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## Protective effect of hydroalcoholic *Rheum ribes* L. extract in male rat model of lead acetate-induced nephrotoxicity

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### ABSTRACT

**Background:** In the recent years, the prevalence of metal-induced toxicity, especially lead-induced toxicity, has increased considerably. Metals including lead cause oxidative stress. Therefore *Rheum ribes* L. as a medicinal plant with antioxidant activity may reduce the complications of these toxicities.

**Objectives:** The aim of the current study was to investigate the protective effect of hydroalcoholic *R. ribes* L. extract in male rat model of lead acetate-induced nephrotoxicity.

**Materials and Methods:** In this experimental study, 40 male Wistar rats were randomly assigned to four groups: Normal saline (control) group, positive control group treated with 0.6 g/L/d of lead acetate in drinking water, and two *R. ribes* groups treated with 200 and 400 mg/kg/d of *R. ribes* extract in addition to lead acetate for 10 days. After treatment, the rats were anesthetized, blood samples were taken, and kidneys were removed and fixed in 10% formalin. Then, serum antioxidant capacity was measured and kidney tissue specimens histopathologically studied.

**Results:** Serum antioxidant capacity was significantly different between lead acetate group and the group treated with lead acetate + *R. ribes* extract (400 mg/kg). Additionally mice receiving lead acetate + *R. ribes* extract (400 mg/kg) had significantly higher antioxidant capacity than the control group ( $P < 0.05$ ). Treatment of lead acetate-treated rats with *R. ribes* extract significantly prevented damage to their kidney tissues.

**Conclusions:** *Rheum ribes* extract can prevent lead-induced kidney damage, and therefore exposure to lead acetate by people are recommended to consume this extract.

### *Implication for health policy/practice/research/medical education:*

*Rheum ribes* L. extract could significantly prevent lead acetate-induced nephrotoxicity in rat.

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### 1. Background

Lead is the most well-known toxic heavy metal and one of the most important environmental pollutants. Lead is used as a fixing and drying agent in a variety of colors, lacquers, colored inks, and coatings of other metals as well as in gasoline for combustion (1). The amounts of over 0.3 mg/mL of lead in human and animal blood can lead to multisystem toxic effects (2). Lead affects all organs and systems of the body. This metal is accumulated in the tissues of the body, including

the liver, kidneys, bones, the circulatory system, and the reproductive system leading to certain complications in them.

Lead affects certain biochemical and physiological activities of the body including the kidney. The kidney is one of the organs involved in the early accumulation of the absorbed lead. A number of lead-binding proteins have been identified in the kidney by experimental studies. It seems that these proteins serve as receptors of lead, and mediate its function and activity in target tissues

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(3). Chronic lead poisoning in human leads to certain lesions such as renal tubular atrophy and fibrosis (4). A study showed that exposure to lead caused a significant increase in kidney weight in rat. In lead-treated samples, certain tissue damage such as renal tubular atrophy, necrosis, and increased proportion of the glomerular cells were observed (5).

Rhubarb, botanically referred to as *Rheum ribes* L., is a plant from the family Polygonaceae (6). *R. ribes* contains flavonoids, vitamins A, E, and C, oxalic acid, cinnamic acid, a compound called rhein, fatty substances, a small amount of essential oil, a large amount of starch, and a bitter compound called  $\beta$ -glucagon (6,7). Studies on *R. ribes* have demonstrated different properties of this plant including anti-platelet accumulation, hypolipidemic, and amelioration of kidney failure. Additionally, in diabetics and liver dysfunction, this herbal drugs had ameliorative impacts (8-13).

## 2. Objectives

In the present study, the protective effect of *R. ribes* against kidney histopathological damage as well as its effect on serum antioxidant capacity in rat with lead poisoning was studied so that lead can be used as a complementary therapy for lead poisoning-induced kidney damage, if its optimal effects on this damage are confirmed.

## 3. Materials and Methods

### 3.1. Animals

Forty male Wistar rats weighing 150-200 g were purchased from the Pasteur Institute of Iran and housed in Animals House of the Medical Plants Research Center of the Shahrekord University of Medical Sciences in controlled ( $23 \pm 2^\circ\text{C}$ ) temperature, 12-hour light/dark cycle, and relative ( $55 \pm 5\%$ ) humidity. All rats had completely free access to standard food and water. The rats were adapted to the new environment by being kept in this environment for one week before tests.

### 3.2. Plant-based materials and extraction

Fresh samples of *R. ribes* were purchased from the Esfahan Agriculture And Natural Resources Research Center and then identified as *R. ribes* L. by a botanist at the Herbarium of the Shahrekord University of Medical Sciences (herbarium no. 527). Then, the samples were dried and pulverized for extraction. In this study, extraction was conducted by maceration. For this purpose, pulverized plant was mixed with 70% ethanol and the resultant mixture was kept at room temperature in the dark for 48 hours. Then, the mixture was filtered

using Whatman filter paper grade 1, concentrated using rotary evaporator, and incubated at  $37^\circ\text{C}$  to dry completely. The extracts were kept at  $5^\circ\text{C}$  till use. In this study, 200 and 400-mg/kg concentrations of the extract in normal saline were prepared and used (14).

### 3.3. Determining serum antioxidant capacity

Serum antioxidant capacity was determined by the ferric reducing/antioxidant power (FRAP) assay. In this method, the complex formed between TPTZ and  $\text{Fe}^{2+}$  produces a blue color at 593nm wavelength.  $\text{FeSo}_4 \cdot 7\text{H}_2\text{O}$  in 100-1000  $\mu\text{M}$  concentrations was used as the standard in FRAP assay (15).

### 3.4. Histopathological studies

Immediately after the rats were sacrificed, their kidney tissues were removed and fixed in 10% formalin. After preparing the kidneys (dehydration with 100%-50% ethanol and clearing or purging with xylene), they were fixed in paraffin waxes and divided into sections of 3-micron thickness using microtome. The specimens were stained with hematoxylin and eosin to be examined under an optical microscope. The lams were numbered and examined by a person who was blind to the grouping of the rats. The debris, vacuolization, degeneration, and flattening of the specimens were studied and semi-quantitatively ranked between 1 and 5. The score 0 was considered to represent lack of tissue damage (16).

### 3.5. Ethical issues

All experimental protocols and steps of the tests were conducted in compliance with the regulations of the Research Ethics Committee of the university and Iranian Ethical Guidelines for the Use of Animals in Research (approval code; IR.SKUMS.REC.1395. 222).

### 3.6. Data analysis

Data were expressed as mean (standard deviation). For inter-group comparisons, one-way analysis of variance (ANOVA) followed by Tukey's test was used. The level of statistical significance was considered  $< 0.05$ . Data analysis was conducted by SPSS 16.

## 4. Results

### 4.1. Serum antioxidant capacity

Serum antioxidant capacity was not significantly different between lead acetate group and control group, but was significantly different between lead acetate group and the group treated with lead acetate + *R. ribes* extract (400 mg/kg). Additionally mice receiving lead acetate + *R. ribes* extract (400 mg/kg) had significantly higher

antioxidant capacity than the control group (Figure 1).

#### 4.2. Kidney tissue damage

Histopathological findings showed significant differences among the groups. Degeneration, vacuolization, flattening, and cell destruction in the groups treated with lead acetate + *R. ribes* extract (200 and 400 mg/kg) were significantly lower compared to lead acetate group. Also lead acetate group and both lead acetate + *R. ribes* extract groups had a significant difference with the control group (Figure 2).

### 5. Discussion

As a heavy metal, lead causes many dangers to human health and environment, and it is therefore essential to prevent lead poisoning (17). Currently, complementary and traditional medicine can be used to treat many diseases. The popularity of such treatments has increased compared to synthetic drugs due to comparatively fewer side effects and lack of causing poisoning (18).

*Rheum ribes* is frequently used to treat certain diseases and

conditions such as platelet accumulation, hyperlipidemia, kidney failure, and hepatic fat accumulation. In this study, the effect of *R. ribes* on poisoning due to exposure to lead acetate was examined.

In this study, we observed that treatment with 0.6 g/L/d of lead acetate in drinking water for 10 days had significant effect on serum antioxidant capacity and was associated with changes in kidney tissue including degeneration, vacuolization, flattening, and debris.

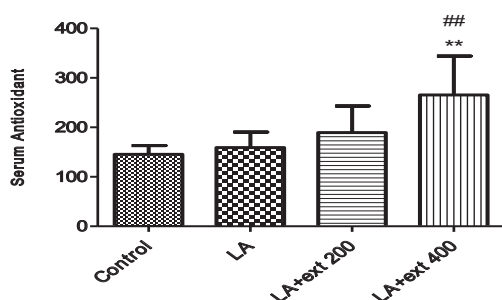
Treatment with *R. ribes* extract at 400-mg/kg concentration significantly increased serum antioxidant capacity in the rat exposed to lead acetate. While antioxidant and anti-inflammatory properties of this extract have been demonstrated (6,19,20), it can be argued that *R. ribes* extract prevents cell damage and dysfunction through decreasing inflammatory cytokines, inhibiting free radicals and reactive oxygen species (ROS), and increasing tissue and serum antioxidant capacity. It is worth mentioning that in our study, lead acetate did not decrease antioxidant capacity but *R. ribes* extract increased it. Therefore, the role of antioxidant effects of this plant against lead adverse effects remains unknown.

Kidney tissue damage following exposure to lead acetate has already been reported (21). It was observed that intraperitoneal administration with 500 ppm of lead acetate caused kidney tissue damage including renal tubular atrophy and necrosis in pregnant mice (5). This finding is consistent with the current study. In our study, co-treatment with lead acetate and *R. ribes* extract caused significant improvement of kidney tissue damage. In addition, protective effects of this extract on cisplatin-induced nephrotoxicity and diabetes have already been demonstrated (19,22), which is in agreement with our study.

Consistent with previous findings, lead acetate caused degeneration, vacuolization, flattening, and debris of the kidney, and *R. ribes* treatment led to the improvement of lead acetate-induced tissue damage.

The study of Thamilselvan et al, demonstrated oxalate-induced toxicity in the kidney (23). It is therefore recommended to investigate the toxic doses of *R. ribes* extract for rat kidney in additional studies because *R. ribes* contains oxalic acid.

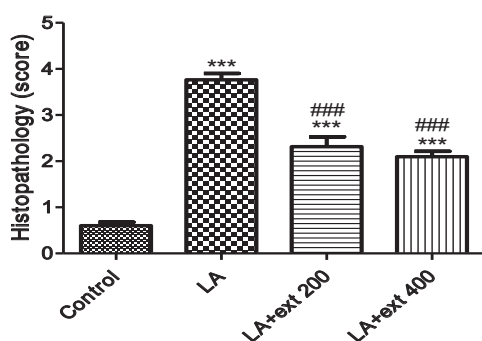
Metals including lead cause increase in free radicals and induce oxidative stress. Therefore *R. ribes* L. as a medicinal plant with antioxidant activity was expected to reduce the complications of lead acetate toxicities. In this study lead acetate with the used concentration damaged kidney tissue, however, did not change serum antioxidant level. *R. ribes* increased antioxidant activity



**Figure 1.** The effect of *Rheum ribes* L. extract (ext) on serum antioxidant capacity in rats treated with lead acetate (LA).

\*\*  $P < 0.01$ ; Compared with control group.

##  $P < 0.01$ ; Compared with Lead acetate group.



**Figure 2.** The effect of *Rheum ribes* L. extract (ext) on kidney necrosis in rats treated with lead acetate (LA).

\*\*\*  $P < 0.001$ ; Compared with control group.

##  $P < 0.001$ ; Compared with Lead acetate group.

and reduced lead damage. Hence, it is not clear to what extent the antioxidant capacity of *R. ribes* attributes to reduced toxicity of lead acetate. Medicinal plants have various therapeutic effects (24-27). Although, each plant has its own effects due to its components, but in high extent their effects attributed to their antioxidant activities.

## 6. Conclusions

This study showed that *R. ribes* could significantly prevent lead acetate-induced nephrotoxicity in rat. With regards to the nutritional value of *R. ribes*, this plant can be used in the diets of the people that are exposed to lead acetate to prevent or decrease lead acetate-induced damage. However, it is recommended to conduct human subject research to investigate the actions and extract of this plant.

## Author's contribution

MRK, ZL and SA designed, conducted, supervised and analyzed the research and prepared the first draft of the article. EB and HH participated in the performance of the research and collected the data. MRK prepared the paper. All authors read and signed the final paper.

## Conflicts of interest

The authors declared no competing interests.

## Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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