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Probiotic bacteria attenuates cisplatin-induced nephrotoxicity through modulation of oxidative stress, inflammation and apoptosis in rats

Emin Sengul[1]✉, Sevda Urçar Gelen[2], Serkan Yıldırım[3], Fikret Çelebi[1], Ali Çınar[1]

[1]*Department of Physiology, Faculty of Veterinary, Ataturk University, Erzurum, Turkey*

[2]*Department of Food Hygiene and Technology, Faculty of Veterinary, Ataturk University, Erzurum, Turkey*

[3]*Department of Pathology, Faculty of Veterinary, Ataturk University, Erzurum, Turkey*

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ABSTRACT

Objective: To investigate the effects of probiotic bacteria on cisplatin (CP)-induced nephrotoxicity. **Methods:** In the present study, 50 Sprague-Dawley rats were used and randomly divided into five groups including control, CP, probiotic bacteria treatment groups with different doses (0.5 and 1 mL) and only probiotic bacteria group. After CP and probiotic administration on seven days, rats sacrificed under anesthesia on the eighth day. The serum urea, creatinine, and blood urea nitrogen levels were analyzed. In renal tissue, malondialdehyde levels, superoxide dismutase and glutathione activity, interleukin-8, interleukin-1 β and tumor necrosis factor-alpha levels were determined and histopathological and immunohistochemical changes were also examined. **Results:** According to results, urea, creatinine and blood urea nitrogen levels as well as kidney weights increased in CP group. Also, CP induced inflammation, oxidative stress, DNA damage and apoptosis in kidney tissue and caused histopathological changes. Administration of the high dose of probiotic bacteria could prevent these changes and damages. **Conclusions:** This study reveals that probiotic bacteria has protective effects on CP-induced renal damage in rats.

1. Introduction

Cisplatin (CP) is one of the most effective anticancer agents, and its activities have been demonstrated in neoplasms, such as testicular, bladder, and other types of cancer[1-3]. The impairment of kidney functions after CP treatment is also known as a major side effect, and it is the primary dose-limiting condition[4]. The kidneys eliminate a wide range of endogenous and exogenous substances including drugs and are the major targets for the toxic effects of these substances. These substances accumulate in large amounts, especially in the proximal tubules. The CP concentration in the proximal tubules has been determined to be approximately 5 times higher than the CP concentration in the serum[5]. This excessive accumulation of CP causes potent toxicity in the proximal tubular cells[6]. CP-induced nephrotoxicity creates renal function alterations, such as increased blood urea nitrogen (BUN) levels and creatinine[7]. Increasing evidence has demonstrated that CP-induced kidney damage is associated with various mechanisms, such as mitochondrial dysfunction, increased generation of free radicals, apoptosis, and increased activity of nitric oxide synthase[8,9].

Although CP has side effects, it is the preferred anticancer agent

✉First and corresponding author: Emin Sengul, Department of Physiology, Faculty of Veterinary, Ataturk University, Erzurum, 25100, Turkey.
E-mail: emin.sengul@atauni.edu.tr

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in chemotherapy. Therefore, the development of preventive or therapeutic methods in CP-induced organ toxicities is extremely important. For this purpose, antioxidant and anti-inflammatory compounds are frequently used in chemotherapeutic agent-induced toxicity[10,11]. Probiotics have regulatory, stimulatory, and antioxidant effects on the immune system[12-14]. Probiotics also have a protective effect against oxidative stress and accumulation of ROS[15]. Furthermore, probiotics are used to reduce the organ toxicity of anticancer agents. It was determined that probiotic administration decreases doxorubicin-induced cardiomyopathy[16], and it has protective effects against the cyclophosphamide-induced immunosuppression and bone marrow supression in mice[17]. In light of these findings, this study aimed to evaluate the protective effect of probiotics on CP induced renal damage.

2. Materials and methods

2.1. Animals

In this study, 50 Sprague-Dawley rats were used, and the average weight of the rats was 220-250 g. Rats were supplied from the Animal Laboratory at the Experimental Research Centre of Ataturk University, Erzurum, Turkey. All the animals were housed in standard environmental conditions and were allowed access to a standard diet and drinking water *ad libitum*. This study was approved by the Local Ethics Committee of Ataturk University for Animal Experiments (Protocol no: 2018/189).

2.2. Probiotic preparation

For the isolation of *Lactobacilli* strains, the drop-plate method by de Man, Rogosa, and Sharpe (MRS, Merck, Germany) was used. An agar medium was planted, and plaques were incubated at 30 ℃ for 48 h as anaerobe. At the end of incubation, a catalase test was performed and catalase negative colonies were identified by API CH50[18]. At the end of identification, *Lactobacillus rhamnosus* (*L. rhamnosus*), *Lactobacillus fermentum* and *Lactobacillus brevis* were isolated. The bacteria were separated from the supernatant culture by centrifugation, washed with phosphate saline buffer, and resuspended in phosphate buffer saline. The final concentration of the mixture was adjusted to contain 10^5 lactic acid bacteria in 1 mL.

2.3. Experimental design

The rats were divided into 5 groups. The control group was orally administered a saline for 7 d. The CP group received intragastric injections of the saline solution for 4 d and intraperitoneal injections of CP (7.5 mg/kg) for the next 3 d. The Probiotic 1+CP and Probiotic 2+CP groups were orally administered 0.5 mL and 1 mL of probiotic, respectively, for 7 d. They were also injected with CP (7.5 mg/kg, *i.p.*) for the following 3 d by starting on the fifth day. The Probiotic 2 group was orally administered probiotic (1 mL) for 7 d. On the eighth day of the experiment, body weights of rats were weighed and the intracardiac blood samples were taken from the rats under sevoflurane anesthesia, and rats were then sacrificed. The weights of kidneys were weighed and blood and kidney tissues were collected for biochemical analysis, histopathological and immunohistochemical examination. The kidney/body weight ratio was evaluated among experimental groups.

2.4. Serum analysis

The blood was centrifuged at 2 500 ×*g* for 15 min, and the serum was separated. The serum samples were analyzed by using an auto analyzer to measure the urea, creatinine, and BUN parameters. These parameters were through standard procedures[19].

2.5. Biochemical assays

The kidney tissues were homogenized. The malondialdehyde (MDA) levels in the kidney homogenate were measured using the thiobarbituric acid reaction according to the method of Placer *et al*.[20]. The production of superoxide radicals was used to measure superoxide dismutase (SOD) activity[21]. Moreover, the glutathione (GSH) content of the kidneys was measured[22].

2.6. Determination of interleukin-8 (IL-8), interleukin-1 β (IL-1 β) and tumor necrosis factor-alpha (TNF- α) levels

Renal tissue IL-8, IL-1 β, and TNF- α levels were measured in a renal homogenate using an enzyme-linked immunosorbent assay kit (rat IL-8 ELISA kit, rat IL-1 β ELISA kit, rat TNF- α ELISA kit, Sunred Biological Tecnology) with regard to the manufacturer's protocol.

2.7. Histopathological and immunohistochemical examination

The kidney tissues were routinely processed and then buried in blocks of paraffin. Tissue sections cut to 4 μm thickness were taken from each block, placed on the slides, stained with hematoxylin-eosin, and examined under a Laboratory Microscope (Leica DM 1000, Germany) to perform an accurate assessment of the hematoxylin-eosin and fibrous tissue staining and adhesion. Bcl-2 and 8-OHdG stainings were conducted with respect to renal immunohistochemistry protocol of previous studies[23,24]. Tissue sections were evaluated using the following ratings: non-positive (-), mild (+), moderate (++), and severe (+++). Bcl-2 and 8-OHdG-positive cell intensity were evaluated by using the following: weak = (+), moderate = (++), and strong = (+++).

2.8. Statistical analysis

All data were analyzed with one-way ANOVA by using the SPSS 20 program. A *post-hoc* Duncan test was used to compare the values between the groups. Data were expressed as the mean±standard deviation (SD) and were considered statistically significant when $P < 0.05$.

3. Results

3.1. Effects of probiotic on kidney and body weight

The ratio of kidney weight to body weight of rats in the CP group was higher than that of the control and it was found that the ratio in the high-dose groups of probiotic was similar to that of the control

group (Table 1).

Table 1

Ratio of kidney weight to body weight in experimental groups (mean ± SD).

Experimental groups	Ratio (g/kg body weight)
Control	0.150 ± 0.004[a]
CP	0.210 ± 0.003[b]
Probiotic 1+CP	0.190 ± 0.002[b]
Probiotic 2+CP	0.160 ± 0.001[a]
Probiotic 2	0.170 ± 0.003[a]

Different letters indicate the statistically significant differences between groups ($P < 0.05$).

3.2. Effects of probiotic bacteria on kidney functions

Serum urea, creatinine, and BUN levels were significantly elevated ($P < 0.05$) in the CP group compared to the control (Table 2). The urea, creatinine, and BUN levels in the Probiotic 1+CP group were lower than those in the CP group, but the differences were not statistically significant. Treatment of high dose of probiotic significantly inhibited increases in these parameters ($P < 0.05$). Urea, creatinine, and BUN levels in Probiotic 2 group were similar to control (Table 2).

Table 2

Serum kidney parameters for all groups (mean ± SD).

Experimental groups	Urea (mg/dL)	Creatinine (mg/dL)	BUN (mg/dL)
Control	35.22 ± 6.52[a]	0.43 ± 0.06[a]	18.21 ± 1.97[a]
CP	52.26 ± 5.71[b]	0.65 ± 0.11[b]	31.24 ± 4.34[b]
Probiotic 1+CP	45.36 ± 6.56[ab]	0.57 ± 0.15[ab]	27.56 ± 3.15[b]
Probiotic 2+CP	38.78 ± 4.86[a]	0.45 ± 0.08[a]	21.16 ± 2.09[a]
Probiotic 2	33.71 ± 7.63[a]	0.42 ± 0.07[a]	20.21 ± 2.96[a]

Different letters indicate the statistically significant differences between groups ($P < 0.05$).

3.3. Effects of probiotic on kidney oxidative stress

Oxidative stress was assessed by measuring renal MDA levels, GSH levels, and SOD activities. MDA levels increased in the CP group compared to other groups (Figure 1A), and the administration of probiotic decreased the MDA level compared to the CP group. MDA levels were not different from control in Probiotic 2+CP and Probiotic 2 groups (Figure 1A). The SOD activities and GSH levels are shown in Figures 1B and 1C, which demonstrate significant decreases in the kidney tissues of the CP group compared to the control group. Treatment with probiotic bacteria (especially with a high dose) significantly prevented ($P < 0.05$) decreases of these enzymes. The SOD activities and GSH levels in Probiotic 2+CP and Probiotic 2 group were similar to control (Figures 1B and 1C).

3.4. Biochemical cytokine (IL-8, IL-1 β, and TNF- α) levels in kidney tissue

IL-8, IL-1 β, and TNF- α levels were assessed as markers of inflammation. Their levels significantly increased ($P < 0.05$) in the CP group compared to the control group. The IL-8 level decreased ($P < 0.05$) in the Probiotic 2+CP group compared to the CP and Probiotic 1+CP groups (Figure 2A). The high-dose probiotic administration in the CP-treated rats reduced the IL-1 β and TNF- α levels, but this reduction for IL-1 β was not significant ($P > 0.05$) (Figures 2B and 2C). The application of the probiotic only did not cause a significant change in these parameters compared to the control (Figures 2A, 2B and 2C).

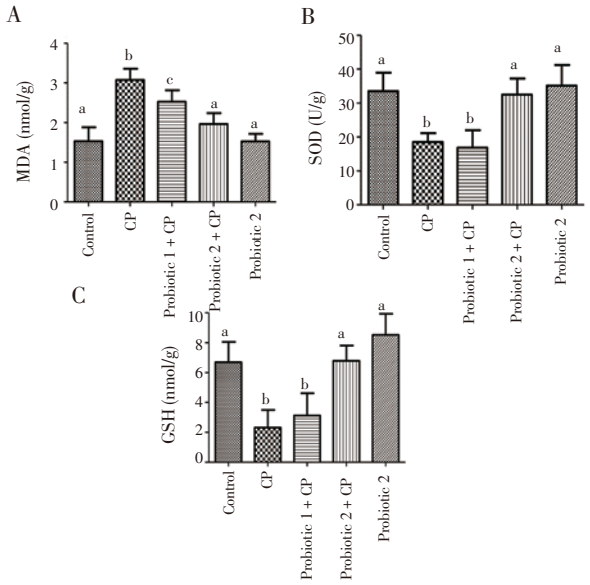

Figure 1. Levels of oxidative stress parameters (MDA, SOD and GSH) for all groups in the kidney tissue.

A: MDA levels, B: SOD activity and C: GSH levels. Different letters indicate significant difference among groups ($P < 0.05$), and the results are expressed as mean ± SD.

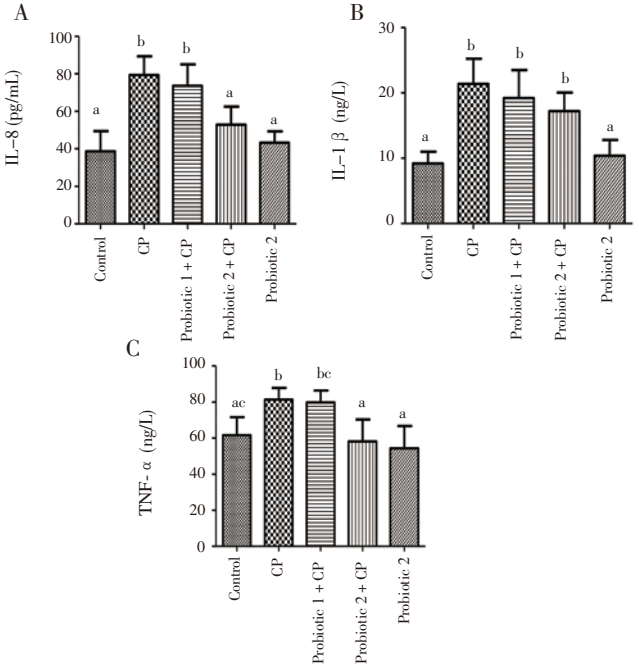

Figure 2. Pro-inflammatory cytokines levels in kidney tissues of all groups.

A: IL-8, B: IL-1 β; C: TNF- α; Different letters indicate the statistical differences among groups ($P < 0.05$, n=10). The results are expressed as mean ± SD.

3.5. Histopathological examination

The control group displayed a normal histopathological structure of renal parenchyma and serosa (Figure 3A). The CP group exhibited severe coagulation necrosis and atrophy in the glomeruli, dilatation in some tubules, hydropic degeneration in the tubular epithelium, and hyperemia in the interstitial vessels (Figure 3B). The Probiotic 1+CP group showed hydropic degeneration, hyperemia in the interstitial vessels, and mild coagulation necrosis (Figure 3C). In the renal tissues of the Probiotic 2+CP group, necrotic epithelium was not present, while mild hydropic degeneration was present (Figure 3D). In the Probiotic 2 group as in control, normal histopathological structures of the renal cortex, medulla, and serosa were observed (Figure 3E).

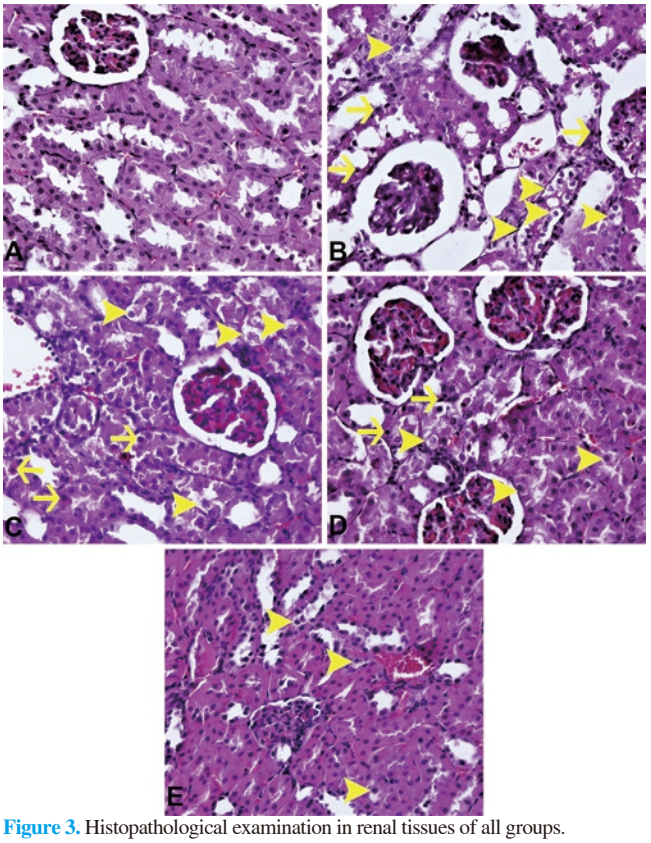

Figure 3. Histopathological examination in renal tissues of all groups.
Control (A): Normal histological appearance, CP (B): Severe coagulation necrosis (arrows) hydropic degeneration (arrowheads) in tubular epithelium, atrophy in glomeruli, dilatation in some tubules, hyperemia in interstitial vessels, Probiotic 1+CP (C): Hydropic degeneration (arrowheads), mild coagulation necrosis (arrows) and hyperemia in interstitial vessel, Probiotic 2+CP (D): mild hydropic degeneration, Probiotic 2 (E): Normal histological appearance,40 ×, H&E.

3.6. Apoptosis and DNA damage

During the immunohistochemical examination of the renal tissues, Bcl-2 and 8-OHdG expressions were not detected in the tubular epithelium of the kidney tissues in the control group (Figure 4A, 5A) and Probiotic 2 group (Figure 4E, 5E). In the CP group, slight cytoplasmic Bcl-2 (Figure 4B) and strong 8-OHdG expressions (Figure 5B) were detected in the tubular epithelium. The Probiotic 1+CP group was observed having medium levels of intracytoplasmic Bcl-2 expression (Figure 4C) and mild cytoplasmic expression of 8-OHdG in the tubular epithelium (Figure 5C). When kidney tissues were examined in the Probiotic 2+CP group, there were strong cytoplasmic Bcl-2 expressions in the tubular epithelium (Figure 4D) and slight levels of cytoplasmic 8-OHdG expressions in the tubular epithelium (Figure 5D, Table 3).

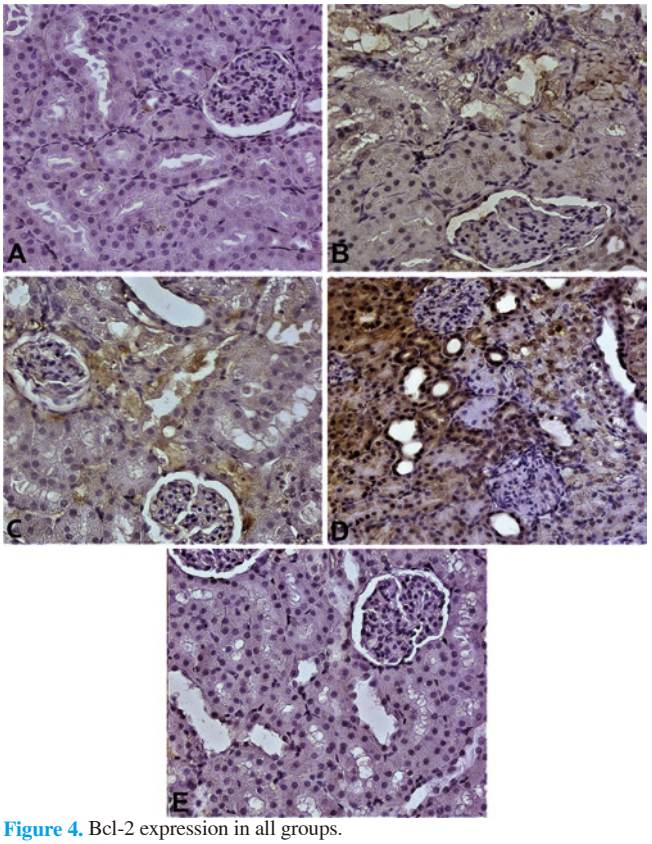

Figure 4. Bcl-2 expression in all groups.
Negative Bcl-2 expression in control group (A), slight Bcl-2 expressions in CP group (B), medium levels of Bcl-2 expression in Probiotic 1+CP group (C), strong level of Bcl-2 expression in Probiotic 2+CP group (D), negative Bcl-2 expression in Probiotic 2 (E), 40 ×, immunohistochemistry-peroxidase (IHC-P).

Table 3
Histopathological-immunohistochemical findings in kidney tissues of rats in experimental groups.

Findings	Control	CP	Probiotic 1 + CP	Probiotic 2 + CP	Probiotic 2
Hydropic degeneration in tubule epithelium	-	+++	++	+	-
Coagulation necrosis in tubule epithelium	-	+++	+	-	-
Hyperemia in interstitial vessels	-	+++	+++	++	-
Bcl-2	-	+	++	+++	-
8-OHdG	-	+++	++	+	-

None = -; weak = +; moderate = ++ and strong = +++.

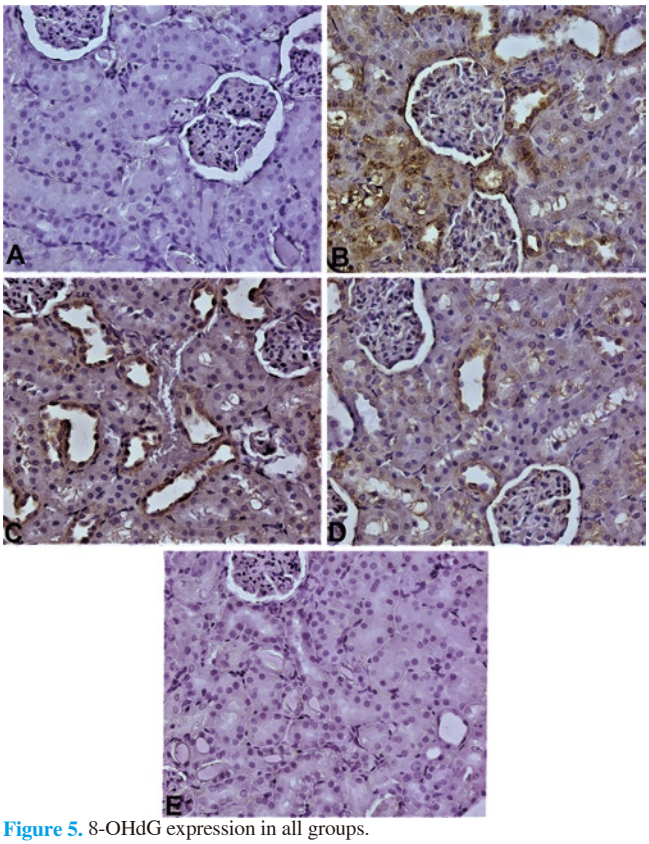

Figure 5. 8-OHdG expression in all groups.
Control (A): Negative 8-OHdG expression, CP (B): Strong 8-OHdG expression, Probiotic 1+CP (C): Mild level of 8-OHdG expression, Probiotic 2+CP (D): Slight 8-OHdG expression, Probiotic 2 (E): Negative 8-OHdG expression, 40 ×, IHC-P.

4. Discussion

When CP is used in chemotherapy, it induces renal injury and acute renal failure by inducing inflammation, oxidative stress, and apoptosis[25]. Although the effects of many compounds on organ toxicity caused by CP have been investigated[23,26], more researches are required to find a possible agent that can prevent CP induced nephrotoxicity without affecting its anti-cancer activity. Therefore, we investigated the effects of probiotic bacteria against CP induced nephrotoxicity in this study.

The major limiting factor in the use of CP is the side effects such as ototoxicity, neurotoxicity, vomiting, and nephrotoxicity[27]. Nephrotoxicity occurs in approximately 1/3 of CP-treated patients[1,28]. Exposure to CP of kidney tubular cells activates complex signaling pathways that cause tubular cell injury and death. A potent inflammatory response is induced, and this situation aggravates renal tissue damage. Also, CP leads to damage in the renal vasculature and, consequently, decreases blood flow to the renal tissue and reduces the glomerular filtration rate in ischemic renal injury. Depending on the lower glomerular filtration rate, creatinine, urea, and BUN levels can significantly increase with increased kidney weight[1,29–31]. In our study, it was determined that creatinine, urea, and BUN levels increased in the CP group, and a high dose of probiotic significantly prevented the increases in these parameters as well as increases in the kidney to body weight ratio. Also, it was determined that only probiotic application in Probiotic 2 group did not cause a negative effect on renal function and show any effect on kidney and body weight. These effects of the probiotic are believed to be due to its modulating immune responses[12]. In a previous study, we determined that probiotic had immunostimulatory effects and was important for stimulating pro-inflammatory and regulatory responses to rapidly decrease inflammation[13]. IL-1 β, IL-8, and TNF-α levels are acceptable as inflammation markers[32,33]. CP treatment induces remarkable up-regulation of TNF-α, IL-1 β, and IL-8 in the kidneys[34,35]. The increase in TNF-α and IL-1 β levels are often elevated in parallel and the increase of one marker elevates another[36]. TNF-α is a main pro-inflammatory cytokine generated by glomerular, endothelial, and renal tubular cells[37]. The levels of TNF-α, renal tissue, and urine increase following CP administration[38]. These cytokines induce the cytotoxicity or inflammatory reactions *via* various mechanisms such as increased production of ROS that causes damage in cell components like protein, lipid, and DNA. In our study, CP administration increased the IL-1 β, TNF-α, and IL-8 levels. A high dose of probiotic significantly prevented rises in levels of IL-8 and TNF-α, but this decline was not significant for IL-1 β. It was observed that only probiotic application did not induce inflammation in the kidneys and pro-inflammatory cytokine levels were similar to the control group. Contrary to our findings, Chabot *et al*.[39] determined that *L. rhamnosus* increased TNF-α levels, and another study showed that *L. rhamnosus* caused a decrease in TNF-α levels and had no effect on IL-1 β levels. Jang *et al*.[40] argued that *Lactobacillus brevis* inhibited the increase of TNF-α and IL-1 β levels in colitic mice.

Several mechanisms such as inflammation, oxidative stress, DNA damage, and apoptosis have been found to contribute to the pathogenesis of CP-induced nephrotoxicity[1,41,42]. The production of oxidants in the cell is controlled by antioxidant systems. The deterioration of the balance between antioxidant and oxidant systems is defined as oxidative stress. The ROS generated as a product of oxidative metabolism usually damages cellular structures such as protein, lipids, and DNA[43]. Oxidative stress plays a very important role in the pathophysiology of many diseases[44], and it has been recognized as an important factor in CP-induced nephrotoxicity[45]. CP-treatment increases various ROS at renal tubular cells. A thiobarbituric acid reactive substances like MDA, degradation is known as an index of lipid peroxidation. CP-induced nephrotoxicity significantly increased the MDA levels in the kidneys[31]. The decrease in SOD activity after CP treatment might be owed to the loss of zinc and copper, both of which are required for enzyme activity[46]. As a result of reduced SOD activity, it is insufficient to scavenge the superoxide anions produced during nephrotoxicity. The GSH depletion by CP has been reported in many studies[47,48]. The depletion of GSH appears to be the main factor that allows lipid peroxidation. In other words, increased lipid peroxidation in CP treatment is a result of GSH depletion and disrupted antioxidant enzyme activities[49]. In line with the literature, our study found that CP treatment increased renal MDA levels, decreased SOD activities and GSH levels. Exogenous antioxidants protect against oxidative stress because they strengthen antioxidant defense. In our study, we demonstrated for the first time that probiotic bacteria significantly attenuated the CP-induced nephrotoxicity in rats by reducing kidney oxidative stress and DNA damage. Besides, we detected that only probiotic application in Probiotic 2 group did not cause renal oxidative stress. Forsyth *et al*.[50] has established that *L. rhamnosus* treatment prevented alcohol-induced tissue and systemic oxidative

stress. The formation of MDA induces DNA oxidative damage, and a parallel effect is seen in 8-OHdG generation[51]. Renal 8-OHdG is a possible repairing agent of oxidative DNA lesions[52]. In our study, the 8-OHdG expression in the CP group increased as in other studies[51,53], and the probiotic prevented DNA damage by reducing the MDA level in the kidneys.

CP-induced nephrotoxicity characterized by tubular cell death is a common histopathological feature. Cell death in the renal tissue is an identified form of necrosis and apoptosis[41]. The CP treatment group showed a structurally disturbed histological pattern of kidney tissue characterized by increased glomeruli space, a disturbed structure of glomeruli, and edema[54]. In our study, it was observed that there was severe coagulation necrosis, hydropic degeneration in the tubular epithelium, atrophy in the glomeruli, dilatation in some tubules, and hyperemia at the interstitial vessel. Probiotic administration reduced these pathologies in the CP-treatment groups and only probiotic application did not cause any histopathological change in kidney tissue. The Bcl-2 proteins belonging to the Bcl-2 family regulate and execute many cellular intrinsic apoptotic pathways, and they are the best-characterized group among apoptosis-regulating factors. Bcl-2, an anti-apoptotic molecule, plays a role in the regulation of CP-induced apoptosis[55]. Neamatallaha *et al*.[24] have determined that Bcl-2 levels in kidney tissues were significantly lower in the CP-administered rats than those of other groups. In the present study, Bcl-2 level in the CP group was lower than those in the treatment groups. Most notably, a high dose of probiotic was higher than CP.

In conclusion, this study found that the administration of probiotic bacteria (especially high dose) in CP induced nephrotoxicity in rats has protective effects by decreasing oxidative stress, inflammation, apoptosis, DNA, and histopathological damage in the kidney tissue. These effects are due to the potent anti-inflammatory and antioxidant effects of probiotics. Besides, it was found that the use of probiotic alone did not have any negative effect on the renal functions, inflammation, oxidative stress, apoptosis and histological structure of the kidney.

Conflict of interest statement

The authors declare no conflict of interest.

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