Effects of a Food Ingredient Group on Oxidative Stress in Lead-Poisoned Mice

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Key Words
Food ingredients · Lead-poisoned mice · Oxidative stress

Abstract

Objective: According to the principle of compatibility of traditional Chinese medicine, a food ingredient group was used to treat lead poisoning of mice. Methods: Ninety Kun-Ming male mice (18–20 g) were randomly divided into the following 6 groups: negative control group (NCG); lead acetate model group (LAG); positive drug group (PDG), and test substance groups with low-, medium-, and high-dose food ingredient group (LDG, MDG, and HDG). The test substance groups were fed with oral gavages containing the food ingredient group. The PDG were fed with gavages containing dimercaptosuccinic acid solution. The LAG and NCG were fed with gavages with deionised water. Results: One month later, both the medium and the high dose of the ingredient group had effectively increased the superoxide dismutase and glutathione peroxidase activity and decreased the malondialdehyde content in the blood, liver, kidney, and brain of the tested mice (p < 0.05). Compared to the PDG, we found that the MDG and HDG recovery from the lead-induced oxidative stress might be attributed to the induction of antioxidant enzyme sensitivity by the dietary supplement. Conclusion: Our findings provide evidence that the proposed food ingredient group has significant impacts on recovering from oxidative damage in lead-poisoned mice.

Yu-Xiu Shang and Yi Zhao contributed equally to this work.
### Introduction

Lead is a highly toxic metal. It is one of the major pollutants to the environment and may represent a serious health hazard to humans and animals. Lead accumulation depends on its exposure duration and concentration. It reacts with a series of functional groups (e.g., sulfhydryl) in proteins, enzymes and amino acids in the body, interfering with many physiological and biochemical activities [1]. Research shows that lead can change the anti-oxidative activities of some enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) by suppressing the sulfhydryl. Malondialdehyde (MDA), SOD and GSH-PX might be the targets of lead toxicity [2, 3].

Lead poisoning causes damages to major organ systems. The kidney, blood, liver and brain are the major sites of lead accumulation and damages [4]. Research has shown that a blood lead concentration of >0.48 μmol/l can cause an irreversible change of the nervous system in Chinese children [5]. An inverse correlation has been shown between children's intelligence quotient and the blood lead level. Children's cognitive ability was declined by inducing low-level exposure to lead [7]. At present, in China, the lead poisoning rate is 23.5%, and the average concentration of blood lead in children was 76.5 μg/l over a 5-year study period [8]. Based on these results, we can conclude that lead poisoning is still at a relatively high level.

In the treatment of lead poisoning, chelating agents with the sulfhydryl group were used. However, they have side effects since they cannot only cause damages to the kidneys but they can also cause the absence of other essential elements and the metabolic disorder of antioxidant. It is necessary to develop a lead-excreting food that has fewer side effects to replace the standard lead excretion drugs. Research has shown a potential for *Lycium barbarum* polysaccharide (LBP) and *Glycyrrhiza uralensis* Fisch extract to significantly affect the antioxidant enzymes SOD and GSH-PX [9, 10]. Numerous studies have shown that increased mung bean and jujube supply may reduce the oxidative damages of the liver [11, 12]. The effective lead excretion components were firstly synthesized using the food ingredient group from *L. barbarum*, *G. uralensis* Fisch, mung bean, and jujube based on the compatibility principle of traditional medicine, i.e. the food ingredient group can decline lead accumulation and toxicity while they follow Chinese herbs. However, the impact of the food ingredient group on the oxidative damages of major organ systems in experimental animals was not reported in our country. The present study was designed to evaluate the impact of food ingredient group on the antioxidant system as well as the protective role of the food ingredient group.

### Materials and Methods

**Test Substance**

The test substance was a powder mixture of mung bean, *L. barbarum*, jujube, and *G. uralensis* Fisch at a ratio of 8:6.25:7.5:2.5 [13]. It was prepared as an oral suspension in doses of 5, 10, and 20 times of 0.60 g/kg body weight/day, as recommended for humans. The suspension was fed to the lead-administrated mice with an oral gavage. The mung bean powder was obtained from Heilongjiang Kangpaier Food Company, the *L. barbarum* powder from Ningxia Beirui Bio-Food Company, the jujube powder from Hebei Cangzhou Century Food Company, and the *G. uralensis* Fisch powder from Fujian Zhangzhou Damin Food Company.

**Animal Treatment**

Ninety male clean-grade Kunming mice (body weight 18–20 g) were provided by the Laboratory Animal Centre, Ningxia Medical University [quality certificate No. SCXK (Ning) 2005-0001; animal room certificate No. SYXK (Ning) 2005-0001]. The mice were treated at a temperature of 22–25°C and a humidity of 45–55%. This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on
the Ethics of Animal Experiments of Ningxia Medical University. Surgery was performed under sodium pentobarbital anesthesia, and every effort was made to minimize suffering.

The mice could freely eat pellets and drink water and were fed and observed for 1 week. Then, they were randomly divided into the following 6 groups of 15 mice each and received i.p. feeds as follows: negative control group (NCG): 12.50 μl/l acetic acid deionised water during 30 days; lead acetate model group (LAG); positive drug group (PDG); low-dose group (LDG); medium-dose group (MDG), and high-dose group (HDG). The LAG, PDG, and test substance group (TSG; included LDG, MDG, and HDG) were fed with lead acetate solution (3.00 g lead acetate trihydrate dissolved in 3,000 ml deionised water; equivalent to Pb²⁺ 546.20 mg/l) during 30 days. We added 12.5 μl/l acetic acid to acidify the solution to prevent the lead salt and the lead hydroxide from cohering [14].

After 30 days, TSG (LDG, MDG, and HDG) were fed with oral gavages containing the food ingredient group in doses of 3, 6, and 12 g/kg body weight/day, respectively, during 30 days, which is equivalent to 5, 10, and 20 times the dose of 0.60 g/kg body weight/day recommended for humans. For the PDG, the dimer-captosuccinic acid capsules were used. Based on the oral dose for a human adult, 1.5 g/60 kg body weight/day, we fed the mice a dose of 0.5 mg/20 g body weight/day. The drug usage and doses were the same as in clinical use for humans and the drug was given for 3 days and then stopped for 4 days. For the NCG and LAG, the deionised water was fed with an oral gavage. As the weight increased, an extra feed was added every week. Except for the PDG, all groups were fed once a day for 30 days.

Chemicals
We used the SOD detection kit, GSH-PX detection kit, and MDA detection kit (all from Nanjing Jiancheng Bioengineering Institute, China). All reagents and chemicals were of analytical grade or higher purity. The deionised water was obtained from the water purification Milli-Q system (Millipor Corporation, USA).

Outcome Measurement
At the end of experimentation, animals were sacrificed by decapitation under anesthesia. The fresh blood was immediately collected in an anticoagulation tube. The liver, kidney, and brain were picked up, washed with saline and homogenized separately. Then the concentrations of SOD, GSH-PX and MDA in the blood, liver, kidney, and brain were measured.

Statistical Analysis
The data are presented as means ± SEM. The single-factor analysis of variance approach was applied to the experiment data, and the least significant difference method was used to compare all group pairs. Statistical significance was defined as a two-tailed p < 0.05. All statistical analyses were performed using SPSS 11.5 for Windows.

Results

Impact of the Lead Removal Product on the SOD, GSH-PX, and MDA Content in the Blood
Compared to the LAG, all the TSG showed significantly higher SOD levels (p < 0.05), and the MDG and HDG demonstrated significantly higher GSH-PX levels (p < 0.05) and significantly lower MDA levels (p < 0.05). Compared to the PDG, all the TSG displayed no statistical differences in SOD level measurements (p > 0.05), both the MDG and HDG showed significantly higher GSH-PX levels (p < 0.05), and the HDG showed significantly lower MDA levels (p < 0.05) (table 1).

Impact of the Lead Removal Product on the SOD, GSH-PX, and MDA Contents in the Liver
Compared to the LAG, the MDG and HDG showed significantly higher SOD levels (p < 0.05) and significantly lower MDA levels (p < 0.05), and all the TSG demonstrated significantly higher GSH-PX levels (p < 0.05). Compared to the PDG, the MDG and HDG demonstrated significantly higher SOD levels (p < 0.05), the MDG showed significantly higher GSH-PX levels (p < 0.05), and all the TSG displayed no statistical differences in MDA levels (p > 0.05) (table 2).
Impact of the Lead Removal Product on the SOD, GSH-PX, and MDA Contents in the Kidney

In comparison to the LAG, the MDG and HDG showed significantly higher SOD levels (p < 0.05) and GSH-PX levels but significantly lower MDA levels (p < 0.05). Compared to the PDG, the MDG and HDG demonstrated significantly higher SOD levels (p < 0.05) and significantly lower MDA levels (p < 0.05), and the HDG showed significantly higher GSH-PX levels (p < 0.05) (table 3).

Impact of the Lead Removal Product on the SOD, GSH-PX, and MDA Contents in the Brain

Compared to the LAG, all the TSG showed significantly higher SOD levels and lower MDA levels (p < 0.05), and the MDG and HDG showed higher GSH-PX levels (p < 0.05). Comparing with the PDG, all the TSG displayed no statistical differences in SOD and MDA levels (p > 0.05). The MDG and HDG showed significantly higher GSH-PX levels (p < 0.05) (table 4).

Discussion

Lead exposure and oxidative damage are closely related. Lead can induce lipid peroxidation and reduce cell antioxidative capability. It also causes change in the redox state of cells and increases the tissue and cells to produce free radicals, which affect the cell oxidation and antioxidative capabilities. Lead exposure can also damage cell enzymes and the nonenzymatic antioxidant defense system [15, 16]. Research has shown that the liver, kidney and brain are considered to be the major target organs of lead poisoning [4]. El-Sokkary et al. [17] found that lead increased neuronal cell lipid peroxidation activity, decreased the activity of the GSH enzyme and SOD, and damaged neuron cells and reduce the cell density of the hippocampus and striatum (regional). In mice, lead exposure reduced the GSH level and total

Table 1. SOD, GSH-PX, and MDA contents in the blood

| Group | SOD     | GSH-PX   | MDA     |
|-------|---------|----------|---------|
| NCG   | 281.50±21.46 | 79.17±10.02 | 7.75±1.22 |
| HDG   | 253.93±19.17a,b | 64.75±11.96a-c | 7.87±1.27b,c |
| MDG   | 252.51±14.48a,b | 76.68±7.72b,c | 8.81±2.48b |
| LDG   | 238.98±18.01a,b | 30.88±9.63a | 9.53±1.79a |
| PDG   | 238.15±14.86a,b | 30.11±8.51a | 10.22±2.13a |
| LAG   | 222.09±16.59a | 29.35±5.03a | 10.40±1.20a |

The mean ± SEM from 15 animals are shown in each group.

a p < 0.05 versus NCG; b p < 0.05 versus LAG; c p < 0.05 versus PDG.

Table 2. SOD, GSH-PX, and MDA contents in the liver

| Group | SOD     | GSH-PX   | MDA     |
|-------|---------|----------|---------|
| NCG   | 282.91±14.50 | 30.20±4.31 | 31.75±9.37 |
| HDG   | 273.53±18.41b,c | 27.64±3.13b | 38.67±10.63b |
| MDG   | 270.30±14.81b | 30.05±1.69b,c | 37.96±4.41b |
| LDG   | 257.23±14.19a | 25.82±3.33a,b | 40.60±10.53a |
| PDG   | 256.94±22.83a | 25.73±2.41a,b | 41.15±8.34a |
| LAG   | 242.66±19.06a | 22.20±3.14a | 47.88±8.46a |

The mean ± SEM from 15 animals are shown in each group.

a p < 0.05 versus NCG; b p < 0.05 versus LAG; c p < 0.05 versus PDG.
oxygen free radical scavenging capacity values slightly; GSH reductase, catalase, and SOD activity was inhibited. Lead damaged the antioxidant system in the mouse liver especially in the group exposed to high-dose lead [18]. Research has also shown that the mechanism of oxidative damage may be associated with nitric oxide (NO) metabolism. Lead showed neurotoxicity through the influence of NO and NO synthase. Lead inhibited NO synthase activity, and the degree of inhibition depends on the time and concentration of lead exposure [19, 20].

Research shows that (1) the mung bean contains a water-soluble pigment component, which has certain antioxidant activity, and its oxygen free radical removal rate can reach up to 70–80%. The extracts of the mung bean contain protein, peptide, tannic acid, and flavonoids [21]. (2) LBP has antimutation and antioxidant effects. Providing a group exposed to lead acetate with different doses of LBP, it was found that the viabilities of SOD and GSH-PX increased with the increase of the LBP dose. The hippocampus is a part of the brain system that is responsible for studying and the spatial memory. LBP can also improve the memory of lead-administrated mice [22, 23]. (3) Jujube polysaccharide has the effects of scavenging $\text{O}_2^–$ and $\text{OH}^–$. In the laboratory environment, its best scavenging rates are 19.34 and 47.30%, respectively [24]. (4) *G. uralensis* Fisch has an antimutation effect. For example, it can suppress the sperm abnormality of mice caused by exposure to lead acetate. The extraction of *G. uralensis* Fisch has the antioxidant effect of removing the free radicals [25, 26]. In our research (preorthogonal experiments and lead excretion experiments), we found that the food ingredient group showed significant effects for lead excretion. We also found that *L. barbarum* and *G. uralensis* Fisch play a major role in excreting lead [27]. In this study, the food ingredient group was designated following the combination principles of traditional Chinese medicine and by synthesizing the effective lead excretion components in the medicinal and edible products of the mung bean, *L. barbarum*, jujube, and *G. uralensis* Fisch. In our study, both the medium and the high dose of the food ingredient group effectively increased the SOD and

### Table 3. SOD, GSH-PX, and MDA contents in the kidney

| Group | SOD   | GSH-PX | MDA       |
|-------|-------|--------|-----------|
| NCG   | 117.88±4.50 | 4.71±0.33 | 30.84±14.44 |
| HDG   | 111.44±6.15 $^{a-c}$ | 4.38±0.40 $^{b,c}$ | 38.24±12.70 $^{b,c}$ |
| MDG   | 110.92±6.64 $^{a-c}$ | 4.25±0.57 $^{b}$ | 42.70±13.86 $^{b,c}$ |
| LDG   | 104.31±6.63 $^{a}$ | 3.86±0.92 $^{a}$ | 72.46±16.88 $^{a}$ |
| PDG   | 104.29±7.17 $^{a}$ | 3.79±0.43 $^{a}$ | 72.07±8.55 $^{a}$ |
| LAG   | 98.91±5.29 $^{a}$ | 3.45±0.53 $^{a}$ | 80.99±15.64 $^{a}$ |

The mean ± SEM from 15 animals are shown in each group.  
$^{a}$ p < 0.05 versus NCG; $^{b}$ p < 0.05 versus LAG; $^{c}$ p < 0.05 versus PDG.

### Table 4. SOD, GSH-PX, and MDA contents in the brain

| Group | SOD   | GSH-PX | MDA       |
|-------|-------|--------|-----------|
| NCG   | 169.39±36.96 | 38.76±8.08 | 175.33±45.47 |
| HDG   | 151.11±21.39 $^{b}$ | 33.52±6.82 $^{b,c}$ | 222.98±38.04 $^{a,b}$ |
| MDG   | 152.05±20.06 $^{b}$ | 31.02±9.12 $^{b,c}$ | 230.61±22.78 $^{a,b}$ |
| LDG   | 140.18±25.40 $^{a-b}$ | 21.09±9.83 $^{a}$ | 246.55±28.42 $^{a,b}$ |
| PDG   | 134.18±28.05 $^{a}$ | 14.35±2.91 $^{a}$ | 250.07±32.44 $^{a,b}$ |
| LAG   | 113.71±14.69 $^{a}$ | 12.51±1.06 $^{a}$ | 355.91±30.42 $^{a}$ |

The mean ± SEM from 15 animals are shown in each group.  
$^{a}$ p < 0.05 versus NCG; $^{b}$ p < 0.05 versus LAG; $^{c}$ p < 0.05 versus PDG.
GSH-PX activity and decreased the MDA content in the blood, liver, kidney, and brain of the tested mice. We also showed that with the medium dose of the test substance, i.e. 6 g/kg body weight/day, SOD and GSH-PX levels were significantly increased and the MDA content in the blood was significantly decreased. Compared to the PDG, we found that the HDG and MDG, as treatment of lead excretion, should have been induced antioxidant enzyme sensitivity. Therefore, the food ingredient group in our study had a better effect on antioxidant, reducing the organ damage caused by the lead poisoning and providing certain treatments.

In our study, the food ingredient group (test substance) was medicinal and edible food (a kind of natural food). Many studies have confirmed that medicinal and edible foods could prevent and treat disease with fewer side effects. In accordance with the principle of formulating a prescription and by analyzing the nature and flavor of food and medicine, we designed the formula mainly with a sweetish taste. With a combination of two herbs of neutral nature, one of warm nature and one of cold nature, the formula possesses a nature of mildness. From the nutritional point of view, it is high in protein and rich in trace elements, which is similar to human food, while the oxidative damage caused by lead poisoning has a therapeutic effect. As is well known, the standard lead excretion drugs, such as calcium disodium EDTA and D-penicillamine, have serious side effects and are nonspecific. These drugs do not only promote lead excretion but could also promote the excretion of certain essential metal elements. In addition, these chelating agents cause obvious toxicity for the kidney. In clinical 1-, 2-, 3-level lead poisoning in children, it was not recommended to use those drugs to drive the lead treatment. Instead, it was suggested to intake diet or supplementary nutrients to promote the body to excrete lead. In summary, the medicinal and edible food (food ingredient group) is either anti-oxidation, lead excretion, and can also supplement the lack of nutrients. The most important role of those foods is that they have fewer side effects.

Our findings provide evidence that the proposed food ingredient group has significant impacts on recovering from the oxidative damage in the lead-poisoned mice.

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Disclosure Statement

All authors have approved the paper and declare no competing financial interests.

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