wnt3A and Lrp5 mRNA transcription levels in rat liver tissues (n=7). Values are given as mean ± SD. Statistical significance (Control and others: *p<0.05, **p<0.01, ***p<0.001; CP and others: #p<0.05, ##p<0.01, ###p<0.001; CP+BTN125 vs. CP+BTN250: +p<0.05, ++p<0.01, +++p<0.001.) was analyzed using One-Way ANOVA.*

Apoptosis Findings

Cyclophosphamide administration significantly upregulated the pro-apoptotic markers caspase-3 and Bax mRNA ($p<0.001$), while downregulating anti-apoptotic Bcl-2 expression ($p<0.001$). Betaine co-treatment reversed this apoptotic imbalance by increasing Bcl-2 and suppressing Bax and caspase-3 expression compared with CP alone ($p<0.01$ and $p<0.001$; Fig. 2). No significant inter-dose differences were observed between the BTN groups (125 vs. 250 mg/kg; Fig. 2).

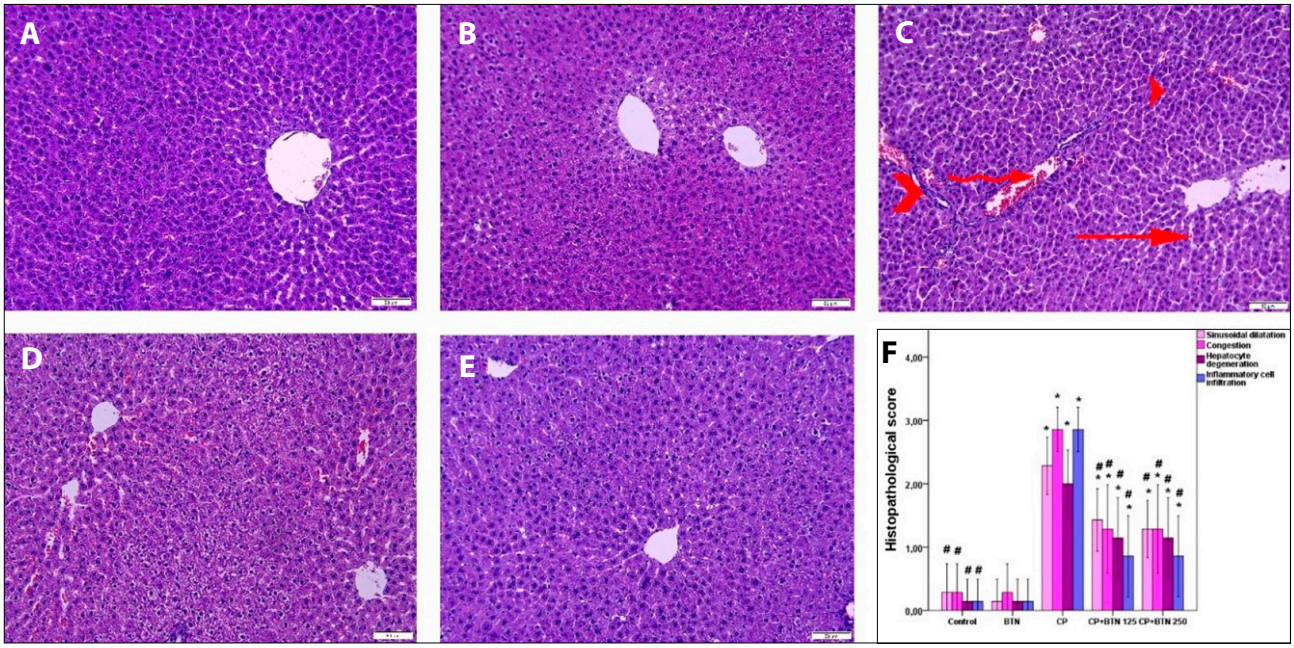

Figure 4. *Representative photomicrographs (H&E, 200×) of liver tissues in all experimental groups (n=7). (A) Control and (B) Normal morphology of liver tissues in BTN groups, (C) vascular congestion (curved arrow), inflammatory infiltrate (arrowhead), sinusoidal dilatation (arrow), degeneration of hepatocyte cells (arrowhead) in the livers of rats treated with CP, decreased histopathological changes in CP+BTN 125 (D) and CP+BTN 250 (E) groups, and morphometric changes in all groups (F). Control vs others: *p <0.05, CP vs others: #p <0.05, CP+BTN 125 vs CP+BTN 250: +p< 0.05*

Wnt3a/Lrp5-Mediated Regeneration And Fibrosis-Related Findings

Activation of Wnt3A (Wingless-Type MMTV Integration Site Family, Member 3A) and Lrp5 (Low Density Lipoprotein Associated Protein 5) pathways, which play a role in liver regeneration and malignancy, was significantly reduced in the CP group (p<0.001). There was an increase in the expression of mRNA of these pathways in the CP+BTN250 group compared to the CP group (p<0.05, Fig. 3). Among BTN doses, the higher dose was more effective but not significantly

Histopathologic Findings

Liver sections from the control (Fig. 4a) and BT Liver sections from the control (Fig. 4a) and BTN (Fig. 4b) groups showed the typical central vein, well-organized hepatic cords, and well-preserved cell membranes and nuclei of hepatocyte cells. In the CP (Fig. 4c) group, vascular congestion, hepatocyte degeneration, sinusoidal dilatation and inflammatory cell infiltration were noted. On the other hand, in the groups treated with BTN (Fig. 4 d,e) along with CP, they showed mild sinusoidal dilatation, mild inflammatory cells, and preserved histological structure with vascular congestion. According to the results of morphometric and semi-quantitative scoring, there was significantly increased inflammatory cell infiltration, hepatocyte degeneration, vascular congestion and sinusoidal dilatation in the CP group compared to the control (p<0.05). The histological damage score in the CP+BTN125 and CP+BTN250 groups was significantly lower than in the CP group (p<0.05). No significant difference was observed between the CP+BTN125 and CP+BTN250 groups (Fig. 4f).

Discussion

Identifying adjunctive strategies that limit chemotherapy-related organ toxicity without blunting antitumor activity has become an increasingly relevant research objective. Cyclophosphamide is well recognized for its broad toxic profile, affecting the liver, kidneys, heart, gonads, nervous system, and skin, which makes it a subject of sustained pharmacological investigation (16). The present study assessed whether BTN could confer hepatoprotection against CP-induced injury in a rat model.

As an inactive prodrug, CP is bioactivated in the liver by CYP450 isoenzymes —particularly CYP2B6, CYP3A4, and CYP2C9— producing phosphoramide mustard, which cross-links

20). ROS-driven lipid peroxidation disrupts hepatocyte membrane integrity, leading to leakage of cytosolic enzymes and the elevation of serum ALT and

AST observed in the CP group (14,21,22). Betaine is known to preserve membrane structure and maintain intracellular osmotic equilibrium under toxic insult (8,11), and in line with earlier reports, its administration in this study attenuated hepatocellular enzyme release in a dose-dependent manner (21,23,24).

The oxidative consequences of CP toxicity extend beyond membrane damage. SOD converts superoxide anions to H_2O_2, which is then neutralized by GPx in a reaction that consumes GSH; CP metabolites impair this chain by depleting GSH through conjugation, leaving cells unable to contain ROS accumulation (11,14,25). MDA, a stable end-product of lipid peroxidation, rises accordingly and serves as a reliable index of oxidative injury (14,15). Betaine replenishes GSH by increasing cysteine availability, thereby re-establishing enzymatic antioxidant activity and suppressing MDA formation (8,11). The CP+BTN250 group showed a more complete restoration of these parameters than the lower-dose group, confirming a dose-dependent antioxidant response.

CP-derived ROS also converge on NF-κB, activating it via a mechanism resembling p53-driven induction during genotoxic stress (4,6,14). Downstream consequences include the upregulation of TNF-α, IL-6, and IL-1β, as well as enhanced recruitment of neutrophils and monocytes to the hepatic parenchyma (16,20,25). Betaine interrupted this sequence by inhibiting NF-κB, reducing pro-inflammatory cytokine output, and tempering Kupffer cell overactivation. These effects were more pronounced at 250 mg/kg than at 125 mg/kg, underscoring the dose-responsiveness of BTN's anti-inflammatory activity (22,25).

CP-induced DNA cross-linking triggers p53-mediated apoptosis by shifting the Bax/Bcl-2 balance toward cell death: Bax is upregulated, Bcl-2 is suppressed, mitochondrial permeability increases, and cytochrome c is released, ultimately activating caspase-9 and caspase-3 (26,27). Betaine reversed these changes—raising Bcl2 while lowering Bax and caspase-3—at both doses tested (6,24,26). However, no significant difference was found between the 125 and 250 mg/kg groups for any apoptotic marker, suggesting that BTN reaches its maximum anti-apoptotic effect at the lower dose. This plateau may reflect restoration of cellular homeostasis at 125 mg/kg, beyond which additional dose increments yield no further modulation of the intrinsic apoptotic pathway within the experimental timeframe.

A sustained acute insult can set the stage for chronic liver damage, and changes in Wnt3A/Lrp5 signaling have been proposed as early molecular indicators of this transition (28). Under normal conditions, Wnt3A engages frizzled receptors and the Lrp5/6 co-receptor to stabilize β-catenin, supporting hepatocyte regeneration and HSC quiescence. Cyclophosphamide suppresses this axis via p53 activation, weakening regenerative capacity and predisposing the liver to fibrosis (28,29). The clinical significance of this suppression is highlighted by reports of 4.6-, 7.4-, and 10.4-fold increases in Wnt3A mRNA at the stages of liver degeneration, precancerous lesion, and hepatocellular carcinoma, respectively (30). In the present study, CP markedly downregulated Wnt3A and Lrp5 expression, whereas BTN treatment dose-dependently restored both, with the CP+BTN250 group showing the greatest recovery—suggesting a protective role in limiting progression toward chronic hepatic injury. Histopathological analysis of CP-treated livers revealed vascular congestion, sinusoidal dilatation, hepatocyte degeneration, and inflammatory infiltration consistent with apoptotic and necrotic cell loss. Co-treatment with BTN substantially reduced these changes, and tissue architecture was largely maintained, corroborating the biochemical and molecular evidence of hepatoprotection mediated through anti-inflammatory and anti-apoptotic pathways (Fig. 4).

Conclusion

Histopathological damage caused by CP in multiple organs, especially the liver and kidney, is significantly reduced thanks to the antioxidant, anti-inflammatory and anti-apoptotic mechanisms of BTN. These findings suggest that BTN may be considered as a potential adjuvant treatment option in alleviating chemotherapy-induced organ toxicity.

Limitations of the Study

This study was conducted under controlled experimental conditions; therefore, the direct applicability of the findings to clinical settings remains limited. The animal model used may not fully replicate human physiological responses. Although the potential effects of alternative dosing regimens were not explored. Furthermore, the study focused primarily on biochemical and histopathological parameters, without an in-depth investigation of molecular mechanisms.

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