Potential of two probiotics Bacillus coagulans and Lactobacillus plantarum with inulin on mercury absorption and oxidative stress in rats

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Abstract

The purpose of the present study was to assess the efficacy of two probiotics (*Bacillus coagulans* and *Lactobacillus plantarum*) along with inulin (as a prebiotic) in inhibiting the toxic impact of mercury and oxidative stress in rats. Twenty-four Wistar rats were randomly divided into 4 groups and the treatments were performed as follows: control, mercury only, mercury along with *B. coagulans* and inulin, and mercury along with *L. plantarum* and inulin. Mercury solution and probiotics were fed to the treated rats by gavage for 42 days. During the test period, body weights were taken and recorded every week. Some enzymes and biochemical factors of serum, such as SOD, GPx, MDA, AST, ALT, creatinine, BUN, and total bilirubin, as well as liver SOD, were evaluated to measure the alterations in oxidative stress. Results indicated that the synbiotic diet in mercury-treated rats significantly increased the body weight, GPx, liver and serum SOD values and diminished ALT, AST, BUN, total bilirubin, and mercury accumulation in the kidney and liver in comparison with the mercury-treated group ($P < 0.05$). There was no significant difference in MDA values between all treated groups ($P > 0.05$). Hence, our results revealed that synbiotic diets including probiotics (*B. coagulans* and *L. plantarum*) and the prebiotic (inulin) can effectively reduce the amount of mercury in the kidney and liver and alleviate the oxidative damage of acute mercury poisoning in a rat model.

Introduction

The element mercury (Hg) is a nonessential metal, recognized as a global pollutant, and exists naturally in the environment. Hg enters the environment due to the natural decomposition of minerals in soils and rocks via exposure to water and wind. The release of Hg from natural resources has been relatively uniform over the years [1]. Hg is not naturally present in food products, while it may be distributed in food chains by smaller organisms used by humans, such as fish. Shellfish and fish are the major sources of the mentioned element [2]. Hg is not commonly found in plants; however, when mercury-containing sprays are used in agriculture, it can enter the human body through crops and vegetables [3]. This toxic element can be present in metal form, as an organic mercury compound, or as a mercury salt. Studies have shown that exposure to high levels of Hg can damage the heart, kidneys, lungs, brain, and immune system [4, 5]. FAO and WHO specify the exposure level of mercury as a Provisional Tolerable Weekly Intake (PTWI) of 1.6 μg/Kg of bodyweight [6]. Many studies have revealed that the mentioned metal generates oxygen radicals and induces oxidative stress [7, 8]. Oxidative stress is a phenomenon that occurs as a consequence of an imbalance between the production of free radicals and antioxidant defences. Hg affects the antioxidant defence system through depletion of glutathione, affecting several enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx), and increasing DNA, protein, and lipid oxidation [9, 10]. Chelation therapy is used to remove heavy metal ions that are toxic to the human body. According to the side effects of chemical chelators (like vomiting, appetite loss, nausea, anorexia, and diarrhea) [11], research efforts have been directed towards bioremediation of heavy metal pollution.

The term “symbiotic” represents a dietary supplement combining both prebiotics and probiotics, which work synergistically to increase the colonization and survival of intestinal microbiota as well as probiotic microorganisms in the gastrointestinal (GI) tract [12].

Prebiotics are food components that pass undigested through the GI tract and have health benefits for the host by stimulating the activity and growth of advantageous microbiota, especially probiotics. Inulin is a group of fructooligosaccharides which is extracted from the chicory root and are recommended as a prebiotic compound [13].

Probiotics are living microorganisms that sufficient amounts consumption of them causes health benefits for the consumer [14]. A number of lactic acid bacteria (LAB) like *Bifidobacterium, Lactobacillus plantarum*, and *Lactobacillus acidophilus* are recognized as the most common and important probiotics. Because of the nature and membrane compositions of *lactobacilli*, they are known as natural adsorbents for metals. Recently, much of the concern has been focused on the sensitivity of these probiotic species to the normal physiological conditions, including bile salts and acidity of the stomach [15]. Therefore, a new probiotic has been introduced that is able to withstand the extreme conditions of the GI tract. It has been indicated that *Bacillus coagulans* is resistant, can survive in harsh conditions through spore production, and is also recognized as safe by the Food and Drug Administration (FDA) [16, 17].

Several studies have indicated the heavy metal biosorption ability and antioxidant impact of probiotic bacteria. It is stated that *L. plantarum* ID9263 and *L. rhamnosus* LC105 exhibited the potential to bind copper, cadmium, and lead in polluted water [18, 19]. Majlesi et al. [8] introduced *B. coagulans* and *L. plantarum* as suitable candidate species for the reduction of mercury toxicity in rats. Furthermore, Al-Wabel et al. [20] demonstrated the protective effects of feeding synbiotic fermented milk against the toxicity of lead acetate in rats. They demonstrated that this synbiotic diet could effectively protect the liver against oxidative damage by enhancing the activity of glutathione-S-transferase. In our previous study, we indicated the effectiveness of a synbiotic diet in preventing acute cadmium toxicity in a murine model [21]. Hence, the objective of the current study was to compare the impacts of two probiotics, *B. coagulans* and *L. plantarum*, along with a prebiotic, inulin, to alleviate the toxic impacts of mercury in rats.

Materials And Methods

Ethics

All the protocols used in the present study followed the ethical guidelines for animal welfare and were carried out with the approval of the Ethics Committee of the School of Veterinary Medicine, Shiraz University, Shiraz, Iran (Ethical approved number: 1392/905659).

Probiotic Strains, media and culture

B. coagulans and *L. plantarum* CNR273 were used as probiotic bacteria in this study. A lyophilized probiotic strain of *B. coagulans* was obtained from the Pardis Roshd Mehregan Company, Iran. The spores of *B. coagulans* were prepared according to the procedure of Jafarpour et al. [22]. Briefly, the bacteria were cultured in nutrient yeast extract salt medium (NYSM) agar (0.5% peptone, 0.3% beef extract, 0.5% NaCl, 0.05% yeast extract, 0.02% $MgCl_2$, 0.01% $CaCl_2$, 1% glucose, 0.001% $MnCl_2$ and 1.5% agar) at 37°C for 24 h. Then, a single colony was inoculated into NYSM broth and incubated at 37°C with shaking for 48 h. Vegetative cells were obtained by centrifugation at 3000 ×*g* for 20 min at 4°C. Subsequently, the bacterial spores were achieved by heating at 80°C for 15 min. The final suspension of spores was prepared at a concentration of 1×10^9 spores/ml in sterile saline and stored at refrigerator temperature for up to a week [22].

L. plantarum CNR273 was prepared from the culture collection of the Department of Food Science and Technology, Shiraz University, Iran. The probiotic was cultured in De Man Rogosa Sharpe (MRS) agar (Merck, Germany) at 37°C for 48 h. A single colony was transferred into MRS broth and kept in a shaker incubator at 37°C for 48 h. Then, the cultured biomass was centrifuged at 3000 ×*g* for 20 min. According to our previous study [22], the final pellets were resuspended in sterile saline to obtain 1×10^9 CFU/mL. The resulting suspension was stored at 4°C for up to a week.

Mercury solution preparation

The Hg solution was prepared according to the method of Nwokocha et al. [26]. In fact, the molecular weight of $HgCl_2$ (Merck, Germany) was divided by the molecular weight of mercury (271.52/200.59) to achieve 1.35 g as the weight of 1 part of mercury in $HgCl_2$. Finally, the mercury chloride solution was made at a concentration of 10 µg/ml.

Experimental design

Twenty-four male adult Wistar rats weighing 170 ± 10 g were supplied by the Razi Vaccine and Serum Research Institute, Shiraz, Iran. They were kept in standard polypropylene cages in a temperature (25 ± 2°C) and humidity (38%) controlled room with 12 h light/dark cycles. The diet that was consumed during the treatment period consisted of a standard rat chow containing 48.5% starch, 17.9% protein, 4.9% sugar, 5.3% crude fiber, 7.1% ash, and 4.6% fat. Drinking water and a diet were available *ad libitum* throughout the experimental periods.

After an acclimation period of 7 days, they were randomly divided into four groups of six rats each. The animal groupings and experimental designs are summarized in Table 1. Half of the rats in each group were treated for the first 21 days, and the treatment of the rest was continued until the end of the experiment period (42 days). Individual rat weight was recorded every week throughout the experimental duration using a balance scale (Shimadzu, model UW4200H, Japan).

Table 1
Treatments performed on the experimental groups

Treatment groups	Feeding	Gavaging (1 ml volume, once daily)
Control	Standard diet	Normal saline
Hg	Standard diet	Hg (10 µg/ml)
HgLpl	Standard diet + 5% inulin*	*L. plantarum* (1×10^9 CFU/ml) + Hg
HgBcl	Standard diet + 5% inulin	*B. coagulans* (1×10^9 spore/ml) + Hg
* Long-chain Chicory based inulin (Roosendaal, The Netherlands)		

Collection of Blood and Organ Samples

On days 21 and 42 of the experiment, three rats were randomly selected from each treatment group and sacrificed under anaesthesia after overnight fasting. Fresh blood samples were immediately collected from the right side of the heart into centrifuge tubes and permitted to coagulate for 30 min at 37°C. The clear serum was separated at 2000× *g* for 10 minutes and used for biochemical assays. A second blood fraction was collected into heparinized test tubes for the preparation of red blood cell hemolysate for GPx and SOD analysis. The kidneys and livers of the sacrificed rats were excised, washed with ice-cold normal saline, and kept at a temperature of -80°C for further biochemical tests and measurement of mercury.

Measurement of MDA, GP_X and SOD level

Lipid peroxidation in the serum was monitored by measuring the malondialdehyde (MDA) level using a direct photometric method. The method was based on the formation of a coloring compound (MDA–TBA) by the reaction of MDA with thiobarbituric acid and analyzed with a UV-visible spectrophotometer at 532 nm [21]. The obtained results were represented as mmol L^{-1}.

GPx activity in heparinised whole blood samples was measured by using the GPx detection RANSEL kit (Randox Com, Crumlin, United Kingdom) based on the decrease in absorbance at 340 nm, caused by oxidation and reduction of glutathione, and represented as a unit per gram of hemoglobin (U/g Hb).

Total SOD activity in both red blood cell hemolysate and the liver was measured using SOD detection RANSOD kit (Randox Com, Crumlin, United Kingdom) according to the manufacturer's instructions. Levels of SOD were recorded spectrophotometrically at 505 nm and the activity was expressed as units per gram of hemoglobin or liver tissue.

To prepare the liver samples, 0.5 g of them was homogenized with 0.5 M cold phosphate buffer pH 7.1 (yellow base homogenizer of DI18 line, IKA, Germany). The homogenates were then centrifuged at 3000× *g* for 15 minutes at 4°C, and the resulting supernatants were obtained and used to determine SOD activity.

Biochemical Assay

Hepatic enzymes including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured at 37°C according to the method of Reitman-Frankel [23], and the results have been presented in units per liter. Other biochemical properties of serums, including creatinine, urea, and bilirubin, were evaluated by a commercial kit (Pars Azmoon Co., Tehran, Iran). Biochemical analyses were assessed by using a standard autoanalyzer and veterinary software (ABX-Diagnostics, Cobas-Mira, Japan).

Mercury measurement

On day 42, about 1 gr of the kidney and liver samples were ground and homogenized. Then, homogenized samples were digested in a polypropylene tube heated overnight using 10 ml of concentrated HNO_3 [24]. After digestion, the mercury content of tissues was measured by cold vapor atomic absorption spectrometry (FIMS-400, Perkin-Elmer Inc., USA).

Statistical analysis

A two-way analysis of variance (ANOVA) was applied to determine the differences among the treatments, and when the differences were detected, a Duncan's post hoc test was utilized to differentiate the means. The statistical analysis was carried out using SPSS software (version 23, SPSS Inc.). P-values of 0.05 or less were considered statistically significant.

Results

Body weight changes

All the animals except the mercury treated group remained alive and healthy throughout the period of the experiment. The changes in the body weight of treated rats during the experiment are depicted in Fig. 1. According to the results, the body weight of all groups gradually increased during the experimental period, while in mercury group animals a significant reduction was seen in the first week of treatment ($P < 0.05$). On the other hand, the mercury-treated rats showed lethargic behavior and had the lowest body weight among all treated groups. Also, our results indicated that the application of synbiotic in rats' diet which exposed to mercury, markedly ($P < 0.05$) affected the weight loss (271.7 ± 7.6 g and 275.3 ± 22.3 for the HgBcI and HgLpI groups, respectively) and caused increase in comparison to the Hg group (246.3 ± 9.5 g).

Biochemical status test

The impacts of synbiotic diets on oxidative stress indicators (MDA, GP_X and SOD) are illustrated in Table 2. At the end of the experimental time (day 42), animals administered mercury orally exhibited a notable decrease in the SOD activity both in the liver and RBC (2.6 ± 0.0 U/g Hb and 273.2 ± 2.8 U/g Hb, respectively) when compared to other experimental rats. On the other hand, administration of synbiotic diets in mercury-induced groups significantly increased liver SOD (2.7 ± 0.0 and 2.7 ± 0.0 U/g tissue,

respectively, for the HgLpI and HgBcI groups) and blood SOD (290.8 ± 1.6 and 289.7 ± 1.6 U/g Hb, respectively, for the HgLpI and HgBcI groups) when compared to rats administered mercury only (P< 0.05). A similar trend was observed for GP_X activity, as noticed for SOD. The level of GP_X activity in the control group on day 42 of the experiment was 1121.6 U/g Hb and the lowest activity of this enzyme was observed in the mercury group (925.5 U/g Hb). In addition, our data showed that the diets given to rats containing *B. coagulans* and *L. plantarum* along with inulin resulted in a significant increase in GP_X activity, so that at day 42, its value reached to 1093.4 and 1076.7 U/g Hb in the HgBcI and HgLpI, respectively.

Table 2
Impacts of mercury and synbiotic diet on levels of glutathione peroxidase (GPx), serum superoxide dismutase (SOD), liver superoxide dismutase (liver SOD) and malondialdehyde (MDA) of treated rats at days 21and 42 of treatments

Treatments	GP_X (U/g Hb)		SOD (U/g Hb)		Liver SOD (U/g tissue)		MDA (mmol/L)	
	Day21	Day42	Day21	Day42	Day21	Day42	Day21	Day42
Control	1177.7 ± 68.2^{aA}	1121.6 ± 57.0^{aA}	300.9 ± 2.8^{aA}	301.8 ± 1.6^{aA}	2.7 ± 0.0^{aA}	2.9 ± 0.0^{aB}	5.0 ± 0.1^{aA}	5.0 ± 0.0^{aA}
Hg	953.9 ± 45.1^{cA}	925.5 ± 34.3^{cA}	269.2 ± 4.2^{cA}	273.2 ± 2.8^{cA}	2.6 ± 0.0^{cA}	2.6 ± 0.0^{cA}	5.0 ± 0.1^{aA}	5.0 ± 0.1^{aA}
HgBcI	1051.8 ± 67.1^{bA}	1093.4 ± 50.1^{bA}	285.6 ± 1.6^{bA}	289.7 ± 1.6^{bA}	2.7 ± 0.1^{bA}	2.7 ± 0.0^{bA}	5.0 ± 0.1^{aA}	5.0 ± 0.1^{aA}
HgLpI	1065.7 ± 48.2^{bA}	1076.7 ± 77.1^{bA}	288.7 ± 1.6^{bA}	290.8 ± 1.6^{bA}	2.7 ± 0.1^{bA}	2.7 ± 0.0^{bA}	5.0 ± 0.0^{aA}	5.0 ± 0.1^{aA}

Hg: mercury; HgLpI: inulin, *Lactobacillus plantarum* and mercury; HgBcI: inulin, *Bacillus coagulans* and mercury. Values are expressed as mean ± SD. The different small letters indicate statistically significant differences in columns (P< 0.05). The different capital letters indicate statistically significant differences between days in each parameter (P< 0.05).

The amounts of hepatic enzymes (ALT and AST) and total bilirubin in the Hg group were significantly higher (P < 0.05) than the other treatment groups (Fig. 2). On the other hand, data revealed that when animals were exposed to synbiotic diets (probiotics with inulin) a notable decrease (P < 0.05) in the mentioned parameters was detected as compared to animals that received mercury alone.

The levels of BUN and creatinine in treated groups are represented in Table 3. According to the results, the highest levels of both BUN and creatinine were observed in the mercury treated rats (P< 0.05). Moreover, feeding rats with *B. coagulans* and *L. plantarum* along with inulin in mercury-induced animals significantly decreased (P< 0.05) BUN content, at day 42 of treatment, from 23.21 to 20.09 and 20.01 mg/dL, respectively. Also, the amount of creatinine markedly decreased in the mentioned groups from

1.06 to 0.99 and 0.91 mg/dL, respectively. No significant differences were noticed for both factors between synbiotic-treated groups ($P > 0.05$).

Table 3
Impacts of mercury and synbiotic diet on levels of creatinine and BUN of treated rats at days 21 and 42 of treatments

Treatments	Creatinine (mg/dL)		BUN (mg/dL)	
	Day21	Day42	Day21	Day42
Control	0.72 ± 0.10^{cA}	0.80 ± 0.03^{cA}	18.38 ± 0.73^{bA}	19.91 ± 0.70^{bA}
Hg	1.11 ± 0.07^{aA}	1.06 ± 0.11^{aA}	21.93 ± 0.27^{aA}	23.21 ± 0.70^{aA}
HgBcI	0.92 ± 0.15^{bA}	0.99 ± 0.09^{bA}	19.08 ± 2.78^{bA}	20.09 ± 0.35^{bA}
HgLpI	0.88 ± 0.07^{bA}	0.91 ± 0.09^{bA}	18.96 ± 0.35^{bA}	20.01 ± 0.60^{bA}

Hg: mercury; HgLpI: inulin, *Lactobacillus plantarum* and mercury; HgBcI: inulin, *Bacillus coagulans* and mercury. Values are expressed as mean ± SD. The different small letters indicate statistically significant differences in columns ($P < 0.05$). The different capital letters indicate statistically significant differences between days in each parameter ($P < 0.05$).

The concentration of mercury in the kidney and liver of rats

The levels of Hg in the kidney and liver of treated animals in the presence of synbiotic diets are shown in Fig. 3. Compared with the Hg group, synbiotic diets significantly decreased ($P < 0.05$) mercury accumulation in the kidney in intervention groups from 4.51 ± 0.55 to 1.49 ± 0.30 and 1.00 ± 0.16 µg/g in the HgBcI and HgLpI groups, respectively. A similar trend was observed in the mercury concentration of the liver, as noticed for kidney. According to the results, both studied probiotics were effective in reducing mercury content and no significant differences were observed between them ($P > 0.05$) (Fig. 3).

Discussion

Mercury is one of the harmful heavy metals and its toxicity considered to be of a major concern that causes public health problems. In this study we used a murine model to investigate the effect of *B. coagulans* and *L. plantarum* as probiotic bacteria along with a prebiotic, inulin, on the toxicity of mercury. We found that synbiotic diet had the potential to alleviate mercury toxicity *in-vivo* condition. Protective effects of them were established by decreasing mercury content in blood and tissues, inhibiting changes in the levels of BUN, creatinine, AST, ALT and also elevating antioxidant enzymes (GP_X and SOD) activities by preventing alterations in peroxidation reaction. Many researches proved that LAB could eliminate various heavy metals by the mechanism of absorption [25]. The application of *Lactobacillus fermentum* and *Bifidobacterium longum* as heavy metal binders from water has been proven [19]. In

addition, Tian et al. [11] reported that *L. plantarum* CCFM861 had the potential to reduce the toxicity of lead in mice.

In the current study the impact of mercury exposure on the body weight of treated rats were investigated. We observed that weight losses were significant in rats exposed to mercury, while synbiotic treatment effectively reduced the weight losses. It was stated that rats experiencing heavy metals intoxication generally lose weight [26]. Similar results were observed by Tatara et al. [27] and Nwokocha et al. [26] reporting that garlic compounds induce positive effects on weight loss caused by heavy metal ingestion. This loss of body weight with continuous exposure to mercury might be explained as a result of anorexia which is induced by consumption of heavy metals [28]. Canli and Atli [29] and Majlesi et al. [50] reported that there was a negative correlation between heavy metals contents and the size of fish species. Another possible explanation for the weight loss probably the cachexia as a result of the oxidative stress induced by mercury. Many researches have indicated that oxidative stress due to heavy metal toxicity is associated with muscle wasting and cachexia and cause body weight losses [14, 31].

Our results also indicated that the synbiotic diets increased the SOD enzyme activity as well as GPx. These enzymes are important enzymatic defences against the harmful effects of free radicals and oxygen toxicity. They effectively reduce the oxidative stress by decomposing superoxide radicals (O_2) to produce hydrogen peroxide (H_2O_2). AL-Hashem's research showed that aluminium chloride significantly reduces the SOD activity of rats which exposed to it [32]. Similar findings by Tian et al. [11] have been shown the stimulatory role of GPx and SOD enzymes on oxidative stress reduction. Also, Chang et al. [33] showed that cadmium notably reduced the activities of GPx and SOD enzymes, while consumption of *B. coagulans* SCC-19 increased their activities in treated common carp. In contrast, our results revealed that synbiotic diet had no effect on MDA content as a marker of oxidative stress in comparison with the control group. Our findings were consistent with the reports of Majlesi et al. [8]. Hence, the results revealed that GPx and SOD enzymes play an important role in the reduction of oxidative stress followed by reduction of mercury toxicity.

The impact of rat treatments with synbiotic diets on serum levels of ALT and AST activities were measured in an attempt to evaluate the hepatotoxic potential of the element mercury. These two biochemical markers are considered as sensitive indicators of hepatic injury. Friedman et al. [34] reported that metals exposure induced liver injury and enhanced the activities of serum AST and ALT. The obtained results revealed that the activities of AST and ALT enhanced in animals treated with mercury. Furthermore, synbiotic diets showed hepatoprotective role via decreasing ALT and AST and inhibit liver injury. Our findings are supported by the achievement of Al-Wabel et al. [20]. They demonstrated that probiotic fermented milk along with various prebiotics significantly decreased serum transaminases in animals exposed to lead. Similar findings were achieved by our previous study that synbiotic diet significantly alleviated AST and ALT activities in animals treated with the cadmium [21].

Creatinine and BUN are the most common biochemical markers of renal injury and the enhancement of their concentrations is commonly related to the impairment of renal performance [35]. Data showed that

the values of BUN and creatinine in the Hg group increased, whereas in the HgBcl and HgLpl groups their values decreased. These results confirmed that the probiotic bacteria absorbed Hg and prevented its impact on kidney injury. Ulutaş et al. [36] demonstrated that cadmium, arsenic, and chromium significantly elevate serum creatinine and urea in metal-treated animals and cause renal cell injury. Similar findings were achieved by Raghuvanshi et al. [35].

Our results indicated that rat treatments with the mentioned dose of mercury increased the accumulation of this metal in the liver and kidney and brought about oxidative stress as a result of changes in the balance between pro-oxidant and antioxidant systems and influenced the antioxidant enzymes. Synbiotic diets exert their protective action against mercury-induced renal and hepatic damage in rats through their chelation mechanism. It seems that since the cell walls of gram-positive bacteria, especially LAB and Bacillus spp., contain peptidoglycan, teichoic and teichuronic acids, they have high affinities for various cations, and these anionic groups show high adsorptive capacity toward heavy metals [37, 38]. These findings are in agreement with the report of Tian et al. [11], declaring that *L. plantarum* CCFM861 notably decreased the concentration of lead in the tissue of treated mice. Early studies have achieved the same results as Jafarpour et al. [21] and Jadán-Piedra et al. [39].

Conclusion

The current study revealed that the consumption of a synbiotic diet, which is a combination of probiotics (*B. coagulans* and *L. plantarum*) and inulin as a prebiotic, is a significantly effective method to reduce mercury toxicity *in-vivo*, recover antioxidant enzymes, and prevent kidney and liver damage in acute mercury poisoning in rats. These findings indicate that the above synbiotic diets can be used as adjuncts to therapeutic regimens to prevent chronic mercury poisoning.

Abbreviations

SOD: superoxide dismutase, GPx: glutathione peroxidase, MDA: malondialdehyde, AST: aspartate transaminase, ALT: alanine transaminase, Hg: mercury, HgBcl: inulin, *Bacillus coagulans* and mercury; HgLpl: inulin, *Lactobacillus plantarum* and mercury

Declarations

Author's Contribution

SSS and HRG were contributed to the conceptualization, methodology, writing-review & editing. DJ was responsible for investigation, writing-original draft preparation, and formal analysis. MM was responsible for investigation, writing - review & editing. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

This work was approved by the Ethics Committee of the School of Veterinary Medicine, Shiraz University, Shiraz, Iran (Ethical approved number: 1392/905659).

Conflict of Interest

The authors declare that they have no competing interests.

Availability of Data and Materials

Research data are not shared.

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Figures

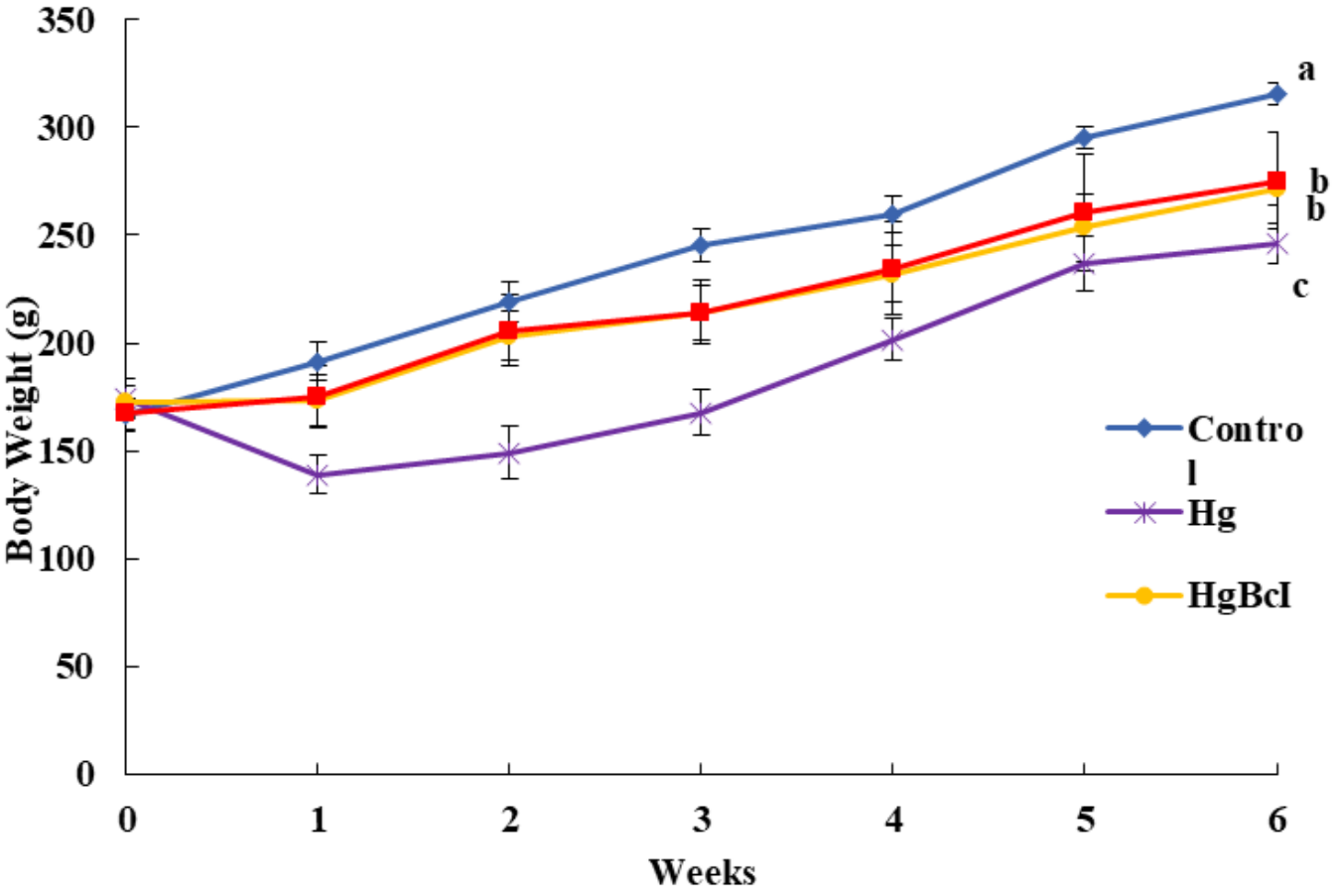

Figure 1

Impacts of mercury and synbiotic diet on body weight alterations in treated rats during 6 weeks of treatments. Hg: mercury; HgLpI: inulin, *Lactobacillus plantarum* and mercury; HgBcI: inulin, *Bacillus coagulans* and mercury. All results are expressed as mean and standard errors. The different letters indicate statistically significant differences ($P < 0.05$).

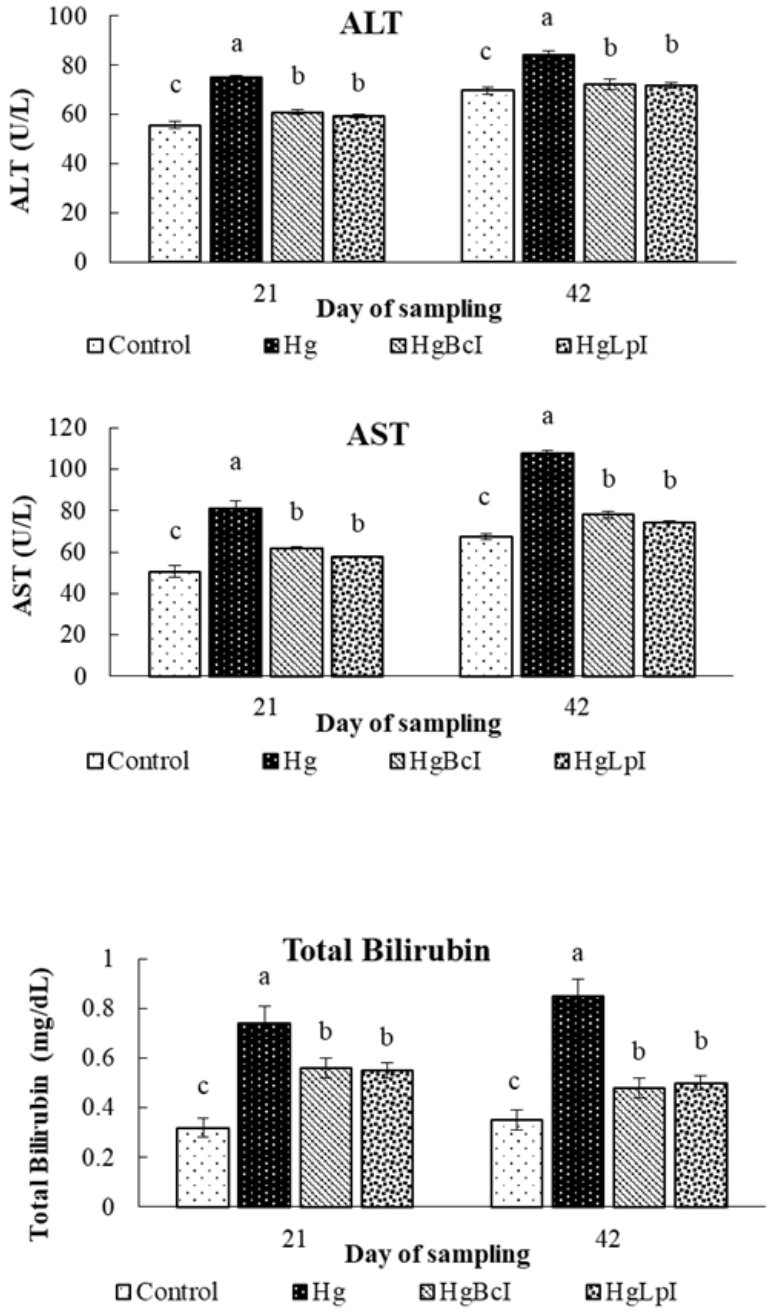

Figure 2

Impacts of mercury and synbiotic diet on levels of ALT, AST and total bilirubin of treated rats at days 21 and 42 of treatments. Hg: mercury; HgLpI: inulin, *Lactobacillus plantarum* and mercury; HgBcI: inulin, *Bacillus coagulans* and mercury. All results are expressed as mean and standard deviation. The different letters indicate statistically significant differences ($P < 0.05$).

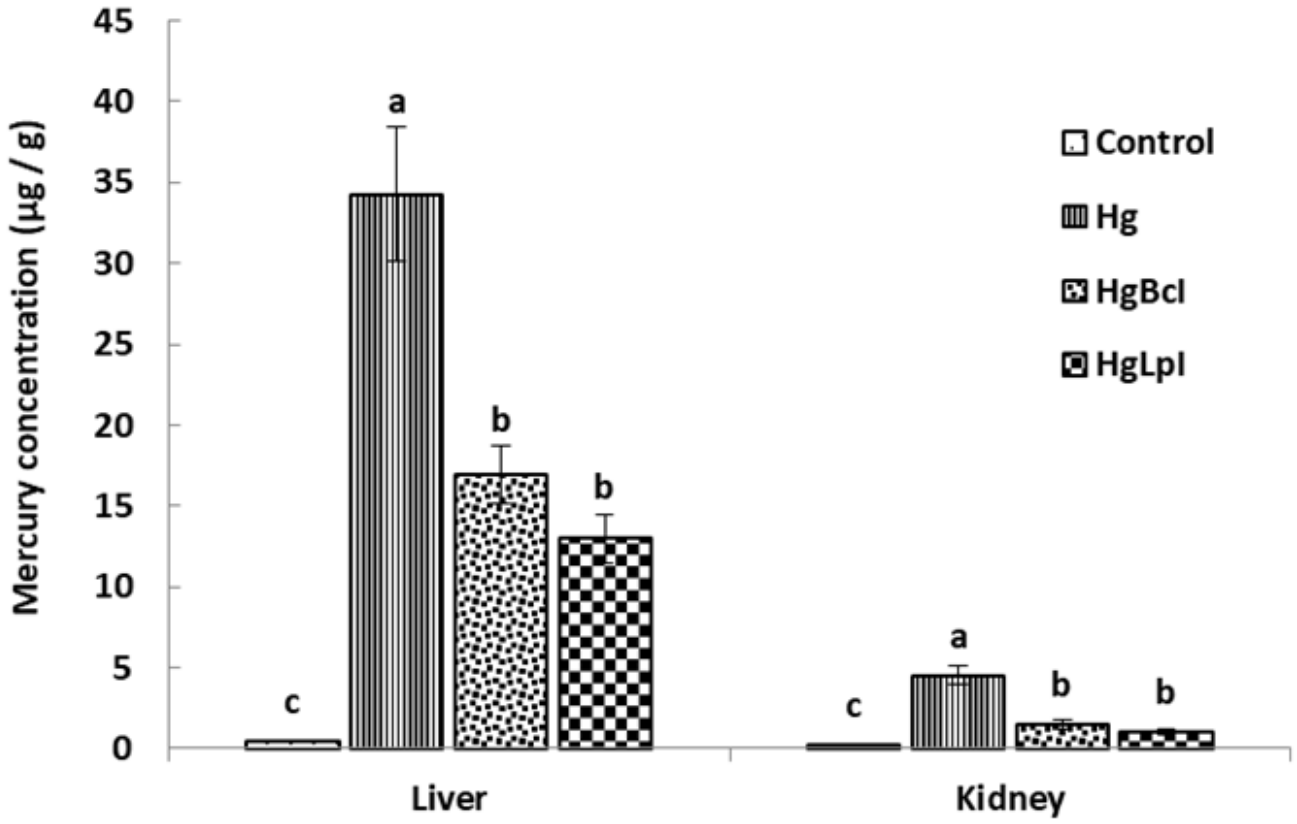

Figure 3

Impact of synbiotic diet on the accumulation of mercury in liver and kidney of treated rats at day 42 of treatments. Hg: mercury; HgBcl: inulin, *Bacillus coagulans* and mercury; HgLpl: inulin, *Lactobacillus plantarum* and mercury. All results are expressed as mean and standard deviation. The different letters indicate statistically significant differences ($P < 0.05$).