Inhibitory ability of *Lactobacillus fermentum* CQPC10 on lead toxicity induced by lead acetate in rats

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Abstract. This study studied the adsorption of *Lactobacillus fermentum* CQPC10 (LF-CQPC10) to induce lead acetate. The animal experiments showed that LF-CQPC10 reduced SD rat serum, liver, kidney and brain lead and maintained the cellular structure and state of liver and kidney tissues. Furthermore, LF-CQPC10 could improve anti-inflammatory cytokinine by reducing anti-inflammatory cytokinine IL-1β, IL-6, TNF-α and IFN-γ and raising IL-10. At the same time, LF-CQPC10 can also enhance the δ-ALAD enzyme activity and decrease the enzyme activities of ATL and AST, and decrease the contents of serum BUN and CRE of rats with lead toxicity injury. Compared with EDTA, LF-CQPC10 not only has better lead ion degradation ability, but also has better anti-inflammatory and organ protection effects. As a lactic acid bacteria, which can be used in food industry, LF-CQPC10 reduce the lead content and lead toxicity in food.

1. Introduction
Heavy metals are not biodegradable, can be food chain enrichment and converted into more toxic organic metal compound [1]. In modern society, heavy metal pollution seriously affects human health. Lead is a kind of toxic substance with affinity and accumulation. Excessive accumulation will damage brain nerve tissue; If it accumulates in the kidney and liver, it will cause acute and chronic diseases in the kidney and liver, and eventually lead to damage to various organ systems [2].

Fermented food is rich in lactic acid bacteria, which can not only degrade nitrite in food, but also play various roles in reducing cholesterol, antioxidant, regulating intestinal health, etc. With the study of lactic acid bacteria, some studies show that some lactic acid bacteria have good resistance and adsorption performance to heavy metals [4]. Heavy metal poisoning biorepair has the advantages of wide material sources, low cost, simple operation, environmental protection and does not cause secondary damage [5]. In this study, LF-CQPC10, an edible lactic acid bacteria isolated from traditional fermented pickles in the laboratory, was used to test the lead adsorption capacity of LF-CQPC10 to determine the lead adsorption capacity of LF-CQPC10 so as to avoid the effects of lead toxicity damage in rats.

2. Materials and methods

2.1. Materials and reagents
SD rats were purchased from Chongqing Medical University, Chongqing, China.
**Lactobacillus fermentum** CQPC10, patent China Center for Type Culture Collection.

EDTA, Sigma, USA; Interleukin 6 (IL-6), IL-10, IL-1β, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), Beijing Solaibo Company; the other reagents were all domestic analytical pure.

### 2.2. Instruments and equipment

Thermo Varioskan Lux Multifunctional Microplate, Thermo Fisher Scientific, USA.

### 2.3. Experiments on animals

Forty-eight 6-week-old SPF male Sprague-Dawley rats were used for this experiment. One week after adaptive feeding, the rats were randomly divided into 4 groups after adaptive feeding for one week: normal group (n = 12), lead induced group (n = 12), EDTA group (n = 12) and LF-CQPC10 group (n = 12). During experiments, normal rats freely got AIG-93G feed and drink water without lead acetate.

The other three groups of rats were free to eat the AIG-93G feed, and at the same time, they were free to drink 200mg/L lead acetate solution instead of drinking water from the first week to the 12th week. Rats in the EDTA group were injected with 50mg/L EDTA daily from week 8 to week 12, and LF-CQPC10 group was gavaged with 1×10⁹ CFU/kg (B.W) LF-CQPC10 daily from week 1 to week 12. After 12 weeks of fasting, all rats were anesthetized with ether after 12 h of fasting, and sacrificed after blood was collected through orbital vein. The heart, liver, kidney and brain tissues of rats were collected with liquid nitrogen and stored at -80°C for use.

### 2.4. Determination of lead in blood, liver, kidney and brain of SD rats

Precisely measure 0.0, 0.4, 0.8, 1.2, 1.6 and 2.0 mL of lead standard solution into a 50 mL volumetric flask, then add 2mL of mixed solution containing 12.5% ammonium dihydrogen phosphate and 2.5% magnesium nitrate, respectively, and then constant volume with 2% nitric acid. The absorbance of 20 μL of the above standard solutions with different concentrations was measured in the graphite furnace atomizer and the standard curve was made. 500 μL of the collected blood and 50mg of each tissue were placed in the tetrafluoroethylene digestion tank respectively, and 5mL of nitric acid was added for digestion. After cooling, 1mL of the mixed solution containing 12.5% ammonium dihydrogen phosphate and 2.5% magnesium nitrate was added and the solution with constant volume of nitric acid was added to 25 mL, and the solution with constant volume of 20 μL was added to the graphite furnace atomizer to measure the absorbance. Calculate the lead content by standard curve.

### 2.5. Determination of serum indexes in SD rats

Serum interleukin-6 (IL-6) and IL-10 were determined by kit operation method. IL-1β, tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) cytokine levels and serum Δ-amino-γ-keto-valerate dehydrase (δ-ALAD), alanine aminotransferase (ALT), glutamic-oxalacetic transaminase (AST), creatinine (CRE) and blood urea nitrogen (BUN) levels were determined according to the kit operation method.

### 2.6. Statistical analysis

Experiment samples were measured three times simultaneously and the average value was calculated. The data was averaged using SPSS software. Multiband Duncan equation test was estimated using single factor variance analysis. The statistical level is $P < 0.05$.

### 3. Results and analysis

#### 3.1. Analysis of lead in blood, liver, kidney and brain of SD rats

As can be seen from the data presented in the table, the amount of lead in normal rat blood, liver, kidney and brain tissues without induction by lead acetate solution was the lowest among all groups (Table 1). In contrast, the highest levels of lead were found in blood, liver, kidney and brain tissues of lead induced rats. After treatment with EDTA and LF-CQPC10, the lead contents in blood, liver, kidney and brain of rats were significantly decreased, and the intervention effect was better than that of EDTA. In
addition, data contrast also found that lead acetate induced the highest amount of lead in rat blood, which was much higher than that in its tissue organs. Probiotics can better adsorb lead ions from solution in the process of adsorbing lead, and bacterial surface groups containing C and N are involved in the adsorption process. After a large amount of lead ions are adsorbed, the morphology of some lactic acid bacteria is destroyed or the interior changes, even ruptured, and the O elements in the cells are dissolved, resulting in a large increase in the O element content [6]. It can be concluded that LF-CQPC10 was a high-quality strain of adsorbed lead lactic acid bacteria.

| Group                  | Blood lead (μg/L) | Liver lead content (μg/g) | Kidney lead content (μg/g) | Brain lead content (μg/g) |
|------------------------|-------------------|--------------------------|---------------------------|--------------------------|
| Normal group           | 1.38±0.29d        | 1.07±0.12d               | 1.32±0.13d                | 0.39±0.06d               |
| Lead induced group     | 26.08±1.44a       | 15.98±0.41a              | 24.32±2.97a               | 5.44±0.23a               |
| EDTA group             | 18.32±1.50b       | 12.36±0.29b              | 20.23±2.03b               | 3.22±0.31b               |
| LF-CQPC10 group        | 12.03±1.09c       | 6.11±0.23c               | 10.89±1.22c               | 2.12±0.25c               |

Note: a-d Different letters indicate significant differences between groups at P < 0.05 level. Tables 2 and 3 are the same as Table 1

3.2. Analysis of inflammatory indexes in serum of SD rats
Table 2 shows the data of serum inflammation index of SD rats. The results showed that serum IL-1 level increased significantly, and the levels of β, IL-6, TNF-α and IFN-γ in normal group were the lowest, while IL-10 level was the highest. The levels of interleukin-10, interleukin 1β, interleukin-6, tumor necrosis factor-α and interferon-γ in lead-induced group were the lowest, while the levels of interleukin-6, tumor necrosis factor-α and interferon-γ were the highest. The levels of interleukin-1β, interleukin-6, tumor necrosis factor-α and interferon-γ in EDTA group and LF-CQPC10 group were lower than those in lead-induced group, while the level of interleukin-10 in LF-CQPC10 group was higher than that in lead-induced group, but the downward and upward trend of LF-CQPC10 group was more obvious than that in EDTA group. Heavy metals enter the body and cause inflammation. Interleukin-1β, interleukin-6, interleukin-10, tumor necrosis factor-α, and interferon-γ are all important inflammatory cytokines that can reflect the degree of inflammation in vivo [7]. The results of this study indicate that LF-CQPC10 can avoid the inflammatory damage caused by lead poisoning in rats by regulating the above-mentioned inflammatory factors.

| Group                  | IL-1β (μmol/L) | IL-6 (μmol/L) | IL-10 (μmol/L) | TNF-α (μmol/L) | IFN-γ (μmol/L) |
|------------------------|----------------|---------------|----------------|----------------|----------------|
| Normal group           | 15.23±1.20d    | 40.79±2.58d   | 45.56±1.47a    | 165.91±22.09d  | 26.51±2.37d    |
| Lead induced group     | 32.36±2.03a    | 70.36±3.21a   | 20.65±1.71d    | 375.65±25.36a  | 84.91±3.22a    |
| EDTA group             | 25.39±2.12b    | 55.97±2.54b   | 27.82±2.09f    | 275.49±26.63b  | 65.63±3.00b    |
| LF-CQPC10 group        | 20.50±1.13c    | 45.12±1.08c   | 36.10±1.96b    | 200.35±20.89c  | 41.03±2.07c    |

3.3. Analysis of ALT, AST, BUN, CRE and δ-ALAD in serum of SD rats
Lead can cause liver lesions of different degrees, cause serious inflammatory reaction, affect ALT, AST, Cre and BUN index, and eventually lead to liver injury [8]. Table 3 shows the activities of glutamic
pyruvic transaminase, glutamic oxaloacetic transaminase, δ-ALAD enzyme and the contents of urea nitrogen and creatinine in rat serum. Among the four groups of rats, the activities of ATL and AST in normal group were the lowest, the activities of δ-ALAD enzyme were the highest, and the contents of BUN and CRE were the lowest. In lead-induced group, the activities of ATL and AST related to liver were the lowest, while the activities of δ-ALAD enzyme and the contents of BUN and CRE related to kidney were the highest. The activity trends of three enzymes and the contents of urea nitrogen and creatinine in EDTA group and LF-CQPC10 group were similar to those in normal group. In terms of enzyme activity, urea nitrogen and creatinine content, the intervention effect of LF-CQPC10 is better than that of EDTA.

Table 3 The contents of ALT, AST, BUN, Cre and δ-Alad in SD serum (μmol/L).

| Group                | ALT       | AST       | BUN        | CRE        | δ-ALAD     |
|----------------------|-----------|-----------|------------|------------|------------|
| Normal group         | 21.03±2.00d | 50.36±3.01d | 1457.02±50.33d | 26.54±1.82d | 523.02±16.78a |
| Lead induced group   | 63.02±2.90a | 91.06±3.74a | 2778.92±54.89a | 51.26±2.63a | 306.36±18.36d |
| EDTA group           | 46.79±2.99b | 82.68±2.92b | 2054.36±48.93b | 38.36±2.03b | 379.36±26.35c |
| LF-CQPC10 group      | 31.06±2.09c | 66.37±2.44c | 1812.19±42.03c | 32.55±1.97c | 412.34±25.80b |

4. Conclusion
In conclusion, LF-CQPC10 can adsorb lead ions in the body and reduce lead content in blood and viscera. In addition, LF-CQPC10 can alleviate the exacerbation of inflammation and liver and kidney injury caused by lead ions entering the body, which can alleviate the damage caused by lead ions to the body. In conclusion, LF-CQPC10 is an excellent strain with strong lead adsorption capacity. In the future, LF-CQPC10 still has more research value, and it has great potential and research value in terms of oxidative stress on lead ions in human body and mitigation of toxicity of other heavy metals.

Acknowledgments
Xin Zhao and Qin Li contributed equally to this work. This research was supported by Chongqing University Innovation Research Group Project (CXQTP20033), China.

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