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

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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 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.
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 suppression 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 °C 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 10^8 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 xg 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–1β (IL–1β) and tumor necrosis factor–α (TNF–α) levels

Renal tissue IL–8, IL–1β, and TNF-α levels were measured in a renal homogenate using an enzyme-linked immunosorbent assay kit (rat IL–8 ELISA kit, rat IL–1β ELISA kit, rat TNF-α ELISA kit, Sunred Biological Tectno) 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 renal 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           |
| CP                  | 0.210 ± 0.003           |
| Probiotic 1 + CP    | 0.190 ± 0.002           |
| Probiotic 2 + CP    | 0.160 ± 0.001           |
| Probiotic 2         | 0.170 ± 0.003           |

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 (Figures 1B and 1C).

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| 0.43 ± 0.06        | 18.21 ± 1.97|
| CP                  | 52.26 ± 5.71| 0.65 ± 0.11        | 31.24 ± 4.34|
| Probiotic 1 + CP    | 45.36 ± 6.56| 0.57 ± 0.15        | 27.56 ± 3.15|
| Probiotic 2 + CP    | 38.78 ± 4.86| 0.45 ± 0.08        | 21.16 ± 2.09|
| Probiotic 2         | 33.71 ± 7.63| 0.42 ± 0.07        | 20.21 ± 2.96|

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 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-1β, and TNF-α) levels in kidney tissue

IL-8, IL-1β, 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-1β and TNF-α levels, but this reduction for IL-1β 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-1β; 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](image)

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](image)

Table 3

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

None = -; weak = +; moderate = ++ and strong = +++.
as ototoxicity, neurotoxicity, vomiting, and nephrotoxicity in this study. We investigated the effects of probiotic bacteria against CP induced nephrotoxicity without affecting its anti-cancer activity. Therefore, are required to find a possible agent that can prevent CP induced nephrotoxicity.

Increased kidney weight, creatinine, urea, and BUN levels can significantly increase with renal injury. Depending on the lower glomerular filtration rate, kidney oxidative stress and DNA damage. Besides, we detected 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 bacteria 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-1β, IL-8, and TNF-α levels are acceptable as inflammation markers [32,33]. CP treatment induces remarkable up-regulation of TNF-α, IL-1β, and IL-8 in the kidneys [34,35]. The increase in TNF-α and IL-1β 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-1β, 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-1β. 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-1β levels. Jang et al. [40] argued that Lactobacillus brevis inhibited the increase of TNF-α and IL-1β 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

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

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.
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study, the 8-OHdG expression in the CP group increased as in other
induced apoptosis
The Bcl-2 proteins belonging to the Bcl-2 family regulate and
application did not cause any histopathological change in kidney
hyperemia at the interstitial vessel. Probiotic administration reduced
severe coagulation necrosis, hydropic degeneration in the tubular
glomeruli, and edema
characterized by increased glomeruli space, a disturbed structure of
kidney. The CP-induced nephrotoxicity characterized by tubular cell death is a
showed a structurally disturbed histological pattern of kidney tissue
identified form of necrosis and apoptosis
of the kidney.

Conflict of interest statement
The authors declare no conflict of interest.

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