Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2017.09.001

Ameliorating effects of *Raphanus sativus* leaves on sodium arsenite-induced perturbation of blood indices in Swiss albino mice

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ARTICLE INFO

Article history:
Received 18 Aug 2017
Accepted 8 Sep 2017
Available online 9 Sep 2017

Keywords:
Sodium arsenite
Raphanus sativus leaves
Ameliorating effect
Serum indices

ABSTRACT

Objective: To evaluate the ameliorating effects of *Raphanus sativus* leaves (RSL) against sodium arsenite (Sa)-induced adverse effects through mice experiments.

Methods: Swiss albino mice were divided into four equal groups: control, Sa, RSL, RSL + Sa. Sa (10 mg/kg body weight/day), and powder form of RSL (50 mg/kg body weight/day) were provided as food supplement orallty. Blood indices were measured using commercially available kits through colorimetric methods.

Results: It was observed that lactate dehydrogenase, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase activities were significantly ($P < 0.05$) higher in Sa-treated mice than those in the control group. RSL significantly reduced Sa-induced elevation of the activities of these enzymes in serum significantly ($P < 0.05$). Serum butyrylcholinesterase activity and high density lipoproteins cholesterol levels in Sa-treated mice were significantly ($P < 0.05$) lower than the control group, and the food supplementation of RSL could significantly ($P < 0.05$) prevent the reduction of Sa-mediated serum butyryl cholinesterase activity and high density lipoproteins cholesterol levels. RSL could also reduce the Sa-induced elevation of serum urea level significantly ($P < 0.05$).

Conclusions: Results of this study suggest the protective or ameliorating effects of RSL on Sa-induced perturbation of blood indices are related to the hepatic, cardiovascular and kidney dysfunction. Therefore, RSL may be useful to reduce arsenic toxicity in human in the future.

1. Introduction

Since decades, chronic arsenic toxicity is a widespread global problem affecting millions of people in many countries including Bangladesh and India. In Bangladesh, it has become a great public health concern. This problem has not only created human sufferings and death but also made a socio-economic burden of the country. It has been reported that 61 out of 64 districts of Bangladesh are affected by arsenic contamination [1]. Recent surveys showed that approximately 80–100 million people of the country are at the risk of arsenic poisoning [2]. Arsenic contamination of groundwater poses a serious threat to the agricultural sustainability in this country. Besides domestic use, significant quantities of water from shallow aquifers are being used in the dry season particularly for irrigating rice and other crops in the country. Because of the use of arsenic-contaminated groundwater for the irrigation

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Foundation project: This work was financially supported by the University of Rajshahi [No. A-892-5/52/BIMOK/BIGGAN (1)/2011].

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

purposes, arsenic has entered the food chain [3]. Therefore, exposure to arsenic is unavoidable.

Several approaches including the filtering machine that can remove arsenic from drinking water have been developed. However, filtering machine is not sufficient for huge daily requirement of water for household purposes and for the irrigation of agricultural production. Therefore, alternative approaches are required to reduce the arsenic toxicity in human. Some synthetic drugs that include meso-2,3dimercaptosuccinic acid, succimer, chemet, sodium 2,3-dimercapto-1-propane sulfonic acid, dimaval, D-penicillamine have been tried for therapeutic purposes but they are not effectively useful for their adverse side effects on human health [4]. Arsenic breaks the anti-oxidant system and exhibits its adverse effect through reactive oxygen species-sensitive pathways [5,6]. Plant materials having antioxidant and free radical scavenging activity may be a plausible target to remediate or prevent arsenic toxicity. Trends on applying nutritional antioxidants in diseases related to oxidative stress have gained immense interests in recent years. Plant products are known to exert their protective effects by scavenging free radicals and modulating antioxidant defense system. Recently some plant materials have shown their potential to reduce arsenic toxicity *in vitro* and *in vivo* models. Previously, we demonstrated the ameliorating effects of turmeric powder against the sodium arsenite (Sa)-induced perturbation of blood indices [7]. Curcumin, an active ingredient of turmeric has been reported to reduce arsenic toxicity [8]. Verma *et al*. showed that *Parachartergus fraternus* (commonly known as Bhumyamalaki), *Terminalia arjuna* (commonly known as Arjuna) and *Moringa oleifera* (commonly known as Sajna) seed had ameliorating effects on arsenic toxicity [9]. Chowdhury *et al.* demonstrated that garlic (*Allium sativum*) could have potential to prevent arsenic toxicity [10]. More recently, protective effects of *Moringa oleifera* Lam. leaves and rhizomes of *Zingiber zerumbet* Linn. on the Sa-induced changes of several blood indices have been reported [11,12]. All these studies suggest the potential application of plant materials against arsenic toxicity.

Radish [*Raphanus sativus* (*R. sativus*)] is a good source of polyphenols and other natural compounds that have antioxidant and free radical scavenging activity [13]. Since the ancient times it has been used as a natural drug against many toxicants [14]. Because of the potential antioxidant and free radical scavenging activities, it was hypothesized that *R. sativus* leaves (RSL) might have protective effects against arsenic toxicity. Therefore, this study was undertaken to check whether RSL could show protective effects against arsenic toxicity in mice.

2. Materials and methods

2.1. Ethical permission

Ethical permission for this study was taken from the Institute of Biological Sciences, University of Rajshahi (No. 21/320-IAMEBBC/IBSc).

2.2. Preparation of RSL powder

RSL were collected from the local farmers near the University of Rajshahi, Bangladesh. Before collection from the local farmers, it was confirmed that no insecticides or pesticides were used. Fresh leaves of 3–4 weeks of ages were collected. After collection from the local farmers, RSL were cleaned and washed repeatedly with distilled water. Leaves were then shade-dried. Finally, powder form was obtained by grinding the dried leaves. The powder was kept at 4 °C with sealed plastic packet to avoid the microbial contamination.

2.3. Animal maintenance

Adult healthy (four weeks of age) Swiss albino male mice with average body weight (b.wt) of (20–22) g were purchased from International Centre for Diarrhoeal Disease Research, Bangladesh. The animals were randomly selected and housed in polycarbonate cages with steel wire tops and wood-cube bedding (six mice per cage). Animals were divided into four equal groups named control, RSL, Sa, RSL + Sa. They were maintained with 12 h:12 h dark–light cycle. The mice experiments were conducted for 16 weeks after one week of acclimation. All experimental mice had free access to food pellets and distilled water *ad libitum* throughout experimental periods. Sa was given to mice with distilled water and RSL powder (50 mg/kg b.wt/day) was added (as a supplement) to the normal diet. The water consumed by each group of mice was recorded twice a week and the final doses of Sa was 10 mg/kg b.wt/day.

2.4. Collection of serum from experimental mice

Four groups of experimental mice were maintained for 16 weeks from the starting day of the experiment. Then blood specimens were collected from the thoracic arteries of the mice after anaesthetization with diethyl ether. For coagulation, blood was kept about 30 min at room temperature. After centrifugation at 6 000 rpm for 15 min at 4 °C, serum was drawn off and stored at − 80 °C until the experiments were performed.

2.5. Laboratory examination

Serum activities of lactate dehydrogenase (LDH), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), high density lipoprotein of cholesterol (HDL-C) and urea levels were measured by commercially available kits (Human Diagnostic, Germany) and butyrylcholinesterase (BChE) activity was measured using BChE kit (Randox, UK) according to the manufacture's protocol with an analyzer (Humalyzer 3000, USA). All samples were analyzed in duplicate, and the mean values were taken.

2.6. Statistical analysis

Statistical analysis for this study was performed using software of statistical packages for social sciences. Data were expressed as mean ± SE. Blood parameters among the different groups of mice were analyzed by independent sample *t*-test.

3. Results

3.1. Effect of RSL on Sa-induced alteration of serum LDH activity

Serum LDH activities were found to be increased significantly after Sa treatment ($P < 0.05$) (Table 1). Food supplementation of RSL significantly inhibited the Sa-induced elevation of serum LDH activity ($P < 0.05$).

Table 1
Activities of liver enzymes in serum of experimental mice (Mean ± SE, $n = 6$) (U/L).

Groups	LDH	BChE	ALP	ALT	AST
Control	119.56 ± 13.20	11 743.60 ± 959.19	121.73 ± 4.73	36.23 ± 1.72	38.70 ± 4.89
Sa	231.46 ± 32.03[a]	8 318.40 ± 564.86[a]	330.23 ± 19.44[a]	48.58 ± 6.45[a]	61.38 ± 6.61[a]
RSL	106.87 ± 28.43	11 390.00 ± 1 370.45	107.36 ± 12.35	27.99 ± 1.31	36.37 ± 4.19
RSL + Sa	135.56 ± 25.96[b]	10 930.00 ± 270.94[b]	245.62 ± 6.01[b]	40.00 ± 7.11[b]	46.95 ± 8.61[b]

[a]Significantly different from control at $P < 0.05$ and [b]significantly different from Sa-treated group at $P < 0.05$.

Table 2
Serum HDL-C and urea levels of all experimental mice (Mean ± SE, $n = 6$) (mg/dL).

Groups	HDL-C	Urea
Control	24.54 ± 2.29	21.67 ± 0.63
Sa	18.91 ± 3.75[a]	31.67 ± 1.97[a]
RSL	33.47 ± 1.46	20.56 ± 1.43
RSL + Sa	30.75 ± 1.05[b]	22.40 ± 1.27[b]

[a]Significantly different from control at $P < 0.05$ and [b]significantly different from Sa-treated group at $P < 0.05$.

3.2. Effect of RSL on Sa-induced alteration of serum BChE activity

The results indicated that BChE activity was significantly lower in Sa group compared to the control group ($P < 0.05$). Intriguingly, it was observed that supplementation of RSL significantly abrogated the Sa-induced perturbation of BChE activity ($P < 0.05$) (Table 1).

3.3. Effect of RSL on Sa-induced alteration of serum hepatic enzymes used for liver function test

In this study, ALP, ALT, and AST activities in all groups of experimental mice were assessed. It was observed that Sa treatment significantly increased the activities of all the three enzymes in serum ($P < 0.05$) (Table 1). Intriguingly, supplementation of RSL significantly afforded the protection against Sa-induced perturbation of serum ALP, ALT and AST activities ($P < 0.05$).

3.4. Effect of RSL on Sa-induced alteration of HDL-C levels

Results demonstrated that Sa treatment significantly decreased serum HDL-C levels ($P < 0.05$). Intriguingly, RSL showed significant protection against Sa-induced perturbation of serum HDL-C levels (Table 2) ($P < 0.05$).

3.5. Effect of RSL on Sa-induced serum urea level

Serum urea levels were significantly increased in Sa-treated mice compared to the control group ($P < 0.05$). RSL supplementation could significantly abrogated the Sa-induced elevation of serum urea level (Table 2) ($P < 0.05$).

4. Discussion

Arsenic exposure has been reported to be associated with dermatitis, a variety of cancers, cardiovascular diseases, diabetes, peripheral neuropathy, and hepatic and renal dysfunction [15,16–24]. Due to the adverse effects on human health, arsenic has become a major threat to the public health in Bangladesh and some other countries in the world. Widespread contamination of arsenic including foods and drinking water indicates that exposure to arsenic is unavoidable. Till today, no effective drugs have been developed to reduce arsenic toxicity. Phytobioremediation may be a plausible approach for the reduction of arsenic toxicity in human. Therefore, this study was designed to check the efficacies of radish leaves against Sa-induced perturbation of blood indices associated with organ damages and diseases.

Several soluble enzymes, proteins or other metabolites of serum have been considered as indicators of the organ damage, cardiovascular diseases, diabetes, and hepatic and renal dysfunctions. Pathogenic condition as well as organ dysfunction can be diagnosed by the alteration of serum indices. Generally high concentrations of LDH are found in liver, heart, kidney, erythrocyte and skeletal muscle [25]. Consequently, diseases affecting those organs such as renal dysfunction, hepatic disorders and myocardial infarction have been reported to be associated with significant elevation in total serum LDH activity. Usually, if tissue damage occurs, LDH is leaked from the damaged tissue or organ to blood where it is measured. It has been reported that arsenic exposure increases serum LDH activity [7,26,27]. In this study, it was observed that oral administration of Sa increased the activity of serum LDH levels in mice. This result was consistent with the previous study that was conducted on human population [27]. RSL showed significant ($P < 0.05$) protection against Sa-induced elevation of LDH activity suggesting that RSL could have protective effects against Sa-induced organ damage. It is reported that arsenic exposure decreased the BChE activity in human [28]. Decreased BChE activity has been reported to be observed in hepatic dysfunction and neurotoxicity [28–30]. Serum BChE activity were decreased in Sa-treated mice significantly. Effect of Sa exposure on serum BChE activity observed in this study were in good agreement with the results of our previous study conducted in arsenic-endemic human subjects [28]. BChE is considered to be a clinically important enzyme because it is involved in both liver function abnormalities and neurotoxicity by several toxic chemicals [31–34]. Intriguingly, RSL showed protective effect on Sa-induced serum BChE activity. The protective effect of RSL on Sa-induced serum BChE activity is noteworthy since liver dysfunction and neurological disorders are the major adverse health effects of chronic exposure to arsenic [28,17,35]. Liver is the primary target organ for arsenic metabolism [36,37]. Protective effect of RSL on Sa-induced liver dysfunction was further confirmed by the evaluation of the activities of other serum enzymes (ALP, ALT, and AST) used for liver function test in the experimental mice. Results showed that Sa treatment significantly increased the activities of serum ALP, ALT, and AST. Intriguingly, RSL exhibited significant protection against Sa-induced perturbation of the activities of those enzymes.

HDL-C has been reported to prevent atherosclerosis through its anti-inflammatory activity [38,39]. Proatherogenic roles of arsenic have been well documented [15,40,41]. A population based study conducted in the arsenic-endemic and non-endemic areas in Bangladesh showed that arsenic exposure decreased the circulating HDL-C levels [15]. In this study, it was observed that serum high density lipoproteins cholesterol (HDL-C) levels were significantly lower in the mice treated with Sa than the non-treated control mice. Decreased serum HDL-C levels observed in this study were in good agreement with the previous results in arsenic-exposed human subjects [15,42]. HDL-C removes deposits of cholesterol from the artery walls and returns it to the liver where they are broken down and eliminated from the body. For this reason HDL-C is considered to be protective against cardiovascular disease and is often referred to as 'good' cholesterol [38,39]. HDL-C transports cholesterol from blood to liver where it is broken down [48,49]. Further, HDL-C is an anti-atherogenic factor with an antioxidant and anti-inflammatory properties. Through its antioxidant properties, HDL-C inhibits the conversion of low density lipoproteins cholesterol (LDL-C) to oxidized LDL-C [43]. The conversion of LDL-C to oxidized LDL by reactive oxygen species is now recognized as a predictive biomarker for the subclinical development of atherosclerosis and accepted as a key biochemical reaction in the initiation, progression and development of the atherosclerotic disease process [44–46]. Through anti-inflammatory properties, HDL-C also inhibits the secretion of different kinds of pro-inflammatory cytokines and molecules responsible for the development of atherosclerotic lesions by macrophage and endothelial cells [47]. Interestingly, in this study, it was observed that food supplementation of RSL provided significant protection against Sa-mediated perturbation of serum HDL-C. Therefore, ameliorating effect of RSL on Sa-induced perturbation of HDL-C might be important for human in order to reduce the risk of arsenic-exposure related cardiovascular diseases.

Elevated serum urea level is correlated with an increased protein catabolism in mammalian body or from more efficient conversion of ammonia to urea as a result of increased synthesis of enzyme involved in urea production in liver. Urea is excreted by kidney. Elevated level of serum urea is an indicator of renal dysfunction [48–51]. In this study, it was found that Sa treatment increased the serum urea levels in mice indicating the excessive catabolism of protein and renal dysfunction. RSL significantly inhibited the Sa-induced elevation of serum urea levels, suggesting that RSL could have protective effect against Sa-induced renal dysfunctions.

This study tested the protective effects of RSL against Sa-induced perturbation of blood indices. In this study, we could not clarify how RSL affords the protection against Sa-induced changes of blood indices. RSL contains active polyphenolics, (catechin, ferulic acid, protocatechuic acid, vanilic acid and sinapic acid) which have antioxidant and free radical scavenging activity [13]. Previous reports suggested that arsenic-induced cellular dysfunction was mediated through the production of ROS [5,6]. Therefore, we argue that polyphenolic compounds present in RSL might inhibit Sa action through their antioxidant and free radical scavenging activities. More detailed study, however, is needed in future to investigate what component(s) and how RSL reduces Sa-induced adverse effects. RSL is a kind of very cheap and edible vegetable and nontoxic to human. Therefore, ameliorating effects of RSL against the adverse effects of Sa observed in this animal study could pave the ways for the future application of RSL against arsenic toxicity in human.

This study had some limitations that warranted further discussion. The dose of Sa was 10 mg/kg b.wt/day for this study. However, before selecting the dose of Sa, a dose-dependent experiment should have been conducted to select minimum dose of Sa that could significantly change the blood indices. This minimum dose of Sa could be more relevant to the human exposure to environmental arsenic. Five times higher amount of plant materials than the dose of Sa were used in this study, however, it was not known whether low dose of RSL could reduce arsenic toxicity. Therefore, dose-dependent experiment of RSL against arsenic-toxicity is required in future. This study could not demonstrate what components of RSL ameliorated Sa-induced perturbation of hepatic, cardiovascular and renal biomarkers in blood. Extracting several components of RSL and then checking the efficacies of those components against arsenic toxicity should be future expansion of this study.

In conclusion, this study tested the efficacies of RSL against the Sa-induced perturbation of serum indices in mice. RSL significantly could reduce the Sa-induced serum LDH activity. RSL also afforded protection against Sa-induced changes of BChE, ALP, ALT, and AST activities and serum HDL-C and urea levels. Thus, the protective effect of RSL against Sa-induced perturbation of blood indices related to hepatic, cardiovascular and renal dysfunction observed in this study suggested the future application of RSL against arsenic toxicity in human.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was financially supported by the University of Rajshahi (No. A-892-5/52/BIMOK/BIGGAN (1)/2011).

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