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Influence of *Lactobacillus plantarum* administration on some serum/tissue (uterus) antioxidant and cytokine levels in female rats exposed to cadmium (Cd)

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Abstract: The study aimed to investigate the effects of oral *Lactobacillus plantarum* (Lp) administration on some serum/tissue (uterus) antioxidant and cytokine levels as well as histopathologic changes in female rats exposed chronically to Cd. For this purpose, rats were separated into four groups including control (C, n = 8), cadmium (Cd, n = 8), Lp (n = 8), and Cd + Lp (n = 8). Cd (dissolved in water at a dose of 2.04 mg/mL) was orally administered to Cd and Cd + Lp groups for 28 days. Besides, active-live Lp, which was grown at a level of approximately 10^8–10^9 cfu/mL in skim milk powder medium, was administered orally to Lp and Cd + Lp group animals on the same days. Only fresh water and standard rat food were given ad libitum to the animals in the C group. After the administration period, they were decapitated and serum/tissue (uterus) samples were taken for measurement of Cd and GSH levels, CAT and SOD enzyme activities, MDA values, TNF-α, IL-6, IL-1ß, and IL-10 levels as well as histopathologic changes. Administration of the Lp suspension decreased the Cd accumulation in the serum samples compared to the Cd group ($p < 0.05$). Also, the administration of Lp led to a decrease in the serum MDA levels in the Cd + Lp group when compared to the Cd group ($p < 0.05$). In addition, serum IL-10 levels decreased in the Cd group compared to the C group ($p < 0.05$). Conversely, we did not determine any significant alterations among the groups regarding tissue IL-6, IL-10, and, IL-1ß levels. However, uterus TNF-α concentrations were higher in both Cd given groups. Also, uterus SOD activity decreased in the Cd group compared to the C group. In conclusion, Lp partially protects female rats from the harmful effects of chronic Cd toxicity.

Key words: *Lactobacillus plantarum*, cadmium, female rats, cytokine, antioxidant, uterus

1. Introduction

The widespread use of toxic heavy metals such as copper (Cu), lead (Pb), mercury (Hg), and cadmium (Cd) in industry, agriculture, and mining areas is one of the most crucial reasons for environmental pollution around the world. These heavy metals can get into the water, be taken up by plants, and released into the atmosphere as gases, or adsorbed by soil components [1, 2].

One of these toxic heavy metals is Cd, a naturally occurring metal, that is substantially obtained from zinc (Zn) and lead (Pb) byproducts. Other essential sources of Cd are listed as manufacturing batteries, pigments, corrosion-protection coating, platings, solar cells, plastic stabilizers, neutron absorbers, and cosmetics by World Health Organization (WHO) [3]. The exposure to Cd, which is defined as a class 1 carcinogen by the International Agency for Research on Cancer (IARC) [4], in humans and animals, mostly occur through the oral route but also through inhalation or skin contact [5-7]. Ingestion of Cd-contaminated water and other foods (especially shellfish), smoking, and working in metal-producing centers can be given as examples of major Cd exposure [8].

Long-term Cd accumulation mainly induces epigenetic changes in DNA expression, inhibition of cell metabolism, oxidative stress (OS), and increased lipid peroxidation (LPO) in the body [9]. It has been reported that Cd exposure may cause irreversible damage in several vital organs including the kidney, liver, lung, brain, bone, duodenum, placenta, and blood [10-12].

Previous studies have revealed that the detrimental effects of Cd on different tissues can be attenuated by different antioxidants, chelating agents, and probiotics

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including melatonin, chitosan, selenium (Se), ß-carotene, chitosan oligosaccharides, quercetin, and *Lactobacillus*/*Bifidobacterium* strains [13-17]. Recently, researchers suggested that some lactic acid bacteria (LAB) strains (*Lactobacillus rhamnosus*, *L. plantarum*, and *L. brevis*) can bind and remove Cd in in vivo and also in in vitro conditions. *L. plantarum*, is a versatile lactic acid bacterium that exists as part of the microbiota of many foods and feeds (meat, fish, and vegetable fermented products). It is a natural inhabitant of human and also animal body [18, 19]. Apart from the high nutritional value of probiotics containing these mentioned bacterial species, their antioxidant activities and immune-modulator effects are also well known [19-21].Although the kidney, liver, and lung are the most influenced organs by Cd accumulation, its degenerative effects have been also determined in the reproductive organs (ovarian follicles, tuba uterine, and uterus) in recent years [22, 23]. Candan et al. [24] reported that Cd administration caused degeneration in ovarian follicles, tuba uterine, endometrium, and uterine epithelium. Malondialdehyde (MDA) is a byproduct of polyunsaturated fatty acids (PUFA) peroxidation in human and also animal cells, which is a crucial marker of LPO [25]. Some enzymatic [superoxide dismutase (SOD), catalase (CAT)] and nonenzymatic [glutathione (GSH)] antioxidant defense systems are utilized by different cells to protect the organism against the harmful effects of free radicals (FR) [26]. In addition, it was reported that the MDA levels increased; however, glutathione peroxidase (GSPx) and CAT enzyme levels were negatively affected due to Cd accumulation in the reproductive organs [24]. Inflammation is a preventive response of the human and also animal body to different injuries including physical, chemical, or infection types. In the inflammation process, many types of immune cells including macrophages, B/T lymphocytes, mast/endothelial cells produce different biochemical compounds modulating immune reactions called cytokines [27, 28]. Cytokines are mainly allocated to two groups as pro- and antiinflammatory. Interleukin-1β (IL-1β) is a proinflammatory cytokine, expressed by monocytes, macrophages, and dendritic cells that are crucial for host-defense system responses to different types of infection and also injury [29]. Another proinflammatory cytokine is interleukin-6 (IL-6), which plays a crucial role in the defense system of the organism (human or animal) including acute phase response, B cell proliferation, and thrombopoiesis [27, 30]. Also, one of the important proinflammatory cytokines is tumor necrosis factor-alpha (TNF-α), which plays a significant role in microbial infections, cell death, and inflammation. Interleukin-10 (IL-10), an antiinflammatory cytokine, is especially necessary for maintaining the balance of the immune response [29, 30]. In some of the previous studies, it was claimed that Cd accumulation affects the levels of certain cytokines in various tissues and blood [11, 17, 31, 32]. The alteration of steroidogenesis, delayed puberty-menarche, the loss of pregnancy, irregularity of the menstrual cycle, and premature birth can be given as other negative-effects of Cd in the female reproductive system [33]. In addition, Adams et al. [34] confirmed that dietary Cd is a risk factor for breast, endometrial, or ovarian cancers in postmenopausal women.

We investigated the effects of Lp administration on some serum-tissue (uterus) antioxidant and cytokine levels as well as histopathologic changes in female rats exposed chronically to Cd.

2. Materials and methods

2.1. Ethical statement

All experimental procedures were approved by Balıkesir University Experimental Animal Ethics Committee (Approval no: 2021/3-5).

2.2. Animals and study design

In our research, thirty-two female Wistar rats (with an average weight of 100–120 g, 6–7 weeks old) obtained from Balıkesir University Experimental Animal Production, Care, Application, and Research Center were used. They were divided into four equal groups as control (C), cadmium (Cd), *L. plantarum* (Lp), and Cd + Lp. Although the rats were separated into different groups, they were kept in individual cages. After the 2-week adaptation period (rats were fasted for 12 h before exposure to heavy metal in the first application), the following applications were performed on the rats;

a) C group: Standard rat food and fresh water were given to animals ad libitum during the experiment.

b) Cd group: Cd ($CdCl_2$) was dissolved in fresh drinking water at a dose of 2.04 mg/mL. Oral administrations were then performed to rats with drinking water for 28 days [19].

c) Lp group: Active-live *L. plantarum*, which was grown at a level of approximately 10^8–10^9 cfu/mL in skim milk powder medium, was administered orally to the rats for 28 days [19].

d) Cd + Lp group: They received cadmium and active-live cultures of *L. plantarum*, at a concentration of 10^8–10^9 cfu/mL in skim milk powder medium for 28 days. All applications were made in an end-of-day controlled manner.

At the end of the 28-days, animals were terminated with cervical dislocation technique under general anesthesia [ketamine/xylazine (0.1 mL/100 mg/b.w.)]. For obtaining serum, blood samples were collected from the heart by cardiac puncture into serum tubes, and then centrifuged (3000 rpm, 25 min, Heinrich, Germany). Collected serum samples were stored at –80 °C until the analysis. Also, the uterus tissue of the rats was taken and

weighed after separating from the ovaries and tuba uterine. Then, they were fixed in 10% neutral buffered formalin for histopathological investigations.

2.3. Vaginal smear applications

A small amount of physiologic saline (0.2 mL) was given into the vagina using different Pasteur pipettes for each rat. Two drops of cell suspension on a smear slide were examined to determine the stages of the estrous cycle. Thirty-two rats have been included in the present study following the determination of proestrus and/or estrous stages depending on cornified cells, and rounded/nucleated epithelial cells [35].

2.4. Determination of resistance to Cd

Lp strain with the highest resistance in terms of Cd was used in the study. To determine, the concentration of minimal inhibitory (MIC) and minimal lethal (MLC) trials were performed on 4 different strains of Lp. By making 10-fold dilutions of Cd, dilutions containing gradually decreasing concentrations of Cd were obtained. Lp test microorganisms were cultivated in the dilutions and incubated at 37 °C for 24–48 h. Lp Biofen (dsmz16627), which was determined to grow in the dilution containing the highest rate of Cd after incubation, was used in our study [19].

2.5. Preparation of Lp diet

In the present study, Lp was grown in a medium containing 10 mL of De Man Rogosa and Sharpe Broth (MRS Broth) at 30 °C for 18–20 hours. At the end of the incubation period, MRS Broths (containing approximately 10^{9}–10^{10} cfu/mL bacterial culture) were centrifuged at 5000 rpm in a cooled centrifuge for 5 min. After, the supernatant was discarded. Then, the remaining bacterial pellets were dissolved in 10 mL skim milk powder (Merck-115363), and 100 ml was completed. Then, it was administrated to the rats [19, 36].

2.6. Determination of the serum Cd concentrations

Mineralization of the whole blood samples was achieved using a wet ashing digestion procedure at atmospheric pressure. Accordingly, a known amount of blood sample (1 mL) was transferred in a beaker and 5 mL of concentrated nitric acid (HNO_3) and 2 mL of hydrogen peroxide (H_2O_2) were added. The vessels were heated to 90 °C without splashing. The mineralization was maintained until clear solutions were obtained. The clear samples were taken in volumetric flasks and diluted with purified water up to 10 mL. The digested samples were stored at + 4 °C until analysis. Blank samples were also prepared using the abovementioned procedure without a blood matrix. Analysis was performed in ICP-OES Perkin Elmer Optima 7300, USA [37].

2.7. Determination of the MDA values and antioxidant enzyme levels in serum and tissue (uterus) samples

The obtained tissue samples (100 mg uterus sample in 900 µL phosphate buffered saline) were homogenized at 7000 rpm by using an ultrasonic homogenizer (Isolab, Merck, Germany). After, they were centrifuged at 4025 × *g* for 15 min at room temperature (Sigma18 - K, Newtown, Shropshire, UK) to get supernatants. Also, serum, tissue, and certain standards were extracted, derivatized, and subjected to ELISA in MDA, GSH, CAT, and SOD precoated microtiter strips. The absorbance of the solution in the wells was read at 450 nm within 15 min using a microplate reader (Biotek ELX800, USA). The optical density was used to calculate the enzyme levels using a standard curve. MDA (Code:SH0020) values, GSH (Code: EA0142Hu) levels, CAT (Code:E0869Ra), and SOD (Code:E1444Ra) enzyme activities were determined in serum and tissue (uterus) samples using commercial kits (BT - LAB; Shanghai, China) by Elisa device (Biotek ELX800, USA).

2.8. Detection of some cytokine levels in serum and tissue (uterus) samples

From the above-mentioned tissue (uterus) and serum samples, IL-1ß (Code:E0119Ra), TNF-α (Code:E0764Ra), IL-6 (Code:E0135Ra), and IL-10 (Code:E0108Ra) cytokine levels were measured with commercial Elisa kits (BT-LAB, Shanghai, China) taking into account the user manuals by Elisa device (Biotek ELX800, USA).

2.9. Histopathological examination

The uterus tissues of sacrificed animals were placed immediately in a 10% formalin solution. The tissues were processed and then embedded in paraffin blocks, sectioned at 4.5 µm, and stained with Hematoxylin and Eosin (H&E) stain for light microscopic evaluation. Arrangement of the luminal epithelium (LE) cells and the glandular epithelium (GE) cells, degeneration, necrosis, and inflammation were examined under a light microscope (Nikon Eclipse Ni, Tokyo, Japan) [11].

2.10. Statistical analysis

The SPSS 25.0 program (SPSS, Inc., Chicago, IL) was used for all of the statistical analyses. All data were expressed as mean ± SEM. A Shapiro-Wilk test was used to determine the distribution characteristics of variables and variance homogeneity. Parameters were analyzed by using the analyses of variance (ANOVA) followed by Duncan's test. The obtained data were considered statistically significant at $p \leq 0.05$.**3.**

3. Results

3.1. Serum Cd levels of the experimental groups

Serum Cd levels were measured the highest in the Cd group (83.96 ± 8.48 µg/L) compared to C group animals ($p < 0.05$). On the contrary, administration of the Lp diet decreased the Cd accumulation in the serum samples of the Cd + Lp group (42.64 ± 5.83 µg/L) ($p < 0.05$), shown in Figure 1. The mean levels of the Cd in the C group were 7.57 ± 0.22 µg/L.

3.2. Serum MDA values and antioxidant enzyme activities

It was observed that Cd increased MDA levels when compared to the C and Lp groups, but the addition of Lp decreased MDA levels even though they did not reach control levels ($p < 0.05$). No significant changes were detected among the groups according to serum GSH levels, SOD, and CAT enzyme activities ($p > 0.05$), shown in Table 1.

3.3. Serum cytokine levels

It was not found any significant alterations among the groups regarding serum TNF-α and IL-1ß levels ($p > 0.05$). Conversely, IL-6 levels were determined higher in the Cd group compared to other groups ($p < 0.05$). In addition, IL-10 levels were lowest in the Cd group and the decline was significant compared to the C group ($p < 0.05$). On the other hand, changes in both serum IL-6 and IL-10 in animals receiving Cd returned to normal values with the addition of Lp ($p < 0.05$), shown in Table 2.

3.4. Uterus MDA values and antioxidant enzyme activities

Although tissue MDA levels tend to increase in the Cd group, any significant alterations were determined among the groups ($p > 0.05$). Besides, we could not define any significant alterations among the groups regarding tissue GSH levels, and CAT enzyme activities ($p > 0.05$). On the contrary, tissue SOD enzyme activity decreased in the Cd group compared to C group in the present study ($p < 0.05$), shown in Table 3.

3.5. Some cytokine levels in the uterus

Tissue TNF-α levels were found the highest in the Cd and Cd + Lp groups compared to the C group in the present study ($p < 0.05$). In addition, we did not determine any significant alterations among the groups regarding tissue IL-6, IL-10, and IL-1ß levels, as shown in Table 4.

3.6. Histopathological findings

In the microscopic examination, the uterus in the C group was detected with high columnar epithelial cells lining the uterine lumen and glands, also the stroma displayed active glands (Figure 2a). In the Cd group, severe changes were seen in LE cells and GE cells. Vacuolar degeneration and necrosis were most prominent in LE cells, also in GE cells was seen vacuolar degeneration, necrosis, and desquamation (Figure 2b). In 5 sections in the Cd group, intraglandular and periglandular neutrophil leukocytes and a small number of mononuclear cell infiltration was observed. In the Cd + Lp group, vacuolar degeneration and necrosis in LE cells and GE cells also in 4 sections in this group inflammation was noted with a predominance of neutrophil leukocytes (Figure 2c). There is no histopathological alterations were observed in Lp only given group (Figure 2d).

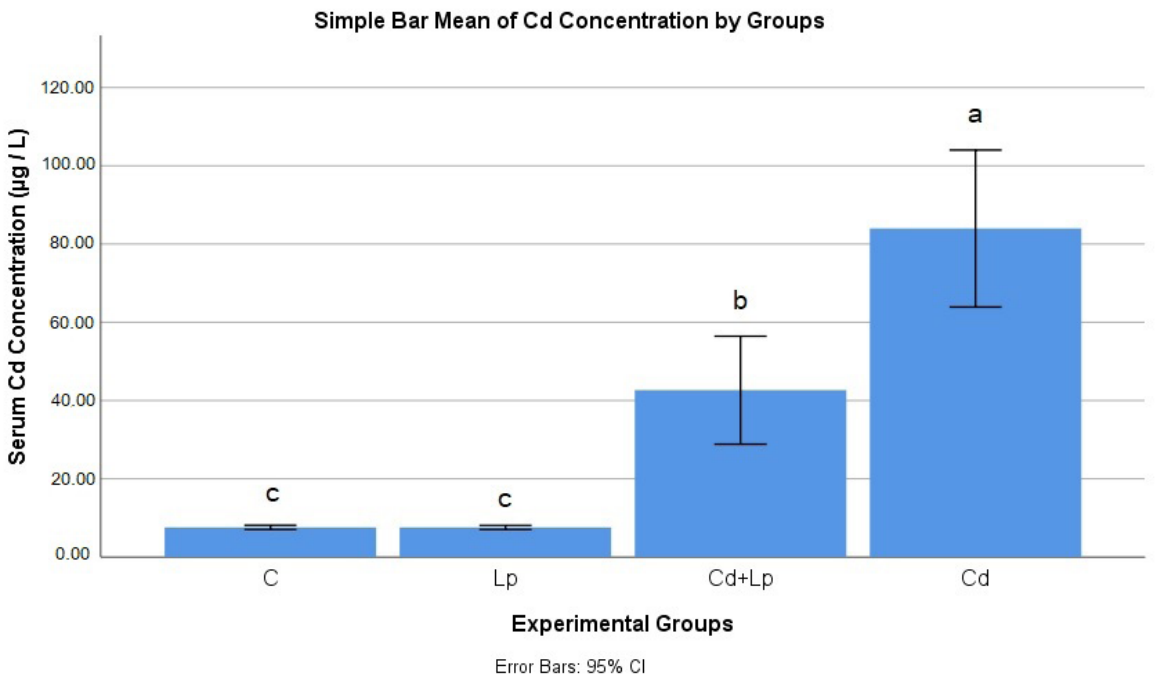

Figure 1. Serum Cd concentrations of the experimental groups. C: Control; Lp: *Lactobacillus plantarum*; Cd: cadmium. [a, b, c] Different superscripts differ ($p < 0.05$) among bars.

Table 1. Serum MDA values and antioxidant enzyme activities of the experimental groups.

Groups	*n*	MDA (nmol/mL) mean ± SEM	SOD (ng/mL) mean ± SEM	GSH (mmol/L) mean ± SEM	CAT (CU/L) mean ± SEM
Cd + Lp	8	2.14 ± 0.31[b]	2.93 ± 0.65	0.26 ± 0.00	250.59 ± 68.43
Cd	8	2.24 ± 0.35[a]	2.74 ± 0.32	0.25 ± 0.01	216.84 ± 39.16
Lp	8	1.65 ± 0.26[c]	3.55 ± 0.47	0.28 ± 0.01	293.40 ± 39.08
C	8	1.68 ± 0.22[c]	3.55 ± 0.39	0.27 ± 0.00	319.09 ± 40.08

[a,b,c]$p < 0.05$, The means with different letters in the same column are significantly different from each other. C: control; Cd: cadmium; Lp: *Lactobacillus plantarum*; MDA: malondialdehyde; SOD: superoxide dismutase; GSH: glutathione; CAT: catalase; SEM: standard error of mean.

Table 2. Serum cytokine levels of the groups.

Groups	*n*	TNF-α (ng/L) mean ± SEM	IL-6 (ng/L) mean ± SEM	IL-10 (pg/mL) mean ± SEM	IL-1ß (pg/mL) mean ± SEM
Cd + Lp	8	187.62 ± 21.62	15.49 ± 1.75[b]	238.28 ± 19.08[a]	2034.54 ± 167.95
Cd	8	223.05 ± 38.97	22.96 ± 2.68[a]	156.28 ± 24.38[b]	2401.58 ± 211.37
Lp	8	132.45 ± 31.80	12.37 ± 2.46[b]	266.18 ± 28.20[a]	1909.13 ± 214.30
C	8	173.64 ± 50.24	13.19 ± 1.67[b]	285.36 ± 20.42[a]	1838.94 ± 225.03

[a,b]$p < 0.05$, The means with different letters in the same column are significantly different from each other. C: control; Cd: cadmium; Lp: *Lactobacillus plantarum*; TNF-α: Tumor necrosis factor-alpha; IL-6: Interleukin-6; IL-10: Interleukin-10; IL-1ß: Interleukin 1-beta.

Table 3. Uterus MDA values and antioxidant enzyme activities of the experimental groups.

Groups	*n*	MDA (nmol/mL) mean ± SEM	SOD (ng/mL) mean ± SEM	GSH (mmol/L) mean ± SEM	CAT (CU/L) mean ± SEM
Cd	8	3.58 ± 0.43	3.43 ± 0.63[b]	0.22 ± 0.01	293.30 ± 66.47
Lp	8	3.08 ± 0.36	4.57 ± 0.59[ab]	0.25 ± 0.01	407.08 ± 52.32
C	8	3.10 ± 0.43	5.93 ± 1.03[a]	0.26 ± 0.01	476.91 ± 62.28
Cd + Lp	8	3.22 ± 0.52	4.32 ± 0.75[ab]	0.25 ± 0.01	382.69 ± 70.86

[a,b]$p < 0.05$, The means with different letters in the same column are significantly different from each other. C: control; Cd: cadmium; Lp: *Lactobacillus plantarum*; MDA: malondialdehyde; SOD: superoxide dismutase; GSH: glutathione; CAT: catalase; SEM: standard error of mean.

Table 4. Uterus cytokine levels of the groups.

Groups	*n*	TNF-α (ng/L) mean ± SEM	IL-6 (ng/L) mean ± SEM	IL-10 (pg/mL) mean ± SEM	IL-1ß (pg/mL) mean ± SEM
Cd	8	748.31 ± 22.05[a]	21.73 ± 1.00	231.20 ± 38.79	3060.22 ± 142.10
Lp	8	653.63 ± 43.85[ab]	17.42 ± 2.25	340.89 ± 59.24	2355.40 ± 358.56
C	8	619.29 ± 34.93[b]	17.19 ± 2.93	322.82 ± 26.60	2189.71 ± 286.84
Cd + Lp	8	727.29 ± 23.14[a]	19.04 ± 1.94	306.92 ± 26.59	2777.50 ± 402.48

[a,b]$p < 0.05$, The means with different letters in the same column are significantly different from each other. C: Control; Cd: Cadmium; Lp: *Lactobacillus plantarum*; TNF-α: Tumor necrosis factor-alpha; IL-6: Interleukin-6; IL-10: Interleukin-10; IL-1ß: Interleukin 1-beta.

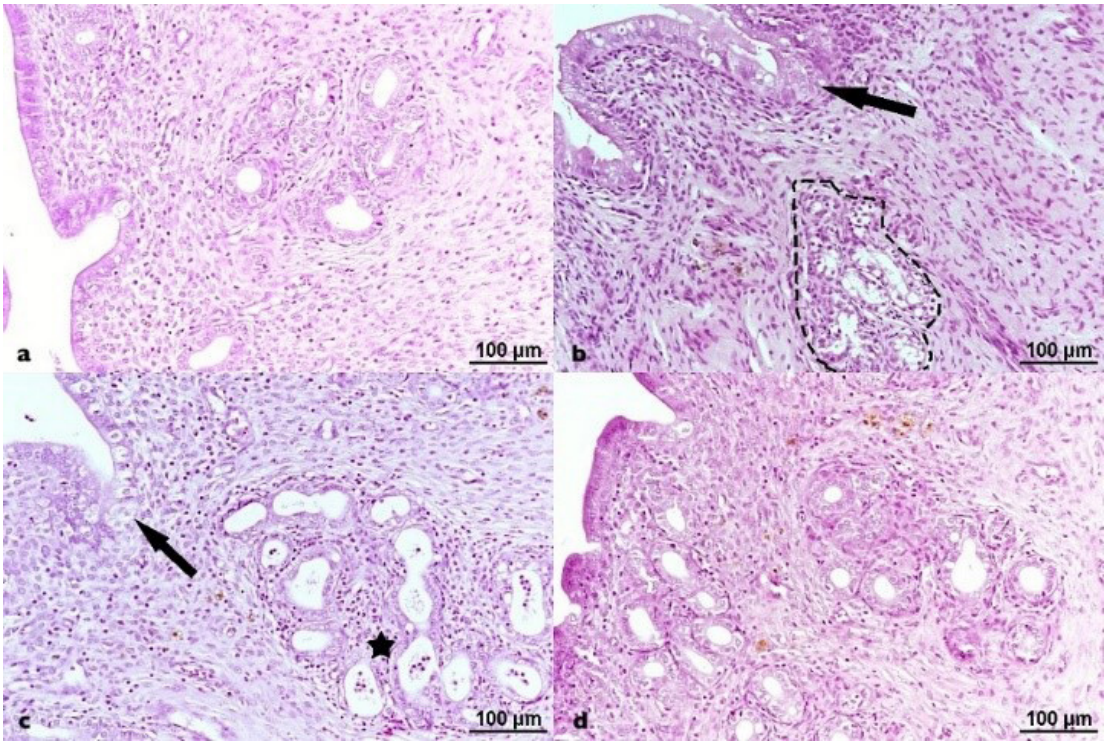

Figure 2. Microscopic view of the uterus sections of all experimental groups **(a)** normal histological appearance of C group; **(b)** necrotic LE cells (arrow), hydropic degeneration and necrosis of GE cells (dotted areas) in the Cd group; **(c)** hydropic degeneration in LE cells (arrow), inflammatory cells in intraglandular and periglandular area (star) in the Cd + Lp group; (d) appeared structurally normal in the Lp group (H & E).

4. Discussion

Occupational exposure, living-working in industrial regions, old age, cigarette smoking, dietary intake, female sex, administration, and iron deficiency was identified as the main risk factors for elevated blood Cd in the literature [38, 39]. In our study, administration of the Cd (2.04 mg/mL for 28 days, p.o.) to Cd and Cd + Lp group animals increased the serum Cd levels in female rats. Nasiadek et al. [22] reported that oral Cd administration (at doses of 0.09–4.5 mg/kg b.w. for 30 days) led to a significant dose-dependent increase in the blood Cd concentration of female rats. Liu et al. [40] also informed that compared with the C group, Cd administration (10 mg/L cadmium chloride for 52 weeks, p.o.) caused a significant increase in the Cd levels in the blood of the mice. Conversely, an oral Lp (approximately 10^{9}–10^{10}/mL bacterial culture) diet led to a decrease in the Cd accumulation in the serum samples of Cd + Lp group animals in the present study. Similarly, Zhai et al. [41] reported that 8-week oral administration of a probiotic strain Lp CCFM8610 (at 1×10^{9} cfu), markedly decreased the blood Cd levels in volunteers that had increased Cd levels due to Cd administration. In addition, the Lp CCFM8610 treatment led to an important reduction in the levels of Cd in the blood of mice consistent with our study [40]. The changes that we defined may be interpreted as the metal-binding activity of the Lp [19].

Cd-induced damage in the blood and different types of tissue is implicated in the generation of reactive oxygen species (ROS) and the depletion of antioxidants [11, 17]. Serum MDA values were detected as the highest in Cd group animals when compared to other groups in our study. These findings were consistent with the previous studies [42 - 44]. On the other hand, the administration of an oral Lp diet caused a decrease in the serum MDA levels in the present study. On the contrary, no significant differences were defined with regard to MDA contents in neither Cd nor other experimental groups (*Bacillus coagulans*, *L. plantarum*, and inulin) during the 42 days in rats [45]. It was also confirmed that the administration of 200 µL of Lp TW1-1 suspension (10^{9} cells) restored the serum MDA concentrations in male mice administered endocrine disruptor diethylhexylphthalate (DEHP) [46]. In the present study, administration of Cd (2.04 mg/mL for 28 days, p.o.) did not influence the serum, GSH levels, SOD, and CAT enzyme activities in female rats. In addition, we could not find any significant changes

regarding the enzyme activities in serum samples among the experimental groups (C, Lp, and Cd + Lp). Abdel-Wahab et al. [47] demonstrated that Cd administration (50 mg cc/kg for 1 month, p.o.) significantly decreased the serum level of total antioxidant capacity (TAC) in female rats. Xue et al. [48] reported that serum CAT activities and GPx levels were significantly decreased; however, serum SOD activity was not altered in the Cd group ($CdCl_2$, 6 mg/kg, b.w.), in comparison with the C group in Sprague–Dawley female rats. Tian et al. [46] also suggested that Lp TW1-1 (200 µL of Lp TW1-1 suspension at the final dose of 10^9 cells) ameliorated the values of GSH and LPO, the enzymatic activities of SOD and CAT in the serum of DEHP-administered male mice. Our and other studies' findings show that the antioxidative effects of Lp may vary according to the probiotic strain and application time [19].

Recently, researchers determined that Cd administration affects certain pro and antiinflammatory cytokine levels in the blood [32]. In the present study, serum IL-6 levels were measured the highest in the Cd group compared to the other groups. Kataronovski et al. [49] found that acute Cd treatment (1 or 2 mg/kg/day, i.p.) caused an increase in blood IL-6 levels in female rats. Besides, the IL-6 concentration in the circulation was noted significantly higher in male rats than in females by the same researchers. In a previous study, it was reported that Cd (2 mg/kg/day, three times a week, p.o.) administration did not change serum IL-6 levels in male rats [32]. El-Boshy et al. [50] informed that Cd (40 mg/L in drinking water, for 30 days) treatment increased the serum IL-6 levels in male rats. Different sex, dose, time, or application method may affect these differences [19]. On the other hand, Lp treatment ameliorated the serum IL-6 levels in the Cd + Lp group when compared to the Cd group in our study. Tian et al. [46] reported that Lp TW1-1(200 µL of *L. plantarum* TW1-1 suspension at the final dose of 10^9 cells) significantly decreased serum IL-6 levels in DEHP-induced testicular damaged male mice. However, it was not determined any significant changes among the experimental groups (C, Lp, Cd, and Cd + Lp) regarding serum TNF-α and IL-1ß levels in the present study. It was also suggested that Cd (2 mg /kg/p.o. for 4 weeks) application did not cause any remarkable change in serum IL-1β; however, it increased the TNF-α levels in male Wistar rats [32]. Conversely, TNF-α, IL-1β, and the gene expression of these inflammatory factors were defined as higher in DEHP-administered male mice compared to Lp TW1-1–administered group [46]. In addition, serum IL-10 levels were defined as lowest in the Cd group compared to the C group in the present study. Similar to our results, Cd exposure can significantly suppress IL-10 secretion in previous studies [51, 52]. However, it was also found that IL-10 can increase under Cd stress within different conditions [53]. It was reported that the expression of antiinflammatory cytokines was highly dependent on the administration methods of Cd and the age of employed animals. This can be expressed as a diverse mechanism towards Cd treatment which might be attributed to immune cellular function [53]. We could not find any research about the potential protective effects of oral Lp administration on serum cytokine levels in female rats exposed to Cd toxicity in the literature. These results can be obtained due to the partial immune-modulatory effects of Lp [21].In the present study, Cd administration (2.04 mg/mL for 28 days, p.o.) did not affect the uterus MDA values, and also GSH levels-CAT enzyme activities in rats. However, tissue SOD activity decreased in Cd group animals. It has been reported that Cd accumulation in the uterus and ovaries of rats, decreased SOD, CAT, GPx, and GSH, however, raised the values of MDA and H_2O_2 [54]. In a previous study [22], administration of Cd (4.5 mg/kg/b.w.) significantly increased MDA values in the uterus only after 6 months of application. Besides, the same dose also decreased the activity of the uterine CAT in female Wistar rats. In addition, no changes in the level of GSH in uterus tissue were determined by researchers in the same study [22]. On the other hand, Lp administration did not cause any alteration in MDA values, also GSH levels-CAT enzyme activities except SOD among the experimental groups in our study. Oral Lp administration partially restored the SOD activity in the Cd + Lp group compared to the Cd in the present study. It was also found that the living cells of *Lactiplantibacillus plantarum* ML05 had a major reversal implication on the changes of SOD, CAT, GSH, and MDA in the cadmium-treated groups in the livers and kidneys of rats [19]. Although there are various studies about the potential protective effects of Lp administration on the different tissues (kidney, liver, and intestine) [20, 41], we could not find any literature about the effects of Lp on female reproductive organs in rats. Changes may be observed due to different probiotic strains, application routes, doses, or animal species, and strains.

In our study, uterus TNF-α levels markedly increased due to oral Cd administration in both Cd groups when compared to the C group. Besides, we did not find any alterations among the groups according to tissue IL-6, IL-10, and IL-1ß levels. Paniagua et al. [55] reported that Cd administration (0.6, 1.2, 2.5 µM for 24 h) caused IL-6 production via ROS-dependent activation of the extracellular signal-regulated protein kinase (ERK1/2) but independent of Jun amino-terminal kinases (JNK) signaling pathway in human placental JEG-3 trophoblast cells. Da Costa et al. [56] offered that Cd treatment (100 ppm in drinking water for 30 days) led to reproductive tract (RT) inflammation, OS, and fibrosis. Pillet et al. [57]

also suggested that females may have a greater risk than males for Cd-induced immunotoxicity. On the other hand, Lp treatment did not cause any amelioration in uterus TNF-α levels of the Cd + Lp group animals compared to the Cd group. Lp administration also did not cause any alteration in other cytokine levels in our study. These results may have been achieved due to the inability of Lp to fully exert its immunomodulatory effect in the uterus tissue at the dose used [21].

When the microscopic examination of the uterus in the Cd group was evaluated, severe changes were observed in LE and GE cells. Vacuolar degeneration and necrosis were most prominent in LE cells. In addition, vacuolar degeneration, necrosis, and desquamation were defined in GE cells. Besides, intra- and periglandular neutrophil leukocytes and also mononuclear cell infiltration were determined compared to the C group. Sapmaz Metin et al. [58] reported that Cd exposure increased the mast cell count: however, it decreased the eosinophilia in the endometrium of mice. Besides, when the same researchers examined the luminal and glandular epithelia, they determined a higher apoptotic index (AI) but a lower proliferation index (PI) in the Cd group compared to C. Massanyi et al. [23] also informed that Cd exposure caused a significant change in the relative volume of GE in the uterus. Also, an increase in stroma was a sign of uterus oedamatization caused by Cd-induced damage in the wall of blood vessels and subsequent diapedesis. On the other hand, Höfer et al. [59] offered that the administration of Cd did not influence the uterine weight and also histology in ovariectomized animals because of the lower hormonal potency. In the Cd + Lp group, vacuolar degeneration and necrosis were seen in LE and GE cells. Also, inflammation was noted with a predominance of neutrophil leukocytes in this group. Any histopathological changes were observed in the Lp group when compared to the C group. In addition, we could not find any histopathologic examination about the effects of Lp administration on uterus tissue in female rats exposed to Cd. On the contrary, the protective effects of Lp were shown in different organs and animals in previous studies [20, 40, 41, 60].

5. Conclusion

The results of the study show that Lp can be used as a potential metal-binding agent for the elimination of Cd toxicity, especially in the blood, and provide notable findings for its use against metal intoxications in females. Therefore, further scientific researches are needed for the clarification of the abovementioned crucial interactions.

Conflict of interest

The authors of this study have no conflicts of interest.

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