Cell J
Cell Journal(Yakhteh)
Homepage: https://www.celljournal.org/
Original Article
Vol 25, No 9, September 2023, Pages: 645-654

β-Glucan Regulates Lipopolysaccharide Induced Genotoxic Damage to The Liver through The Induction of BRCA1 Protein Expression

Gözde Aydoğan Kılıç, Ph.D.*, Mojahed Alsafi, Ph.D.

Department of Biology, Faculty of Science, Eskişehir Technical University, Eskişehir, Turkey

Abstract

Objective: The present study aims to investigate the role of breast cancer-susceptibility gene 1 (*BRCA1*) protein in the β-Glucan (βG) molecule mediated regulation of lipopolysaccharide (LPS)-induced liver genotoxicity.

Materials and Methods: In this experimental study, totally, 32 male Swiss Albino mice were randomly divided into 4 equal groups: control (C), LPS-administered (LPS), βG-administered (βG) and βG-pre-administered/LPS-administered (βG+LPS). The βG was injected at the dose of 150 mg/kg/day intraperitoneally (i.p.) for 3 days. A single dose of 4 mg/kg (i.p.) LPS was administered 24 hours after the last βG injection. BRCA1 expression was determined by western blot analysis and confirmed by quantitative immunofluorescence. Proliferating cell nuclear antigen (PCNA), nuclear factor erythroid 2–related factor (Nrf2) and 8-OHdG protein levels were also determined by the immunofluorescence analysis. The alkaline comet assay was performed. superoxide dismutase (SOD), catalase (CAT) and membrane lipid peroxidation were biochemically measured, and light microscopic histology was evaluated.

Results: The BRCA1 expression level was significantly decreased in the LPS group. However, in the βG+LPS group, expression of BRCA1 protein was over 2 folds higher than the control. After the LPS induction, the DNA strand breaks, oxidative DNA lesions and abnormal proliferation of the liver cells were almost entirely suppressed in βG pre-administrated animals, indicating the BRCA1 mediated ubiquitination of PCNA and activation of the DNA damage repair pathways. Activation of Nrf2 in the βG+LPS group resulted in an increase in the levels of Nrf2 pathway dependent antioxidant enzymes SOD and CAT, prevented the peroxidation of membrane lipids and maintained the histological architecture of the liver.

Conclusion: The results manifested that the βG is a strong inducer of the BRCA1 protein expression in the LPS-induced hepatic stress and the protein constitutes the key component of a βG mediated liver protection against an LPS-induced genotoxic and pathological damage.

Keywords: Beta-Glucan, BRCA1, Genotoxicity, Lipopolysaccharide, Liver

Citation: **Kılıç GA, Alsafi M. β-Glucan regulates lipopolysaccharide induced genotoxic damage to the liver through the induction of BRCA1 protein expression. Cell J. 2023; 25(9): 645-654. doi: 10.22074/CELLJ.2023.1989382.1226**

Introduction

The lipopolysaccharide (LPS) is the major surface membrane component of the gram-negative bacterial cell wall and the main pathogenic factor of endotoxemia. The liver is particularly susceptible to an LPS-induced damage since hepatocytes are the main players in the elimination of the endotoxin (1, 2). LPS-induced damage to the liver is a result of the dysregulated immune response to the pathogenic factor, and the following induction of oxygen free radicals such as superoxide anion radical (O_2•-), and non-radical but highly reactive molecules like hydrogen peroxide (H_2O_2). The resulting oxidative stress (OS) leads to the depletion of an intracellular storage of endogenous antioxidants, while inducing a damage to protein, membrane lipid and DNA (3, 4). LPS-induced single (SSB) and double (DSB) strand breaks can cause malfunctions in the cell cycle. These changes can constitute the potential cause of carcinogenesis after severe infections (5, 6). Compounds of anti-oxidative and anti-genotoxic are considered a potent agent can prevent the LPS-induced hepatic injury and the risk of developing a subsequent cancer (7, 8).

Beta-glucans (βGs) are a group of biologically active natural compounds that commonly are found in the cell walls of fungi, yeast, algae, and cereals. They are well-known for their immune-modulator and anti-oxidative functions (9). The anti-genotoxic potential of βGs have been subjected to previous studies, their controversial results led to need a better understanding of the mechanism of βGs (10, 11). The βGs have been shown to have an anti-proliferative action on different cancer cell types (12-14). As a suitable target for cancer prevention, breast cancer susceptibility gene 1 (*BRCA1*) gene has potentially associated with DNA replication, damage, repair, cell proliferation and

Received: 07/February/2023, Revised: 20/April/2023, Accepted: 14/May/2023
***Corresponding Address: P.O.Box: 26470, Department of Biology, Faculty of Science, Eskişehir Technical University, Eskişehir, Turkey**
Email: gozdea@eskisehir.edu.tr

Royan Institute
Cell Journal (Yakhteh)

antioxidant pathways. The effects of dietary compounds on the expression of BRCA1 protein have been subjected to some of the recent studies. The necessity of further investigation on the role of dietary compounds involved in the regulation of the expression of the gene has been pointed out (15-17).

To the best of our knowledge, an anti-genotoxic mechanism of the βG has not been evaluated in association with the functions of BRCA1 protein. Besides, an anti-genotoxic potential of the βG against the LPS-induced hepatic damage has not been subjected to previous studies. Hence, the present study aims to investigate the multifunctional role of the BRCA1 protein on the βG mediated regulation of genotoxic damage. For this aim, LPS-induced liver is chosen as the organ that can firstly reflect the effects of toxic compounds, and due to the risk of subsequent carcinogenesis development.

Materials and Methods

Ethics approval code

The present experiments were approved by the Local Ethical Committee at Animal Experimentation of the Anadolu University, Eskisehir, Turkey (2018-1) in accordance with the National Institutes of Health Guidelines for the use of Laboratory Animals.

Materials

In this experimental study, LPS (L2630) and *Saccharomyces cerevisiae* β-glucan (from baker's yeast) (9012-72-0) were both purchased from Sigma (St Louis, MO, USA). Primary antibodies for BRCA1 (9010S) and proliferating cell nuclear antigen (PCNA, 2586S) were purchased from Cell Signaling Technology (Danvers, MA, USA) and Nrf-2 (NBP1-32822) was from Novus Biologicals (Littleton, CO, USA). The 8-oxoguanine (8- oxoG) antibody was obtained from Santa Cruz Biotechnology (CA, USA).

Animals and treatment

Totally, 32 Male Swiss Albino mice were obtained from the Experimental Animals Research Center, Anadolu University, Eskişehir, Turkey. Animals with a weight between 30-40 g were acclimatized to the laboratory conditions for 3 days before the experiments onset. During this period, all animals were kept at the 25 ± 3˚C with a 12 hours light-dark cycle condition and fed ad libitum with Purina Chow pellets (022-5022 Nestlé Purina. PetCare, St Louis. MO, USA). In succession, all animals were randomly divided into four equal groups (n=8); including control (C), LPS-administered (LPS), β-Glucan-administered (βG) and βG-pre-administered/LPS-administered (βG+LPS). The total treatment period lasted for 6 days. The control group received vehicle, 0.9% saline (S0817 Merck, Darmstadt, Germany) intraperitoneally (i.p.). The prophylactic βG (9012-72-0, Sigma, St Louis, MO, USA) was injected to both groups, βG and βG+LPS, at the dose of 150 mg/kg/day intraperitoneally (i.p.) for 3 days. A single dose of LPS (4 mg/kg i.p.) (Cat No: L2630, Sigma, St Louis, MO, USA) was injected into the animals in the LPS group and βG+LPS group on the fourth day of the experimental period (The βG+LPS group received its LPS dose 24 hours after the last βG injection). All the animals in control and experimental groups were sacrificed at the end of the experimental period under ketamine (K-113, Sigma, St Louis, MO, USA) anesthesia (80 mg/kg i.p.) and their livers were dissected for the analysis by using standard procedure. The treatment strategies, doses, days and duration of the βG and LPS intervention were conducted based on previous studies (18, 19).

BRCA1 protein western blot assay

The protein lysates of the liver tissue were subjected to western blot analysis with both a specific rabbit polyclonal antibody BRCA1 (9010S, Cell Signaling Technology, Danvers, MA, USA), against BRCA1 protein as well as an appropriate HRP-conjugated secondary antibody (7074, Cell Signaling Technology, Danvers, MA, USA). Using Bradford technique, the total protein content of all samples was measured spectrophotometrically at 595 nm (Shimadzu UV-2101PC, Japan) (20). Upon being quantified, samples (60 µg of each) were resolved by SDS-PAGE (Bio-Rad Mini Protean System, USA) and transferred to the specific membrane (88018, Thermo Fisher Scientific, USA) by a Dry Blotting System (I Blot 2, Thermo Fisher Scientific, Waltham, MA, USA). Successively, the administration of antibodies to the membrane was performed (Invitrogen™ iBind™ Flex Western device, Thermo Fisher Scientific, USA). During this process, all applied solutions were checked for their compatibility with the device before being applied to the device. β-actin was measured as a loading control (21). An ECL system (GEN-BOX imagER CFx, ER Biotech Ltd., Ankara, Turkey) was used to visualize the protein bands. The band intensity of BRCA1 was measured with the ImageJ software (version 1.53t, NIH, USA).

Immunofluorescence assay

In a first step, 5 µm thick sections were taken from the tissue blocks which were previously prepared for the histological examinations (22). An indirect immunofluorescence method was applied to the samples by using a rabbit polyclonal antibody against BRCA1 (9010S, Cell Signaling Technology, Danvers, MA, USA), a rabbit monoclonal antibody against PCNA (2586S, Cell Signaling Technology, Danvers, MA, USA) and a mouse polyclonal antibody against Nrf-2 (NBP1-32822, Novus Biologicals, Littleton, CO, USA) (23). The 8- oxoguanine (8- oxoG) labelling protocol was applied by using a mouse 8- oxoguanine (8- oxoG) antibody (sc-516176, Santa Cruz Biotechnology, CA, USA) according to the method of Kemeleva et al. (23). For the immunofluorescence assay, the liver sections were incubated in the blocking solution for 30 minutes at room temperature. Tissues were incubated together with the primary antibody at an appropriate degree of dilution (1:200 v/v) with a 1% BSA (Cat No: A3294, Sigma, St Louis, MO, USA) in the PBS buffer (P7059, Sigma, St Louis, MO, USA) and at a consistent temperature of 4°C overnight. After washing with PBS (3×5

minutes), liver sections were incubated with the appropriate secondary antibody (labelled with Alexaflor 488) (ab150113, ab150077, Ab Chem, Cambridge, UK) at an appropriate degree of dilution (1:1000 v/v) in PBS buffer for one hour, at consistent room temperature and in the dark. Positive controls were mouse brain, regenerating rat liver, piceatannol induced mouse liver and Al-induced rat liver for BRCA1, PCNA and Nrf-2 proteins and 8- oxoG lesion sites respectively. Negative controls were the same tissues that were prepared by avoiding the use of primary antibodies. Subsequently, immunofluorescence examinations were performed under a Leica DM 6000B fluorescence motorized microscope (Leica microsystems, Wetzlar, Germany). At least 20 different areas from the liver sections of all animals were randomly selected from each experimental group (n=160) and analyzed using the Image J software (version 1.53t, NIH, USA).

Comet assay procedure

The 0.1 g of liver tissues was homogenized in the Merchant EDTA buffer (0.5 mM NaEDTA, 10 % DMSO in phosphate buffered saline, pH=7.4) with a Potter Elvehjem homogenizator. Totally, 1×10^5/ml liver tissue cells were suspended in a low melting point agarose (75 µl of 1%, w/v) (Cat No: A9095, Sigma, St Louis MO, USA). A microgel was formed on a microscope slide (precoated with 1% normal melting point agarose) by using 85 µl of the cell suspension and allowed to set at 4°C for 5 minutes. Samples were treated with the cell lysis buffer (2.5 mol l^{-1} NaCl, 100 mmol l^{-1} EDTA, 10 mmol l^{-1} Tris-HCl, pH=10.0, containing 1% Triton X-100 added just before use, and 40 mmol l^{-1} dithiothreitol) for 24 hours at room temperature. To remove salt and detergent, slides were washed with deionized water (C7684, Merck- Milipore, Darmstadt, Germany) three times in a 10 minutes intervals between washes. Slides were allowed to equilibrate for 20 minutes in a horizontal electrophoresis unit with a running buffer (500 mmol l^{-1} NaCl, 100 mmol l^{-1} Tris-HCl and 1 mmol l^{-1} EDTA, pH 9.0) before electrophoresis (0.60 V cm^{-1}, 250 mA) for 30 minutes. They were then neutralized with the 0.4 mol l^{-1} Tris (pH=7.5) and stained with SYBR Green I (1:10.000, S9430 Sigma, St Louis, MO, USA) for 1 hour. Fluorescent microscopic examinations were performed under a Leica DM 6000B microscope (Leica microsystems, Wetzlar, Germany). Analysis of the gels was performed by using the Comet IV Software (Perceptive Instruments, Wiltshire, UK). An average of 100 cells from each slide was counted. The results were given as 'tail moment' which is a product of the tail length and the tail DNA% (Tail moment=tail length× % of DNA in the tail) (24).

Biochemical evaluation

For the superoxide dismutase (SOD) activity measurement, an experiment was conducted in accordance with the method of Nebot et al. (25), which was previously standardized. This method is based on the measurement of the formation rate of the chromophore resulted from the SOD enzyme-dependent autooxidation of 5,6,6a,11btetrahydro-3,9,10-trihydroxybenzo[c]fluorine (BXT-01050) in an aqueous alkaline solution. One unit of an enzyme is defined as the amount of enzyme that allows the autoxidation of one micromole of the BXT-01050 reagent at 37°C, pH=8.8 in one minute. The catalase (CAT) activity was determined according to the method of Beers and Sizer (26). The method is based on the absorbance measurement of the hydrogen peroxide at 240 nm. Therefore, one unit of an enzyme is defined as the amount of an enzyme that is degraded to a 1.0 micromole of the hydrogen peroxide in one minute at 25°C and pH=7.0. Furthermore, the lipid peroxidation (LP) level was measured at 532 nm, according to the method of Ohkawa and Ohishi from the n-butanol and pyridine (15:1, v/v) extract of the malondialdehyde (MDA) (27).

Histological assay

All tissues were fixed in a solution containing 4% paraformaldehyde in PBS buffer (EMS 15712, Electron Microscopy Sciences Hatfield, PA, USA) (pH=7.2-7.3). Then, each sample was passed through a graded series of the alcohol (1012768, Sigma, St Louis, MO, USA) to remove the water. In addition, the samples were infiltrated with a mixture of LR White medium grade kit (14380, Electron Microscopy Sciences Hatfield, PA, USA) and 70% ethanol (2: 1; v/v) with a following treatment in pure LR White. Consecutively, all tissue blocks were polymerized in an 60°C oven overnight and sectioned (700 nm) by using a Leica EM UC7 ultramicrotome. These sections prepared for light microscopic observations were then stained with 1% toluidine blue/borax (pH=8.4, 104172, Merck, Germany). As a final step, light microscopic examinations were performed under a Leica DM 6000B light microscope (Leica microsystems, Wetzlar, Germany) (22).

Statistical analysis

Statistical analyses were performed by using SPSS 20.0 Software (IBM Corp., NY, USA). The one-way analysis of variance (ANOVA) was followed by the Dunnett's T3. Values of $P<0.05$ were considered as statistically significant. All data are expressed as the mean ± standard error (SE).

Results

BRCA1 western blot analysis results

BRCA1 western blot analysis was performed in order to compare the expression levels of the protein among groups (Fig.1A). The protein expression of *BRCA1* gene of the LPS group was significantly lower than the control group ($P<0.05$). The BRCA1 protein expression level was not significantly different in the βG group in comparison with the control group, while the βG+LPS group showed an over 2-fold increase in comparison with the control group ($P<0.05$). This increase in the BRCA1 protein expression level, particularly takes place in the Kupffer cells. Our immunofluorescence findings also confirmed our Western blot results (Fig.1B). As it is demonstrated in Figure 1, βG pre-administration resulted in an overexpression of *BRCA1* gene. βG-stimulated significant increase in the level of BRCA1 protein took place only in LPS-induced stress conditions.

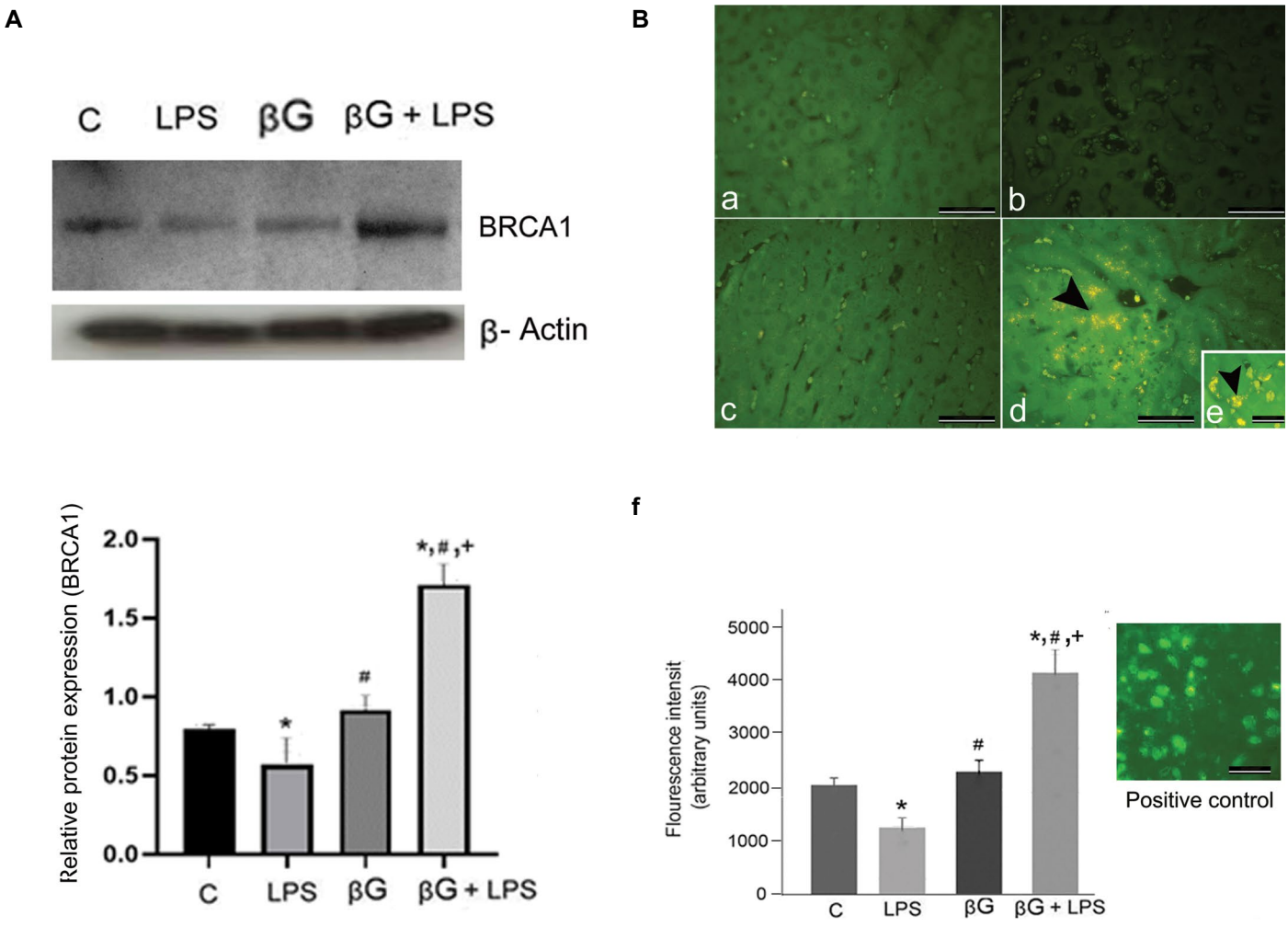

Fig.1: BRCA1 protein expression and immunofluorescence results**. A.** Western blot analysis results of BRCA1. **B.** Representative images of immunohistochemical staining of BRCA1 protein. a; Control (0.9% saline), b; LPS (4 mg/kg single dose i.p.), c; βG (150 mg/kg/d for 3 days i.p.), d; βG+LPS (150 mg/kg/d for 3 days βG i.p. and 4 mg/kg single dose LPS i.p), e; Increased fluorescence signal in the Kupffer cells, and f. Fluorescence intensity graph of the Image J analysis (scale bar: 100 µm). C; Control, LPS; Lipopolysaccharide, βG; Beta glucan, Black arrow; Increased fluorescence signal of BRCA1 protein, *; Significant differences when compared to control, #; Significant differences when compared to the LPS group, +; Significant differences when compared to the βG group.

BRCA1, PCNA and Nrf2 immunofluorescence

Using immunofluorescence assay, we confirm our western blot results for BRCA1 protein expression. On the other hand, PCNA is a multifunctional essential protein for DNA replication and repair that is also in association with the BRCA1 protein at various levels. Therefore, PCNA immunofluorescence levels were analyzed to determine the levels of liver cell proliferation. The Nrf2 immunofluorescence was also examined due to its role in mediating the expression levels of endogenous antioxidants in association to BRCA1. A base level of signal was observed in the control group that confirmed our western blot outcomes (Fig.1Ba). The fluorescence signal in the LPS group was significantly lower than the control group ($P<0.05$). In the βG group, the signal was equal with the control group (Fig.1Bb, c, f). However, the fluorescence signal intensity of the βG+LPS group was over 2-fold higher than the control group ($P<0.05$), and the fluorescence intensity was observed to be higher in the Kupffer cells (Fig.1Bd). Figure 2A shows the PCNA immunofluorescence assay results as a marker of a hepatocellular proliferation. There were not any observable nuclear signals in the control group (Fig.2Aa). A fluorescence signal was observed in the tissue sections of the LPS group, while indicating approximately 4 folds higher active proliferation rate of hepatocytes, in comparison with the control group ($P<0.05$, Fig.2Ab, e). However, there was not a significant signal in either βG group or βG+LPS group (Fig.2Ac, d). The basal level of cytoplasmic expression of Nrf2 protein in hepatocytes was observed in the control group (Fig.2Ba). In the LPS group of animals, low level but significant induction of Nrf2 protein expression was observed ($P<0.05$, Fig.2Bb). The βG+LPS group showed a significant increase in the Nrf2 protein expression level (over 2-fold) ($P<0.05$), while there was not a significant difference between control and βG groups (Fig.2Bc, d). Taken together, the results of the immunofluorescence findings revealed that the βG stimulated the overexpression of BRCA1 protein, normalized the protein level of PCNA and significantly induced the protein level of Nrf2 in the βG+LPS group of animals.

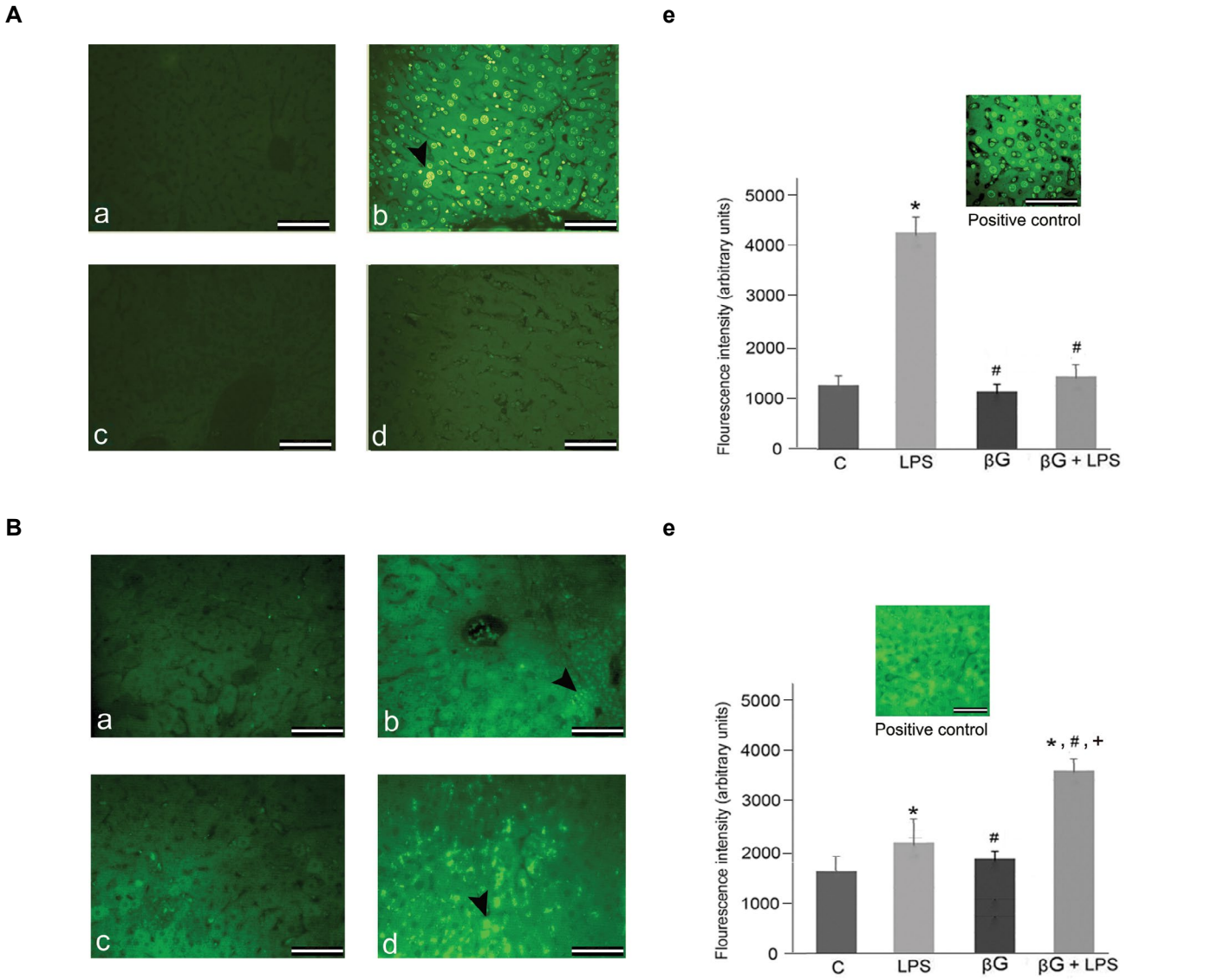

Fig.2: Results of PCNA and Nrf2 immunofluorescence analysis. **A.** Representative images of immunofluorescence staining of PCNA. a; Control (0.9% saline), b; LPS (4 mg/kg single dose i.p.), c; βG (150 mg/kg/d for 3 days i.p.), d; βG+LPS (150 mg/kg/d for 3 days i.p. βG and 4 mg/kg single dose i.p. LPS), and e; Fluorescence intensity graph of Image J analysis**. B.** Representative images of immunohistochemical staining of Nrf2 analysis. a; Control (0.9% saline), b; LPS (4 mg/kg single dose i.p.), c; βG (150 mg/kg/d for 3 days i.p.), d; βG+LPS (150 mg/kg/d for 3 days i.p., βG and 4 mg/kg single dose i.p. LPS), and e; Fluorescence intensity graph of Image J analysis (scale bar: 100 µm). C; Control, LPS; Lipopolysaccharide, βG; Beta glucan, Black arrow; Increased fluorescence signal of PCNA and Nrf2 proteins, *; Significant differences when compared to control; #; significant differences when compared to the LPS group, and +; Significant differences when compared to the βG group.

DNA damage results

Single (SSB) and double (DSB) strand breaks and alkali labile sites of the DNA damage were analyzed by the alkaline comet assay. The 8-oxoG lesion was also analyzed in order to determine the oxidative stress-induced lesions in DNA (Fig.3). Typical control nuclei were observed in Figure 3Aa characterized by a regular round structure. Based on tail moment calculations, LPS pre-administration resulted in a significant DNA damage which is to about three-fold in comparison with the control group ($P<0.05$, Fig.3Ab, e). In the βG group, there was no significant difference between tail moments in comparison with the control group (Fig.3Ac, e). The βG pre-administration significantly regulated DNA damage with a reduction of ~ 63 % in comparison with the LPS group of animals ($P<0.05$). Furthermore, there was no significant difference in comparison with the control group (Fig.3Ad, e). Figure 3B represents the results of 8-oxoG immunofluorescence. Our control group showed a pale background immunofluorescence (Fig.3Ba). In the LPS group of animals, the overall fluorescence intensity was significantly increased. Also, 8-oxoG DNA lesions in the LPS group were about two times higher than the control group ($P<0.05$). Lesions were particularly observed in Kupffer cells and hepatocytes upon being localized at the damaged sites of the liver tissue (Fig.3Bb, e). The βG group did not show significant differences in the fluorescence intensity rate in comparison with the control group (Fig.3Bc, e). We observed a decrease of ~34 % in fluorescence intensity in the βG+LPS group in comparison with the LPS group ($P<0.05$). The difference in fluorescence

intensity between βG+LPS group and control group was not significant, very rare signals were mostly observed in the Kupffer cells (Fig.3Bd, e). As a result, a βG pre-administration was evidently efficient in protecting against DNA damage in both analyses.

Biochemical findings

SOD and CAT levels were evaluated to demonstrate the capacity of endogenous antioxidants. The LP was evaluated in order to show the OS levels (Fig.4). The LPS administration resulted in a significant decrease in the activities of SOD and CAT, 52% and ~57%, respectively, in comparison with the control group (P<0.05). The levels of SOD and CAT, (~13 and ~31% respectively) were observed significantly higher in the βG group in comparison with the control group (P<0.05) (Fig.4A, B). In the LPS group, the LP level was significantly increased (~60 %) in comparison with the control (P<0.05, Fig.4C). In the βG+LPS group, a decrease was observed in the LP rate, that it reached to the control group level (Fig.4C).

Histological findings

Light microscopic observations were performed to demonstrate the histopathological differences among groups (Fig.5). The control group showed a normal histological architecture of the liver (Fig.5A). Steatosis was observed in the LPS group sections, accompanied by apoptotic and necrotic changes (Fig.5B). In the βG group, there were no observable changes in the pattern of hepatocytes (Fig.5C). In βG+LPS group, mild microvesicular fatty change and rare apoptotic events were observed (Fig.5D). Generally, normal histological architecture of the liver was preserved in the βG pre-administered group.

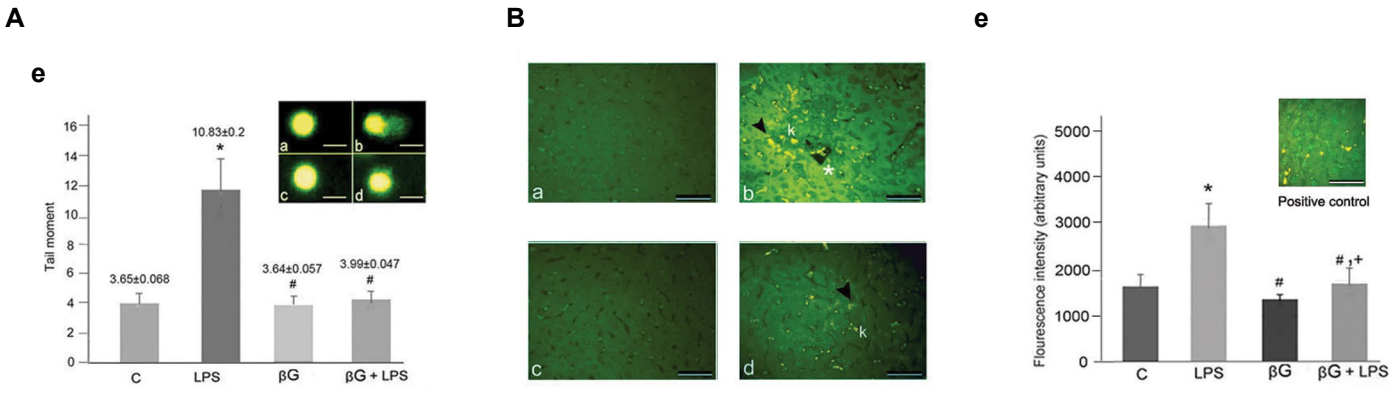

Fig.3. Comet assay analysis and 8-oxoG labelling results**. A.** Representative images of the comet assay results and graphical illustration of tail moment comparisons. a; Control (0.9% saline i.p.), b; LPS (single dose of 4 mg/kg i.p.), c; βG (150 mg/kg/day i.p.), d; βG+LPS (150 mg/kg/day i.p. for 3 days; 24 hours later, a single 4 mg/kg dose of LPS i.p.), and e; Tail moment graph. **B.** Representative images of 8-oxoG labelling. a; Control (0.9% saline i.p.), b; LPS (single dose of 4 mg/kg i.p.), c; βG (150 mg/kg/day i.p.), d; βG+LPS (150 mg/kg/day i.p. for 3 days; 24 hours later, a single 4 mg/kg dose of LPS i.p.), and e; Fluorescence intensity graph of Image J analysis. C; Control, LPS; Lipopolysaccharide, βG; Beta glucan, Black arrow; 8-oxoG lesion Immunofluorescence, White star; Damaged sites, k, Kupffer cells, *; Significant differences when compared to control, #; Significant differences when compared to the LPS group, and +; Significant differences when compared to the βG group.

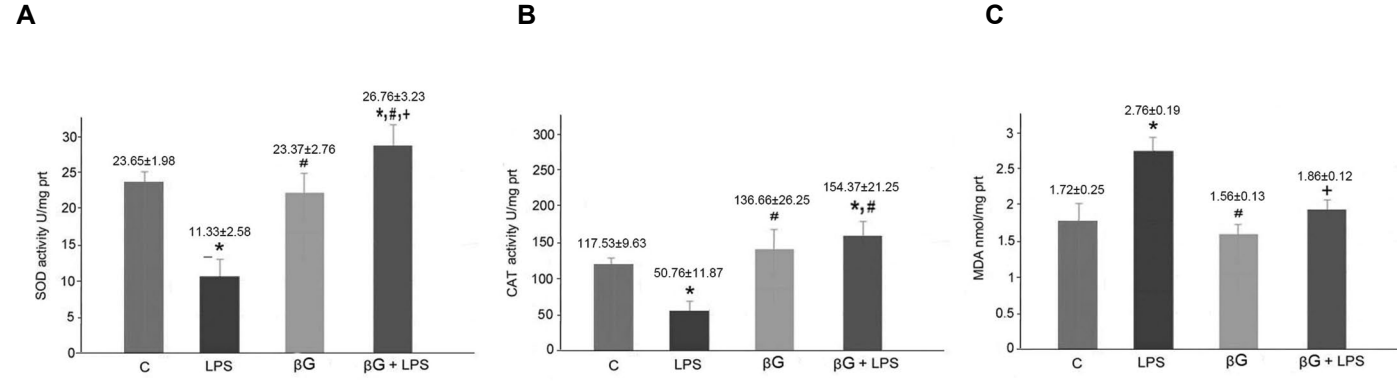

Fig.4: Results of the biochemical measurements. **A.** SOD activity (U/mg/prt). **B.** CAT activity (U/mg prt). **C.** MDA levels (nmol/mg/prt). SOD; Superoxide dismutase, CAT; Catalase, MDA; Malondialdehyde, C; Control, LPS; Lipopolysaccharide, βG; Beta glucan, *; Significant differences when compared with the control group (P<0.05), #; Significant differences when compared with LPS group (P<0.05), and +; Significant differences when compared with βG group.

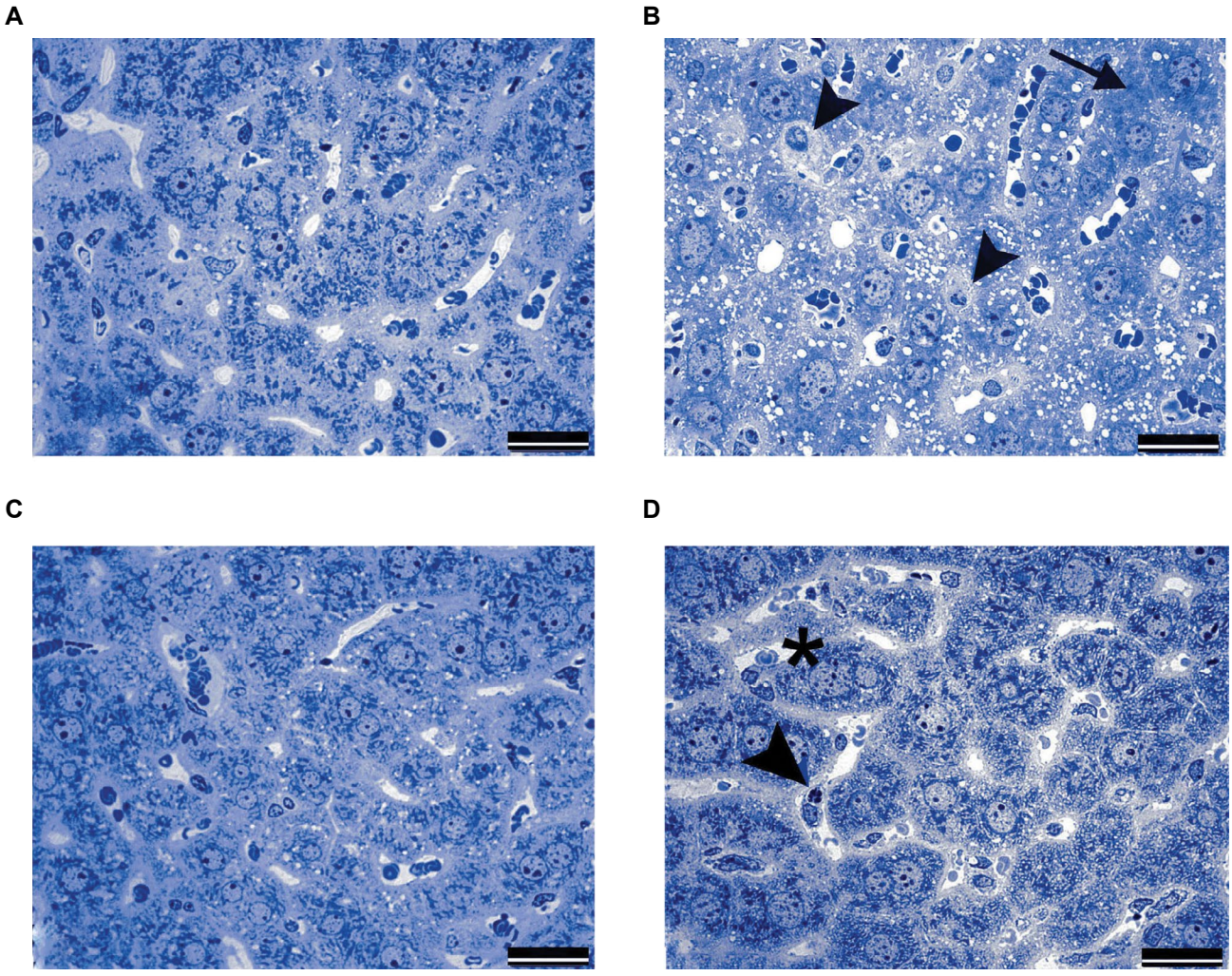

Fig.5: Representative images of light microscopic observations of the liver tissue. **A.** Control (0.9% saline i.p.): normal histological architecture of the liver tissue. **B.** LPS (single dose of 4 mg/kg i.p.): steatosis with discernible apoptotic (►) and necrotic (→) appearance in individual hepatocytes. **C.** βG (150 mg/kg/day i.p.): liver tissue with normal histological appearance. **D.** βG+LPS (150 mg/kg/day i.p. for 3 days: 24 hours later, a single 4 mg/kg dose of LPS i.p.): liver tissue with no observable pathologies with the exception of mild swelling of hepatocytes (*) and rare apoptotic events (scale bar: 20 µm).

Discussion

The LPS-induced damage to DNA is an early event in severe bacterial infections. The administration of compounds with a natural origin, applies to managing damages and subsequent complications, such as cancer formation (28, 29). As a gatekeeper of genome stability, *BRCA1* is considered to be a critical target of regulation. Although a variety of bioactive food compounds, such as βGs, have been reported as a gene modulator in cancer cells (30, 31), there is a lack of evidence on the βGs molecular effect on the *BRCA1* gene, in normal and stress-induced cells. According to the present study, it seems that the BRCA1 protein expression is significantly down-regulated with a mechanism that reversely is controlled by the *lncRNA MALAT1* in the LPS group of animals. The mechanism of this down-regulation was formerly reported by Yong et al. in their study (32).

However, a dramatic increase in the expression of BRCA1 protein was observed in the βG+LPS group (over 2-fold when compared with the control). The fluorescence intensity was observed to be higher in the Kupffer cells in this group. The LPS is a potent stimulator of the Kupffer cell tumor necrosis factor alpha (TNF-α) production and the following increase in the inflammation and oxidative stress. Therefore, Kupffer cells become more susceptible to damage. Bennett et al. (33) provided evidence that Kupffer cells give the first hand response to an LPS intoxication which is consistent with our findings. In the βG group of animals, BRCA1 protein expression level which was not statistically different from the control, indicated an induction of the gene by βG only in stress conditions. BRCA1 is a stress response protein which mediates both cell-cycle arrest and apoptosis, beside regulating DNA damage repair following different types of injury in a cell (34).

LPS-induced damage to the DNA was observed to be effectively decreased by the pre-administration of βG as long as the non-significant difference in the DNA

damage between the βG+LPS group and the control group are considered. Associated with its potential to trigger significant damage to DNA, LPS can promote hepatocellular carcinoma by activating TLR-4 mediated proliferative and anti-apoptotic signals (35). In parallel with the downregulation of BRCA1 protein expression, we observed an abnormal proliferation rate of the hepatocytes that was exerted with the significant increase in the protein levels of PCNA in the LPS group of animals. In the βG+LPS group, the βG treatment mediated BRCA1 protein expression increase was accompanied by the suppression of abnormal hepatocyte proliferation. Besides being a useful marker of cells with proliferative potential, PCNA protein is an essential tool for the DNA replication pathways. It is involved in the DNA excision repair pathways as well as mismatch repair pathways, when it is ubiquitinated with the mechanisms promoted by *BRCA1* (36). BRCA1 protein functions in a number of cellular pathways in the nucleus and cytoplasm in association with PCNA at various levels. By the control of DNA damage-induced cell cycle checkpoint activation and repair of the damaged DNA, these pathways maintain stability of the genome. The products of these genes also function in the ubiquitination of the proteins, chromatin remodeling, transcriptional regulation, apoptosis and cell survival in relation to each other. The *BRCA1* gene mediated PCNA ubiquitination results in the replication blockade, and also increases the translesion polymerases recruitment and DNA damage repair (37). The present study findings manifest that the βG molecule acts its role by its ability to stimulate BRCA1 protein expression. The ubiquitination of G2/M cell cycle proteins, such as PCNA, and transcriptional regulation of the genes in the DNA repair pathways of the BRCA1 protein are suggested to be the primary routes of the βG's molecular protection against LPS-induced genotoxicity in the liver cells.

On the other hand, we observed an LPS-driven decrease in the antioxidant capacity that was balanced with the βG administration. The endogenous antioxidant system was fairly insufficient to deal with the increased OS in the LPS group of animals, as shown by the increase in the LP level and a significant decrease in the levels of antioxidant enzymes SOD and CAT. However, in the βG+LPS group SOD and CAT enzyme levels were higher than the LPS group and also slightly but significantly higher than the control as a sign of a regulation in the antioxidative defense system. The LP, which was observed in control levels in the βG+LPS group of animals is concrete evidence that the βG pre-administration treatment efficiently regulated these enzymes to overcome the increasing radical formation. The BRCA1 induction in this group is also suggested to be involved in the mechanisms of βG molecules to overcome LPS-induced oxidative stress. Previous studies revealed that BRCA1 protein plays an active role in the regulation of OS to prevent carcinogenesis in normal cells by interacting with the transcription factor nuclear factor erythroid 2-like 2 (Nrf2). Gorrini et al. (38) reported that the BRCA1 protein binds to the Nrf2 protein to control the Keap1-mediated ubiquitination activity, while this binding results in the increased stability and activation of Nrf2 protein. As a master regulator of anti-oxidative responses, an increase in the Nrf2 protein expression, enhances the expression of the endogenous antioxidants (39).

We observed a low significant increase in the Nrf2 protein expression in the LPS group. However, the stability and activity of the Nrf-2 protein seem to be declining as a result of decreased BRCA1 protein level in this group. The decrease in the levels of SOD and CAT supported this observation, indicating the impairment of Nrf2-driven antioxidant signaling in LPS-exposed animals. On the other hand, the βG pre-administration resulted in a significant increase in BRCA1/Nrf2 protein expressions and SOD/CAT enzyme levels resulting in the reduced levels of OS and DNA damage. Based on the supporting histological evidence, the βG pre-administration treatment prevented an intense cytoplasmic vacuolation, inflammatory infiltrates, hyperplasia, and necrosis of the individual hepatocytes which are the common signs of the LPS intoxication in the liver. Apart from having very few vacuoles and rare apoptotic events for the elimination of the damaged cells, livers from the βG+LPS group of animals were almost always normal in the term of their tissue architecture. Herein, in the βG pre-administered group, βG-induced BRCA1 protein expression increased the antioxidant capacity of the liver. It seems that BRCA1 interacting with the Nrf2, promotes the stability and activation of the Nrf2 protein. Although, the βG molecule, itself could serve as a molecular scavenger of free radicals and support the endogenous antioxidant capacity of the liver (40), the replenishment of the antioxidant enzymes by the mechanisms shaped on the βG-driven induction of BRCA1 protein interaction of the molecule is manifested to be the most efficient route of OS reduction in LPS-exposed animals. The present study findings strongly suggest that the BRCA1 protein expression stimulation by the βG molecule is the underlying factor in the protection against an LPS-induced DNA damage to the liver. However, mechanisms of this protection are needed to be further investigated, since many other related molecular pathways could contribute to this protection in different ways.

Conclusion

The present study demonstrated that prophylactic βG protects against the LPS-induced genotoxicity and pathological damage of the liver tissue, through the induction of BRCA1 protein expression. The βG molecule alone do not change the expression of BRCA1 protein while, the protein level solely arises during the LPS-induced hepatic stress conditions. In the LPS-exposed liver with an increase in DNA damage and hepatocytes with abnormal proliferation, a βG-stimulated BRCA1 protein as a key factor of the protection, mediates the ubiquitination of PCNA which results in the arrest of the cell-cycle at the S-phase. The BRCA1-promoted pathway dependent repair of the damaged DNA efficiently reduces LPS-induced DNA strand breaks and maintains

the genomic stability of the liver. The βG molecule also regulates and supports the antioxidant capacity of the liver via BRCA1 interaction with Nrf2, that results in the activation and stabilization of the transcription factor. Activation of Nrf2-driven antioxidative pathway supports the endogenous antioxidative capacity of the liver and prevents the LPS-induced oxidative stress, associated DNA lesions, membrane lipid damages and also pathological damage to the liver. These results support the potential use of the βG as an early management tool to prevent a genotoxic damage and subsequent carcinogenic nature in the endotoxemia liver tissue. The present study provides knowledge for future studies subjected to the development of the βG-based drugs that aims *BRCA1* gene targeted therapy. Further studies on other possible molecular targets and pathways could be useful for the development of different therapeutic strategies.

Acknowledgements

This study was supported by the Eskisehir Technical University Scientific Research Projects Comission under the grant no:1705F229. The authors gratefully acknowledge to Şeyda Uçarcan for her laboratory technical assistance. The authors declare that they have no conflicts of interest in this study.

Authors' Contributions

G.A.K.; Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – Original Draft. M.A.; Writing- Reviewing and Editing, Visulalization, and Investigation. All authors have read and approved the manuscript.

References

1. Yan J, Li S, Li S. The role of the liver in sepsis. Int Rev Immunol. 2014; 33(6): 498-510.
2. Guerville M, Boudry G. Gastrointestinal and hepatic mechanisms limiting entry and dissemination of lipopolysaccharide into the systemic circulation. Am J Physiol Gastrointest Liver Physiol. 2016; 311(1): G1-G15.
3. Xu T, Liu R, Zhu H, Zhou Y, Pei T, Yang Z. The inhibition of LPS-induced oxidative stress and inflammatory responses is associated with the protective effect of (-)-Epigallocatechin-3-Gallate on bovine hepatocytes and murine liver. Antioxidants (Basel). 2022; 11(5): 914.
4. van Elsland D, Neefjes J. Bacterial infections and cancer. EMBO Rep. 2018; 19(11): e46632.
5. Liu Z, Mahale P, Engels EA. Sepsis and risk of cancer among elderly adults in the United States. Clin Infect Dis. 2019; 68(5): 717-724.
6. Samivel R, Subramanian U, Ali Khan A, Kirat O, Masmali A, Almubrad T, et al. Lipopolysaccharide enhances genotoxicity by activating GADD45G and NF-κB in human corneal epithelial cells. Oxid Med Cell Longev. 2022; 2022: 4328116.
7. Hamzawy MA, El-Denshary ESM, Abdel-Wahhab MA. Effects of natural compounds in treatment and prevention of hepatotoxicity and hepatocellular carcinoma. Hepatoma Res. 2015; 1: 111-118.
8. Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, et al. The role of oxidative stress and antioxidants in liver diseases. Int J Mol Sci. 2015; 16(11): 26087-26124.
9. Kofuji K, Aoki A, Tsubaki K, Konishi M, Isobe T, Murata Y. Antioxidant activity of β-glucan. ISRN Pharm. 2012; 2012: 125864.
10. da Silva AF, Sartori D, Macedo FC Jr, Ribeiro LR, Fungaro MH, Mantovani MS. Effects of β-glucan extracted from Agaricus blazei on the expression of ERCC5, CASP9, and CYP1A1 genes and metabolic profile in HepG2 cells. Hum Exp Toxicol. 2013; 32(6): 647-654.
11. Madrigal-Bujaidar E, Morales-González JA, Sánchez-Gutiérrez M, Izquierdo-Vega JA, Reyes-Arellano A, Álvarez-González I, et al. Prevention of aflatoxin B□-induced DNA breaks by β-D-glucan. Toxins (Basel). 2015; 7(6): 2145-2158.
12. Oliveira RJ, Salles MJ, da Silva AF, Kanno TY, Lourenço AC, Leite Vda S, et al. In vivo evaluation of the antimutagenic and antigenotoxic effects of β-glucan extracted from Saccharomyces cerevisiae in acute treatment with multiple doses. Genet Mol Biol. 2013; 36(3): 413-424.
13. Queiroz EA, Fortes ZB, da Cunha MA, Barbosa AM, Khaper N, Dekker RF. Antiproliferative and pro-apoptotic effects of three fungal exocellular β-glucans in MCF-7 breast cancer cells is mediated by oxidative stress, AMP-activated protein kinase (AMPK) and the Forkhead transcription factor, FOXO3a. Int J Biochem Cell Biol. 2015; 67: 14-24.
14. Binmama S, Dang CP, Visitchanakun P, Hiengrach P, Somboonna N, Cheibchalard T, et al. Beta-glucan from S. cerevisiae protected AOM-induced colon cancer in cGAS-deficient mice partly through dectin-1-manipulated macrophage cell energy. Int J Mol Sci. 2022; 23(18): 10951.
15. Lagunas-Rangel FA, Bermúdez-Cruz RM. Natural compounds that target DNA repair pathways and their therapeutic potential to counteract cancer cells. Front Oncol. 2020; 10: 598174.
16. Kang HJ, Hong YB, Kim HJ, Wang A, Bae I. Bioactive food components prevent carcinogenic stress via Nrf2 activation in BRCA1 deficient breast epithelial cells. Toxicol Lett. 2012; 209(2): 154-160.
17. Dalmasso B, Puccini A, Catalano F, Borea R, Iaia ML, Bruno W et al. Beyond BRCA: the emerging significance of DNA damage response and personalized treatment in pancreatic and prostate cancer patients. Int J Mol Sci. 2022; 23(9): 4709.
18. Oliveira RJ, Salles MJ, da Silva AF, Kanno TY, Lourenço AC, Freiria GA, et al. Effects of the polysaccharide beta-glucan on clastogenicity and teratogenicity caused by acute exposure to cyclophosphamide in mice. Regul Toxicol Pharmacol. 2009; 53(3): 164-173.
19. Hamesch K, Borkham-Kamphorst E, Strnad P, Weiskirchen R. Lipopolysaccharide-induced inflammatory liver injury in mice. Lab Anim. 2015; 49(1): 37-46.
20. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976; 7 (72):248-54.
21. Tu H, Zhang ZW, Qiu L, Lin Y, Jiang M, Chia SY et al. Increased expression of pathological markers in Parkinson's disease dementia post-mortem brains compared to dementia with Lewy bodies. BMC Neurosci. 2022; 23(1): 3.
22. El Shawi OE, Abd El-Rahman SS, El Hameed MA. In: Bancroft JD, Gamble M, editors. Theory and practice of histological techniques. 6th Ed. China: Churchill Livingstone, Elsevier; 2008.
23. Kemeleva EA, Sinitsyna OI, Kolosova NG, Vasyunina EA, Zharkov DO, Conlon KA, et al. Immunofluorescent detection of 8-oxoguanine DNA lesions in liver cells from aging OXYS rats, a strain prone to overproduction of free radicals. Mutat Res. 2006; 599(1-2): 88-97.
24. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res. 1988; 175(1): 184-191.
25. Nebot C, Moutet M, Huet P, Xu JZ, Yadan JC, Chaudiere J. Spectrophotometric assay of superoxide dismutase activity based on the activated autoxidation of a tetracyclic catechol. Anal Biochem. 1993; 214(2): 442-451.
26. Beers RF Jr, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem. 1952; 195(1): 133-140.
27. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95(2): 351-358.
28. Aisa-Alvarez A, Soto ME, Guarner-Lans V, Camarena-Alejo G, Franco-Granillo J, Martínez-Rodríguez EA, et al. Usefulness of antioxidants as adjuvant therapy for septic shock: a randomized clinical trial. Medicina (Kaunas). 2020; 56(11): 619.
29. Gugliandolo E, Cordaro M, Siracusa R, D'Amico R, Peritore AF, Genovese T, et al. Novel combination of COX-2 inhibitor and antioxidant therapy for modulating oxidative stress associated with intestinal ischemic reperfusion injury and endotoxemia. Antioxidants (Basel). 2020; 9(10): 930.
30. Khan H, Labanca F, Ullah H, Hussain Y, Tzvetkov NT, Akkol EK et al. Advances and challenges in cancer treatment and nutraceutical prevention: the possible role of dietary phenols in BRCA regulation.

Phytochem Rev. 2022; 21: 385–400.
31. Huff MO, Klinge CM, Regulation of gene expression by β-Glucans. Am J Immunol. 2017; 13(1): 1-10.
32. Yong H, Wu G, Chen J, Liu X, Bai Y, Tang N, et al. lncRNA MALAT1 accelerates skeletal muscle cell apoptosis and inflammatory response in sepsis by decreasing BRCA1 expression by recruiting EZH2. Mol Ther Nucleic Acids. 2020; 19: 97-108.
33. Bennett H, Troutman TD, Sakai M, Glass CK. Epigenetic regulation of kupffer cell function in health and disease. Front Immunol. 2021; 11: 609618.
34. Gilmore PM, Quinn JE, Mullan PB, Andrews HN, McCabe N, Carty M, Kennedy RD, Harkin DP. Role played by BRCA1 in regulating the cellular response to stress. Biochem Soc Trans. 2003; 31(Pt 1): 257-262.
35. Singh A, Koduru B, Carlisle C, Akhter H, Liu RM, Schroder K, et al. NADPH oxidase 4 modulates hepatic responses to lipopolysaccharide mediated by Toll-like receptor-4. Sci Rep. 2017; 7(1): 14346.
36. Thakar T, Dhoonmoon A, Straka J, Schleicher EM, Nicolae CM, Moldovan GL. Lagging strand gap suppression connects BRCA-mediated fork protection to nucleosome assembly through PCNA-dependent CAF-1 recycling. Nat Commun. 2022; 13(1): 5323.
37. Tarsounas M, Sung P. The antitumorigenic roles of BRCA1–BARD1 in DNA repair and replication. Nat Rev Mol Cell Biol. 2020; 21(5): 284-299.
38. Gorrini C, Baniasadi PS, Harris IS, Silvester J, Inoue S, Snow B et al. BRCA1 interacts with Nrf2 to regulate antioxidant signaling and cell survival. J Exp Med. 2013; 210(8): 1529-1544.
39. He F, Ru X, Wen T. NRF2, a transcription factor for stress response and beyond. Int J Mol Sci. 2020; 21(13): 4777.
40. Giese EC, Gascon J, Anzelmo G, Barbosa AM, da Cunha MA, Dekker RF. Free-radical scavenging properties and antioxidant activities of botryosphaeran and some other β-D-glucans. Int J Biol Macromol. 2015; 72: 125-130.