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Protective effects of *Moringa oleifera* Lam. leaves against arsenic–induced toxicity in mice

Afzal Sheikh, Fouzia Yeasmin, Smita Agarwal, Mashiur Rahman, Khairul Islam, Ekhtear Hossain, Shakhawoat Hossain, Md Rezaul Karim, Farjana Nikkon, Zahangir Alam Saud*, Khaled Hossain

Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi–6205, Bangladesh

PEER REVIEW

Peer reviewer

Dr. Anwarul Azim Akhand, Professor, Department of Genetic Engineering and Biotechnology, University of Dhaka, Dhaka–1000, Bangladesh.
Tel: +880–1911176126
E–mail: akhand66@yahoo.com

Comments

In this study, the authors showed protective effects of *M. oleifera* leaves on arsenic–induced adverse effects in mice model. Orally administered *Moringa* leaves blocked various toxic effects of arsenic in mice. The results are promising for therapeutic application of these leaves for treating arsenic–exposed human.

Details on Page S356

ABSTRACT

Objective: To evaluate the protective role of leaves of *Moringa oleifera* (*M. oleifera*) Lam. against arsenic–induced toxicity in mice.

Methods: Swiss albino male mice were divided into four groups. The first group was used as non–treated control group while, the second, third, and fourth groups were treated with *M. oleifera* leaves (50 mg/kg body weight per day), sodium arsenite (10 mg/kg body weight per day) and sodium arsenite plus *M. oleifera* leaves, respectively. Serum indices related to cardiac, liver and renal functions were analyzed to evaluate the protective effect of *Moringa* leaves on arsenic–induced effects in mice.

Results: It revealed that food supplementation of *M. oleifera* leaves abrogated the arsenic–induced elevation of triglyceride, glucose, urea and the activities of alkaline phospatase, aspartate aminotransferase and alanine aminotransferase in serum. *M. oleifera* leaves also prevented the arsenic–induced perturbation of serum butyryl cholinesterase activity, total cholesterol and high density lipoprotein cholesterol.

Conclusions: The results indicate that the leaves of *M. oleifera* may be useful in reducing the effects of arsenic–induced toxicity.

KEYWORDS
Moringa oleifera leaves, Arsenic toxicity, Serum indices

1. Introduction

Arsenic, a naturally occurring toxicant, is present in food, soil and water. People are generally exposed to arsenic via ingestion of drinking water contaminated with arsenic. Arsenic intoxication is associated with severe health hazards including dermatitis, hyperkeratosis, gangrene, and skin cancer[1,2] despite of its few beneficial roles in the treatment of certain tropical diseases[3]. Severe diabetic disorders have been found in arsenic–intoxicated humans[4,5]. The most common forms of arsenic are water–soluble arsenite (As III) and arsenate (As V), and trivalent arsenic is known to be more toxic than the pentavalent arsenic. Moreover, inorganic forms of trivalent arsenic are more toxic than its organic forms[6]. It is reported that the inorganic As (III) form (H_2AsO_3) is 40–60 times more toxic than As (V) form (H_2AsO_4) [7]. Arsenic toxicity has caused an environmental tragedy in Bangladesh and West Bengal of India where millions of people have been affected due to drinking of arsenic contaminated ground water[8–10]. A huge number of toxicity cases have been already reported in the north–west region of Bangladesh.

*Corresponding author: Zahangir Alam Saud, Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi–6205, Bangladesh.
Tel: +88–0721–711109
Fax: +88–0721–750064
E–mail: zasaud@ru.ac.bd

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Although several hypotheses have been proposed, the exact mechanism of arsenic toxicity has not yet been clearly defined. Several studies suggest that higher concentrations of arsenic causes oxidative stress, increased generation of reactive oxygen species and nitric oxide, inhibition of enzyme and mitochondrial function, and induction of several stress genes[11-13]. Considering arsenic toxicity as one of the serious problems worldwide, its specific, reliable and safe treatment still remained mostly unknown. Various medicinal plants with high antioxidant properties have created increased interest for their therapeutic potential in reducing free radical-induced tissue injury[14,15]. In recent years, attention is focused worldwide on the potentiality of dietary antioxidants in reducing free radical-induced cellular impairment during stress[16]. Many plant products exert their protective effects against oxidative stress-mediated diseases by scavenging free radicals.

Moringa oleifera (*M. oleifera*) Lam. (local name Sajna) belongs to the Moringaceae family[17]. It is a multipurpose tree widely distributed in Bangladesh, India, Pakistan, Sri Lanka, Myanmar, Malaysia, Singapore, the Philippines, Thailand, Cuba, Jamaica and Nigeria[18]. *M. oleifera* is valued mainly for the young leaves and tender pods which are esteemed as very common vegetable in Bangladesh and India. It is reported that *Moringa* leaf is a potential source of natural antioxidants such as total phenolics and antioxidant vitamin A, C and E, ascorbic acid oxidase, polyphenol oxidase and catalase[19,20]. The leaves are a rich source of essential amino acids such as methionine, cysteine, tryptophan, lysine, vitamins and minerals[21]. Fresh leaves are used as an herbal medicine in treating a wide variety of diseases in Bangladesh and India. *Moringa* leaves have been reported to act as a hypocholesterolemic agent, thyroid hormone regulator, antidiabetic agent, antitumor agent and hypotensive agent[22-26]. Despite the above mentioned beneficial effect of *Moringa* leaves, its efficacy in reducing metal toxicity in general and arsenic toxicity in particular, has not yet been studied. It is reported that *Moringa* seeds and flowers provide protection against arsenic induced toxicity in albino rats[27-29], but still there is no report about *Moringa* leaves against arsenic toxicity. Therefore, we aimed to investigate the efficacy of *M. oleifera* leaves on arsenic toxicity in mice model. To the best of our knowledge, this is the first study that demonstrates the beneficial effect of the leaves of *M. oleifera* against arsenic toxicity.

2. Materials and methods

2.1. Chemicals and kits

Sodium arsenite ($NaAsO_2$), diethyl ether (Merck, India) reagent and triglyceride (TG), total gholesterol (TC), glucose, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, butyryl cholinesterase (BChE) detection kits and high density lipoprotein cholesterol (HDL-C) precipitation reagent were used for this study.

2.2. Animal maintenance

Adult healthy (4 weeks of age) Swiss albino male mice with average body weight 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). The animals prior to use were acclimatized for 7 d and were divided into four equal groups named control, sodium arsenite, *M. oleifera* leaves, sodium arsenite plus *M. oleifera* leaves. Sodium arsenite was given to the mice with (10 mg/kg body weight) drinking water and *Moringa* leaves powder (50 mg/kg) was added to the normal diet (as a supplement). The doses were selected on the basis of previously published reports and experiments were conducted for 16 weeks before sacrifice. Mice were maintained with 12 h:12 h dark light cycle with available supply of distilled water and food. Ethical permission of this study was obtained from the Institute of Biological Sciences, University of Rajshahi, Bangladesh (21/320-IAMEBBC/IBSc).

2.3. Preparation of Moringa leaves powder

Fresh young *M. oleifera* leaves were collected from Rajshahi University Campus, washed properly with tape water and sun dried. Finally leaves powder was obtained by grinding the sun dried leaves and kept at 4 °C with sealed plastic packet until experiment to avoid the microbial contamination.

2.4. Sample collection and preparation of serum

Blood specimens were collected from thoracic arteries of mice after anaesthetization with diethyl ether. Blood was kept about 30 min at room temperature for coagulation followed by centrifugation at 4000 r/min for 15 min at 4 °C. Serum were drawn off and stored at -80 °C until the experiments were performed.

2.5. Biochemical assay

The analyzer (CHEM-5 V3, Erba, Mannheim, Germany) was used for the measurement of serum indices using commercially available kits according to the manufacture's protocol. TG, TC, HDL, urea, glucose were measured; ALP, AST and ALT activities were determined by the kits from Human, Germany. BChE activity was measured using butyryl cholinesterase (ChE) kit (RANDOX, UK). All serum samples were analyzed in duplicate and then mean values were taken.

2.6. Statistical analysis

Statistical analyses were performed with SPSS for windows, version 15.0 (SPSS, Chicago, IL). Data are expressed as mean ±SD. Differences between the serum indices of different groups of mice were analyzed by using *t*-test. A value of $P<0.05$ was considered statistically significant.

3. Results

Elevated level of TG is very often associated with cardiovascular diseases[30], and cardiovascular disease is one of the major causes of arsenic-related mortality[31]. In this study, we determined the serum TG levels in the four groups of experimental mice. The TG levels (mean±SD) of the control, *Moringa* leaves, arsenic and arsenic plus *Moringa* leaves were (80.30±5.90), (78.5±5.38), (125.47±12.78) and (85.46±8.80) mg/dL, respectively (Table 1).

Table 1

Serum TG, TC, HDL, glucose and urea levels of the experimental mice.

Groups	Serum indices (mg/dL)				
	TG	TC	HDL-C	Glucose	Urea
Group I	80.30±5.90	121.60±11.69	54.16±1.45	121.54±7.01	40.15±2.11
Group II	78.50±5.38	103.50±4.46	58.88±4.38	111.09±11.7	39.74±0.50
Group III	125.47±12.78[a]	85.78±5.23[a]	40.81±1.57[a]	160.00±0.65[a]	46.37±2.01[a]
Group IV	85.46±8.80[b]	90.40±7.86	57.00±5.02[b]	120.86±8.09[b]	42.63±2.01[b]

Values are expressed as mean±SD, n=6. [a]: significantly different from control at $P<0.05$; [b]: significantly different from the arsenic-treated group at $P<0.05$. Group I: control; Group II: *M. oleifera* leaves; Group III: sodium arsenite; Group IV: sodium arsenite+*M. oleifera* leaves.

Compared with control, we found that arsenic exposure significantly ($P<0.05$) increased serum TG levels. Administration of food supplemented with *Moringa* leaves showed significant ($P<0.05$) protection against arsenic-induced elevation of TG levels in blood. We also observed a significant ($P<0.05$) decrease of TC levels in mice exposed to arsenic compared to control. The serum TC levels were (121.6±11.69), (103.50±4.46), (85.78±5.23) and (90.40±7.86) mg/dL for the control, *Moringa* leaves, arsenic and arsenic plus *Moringa* leaves groups, respectively (Table 1). Food supplementation of *Moringa* leaves could prevent arsenic-induced decrease of TC levels although this protection was not statistically significant. Atherosclerosis risk is inversely related to circulating levels HDL-C and low HDL-C levels are independent predictive marker of cardiovascular risk[32]. In this study, we evaluated the serum HDL-C levels in the four groups of experimental mice which were (54.16±1.45), (58.88±4.38), (40.81±1.57), and (57.00±5.02) mg/dL for control, *Moringa* leaves, arsenic and arsenic plus *Moringa* leaves groups, respectively (Table 1). We have found that *Moringa* leaves significantly ($P<0.05$) protected the lowering tendency of HDL-C levels in arsenic-exposed mice.

It is reported that arsenic induces an increase of the serum glucose level both in human and mice[33-35]. In this mouse model experiment, we also found that arsenic caused an increase of the serum glucose levels. Serum glucose levels (mean±SD) of the control, *Moringa* leaves, arsenic and arsenic plus *Moringa* leaves groups were (121.54±7.01), (111.09±11.70), (160.00±9.65), and (120.86±8.09) mg/dL, respectively (Table 1). Interestingly, co-administration of *Moringa* leaves significantly ($P<0.05$) reduced arsenic-mediated elevation of glucose levels in the arsenic-treated mice. *Moringa* leaves alone could also decrease serum glucose levels indicating its ability of possessing glucose lowering effects.

Kidney dysfunction is one of the major health effects of the long term arsenic exposure, and elevated levels of serum urea have been reported to be associated with renal dysfunction and excessive protein catabolism[35,36]. We have found that arsenic exposure significantly ($P<0.05$) increased serum urea levels compared with control. Food supplementation of *Moringa* leaves significantly ($P<0.05$) protected arsenic-induced elevation of serum urea levels (Table 1).

In order to confirm the hepatocellular degeneration of hepatic tissue, activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT) were then estimated. Usually the serum levels of all these three enzymes are increased in liver injury. We observed that these enzyme activities were significantly increased ($p<0.05$) in arsenic-treated mice group when compared to the normal control group. A significant ($p<0.05$) lower levels of these altered enzymatic activities were observed in arsenic-treated mice supplemented with *moringa oleifera* leaves (Table-2). Previously it has been reported that arsenic exposure decreased plasma BChE activity in mice and humans[33,37]. In this study, we investigated whether moringa leaves could prevent the arsenic-induced decrease of serum BChE activity. Serum BChE activity (mean ± SD) were measured for control, moringa leaves, arsenic and arsenic plus moringa leaves as 13071.00 ± 593.35, 12875.83 ± 390.74, 10602 ± 654.75, and 12551.13 ± 654.74 U/L, respectively (Table-2). The enzyme activity was significantly ($p<0.05$) decreased in the group of mice exposed to arsenic compared with the control group. Intriguingly, we observed that supplementation of moringa leaves in mice food prevented the arsenic-induced perturbation of BChE activity. However, no significant differences in serum BChE activity was observed between the control and moringa leaves groups.

Table 2

Effect of *Moringa* leaves on sodium arsenite-induced liver enzymes activity.

Groups	Serum indices (IU/L)			
	ALP	AST	ALT	BChE
Group I	137.10±5.53	86.22±9.08	86.22±9.08	13071.00±593.35
Group II	126.89±2.51	75.33±6.89	39.02±4.94	12875.83±390.74
Group III	210.16±7.16[a]	118.28±7.87[a]	50.42±1.96[a]	10602.00±654.75[a]
Group IV	123.98±1.61[b]	89.19±9.09[b]	41.84±2.51[b]	12551.13±654.74

Values are expressed as mean±SD, n=6. [a]: significantly different from control at $P<0.05$; [b]: significantly different from the arsenic-treated group at $P<0.05$. Group I: control; Group II: *M. oleifera* leaves; Group III: sodium arsenite; Group IV: sodium arsenite+*M. oleifera* leaves.

4. Discussion

The present study evaluated the effect of crude leaves of *M. oleifera* on arsenic-induced toxicity and the results of the present investigation revealed that treatment with the *Moringa* leaves significantly protect animals from the toxic effects of arsenic. From the ancient time, *Moringa* leaves are used as vegetable in Bangladesh and Indian subcontinent. Due to the deleterious effects of arsenic on human body, there is an increasing interest in the development of preventive therapy for reducing arsenic toxicity in human. *Moringa* leaf is a safe natural antioxidant containing vegetable and is found as a potential source of four natural antioxidants such as total phenolics antioxidant, vitamin A, C, and E[38,39].

We observed that serum TC and HDL-C levels were significantly lower in the mice treated with arsenic than in the non-treated control mice. Decreased serum TC and HDL-C levels observed in this study were in agreement with the previous results in arsenic-exposed human subjects demonstrated by Nabi *et al*.[40] and Karim *et al*[41]. HDL-C removes deposition of cholesterol from the artery walls and returns them to the liver where they are broken down and eliminated from the body[42]. Therefore, decreased HDL-C observed in this study led us to make a hypothesis that HDL-C lowering effect of arsenic might be a key event for the development of arsenic-induced atherosclerosis. Interestingly, we found that food supplementation of *Moringa* leaves provided protection against arsenic-induced alteration of serum TC and HDL-C. Several soluble enzymes, proteins or other metabolites of serum have been considered as potential indicators of cardiovascular diseases, diabetes, hepatic and kidney dysfunctions. Arsenic-induced elevation of serum glucose levels observed in this study is consistent with the result of Tseng *et al*.[35], which showed that chronic arsenic exposure increased blood glucose levels in human population. Intriguingly, *Moringa* leaves provided significant protection against arsenic-induced elevation of blood glucose levels.

The blood urea becomes raised when the kidney tubules are prevented from removing the urea and other waste products from the blood[37]. In this study, we found that arsenic treatment increased the serum urea levels in mice that might be an indication of the adverse effects of arsenic on kidney and liver. *Moringa* leaves potentially inhibited the arsenic-induced elevation of serum urea levels. Present study clearly indicated the ameliorating effects of *Moringa* leaves on the arsenic-induced changes of serum indices. However, we did not clarify how *Moringa* leaves showed the protection against arsenic action. One possibility was that the phenolic acids, the active ingredients of *M. oleifera* leaves[43], might inhibit arsenic action by perturbation of the arsenic-mediated signal transduction pathways or by scavenging free radicals through its antioxidant property. It has been well documented that reactive oxygen species produced by arsenic acts as a second messenger for transducing intracellular signals. Probably ingredient of the *Moringa* leaves inhibited arsenic-induced reactive oxygen species production which ultimately abrogated the signaling pathways associated with toxicity of arsenic. Further study is needed to identify the possible ingredients in *Moringa* leaves that are involved in preventing toxic effects of arsenic.

ALP, AST and ALT are the major serum hepatic enzymes used for liver function test. The elevated activities of these enzymes in serum are an indication of liver damage. In this study, we found that arsenic administration substantially increased serum ALP, AST and ALT activities and all these results were consistent with the results showed by Islam *et al*[44]. Elevated activities of serum ALP are found together with elevated activities of serum AST and ALT, which are more specific to liver damage. Co-administration of *Moringa* leaves as a food supplementation significantly reduced arsenic-induced elevation of ALP, AST and ALT activities. These results indicated that *Moringa* leaves had a protective effect on arsenic-induced liver injury. Decreased serum/plasma BChE activity is associated with hepatitis, hepatic metastases, heart attack and neurotoxicity[45-49]. Recently, it is reported that BChE activity significantly decreases in human population exposed to arsenic chronically[30]. Interestingly, in our mice model experiment, we also observed that serum BChE activity decreased in arsenic treated mice, and food supplementation of *Moringa* leaves prevented this decrease of arsenic-induced BChE activity.

Therefore, this study showed the potentiality of *M. oleifera* leaves to reduce arsenic toxicity in mice model. Thus, the results suggested that *M. oleifera* leaves could be useful therapeutically in future to reduce or prevent the toxic effect of arsenic in humans.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Arsenic, a devastating environmental pollutant, is exposed to millions of people through drinking water causing them from suffering various diseases including dermatitis, cardiovascular diseases, liver dysfunction, renal failure, diabetes and a variety of cancers. Therefore, there is a need for innovation of potent, cheap and available therapy to treat huge number of patients suffering from arsenic exposure.

Research frontiers

This study evaluated the protective role of leaves of *M. oleifera* against arsenic-induced toxicity in mice model. The authors showed that *Moringa* leaves, when orally administered, protected mice from various adverse effects of arsenic.

Related reports

M. oleifera is reported to be known as a potential source of natural antioxidants. The leaves of this plant are rich in essential amino acids, vitamins and minerals. Also the leaves have the potentiality to act as a hypocholesterolemic agent, thyroid hormone regulator, antidiabetic agent, antitumor agent and hypotensive agent.

Innovations and breakthroughs

The authors have demonstrated that food supplementation of *M. oleifera* leaves abrogated the arsenic-mediated elevation of various serum parameters including TG, TC, glucose, urea and the activities of various enzymes including ALP, AST, ALT and BChE.

Applications

M. oleifera is known as a common vegetable in South Asia, and therefore it is safe for human and other animals. The results of this study suggested that *Moringa* leaves could be useful therapeutically in future to reduce or prevent the toxic effect of arsenic in humans.

Peer review

In this study, the authors showed protective effects of *M. oleifera* leaves on arsenic-induced adverse effects in mice model. Orally administered *Moringa* leaves blocked various toxic effects of arsenic in mice. The results are promising for therapeutic application of these leaves for treating arsenic-exposed human.

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