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Anti-inflammatory and anti-apoptotic potential of beta-glucan on chemotherapy-induced nephrotoxicity in rats

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Abstract

Background/Aim: Cyclophosphamide (CP) is an anti-cancer agent that mediates nephrotoxicity. Beta (β)-glucan has restorative effects on kidney toxicities through its antioxidant potential; however, the effects of β-glucan on CP-induced renal injury remain unknown. In an experimental nephrotoxicity model using rats, we sought to examine the potential protective action of β-glucan on kidney histomorphology, apoptosis, and TNF-α expression.

Methods: Male albino Wistar rats were divided equally into four groups: control, CP, β-glucan, and CP+β-glucan. The kidney tissues of the rats were examined for TNF-α and caspase-3 immunostaining to evaluate inflammation and apoptosis, respectively. Hematoxylin and eosin (H&E) and periodic acid–Schiff (PAS) staining were used for histopathological analyses.

Results: The CP group showed severe histopathological damage in the renal tissues of rats.

In the renal tissue of the CP group, immunoreactivities for TNF-α (1.25 [0.079] and caspase-3 (1.506 [0.143] were also higher than the control group (0.117 [0.006] and 0.116 [0.002], respectively; $P<0.001$). In the CP+β-glucan group, the histopathological changes significantly improved.

Conclusion: Beta-glucan has therapeutic potential against CP-induced nephrotoxicity in rat kidney.

Keywords: β-glucan, nephrotoxicity, oxidative stress, caspase-3, TNF-α

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Ethics Committee Approval
The study was approved by the Local Animal Experiments Ethical Committee of Kahramanmaras Sutcu Imam University, Protocol no: 2022/02. All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

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Introduction

Cyclophosphamide (CP), an alkylating drug, is frequently used as an immunosuppressant during organ transplantation and is used against many types of cancers, autoimmune disorders, and other conditions [1, 2]. However, the therapeutic use of CP is restricted due to the side effects it produces, such as nephrotoxicity, cardiotoxicity, and hepatotoxicity [3]. Renal damage induced by CP includes apoptosis and necrosis of tubular epithelial cells [3], inflammation [4], fibrosis [5], and decreased lysosomal enzyme activity [6]. The toxic mechanism of CP is attributed to its main metabolites, phosphoramide mustard and acrolein, which cause an increase in free radicals [7]. Acrolein is responsible for inducing the reactive oxygen species (ROS) production that is crucial for DNA interstrand cross-links and the activation of multiple signaling molecules and inflammatory markers, specifically tumour necrosis factor-alpha (TNF-α) and interleukins (ILs); IL-1β and IL-6 [8,9]. It also leads to mitochondrial and endoplasmic reticulum dysfunction, as well as cell membrane disruption, resulting in apoptosis [10].

Beta (β)-glucans are beneficial and natural food ingredients that can be found in yeast, cereal, seaweed, and mushroom [11]. Its many biological properties, such as anti-inflammatory [12], antioxidant [13], anticancer [14], ROS-scavenging [11], and immune-modulating properties [15], have been reported. As evidenced by previous reports, β-glucan exhibited cytoprotective action against uranyl acetate–induced nephrotoxicity [16], chronic nicotine toxicity [17], and renal ischemia-reperfusion injury [18] in rodents.

TNF-α is a crucial mediator produced during inflammatory processes in acute and chronic kidney diseases. Blocking TNF-α with neutralizing antibodies or specific antibodies has been highly effective in the treatment of inflammatory disorders [19]. Previous studies have shown that dietary β-glucan reduces inflammation in various tissues by reducing the expression of pro-inflammatory cytokines [20,21].

The preventive properties of β-glucan against CP-induced renal damage have not, as far as we know, been studied. The purpose of this investigation was to determine whether β-glucan would help protect rats from CP-induced kidney injury by doing a histopathological analysis and utilizing an immunohistochemistry technique to quantify TNF-α and caspase-3 levels.

Materials and methods

Experimental procedures and groups

Twenty-eight male albino Wistar rats (200–250 g) from the Kahramanmaras Sutcu Imam University Animal Care and Research Unit (Kahramanmaras, Turkey) were housed under optimal laboratory conditions (12 h of light, 12 h of darkness, temperature of 22±2 °C) and had water and standard rodent food available *ad libitum*. The Kahramanmaras Sutcu Imam University's Ethics Committee granted permission for the animal experimentation procedure (Approval number: 2022/02-01). The four groups (n=7 each) were the control, CP, β-glucan, and CP+β-glucan groups. The control group was given no treatment for 7 days. On day 2, the CP group received a single intraperitoneal (i.p.) dosage of 200 mg/kg CP to induce nephrotoxicity [8]. β-glucan group received β-glucan dissolved in distilled water at a dose of 50 mg/kg by oral gavage (o.g.) [22] for 7 days. The CP+β-glucan group, however, was given 200 mg/kg (i.p.) CP on day 2 and 50 mg/kg (o.g.) β-glucan, both as a single dose, for 7 days.

On the eighth day, the animals were sacrificed under anesthesia using ketamine/xylazine HCl (75/10 mg/kg, i.p.). All kidney tissues were collected for histomorphological analysis and fixed in 10% buffered formalin solution.

Histopathological examination

The fixed kidney tissues were processed, and 5 µm thick sections were stained with hematoxylin–eosin (H&E) and periodic acid–Schiff (PAS) for histopathological evaluation. The prepared slides were examined by an observer in a blind manner under a light microscope (Carl Zeiss Axio Imager A2 microscope, Germany) with ×20 magnification. The following degenerative changes were scored within each slide in 10 histological fields [4]: 0=no damage, 1=10% of the histopathology damage, 2=10–25% damage, 3=25–50% damage, 4=50–75% damage, 5=>75% damage.

Immunochemistry

Sections taken on adhesive slides were deparaffinized and boiled by a microwave oven in Tris-EDTA buffer for antigen retrieval. The slides were treated with 3% H_2O_2 solution and then blocked with normal goat serum. The slides were incubated with primary antibodies against anti-TNF-α (1: 200, ab220210, Abcam) and anti-caspase-3 (1: 200, ab184787, Abcam) at +4 °C overnight. The slides were rinsed with PBS before being incubated for 30 minutes with anti-rabbit IgG secondary antibody (1: 200, 65-6140, Thermo Scientific), washed, and incubated for 10 minutes with horseradish peroxidase (HRP; 1: 200, 43-4323, Thermo Scientific). After placed in diaminobenzidine (DAB), the slides were counterstained with Mayer's hematoxylin. The histoscore was calculated using the following rating scale: 0.1: <25%, 0.4: 26–50%, 0.6: 51–75%; 0.9: 76–100%, and intensity of immunoreactivity as 0: unstained, +0.5: very low, +1: low, +2: moderate, +3: severe. The score was calculated using the staining intensity × prevalence [23].

Statistical analysis

The software SPSS v. 25.0 was used to conduct statistical analysis (IBM, Chicago, IL). The Shapiro–Wilk test was used for data with a normal distribution. One-way analysis of variance (ANOVA) or the Kruskal–Wallis test were applied to compare the groups, as necessary. For the significant group comparisons, Tukey's multiple range test or the Mann–Whitney *U* test were used, with the Bonferroni correction and an adjusted α value (5C2=0.05 / 10=0.005). The results were presented as mean (standard deviation [SD]) or a median (min–max). Statistics were deemed significant at $P<0.05$.

Results

Effects of β-glucan on renal histological changes

The kidney sections in the control and β-glucan groups showed typical tubular and glomerular histoarchitecture (Figures 1a and 1b). The CP group revealed degenerative changes including desquamation of tubular epithelial cells, hyaline casts, cellular vacuolization, focal inflammatory cells, tubular

degeneration, and coagulation necrosis of some tubular epithelium (Figures 1c and 1d). However, these histopathological changes were markedly attenuated with concomitant treatment by β-glucan (Figure 1e). As shown in Table 1, the injury score showed significant increase in the CP group compared to the control group (P<0.001). The tubular injury score in the CP+β-glucan group was significantly lower than in the CP group (P<0.001).

Figure 1: Photomicrograph of H&E stained renal sections. The control (a) and ß-glucan (b) groups show typical glomerule (g), proximal (p) and distal (d) tubule. (c,d) Sections from the CP group show degeneration in tubule epithelial cells (black asterisks), hyaline cast (black arrow), tubular lumen with sloughed epithelial cells (black arrowhead), cellular vacuolation (white arrow), coagulation necrosis of tubular epithelium (curved arrow). (e) CP+β-glucan group, (a,b,c,d X400).

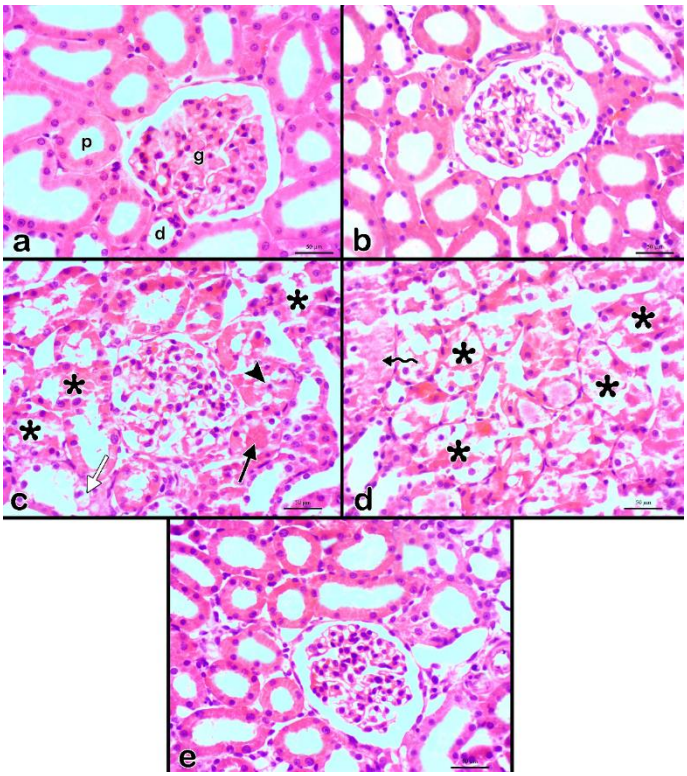


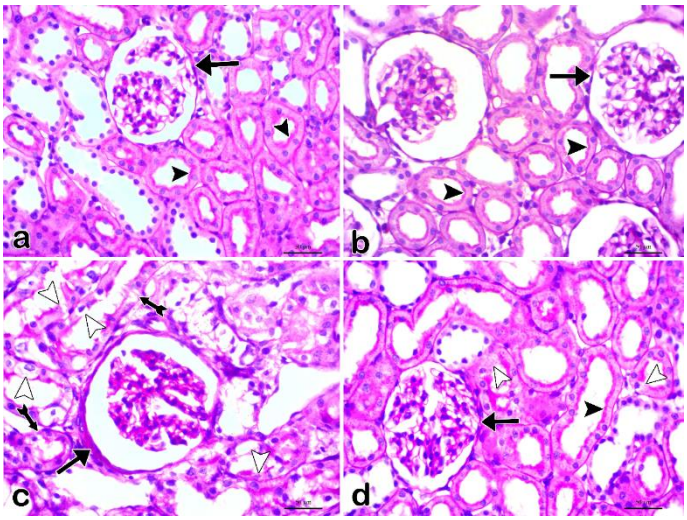
Table 1: Histopathological scores of all groups in renal tissues

Groups	Median (min-max)
Control	0[a] (0-1)
β-glucan	0[a] (0-1)
CP	4[b] (3-5)
CP+β-glucan	1[c] (0-2)
***P*-value [Θ]**	<0.001

[Θ]: Kruskal Wallis H test was used. Mann Whitney U Test was used to pairwise comparisons and an adjusted alpha value for Bonferroni correction was 0.008. [a]: The control and β-glucan groups did not differ significantly (P=0.189). [b]: Significant difference observed between control, β-glucan, CP+β-glucan and CP groups (P<0.001). [c]: Significant difference observed between control, β-glucan and CP+β-glucan groups (P<0.001)

PAS-stained slides from the control and β-glucan groups showed PAS-positive reaction of the brush border of the proximal tubule and parietal layer of Bowman's capsule (Figures 2a and 2b). The basal lamina of a parietal layer of Bowman's capsule thickened in the CP group. The proximal tubules had a disrupted brush border, and irregularity and disruption of proximal basal lamina was determined (Figure 2c). The CP+β-glucan group displayed nearly normal basal lamina of tubules and parietal layer of Bowman's capsule and the brush border for PAS reaction as compared to the CP group. However, some proximal tubules showed only a mild reaction along their brush border (Figure 2d).

Figure 2: Photomicrograph of PAS-stained renal sections. The parietal layer of Bowman's capsule (black arrow) and the brush border (black arrowheads) of the proximal tubule both show strong PAS positive reactions in the control (a) and (b) ß-glucan groups. The CP group (c) exhibits disruption of the proximal basal membrane (tailed arrows), strong PAS positive reactions in the parietal layer of the Bowman's capsule that is thickened (black arrow), and a weak reaction along the disrupted brush border of the proximal tubules (white arrowheads). (d) CP+β-glucan group shows strong positive reaction in the brush border of nearly most of proximal tubules (black arrowhead), along parietal layer of Bowman's capsule (black arrow) and also weak reaction along the disrupted brush border of some proximal tubules (white arrowheads), (a,b,c,d X400).



Immunohistochemical findings of TNF-α and caspase-3 for kidney

Figure 3 and Table 2 show that the CP group had significantly higher TNF-α and caspase-3 expression than the control group (P<0.001). The administration of β-glucan along with CP led to the decreased expression of these proteins when compared to the group that had only received CP (P<0.001).

Figure 3: Representative images of TNF-α (a-d) and caspase-3 (e-h) immunostained kidney sections. (a,e) Control group, (b,f) β-glucan group, and (c,g) CP group: Intense TNF-α and caspase-3 expression (black arrows), respectively. CP+β-glucan group (d,h): Mild TNF-α and caspase-3 expression (black arrows), respectively, (a-h X400).

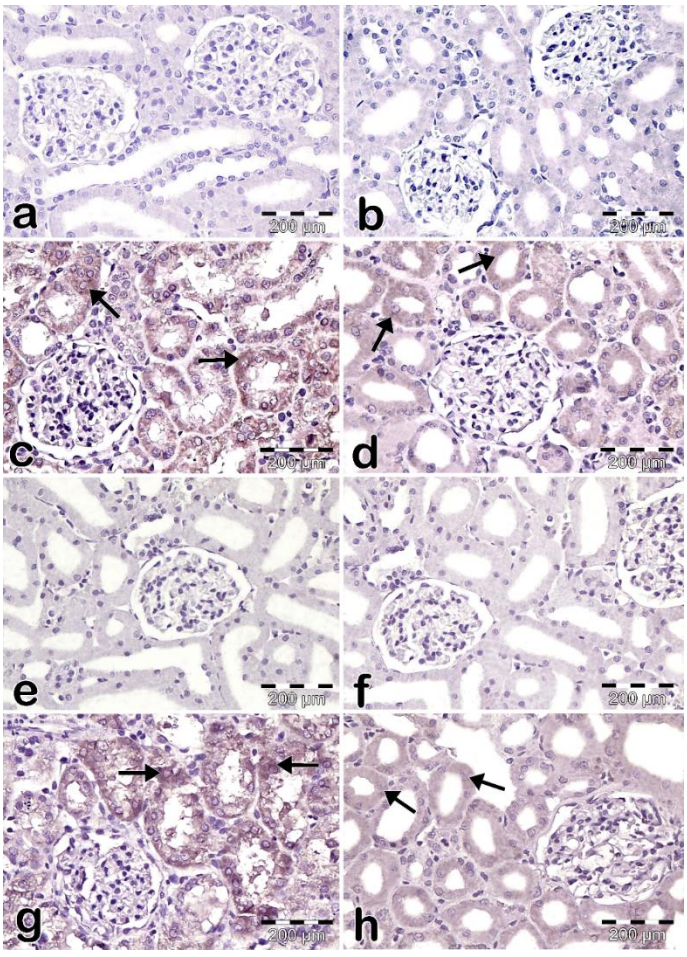


Table 2: Histoscores of TNF-α and caspase-3 immunoreactivity of all groups

Groups	TNF-α Mean (SD)	Caspase-3 Mean (SD)
Control	0.117[c] (0.006)	0.116[c] (0.002)
β-glucan	0.110[c] (0.010)	0.893[c] (0.030)
CP	1.250[a] (0.079)	1.506[a] (0.143)
CP+β-glucan	0.75[b] (0.114)	1.31[b] (0.07)
***P**-values**	< 0.001	< 0.001

*: One Way ANOVA; Tukey test was used to pairwise comparisons, SD: Standard deviation. In terms of differences for TNF-α and caspase-3 immunoreactivity; [a]: CP group was significantly higher in all groups (P<0.001); [b]: CP+β-glucan group was higher than both control and β-glucan (P<0.001); [c]: Control and β-glucan groups did not differ significantly (P=0.998).

Discussion

Kidneys play a fundamental role in basal metabolism in addition to maintaining homeostasis and eliminating toxins and drugs [24]. The antineoplastic drug CP, which is used to manage chemotherapy [25], is known to cause serious nephrotoxicity. CP toxicity has been reported to cause degenerative changes to the kidney, worsened renal injury markers, and oxidative stress parameters in rats [10].

A previous study indicated that CP causes necrosis, Bowman's capsule injury, and inflammatory cells [6]. Another study on rats found inflammatory cell infiltration, congestion, glomerular and tubular distortion, along with thickening of Bowman's capsule, of the wall of blood vessels, and of the tubular basal lamina [26]. These outcomes are consistent with the nephrotoxicity observed in the renal tissues of rats treated with CP in the current study. Several natural and other antioxidants are beneficial and effective source for preventing renal damage [27]. Previous studies have revealed the beneficial potential of β-glucan, a natural product on kidney [16-18]. Additionally, β-glucan has been reported to exhibit beneficial effects on gut microbiota, intestinal barrier function, and enhanced signaling in significant brain regions of C57BL/6 J male mice with cognitive impairment [20].

In the current study, β-glucan administration reduced CP-induced nephrotoxicity, due to its antioxidant activity, when combined with CP [13]. The results of this research, therefore, support the therapeutic benefit of β-glucan in the treatment of CP-induced nephrotoxicity.

The accumulation of CP in the cell may disrupt the antioxidant defense mechanisms and increase the generation of reactive compounds. In the course of CP metabolism and degradation of its metabolites, there is production of ROS encompassing superoxide anions, hydroxyl radicals, and hydrogen peroxide [28]. The production of oxidative stress leads to the activation of inflammatory cascades in damaged renal tissue [3,29]. ILs and TNF-α are important inflammatory mediators in the pathogenesis of CP-mediated inflammation [1]. TNF-α levels in rat renal tissues were found to be significantly higher after CP administration, according to previous research [8, 30]. In the current study, rats with CP-induced kidney damage had higher expression of the pro-inflammatory cytokine TNF-α than did control rats. Beta-glucan has anti-inflammatory activities and numerous studies have shown the positive effects of dietary β-glucans on various tissues [21,31,32]. Different types of inflammatory mediators, including TNF-α, ILs, nitric oxide, interferon gamma, and the non-cytokine mediator prostaglandin E2, play important roles in the anti-inflammatory effects of β-glucans [33]. Consistent with previous research, we found that β-glucan induced an anti-inflammatory effect by significantly reducing the expression of TNF-α in the kidney tissue of the CP+β-glucan group compared to the CP group.

Kidney inflammation and apoptosis have been linked to CP toxicity [30]. Apoptosis is crucial for maintaining tissue homeostasis by removing damaged or infected cells, and caspases, which are sensitive to the redox state of the cell, play a major role in the apoptotic process [34]. CP significantly increased caspase-3 expression in rats compared to controls in the current study. This finding corroborates the reports that CP-induced oxidative stress leads to apoptosis through caspase-3 activation in kidney tissues of rats [8,30].

In contrast, the anti-apoptotic properties of β-glucan [22,35,36] protected against apoptosis by inhibiting caspase-3 expression and significantly reduced its activity relative to the CP-treated rats. We believe that this is the first work to demonstrate the anti-apoptotic properties of β-glucan against CP-induced apoptosis in rat kidney tissues.

Conclusion

In conclusion, β-glucan restored CP-induced nephrotoxicity in rats by improving histopathological damage, suppressing the TNF-α and apoptosis, allowing for the possibility of blocking nephrotoxicity mediated by the anti-inflammatory and anti-apoptotic properties of β-glucan. Combining β-glucan with chemotherapy is encouraged to reduce the nephrotoxicity caused by CP in kidney tissue.

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