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ORIGINAL ARTICLE

Protection of probiotic *Bacillus coagulans* and *Lactobacillus plantarum* against cadmium toxicity in rats

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Abstract

Cadmium (Cd) is an environmental pollutant that can cause harmful effects on human and animal health due to its transfer to the food chain. Our previous work showed that probiotic candidates of *Bacillus coagulans* and *Lactobacillus plantarum* can relieve mercury oral harmful effects in rats. Hence, this follow-up study investigated the effect of these two bacteria on chronic cadmium toxicity. Forty-eight Wistar rats have been studied for 48 days, so that cadmium receiving groups received 100 µg/mL of cadmium chloride per day through gavage. Daily administration of 1×10^9 CFU/spore/mL of *B. coagulans* and *L. plantarum* bacteria increased cadmium excretion through feces and reduced the metal absorption in liver and kidney tissues. The probiotic treatment has led to the revival of superoxide dismutase (SOD) and glutathione peroxidase (GPx) as oxidative stress parameters in rats that have been significantly reduced due to cadmium exposure. Both probiotics also reduced renal and hepatic damage bio-markers including urea, creatinine, bilirubin, alanine transaminase (ALT), and aspartate transaminase (AST) in rats'' blood. The results summarize the potential protective effects of probiotic bacteria *B. coagulans* and *L. plantarum* against cadmium toxicity.

Keywords Cadmium · *Bacillus coagulans* · *Lactobacillus plantarum* · Oxidative stress · Rat

Introduction

Cadmium is a heavy metal that enters the human food chain due to a large amount of transfer from soil to plants; therefore, the diet is the primary source of exposure for non-smokers and those who are not exposed to cadmium (Clemens 2006; Franz et al. 2008). Insufficient protection of food and industrial pollution from contamination with cadmium caused a significant increase in the prevalence of its chronic toxicity (Nordberg et al. 2014).

The most undesired effects of cadmium occur in the liver and kidneys leading to hepatotoxicity and nephrotoxicity (Liu et al. 2015; Nordberg 2009), followed by skeletal (James and Meliker 2013), cardiovascular (Tellez-Plaza et al. 2012), reproductive (Thompson and Bannigan 2008), neural (Wang et al. 2016) and intestinal systems disorders (Jumarie et al. 1999; Blais et al. 1999). The suggested mechanism for cadmium toxicity is oxidative stress due to increase in reactive oxygen species (ROS) including superoxide and hydrogen peroxide ions and decrease in antioxidant defense system (Valko et al. 2006; Pathak and Khandelwal 2006; Oh and Lim 2006). The main strategy for the treatment of heavy metals toxicity is chelation therapy, which is associated with the side effects such as impairment of the absorption of essential metals required by the body and direct effect on physiological processes (Sears 2013; Gaur et al. 2014). In the host body system, gastrointestinal (GI) microbiota prevents the absorption of metals and only 40–60% of the entered metals can pass through the intestinal wall (Wester et al. 1992; Zubero et al. 2010).

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Probiotics are recognized as live microorganisms that if administered adequately, have beneficial effects on consumer's health (Hotel and Cordoba 2001). A significant number of in vitro (Li et al. 2017; Zhai et al. 2015; Milanowski et al. 2017; Yi et al. 2017; Gasong et al. 2017; Halttunen et al. 2007) and in vivo studies have reported the efficacy of probiotics (mainly lactic acid bacteria (LAB)) in absorption of heavy metals (Yi et al. 2017; Yu et al. 2017; Tian et al. 2012; Zhai et al. 2016).

In our previous study, we reported the potential of probiotics *Lactobacillus plantarum* and *Bacillus coagulans* in increasing mercury excretion through feces and decreasing the toxic effects of mercury in rats (Majlesi et al. 2017).

Considering the ability of *B. coagulans* and *L. plantarum* in the absorption of cadmium and their effect on the reduction of oxidative stress induced by heavy metals, the beneficial effects of these two probiotics on chronic cadmium toxicity have been investigated in rats.

Material and methods

Preparation and culture of bacteria

Lyophilized *L. plantarum* CNR 273 was provided from the Department of Food Science and Technology, Shiraz University, Iran. The strain was cultivated under constant conditions in MRS liquid medium at 37 °C for 48 h. Fresh cultures were washed with ultra-pure water several times and stored in lyophilized skim milk at − 20 °C to provide sufficient biomass. The live bacterial count in the biomass was approximately 1×10^9 CFU/mL before oral administration to the rat. Lyophilized *B. coagulans* was obtained from Pardis Roshd Mehregan Company, Iran, and incubated in NYSM medium (nutrient yeast extract salt medium) at 37 °C for 48 h. The suspension was then centrifuged three times at 3000 × g for 20 min and washed with sterilized normal saline. For the spore count, *B. coagulans* biomass was heated at 80 °C for 15 min and then cultured in a solid NYSM medium. The final spore suspension containing 1×10^9 Spore/mL was maintained in sterilized normal saline at 4 °C. The bacterial suspension for oral administration was prepared daily. Furthermore, groupings were applied to study the simultaneous effects of cadmium and probiotics. One mL of a double concentrated suspension (2×10^9 CFU/spore/mL and 200 µg/mL $CdCl_2$) (0.5 mL of each solution) was administrated to the rats by gavage tube.

Animal experiments

Forty-eight adult Wistar rats were obtained from Razi Vaccine and Serum Research Institute, Shiraz, Iran. The rats were placed in separate cages (eight in each cage) and quarantined for 1 week. They were held in an automatic 12 h of light/dark cycle under constant temperature (25 °C) and moisture conditions (60 ± 5%). Rats received normal rat chow and drinking water during the experiment. This protocol was approved by the State Committee for Animal Ethics, Shiraz University, Shiraz, Iran.

As shown in Table 1, 48 rats were randomly assigned to the control ($n=8$), cadmium (Cd), *B. coagulans* (BC), *L. plantarum* (LP), combined cadmium and *B. coagulans* (Cd + BC) and combined cadmium and *L. plantarum* (Cd + LP) groups. According to the literature, the concentration of cadmium chloride was 100 µg/mL, considered for oral administration to the rats (in the groups administered probiotic with cadmium simultaneously, the volume of cadmium solution was halved, and the concentration was doubled). The oral dose of $CdCl_2$ at a concentration of 100 µg/mL in drinking water is considered for environmental study as exposure to a low concentration of cadmium (Thijssen et al. 2007; Satarug and Moore 2004; Damek-Poprawa and Sawicka-Kapusta 2003; Thijssen et al. 2007). The solution containing $CdCl_2$ was prepared twice a week and a gavage tube was applied for administration of suspension containing cadmium and probiotics to the rats. Only normal saline and 100 µg/mL cadmium were given to the control and cadmium groups, respectively. The probiotic-treated groups received 1×10^9 CFU/mL of *L. plantarum* and 1×10^9 spore/mL of *B. coagulans* daily.

Blood and tissue samples

To take blood and tissue samples on the 24th and 48th days of the study, four rats were selected from each group and after anesthesia; blood samples were collected through heart puncture and euthanized with a gradual increase in CO_2 levels. In order to separate the serum, the blood sample was centrifuged at 3000 × g for 15 min, and the blood sera were stored at − 80 °C for further biochemical analysis. Livers and kidneys of sacrificed rats were excised, washed with cold normal saline, and stored at − 80 °C. Before killing the

Table 1 Treatment groups in the animal study

Groups	Gavage treatment (1 mL volume- once daily)
Control	Normal saline
Cd	$CdCl_2$ (100 µg/mL)
LP	*L. plantarum* (1×10^9 CFU/mL)
BC	*B. coagalans* (1×10^9 spore/mL)
Cd + LP	*L. plantarum* (1×10^9 CFU/0.5 mL + $CdCl_2$ (100 µg/0.5 mL)
Cd + BC	*B. coagulans* (1×10^9 spore/0.5 mL + $CdCl_2$ (100 µg/0.5 mL)

rats, they were individually placed in separate cages for 1 h, and the fresh feces samples were taken and stored at 4 °C.

Determine cadmium in tissues and feces

The liver, kidney, and feces samples were digested in concentrated HNO_3 with a microwave digestion system (MARS XPress), and cadmium concentration was then determined using a graphite furnace atomic absorption spectrophotometer (FIMS 400 Perkin Elmer Inc., USA). The cadmium content of feces and tissues was expressed in µg/g of the fresh weight of the sample.

Bacterial analysis of feces

Feces samples taken on days 24, and 48 were immediately transferred to the laboratory, kept at 4 °C and analyzed under sterile conditions within 2 h. Feces samples were diluted 1:10 (w/w) and homogenized in 0.1 M phosphate-buffered saline, pH 7.4 (Oxoid, Hampshire, UK). Each sample was cultured twice using MRS agar (Merck, Darmstadt, Germany), PCA agar (Oxoid Ltd, Basingstoke), NYSM agar, and selective media, respectively, to enumerate anaerobically LAB and anaerobic bacteria count, aerobically total count, *B. coagulans*, and *L. plantarum*. The results of the bacterial count were expressed as log CFU/g of fresh weight of feces.

Oxidative stress parameters

To evaluate the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in rat blood serum, SOD and GPx determination RANSEL kits (Randox Lab., CrumLin, Uk) were used, respectively. Each unit (U) of GPx activity was referred to as the amount of enzyme that converts 1 µmol of NADPH to $NADP^+$ per minute. The amounts of SOD and GPx activity were expressed as unit per gram of hemoglobin (U/g Hb).

Serum biochemistry analysis

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, urea, and creatinine of serum were determined using Pars Azmoon Co. Tehran, Iran, kits according to the manufacturer's standard protocol and measured at 37 °C.

Statistical analysis

Data were analyzed using SPSS software, version 16.0 (SPSS Inc., Chicago, Illinois). For comparison of different parameters among experimental groups, two-way ANOVA and follow-up Duncan's test were used. A *P*-value of less than 0.05 was considered statistically significant.

Results

Determine cadmium in tissues and feces

The effects of *B. coagulans* and *L. plantarum* on the accumulation of cadmium in the liver, kidney, and feces in rats are presented in Fig. 1A–C. The cadmium content in feces of rats that received *B. coagulans* and *L. plantarum* was significantly higher than that of the Cd-only treated group ($P < 0.05$) (Fig. 1A). It seems that probiotics increase the excretion of cadmium through feces. This

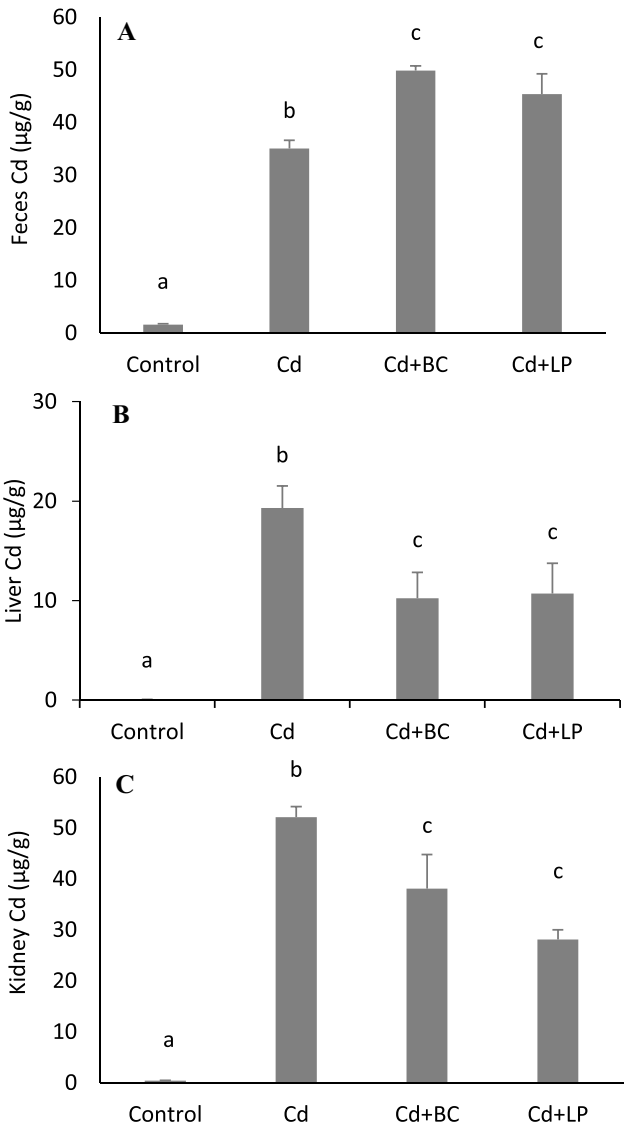

Fig. 1 Effects of *B. coagulans* and *L. plantarum* on Cd levels in the feces **A** liver, **B**, and kidney **C** of rat. Cd, cadmium. BC, *B. coagulans*. LP, *L. plantarum*. Values are expressed as mean ± SD. Superscript letters indicate groups with different letters differ significantly ($P < 0.05$)

heavy metal accumulation in the liver of Cd group rats was 19.3 ± 2.67 µg/g which indicated a significantly higher level, compared to the other groups (Fig. 1B) ($P < 0.05$). The changes in the cadmium amount in the kidney (Fig. 1C) have shown a similar trend to the liver, and the highest measure of metal was recorded in the Cd group. *B. coagulans* and *L. plantarum* compared to the Cd-only received rats reduced the cumulating of cadmium in kidney tissue by 26.93 and 46.14%, respectively.

The effect of cadmium and probiotics on feces microbial population

Feces bacterial population count on days 24 and 48 is shown in Fig. 2. Stool total count (Fig. 2A) in Cd group has been significantly reduced, compared to the other groups ($P < 0.05$). When probiotic bacteria were administered lonely (BC or LP), the total count of feces was significantly higher than in animals who received cadmium alone or along with the probiotics ($P < 0.05$). *L. plantarum* and *B. coagulans* remarkably reduced the number of anaerobic bacteria (Fig. 2A) ($P < 0.05$). These two probiotics resulted in a considerable increase in LAB, compared to Cd and control groups (Fig. 2C) ($P < 0.05$). The population of LAB in rats treated with Cd-only was significantly lower than other groups ($P < 0.05$).

As expected, at the end of the study the highest number of *B. coagulans* (6.88 ± 0.06 Log CFU/mL) and *L. plantarum* (7.11 ± 0.33 Log CFU/mL) has been observed in feces of groups BC and LP, respectively (Fig. 2E, D).

The effects of cadmium and probiotics bacteria on oxidative stress parameters

As shown in Table 2, on day 24 of the study the activity of SOD in blood serum of rats treated only with cadmium (1142.7 ± 30.9 U/g Hb) was significantly lower than the control group (1262.1 ± 26.2 U/g Hb) ($P < 0.05$). However,

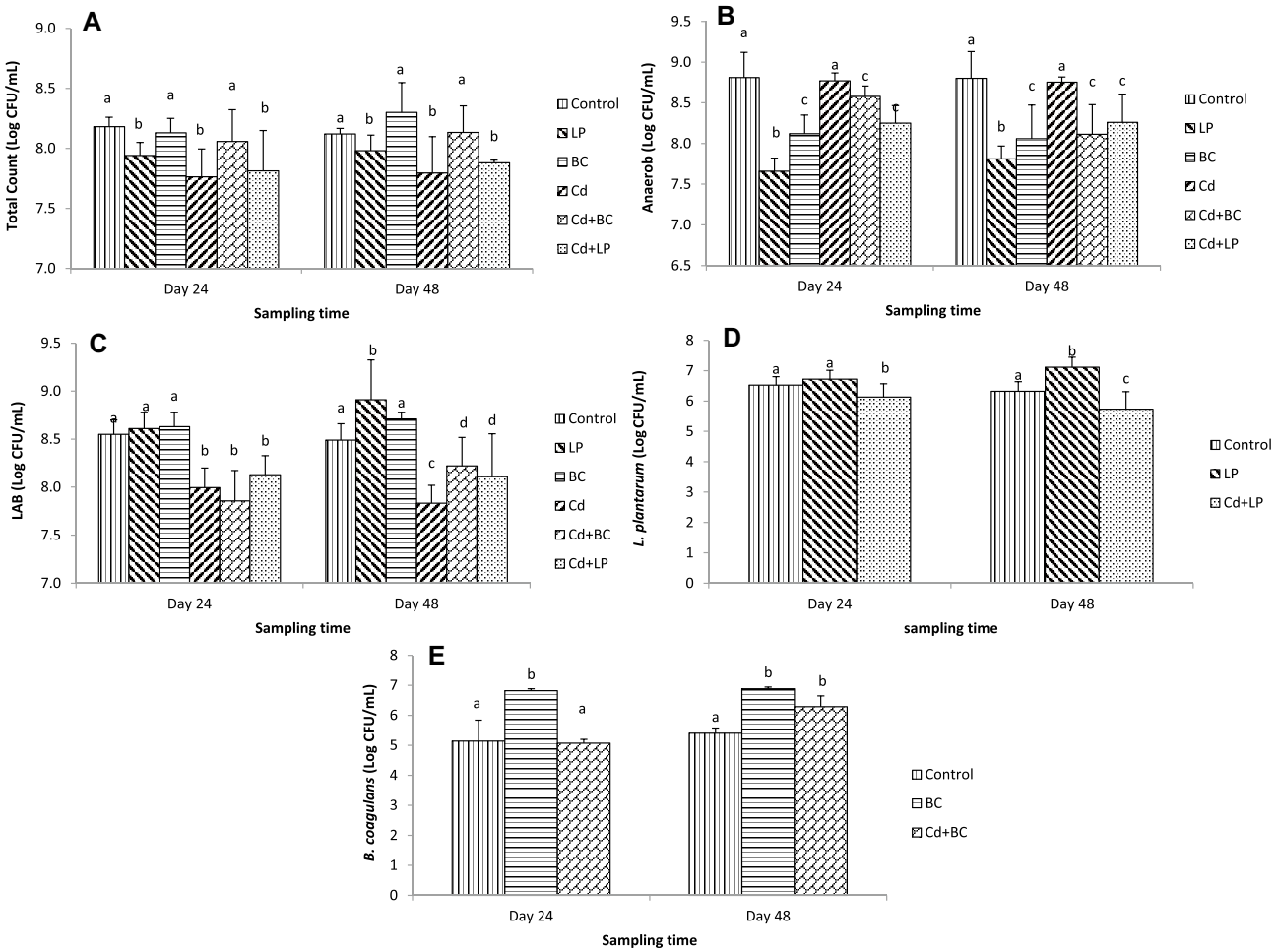

Fig. 2 The population of fecal bacteria in Cd and probiotics exposed rats. (**A** total count, **B** anaerobe, **C** LAB, **D** *L. plantarum*, **E** *B. coagulans*). Values are expressed as mean ± SD. Superscript letters indicate groups with different letters differ significantly ($P < 0.05$)

Table 2 Effects of *B. coagulans* and *L. plantarum* on cadmium-induced alterations of the activities of SOD and GPx in blood serum of rats

Treatment groups	SOD (U/g Hb)		GPx (U/g Hb)	
	Day 24	Day 48	Day 24	Day 48
Control	1262.1 ± 26.2^{Aa}	1274.9 ± 5.3^{Aa}	32.17 ± 0.53^{Aa}	31.52 ± 0.75^{Aa}
Cd	1142.7 ± 30.9^{Ab}	1233.6 ± 26.3^{Bb}	31.13 ± 0.24^{Ab}	32.09 ± 0.25^{Bb}
BC	1229.96 ± 30.71^{Aa}	1136.60 ± 73.56^{Bc}	32.92 ± 0.24^{Aa}	30.68 ± 0.67^{Bc}
LP	1232.40 ± 13.23^{Aa}	1198.01 ± 10.83^{Bc}	32.83 ± 0.76^{Aa}	30.85 ± 0.55^{Bc}
Cd + LP	1238.7 ± 22.3^{Aa}	1228.7 ± 8.6^{Ab}	32.66 ± 0.18^{Aa}	31.63 ± 0.21^{Aa}
Cd + BC	1237.1 ± 9.8^{Aa}	1320.7 ± 49.0^{Bd}	32.40 ± 0.46^{Aa}	31.12 ± 0.12^{Aac}

Values are expressed as mean ± SD. Superscript lowercase and uppercase letters respectively indicate significant differences between groups and days ($P < 0.05$)

SOD superoxide dismutase, *GPx* glutathione peroxidase, *Cd* Cadmium, *LP L. plantarum*, *BC B. coagulans*

two administered probiotics significantly increased the SOD level, compared to the Cd group ($P < 0.05$). The SOD change trend has been observed in the subject of GPx activity. This oxidative stress parameter in Cd group rats exhibited a significant depletion, compared to the untreated group, but the probiotic-treated group which was exposed to cadmium simultaneously revealed higher GPx activity when checked to the Cd-only group ($P < 0.05$). The result also showed that there were no significant differences in the GPX and SOD values when control group, compared to groups LP and BC.

The effects of cadmium and probiotics on biomarkers of liver and kidney damage

The indicators of the liver (bilirubin) and renal (urea and creatinine) damage are presented in Table 3. The level of blood serum bilirubin in Cd + BC and Cd + LP groups was significantly lower than Cd-only group ($P < 0.05$). On day 48 of the experiment, bilirubin concentration in the control group was 0.34 ± 0.12 mg/dL which was significantly lower than that of the rats who received cadmium alone (1.19 ± 0.06 mg/dL). After 48 days of the study, the blood urea in control, Cd, Cd + BC, and Cd + LP groups was 17.04 ± 2.08, 100.60 ± 4.12, 53.89 ± 5.42, and 55.33 ± 4.94 mg/dL, respectively. The serum creatinine level was significantly higher in the Cd group than in the other groups at both time intervals. The probiotics applied in this study when administrated with cadmium noticeably alleviated the serum creatinine level increase induced due to the heavy metal exposure ($P < 0.05$). These findings indicated the significant influence of cadmium on the elevation of kidney biomarkers damage and the efficacy of probiotics *B. coagulans* and *L. plantarum* on decreasing them ($P < 0.05$).

Cadmium has a considerable effect on plasma ALT and AST (Table 4). On the last day of the study (day 48), the levels of ALT and AST in the Cd group were 147.67 and 143.43 U/L higher than the control group, respectively. However, probiotics have significantly reduced these two liver damage indexes in plasma ($P < 0.05$). For example, *L. plantarum* at the end of the study caused a reduction in plasma ALT and AST in the intervention group in comparison to Cd-only by 59.09 and 75.58, respectively. However, regarding the renal and liver biomarkers levels, there were no significant differences between control and probiotic-only-treated groups.

Table 3 Effects of *B. coagulans* and *L. plantarum* on cadmium-induced alterations of levels of bilirubin, urea, and creatinine in blood serum of rats

Treatment groups	Bilirubin (mg/dL)		Urea (mg/dL)		Creatinine (mg/dL)	
	Day 24	Day 48	Day 24	Day 48	Day 24	Day 48
Control	0.32 ± 0.11^{Aa}	0.34 ± 0.12^{Aa}	16.83 ± 1.96^{Aa}	17.04 ± 2.08^{Aa}	1.61 ± 0.59^{Aa}	1.60 ± 0.58^{Aa}
Cd	1.06 ± 0.09^{Ac}	1.19 ± 0.06^{Ac}	96.56 ± 4.33^{Ac}	100.60 ± 4.12^{Ac}	5.31 ± 0.72^{Ac}	5.50 ± 0.70^{Ac}
BC	0.32 ± 0.09^{Aa}	0.36 ± 0.1^{Aa}	16.47 ± 2.6^{Aa}	16.68 ± 2.52^{Aa}	1.56 ± 0.45^{Aa}	1.5 ± 0.47^{Aa}
LP	0.35 ± 0.11^{Aa}	0.36 ± 0.07^{Aa}	16.4 ± 2.27^{Aa}	16.71 ± 2.13^{Aa}	1.53 ± 0.49^{Aa}	1.56 ± 0.51^{Aa}
Cd + LP	0.64 ± 0.12^{Ab}	0.60 ± 0.12^{Ab}	58.12 ± 5.07^{Ab}	55.33 ± 4.94^{Ab}	3.08 ± 0.13^{Ab}	3.03 ± 0.121^{Ab}
Cd + BC	0.68 ± 0.10^{Ab}	0.61 ± 0.09^{Ab}	59.48 ± 5.96^{Ab}	53.89 ± 5.42^{Ab}	3.18 ± 0.15^{Ab}	3.11 ± 0.11^{Ab}

Values are expressed as mean ± SD. Superscript lowercase and uppercase letters respectively indicate significant differences between groups and days ($P < 0.05$)

Cd cadmium, *LP L. plantarum*, *BC B. coagulans*

Table 4 Effects of *B. coagulans* and *L. plantarum* on cadmium-induced alterations of levels of ALT and AST in blood serum of rats

Treatment groups	ALT (U/L)		AST (U/L)	
	Day 24	Day 48	Day 24	Day 48
Control	11.20 ± 6.46^{Aa}	12.08 ± 6.97^{Aa}	42.94 ± 10.16^{Aa}	43.57 ± 10.95^{Aa}
Cd	150.96 ± 5.87^{Ac}	159.75 ± 7.69^{Bc}	178.09 ± 3.95^{Ac}	187.00 ± 0.51^{Bc}
BC	12.03 ± 6.94^{Aa}	$.8.40 \pm 4.85^{Aa}$	43.92 ± 8.90^{Aa}	46.82 ± 8.37^{Aa}
LP	$14.5 \pm .42^{Aa}$	17.21 ± 9.94^{Aa}	43.4 ± 12.11^{Aa}	44.38 ± 12.44^{Aa}
Cd + LP	106.53 ± 5.25^{Ab}	100.66 ± 4.52^{Ab}	117.55 ± 6.89^{Ab}	111.42 ± 7.54^{Ab}
Cd + BC	109.69 ± 7.06^{Bb}	89.86 ± 3.90^{Ab}	115.50 ± 5.88^{Ab}	107.34 ± 1.93^{Ab}

Values are expressed as mean ± SD. Superscript lowercase and uppercase letters respectively indicate significant differences between groups and days ($P < 0.05$)

AST aspartate transaminase, *ALT* alanine transaminase, *Cd* cadmium, *LP L. plantarum*, *BC B. coagulans*

Discussion

Accumulation of cadmium in the liver, kidney, and feces

In our previous study, probiotics *L. plantarum* and *B. coagulans* decreased the accumulation of mercury in the liver and kidney and improved the oxidative stress condition in rats which demonstrated the ability of these bacteria to reduce the toxicity effects of heavy metals (Majlesi et al. 2017). Hence, this study evaluated the effects of these two probiotics on diminishing the cadmium toxicity caused by oral exposure in rats. In many studies, the characteristics of biosorption of heavy metals by *B. coagulans* (Majlesi et al. 2017; Jafarpour et al. 2017) and *L. plantarum* (Zhai et al. 2015; Yu et al. 2017; Jafarpour et al. 2017; Zhai et al. 2019) have been reported. Jafarpour et al. (2017) investigated the symbiotic intervention on acute cadmium poisoning, while in this study only probiotics were administrated in a relatively longer time to examine the toxicity outcomes in rats (34). The probiotics such as LABs have a special ability to bind to cadmium (Mrvčić et al. 2012), which can apply to reduce the absorption of cadmium in the intestine; the primary target organ of heavy metal toxicity. The liver and kidney are critical target organs for cadmium adverse effects due to their high content of metallothionine which has a great tendency to bind to heavy metals such as cadmium (Klaassen et al. 2009). Probiotics and lactic acid bacteria, through biosorption and bioaccumulation (Mrvčić et al. 2012) relieve the toxic effects of heavy metals. Hydroxyl groups of peptidoglycan and teichoic acid in the cell wall of gram-positive bacteria and the presence of functional groups such as carboxylate groups of proteins cause the binding of heavy metals to LAB (Mrvčić et al. 2012; Gerbino et al. 2011). Studies reported the protective effects of diverse strains of *L. plantarum* on several heavy metals accumulation in different tissues such as aluminum in the brain (Yu et al. 2017) and cadmium in the liver and kidney (Zhai et al. 2016; Zhai et al. 2019) which is often accompanied by increasing the excretion of metals through feces. These researches support our findings concerning the effect of *L. plantarum* on reducing the accumulation of liver and kidney cadmium and increasing excretion of this metal by defecation. The isolated strains of *Bacillus* sp. from sewage revealed a high potential for absorbing heavy metals such as cadmium (García et al. 2016). Oral administration of *B. coagulans* in rats caused a remarkable reduction in the renal and hepatic accumulation of Hg (Majlesi et al. 2017) and Cd (Jafarpour et al. 2017). Regarding the noteworthy capacity for metal sequestration, probiotics treatment lessens the permeability of the intestinal cell to cadmium which leading to the increasing fecal cadmium excretion and decreasing this metal accumulation in tissues of rat (Zhai et al. 2016).

Prior to the cadmium absorption in the intestinal tract, *L. plantarum* by sequestration mechanisms and effective binding to cadmium (Zhai et al. 2019) caused the fecal elimination of this toxic metal. As exhibited in the discussed literatures, the bioremediation process is quite complex and the type of applying microorganisms, kind of heavy metals, and even the environmental conditions are effective.

Effect on fecal microbiota

The growth rate and population of intestinal microbial flora are reduced by the administration of different concentrations of cadmium. Different doses of cadmium reduce the normal microflora of the small and large intestine, and rectum, which mentioned that metal influence on protein synthesis, is the regular mechanism of toxic effects of cadmium on microorganisms' growth (Liu et al. 2014). *B. coagulans* and *L. plantarum* have shown decreasing and increasing effects on the population of anaerobic bacteria and LABs, respectively. Aminlari et al. (2018) reported that the number of Enterobacteriaceae in the feces of the rat was reduced by the administration of probiotic bacteria while *L. plantarum* and *B. coagulans* count increased with an effective pass to the intestine (Aminlari et al. 2018). The intestinal microflora can be considered the

first contact site for toxic metal compounds which directly interfere with the bioavailability of metals in GI (Breton et al. 2013).

In a study, Jama et al. (2012) reported that oral intake of cadmium in rats resulted in a reduction in the number of *Lactobacilli* including *L. acidophilus* and *L. rhamnosus*, while simultaneous administration of probiotics with this toxic metal has led to an increasing trend on *Lactobacilli* population (Jama et al. 2012). Applying probiotics in the diet increases the number of *Lactobacilli* and leads to improving microbial balance in the intestine (Lin et al. 2011). Probiotics plus their own role in the reduction of heavy metals entry into the body seem to be supported by increasing the number of *Lactobacilli* in GI.

The effect of probiotic bacteria on oxidative stress parameters

Applying antioxidant treatment plays a major role in cadmium toxicity alleviation. Recently, the safety of synthetic antioxidants has been questioned due to hepatocellular damages and carcinogenicity effects (Luo and Fang 2008). Nevertheless, the evidence suggests that probiotics have a significant antioxidant capability in vitro and in vivo (Persichetti et al. 2014). Hence, the antioxidant ability is probably one of the most important mechanisms of probiotic bacteria in relieving cadmium toxicity. *L. plantarum* CCFM 8610 significantly inhibited the production of reactive oxygen species (ROS) caused by cadmium toxicity, which indicates the direct protection of this probiotic against oxidative stress (Zhai et al. 2016). The data revealed that the enzymatic activity of SOD and GPx has been reduced because of Cd exposure. The formation of large amounts of ROS due to cadmium's adverse effects leads to the antioxidant mechanism discharge and depletion in enzyme activities (Thijssen et al. 2007).

In this study, *B. coagulans* and *L. plantarum* have provided a protective effect on the antioxidant activity of SOD and GPx. This innate activity of probiotic bacteria in cadmium toxicity has also been reported in the other studies (Zhai et al. 2019). Probiotics can stimulate and increase the efficacy of the antioxidant system in the host. Many reports showed the positive effects of probiotics on the enhancement of SOD and GPx activity when investigated in different hosts such as humans (Ejtahed et al. 2012).

The effect on liver and kidney damage indicators

ALT and AST are known as the bio-markers of liver damage created by heavy metals such as cadmium (Mohammad 2009). Both ALT and AST enzymes are released from hepatocytes and enter the extracellular liquids when the liver is damaged. Exposure to cadmium resulted in the higher levels of both ALT and AST in rat blood plasma that mutually has been reduced effectively by the administration of *L. plantarum* (Mrvčić et al. 2012) which is consistent with our findings that report the contrariwise effect of Cd and probiotics on these two enzyme activity. Djurasevic et al. (2016) reported that exposure to cadmium increases serum ALT and AST activity and in opposite the administration of probiotic supplements along the cadmium causes a reduction in activities of enzymes that increased due to cadmium contact (Djurasevic et al. 2017).

Bilirubin is another indicator of liver damage that increases cadmium toxicity that shows a degenerative hepatic injury (Albasha and Azab 2014). In this study, probiotic bacteria in the intervention groups caused a reduction in the bilirubin level. ROS is created by heavy metals, i.e., cadmium leading to the renal tubule damage, thereby reducing the glomerular filtration that results in an increase in the level of urea and creatinine in the blood (Albasha and Azab 2014). Oxidative stress, apoptosis, and necrosis are considered the main mechanisms for nephrotoxicity that occur by heavy metals in vivo and in vitro (Sabolić 2006). Fortunately, surveys suggest that probiotic bacteria can delay the progression of renal failure and reduce inflammatory markers. The renal function biomarkers such as blood urea are reduced by probiotics (Miranda Alatriste et al. 2014; Dehghani et al. 2016). Oral administration of probiotic bacteria in patients with chronic kidney disease has led to a reduction of 63% in urea and 43% in creatinine of serum (Ranganathan et al. 2010). The reduction of the level of these biological parameters indicates the protective effects of *B. coagulans* and *L. plantarum* on hepatic and renal damages that happened by chronic cadmium toxicity in rats.

Conclusion

The results of this study show that probiotics including *B. coagulans* and *L. plantarum* may have beneficial effects in the alleviation of humans and animals' toxicity in high cadmium-exposed environments. However, many aspects of metal-microbe interactions remain unexploited in biotechnology, and further study and application are necessary.

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Data availability Data will be made available on request.

Compliance with ethical standards

Funding This study was not supported by any funding.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All the applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent Informed consent was obtained from all the individual participants included in the study.

Consent for publication Consent for publication was obtained for every individual person's data included in the study.

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