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Lactobacillus rhamnosus GG alleviates bisphenol-A induced oxidative stress in serum

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ABSTRACT: The objective of this investigation was to identify changes in the serum oxidant-antioxidant balance of rats exposed to bisphenol A (BPA) and to investigate the impact of *Lactobacillus rhamnosus* GG (LGG) administration on those changes. Twenty-four rats (Wistar Albino, 250-300 grams, male) were divided into control, BPA, and BPA+LGG groups with an equal number of rats. BPA and LGG were applied to the rats in the relevant groups for six weeks, five days each week. Six weeks later, the blood samples were withdrawn and serum samples were prepared. Total oxidant and antioxidant status (TAS), glutathione, and lipid peroxidation determinations were determined in serum samples, and the oxidative stress index was calculated. BPA exposure decreased serum total antioxidant status and increased serum total oxidative status, oxidative stress index, and lipid peroxidation level compared to the control group. LGG administration improved the increased serum oxidative stress caused by BPA. Administration of LGG to BPA-treated rats reversed oxidative stress-induced changes. In conclusion, administration of *Lactobacillus rhamnosus* GG to rats for 30 consecutive days prevented oxidative stress in serum caused by bisphenol A.

KEYWORDS: Bisphenol A; *Lactobacillus rhamnosus GG*; probiotic; oxidative stress; blood.

1. INTRODUCTION

Bisphenol A (BPA) is a chemical molecule that has been shown to have adverse effects on human health yet is nevertheless permitted to be used in the manufacturing of numerous items. It can disrupt the endocrine system by structurally mimicking various hormones in living organisms, especially estrogen, one of the female sex hormones [1]. It is frequently observed in pet and plastic materials, food and beverage packaging, heated and processed thermal papers in cans and jar lids, and dental materials [2]. BPA has been found to reduce sperm production and quality in men and cause breast cancer and menstrual irregularities in women [3]. In addition, it is possible that BPA, exposed to high doses, causes tissue and organ damage in both genders and may increase oxidative stress in tissues and organs of metabolism, especially the liver, heart, and kidney [4]. Food consumption, inhalation, skin and eye contamination, or maternofetal transmission are the most common routes of exposure to Bisphenol A [5]. BPA toxicity has been linked to oxidative stress and associated markers in various experimental animal models and humans. [6]. BPA toxicity significantly promotes the production of oxidants and decreases antioxidant defense capacity, altering the mitochondrial and intracellular oxidative balance [7]. Studies on animals have demonstrated that BPA can cause cellular damage to the liver through oxidative stress and trigger oxidative stress in organs such as the kidney, ovary, and testis [8-10]. Many follow-up human studies have also reported associations between oxidative stress and long-term BPA exposure. Exposure to BPA throughout pregnancy has been associated with adverse postnatal outcomes or an increase in oxidative stress parameters that may accompany fetal development [11]. Studies continue on substances that may have protective and preventive properties against damage caused by BPA. Accordingly, in this study, the effect of *Lactobacillus rhamnosus* (LGG), a probiotic, on BPA-induced oxidative stress was examined in serum samples. Probiotics are groups

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of live microorganisms that contain various bacterial species that are beneficial to the living microbiota and metabolism [12]. Although there are many different types of probiotics, probiotics must have certain properties for the body to get maximum benefit from probiotics taken with food or supplements. Probiotic species resistant to stomach acid are among the most ideal probiotics. *Lactobacillus rhamnosus* GG is a type of probiotic produced to obtain ideal probiotics [13]. In humans, they endogenously constitute an important component of the microbiota in many body regions, like the digestive, genital, and urinary systems [14, 15]. Many scientific studies have proven its positive effects on metabolism and other tissues, in addition to the intestinal microbiota. To protect the human body from oxidative damage, different types of antioxidant supplementation are recommended. Some studies have reported that prebiotic and probiotic bacterial supplementation can reduce free radicals and their formation due to the antioxidant production capacity of the organism, thus reducing oxidative stress [16]. It has been reported that LGG strains can produce many antioxidant mechanisms, including antioxidants like glutathione and superoxide dismutase enzyme, and can reduce the elevated total oxidant status [17]. In this study, the possible effects of LGG, which is stated to contribute to antioxidant activity, on the serum oxidant-antioxidant balance of rats exposed to BPA, were investigated.

2. RESULTS

2.1. Total Oxidant Status, Total Antioxidant Status, and Oxidative Stress Index

It was determined that serum total oxidant status and oxidative stress index significantly increased and the total antioxidant status of the BPA group decreased compared to the control group (Table 1). LGG application to the BPA group significantly decreased total oxidant levels and oxidative stress index compared to the BPA groups (Table 1). LGG administration in the BPA group also significantly increased the total antioxidant status in serum samples compared to the control group and the BPA group (Table 1).

Table 1. Serum Total Antioxidant Capacity, Total Antioxidant Capacity, and Oxidative Stress Index

	Control		BPA		BPA+LGG		P_{Anova}
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
TAS (µmol/L)	1.76	0.04	1.50 +	0.11	2.50 + •	0.23	0.0001
TOS (µmol/L)	5.95	0.32	11.25 +	0.45	5.28 •	0.65	0.0001
OSI	0.034	0.002	0.076 +	0.005	0.021+ •	0.003	0.0001

TOS: Total Oxidant Status, **TAS:** Total Antioxidant Status **OSI:** Oxidative Stress Index **BPA:** Bisphenol A, **LGG:** *Lactobacillus rhamnosus* GG
+: p<0.001 compared to the control group
•: p<0.001 compared to the BPA group

2.2. Glutathione and Malondialdehyde Level

A significant increase was determined in the malondialdehyde (MDA) level of the BPA group compared to the control group (Fig. 1). Meanwhile, there were no significant differences when the same group was compared with the control group in terms of glutathione levels (Figure 2). The MDA levels of the administered BPA group decreased significantly compared to the BPA group (Figure 1). It was observed that the glutathione levels in the LGG-administered BPA group significantly increased compared to both the control group and the BPA group (Figure 2).

3. DISCUSSION

Bisphenol A, an endocrine disrupter, has been shown to increase total oxidant status levels and oxidative stress index and decrease total antioxidant status levels [18, 19]. Similarly, in this study, it was observed that total oxidant status and the oxidative stress index increased and serum total antioxidant status decreased significantly as a result of bisphenol A exposure compared to the control group.

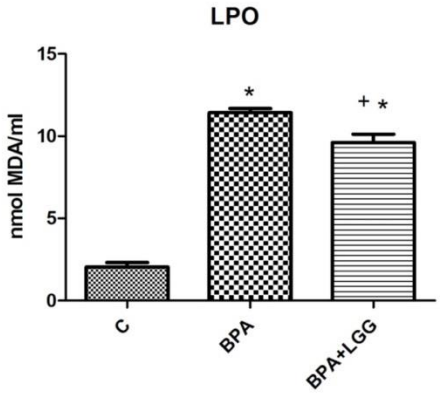

Figure 1. Serum Malondialdehyde Levels. BPA: Bisphenol A, LGG: *Lactobacillus rhamnosus* GG, * p<0.05 compared to the control group, +: p<0.05 compared to the BPA group

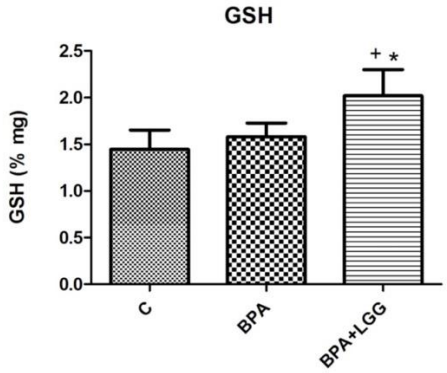

Figure 2. Serum Glutathione Levels, BPA: Bisphenol A, LGG: *Lactobacillus rhamnosus* GG * p<0.05 compared to the control group +: p<0.05 compared to the BPA group

Lactobacillus is regarded as a potential candidate in the food and pharmaceutical industries, demonstrating antioxidant properties [20]. Many antioxidant scavenging studies applying Lactobacillus have indicated a mechanism involving hydroxyl radicals and superoxide anions. Several in-vitro experiments have shown that Lactobacillus has significant antioxidant properties by neutralizing free reactive oxygen radicals [21]. Considering the results obtained in this study regarding the positive effects of *Lactobacillus rhamnosus* GG on the oxidant damage caused by bisphenol A administration. It was determined that the total oxidant capacity and oxidative stress index decreased compared to the bisphenol A administered group, and the total antioxidant capacity increased compared to the bisphenol A applied group. The influence of bisphenol A (BPA) exposure on blood glutathione levels has received a lot of interest because of the possible consequences for human health. Glutathione, a critical antioxidant, protects cells from oxidative damage and maintains redox homeostasis. Although most studies indicate that bisphenol A reduces glutathione levels in tissues, there are varying results. In a study, it was stated that BPA exposure (50 mg/kg for 30 days) caused a decrease in glutathione levels in liver tissue [22]. Gualteri et al. [23] observed a significant increase in sertoli cell line glutathione levels exposed to BPA. Nasonova et al. [24] stated that application of bisphenol A to PC-12 cells did not change the glutathione level. In this study, no significant change was found in serum glutathione levels following BPA exposure in rats, but antioxidant capacity decreased. This indicates that there may be changes in the direction of decrease in other enzymatic or non-enzymatic antioxidants. While no significant difference was observed in GSH levels between the control and BPA groups in this study, the application of LGG to the BPA group increased the GSH level. When the results of studies on lipid peroxidation levels after bisphenol A exposure were examined, it was found that Bisphenol A application increased lipid peroxidation levels in all tissues examined compared to the control group [25-27]. Similarly, in this study, serum lipid peroxidation levels of the Bisphenol A group were found to be significantly higher than the control group. LGG application after BPA exposure reduced lipid peroxidation. This result shows that LGG increases serum antioxidant capacity and thus reduces lipid peroxidation.

4. CONCLUSION

Administration of *Lactobacillus rhamnosus* GG to rats was found to reduce BPA-induced oxidative stress in serum significantly. However, since the mechanism of probiotics for the elimination of free radicals has not been fully elucidated, the findings of this study will contribute to this issue and can serve as an acceptable basis for further investigations on probiotics as a nutritional supplement.

5. MATERIALS AND METHODS

5.1. Experimental Animals

In this study, twenty-four Wistar Albino male rats, weighing an average of 250-300 grams, were used. Rats were kept under conventional conditions throughout the experimental period. Marmara University Animal Care and Use Ethics Committee approved the experimental stages of this study (Protocol Number: 56.2023 mar.).

5.2. Experimental Procedure

The rats were divided into control (n= 8), BPA (n= 8), and BPA + LGG (n= 8) groups. 50 mg/kg BPA [28] was administered intraperitoneally to the BPA and BPA+LGG groups. 1 x 10^9 CFU/day *Lactobacillus rhamnosus* GG [28] was administered to the rats of the BPA+LGG group. BPA and LGG were administered 5 days a week for 6 weeks.

5.3. Preparation of Serum Samples

Trunk blood samples taken after decapitation were used to prepare serum samples. The blood samples were first centrifuged at 100 x g for 10 minutes and then at 200 x g for 5 minutes, and serum samples were separated.

5.4. Biochemical Analysis

5.4.1. Total Antioxidant Status Determination

Serum Total Antioxidant Status was performed by using Rel Assay Diagnostic's kit (RL0017, Turkey). This technique allows for measuring the serum sample's antioxidative protection against strong free-radical reactions sparked by the generated hydroxyl radical. Trolox equivalents (mmol) per liter were used to express results.

5.4.2. Measurement of Total Oxidant Status

Total oxidant status (TOS) was performed using an imaging commercial kit (Rel Assay Diagnostics, RL0024, Turkey). The total amount of oxidant molecules in the serum sample is proportional to color intensity, which can be determined spectrophotometrically. The test is calibrated using hydrogen peroxide, and results are provided in μmol H_2O_2 eq/L.

5.4.3. Oxidative Stress Index

The ratio between total oxidant and total antioxidant status, given as a percentage, is the definition of the oxidative stress index (OSI). The following formula was used to calculate the OSI value;

*OSI= [(Total oxidant status (*m*mol H_2O_2 Eq/L)/ Total antioxidant status (*m*mol Trolox Eq/L)]x100*

5.4.4. Determination of Glutathione Level

In this method, the sulfhydryl groups of glutathione (GSH) react with the 5,5-dithio-bis-2-nitrobenzoic acid. The generated yellow product was evaluated spectrophotometrically at 420 nm. The findings were expressed as mg% GSH [29].

5.4.5. Determination of Lipid Peroxidation Level

The oxidant parameter used to measure the amount of lipid peroxidation was determined as malondialdehyde (MDA) levels. This technique uses thiobarbituric acid and MDA to form a pink molecule, which is then measured spectrophotometrically for absorbance. The equal amount of MDA is expressed by an extinction coefficient of 1.56x105 M^{-1} cm^{-1}, and the findings were given as nmol MDA/mL [30].

5.5. Statistical Analysis

Graphpad Prism 6.0 (Graphpad Software, San Diego, CA, USA) and SPSS 29 (IBM, USA) were used to evaluate the data. The standard deviation ± mean was used to express all data.

Analysis of variance (ANOVA) and the Tukey test were used to analyze biochemical data sets. Statistical significance was defined as $p<0.05$.

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