Effect of *Momordica charantia* on the removal of arsenic from different organs of arsenic-treated rat

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Abstract

The present study was designed to examine the effect of *Momordica charantia* ethanol extract on the removal of arsenic from different organs of the arsenic-loaded rat. The rats (n=30) were divided into 5 groups: a) control, b) arsenic-treated (200 µg/L), c) extract-treated (50 mg/day), and d) arsenic plus extract-treated (50 or 200 mg/kg). The rats were sacrificed on day 61. The study revealed that there were significant accumulation of arsenic in the liver, kidneys, intestine and skin. On the other hand, there was significant decrease in glutathione level in the liver. The administration of extract significantly decreased the amount of arsenic in all the organs tested and increased the glutathione level significantly in the liver. The maximum reduction of arsenic was found in skin (100%), liver (91.2%), intestine (89.2%) and kidney (83.1%). The maximum elevation of glutathione in liver was 48.3%. In conclusion, *M. charantia* reduces the arsenic level from different organs of the arsenic-loaded rat.

Introduction

Arsenicosis is usually due to chronic ingestion of high concentration of arsenic through drinking water. However, it has no specific treatment. Some investigators suggest the effectiveness of retinal, beta-carotene,[1] zinc,[2] ascorbic acid,[3] selenium,[4] tocopherol[5] and spirulina.[6] These treatments require several months to get some improvement. However, long duration of treatment may affect the patient compliance. In addition, recurrence occurs after the stoppage of treatment. For this reason, new treatment option is required.

Momordica charantia is used as a vegetable. It has the medicinal properties such as antidiabetic, antitumour, antibacterial, antiviral, antihelmintic and abortifacient.

Chronic arsenic toxicity decreases the intracellular glutathione level. *M. charantia* is a rich source of flavonoids. Flavonoids increase the intracellular glutathione level by transactivation of the γ-glutamylcysteine.[7] Glutathione promotes the methylation capacity in liver which is the main metabolic pathway of arsenic and facilitates the excretion of arsenic that consequently reduces the arsenic burden.[8]

In this study, it was examined whether the extract of *M. charantia* could remove the arsenic from different organs of arsenic-treated rat.

Materials and Methods

The study was carried out from September 2016 to January 2018. The *M. charantia* (30 kg) was purchased from the local market. They were washed and chopped without seeds. They were, then, dried in open air at room temperature for 3 days.

Extraction procedure

M. charantia was taken in an amber colored container. They were soaked in 80% ethanol at room temperature. After 24 hours, this was filtered by Whatman filter paper. The filtrate was condensed in a rotatory vacuum evaporator at 60°C and at rotations of 100 rpm. Then, the condensed thick extract was obtained (130 g).

Brine shrimp lethality assay

Serial dilution of the M. charantia extract: Two milligram of *M. charantia* extract was measured using an analytical balance. The test tubes were taken and labeled. The extract dissolved in 2 mL solution to prepare the stock solution. Serial dilution of the stock solution was done to prepare the concentration of 1000, 200, 50 and 1 µg/mL. These prepared solutions were taken into the four test tubes labeled as 1-4 that contained 10 nauplii and 1 mL of seawater.

Hatching of brine shrimp: *Artemia salina* (brine shrimp) eggs were collected from the local

market. A rectangular jar was filled with 3 L of water.[9] Twenty-seven gram of table salt was to it and mixed with the jar water using a spatula. Proper aeration maintained by putting the tip of an airline from an air pump into the bottom of the jar. Brine shrimp eggs (15 g) were mixed into the jar water. A light (60 watt bulb) was switched on and placed a few inches away from the jar.

After 72 hours, the nauplii were hatched. Ten actively moving nauplii were transferred to each test tube containing different concentration of *M. charantia* extract by using Pasteur pipette and motility was observed after 0, 2 and 24 hours.

Experimental procedure

Maintenance of the rat house: The environment of the rat house was properly maintained where the dark/light cycle 12/12 hours, temperature 25-30°C. Wasted products were removed regularly. It was essential for animal well-being, the quality of animal research and also the health and safety of the investigator.

Prepare the rat cases: The polypropylene plastic cages were needed for keeping the rats. A layer of wood shavings was placed on the floor of the cages. Each cage was properly labeled for identification of different groups. The cage was cleaned regularly during the experimental period.

Isolation of the experimental rats: Thirty rats (Long-Evans Norwegian strains, adult healthy male) were isolated for this study. The weight of the rats was 200-250 g. The rats were divided into five groups: a) control, b) arsenic-treated (200 µg/L), c) extract-treated (50 mg/day), and d) arsenic plus extract-treated (50 or 200 mg/kg) (Table I). The control group received only food and water *ad libitum* every day for 60 days. The arsenic group received arsenic contaminated water 200 µg/L *ad libitum* for the first 30 days followed by normal diet and water *ad libitum* for another 30 days. The extract group received normal diet and water *ad libitum* for the first 30days followed by *M. charantia* extract 200 mg/day for another 30 days. The arsenic plus extract (50 or 100 mg) exposed group received arsenic contaminated water for the first 30 days followed by *M. charantia* extract 50 or 100 mg/day for another 30 days. Rats were sacrificed on day 61.

Preparation of arsenic solution (1 mg/mL): One hundred and thirty-two milligram of arsenic trioxide (E. Merck, Germany) was required to prepare arsenic trioxide stock solution (1 mg/mL). It was taken in a measuring cylinder (100 mL) and 100 mL of distilled water was added to it. The arsenic was not completely dissolved in water. So, 5% sodium hydroxide was added drop by drop until the arsenic trioxide dissolves completely. Then, it was kept in the refrigerator at 0-4°C before use.

Administration of arsenic contaminated water: Arsenic contaminated drinking water (200 µg/L) was prepared from the stock solution. Then, about 200 mL of arsenic water was given every day in the concerned groups of rats by water feeding bottle. Each rat took water *ad libitum*. In an average each rat took 30 mL of arsenic contaminated water per day.

Dilution of M. charantia extract in the solvent: *M. charantia* extract (3 g) was dissolved in 15 mL solvent (1.5 mL ethanol + 13.5 mL distilled water) to obtain the concentration of extract 200 mg/mL. *M. charantia* extract (400 mg) was dissolved in 8 mL solvent (0.8 mL ethanol + 7.2 mL distilled water) to obtain the concentration of 50 mg for each rat.

Administration of M. charantia extract: Each rat of two groups (arsenic plus extract 200 mg group and extract group) was fed 200 mg/mL extract orally everyday for one month at 9.00-10.00 am. Another group of arsenic plus extract (50 mg) was given 50 mg/mL extract orally everyday for one month at the same time.

Sacrifice of animals

The rats of all groups were sacrificed on day 61. Sacrifice procedure was performed under light anesthesia with chloroform.

Table I

Experimental study design

Groups	n	Treatment schedule		Sacrifice day 61
		Day 1 to day 30	Day 31 to day 60	
Control	6	Food and water *ad Libitum*	Food and water *ad libitum*	
Arsenic	6	Arsenic contaminated drinking water (200 µg/L) + food *ad libitum*	Food and water *ad libitum*	
Extract	6	Food and water *ad libitum*	Extract (200 mg/day) + food and water *ad libitum*	
Arsenic plus extract	6	Arsenic contaminated drinking water (200 µg/L) + food *ad libitum*	Extract (50 mg/day) + food and water *ad libitum*	
Arsenic plus extract	6	Arsenic contaminated drinking water (200 µg/L) + food *ad libitum*	Extract (200 mg/day) + food and water *ad libitum*	

Collection and preservation of organs

Organs (liver, kidneys, intestine and skin) were collected after opening the abdomen of rats by midline incision. Then, these were packed in separate polyethylene packets with labeled and preserved in a deep freeze until analysis.

Preparation of tissue homogenate

At first, the tissue was taken separately in a petri dish containing distilled water (resting in an ice bath) and clean properly. Then, 500 mg of each experiment tissue after blotting on filter paper was weighted. This was chopped into small pieces (1-2 mm in size). Two milliliter of distilled water and chopped tissue were taken in a hand homogenizer that placed in an ice bath. The tissue was homogenized. Carefully transferred homogenates to the labeled amber color container and kept at -20°C before use

Results

The acute toxicity study using the brine shrimp shows that *M. charantia* extract had little effect (Table II).

Table II

Number of live nauplii at different concentrations of the extract

Concentration of extract (µg/mL)	Hour 0	Hour 2	Hour 24
0	10 ± 0.0	10 ± 0.0	8 ± 1.5
1	10 ± 0.0	9 ± 1.5	7 ± 1.0
50	10 ± 0.0	7 ± 1.0	5 ± 0.5
200	10 ± 0.0	8 ± 1.1	6 ± 0.5
1000	10 ± 0.0	8 ± 1.0	7 ± 0.5

Liver

The mean (± SD) amount of arsenic in the liver in the control group was 5.6 ± 4.8 µg/g which was increased to 12.5 ± 6.5 µg/g after ingestion of arsenic for 30 days (Table III). The administration of 50 mg *M. charantia* extract (arsenic plus extract 50 mg) reduced the mean amount of arsenic to 1.1 ± 2.5 µg/g which was 91.2% less than the arsenic group. This change was statistically significant (p=0.002). The administration of 200 mg *M. charantia* extract (arsenic plus extract 200 mg) reduced the mean amount of arsenic to 3.4 ± 4.0 µg/g which was 72.8% reduction when compared to the arsenic group. This change was also statistically significant (p=0.015).

Kidneys

The exposure of rat to high concentration of arsenic lead to the amount of arsenic in kidneys 17.2 ± 9.0 µg/g from the control value of 3.5 ± 4.2 µg/g (Table III). The administration of *M. charantia* extract (50 mg) reduced the mean amount of arsenic to 2.9 ± 3.4 µg/g (83.1% reduction). This change was statistically significant (p=0.004). The administration of 200 mg *M. charantia* extract reduced the mean amount of arsenic to 3.5 ± 4.9 µg/g which was 79.7% reduction when compared to arsenic group. This change was also statistically significant (p=0.008).

Intestine

The mean (± SD) amount of arsenic in the intestine in control group was 0.2 ± 0.2 µg/g. In arsenic-treated group, the mean amount of arsenic in

Table III

Effect of different concentrations of *M. charantia* extract on the removal of arsenic from rat liver

Tissue		Control	Arsenic	Extract	Arsenic plus extract (50 mg)	Arsenic plus extract (200 mg)
Liver	Arsenic concentration (µg/g)	5.6 ± 4.8	12.5 ± 6.5	4.8 ± 4.5	1.1 ± 2.5	3.4 ± 4.0
	%Removal	-	-	-	91.2	72.8
	p value	-	-	-	0.002[a]	0.015[b]
Kidney	Arsenic concentration (µg/g)	3.5 ± 4.2	17.2 ± 9.0	3.9 ± 4.0	2.9 ± 3.4	3.5 ± 4.9
	%Removal	-	-	-	83.1	79.7
	p value	-	-	-	0.004[a]	0.008[b]
Skin	Arsenic concentration (µg/g)	0	6.2 ± 3.5	0	0	0
	%Removal	-	-	-	100	100
	p value	-	-	-	0.001[a]	0.001[b]
Intestine	Arsenic concentration (µg/g)	0.2 ± 0.2	13.9 ± 7.5	0.7 ± 1.3	1.5 ± 2.9	1.6 ± 1.8
	%Removal	-	-	-	89.2	88.5
	p value	-	-	-	0.003[a]	0.003[b]

Data were presented as mean ± SD; Unpaired t-test was done to be comparison: [a]Arsenic vs. arsenic plus extract (50 mg) group, [b]Arsenic vs. arsenic plus extract (200 mg) group

intestine increased to 13.9 ± 7.5 µg/g (Table III). The administration of 50 mg *M. charantia* extract reduced the mean amount of arsenic in the intestine to 1.5 ± 2.9 µg/g which was 89.2% less than the arsenic -treated group. This change was statistically significant (p=0.003). The administration of 200 mg *M. charantia* extract reduced the mean amount of arsenic to 1.6 ± 1.8 µg/g which was 88.5% reduction when compared with arsenic group. This change was also statistically significant (p=0.003).

Skin

There was no detectable amount of arsenic in the skin of control group. It increased to 6.2 ± 3.5 µg/g in arsenic-treated group. The administration of 50 mg *M. charantia* extract reduced the mean amount of arsenic in the skin to 0 µg/g (Table III).

Accumulation of glutathione in rat liver

The mean (± SD) amount of glutathione in the liver of control group was 13.6 ± 0.7 mg/g. The administration of arsenic for 30 days in the arsenic-treated group decreased the mean amount of glutathione to 5.8 ± 0.5 mg/g. The administration of 50 mg *M. charantia* extract increased the mean amount of glutathione to 7.7 ± 0.3 mg/g which was 32.8% increased than that of arsenic-treated group. This change was statistically significant (p=0.000). The administration of 200 mg *M. charantia* extract increased the amount of glutathione to 8.6 ± 0.2 mg/g which was 48.3% increased when compared to the arsenic-treated group. This change was also statistically significant (p=0.000) (Table IV).

Discussion

The present study has shown the significant effect of *M. charantia* ethanol extract on the removal of arsenic from the different organs of arsenic-treated rat. The percentage of the removal of arsenic by *M. charantia* was 100% in the skin, 91% in the liver, 89% in the intestine and 83% in the kidney.

Table IV

Effect of *M. charantia* extract on glutathione level in rat liver

Groups	Concentration of glutathione in liver (mg/g)	% Increase	p value
Control	13.6 ± 0.7	-	-
Arsenic	5.8 ± 0.5	-	-
Extract	13.3 ± 0.5	-	-
Arsenic plus extract (50 mg)	7.7 ± 0.3	↑32.8	0.00001[a]
Arsenic plus extract (200 mg)	8.6 ± 0.2	↑48.3	0.00001[b]

Data were mean ± SD; Unpaired t-test was done to be comparison; [a]Arsenic plus extract (50 mg) vs. arsenic-treated group; [b]Arsenic plus extract (200 mg) vs. arsenic group

In the present study, the less amount of arsenic was found in different organs of the control group. It is due to the inevitable presence of arsenic in normal drinking water and food. The amount of arsenic was increased in the different organs of rats following administration of 200 µg/L of arsenic for every day. The levels were compared with that of the control group. After arsenic administration, the highest accumulation of arsenic was found in the kidney following liver, intestine, and skin. This finding was similar to another study.[10]

The incidence of kidney pathology in chronic arsenic poisoning is uncommon. There is a causal relationship between arsenic exposure in drinking water and bladder cancer.[11] The prolong exposure to arsenic may cause accumulation within the red blood cell.

As a result, arsenic accumulation is increased in the reticuloendothelial rich organs like the liver. Inorganic arsenic is metabolized and rapidly cleared from tissues through urine.[12] This biomethylation process can easily become saturated. Thus, it leads to the excess inorganic arsenic being deposited in the skin, hair, and nail, where it binds tightly to keratin. In this study, there were two arsenic exposed groups, where the different concentration of extract 50 and 200 mg were given every day. The study showed that arsenic was removed in different concentration from different organs in the different rates. Extract 50 mg was more effective in the removal of arsenic from the kidney and liver. In case of intestine and skin, removal of arsenic by 50 and 200 mg showed the same effect. There was significantly decreased glutathione level in the liver after arsenic administration. Different concentration of the extract was increased the glutathione level, 32.8% in 50 mg and 48.3% in 200 mg.

A number of studies are available on the removal of arsenic from the different organs by the different extract. Corn extract (water) reduced the highest percentage of arsenic from the liver tissue 69% followed by kidney- 64%, skin- 69%.[10] Spinach extract (1%) removed the arsenic from the liver in 76%, kidney- 44%, intestine- 57%, and skin- 63%.[13]

Another study done by Quayum, 2007 showed the effectiveness of the root *Eichhornia crassipes* extract on the removal of arsenic from different tissues in 60% in the liver, 48% in the intestine, 43% in the kidney, and 35% in the skin.[14]

It was observed that removal of arsenic from all the tissues by *M. charantia* was higher than the other extract. In the present study, it was found that 90% arsenic reduced by the *M. charantia* in comparison with the other extract such as 67% by the corn, 60% by the spinach, 46% by the root *Eichhornia crassipes*. Different studies have shown that there are certain phytochemicals of *M. charantia* detoxify many toxic agents. This detoxifying property helps in the remo-

val of arsenic.

Arsenic decreased the glutathione level in liver.[15] Administration of arsenic in rats produced a significant reduction in hepatic glutathione.[16] The present study also showed that there was significantly decreased the glutathione in the liver. Arsenic can reduce the cellular level of glutathione by three mechanisms. At first, reduction of pentavalent to trivalent arsenicals occurred by glutathione which acts as an electron donor. Secondly, arsenic has the high affinity for glutathione. The third one is arsenic -induced free radicals decreased glutathione level by oxidation.

Certain amino acid like cysteine, vitamins like ascorbic acid and minerals have an important role in reducing the oxidative stress. Arsenic exposure causes the higher accumulation of arsenic in different organs. *M. charantia* is rich in vitamins, minerals, and amino acid. So, it is helpful in reducing the oxidative stress and declining the arsenic burden from the different tissues.

Conclusion

M. charantia extract reduces of arsenic level from different organs of the arsenic-loaded rat.

Ethical Issue

Prior to the study conduct, ethics for animal study were followed: a) Animals were only used as a least resort; b) Every practical step was taken to avoid distress or suffering of animal; c) The smallest possible number of animals were used as such only 6 rats taken for each group; and d) The potential benefits had weighed against the cost to the animals, the simplest or least sentient species were used.

Conflict of interest

Authors declare no conflict of interest

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