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Lycium barbarum Formula Biotransformed by *Rhodopseudomonas palustris* Promoted Lead Removing and Anti-Oxidative Damage in Lead-Susceptible Tissues of Lead-Exposed Mice

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We aimed to study the lead-reducing effect of *Lycium barbarum* formula biotransformed by *Rhodopseudomonas palustris* (LFBR) comparing to other lead-removing preparations and preliminarily inferred lead-removing mechanism of LFBR. The lead-reducing effect was investigated via tissue distribution level examination based on the method of graphite furnace atomic absorption spectrometer (GFAAS). Results demonstrated LFBR could effectively reduce lead distribution levels of livers, kidneys, thighbones, brains, heart in mice and protect lead-susceptible tissues against lead poisoning in vivo. LFBR also could prevent the reduction of the activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and reduced glutathione (GSH) in lead-susceptible tissues and indirectly protect these tissues from lead-induced oxidative damage. We found that low LFBR group and high LFBR group exerted the relatively better lead-reducing effect and anti-oxidative damage than mean LFBR group which indicated that the lead removing capacity of LFBR was not based on a linear dose-response manner. In addition, results showed tissue distribution of microelements in mice treated with LFBR had no significant difference with that in mice treated with distilled water. LFBR effectively promoted lead removing and anti-oxidative damage in lead-susceptible tissues of lead-exposed mice but not affected the absorption and distribution of microelements.

Keywords: *Lycium barbarum*, *Rhodopseudomonas palustris*, Lead, Biotransformation, Toxicity.

Lead poisoning is a global environmental disease that induces lifelong adverse health effects[1]. It is well-known that lead exposure is dangerous for human health as the undegradability of lead which induces the massive accumulation both in environment and human body in long term. The accumulation will cause serious health damage especially happened in some lead-susceptible tissues such as livers, brains, kidneys, hearts and thighbones. Research indicated that lead exposure would cause inordinately damage of nerves, hematopoiesis, immune, digestion, growth and internal organs in body, and what is more serious is that lead can cause damage in infants and young children's cognitive and neural behavior and other brain functions[2]. Especially children's lead poisoning widely and frequently occurred in the world which has drawn more attention from the society. Long-term low-dose exposure to lead can exacerbate lipid peroxidation in vivo, over time may induce tumor and even slight lead exposure can impair intellectual and memory[3]. Furthermore, lead accumulated on animal or human kidney, liver, reproductive system, nervous system, blood

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system and so on produces toxic effects in many aspects[4]. Lead poisoning can damage the activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and reduced glutathione (GSH), and enhance the level of malondialdehyde (MAD) leading to accumulation of oxygen free radicals (OFR), which finally can cause lipid peroxidation of biological membranes and indirectly impair cellulated integrity[5]. This is also the main mechanism of lead-induced damage in human body.

Traditional medical treatment available for lead poison therapy is chelation, which can help people suffering severe lead poisoning to keep health. Classical drug used for the lead poisoning is EDTA, $CaNa_2EDTA$, BAL, DMSA, DUSP, DMPS and so on. However, these medicines usually have negative effect on some susceptible tissues, to some extent, in the treatment. They are often effective for short-term therapy and thus can be limited for wide usage[6]. Recent years, more and more people pay attention to *Lycium barbarum* which is one of traditional Chinese medicines and has complex mechanism of lead removing. The extract of *L. barbarum*, especially the polysaccharide ingredient, is regarded as a highly potential antioxidant agent[7]. Research indicated that *L. barbarum* polysaccharides (LBP) can remove oxygen free radicals (OFR) in vivo, improve the activity of endogenous antioxidant enzymes and eventually restrain lipid peroxidation[8]. Moreover, LBP is referred as a good electron supplier. Electron supplied by LBP can make the Fe^{3+} reduce to Fe^{2+} and furthermore the reducing power of LBP will enhance with the concentration increasing within some limits. Meanwhile, some studies showed that LBP can remove OFR, chelate metal ion, block and reverse lead-induced oxidative damage, functioning in maintenance of metal ion normal valence, and thereby protect the activity of SOD in vivo, indirectly or directly restraining lipid peroxidation[9].

Rhodopseudomonas palustris is one of photosynthetic bacteria and has the strong tolerance for heavy metals[10]. Some reports said that *R. palustris* has the effect on lead removing which was based on the glycoproteins existing in *R. palustris* cell walls. The Pb^{2+} could combine with the glycoproteins of cell walls, and then was transmitted into cytoplasm which contained S^{2-} generated by assimilation of sulfate reduction and the effect of cysteine desulfhydrase, and then synthesized PbS precipitation with S^{2-} in the cytoplasm, eventually moved out of the cell[11].

In our previous study, we investigated the effect of *L. barbarum* extract with different concentrations (6.25 $g^{-1}l$, 12.5 $g^{-1}l$, 200 $g^{-1}l$) on the growth of *R. palustris* cultured in conventional medium and the viable count, growth curve and dehydrogenase activity of *R. palustris* were respectively examined. Results showed that low concentration of *L. barbarum* extract could promote growth of *R. palustris*, shorten bacterial cycle and improve bacterial activity. Moreover concentration of *L. barbarum* ranging from 3.125 $g^{-1}l$ to 6.25 $g^{-1}l$ was best for bacterial growth[12]. In addition, some studies also proved that *R. palustris* can really improve the ability of lead reduction[11]. At last, the effect of lead removing, antioxidant damage and protection of lead-susceptible tissues of LFBR in lead-exposure mice was investigated in present study.

MATERIALS AND METHODS

Preparation of conventional medium and *L. barbarum* medium

L. barbarum was purchased from Guoyuan Chinese medicine Ltd in Bozhou and was identified by Prof. Bai Yun'e of Shanxi Medical University as dry ripe fruit of *L. barbarum*. *R. palustris* was identified and donated by Photosynthetic Bacteria Laboratory of Shanxi University. Conventional medium contains the following materials (per liter): 2 g sodium malate, 0.15 g $MgSO_4 \cdot 7H_2O$, 1.2 g yeast extract, and 1.5 g $(NH_4)_2SO_4$ with the pH adjusted to 7.0 before autoclaving. *L. barbarum* medium was prepared by the following procedure: dry matured fruit of *L. barbarum* was aqueously extracted twice, and then all extract properly concentrated by Rotory evaporator was totally added into conventional medium. The final concentration was 0.20 g ml^{-1} of *L. barbarum* in medium. Then pH of this medium was adjusted to 7, finally sterilized for half an hour.

Culture procedures

R. palustris was cultured in culture flasks containing conventional medium under the condition of light and anaerobic environment. These germs were transferred into the flasks under

the aseptic condition. The flasks were placed in the culture room with the illumination intensity of 2500 Lux and temperature of 30!. To culture for 3 days, the *R. palustris* being activated were obtained, and then added the *R. palustris* respectively into conventional medium and *L. barbarum* medium with the ratio of 1/10 in these mediums to culture in the same situation for 20 d.

Animals

Four-week old healthy clean level Kunming male mice (average weight: 20 g, n = 80) were obtained from Shanxi Medical University of Medicine Laboratory Animal Center and used in the present work. Mice were kept in cages (20×15×15 cm, 5 mice each) with free access to basal feed and deionized water under a 12 h light/ 12 h dark cycle in a humidity (55 ± 10)% and temperature (25 ± 1) ! controlled room. Mice were placed in the experimental environment in advance for 1-week adaptation period. This study was reviewed and approved by Shanxi Medical University of Medicine Ethical Review Board.

Establishment of high blood lead level (BLL) models in mice

To establish high BLL models, mice received peritoneal injection (ip) with lead acetate solution (0.02 g kg^{-1} bw, i.e. 0.01 g Pb^{2+} kg^{-1} bw) once daily for 7 d according to previous study[13]. As our previous experiments have proved that this method of peritoneal injection for 7 days for establishing the high BLL models was effective, and results obtained from the pre-experiment all satisfied the requirement of this experiment, thus we used peritoneal injection for 7 days for building mice models in this study.

Experimental groups

The mice (n=10) received peritoneal injection (ip) with normal saline (0.02 g kg^{-1} bw) belonged to negative group (NC). And the mice with eligible high BLL were then randomly divided into 6 groups (n=10): (i) high BLL model control group (MC); (ii) *L. barbarum* group (LB); (iii) *R. palustris* group (GC); (iv) low LFBR (LE); (v) mean LFBR (ME); (vi) high LFBR (HE) (Table 1).

Tissue distribution

To investigate the effect of LFBR on the reduction of lead in lead-susceptible tissues of lead-exposed mice in vivo, mice in all groups were killed after 30-days experimental period. Some tissues which easily accumulate lead toxin (liver, kidneys, thighbone and brain) were separated and their lead, calcium, iron, zinc and magnesium levels were quantified and compared.

Determination of lead, calcium, iron, zinc and magnesium levels in tissues

Lead, calcium, iron, zinc and magnesium levels in tissues were analyzed by graphite furnace atomic absorption spectrometer (GFAAS) method. After killing of mice, liver, kidneys, thighbone, heart and brain samples (0.5ml) were wet-weighed and saline-rinsed. Tissue samples were respectively soaked by concentrated nitric acid (15 ml g^{-1}) for 12 hours. After that, tissue samples were digested on the electric heat until produced mist. Then eligible tissues were diluted 1:10 in 1.0% (v v^{-1}) of dilute nitric acid. Then, tissues samples were deposited in the graphite furnace for quantitative analysis. An atomic absorption spectrometer (Perkin-Elmer AA800) equipped with graphite furnace and autosampler (Perkin–Elmer As-72) was used for the determination of lead, calcium, iron and zinc levels. The method performance of GFAAS analysis was validated via limit of detection (LOD), limit of quantification (LOQ), precision, spiked recovery and quality control (QC) proficiency test according to our previous study.

Evaluation of lead poisoning induced oxidative damage in lead-susceptible tissues

Lead can reduce the activity of catalase and superoxide dismutase in tissues that may be the main cause for tissues oxidative damage[14]. To investigate whether LFBR have the better effect against lead-induced oxidative damage on lead-susceptible tissues, the changes of SOD, GSH-PX and GSH levels in lead-susceptible tissues after giving LFBR in mice were determined. SOD fit, GSH-PX fit and GSH fit were purchased from Nanjing Jiancheng Bioengineering Institute. All experiments were respectively followed the instruction manual provided by fits.

Statistical analysis

All data of samples in experiments were multiple independent and results were showed as means ± SD. Data obtained in GFAAS analysis of calcium, iron , magnesium and zinc levels, as well as SOD activity, GSH-PX activity and GSH concentration, were evaluated using Dunnett-t multiple comparison test. Statistical analysis was performed using the Statistical Product and Service

Solutions (SPSSs) version 13.0 statistical software (SPSS Inc., Beijing, China). Differences were considered significant at $p < 0.05$ in all statistical tests.

RESULTS

Metal standard curves of GFAAS analysis

The standard solution of lead (National center for reference materials), iron (General Research Institute for Nonferrous Metals Beijing), calcium (General Research Institute for Nonferrous Metals Beijing), magnesium (National center for reference materials) and zinc (General Research Institute for Nonferrous Metals Beijing) were respectively prepared according to LOQ. Concentration range of lead standard solution: 0 ¼g l^{-1}, 20¼g l^{-1}, 40¼g l^{-1}, 60¼g l^{-1}, 80¼g l^{-1}, 100¼g l^{-1}; Concentration range of calcium standard solution: 0 mg l^{-1}, 0.5 mg l^{-1}, 1 mg l^{-1}, 2 mg l^{-1}, 4 mg l^{-1}, 8 mg l^{-1}; Concentration range of magnesium standard solution: 0 mg l^{-1}, 0.5 mg l^{-1}, 1 mg l^{-1}, 2 mg l^{-1}, 4 mg l^{-1}, 8 mg l^{-1}; Concentration range of iron standard solution : 0 mg l^{-1}, 0.5 mg l^{-1}, 1 mg l^{-1}, 2 mg l^{-1}, 4 mg l^{-1}, 8 mg l^{-1}; Concentration range of zinc standard solution : 0 mg l^{-1}, 0.125 mg l^{-1}, 0.25 mg l^{-1}, 0.5 mg l^{-1}, 1.0 mg l^{-1}, 2.0 mg l^{-1}. Metal standard curves were obtained via GFAAS analysis (Fig. S1).

Effect of LFBR on reduction of lead in lead-susceptible tissues

Lead distribution levels of selected mice tissues in all research groups were shown in Fig. 1. Results showed that lead distribution levels in all groups varied between different tissues. Lead distribution levels in mice thigh bones were much higher than in other investigated tissues, such as the average lead level in mice thigh bones in high BLL model control group was 1017.33¼g g^{-1}, which was obviously higher than in brain, liver, heart and kidney with lead distribution levels: 4.39¼g g^{-1}, 90.08¼g g^{-1}, 80.12¼g g^{-1} and 93.19¼g g^{-1}respectively. This trend was same in other groups (Table S2), indicating pb^{2+} ion was easily absorbed and mainly accumulated in mice skeleton. Lead distribution levels in mice brain in all groups were much lower than in other investigated tissues (Table S2), probably caused by the existence form of lead in mice blood , which were usually lead ion, the combination of $pb^{2+}$and hemoglobin, and thus hardly passed the blood brain barrier. Compared to high BLL model control group, the lead distribution levels of brain, livers, kidneys, hearts and thighbones in mice in *L. barbarum* group, *R. palustris* group and all LFBR groups exerted significant lead-removing effect ($p<0.05$, Fig. 1). Moreover, low LFBR group and high LFBR group demonstrated better lead-removing effect with relatively high reducing rates in all of selected tissues (low LFBR group: 17.4% in livers, 28.3% in kidneys, 17.9% in thigh bones, 32.2% in heart and 53.5% in brain; high LFBR group: 24.6% in livers, 21.6% in kidneys, 25.3% in thigh bones, 52.2% in heart and 43.0% in brain) among all of five lead-

Table 1. Experimental groups and the level of lead acetate (n=10)

group	Lead acetate (g kg^{-1} bw)[a]	Administered groups[b] Addition level (ml kg^{-1})
Negative control	0.02[c]	5[d]
High BLL model control	0.02	5[d]
L. barbarum	0.02	5[e]
R. palustris	0.02	5
Low LFBR	0.02	2.5[f]
Mean LFBR	0.02	5[f]
High LFBR	0.02	10[f]

[a] Lead acetate was used for the establishment of mice high BLL model and administered via peritoneal injection for 7 days.
[b] All formulations were administered via irrigation for 30 days.
[c] Mice received peritoneal injection (ip) with normal saline.
[d] Mice were administered via irrigation with deionized water.
[e] The concentration of *L. barbarum* was 0.36 g ml^{-1}.
[f] The concentration of *L. barbarum* in LFBR was 0.2g ml^{-1}.

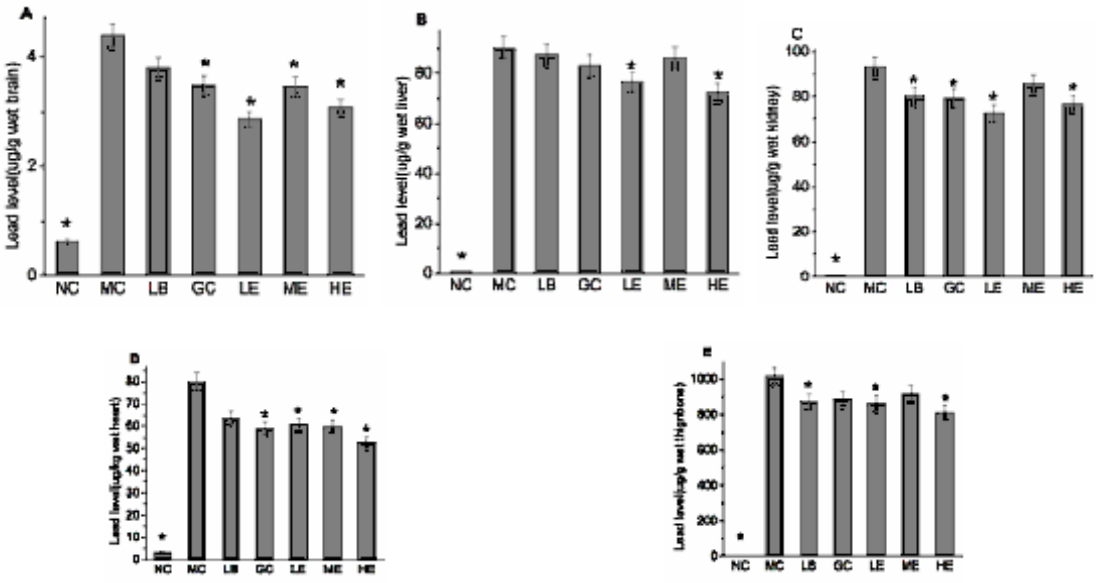

Fig.1. Reduction effect of biotransformation extract on lead distribution levels in (A) brain, (B) liver, (C) kidney, (D) heart and (E) thighbones of mice (n = 10). Group NC, negative control group; Group MC, high BLL model group; Group LB, *L. barbarum* group ; Group GC, *R. palustris* group; Group LE, low LFBR group; Group ME, mean LFBR group; Group HE, high LFBR group.*p<0.05 versus high BLL model group

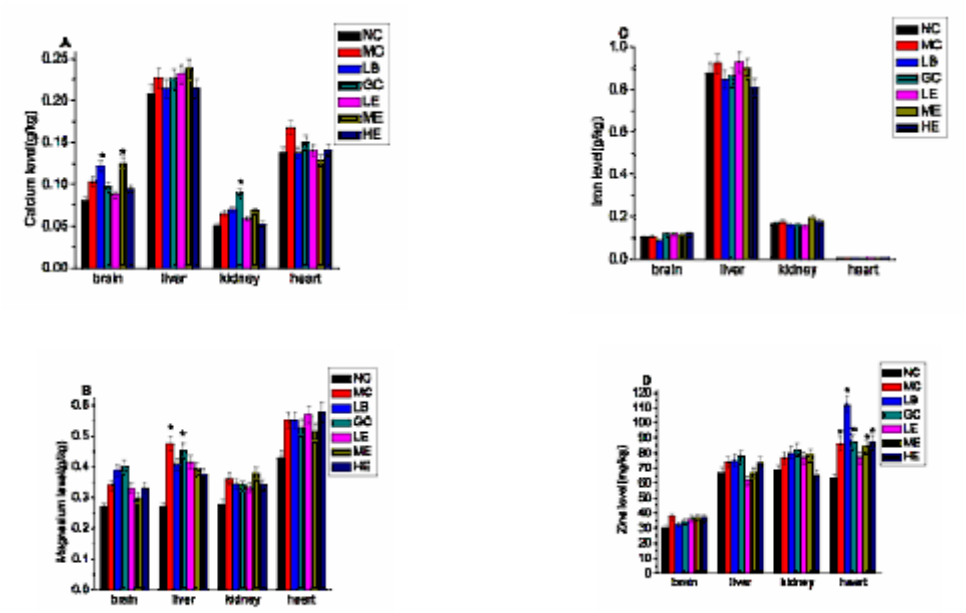

Fig. 2. Calcium, magnesium, iron and zinc level in lead-susceptible tissue (brain, liver, kidney, heart, thigh bone). A: calcium level; B: magnesium level; C: iron level; D: zinc level. Group NC, negative control group; Group MC, high BLL model group; Group LB, *L. barbarum* group ; Group GC, *R. palustris* group; Group LE, low LFBR group; Group ME, mean LFBR group; Group HE, high LFBR group.*p<0.05 versus negative control group

reducing groups. To investigate whether LFBR reduced the distribution levels of essential minerals, calcium, iron, magnesium and zinc levels in mice livers, kidneys, thigh bones, hearts and brains in all groups were determined. Overall, results revealed that there was no significant difference on calcium, iron, magnesium and zinc levels between negative control group and LFBR groups except some outliers ($p<0.05$, Table S3-6 Fig. 2).

Effect of LFBR on lead poisoning induced oxidative damage on selected tissues

Lead-induced oxidative damage on lead-susceptible tissues in mice was mainly based on the oxidative process (lipid peroxidation). Results showed that the concentrations of SOD and GSH-PX in mice livers in all LFBR groups were significantly enhanced, compared to corresponding parameters in high BLL model group($p<0.05$, Fig. 3). The fact that LFBR could protect the enzyme from the oxidative damage induced by lead exposure supported the notion that the activity of SOD and GSH-PX was indeed reduced by lead exposure. Results also demonstrated that the concentration of GSH and GSH-PX in mice kidney in all LFBR groups was significantly enhanced ($p<0.05$, Fig. 4), indicating that the capability of ORF in mice kidney was greatly enhanced which lead-induced oxidative damage would impair the activity of GSH and GSH-PX in lead-susceptible tissues which indirectly lead to produce more ORF. However, more ORF could cause the damage of biological membranes. We also investigated the anti-oxidative effect of LFBR on mice brains, and the results showed that the concentration of SOD and GSH-PX in mice brains in all LFBR groups was significantly enhanced, compared to corresponding parameters in high BLL model group ($p<0.05$, Fig. 5). It was revealed that LFBR groups also exerted significant anti-oxidative effect on mice brain via indirectly protecting the enzymes from oxidative damage. Meanwhile, the activity of aspartate transaminase (AST) was also determined in mice blood in LFBR groups, which demonstrated dramatic reduction compare to high

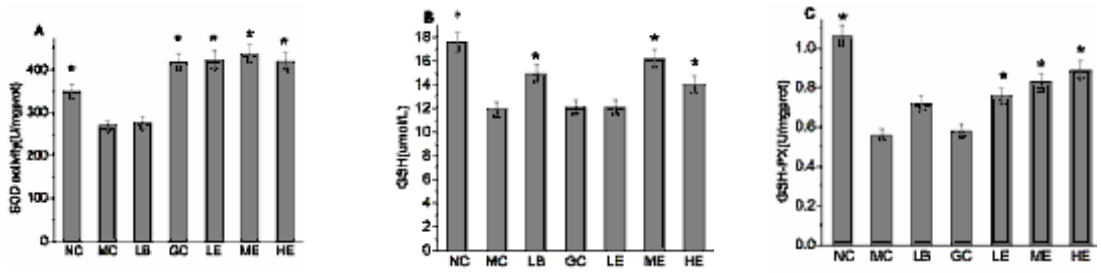

Fig. 3. Prevention effect of LFBR on lead-induced oxidative damage in liver of mice (n = 10). (A) SOD activity; (B) GSH levels; (C) GSH-XP activity. Group NC, negative control group; Group MC, high BLL model group; Group LB, *L. barbarum* group ; Group GC, *R. palustris* group; Group LE, low LFBR group; Group ME, mean LFBR group; Group HE, high LFBR group.*$p<0.05$ versus high BLL model group

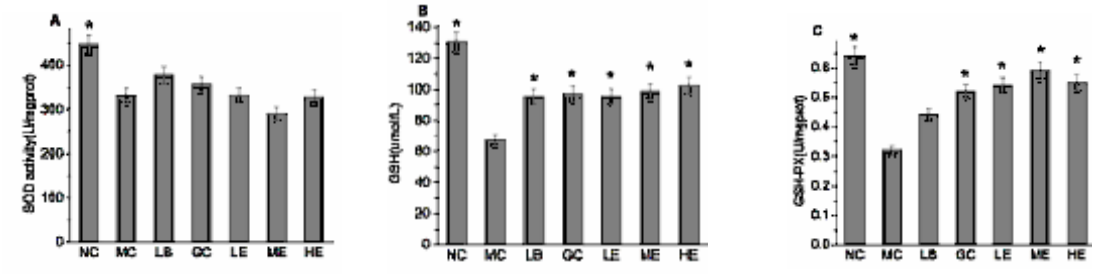

Fig. 4. Prevention effect of LFBR on lead-induced oxidative damage in kidney of mice (n = 10). (A) SOD activity; (B) GSH levels; (C) GSH-XP activity. Group NC, negative control group; Group MC, high BLL model group; Group LB, *L. barbarum* group ; Group GC, *R. palustris* group; Group LE, low LFBR group; Group ME, mean LFBR group; Group HE, high LFBR group.*$p<0.05$ versus high BLL model group

BLL model group ($p<0.05$, Fig. 6). The AST was one of the most effective indexes for evaluating the function of liver, and thus we inferred that the LFBR may play a role in maintaining the function of liver in lead-exposure mice. Moreover, Low LFBR group and high LFBR group demonstrated better anti-oxidative effect with relatively high rates in all of selected tissues among all of other groups, which were in according with the strong lead-reducing effects of low LFBR group and high LFBR group during the above experiment. In summary, the present study showed that LFBR could effectively reduce the risk of lead-induced oxidative damage in lead-susceptible tissues in vivo. All values mentioned above were shown in Table S7-10.

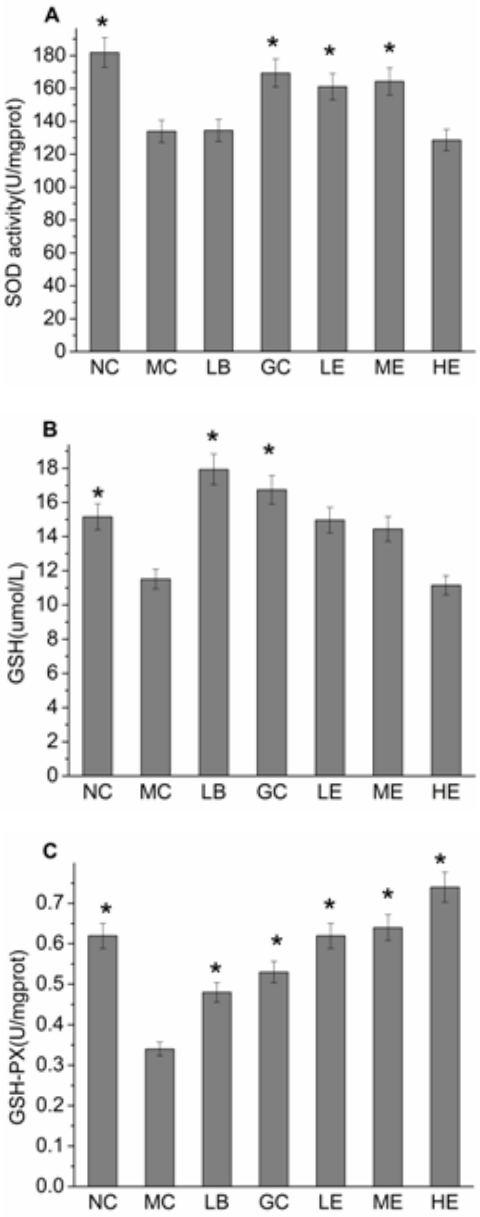

Fig. 5. Prevention effect of LFBR on lead-induced oxidative damage in brain of mice (n = 10). (A) SOD activity; (B) GSH levels; (C) GXP activity. Group NC, negative control group; Group MC, high BLL model group; Group LB, *L. barbarum* group ; Group GC, *R. palustris* group; Group LE, low LFBR group; Group ME, mean LFBR group; Group HE, high LFBR group.*$p<0.05$ versus high BLL model group

DISCUSSION

The aim of this study was to investigate the lead-reducing effect and anti-oxidative damage of LFBR in lead-susceptible tissues of lead-exposed mice and infer probable lead-removing mechanism of LFBR. It has been well-known that natural antioxidants like extract of *L. barbarum* have promising capacity of reducing the level of blood lead in lead-exposed mice, and in some researches, the effect of lead removing and anti-oxidative damage of *L. barbarum* has been proved by experiments and its lead-reducing rate could reach 56% or so[15]. Many studies showed that *L.*

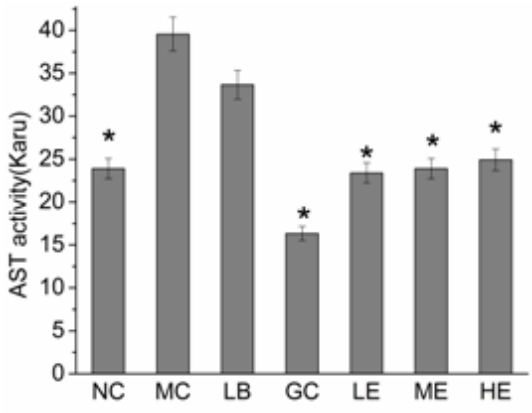

Fig. 6. Effect of LFBR on maintaining the function of liver in lead-exposure mice (n = 10). AST level among all groups were determined as the biomarker for the liver function. Group NC, negative control group; Group MC, high BLL model group; Group LB, *L. barbarum* group ; Group GC, *R. palustris* group; Group LE, low LFBR group; Group ME, mean LFBR group; Group HE, high LFBR group.*$p<0.05$ versus high BLL model group

barbarum as a material used for resistance of lead damage had a good effect. Moreover, Studies indicate *L. barbarum* has antioxidant, anti-aging, neuroprotective, blood glucose regulating, immunomodulative, anti-tumor, cytoprotective and many other effects[15]. Among various constituents in L. barbarum, *L. barbarum* polysaccharides (LBP) with a glycan-O-Ser glycopeptide structure has been researched extensively and is considered to be important for the efficacy of *L. barbarum*, especially for its antioxidant effects. LBP-standardized fruit juice has been shown to have a significant clinical antioxidant effect, as indicated by increased serum levels of SOD and GSH-PX, and reduced MAD levels *in vivo* in a randomized, double-blind, placebo-controlled human clinical study[16]. In the present study, we selected LFBR to sequentially discover the deeper effect of lead removing and anti-oxidative damage of LFBR. Also, we found that low LFBR group and high LFBR group exerted the relatively better lead-reducing effect and anti-oxidative damage than mean LFBR group which indicated that the lead removing capacity of LFBR was not based on a linear dose-response manner. (Fig. 1, Fig. 3-5). Previous studies found there were different responses of lead-induced oxidative stress in various target sites in animal studies from low to high doses of lead exposure[17, 18]. Meanwhile, the lead-reducing effect of LFBR had a correlation with its anti-oxidative damage and bidirectional effect of antioxidants/oxidative balance in vivo might affect the lead distribution level in some tissues. Although the mechanism of antioxidants/oxidative balance in vivo was not completely clear[19], the material which can promote maintenance of the antioxidants/oxidative balance may exert an effect of anti-oxidative damage. In our present study, LFBR could indirectly adjust antioxidants/oxidants balance via changing the activity of some enzymes (SOD, GSH, GSH-PX) involved in this balance process (Fig. 3, 4, 5).

In our study, the effect of lead removing and anti-oxidative damage in the low LFBR group was almost no different with that of high LFBR group, and the dosages of *L. barbarum* administration in two LFBR groups were respectively 0.5g kg^{-1} and 2.0 g kg^{-1} (Table 1), which obviously indicated that this effect was not only based on the additive action of *R. palustris* and *L. barbarum*, but on the new active substances and factors produced during the biotransformation process.

Thus we preliminarily inferred that there existed at least three mechanisms of lead removing and anti-oxidative damage of LFBR in selected tissues. One of the mechanisms was bioabsorption of *R. palustris*. Cell wall surfaces of *R. palustris* were inlaid with some polysaccharides and proteins which could absorb Pb^{2+}. Then parts of Pb^{2+} were transferred into cytoplasm through ABC transporter of cell wall and accumulated for other usages. However, other parts of Pb^{2+} were deposited in the form of conjugates and underwent exocytosis with cell membrane motility. Another mechanism of lead removing of LFBR was biotransformation of *R. palustris*. When Pb^{2+} were accumulated in cytoplasm as mentioned above, they might involved in metabolism pathways of *R. palustris* and then active enzymatic reactions such as sulfate assimilation reactions, which could transfer SO_4^{2-} into S^{2-}. Then Pb^{2+} was synthesized PbS precipitation with S^{2-} in the cytoplasm, eventually moved out of the cell[11]. The last mechanism was comprehensive effect of *L. barbarum* biotransformed by *R. palustris*. As mentioned above, there were many studies confirmed that *L. barbarum* exist the effect of lead removing and antioxidant damage. The extract of *L. barbarum*, especially the polysaccharide ingredient, was regarded as a highly potential antioxidant agent and could have complexation reaction with Pb^{2+} which could be mainly reason for the effect of lead removing and antioxidant damage. In addition, the mechanism of lead removing and anti-oxidative damage in selected tissues was not only based on enhancement of LBP level and devouring effect of *R. palustris* but also on other active substances and factors produced by biotransformation effect of *R. palustris* on *L. barbarum.* We would further study the new substances in our following work. Studies indicated that *R. palustris* as a photosynthetic probiotics could effectively reduced the lead level via devouring the blood or tissue lead and then generating the lead sulfide precipitation finally excreted to the outside of the body[11]. *R. palustris* group showed the significant reduction of lead in tissues compared to high BLL model group ($p>0.05$, Fig. 1), indicating that *R. palustris* really involved

in lead reduction process in lead-exposure mice.

This study mainly demonstrated the effect of lead removing and anti-oxidative damage of LFBR in some representative tissues which easily accumulated lead toxin. Besides the alleviation effect in liver, heart and kidneys, we found that the lead-reducing effect of LFBR was also available in some deep distributed tissues, such as thighbone and brain (Fig. 1). Bone is the major reservoir of body lead, accounting for 75% in children and 90% in adults. Previous work indicated that lead increased bone turnover resulting in weaker cortical bone in adult female mice and suggested that lead might exacerbate bone loss and osteoporosis in the elderly[20]. In present work, lead distribution levels were significantly reduced by LFBR in all of representative tissues. Especially, we need to highlight that low and high LFBR group not only showed a promising lead-reducing effect in livers, hearts, thighbones, brains and kidneys, but also exerted the maintenance of liver functionality (Fig. 6). These results indicated that LFBR could protect susceptible tissues against lead poisoning in long terms which were mainly based on the antioxidants produced by *R. palustris* and *L. barbarum*. Coenzyme Q10 was one of secondary metabolites of *R. palustris* and regarded as the most effective antioxidant among contents of *R. palustris.* There are three redox states of Coenzyme Q10: fully oxidized (ubiquinone), semiquinone (ubisemiquinone), and fully reduced (ubiquinol). The capacity of this molecule to exist in a completely oxidized form and a completely reduced form enables it to perform its functions in the electron transport chain, and as an antioxidant, respectively. In addition, research indicated that antioxidant effect of LBP of *L. barbarum* was better than that of vitamin C[21]. All of these antioxidants could clear free radical produce by lead and protect the body from oxidant damage.

Furthermore, LFBR would not affect the absorption and metabolism of calcium, iron, magnesium and zinc in administered mice in vivo. This evidence suggested that the LFBR used in this study did not only effectively reduce lead distribution levels, but also maintained normal absorption of essential calcium, iron, magnesium and zinc in mice. Many studies have so far focused on the reduction of lead exposure via endogenous or exogenous factors[22, 23, 24]. However, few studies have further investigated whether the absorption of essential elements was also reduced, which was fully considered in the present work.

One of lead poisoning associated complications might be lead-induced oxidative stress and related damage[25]. Some previous studies indicated that lead exposure could reduce the activity of SOD, GSH-PX and GSH in vivo, aroused so great accumulation of ORF in blood or tissues that break the antioxidants/oxidative balance and finally caused the damage of biological membranes[26, 5]. It had been found that the LBP, one of the most effective components in *L. barbarum*, could against the lead-induced reduction of activity of SOD, GSH-PX and GSH, promote the maintenance of antioxidants/oxidants balance and indirectly protect the integrity of cell membranes in blood and tissues. To investigate the effect of LFBR on the above lead toxicity mechanisms, three biomarkers including SOD, GSH-PX and GSH were evaluated. In the present work, the enhancement effect of lead on lipid peroxidation suggested that lead was not able to initiate peroxidation by a direct action on membrane-lipids and, therefore, that the mechanism of lead-induced lipid peroxidation must involve an indirect process[27, 28]. LFBR in this experiment could affect the indirect process to some extent and therefore play a role in protecting the protein and membrane in blood and tissues from lead poisoning. Results from this study demonstrated that the low and high level of LFBR exerted better effect against lead-induced oxidative damage than mean level of LFBR (Fig 3-5). However, the *L. barbarum* group did not show significant effect of lead removing and anti-oxidative damage in lead-susceptible tissues in administered mice.

In this study, the results have demonstrated that LFBR could effectively reduce the lead and lead-induced oxidative damage in selected tissues in lead-exposure mice. Some mechanisms of reduction of lead and protection of membranes of LFBR in administered mice have been discussed above. And we selected the 30 days for experiment to probe the long-term effect of our preparation, but did not investigate the short-term effect of LFBR to lead-exposed mice. In our further experiment, we will deeply study the mechanisms of lead removing and antioxidant

damage effect of LFBR which may involve some new products and factors produced during biotransformation process.

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