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Protective effects of crude polysaccharide from Xinyang red tea against Lead toxicity

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ABSTRACT

To investigate protective effects of polysaccharides from Xinyang red tea against lead toxicity of mice, 60 healthy rats were randomly divided into six groups: control group, lead-poisoning model group and EDTA group, low, middle and high dose groups treated with 100 mg/kg of EDTA,50mg/kg,100mg/kg and 200mg/kg tea polysaccharide, respectively every day. The contents of lead in blood, liver and kidney were determined and the activity of d-aminolevulinic acid dehydratase (ALAD) and the level of reactive oxygen species (ROS) in blood were measured. Furthermore superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and malonaldehyde (MDA) in liver were detected to explore the possible mechanism. Compared with the lead-poisoning group, there was a obvious increase（$P<0.05$）in the activities of blood ALAD, GSH-Px, SOD and CAT and reduction in blood ROS and liver MDA content（$P<0.05$）in EDTA and tea polysaccharide treated groups. Meanwhile the activities of blood ALAD and GSH-Px, SOD and CAT of liver in tea polysaccharides treated groups increased while blood ROS and liver MDA level decreased compared to the EDTA group. Increments in the activities of blood ALAD, liver GSH-Px, SOD and CAT and reduction in blood ROS and liver MDA level appeared with increasing tea polysaccharide dose. It was indicated that polysaccharides from Xinyang red tea could improve the activity of antioxidant enzymes and alleviate oxidative stress induced by lead exposure on rats.

KEYWORDS

Tea polysaccharides; Lead; Toxicity; Protective effects; Antioxidation.

INTRODUCTION

Lead is a ubiquitous pollutant in the ecosystem and is highly toxic to human at any age stages of life, especially for children[1]. Excessive exposure to lead ions results in the metal entering a human body through the digestive path, lung inhalation, and skin, and provoking damage of almost all organs and systems, in particular, the central nervous system, immune system, kidneys, adrenals, and bones[2]. The mechanism of lead toxicity is unclear up to now. Oxidative stress is considered a possible molecular mechanism involved in lead toxicity, which leads to free radical damage by two separate and related pathways: 1) the generation of reactive oxygen species (ROS), including hydroperoxides,singlet oxygen, and hydrogen peroxide, and 2) the direct depletion of antioxidant reserves[3]. Recent studies have shown that lead inhibits the activity of antioxidant enzymes, including glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD)[4]. These findings clearly suggest that antioxidants play an important role in the treatment of lead poisoning.

Generally the mainstays of therapy consist of the elimination of all sources of exposure, nutritional support, and the therapeutic use of specific chelators for binding of metal ions and removal of them from the body with urine or feces[5]. However, the treatment of chelator is accompanied by adverse effects including renal toxicity and loss of other essential metals and causes some side effects such as nausea; vomiting, anorexia, appetite loss, and diarrhea still remain[6]. Therefore, there are an increasing number of studies trying to seek for safe and efficient dietary supplements against lead toxicity. At present attention is given to a group of substances called dietary fibers, including pectins, alginates, chitin and chitosan, carrageenans, lignin and some others, which were shown to exert beneficial influence on different organs and systems and have a capacity to bind heavy metals[7].

Tea is the most widely consumed beverage in the world and has become an important agricultural product. During the past two decades, research has revealed that tea possesses beneficial effects, including hypoglycemic, depression of hypertension, anti-oxidation, protection against cardiovascular disease, and anticancer. These beneficial effects have been partly attributed to its variety of chemical ingredients[8]. Tea polysaccharide was found to be an important water soluble polysaccharide with certain bioactivities in the late 1980s. Many researchers made great efforts to study the biological and pharmacological properties of tea polysaccharide ever since. Some literatures have demonstrated that tea polysaccharide possesses many biological activities as anticancer, anti-mutation, anti-atherosclerotic, anti-radiation damage, anti-oxidant, anti-coagulation, anti-HIV properties, moderating blood glucose, etc[9].

The present study thus was planned to investigate the protective effects of tea polysaccharide in preventing lead-induced alterations in some hematologic variables and assess the effects of tea polysaccharide on anti-oxidative property which might play a potential role against lead toxicity.

MATERIALS AND METHODS

Materials

The dry tea leaves were extracted with 10-times distilled water at 90℃ for 2 h and repeated twice. Then the extracts were centrifuged to remove the contaminants. The supernatant was concentrated via rotary evaporation method and freed from the protein with trichloroacetic acid. Then the solution was precipitated with 95% alcohol and freeze-dried to yield crude polysaccharides powder[10].

EDTA was purchased from New Asiatic Pharmaceutical Company (Shanghai, China). Lead acetate $(CH_3COO)_2Pb{\cdot}3H_2O$ and all other analytical laboratory chemicals and reagents were purchased from Sinopharm Chemical Reagent Company (Shanghai, China). All kits for the assay of GSH-Px, CAT, SOD, MDA and were from Jiancheng Bioengineering Institute (Nanjing, China).

Animals and treatment

Sixty Adult male Kunming mice obtained from the Henan Laboratory Animal Center (Zhengzhou, China) were used in the experiments. The mice were selected strictly by weight (between 28 and 30g) and kept in stainless steel cages in a temperature-and humidity-controlled room that was equipped to maintain a 12-h light/12-h dark cycle. The mice were fed standard commercial rat chow, and water was given ad libitum. All the protocols of the study were approved by the Ethics Committee of Henan University, China. All the procedures of the study were carried out in accordance with European Community guidelines (Directive 2010/63/ EU) for the care and use of experimental animals.

Sixty mice were divided into six groups (ten animals in each group) and treated for 6 weeks: control group, lead-poisoned model group, EDTA-treated group, low dose tea polysaccharides group, middle dose tea polysaccharides group, high dose tea polysaccharides group. Lead-poisoned group were given 0.2% lead acetate water by gavage drink while control were treated with distilled water. EDTA group were treated by gavage with 100mg/kg. Tea polysaccharide of low, middle and high dose groups were treated by gavage with 50,100 and 200 mg/kg（weight）respectively every day. After the last administration, animals were given a 48-h rest period and sacrificed under light ether anesthesia. Blood was collected by cardiac puncture inheparinized tubes. The liver and kindey were removed, rinsed in cold saline, blotted and used for various biochemical variables.

Analysis of lead in blood and different organs

Samples were digested in concentrated HNO_3 by using Microwave Digestion System. Levels of lead were then determined by using graphite furnace atomic absorption spectrophotometer by using graphite furnace atomic absorption spectrometry (Hitachi, Japan)[11].

Blood ALAD assay

The activity of blood d-aminolevulinic acid dehydratase (ALAD) was assayed according to the procedure of Berlin and Schaller[12]. The assay system consisted of 0.2 mL of heparinized blood and 1.3 mL of distilled water. After 10 min of incubation at 37℃ for complete hemolysis, 1 mL of standard d-aminolevulinic acid (ALA) was added to the tubes and incubated for 60 min at 37℃. The reaction was stopped after 1 h by adding 1 mL of trichloroacetic acid. To the supernatant, an equal volume of Ehrlich's reagent was added and the absorbance was recorded at 555 nm after 5 min.

ROS level in blood

The levels of ROS in blood were measured by chemiluminescence. Briefly, 50 μL of tissue extract was added to 830μL Hepes buffer (pH 7.4), and 20mL of horseradish peroxidase was added to the reaction mixture as a catalyst. Then, 100 μL of 5 mmol/L luminol was added to the mixture. Levels of ROS were determined by measuring chemiluminescence for 15 min after the reaction[13].

Analysis of MDA, GSH-Px, SOD and CAT

The levels of malondialdehyde (MDA) and the activities of GSH-Px, SOD and CAT in liver were measured using the assay kit purchased from Nanjing Jiancheng Bioengineering Institute, China.

Statistical analysis

Data were presented as mean±SD for each group with ten mice per group. Statistically significant differences among groups were determined using one-way analysis of variance, followed by Tukey HSD. P values less than 0.05 were considered as significant.

RESULTS AND DISSCUSSION

Lead levels in blood and tissues of mice are shown in TABLE 1. These series of experiments showed that administration of lead acetate for 6 weeks results in dramatically increased lead contents in blood, liver and kidney comparison with control group, which indicated success of lead-exposure. Simultaneous use of EDTA or tea polysaccharide along with lead acetate did significantly change these parameters. Compared with the lead-poisoned model groups, tea polysaccharide treatment significantly decreased lead concentration in blood and tissues, and the protection was more prominent with increasing administration dose. EDTA treatment in intervention groups showed better effect on reducing lead burden than tea polysaccharide.

TABLE 1 : Lead level of blood, liver and kidney in different groups

Groups	lead levels in blood(μg/L)	lead levels in liver(μg/g)	lead levels in kindey(μg/g)
Control	8.32±0.23	0.72±0.09	0.96±0.11
Model	652±10.88	15.44±0.22	19.67±0.37
EDTA	241±6.34	9.42±0.14	8.32±0.22
Low dose	362±7.22	11.36±0.19	13.44±0.17
Medium dose	325±4.33	10.89±0.16	12.76±0.32
High dose	278±4.89	9.78±0.12	11.02±0.21

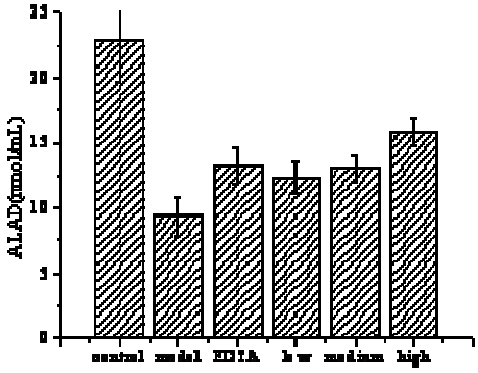

Figure 1 : Effects of tea polysaccharide and EDTA treatments on levels of ALAD in blood of mice

Lead exposure significantly inhibited blood ALAD activity and increased ROS level in all groups (Figure 1, 2). Coadministration of tea polysaccharide treatment showed prominent protective effects on ALAD activity recovery and ROS reduction ($P<0.05$). Better effects of ALAD activity recovery were noticed in high dose tea polysaccharide groups than that in low dose groups. The treatment of EDTA was less effective for recovering the ALAD activity and decreasing ROS level compared with tea polysaccharide treatment. Blood ALAD is an important enzyme that catalyzes the asymmetric condensation of two molecules of ALA to form porphobilinogen. It may be the most sensitive enzyme to lead toxicity because lead could bind SH groups and inhibit the catalytic activity of the enzyme. The inhibition of ALAD activity by lead causes an accumulation of ALA, which will generate ROS and induce oxidative stress.[14,15]. A significant recovery of ALAD activity was showed in the tea polysaccharide-treated groups, which indicated that tea polysaccharide was capable of recovering the inhibition of ALAD activity and protect sulfhydryl groups against oxidation and prevent ROS production caused by lead exposure.

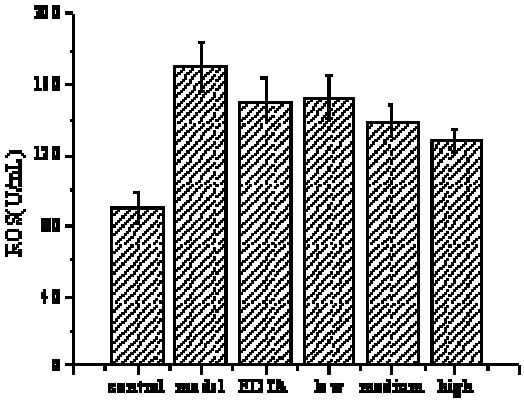

Figure 2 : Effects of tea polysaccharide and EDTA treatments on levels of ROS in blood of mice

Data shown in Figure 3,4 suggested changes in levels of MDA and glutathione (GSH-Px) and the activities of SOD and CAT in the liver. A significant increase in levels of GSH-Px, SOD and CAT activity was noted on lead exposure, whereas MAD values showed a decrease. Tea polysaccharide coadministration significantly prevented an increase in GSH-Px, SOD and CAT, whereas MAD levels responded favorably to tea polysaccharide at all doses. Tea polysaccharide coadministration (all doses) was able to decrease blood lead concentration, although the protection was more prominent at the higher dose. MDA are a byproduct of the lipid peroxidation process and a biomarker of oxidative stress. GSH-Px, SOD and CAT are three families of primary antioxidant enzymes in mammalian cells which are critical to peroxide removal, which were considered to play important roles in antioxidant defense system[16,17]. The decrease in the activities of GSH-Px, SOD and CAT could be due to a feed back inhibition or oxidative inactivation of enzyme protein caused by ROS generation[18]. Therefore the protective effects of tea polysaccharide on the changes in antioxidant enzyme activities such as GSH-Px, SOD and CAT, and alterations in the levels of some molecules related to oxidative stress like ROS, MDA in mice indicated that tea polysaccharide possessed a direct protective effect on lead-induced oxidative stress, accompanied by restoration of deficits in antioxidative defense system. In fact, tea polysaccharides are already reported to have antioxidative effects. However, the recorded increase of enzymatic activity of GSH-Px, SOD and CAT may be due to the increased utilization of tea polysaccharide to counteract lipid peroxidation production.

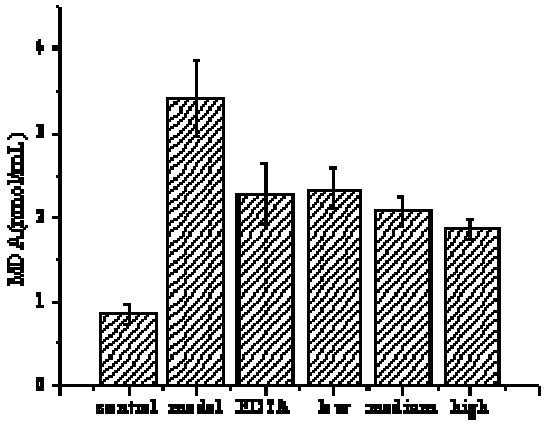

Figure 3 : Effects of tea polysaccharide and EDTA treatments on levels of MDA in liver of mice

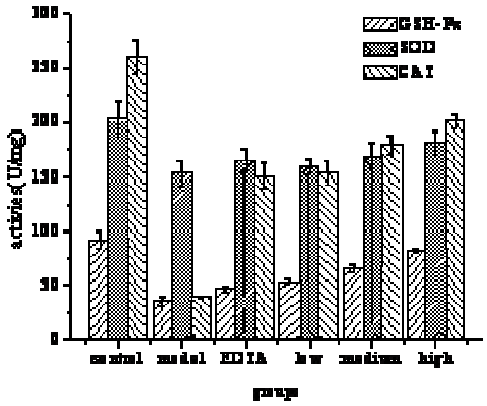

Figure 4 : Effects of tea polysaccharide and EDTA treatments on levels of GSH-Px, SOD and CAT in liver of mice

CONCLUSION

Comparing with control groups treated with EDTA, a well-known chelating agent used in lead intoxication, tea polysaccharide treatment is less efficient in decreasing lead levels in blood and tissues but shows better protective effect on antioxidative defense system, suggesting that tea polysaccharide has a more comprehensive protective effect than EDTA. The antioxidative ability might be one of the main mechanisms for tea polysaccharide to alleviate lead toxicity, which needs to be further confirmed. Moreover, tea polysaccharide can be used as a safe and efficacious nutritional dietary supplement in the daily life to prevent and alleviate lead toxicity. Further understanding of the underlying mechanism of protective effects of tea polysaccharide against lead toxicity needs more study in the future.

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