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ORIGINAL ARTICLE

Hepatoprotective and Antioxidant Potential of Radish Seed Aqueous Extract on Cadmium-induced Hepatotoxicity and Oxidative Stress in Mice

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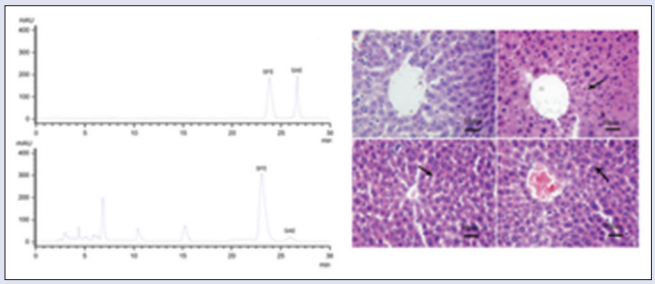
ABSTRACT

Background: Exposure to cadmium (Cd) is a major environmental pollutant that causes injury on many organs and tissues, particularly the liver. However, the protection of radish seed aqueous extract (RSE) against hepatic injury in Cd-exposed mice yet remains unclear. **Objective:** The research aimed to investigate the mitigation effect of RSE against Cd-induced hepatotoxicity in experimental animals. **Materials and Methods:** The mice were administered intraperitoneally with Cd Chloride ($CdCl_2$, 75 mg/kg b. wt) as a positive control to compare RSE. The hepatic function and antioxidant status were assessed in liver tissue of poisoned and control mice. **Results:** Levels of serum hepatic enzymes (aspartate transaminase: AST and alanine transaminase: ALT) as well as total bilirubin were significantly increased in Cd-exposed mice. In addition, Cd exposure elicited enhancement of oxidative stress level. Co-treatment with RSE (200 and 400 mg/kg b. wt) significantly decreased the serum levels of liver function biomarkers. Furthermore, RSE treatment showed a significant reduction of lipid peroxidation and increase of enzyme and glutathione concentrations. Histopathological analysis was parallel to these biochemical findings. **Conclusion:** The results clearly demonstrated that RSE is effective for ameliorating hepatic cytotoxicity and oxidative damage arising from Cd exposure.

Keywords: Cadmium, liver, oxidative stress, radish seed, toxicity

SUMMARY

- The extracts of radish seed contain sulforaphene, sulforaphane, total phenolics and total flavonoids which have shown chemopreventive activities
- The radish seed extract has shown the protection potential against hepatic injury induced by cadmium exposure
- The radish seed extract is effective for ameliorating hepatic oxidative damage in cadmium-treated mice.



Abbreviations used: Cd: Cadmium; ROS: Reactive oxygen species; RS: Radish seed; RSE: Radish seed aqueous extract; HPLC: High-performance liquid chromatography; SAE: Sulforaphene; SFE: Sulforaphene; TFC: Total flavonoids content; TPC: Total phenolics content; AST: Glutamic-oxalacetic transaminase; ALT: Glutamic pyruvic transaminase; TB: Total bilirubin; MDA: Malondialdehyde; CAT: Catalase; GPX: Glutathione peroxidase; NOx: Nitrates and nitrites; NO: Nitric oxide; LRS: Low dose of radish seed extract; HRS: High dose of radish seed extract; LPO: Lipid peroxidation; $ONOO^-$: Peroxynitrite radical; iNOS: Nitric oxide synthase.

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INTRODUCTION

Cadmium (Cd) is a ubiquitous no-degradable heavy metal and widely used in some fast-growing industries such as Cd-nickel battery, electroplating, alloy, pigment, power-generation, and fertilizer.[1] This seriously toxic metal is listed in 126 priority environmental pollutants by the U. S. EPA and tends to prompt a severe global attention as regards its highly bioaccumulation and poisoning to human through contaminated food chain, drinking water, air pollutions as well as cigarette smoke.[2] The liver is regarded to be among the most sensitive target organs because Cd primarily accumulates in this organ.[3] The exposure of Cd initially causes hepatocyte inflammatory cascade through impairment in endothelial cell and activation of Kupffer cells.[4] Moreover, another pathogenesis of Cd-mediated hepatotoxicity is directly relevant with oxidative stress induced by reactive oxygen species (ROS),[5] mainly comprising of superoxide ($\bullet O^2$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\bullet OH$). It has reported that Cd exposure can largely generate the ROS through disrupting the electron transfer chain of hepatic mitochondria.[6] If this excessive production of ROS is not counteracted by *in vivo* antioxidant defense, oxidative injury would certainly be induced, characterized by lipid peroxidation (LPO), membrane protein

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degradation, damaged antioxidant system, and DNA.[7] Hence, ROS generated by mitochondria lead to oxidative stress and best explain the progression of liver diseases, and antioxidant exhaustion may exacerbate the oxidative stress. Conversely, antioxidants supplement shows promise in the pharmacologic therapy of hepatopathy.[8] Mounting evidence suggests that natural agents with antioxidant potential use as dietary supplements perform their protective and therapeutic potential against all of the liver diseases such as fatty liver.[9] Based on the above information, antioxidants are proposed to be valid in precaution against some poisoning substances including Cd. In fact, a previous report has investigated the protection activities of several naturally-occurring antioxidants against Cd-induced acute hepatotoxicity.[10]

Radish (*Raphanus sativus* Linn.) belongs to the family of *Cruciferae*. Different parts (seeds, roots, and leaves) of this plant are generally consumed in a daily diet. Radish seed (RS), known as semen raphani or "NaiFuZi" in traditional Chinese medicine, attracts extensive attentions due to its multiple pharmacological effects.[11] Among the secondary metabolites isolated from RS, many definite antioxidants have been proven to be contained in RS, or its extracts in many documented scientific reports, such as sulforaphene [SAE, Figure 1a], sulforaphane [SFE, Figure 1b], flavonoids, saponins, tannins, alkaloids, and total phenols.[12] Taken together, RS would be speculated to ameliorate Cd-induced hepatotoxicity. However, few comprehensive studies related to this activity are available until now. Therefore, the aim of this study was to complement the protective role of RS against Cd-induced hepatocellular injury in the animal system in terms of the fact that the supplementation of some antioxidants is popularly effective in counteracting the toxicity of Cd.[13]

MATERIALS AND METHODS

Reagents and chemicals

Cd chloride ($CdCl_2$) was bought from Sigma (St. Louis, MO, USA). The commercial kits (glutathione [GSH], superoxide dismutase [SOD], malondialdehyde [MDA], catalase [CAT], and glutathione peroxidase [GPX]) for the analysis in this study were offered by Nanjing Jiancheng Bioengineering Inc., Nanjing, China, the commercial kits (GOT, glutathione S-transferase, and total bilirubin [TB]) were purchased from BioSino Biotechnology and Science Inc., Beijing, China. While all other chemicals and reagents used were of reagent grade quality, RSs (*R. sativus* cv. Bolide) were kindly offered by Tianjin Academy of Agricultural Sciences (Tianjin, China).

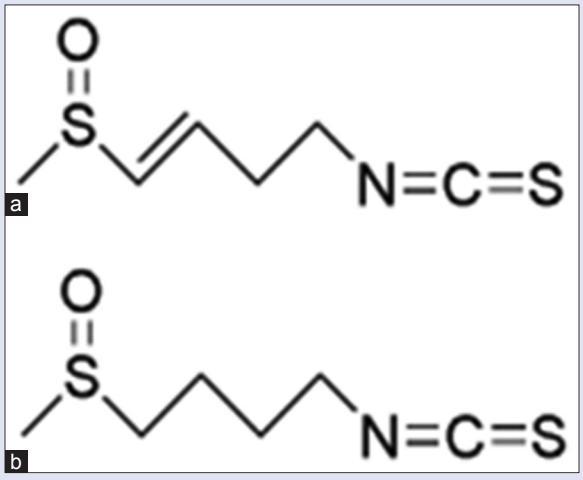

Figure 1: Chemical structures of sulforaphene (a) and sulforaphane (b)

Preparation of radish seed extract

The preparation of RS aqueous extract (RSE) was done using the method of Sangthong with some modification.[14] In brief, 100 g of RSs was added into 1.2 L of water, homogenized, and incubated for 7 h at 35°C to allow the hydrolysis of glucosinolate by endogenous myrosinase. The hydrolyzed mixture was centrifuged for 30 min at 8000 ×g for removing proteins. The supernatant was addressed with dichloromethane (MC) three cycles. The MC layer was collected and evaporated to obtain crude SFE extract with a rotary evaporator (RE-3000B, Rongya Co., Shanghai, China) at 45°C. The purification of RSE was conducted by a preparative liquid chromatography (LC) system (LC-1100, Agilent technology Inc., Shanghai, China) equipped with a C18 column (250 mm × 30 mm, 10 μm, Daisogel™) and a ultraviolet (UV)/visible spectroscopic detector. The mobile phase contained methanol/water (30:70, v/v). The other conditions of preparative high-performance liquid chromatography (HPLC) separation were as follows: 10 mL/min of flow rate, 245 nm of detection wavelength, 5 mL of injection volume. The fraction was collected according to SFE peak in the chromatogram. The SFE solution was filtered, concentrated, and dried to obtain SFE extract for further animal experiment.

Analytical high-performance liquid chromatography analysis and phytochemical analysis

RSE sample was analyzed by HPLC based on SFE and SAE standard. The column was a Cosmosil C18 column (250 mm × 4.6 mm i. d. 5 μm). The HPLC conditions were as follows: the mobile phase consisted of 1% acetonitrile (v/v) and 0.1% (v/v) trifluoroacetic acid in ultrapure water. Column temperature: 30°C, flow rate: 1.0 mL/min, detection: 254 nm. Total phenolics content (TPC) and total flavonoids content (TFC) of RSE were determined using methods described by Lin and Tang.[15]

Animals

Thirty-two male mice weighing 18–22 g were obtained from Academy of Military Medical Sciences (Beijing, China). The mice were housed under standard laboratory conditions (24°C–26°C, relative humidity of 60%–70% and 12 h light/12 h dark cycle), and they were fed standard commercial mice chow and given distilled water *ad libitum*. All animal procedures were approved by the Academic Board of Tianjin Agricultural University in accordance with laboratory animal guideline for ethical review of animal welfare (project no: GB/T 35892-2018, China) and performed in strict accordance to the guidelines for care and use of laboratory animals issued d by the National Institutes of Health.

Experimental design

Animals were randomly assigned to four groups (n = 10). Group Control (Group Con) served as a negative control group and received saline (0.9%) by gavage. Group Cd served as an experimental group and received daily intraperitoneal injection of $CdCl_2$ (75 mg/kg b. wt.) for seven consecutive days. Group low dose of radish seed extract (LRS) and Group high dose of radish seed extract (HRS) were administered with $CdCl_2$ (75 mg/kg b. wt.) along with RSE by gavage for a period of consecutive 28 days at the dose of 200 and 400 mg/kg b. wt., respectively.

Collection of serum and tissue samples

At the end of the experiment, blood samples were collected under ether anesthesia after the animals being fasted for 12 h. All mice were sacrificed by cervical decapitation. Serum was then collected by centrifugation (3000 rpm, 4°C, 10 min) and stored at −20°C until further used. Liver samples were immediately excised, blotted, washed

in ice-cold saline (0.9%), dried and weighed. Thereafter, half-gram liver tissues were homogenized in 4.5 ml of precooling KCl (1.5%) using glass homogenate and centrifuged at 10,000×g for 15 min using a refrigeration centrifuge. Supernatant samples were collected and stored at −20°C for the following analysis.

Serum biochemical assay

Liver injury was assessed by quantifying serum enzymatic activity. Serum glutamic oxalacetic transaminase (AST), glutamic pyruvic transaminase (ALT), and TB were determined on a Glamour 3000 Automatic Analyzer according to the test kit instructions.

Antioxidative enzyme activity in hepatocytes

Liver homogenates were used to identify the status of LPO and oxidative stress. The MDA content and the activities of enzymatic activities of SOD, CAT as well as GPX were assayed using commercial kits. The method was employed to measure the total protein level.[16]

Nitric oxide content

The hepatic concentration of nitrates and nitrites (NOx) was employed as nitric oxide (NO) production and evaluated using Griess reagent. The nitrates in the liver samples were reduced to nitrites by incubating the samples with a reductase. With the Griess reagent, the total level of nitrite forms a purple azo dye, which can be detected spectrophotometrically at 492 nm (UV-8000, Yuanxi Co., Shanghai, China).

Cadmium content

The Cd contents in the biological materials of individual mice were measured, including whole blood, liver, kidney, and feces collected during the last week of the trial. The samples were dried at 80°C and totally digested in 35% (w/v) H_2O_2 and 65% (w/v) nitric acid, followed by diluting the clear digest with ultrapure water (1:5). The tissue's Cd concentrations were determined using an atomic absorption spectrometry with a 1000 mg/L cadmium solution as standard. Each sample was performed in triplicate.

Histological examination

The samples of liver tissue were fixed in 10% neutral formalin for 48 h, flushed with tap water for a night, dehydrated in ethanol series (50%–100%), cleaned in xylene and then paraffin-embedded. A Leica Microsystem microtome (Model RM 2235, Germany) was used to prepare tissue sections (5–6 μm) which were deparaffinized with xylene, rehydrated using a series of alcohol (100%–50%), and finally stained with hematoxylin and eosin (H and E) dye using an auto strainer (XL, Germany). The tissue slides were examined under a light microscope (Olympus 4X-1, Japan) for histopathological analysis.

Statistical analysis

Results are expressed as mean ± standard error. Statistical analysis was performed using SPSS 18.0 for windows (SPSS Inc., Chicago, IL, USA). Differences between the designed groups were estimated through one-way analysis of variance followed by Duncan's (AST, ALT, TB, ALP, NOx, SOD, GSH-Px, CAT) or Tamhane's T2 (MDA, GSH, Cd content of liver, kidney, blood, and feces) multiple tests according to homogeneity test of variance. $P \leq 0.05$ was considered as the criterion of statistically significance.

RESULTS

Phytochemical analysis

The highest levels of SFE and SAE were revealed by the strict screening for bioactive ingredients of RSE. The content of SAE was low. The TPC, TFC, SFE, and SAE were 133.40 ± 0.95 mg GAE/100 g dry wt., 30.27 ± 0.55 mg QE/100 g dry wt., 158.22 ± 1.91 mg/g and 12.13 ± 0749 mg/g, respectively [Table 1].

Cadmium content

The exposure of mice to Cd markedly increased Cd concentrations in serum, feces, and tissues (liver and kidney) as compared to the control mice. It is interesting to note that the average Cd accumulation in the liver is about twice as high as that in the kidney [Figure 2]. These enhanced Cd levels were, to various extents (approximate 1.3–1.4 fold), decreased in both LRS and HRS treated mice in respect to Group Cd. In addition, RSE diminished the similar degree of Cd content in the serum and kidney of mice [Figure 2a]. However, concomitant exposure of mice to Cd and RSE increased Cd levels in the feces, approximately 1.3-fold, compared with the mice treated with Cd only [Figure 2b].

Serum hepatic enzymes and total bilirubin level

The levels of serum AST and ALT in the Cd-treated mice were significantly increased ($P < 0.05$), compared with the control mice. Although Group LRS showed no significant effects ($P > 0.05$) on the activities of the both serum enzymes as compared with Group Cd, adding HRS obviously inhibited ($P < 0.05$) the activities of this two serum hepatic indexes as compared with Group Cd and Group LRS. The serum level of TB was substantially increased ($P < 0.05$) after the Cd treatment, whereas, RS treatment, particularly HRS, significantly suppressed this tendency [Table 2].

Activities of hepatic antioxidant enzymes

Cd exposure significantly ($P < 0.05$) decreased activities of antioxidant enzymes (SOD, GPX, and CAT) as compared to normal mice. However, adding RSE to the Cd-treated mice reduced the liver SOD and CAT activities as compared with those of Group Cd ($P < 0.05$). Maximum changes of liver SOD and CAT were obtained in Group HRS. There were no substantial ($P > 0.05$) changes of liver GPX activities in both Group LRS and Group HRS as compared to Group Cd [Figure 3a-c].

Hepatic glutathione levels

The levels of GSH were decreased by 37.7% ($P < 0.05$), as compared to Group con after the Cd exposure, which may associate with an accommodative response to the liver injury. The effects of both LRS and

Table 1: Total sulforaphene and sulforaphene content of radish seed aqueous extract

RSE	Quantity (mg/100 g dry weight)	Retention time (min)	Percentage RSD	LOD (μg/g)	LOQ (μg/g)
TP	133.40±0.95				
TF	30.27±0.55				
SFE	15.80±0.05	23.01±0.02	0.09	0.38	1.18
SAE	1.21±0.05	25.65±0.03	0.12	0.32	1.02

SAE: Sulforaphene; SFE: Sulforaphene; TP: Total phenolics; TF: Total flavonoids; RSD: Relative standard deviation; LOD: Limits of detection; LOQ: Limit of quantitation; RSE: Radish seed aqueous extract

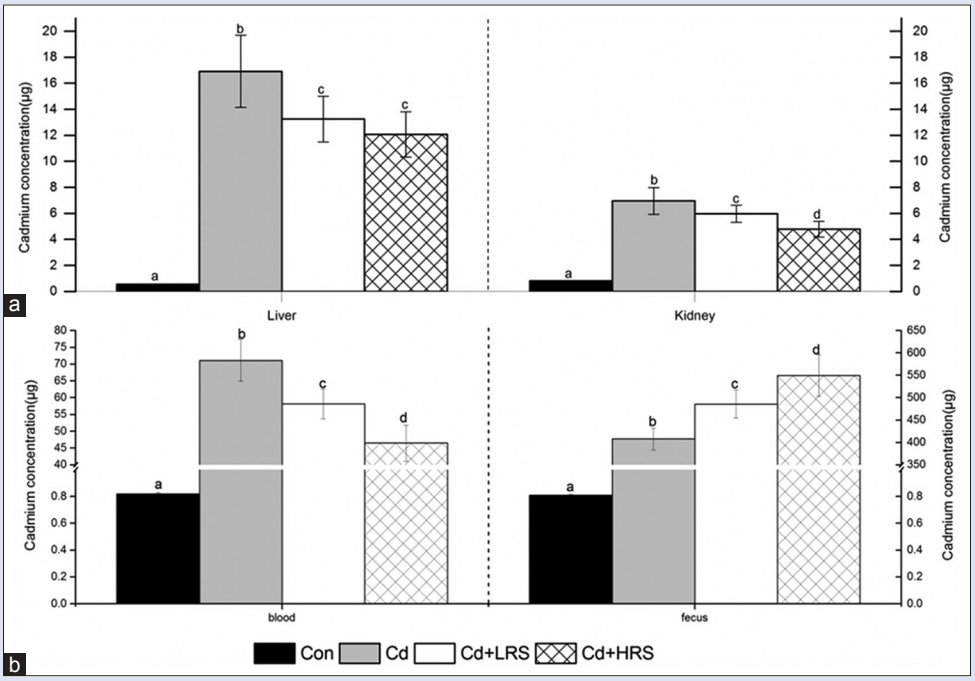

Figure 2: Cadmium concentration (μg/g) in liver/kidney (a) and blood/fecus (b) of the test mice, given as means ± standard deviation (n = 10). The different letters above the bars marks a statistical difference between the groups

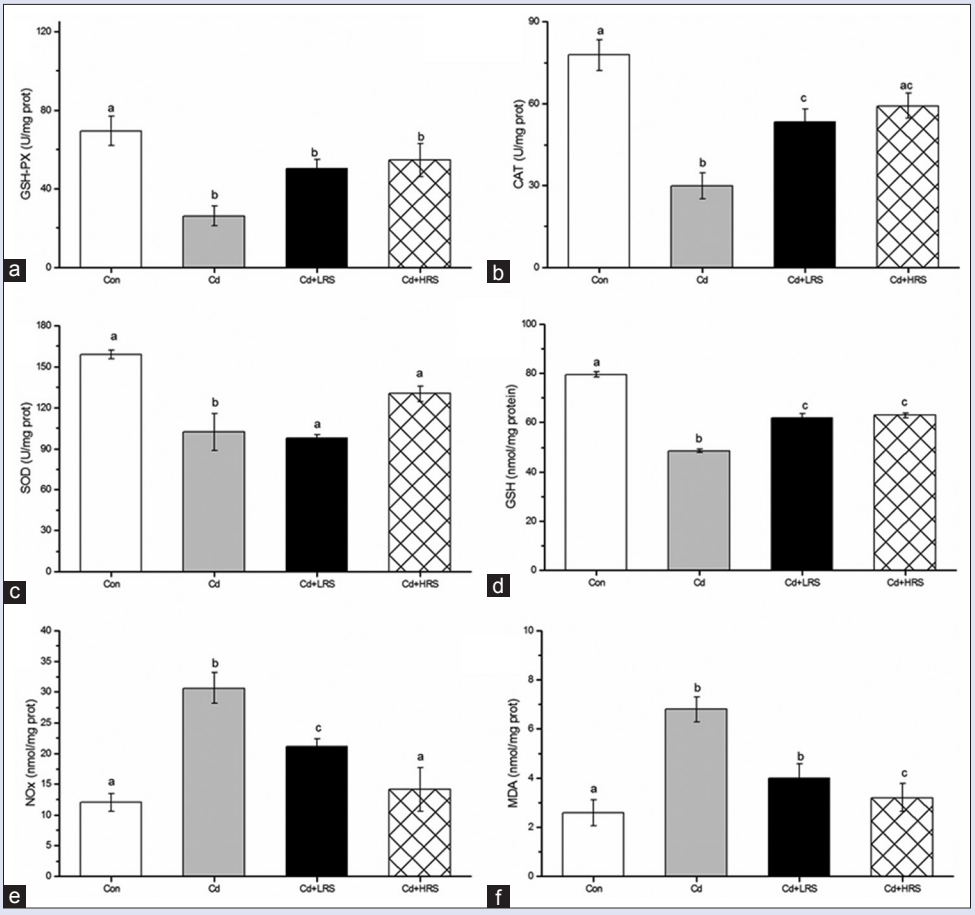

Figure 3: (a-f) Effect of radish seed extract on the liver antioxidant markers, including glutathione-Px, catalase, superoxide dismutase, nitrates and nitrites and malondialdehyde in the mice. Bars present means ± standard deviation (n = 10). Values with different letters differ significantly between the groups ($P < 0.05$)

HRS can increase the contents of GSH to 24.6% and 30.5% ($P < 0.05$ as compared to Group Cd), respectively [Figure 3d].

Nitric oxide content

The serum NOx concentration was significantly higher in the Cd-treated group (30.65 ± 2.55 nmol/L) in comparison with Group Con (12.05 ± 1.45 nmol/L). However, a significant reduction in the serum NOx levels ($P < 0.05$) was observed in both Group LRS (21.08 ± 1.33 nmol/L) and Group HRS (14.16 ± 3.56 nmol/L). No statistical difference in the NOx content was detected between Group Con and Group HRS [Figure 3e].

Hepatic malondialdehyde levels

MDA was utilized for illuminated LPO in liver tissue. Cd exposure distinctly increases ($P < 0.05$) the levels of MDA when compared with Group Con, indicating hepatocellular LPO. The concomitance of Cd with RSE, particularly HRS ($P < 0.05$), reversed the enhanced MDA levels toward Group Con [Figure 3f].

Histopathological observation

Group con showed normal liver histological architecture. Whereas, Cd administration induced extensive morbid alterations in liver tissue, including destruction of parenchymal architecture, peripheral hemorrhage, inflammatory cell infiltration, and hepatocellular swelling [Figure 4a and b]. However, the severity of these pathological lesions was significantly reduced by both of two different RS (LRS and HRS) treatment. Furthermore, HRS treatment afforded the better protective effects as compared with LRS [Figure 4c and d], and the liver sections in Group HRS were almost close to Group Con.

DISCUSSION

The most oral Cd intake is eliminated in feces[17] with the absorbed part transporting in blood. About 50%–70% of this absorbed heavy metal may accumulate in kidney and liver partly because these organs contain a great deal of metallothionein, which can bind many metals including Cd.[18] Our results reveal that Cd levels of the mice (blood, feces, liver, and kidney) were significantly raised on the condition of Cd exposure. By comparison, RSE administration enhanced Cd content in the feces, however, reduced it in the liver, blood, and kidney [Figure 1]. This study suggests that RSE might result in a subsequent reduction of Cd accumulation by promoting excretion of Cd in feces. Since, GSH is regarded as a potent Cd-chelator for endocellular Cd disintoxication,[19] RSE might lighten Cd burden indirectly by raising the amount of GSH.

Cd can definitely stimulate Kupffer cells to generate ROS via displacing and transferring Fe in ferritin,[20] depleting detoxification systems[21] and destroying protein-bound sulfhydryl groups (–SH).[22] The continuous ROS overproduction ultimately leads to oxidative stress by the interaction between ROS with some vital cellular biomolecules, as a consequence of which, the architecture integrity of hepatocytic membrane is undermined, and the subsequent leakage of intracellular marker enzymes (particularly AST and ALT) into the bloodstream is triggered.[23] In like manner, hepatocellular dysfunction, and cholestasis regularly cause more cytoplasmic leaking with the subsequent rise of ALP and TB.[24] Hence, the elevated levels of serum AST, ALT, ALP, and TB are commonly considered as biomarkers in evaluating the viability and membrane permeability of hepatocyte. Our result shows that Cd-induced hepatotoxicity is in accordance with previous study,[25] manifested by the obvious enhancement of serum ALT, AST, and TB. However, the observed increasing levels of the biochemical indexes were reverted by RS treatment. It is conceivable that RS initially might be beneficial for the prevention against Cd-mediated damage on liver function. The observed pharmacological activity of RSE was further corroborated by the histopathology evaluation.

The pathogenesis of liver diseases is attributed to oxidative stress processes. Many factors (medication, heavy metals, and ethanol) can induce oxidative stress via increase of cellular oxidants and depletion of antioxidants in the liver. The vital endogenous enzymatic antioxidant system serves as the first-line defense against ROS and nonoxygen free radicals in the maintenance of cellular redox balance and represents protection against oxidative stress, which primarily consists of SOD, CAT, and GPX. ROS and other generated free radicals are catalyzed by SOD, followed by decomposition regulated by CAT and/or Gpx.[26] The administration of various toxins including Cd may dramatically induce the inhibition of antioxidase activities as well as overproduction of ROS beyond hepatocellular intrinsic antioxidant capacity, followed by an imbalance between oxidant and antioxidant defense system. Consequently, oxidative stress is induced and subsequently destroys many biological molecules in liver cell, mainly including damage of DNA and mitochondrion, LPO, inhibition of protein synthesis, and modulation of functional signaling pathways.[27] Combined with MDA, one of the end products of LPO,[28] decreased levels of enzymatic antioxidants in liver are normally employed to indicate toxin-induced

Table 2: Hepatic markers in the serum of the experimental mice

Groups	AST (U/L)	ALT (U/L)	ALP (IU/L)	TB (mg/dl)
Con	100.98±8.16^{a}	38.11±3.69^{a}	37.95±1.51^{a}	0.88±0.05^{a}
Cd	148.72±3.41^{b}	83.22±6.21^{b}	57.96±5.75^{b}	1.89±0.08^{b}
Cd + LRS	141.02±4.64^{b}	68.92±6.34^{b}	46.03±6.35^{c}	1.45±0.11^{c}
Cd + HRS	119.15±7.64^{a}	43.38±3.76^{a}	36.96±3.31^{a}	1.19±0.10^{a,c}

Effects of RSE on the serum biomarkers of the experimental mice, including AST, ALT, ALP, and TB. Values are presented by means±SD (n=10). Values with different letters in each column differ significantly between the groups (P<0.05). Cd: Cadmium; LRS: Low dose of radish seed extract; HRS: High dose of radish seed extract; AST: Glutamic-oxalacetic transaminase; ALT: Glutamic pyruvic transaminase; ALP: Alkaline phosphatase; TB: Total bilirubin; SD: Standard deviation; RSE: Radish seed aqueous extract

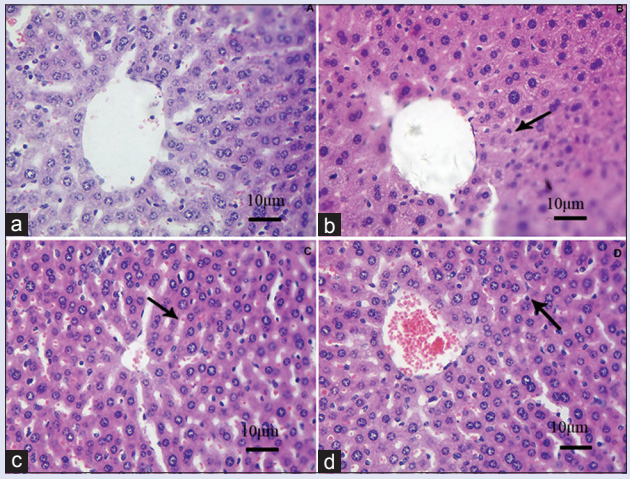

Figure 4: The Protective effects of radish seed extract on histological changes in the liver tissue of acute cadmium administrated mice (×40). (a) Group Con: Showing a severe loss of hepatic architecture and multiple inflammation in hepatocytes. (b) Group cadmium: Control mice with normal histological architecture and clear hepatic cell nucleus; (c and d) Group low dose of radish seed extract and Group high dose of radish seed extract: Mice treated with high dose of radish seed extract (200 mg/kg b. wt. radish seed extract) and low dose of radish seed extract (400 mg/kg b. wt. radish seed extract) with meliorative cellular architecture similar to normal

hepatic injuries/diseases. In this study, our observation that Cd intoxication initiated the formation of liver MDA is in line with previous reports,[29] suggests that Cd administration could compromise the natural antioxidant defense mechanism to scavenge excessive free radicals. The results clearly confirmed that oxidative stress is the mechanism of Cd-induced liver toxicity/diseases. By contrast, the reduced MDA levels close to its normal value in Group HRS and Group LRS might partially be a clue to the efficacy of RSE in mitigating hepatic LPO. Moreover, activities of antioxidant enzymes were significantly decreased to a different degree in Cd-exposed mice as previously reported.[30] Because many trace elements (mainly Zn^{2}+, Mn^{2}+, Cu^{2}+) are considered to be indispensable for the activities of these enzymes,[31] the reasonable explanation for the inhibition of the antioxidant enzymes is the fact that Cd accumulation can displace the essential metals cofactors from their active binding sites of the enzyme protein and arouse the perturbation of enzyme catalytic activity.[32] However, RSE seemed to considerably restore these inhibited enzymes in the liver of Cd-treated mice, which was indicative of the notable activity of RSE in protecting enzymatic antioxidant mechanism *in vivo*.

It is well known that physiological antioxidant system involves two main parts, namely antioxidant enzymes and non-enzymatic antioxidant. GSH is the most vital non-enzymatic antioxidant because of its dual role of neutralizing free radicals such as ROS, RNS, LPO, and detoxifying Cd directly. Mechanistic studies on Cd-induced liver toxicity revealed that the lack of GSH conjugation could decrease the elimination of this toxic metal due to the insufficient of thiol group, which is the major reason of liver pathology. In the present work, the content of GSH in the hepatic cellular fraction of Cd-exposed mice significantly decreased, which indicates the induction of oxidative stress and the debilitative of GSH participation in cellular defense against ROS. The observed depletion of GSH may be associated with the binding reaction of Cd with sulfhydryl groups.[33] The trend of GSH similar to the previous study[34] gives more corroborative evidence of Cd-induced potential hepatotoxicity. However, co-treatment with RSE significantly elevated hepatic GSH concentration to its near-normal levels. The results indicate that RSE can help to eliminate ROS and relieve Cd-induced oxidative damage in the liver. The possible mechanism might be related to the properties of RSE, for instance, displacing Cd from enzymes and chelating Cd.[35]

In addition, a supplementary evidence for the hepatoprotective effect of RS against oxidative stress is involved in NO production in Cd-treated tissues. NO is an endogenous physiological product with highly reactive routinely generated by activated macrophages and heme. Under some pathologic states, NO is considered to be associated with inflammation, oxidative stress as well as apoptosis by reacting with superoxide to generate peroxynitrite radical.[36] In this study, NOx is employed as a measure of NO production. Cd exposure induced the significant enhancement of liver NOx level, which may be attributed to the up-induction of NO synthase by tumor necrosis factor-alpha.[25] The excessive NO positively causes further intense LPO and the exhaustion of intrahepatic GSH facilitating the vulnerability to oxidative damage. Conversely, the pretreatment of RSE diminished the NO overproduction. This might be implicated in the potential hepatoprotective mechanism of RSE. Nevertheless, further research is needed to clarify its precise mechanism.

Considerable interests have been focused on the exploitation of natural antioxidant sources with few side effects due to the adverse effects of synthetic antioxidants for the therapy or prevention of liver diseases. For example, Farzaei reported that curcumin exerts remarkable protective and therapeutic effects of oxidative associated liver diseases through various cellular and molecular mechanisms including suppressing LPO products (MDA) and ameliorating the expression of SOD, CAT, GPx, and GR.[37] Zobeiri reported that naringenin and its nano-formulations exerts therapeutic effects against nonalcoholic fatty liver disease via antioxidant related molecular mechanisms including decrease of biomarkers of LPO, increase of antioxidant defenses, ROS scavenging and modulation of signaling pathways related to fatty acid metabolism which can favor the oxidation of fatty acid.[38] RS is a kind of Chinese folk medicine as well as a potential source for attenuating chemical-induced hepatic injury. The pharmacological effects of RSE may be attributed to the multiple antioxidants. In the current study, phytochemical screening proved four groups of phyto ingredients (e.g., phenolics, flavonoids, SFE, and SAE) in RSE. Earlier literature has reported that both phenolic and flavonoids exert different kinds of pharmacological activities, including anti-oxidation, anti-inflammation, and hepato-protection.[39] The two bioactive ingredients might play a primary role in the aforementioned effects of RSE against $CdCl_2$ induced liver lesion. Further investigations are needed to analyze phenolics and flavonoids in RSE. Furthermore, HPLC analysis of RSE revealed the presence of two peaks with their retention times of 23.04 and 25.96 min [Figure 5a], respectively, which is accord with the HPLC profile of standard SFE (23.80 min) and SAE (26.66 min) [Figure 5b]. Indeed, SAE and SFE have been reported to isolate from RS, and definitely, the two substances have also been certified to possess intense antioxidant activity and other bioactivities.[40] Hence, SAE and SFE [Figure 1] may be partly responsible for the hepatic remedial effects of RSE. Especially, SAE is the most promising isothiocyanates with broad-spectrum bioactivities, and its therapeutic effect on liver injury has been illuminated in previous scientific documents. For example, Baek *et al*. reported that sulfur-radish extract and synthetic SAE may prevent tetrachloromethane-induced liver toxicity, partially by indirectly improving antioxidant function of the detoxification system.[41] Gaona-Gaona *et al*. found that the scavenging ability of SAE against ROS was nearly negligible *in vitro*, the function of SAE against drug-induced hepatic injury was possibly correlated with the preservation of antioxidant enzymes, and the protection against oxidant stress of liver tissue and chondriosome.[42] Nazmy *et al*. also revealed that SAE could significantly prevent sodium valproate-induced hepatocellular toxicity, mainly by its antioxidation and anti-inflammation.[43] Unfortunately for the prepared RSE in this study, the other bio-components that may be responsible for the hepatoprotective characteristics maintain unrevealed and need to be further studied.

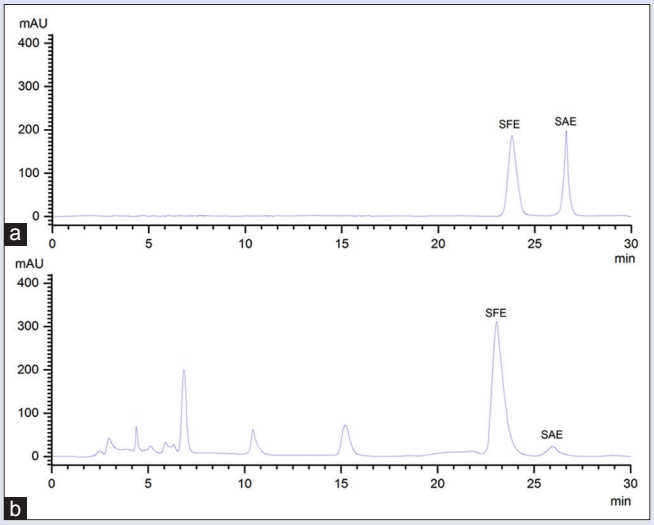

Figure 5: High-performance liquid chromatography chromatograms of analytes detected at 254 nm. Peaks of (a) mixture of standard sulforaphene and sulforaphane, (b) extracts from radish seed with sulforaphene and some unknown peaks

CONCLUSION

In general, the present study suggests that RSE administration could clearly counteract Cd-induced hepatic dysfunction. The possible mechanisms are believed to be associated with RSE alleviating oxidative injury and reinforcing antioxidant defense system. This study might replenish the new clue for the application and research of SRE in the pharmaceutical industry. Further researches are necessary to isolate and analyze major bioactive constituents from RSE extracts and to examine their effects along with functional mechanisms.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Shukla R, Kumar M. Role of *Panax* ginseng as an antioxidant after cadmium-induced hepatic injuries. Food Chem Toxicol 2009;47:769-73.
2. Chen W, Chang AC, Wu L. Assessing long-term environmental risks of trace elements in phosphate fertilizers. Ecotoxicol Environ Saf 2007;67:48-58.
3. Hispard F, de Vaufleury A, Cosson RP, Devaux S, Scheifler R, Coeurdassier M, *et al.* Comparison of transfer and effects of cd on rats exposed in a short experimental snail-rat food chain or to CdCl2 dosed food. Environ Int 2008;34:381-9.
4. Rikans LE, Yamano T. Mechanisms of cadmium-mediated acute hepatotoxicity. J Biochem Mol Toxicol 2000;14:110-7.
5. Saidi I, Chtourou Y, Djebali W. Selenium alleviates cadmium toxicity by preventing oxidative stress in sunflower (*Helianthus annuus*) seedlings. J Plant Physiol 2014;171:85-91.
6. Eneman JD, Potts RJ, Osier M, Shukla GS, Lee CH, Chiu JF, *et al.* Suppressed oxidant-induced apoptosis in cadmium adapted alveolar epithelial cells and its potential involvement in cadmium carcinogenesis. Toxicology 2000;147:215-28.
7. Sugiyama M. Role of cellular antioxidants in metal-induced damage. Cell Biol Toxicol 1994;10:1-22.
8. Ilyas UK, Katare DP, Aeri V. Comparative evaluation of standardized alcoholic, hydroalcoholic, and aqueous extracts of phyllanthus maderaspatensis linn. Against galactosamine-induced hepatopathy in albino rats. Pharmacogn Mag 2015;11:277-82.
9. Sadeghi A, Bahramsoltani R, Rahimi R, Farzaei MH, Farzaei F, Haghighi ZM, *et al.* Biochemical and histopathological evidence on beneficial effects of standardized extract from *Tragopogon graminifolius* as a dietary supplement in fatty liver: Role of oxidative stress. J Diet Suppl 2018;15:197-206.
10. Dkhil MA, Al-Quraishy S, Diab MM, Othman MS, Aref AM, Abdel Moneim AE. The potential protective role of *Physalis peruviana* L. fruit in cadmium-induced hepatotoxicity and nephrotoxicity. Food Chem Toxicol 2014;74:98-106.
11. Ghayur MN, Gilani AH. Radish seed extract mediates its cardiovascular inhibitory effects via muscarinic receptor activation. Fundam Clin Pharmacol 2006;20:57-63.
12. Wu X, Lu Z, Chen J, Wang J, Huang F. Hepatic c-fos expression is independent of oxidative stress and inflammation induced by acute cadmium exposure in rats. Ann Nutr Metab 2007;51:258-63.
13. Karbownik M, Gitto E, Lewinski A, Reiter RJ. Induction of lipid peroxidation in hamster organs by the carcinogen cadmium: Melioration by melatonin. Cell Biol Toxicol 2001;17:33-40.
14. Sangthong S, Weerapreeyakul N. Simultaneous quantification of sulforaphene and sulforaphane by reverse phase HPLC and their content in *Raphanus sativus* L. var. Caudatus alef extracts. Food Chem 2016;201:139-44.
15. Lin JY, Tang CY. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chem 2007;101:140-7.
16. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
17. Satarug S, Garrett SH, Sens MA, Sens DA. Cadmium, environmental exposure, and health outcomes. Environ Health Perspect 2010;118:182-90.
18. Freisinger E, Vašák M. Cadmium in metallothioneins. Met Ions Life Sci 2013;11:339-71.
19. Belcastro M, Marino T, Russo N, Toscano M. The role of glutathione in cadmium ion detoxification: Coordination modes and binding properties – A density functional study. J Inorg Biochem 2009;103:50-7.
20. Valko M, Morris H, Cronin MT. Metals, toxicity and oxidative stress. Curr Med Chem 2005;12:1161-208.
21. Pari L, Shagirtha K. Hesperetin protects against oxidative stress related hepatic dysfunction by cadmium in rats. Exp Toxicol Pathol 2012;64:513-20.
22. Wang J, Hao ML, Liu CG, Liu RT. Cadmium induced apoptosis in mouse primary hepatocytes: The role of oxidative stress-mediated ERK pathway activation and the involvement of histone H3 phosphorylation. Rsc Adv 2015;5:31798-806.
23. Gong P, Chen FX, Wang L, Wang J, Jin S, Ma YM. Protective effects of blueberries (*Vaccinium corymbosum* L.) extract against cadmium-induced hepatotoxicity in mice. Environ Toxicol Pharmacol 2014;37:1015-27.
24. Rajesh MG, Latha MS. Preliminary evaluation of the antihepatotoxic activity of Kamilari, a polyherbal formulation. J Ethnopharmacol 2004;91:99-104.
25. Arafa MH, Mohammad NS, Atteia HH. Fenugreek seed powder mitigates cadmium-induced testicular damage and hepatotoxicity in male rats. Exp Toxicol Pathol 2014;66:293-300.
26. Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: An overview. Methods Enzymol 1990;186:1-85.
27. Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN. Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol 2014;7:60-72.
28. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
29. Liu L, Tao R, Huang J, He X, Qu L, Jin Y, *et al.* Hepatic oxidative stress and inflammatory responses with cadmium exposure in male mice. Environ Toxicol Pharmacol 2015;39:229-36.
30. García-Niño WR, Pedraza-Chaverrí J. Protective effect of curcumin against heavy metals-induced liver damage. Food Chem Toxicol 2014;69:182-201.
31. Radosavljevic T, Mladenovic D, Ninkovic M, Vucevic D, Boricic I, Jesic-Vukicevic R, *et al.* Oxidative stress in rat liver during acute cadmium and ethanol intoxication. J Serb Chem Soc 2012;77:159-76.
32. Casalino E, Calzaretti G, Sblano C, Landriscina C. Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium. Toxicology 2002;179:37-50.
33. Gerson RJ, Shaikh ZA. Differences in the uptake of cadmium and mercury by rat hepatocyte primary cultures. Role of a sulfhydryl carrier. Biochem Pharmacol 1984;33:199-203.
34. Lawal AO, Farombi EO, Lawal AF. Aqueous extract of potato (*Solanum tuberosum*) modulates cadmium-induced liver damage in female Wistar rats. Int J Pharmacol 2011;7:599-607.
35. Renugadevi J, Prabu SM. Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. Exp Toxicol Pathol 2010;62:171-81.
36. El-Habit OH, Abdel Moneim AE. Testing the genotoxicity, cytotoxicity, and oxidative stress of cadmium and nickel and their additive effect in male mice. Biol Trace Elem Res 2014;159:364-72.
37. Farzaei MH, Zobeiri M, Parvizi F, El-Senduny FF, Marmouzi I, Coy-Barrera E, *et al.* Curcumin in liver diseases: A systematic review of the cellular mechanisms of oxidative stress and clinical perspective. Nutrients 2018;10. pii: E855.
38. Zobeiri M, Belwal T, Parvizi F, Naseri R, Farzaei MH, Nabavi SF, *et al.* Naringenin and its nano-formulations for fatty liver: Cellular modes of action and clinical perspective. Curr Pharm Biotechnol 2018;19:196-205.
39. Liu RH. Health-promoting components of fruits and vegetables in the diet. Adv Nutr 2013;4:384S-92S.
40. Lim S, Han SW, Kim J. Sulforaphene identified from radish (*Raphanus satiuus* L.) seeds possesses antimicrobial properties against multidrug-resistant bacteria and methicillin-resistant *Staphylococcus aureus*. J Funct Foods 2016;24:131-41.
41. Baek SH, Park M, Suh JH, Choi HS. Protective effects of an extract of young radish (*Raphanus sativus* L) cultivated with sulfur (sulfur-radish extract) and of sulforaphane on carbon tetrachloride-induced hepatotoxicity. Biosci Biotechnol Biochem 2008;72:1176-82.
42. Gaona-Gaona L, Molina-Jijón E, Tapia E, Zazueta C, Hernández-Pando R, Calderón-Oliver M, *et al.* Protective effect of sulforaphane pretreatment against cisplatin-induced liver and mitochondrial oxidant damage in rats. Toxicology 2011;286:20-7.
43. Nazmy EA, El-Khouly OA, Atef H, Said E. Sulforaphane protects against sodium valproate-induced acute liver injury. Can J Physiol Pharmacol 2017;95:420-6.