

ORIGINAL ARTICLE

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Beta-glucan: A powerful antioxidant to overcome cyclophosphamide-induced cardiotoxicity in rats

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Abstract

Cyclophosphamide (CP) is a common chemotherapeutic drug; however, it does have several toxic side effects, including cardiotoxicity. Beta (β)-glucan is a free radical scavenger and powerful antioxidative molecule. The effects of β -glucan on CP-induced cardiotoxicity were investigated in this experimental study. Twenty-eight Wistar albino rats were divided into the following four equal groups: control (no treatment), CP (200 mg/kg CP, intraperitoneally on day 2), β -glucan (50 mg/kg β -glucan, oral gavage for seven days), and CP+ β -glucan (200 mg/kg CP, intraperitoneally on day 2+50 mg/kg β -glucan, oral gavage for seven days) groups. Histopathology, irisin, and caspase-3 expressions were evaluated in ventricular myocardial tissues. The CP group exhibited considerably more severe cardiac damage than the control group, according to histological evaluation. Immunohistochemically irisin expression was decreased, and caspase-3 expression was much higher in the CP group. β -glucan, on the other hand, improved histopathological changes and exhibited a protective potential against CP-induced cardiac tissue damage when combined with the CP group. Our findings showed that β -glucan could act as an effective cardioprotective molecule against the toxic effects caused by CP.

Keywords: Cyclophosphamide, cardiotoxicity, β -glucan, irisin, caspase-3

Introduction

An anticancer drug, cyclophosphamide (CP), is commonly used to treat cancers such as lymphoma and breast cancer and suppress the immune system in organ transplants, rheumatoid arthritis, and multiple sclerosis. Despite its broad spectrum of applications, cardiotoxicity, nephrotoxicity, hepatotoxicity, and immunotoxicity are all adverse effects of CP [1,2]. When CP is administered, it is immediately converted to 4-hydro-cyclophosphamide (4H-CYP) by cytochrome P450 enzymes, resulting in the metabolites acrolein and phosphoramidate mustard. Phosphoramidate mustard has been reported to be responsible for the antitumoral effect of CP, whereas acrolein has been linked to cardiotoxicity [1]. By mediating oxidative stress, inflammatory response, membrane damage, apoptosis, and formation of reactive oxygen species (ROS), acrolein induces cardiotoxicity [3].

Beta (β)-glucans, glucose polymers found in bacteria, fungus, algae, and some plant cell walls [4]. The β -glucan molecule has been studied for its anticancer, anti-inflammatory, and immunomodulatory properties [4,5]. It reduces serum levels of cholesterol and triglyceride, regulates blood sugar, and facilitates the skin [6]. According to previous studies, β -glucan has anti-diabetic properties [7], inhibits tumor growth [8], and has free radical scavenging, and high antioxidant capacity [9].

Irisin, generated by cleavage of fibronectin type III domain-containing 5 (FNDC5), is a hormone-like myokine secreted mainly by cardiac and skeletal muscle cells, but also by other organs containing adipose tissue, skin, stomach, liver, spleen, brain, and testis [10,11]. Irisin is essential not only in energy metabolism but also in the mechanisms of several diseases, including type 2 diabetes and cardiovascular disease [12]. According to research, irisin has anti-apoptotic, anti-oxidative, and anti-inflammatory properties [13,14].

Myocardial cells are known to start repairing after myocardial injury. Irisin, expressed in the myocardium, contributes to cell repair in cardiovascular diseases by preventing mitochondrial malfunctioning and reducing oxidative stress. The therapeutic relevance of irisin in cardiac damage is growing; due to its

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protective effects [10].

The potential ameliorative effect of β -glucan over CP-mediated cardiac damage has not yet been studied. Thus, the current investigation was designed to determine whether β -glucan could protect rats from CP-induced myocardial injury by studying the irisin and apoptosis.

Material and Methods

Kahramanmaraş Sutcu Imam University Local Ethics Committee of Experimental Animals authorized this experimental research (Approval number: 2022/05-02, date: 18/05/2022). Twenty-eight Wistar albino male rats (200 to 250g) were used to examine the effect of β -glucan on CP-mediated cardiac toxicity. The rats were fed a standard rat diet, given tap water, and kept at $22 \pm 2^\circ\text{C}$ with 12 hours light-12 hours of darkness schedule. The following were the experimental groups:

Groups

- Control group (n=7): No treatment for seven days.
- β -glucan group (n=7): 50 mg/kg β -glucan (Mustafa Nevzat Company, Turkey) was administered by oral gavage (o.g.) for seven days [15].
- CP group (n=7): On the 2nd day of the experiment, a single dose of 200 mg/kg CP was administered intraperitoneally (i.p.), and no other treatment was applied during the experiment [16].
- CP+ β -glucan group (n=7): 50mg/kg β -glucan was administered by o.g. for seven days and on the 2nd day of the experiment, a single dose of 200mg/kg CP was administered i.p.

After 7 days, all rats were sacrificed under anesthesia using ketamine (75mg/kg)+xylazine HCl (10mg/kg). Afterward, ventricular myocardial tissues were collected and fixed with a 10% buffered formalin solution for routine histopathology and immunohistochemistry staining.

Histopathological examination

The formalin-fixed ventricular myocardial tissues were processed routinely and embedded in paraffin. Hematoxylin and Eosin (H&E) staining were used on sections cut at 5 μm . A blind observer evaluated and scored stained sections under a Carl Zeiss Axio Imager A2 microscope (Zeiss Instruments Inc., Germany). The histopathological damage score was calculated using a previously described procedure [17]. Cardiomyopathy score severity was graded as follows: (0) no damage, (1+) mild lesion, (2+) moderate lesion, and (3+) severe lesion.

Immunohistochemical analysis

Irisin and caspase-3 expressions were evaluated within myocardial tissue sections. For immunohistochemical staining, the sections taken on adhesive slides were immunostained with irisin (1:200, Catalog number: H-067-17, Phoenix Pharmaceuticals, Inc.) and caspase-3 (1:200, ab184787, Abcam) primary antibodies and evaluated according to the literature [18]. The histoscore was calculated using the following rating scale to represent the occurrence of irisin and caspase-3 immunoreactivity in heart tissue: 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 $\times$ prevalence.

Statistical Analysis

The SPSS software, version 25.0 (IBM Inc., Chicago, IL), was used for statistical analyses. The Shapiro–Wilk test was applied to determine if the data distribution was normal. One-way ANOVA or the Kruskal Wallis H test was applied to compare the groups where appropriate. For the influential group's comparisons, Tukey's multiple range test or the Mann-Whitney U test (Bonferroni correction was employed for which adjusted alpha value ($5C2=0.05/10=0.005$) were applied. The data was provided as a mean SD or a median (min-max). A p-value of <0.05 was accepted as statistically significant.

Results

Effect of β -glucan on myocardial histopathology

In the microscopical examination, sections of the control (Figure 1a) and β -glucan (Figure 1b) groups indicated the typical histological architecture of myocardial tissues. The CP-treated group (Figure 1c, 1d, 1e) showed severe histopathological alterations such as congested blood vessels, disruption of myofibrils, muscle fiber hypertrophy, myocardial fiber vacuolization, leukocyte infiltration, perinuclear vacuolization, focal areas of myocytic degeneration and myocytes with intensely eosinophilic sarcoplasm in comparison to the control group. Compared to the CP group, all these histopathological changes were almost ameliorated in the CP+ β -glucan group (Figure 1f). Table 1 shows that histopathological scores in the CP group were significantly higher than in the control or treatment group ($p<0.001$). Compared to the CP group, the CP+ β -glucan group had significantly reduced myocardial injury ($p<0.001$).

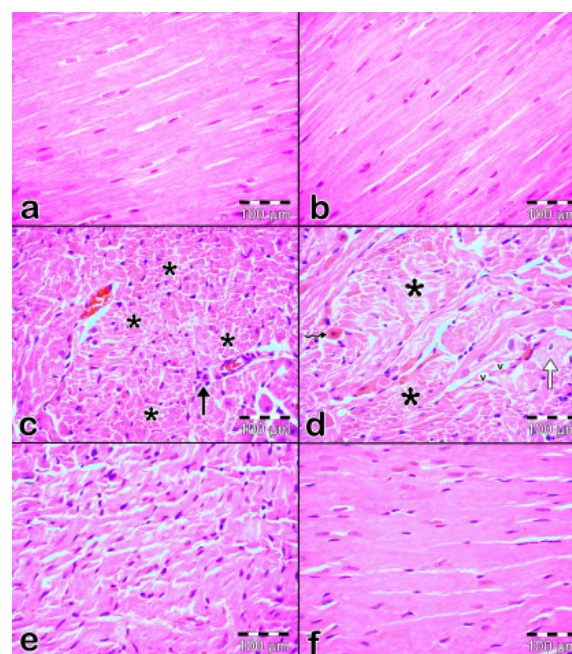


Figure 1. Photomicrographs of H&E-stained myocardial tissue sections from groups ($\times 400$). (a) Control and (b) β -glucan groups with normal myocardium. (c,d) CP group; disruption of myofibrils (asterisks), leukocyte infiltration (black arrow), myocytes with intensely eosinophilic sarcoplasm (curved arrow), hypertrophy (white arrow), and vacuolation (v) in cardiomyocytes. (e) CP group; focal areas of myocytic degeneration. (f) CP+ β -glucan group

Table 1. Histopathologic scores of the experimental groups

Groups	Median (min-max)	p-value Ø
Control	0 ^a (0-1)	<0.0001
β-glucan	0 ^a (0-1)	
CP	3 ^{b,c} (2-4)	
CP+β-glucan	2 ^b (0-3)	

Ø: Kruskal Wallis H test was used.
Mann Whitney U Test was used for pairwise comparisons and an adjusted alpha value for Bonferroni correction was 0.008
^a: No significant difference between control and β-glucan groups (p>0.05)
^b: CP+β-glucan group was significantly higher than the control and β-glucan groups (p<0.001)
^c: CP group was significantly higher than all the other groups (p<0.001)

Table 2. Histoscore of irisin and caspase-3 immunoreactivity of the experimental groups

Groups	Irisin	Caspase-3
Control	0.29 ^{a,b} ±0.06	1.04 ^c ±0.08
β-glucan	0.33 ^a ±0.06	1.11 ^c ±0.07
CP	0.15 ^c ±0.03	1.55 ^a ±0.15
CP+β-glucan	0.23 ^b ±0.04	1.31 ^b ±0.07
p* values	<0.001	<0.001

*: One Way ANOVA; Tukey test was used for pairwise comparisons.
The differences for irisin immunoreactivity;
^a: No significant difference between control and β-glucan groups (p>0.05), β-glucan was significantly higher than both CP and CP+β-glucan groups (p<0.05)
^b: No significant difference between control and CP+β-glucan (p>0.05), it was significantly higher than the CP group (p<0.05)
^c: CP group was significantly lower than all groups (p<0.05)
The differences for caspase-3 immunoreactivity;
^a: CP group was significantly higher in all groups (p<0.05).
^b: CP+β-glucan group was significantly higher than both control and β-glucan (p<0.05)
^c: No significant difference between control and β-glucan (p>0.05).

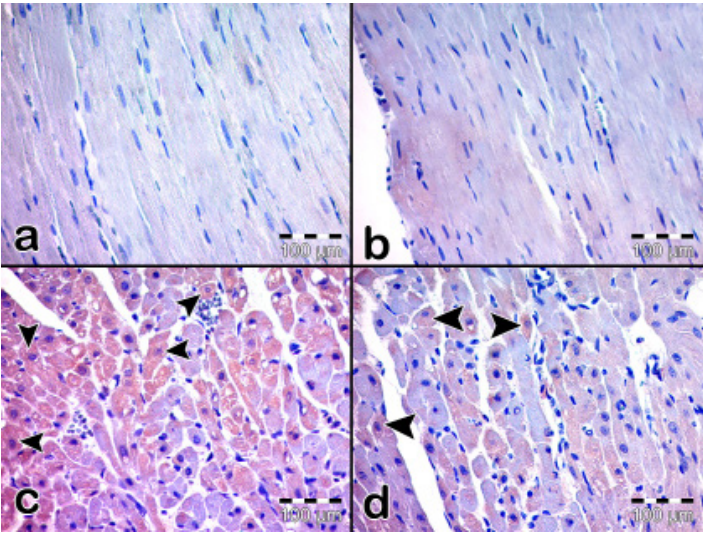


Figure 2. Representative photomicrographs of caspase-3 immunoreactivity (arrows) in rat cardiac sections (x400). (a) Control group, (b) β-glucan group, (c) CP group, (d) CP+β-glucan group

Caspase-3 and irisin expressions

Expressions of irisin and caspase-3 are summarized in Table 2. Similar caspase-3 expression was observed in the control (Figure 2a) and β-glucan (Figure 2b) groups (p>0.05). When compared to the control group, there was a significant increase in caspase-3 immunoreactivity in the CP group (p<0.05); (Figure 2c). Following CP+β-glucan administration, caspase-3 expression decreased significantly in comparison to the CP group (p<0.05); (Figure 2d).

Irisin immunoreactivity was determined both in myocytes and connective tissue between the myocytes. The control (Figure 3a) and β-glucan (Figure 3b) groups revealed similar expressions of

irisin (p>0.05). The CP group exhibited significantly lower irisin immunoreactivity than the control group (p<0.05); (Figure 3c). On the other hand, higher irisin immunoreactivity was observed in the CP+β-glucan group compared to the CP group (p<0.05); (Figure 3d).

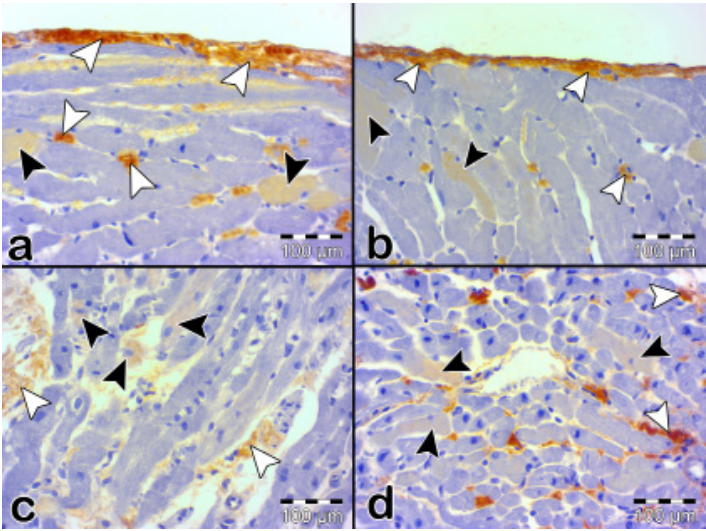


Figure 3. Representative photomicrographs of irisin immunoreactivity in rat cardiac sections (x400). (a) Control group, (b) β-glucan group, (c) CP group, (d) CP+β-glucan group. Myocytes (black arrowheads), connective tissue (white arrowheads)

Discussion

Despite its toxic side effects [19], including cardiac toxicity [20], CP is a widely used chemotherapeutic drug. CP or its metabolite is associated with increased superoxide radical production, inflammation, endothelial cell apoptosis, endoplasmic reticulum, and mitochondrial damage in the heart [1,3]. The cardiac membrane

is damaged by CP, as previously described. The production of OH-free radicals during CP metabolism causes this injury. Because of the lipid peroxidation caused by these free radicals, the cardiac membrane loses its integrity and function. Superoxide dismutase, glutathione, and catalase are critical inhibitors of free radicals produced by lipid peroxidation; CP reduces the amount of these enzymes, resulting in myocardial tissue injury [21]. Cardiotoxic effects of CP administration on cardiac tissue in rats have been described in previous investigations [16,22]. We found that CP caused significant damage to cardiac tissue in rats, as previously described in the literature [23,20]. Antioxidants and related polyphenolic substances have been shown in epidemiological studies to prevent cardiomyopathy [24] effectively. Previous research has revealed that β -glucan has therapeutic effects on heart tissue under oxidative stress conditions [25,26]. In the current study, a natural antioxidant, β -glucan, was found to considerably decrease the side effects of CP-induced cardiotoxicity by restoring cardiac tissue to near normal appearance at a dose of 50 mg/kg. The potential of β -glucan to reduce oxidative stress could be related to its antioxidant and free radical scavenging properties could explain its therapeutic effect [10,27].

A previous study [28] has found a link between CP-induced apoptosis and cardiac inflammation and oxidative stress. The same study also revealed that CP induces myocyte, which results in myocardial infarction, left ventricular hypertrophy, and heart failure. Chronic acrolein exposure can increase myocyte apoptosis in the heart up to six-fold [29], as acrolein has been associated with cardiac damage [1]. CP has elevated cardiac caspase-3 expression in rats [30,31]. Similarly, we determined that a single 200 mg/kg i.p. dose of CP caused apoptosis in rats, as demonstrated by enhanced caspase-3 expression in cardiac tissue of the CP group. In contrast, β -glucan treatment significantly reduced caspase-3 expression in the CP+ β -glucan group. The findings indicate that β -glucan can be safely applied to counteract the apoptotic effect of CP on rat cardiac tissue via its anti-apoptotic properties.

Irisin; produced more by cardiomyocytes than skeletal muscle, affects various cardiovascular functions. Irisin is also secreted mainly in the connective tissue [32]. Irisin expression and circulating irisin were decreased in rats with ischemic cardiomyopathy, heart failure, and isoproterenol-induced myocardial infarction [32,33]. In addition, irisin level was found to be lower in conditions that increase oxidative stress, such as type 2 diabetes [18,34], chronic kidney disease [35], Behçet's disease [36], and chronic obstructive pulmonary disease [37]. As reported in the previous research [18,34,35,36,37], CP-generated oxidative stress resulted from a significant decrease of irisin expression in the myocardial tissue of rats in contrast to the control group in the current study. In contrast to the CP group, the CP+ β -glucan group showed increased irisin immunoreactivity.

It is unclear whether decreased irisin immunoreactivity results from cardiac damage or a protective mechanism against CP-induced cellular stress due to its anti-oxidative and anti-inflammatory properties and energy regulation role [13,33,38].

Conclusion

In conclusion, this research found that β -glucan can be effectively used for preventing damage and alleviating apoptosis caused by

CP in the cardiac tissue. The present results showed that β -glucan administration could serve as a therapeutic strategy for preventing CP-induced cardiotoxicity. More experimental and clinical studies are needed to explore the therapeutic molecular mechanism of β -glucan over CP-induced cardiotoxicity in rats.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical approval

Local Ethics Committee of Experimental Animals at Kahramanmaraş Sutcu Imam University approved this experimental research (Approval number: 2022/05-02, date: May 18, 2022).

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