

The influence of alive or heat - killed *Limosilactobacillus reuteri* administration on some serum antioxidant and cytokine levels in rats exposed to cadmium

Efecto de la administración de *Limosilactobacillus reuteri* vivo o muerto por calor en los niveles de algunos antioxidantes y citocinas en la suero de ratas expuestas al cadmio

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ABSTRACT

This study aimed to examine the effects of alive or heat - killed *Limosilactobacillus reuteri* (*L. reuteri*) administration on some blood antioxidant and cytokine levels in rats exposed to chronic cadmium toxicity. For this purpose, rats (n = 48) were randomly separated into six equal groups : cadmium, alive *L. reuteri*, cadmium + alive *L. reuteri*, dead *L. reuteri*, cadmium + dead *L. reuteri*, and control. cadmium was administered orally to the cadmium, cadmium + alive *L. reuteri*, and cadmium + dead *L. reuteri* groups three times a week for four weeks. Also, alive or heat - killed *L. reuteri* were administered orally to alive *L. reuteri*, dead *L. reuteri*, cadmium + alive *L. reuteri*, and cadmium + dead *L. reuteri* groups at the same periods. After the treatment period, serum samples were obtained from the animals and analyzed for serum cadmium and glutathione peroxidase levels, catalase and superoxide dismutase enzyme activities, malondialdehyde concentration, and tumor necrosis factor - alpha (TNF - α), interleukin - 6 (IL - 6), IL - 10, and IL - 1beta (β) levels. The administration of alive or heat - killed *L. reuteri* suspension decreased the cadmium accumulation in serum samples of alive *L. reuteri* and dead *L. reuteri* (especially) group animals compared to the cadmium group (P < 0.05). While the administration of alive *L. reuteri* lowered serum superoxide dismutase enzyme activity, the dead *L. reuteri* decreased glutathione peroxidase levels in comparison to the control group (P < 0.05). In conclusion, alive and dead *L. reuteri* strains had varied protective effects on the serum cytokine levels of rats, but their effects on oxidative stress indicators were limited.

Key words: Cadmium; cytokines; *L. reuteri*; rat.

RESUMEN

El propósito de este estudio fue examinar cómo la administración de *Limosilactobacillus reuteri* (*L. reuteri*), ya sea en su forma viva o muerta por calor, influye sobre los niveles de ciertos antioxidantes y citocinas en la sangre de ratas expuestas a la toxicidad crónica del cadmio. Para ello, se separó aleatoriamente a las ratas (n = 48) en seis grupos homogéneos : cadmio, *L. reuteri* vivo, cadmio + *L. reuteri* vivo, *L. reuteri* muerto, cadmio + *L. reuteri* muerto y grupo de control. Se administró cadmio por vía oral a los grupos cadmio, cadmio + *L. reuteri* vivo y cadmio + *L. reuteri* muerto tres veces por semana durante cuatro semanas. Se dio *L. reuteri*, ya sea vivo o muerto por calor, por vía oral a los grupos *L. reuteri* vivo, *L. reuteri* muerto, cadmio + *L. reuteri* vivo y cadmio + *L. reuteri* muerto durante el mismo tiempo. Tras el periodo de tratamiento, se obtuvieron muestras de suero de los animales. Se estudiaron los niveles de cadmio y glutatión peroxidasa en la sangre, así como las actividades de las enzimas catalasa y superóxido dismutasa. Se evaluaron también los niveles de malondialdehído (MDA) y los niveles de factor de necrosis tumoral alfa (TNF - α), interleucina - 6 (IL - 6), IL - 10 e IL - 1 β . La administración de *L. reuteri*, ya sea vivo o muerto por calor, redujo la acumulación de cadmio en muestras de suero de los animales de los grupos *L. reuteri* vivo y *L. reuteri* muerto (especialmente) en comparación con el cadmio (P < 0,05). La administración de *L. reuteri* vivo redujo la actividad de la enzima superóxido dismutasa en suero, mientras que, el *L. reuteri* muerto disminuyó los niveles de glutatión peroxidasa en comparación con el grupo control (P < 0,05). En conclusión, las cepas vivas y muertas de *L. reuteri* tuvieron diversos efectos protectores sobre los niveles séricos de citocinas en ratas, pero sus efectos sobre los indicadores de estrés oxidativo se limitaron.

Palabras clave: Cadmio; citocinas; *L. reuteri*; rata.

INTRODUCTION

Cadmium (Cd) toxicity is one of the most common cases of toxic exposure to heavy metals worldwide, particularly in industrialized countries. Worldwide, it has a detrimental effect on human health, animals, and the ecosystem [1]. Cd is released into the environment through natural causes such as volcanic eruptions, forest fires, soil erosion, sea salt aerosols, and the chemical / physical breakdown of rocks containing Cd [2, 3].

The classification of Cd as a toxic, carcinogenic, and stimulant element has resulted in extensive study of its biological significance over the past few decades [4]. The widespread availability of Cd in air, food, feed, and water is an important problem regarding human and animal health. Previous studies have estimated that heavy metals pollute 13 % of farmland and 40 % of the world's rivers and lakes [5].

The binding of Cd to blood erythrocytes (RBC) and albumine (Alb) following its entry into an organism through inhalation, food, feed, or water results in the reaching of different important tissues and organs. Then, it triggers the production of metallothionein mRNA by binding to low molecular weight proteins to form metallothioneins. It enters the bloodstream following renal glomerular filtration processes and is reabsorbed by proximal epithelial cells [6].

Numerous studies suggest that prolonged exposure to Cd can result in numerous diseases that affect humans, including Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, multiple sclerosis, diabetes, cardiovascular disease, decreased kidney function, pancreatic damage, and cancer [7, 8, 9, 10].

Prior research on the topic has revealed that exposure to Cd results in the production of reactive oxygen species (ROS), which subsequently causes oxidative damage to cells. Additionally, it has been demonstrated that Cd mostly binds to antioxidant enzymes that include thiols, which lowers the amounts of these enzymes in the living organism [11]. It has also been observed that Cd exposure affects the malondialdehyde (MDA) concentrations and some antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), in living beings [12, 13].

The process of inflammation is complicated and multifaceted. It is an organism's defense mechanism against injury (from physical stimuli, chemicals, etc.) or infection (from microbes). Its functions include removing the agents that cause harm, preventing tissue damage, starting the healing process, and reestablishing the physiological functions of the tissue or organ that is impacted by the inflammatory process [14].

It has been found that oxidative stress (OS) and inflammation are intimately associated with each other. Previous studies have indicated that Cd causes OS and also affects certain cytokine levels such as tumor necrosis factor alpha (TNF - α), interleukin - 6 (IL - 6), IL - 10, and IL - 1 - beta (β) in different tissues of the body [15, 16].

A variety of approaches have been investigated to reduce the toxicity of Cd, with an increasing amount of focus being placed on the use of chemical agents, herbal remedies, and detoxification strategies [17, 18].

Heavy metals can be eliminated from the body cost-effectively and in an environmentally friendly way using microbiological methods. Microbiological procedures indicate that numerous benefits encompass enhanced processing ability, high environmental adaptability, cost - effectiveness, and ecological sustainability. The cell walls of fungi contain polyphosphoric acid, chitin and other ingredients that can react with elements such as heavy metals. Also, fungi are microorganisms that have the capacity to eliminate heavy metals that are present in the natural environment [19, 20].

In addition to fungi, certain beneficial microorganisms of food origin have attracted attention for their detoxification capabilities. Lactic acid bacteria (LAB), particularly *L. reuteri*, are frequently employed as probiotics in fermented milk and non - dairy foods. These microorganisms not only help to preserve food and increase nutritional quality, but they also improve food safety by lowering hazardous chemicals such as mycotoxins and heavy metals. Therefore, it is important to investigate the ability of *L. reuteri* to regulate cadmium toxicity in the context of public health and food safety [21, 22].

In recent years, researchers have been reported that specific strains of LAB possess the capability to bind and detoxify Cd in both in - vitro and in - vivo circumstances [23]. It was suggested that *Lactobacillus* and *Bifidobacterium* probiotic strains that decrease systemic absorption by binding Cd ions in the gastrointestinal tract [24, 25].

Limosilactobacillus reuteri (*L. reuteri*), which was previously classified within the *Lactobacillus* genus, is naturally found in various systemic areas, including the digestive system, urinary system, skin, and mammary tissue. The amount of *L. reuteri* has been shown to vary from person to person and depending on individual circumstances. Many *L. reuteri* strains have been shown to play different roles in various diseases, such as hypercholesterolemia, skin infections, allergy - induced asthma, periodontitis, and autism spectrum disorders. For example, in allergy-related asthma, *L. reuteri* has been reported to reduce airway inflammation by modulating the immune response. This is reportedly enhancing anti - inflammatory pathways and influencing cytokine balance. It has been suggested that *L. reuteri* supports the immune system in treating skin infections by producing bioactive compounds that exhibit antimicrobial activity against pathogenic microorganisms. It has been reported that, in conditions of periodontitis, it may contribute to the control of dysbiosis by producing antimicrobial compounds such as 3 - hydroxypropionaldehyde, thereby balancing the oral biofilm and improving the balance between pathogenic and commensal microbiota, as well as reducing plaque formation. In addition, it is suggested that it modulates the immune response by reducing levels of the pro-inflammatory cytokines IL - 1 β , TNF - α and IL - 6 while increasing the anti - inflammatory cytokine IL - 10; furthermore, it may reduce the inflammatory response by inhibiting the Nuclear Factor kappa B (NF - κ B) signalling pathway. It is thought that these mechanisms may result in a reduction in periodontal inflammation and tissue degeneration. In addition, they have been found to act as critical probiotics, particularly in gastrointestinal disorders [26, 27, 28].

It has been reported that *L. reuteri* may be effective in the prevention of Cd toxicity in living beings [29]. However, the effect of *L. reuteri* on specific serum cytokine and antioxidant levels in rats (*Rattus norvegicus*) chronically exposed to Cd has not been determined. In this context, the aim of this study was to determine whether *L. reuteri* (alive or dead) had a protective

effect on certain serum cytokine and antioxidant levels in rats exposed to chronic Cd toxicity.

MATERIAL AND METHODS

Ethical committee approval

In the present study, all research procedures carried out on animals were approved by the Experimental Animal Ethics Committee of Balikesir University (Approval no: 2024 / 11 – 11).

Assessment of resistance to Cd

The *L. reuteri* strain exhibiting the strongest resistance to Cd was utilized in this study. Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) were determined for four different *L. reuteri* strains. Each strain was grown in de Man, Rogosa and Sharpe (MRS) broth (Merck, Darmstadt, Germany) supplemented with different concentrations of cadmium chloride (CdCl₂), following the method described by Zhai *et al.* [30] with minor modifications.

Cadmium concentrations ranging from 0.1 to 200 mg / L were used in order to cover a wide range including environmentally relevant levels and concentrations previously reported to affect lactic acid bacteria growth and cadmium interactions with probiotic bacteria [31, 32]. All required concentrations were prepared by ten - fold serial dilutions from a 1.000 mg / L CdCl₂ stock solution, resulting in progressively decreasing Cd concentrations. Each dilution was inoculated with *L. reuteri* strains and incubated at 37 °C for 24 - 48 h in an incubator (Memmert, model E412.0361, Schwabach, Germany).

Bacterial growth was monitored by visual turbidity and optical density measurements at 600 nm (OD₆₀₀) using a microplate spectrophotometer (SPECTROstar Nano, BMG LABTECH GmbH, Ortenberg, Germany). MIC was defined as the lowest Cd concentration that inhibited observable bacterial growth, whereas MLC was determined as the lowest concentration resulting in no growth after subculturing onto fresh Cd - free MRS agar plates. Each experiment was performed in triplicate.

At the end of the incubation period, *L. reuteri* DSM 17938, which showed growth at the highest Cd concentration, was identified as the most Cd - resistant strain and was selected for use in subsequent experiments

Preparation of live and heat - inactivated *L. Reuteri*

L. reuteri was cultivated in 10 mL of de MRS broth and incubated (Memmert, model E412.0361, Schwabach, Germany) at 37 °C for 24 h. At the end of the incubation period, bacterial cultures were centrifuged (Hermle Z380, Germany) at 4200 × g for 5 minutes (min) at 4 °C using a refrigerated centrifuge (Hermle Z380, Germany). The supernatants were discarded and the bacterial pellets were resuspended in 10 mL of sterile skim milk solution (Merck, 115363, Darmstadt, Germany).

For preparation of live *L. reuteri*, the bacterial suspension was adjusted to obtain a final concentration of approximately 10⁹ - 10¹⁰ cfu/mL and directly used as the experimental treatment. The selected bacterial dose (10⁹ - 10¹⁰ cfu/mL) was chosen based on previous studies evaluating the detoxification and binding

capacity of *Lactobacillus* strains and represents a concentration commonly used in probiotic intervention experiments [31, 33].

For preparation of heat - inactivated *L. reuteri*, the bacterial suspension was subjected to heat treatment at 121 °C for 15 min using an autoclave (Hirayama, Hiclave HV85, Japan). Following heat treatment, the suspensions were cooled to room temperature and used as the dead - cell treatment. The absence of bacterial growth after heat treatment was confirmed by plating on MRS agar.

Animals and study design

Study design and administration of Cd were performed according to the method described before by Kisadere *et al.* [17], Almenara *et al.* [34], and Wardani *et al.* [35]. In the present study, 48 healthy male Wistar albino rats (~ 12 - 16 weeks old, weighing (Kern, EW 620 - Germany) 200 ± 30 g) obtained from Balikesir Experimental Animal Production Center, were used. The rats were kept under conventional laboratory conditions (standard plastic rat cages, 12 hours light / 12 hours dark, 23 ± 2 °C, 55 ± 10 % relative humidity, *ad libitum* feeding and watering) during the experiment. The rats were randomly divided into six equal groups : Cd, the alive *L. reuteri* (ALR), the Cd + ALR, the dead *L. reuteri* group (DLR), Cd + DLR, and the control (C) group.

1. Cd ($n = 8$) : CdCl₂ (2 mg / kg / d) was administered to animals via gastric gavage three times a week for four weeks [17, 25].
2. ALR ($n = 8$) : Alive *L. reuteri* solution (containing ~ 10⁹ - 10¹⁰ cfu / mL) was administered to animals via gastric gavage three times a week for four weeks [31, 33].
3. Cd + ALR ($n = 8$) : CdCl₂ (2 mg / kg / d) and alive *L. reuteri* solution (containing ~ 10⁹ - 10¹⁰ cfu / mL) were administered to animals via gastric gavage three times a week for four weeks [17, 25, 31, 33]. At this stage, the rats were first administered CdCl₂ and then, without any waiting time, ALR.
4. DLR ($n = 8$) : Dead *L. reuteri* solution (containing ~ 10⁹ - 10¹⁰ cfu / mL) was administered to animals via gastric gavage three times a week for four weeks [31, 33].
5. Cd + DLR ($n = 8$) : CdCl₂ (2 mg / kg / day) and dead *L. reuteri* solution (containing ~ 10⁹ - 10¹⁰ cfu / mL) were administered to animals via gastric gavage three times a week for four weeks [17, 25, 31, 33]. At this stage, the rats were first administered CdCl₂ and then, without any waiting time, DLR.
6. C ($n = 8$) : The physiological saline solution was administered to animals via gastric gavage three times a week for four weeks.

The rats were given the treatment by way of an oral gavage, with a volume of 1 mL/kg body weight (b.w.). Following the 28 - d experiment, a cardiac puncture was performed to obtain blood samples from the rats' hearts for the study. Rats were euthanised under anaesthesia using an intramuscular injection of Ketamine / Xylazine (0.1 mL / 100 g b.w.) at the end of the 4 - week experimental period. Five milliliters of blood were drawn and placed in tubes without anticoagulants. Centrifugation was used to separate the serum samples (Hermle Z380, Germany, 3500 rpm, 15 min). Before analysis, serum samples were stored at -80 °C (Arçelik 270530EB, Türkiye).

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Assessment of Cd levels in serum samples

The determination of serum Cd levels was carried out using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Perkin Elmer Optima 7300, USA) device in accordance with the methodologies established by Altundag and Tüzen [36] and Tokay and Bağdat [37]. Serum samples (1 mL) were taken into teflon containers. After, H₂O₂ (2 mL) and HNO₃ (5 mL) solutions were added to these containers. Then, the teflon containers were heated to 90 °C. Clear solutions were obtained by continuous mineralization. Clear samples were transferred to balloon vials and diluted to 10 mL with ultrapure water.

Assessment of serum cytokine and antioxidant levels

The serum concentrations of TNF - α [(Bioassay Technology Laboratory (BT Lab), E0764Ra, China)], IL -1β [(BT Lab, E0119Ra, China)], IL - 6 [(BT Lab, E0135Ra, China)], and IL - 10 (BT Lab, E0108Ra, China) were quantified utilising commercially available ELISA kits in line with the protocol outlined by the manufacturer's by using an ELISA reader (SPECTROstar Nano, Germany).

The concentrations of MDA (BT Lab, E0156Ra, China), GPX values (BT Lab, E1172Ra, China), SOD (BT Lab, E1444Ra, China), and CAT (BT Lab, E0869Ra, China) enzyme activities were quantified utilizing commercially available ELISA kits in accordance with the manufacturer's instructions and an ELISA reader (SPECTROstar Nano, Germany).

Statistical analysis

The data was statistically analyzed using SPSS 26 (IBM SPSS Statistics for Windows, Version 22.0, Armonk, New York: IBM Corporation). The mean ± SEM was used to show all data. The Shapiro - Wilk test was employed to assess the distribution characteristics of variables and the homogeneity of variance. Parameters were assessed using analyses of variance (ANOVA) and subsequently evaluated with Duncan's test. A P -value of less than 0.05 indicated statistical significance for the collected data.

RESULTS AND DISCUSSION

In the Cd group, serum Cd levels were found to be significantly higher than in the other groups (P < 0.05). On the other hand, Cd + ALR showed significantly higher serum Cd levels than Cd + DLR in the present study (P < 0.05). Serum concentrations of Cd were not significantly different within the C, ALR, and DLR groups (P > 0.05). The serum Cd levels of the animals are shown in FIG 1.

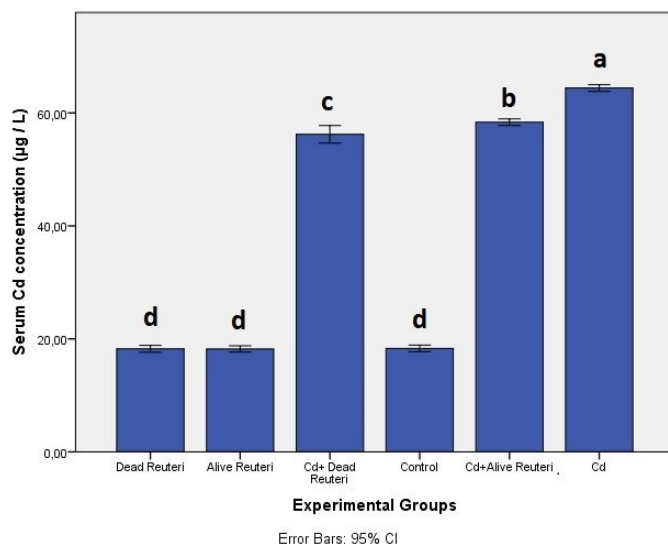


FIGURE 1. Serum Cd levels in rats. ^{a, b, c, d} The bars exhibit a significant difference (P < 0.05) in the superscripts. Cd : Cadmium; µg : Microgram; L : Liter.

Tumor necrosis factor alpha (TNF - α) levels were determined to be higher in the Cd group compared to the C group (P < 0.05). Although the administration of ALR and DLR alone increased TNF - α levels when compared to the C group, this increase was lower than that observed in the Cd group. In addition, the Cd + ALR and Cd + DLR groups observed a decrease in TNF - α levels; however, this difference was not considered statistically significant (P > 0.05). When compared to the C group, IL - 6 levels were significantly higher in the Cd group (P < 0.05). DLR group showed higher IL - 6 levels than ALR group. On the other hand, when compared to the Cd group, the Cd + ALR and Cd + DLR groups showed a decrease in IL - 6 levels that was statistically significant (P < 0.05).

In this study, IL - 10 levels were determined higher in the C group compared to other experimental groups (P < 0.05). The IL - 1β level in the Cd group was lower than that in the C and ALR groups. On the other hand, IL - 1β levels in the Cd + ALR group were elevated compared to the Cd group and comparable to those in the C group (P < 0.05). In contrast, Cd + DLR did not significantly improve IL - 1β levels in rats. The levels of serum cytokines are presented in TABLE I.

TABLE I
Some serum cytokine levels of the rats

Groups	n	TNF - α (ng / L)	IL - 6 (ng / L)	IL - 10 (pg / mL)	IL - 1β (pg / mL)
C	8	1.52 ± 0.24 ^c	0.66 ± 0.11 ^d	267.82 ± 72.09 ^a	47.55 ± 2.80 ^o
ALR	8	2.31 ± 0.02 ^{ab}	0.95 ± 0.09 ^c	144.86 ± 4.63 ^b	50.04 ± 7.53 ^a
DLR	8	2.04 ± 0.11 ^b	1.23 ± 0.05 ^{bc}	100.17 ± 18.60 ^b	35.03 ± 5.33 ^{ab}
Cd	8	2.56 ± 0.12 ^a	2.02 ± 0.11 ^a	116.75 ± 8.82 ^b	21.42 ± 3.26 ^b
Cd + ALR	8	2.32 ± 0.16 ^{ab}	1.08 ± 0.06 ^{bc}	88.17 ± 12.42 ^b	42.79 ± 4.53 ^a
Cd + DLR	8	2.31 ± 0.04 ^{ab}	1.30 ± 0.12 ^b	86.82 ± 8.05 ^b	25.02 ± 4.80 ^b

It means that different letters in the same column are significantly different (P < 0.05). C: Control, ALR: Alive L. reuteri group, DLR : Dead L. reuteri group, Cd : Cadmium, Cd + ALR: Cadmium + Alive L. reuteri group, Cd + DLR : Cadmium + Dead L. reuteri group. TNF - α : Tumor Necrosis Factor - Alpha; IL - 6 : Interleukin - 6; IL - 10 : Interleukin - 10; IL - 1β : Interleukin 1 - Beta; n : Number of animals; ng : Nanograms; L : Liter; pg : Pictograms; mL : Milliliter.

In terms of serum MDA and CAT levels, no significant differences were observed between the groups ($P > 0.05$). In contrast, the group that obtained Cd administration showed a significant decrease in GPx levels, although group C presented the highest levels

The GPx levels in the Cd + ALR and Cd + DLR groups were similar to those in the Cd group and did not show statistically

significant increases. The C group showed the highest SOD levels, while the Cd group exhibited the lowest, with statistically significant differences observed in comparison to the other groups. Furthermore, when compared to the C group, the ALR administration demonstrated significantly lower levels, but when compared to the Cd group, it showed significantly higher levels. Serum oxidant - antioxidant levels are presented in TABLE II.

TABLE II
MDA concentrations and some serum antioxidant levels in the experimental groups

Groups	n	MDA (nmol / mL)	GPX (ng / mL)	SOD (ng / mL)	CAT (ng / mL)
C	8	1.32 ± 0.14	2000.00 ± 488.25 ^a	22.56 ± 3.37 ^a	12.43 ± 5.65
ALR	8	1.25 ± 0.16	1385.00 ± 287.36 ^{ab}	15.30 ± 1.63 ^b	10.73 ± 3.05
DLR	8	1.48 ± 0.02	975.00 ± 15.08 ^b	12.76 ± 1.48 ^{bc}	7.71 ± 0.26
Cd	8	1.69 ± 0.22	898.75 ± 42.71 ^b	6.77 ± 0.43 ^c	7.36 ± 2.11
Cd + ALR	8	1.67 ± 0.23	1042.50 ± 27.17 ^b	12.43 ± 3.20 ^{bc}	9.53 ± 0.58
Cd + DLR	8	1.57 ± 0.08	992.50 ± 54.23 ^b	8.74 ± 2.08 ^{bc}	7.45 ± 1.14

It means that different letters in the same column are significantly different ($P < 0.05$). C: Control, ALR: Alive *L. reuteri* group, DLR: Dead *L. reuteri* group, Cd: Cadmium, Cd + ALR: Cadmium + Alive *L. reuteri* group, Cd + DLR: Cadmium + Dead *L. reuteri* group. MDA: Malondialdehyde; SOD: Superoxide Dismutase; GSH: Glutathione; CAT: Catalase; n: Number of animals; nmol: Nanomol; ng: Nanograms; mL: Milliliter.

It has been suggested that Cd exacerbates metabolic dysfunction and tissue damage by upregulating ROS production and encouraging chronic inflammatory responses, which upset cellular homeostasis [38]. In the present study, serum Cd levels considerably elevated in the Cd group (2 mg / mL for 4 weeks, peros (p.o.)) compared to the other experimental groups. Similarly, Kisadere *et al.* [25] suggested that Cd administration (2.04 mg / mL for 28 days, p.o.) increased the serum Cd levels (83.96 ± 8.48 µg / L) in female Wistar rats. In addition, it was reported by Andjelkovic *et al.* [39] that serum Cd levels rose to 5.42 ± 0.88 and 8.97 ± 1.16 µg / L as a result of a single oral dose of Cd (15 and 30 mg / kg) administration.

The outcomes demonstrate that the utilized dosage regime raises blood Cd levels in a manner consistent with findings from prior investigations. On the other hand, serum Cd levels decreased in the Cd + ALR and Cd + DLR groups after the alive or dead *L. reuteri* administrations in this study. This reduction was observed significantly greater in Cd + DLR group than Cd + ALR group. Zhai *et al.* [40] confirmed that increased blood Cd levels (6.32 ± 4.44 µg / L) following the Cd administration (100 mg / L CdCl₂) were ameliorated by using the probiotic *L. plantarum* CCFM8610 (1 × 10⁹ cfu, administered orally for eight weeks) in rats. In a previous study, it was reported that *L. plantarum* administration led to a decrease of serum Cd levels in female Wistar rats [25]. The changes may have been observed due to the metal - binding activity of DLR (especially) and ALR.

Recently, researchers have reported that Cd administration alters the levels of various pro - and anti - inflammatory cytokine levels in the blood [41]. In the immune system, IL - 6 is a multifunctional cytokine that exhibits both pro - and anti - inflammatory activities. It typically exists in the bloodstream in small quantities, but when the organism is exposed to harmful substances, toxic chemicals, infections, or tissue injury, its levels rise dramatically [42, 43].

The levels of IL - 6 were determined to be the highest in the Cd group (2.02 ± 0.11 ng / L) when compared to the C group (0.66 ± 0.11 ng / L) in the present study. The results were in line with the

results of past studies on IL - 6 levels [29, 44, 45, 46, 47]. On the contrary, Kisadere [41] found that administration of Cd did not change the blood IL - 6 levels in male Wistar rats, interestingly. These differences may be influenced by factors such as gender, dose, time, or administration method [23].

In this study, increased IL - 6 levels (2.02 ± 0.11 ng / L) due to Cd administration were ameliorated by using alive or dead *L. reuteri* in the Cd + ALR (1.08 ± 0.06 ng / L) and Cd + DLR (1.30 ± 0.12 ng / L) groups. Furthermore, this results aligned with the findings of previous studies [44, 45, 46, 47]. The protective effects of *L. plantarum* and *L. reuteri* administrations on IL - 6 levels in the other tissues have also been demonstrated in previous studies [29, 48].

It could be attributed to the immune system - stimulating actions of probiotic - derived structural components. TNF - α, a significant proinflammatory cytokine, is primarily produced by macrophages, monocytes, T lymphocytes, and natural killer (NK) cells. It induces an acute inflammatory response in circumstances of infection or tissue damage [49].

Besides, IL-1β (a proinflammatory cytokine that increases the inflammatory response with TNF - α) plays a crucial role in the body's initial immune response against infection or tissue damage. It is generally produced by monocytes and macrophages, and is crucial for beginning and continuing inflammation [50].

In this study, the administration of Cd increased TNF - α levels but decreased IL - 1β levels in Wistar rats. Interestingly, Li *et al.* [46] suggested that Cd administration (5 mg / kg for 28 d) caused to increase in both TNF - α and IL - 1β levels in rats. Conversely, it was reported that TNF - α and IL - 1β levels were not affected by Cd administration in female rats [25].

On the other hand, Kisadere [41] also concluded that TNF - α levels increased in Wistar rats after CdCl₂ administration (at a dose of 2 mg / kg / day for four weeks). The dose and duration

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of Cd administration, and the use of different commercial kits may explain the differences in the results [51, 52]. This study demonstrated that *L. reuteri* administration, as a probiotic, exhibited a tendency to decrease TNF - α levels in the Cd + ALR and Cd + DLR groups.

In addition, TNF - α levels decreased after the administration of DLR alone in the present study. Also, it was determined that administration of ALR and Cd + ALR increased IL - 1β levels, thereby eliminating the inhibitory effect of Cd. However, it was found that neither DLR nor Cd + DLR administration increased IL - 1β levels in the present study. Moreover, Sajjad *et al.* [29] reported that increased levels of TNF - α and IL - 1β depended on the Cd administration (2 mg / kg) were ameliorated by the *L. reuteri* administration in the breast cancer - detected mice (*Mus musculus*).

Similarly, Li *et al.* [53] found that a novel polysaccharide derived from *Lactobacillus* - fermented *Nostoc commune* Vauch. reduced IL - 6, IL - 1β , and TNF - α levels and mitigated Cd - induced kidney injury in rats. These obtained results indicate that *L. reuteri* has a limited inhibitory effect on inflammation caused by Cd. In addition, alive probiotics can play a regulatory and balancing role in the immune system.

The main function of the cytokine IL - 10 is to reduce inflammation. This characteristic prevents the production of pro - inflammatory cytokines such IL - 1β , IL - 6, and TNF - α . It protects the organism from tissue damage and stops an overabundance of immune response activation [54]. In this study, the C group's higher IL - 10 levels (267.82 \pm 72.09 pg / mL) in comparison to the other groups is suggestive of the system's natural responses that reduce inflammation in physiological conditions.

On the other hand, Cd administration led to a decrease of rat's IL - 10 levels in the present study. Similarly, Cd exposure significantly suppressed the IL - 10 secretion of rats in previous studies [25]. These outcomes might have been brought on by Cd's immunosuppressive function. On the other hand, administration of either the alive (144.86 \pm 4.63 pg / mL) or dead forms (100.17 \pm 18.60 pg / mL) of *L. reuteri* resulted in a significant decrease of IL - 10 levels in rats compared to the C group (267.82 \pm 72.09 pg / mL) in this study. Sajjad *et al.* [29] reported that IL - 10 levels (60.2 \pm 2.0 pg / mL) decreased with the administration of cadmium + tamoxifen + *Lactobacillus reuteri* (20.8 \pm 1.1 pg / mL) in rats. Also, Kisadere *et al.* [25] reported that *L. plantarum* administration decreased the increased serum IL - 10 levels after Cd administration in female Wistar rats. It may be expressed that probiotics have immunomodulatory properties in both their alive and dead forms.

It has been reported that Cd exposure increases ROS and OS parameters by reducing intracellular glutathione (GSH) levels and inhibiting the action of antioxidant enzymes such as SOD and CAT in living beings [55]. MDA, one of the important markers of lipid peroxidation, concentration was not affected by the different administrations in the present study.

Conversely, Cd administration increased the serum MDA concentrations in rats in the previous studies [25, 56, 57]. The findings may have varied depending on the Cd dose, administration period, individual characteristics, or utilized commercial kits. Similar to Cd administration, alive or dead *L. reuteri* treatments did not cause any changes in the serum MDA concentration of rats in the present study. This result

may indicate that the effect of *L. reuteri* administration on lipid peroxidation products is limited. In addition, Cd administration led to a decrease in serum GPx levels (898.75 \pm 42.71 ng / mL) of Cd group animals compared to C group (2000.00 \pm 488.25 ng / mL) in this study.

Galazyn-Sidorczuk *et al.* [58] and Poli *et al.* [59] also determined similar results to this study regarding GPX levels in rats' serum and kidney tissues exposed to Cd. On the other hand, administration of neither alive nor dead *L. reuteri* caused any crucial change in GPX levels of the Cd + ALR (1042.50 \pm 27.17 ng / mL) and Cd + DLR (992.50 \pm 54.23 ng / mL) groups compared to the Cd group (898.75 \pm 42.71 ng / mL) in the present study.

On the contrary, Banwo *et al.* [23] and Li *et al.* [53] observed protective effects of different types of *Lactobacillus* administrations (*Lactiplantibacillus plantarum* ML05 and *Lactobacillus* - fermented *Nostoc commune* Vauch) in the tissue GPX and GSH levels in rats exposed to Cd. Furthermore, Kisadere *et al.* [25] reported that the administration of *L. plantarum* did not affect the serum GSH levels in female rats. Although serum CAT enzyme activity tended to decrease in the Cd group compared to the other groups, it did not cause significant change in this study.

Similarly, it was suggested that administration of Cd (2.04 mg / mL for 28 days, p.o.) did not influence the serum CAT enzyme activities in female rats [25]. In contrast, serum CAT activities in Sprague Dawley female rats were found to be reduced as a result of Cd administration by Xue *et al.* [60].

Also, numerous studies demonstrated that Cd adversely affected the CAT enzyme activity in various tissues and organs [59, 61]. The dose of Cd administration, different genders, or individual properties may explain the different results.

On the other hand, alive or dead *L. reuteri* administration could not affect the serum CAT enzyme activities in the Cd + ALR and Cd + DLR groups compared to only Cd treated group in the present study. In a previous study, Tian *et al.* [48] suggested that Lp TW1 - 1 ameliorated the enzymatic activities of CAT in the serum of diethylhexylphthalate - administered male mice.

These findings are compatible with Kisadere *et al.* [25]'s results regarding to serum CAT enzyme activity. The different antioxidant properties of probiotic strains may explain the various results. In the present study, Cd administration decreased the serum SOD enzyme activity in male Wistar rats.

On the contrary, Kisadere *et al.* [25] confirmed that Cd administration did not lead to an alteration in serum SOD levels in female Wistar rats. Suhartono *et al.* [62] also found similar results to this study regarding to SOD enzyme activity in male Sprague - Dawley male rats exposed to Cd. In other investigations, it has also been established that Cd suppresses the activity of the SOD enzyme in different tissues [45, 63, 64].

In this study, SOD enzyme activity was found markedly lower in the ALR group (15.30 \pm 1.63 ng/mL) when compared to C group (22.56 \pm 3.37 ng / mL), interestingly. It was found that SOD activity was significantly reduced in the CD group (6.77 \pm 0.43 ng/mL) compared to group C (22.56 \pm 3.37 ng/mL). It is thought that this is due to the suppression of the antioxidant defence system resulting from cadmium-induced oxidative

stress. Additionally, the decrease in SOD enzyme activities caused by Cd could not be ameliorated in the Cd + ALR and Cd + DLR groups by the administration of live or dead *L. reuteri*.

Moreover, it was determined that *L. plantarum* administration did not cause any influence on the serum SOD levels of rats exposed to Cd [25]. In contrast, *Lactiplantibacillus plantarum* ML05 and *Lactobacillus* - fermented *Nostoc commune* Vauch administrations showed a positive reversal impact on SOD enzyme activities in animals (rats and mice) whose kidney and liver tissues were exposed to Cd toxicity [23, 53]. This may be due to the low binding rate of Cd ions by *L. reuteri* in the gastrointestinal tract compared to other bacterial strains (*Lactobacillus* and *Bifidobacterium* probiotic strains) [24].

Due to the fact that *L. reuteri* strains are food - derived and are frequently employed as probiotics in the fermentation of dairy products, the Cd binding capacity and effects on cytokine modulation observed in this study indicate a potential role in the enhancement of the safety of food contaminated with heavy metals, as well as in the maintenance of health. These probiotic applications can be regarded as a biotechnological approach to reducing toxic metal residues in the food chain that is both environmentally friendly and food compatible.

CONCLUSIONS

The investigation of the beneficial effects of probiotics can be extended by using cadmium as a toxic substance.

The findings of this investigation showed that dead *L. reuteri* can bind to Cd especially in the bloodstream.

Although the effect on markers of oxidative stress is limited, when cytokine levels are taken into account, the protective effect of both live and dead *L. reuteri* is clear.

Further studies at both the molecular and cellular levels are necessary to better understand the protective effect of *L. reuteri* strains in Cd toxicity. *L. reuteri*, a food - derived bacterium frequently used in functional and fermented foods, shows Cd binding capacity and immunomodulatory effects, suggesting potential applications in the development of probiotic foods and food safety.

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Conflicts of interest

The authors of this article do not have potential conflicts of interest.

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