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ORIGINAL ARTICLE

Removal of Arsenic from Isolated Liver Tissues of Experimental Rat by Kalmishak

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Abstract

Background: Kalmishak has some ability of removal of inorganic matter. **Objective:** The purpose of the present study was to see the ability of Kalmishak for the removal of arsenic from isolated liver tissues of rat. **Methodology:** This animal study was carried out on isolated liver tissues of Long Evans Norwegian adult healthy male rats weighing 160 to 200 g. The rats were 3 to 6 months of age obtained from animal house of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh from 2004 to 2005. Measurements and all tasks were performed in a very careful manner. Atomic Absorption Spectrophotometer with Hydride Generator was used to measure the arsenic level. No arsenic was added in test tube I. 2.5 µg/ml arsenic trioxide was added rest of the test tubes. They all were incubated for 45 minutes at 37^0C. Then the tissues were washed properly. In second incubation, different extracts of kalmishak (C 1and C 2) were added at 20 µl/ml dose. Second incubation was also for another 45 minutes at 37^0C. There were duplicates of all test tubes. **Result:** When the tissues were incubated with no arsenic in both 1st and 2nd incubation the amount of arsenic was found 6.62 ± 3.40 µg / g (mean ± s.e) of protein and it was considered as blank. The liver tissues of rat loaded with 2.5 µg / ml arsenic were incubated for 45 minutes at 37°C with no extract and the amount of arsenic was 98.32 ± 36.10 µg / g (mean ± s.e) of protein and the value was considered as standard. The blank value was then deducted from the standard and the derived value was considered as control. After exposure with Compound -1 (20 µl /ml) in second incubation for 45 minutes at 37°C, the amount of arsenic was 69.57 ± 7.60 µg / g (mean ± s.e) of protein. There was 13.00% removal of arsenic. **Conclusion:** In conclusion Kalmishak has the ability to remove of arsenic from isolated liver tissues of rat. [*Journal of Current and Advance Medical Research, July 2020;7(2):55-59*]

Keywords: Removal; Arsenic; Isolated Liver Tissues; Experimental Rat; Kalmishak

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Introduction

Arsenic is a naturally occurring element in the earth's crust with a long history of use as a constituent of commercial and industrial products as a component in pharmaceuticals and as an agent of deliberate poisoning[1]. Recent commercial applications of arsenic include its use in manufacturing of semi-conductors, wood preservatives, herbicides, cotton desiccants, nonferrous alloys and glass, insecticides and veterinary pharmaceuticals[2]. In some region of the world ground water may contain high level of arsenic that has leached from natural mineral deposits. Arsenic in drinking water in the Ganges delta of India and Bangladesh is now recognized as one of the world's most pressing environmental health problems[3].

Arsenopyrite is non-water soluble, when deep tube wells were introduced, some air enters, which is oxygen[4]. Oxygen oxidizes arsenopyrite and makes free arsenic, which dissolves with water in alkaline media. Thus deep tube wells are largely contaminated with arsenic[5]. Though Bangladesh has so many rivers and ponds, widespread use of ground water was introduced in late sixties. Before introduction of deep tube well, the surface water was the source of water of human use throughout Bangladesh. Surface water is responsible for much water borne disease like cholera, diarrhoea, enteric fever, hepatitis[6]. But as ill luck would have it, this ground water became a source of chronic arsenic poisoning[7].

Arsenic is considered as the major causative agent of black foot disease[4]. Arsenic has shown genetic activity in animal tests. Drinking of water containing (10 mg/l) for 8 weeks increased the frequency of chromosome aberrations in bone marrow of mice[3]. Ingestion of seafood may result in total urinary arsenic level of more than 10μmol/l. While persons without such exposure to arsenic usually have urinary level in range 0.1-0.7 μmol/l. Measurement of inorganic arsenic, MMA and DMA in urine should therefore give a better estimate of exposure to inorganic arsenic than total urinary arsenic[8].

Ipomoea aquatica (water convolvulus) commonly known as kolmishak in our country is an herb rich in iron and with medicinal properties[5]. The plant is highly invasive forming dense mats over the surface water bodies such as lakes, ponds, canals, marshes and ditches. It is also found in the muddy banks along the stream. The weed spreads rapidly from plant fragments and its floating seeds allow effective colonization in new areas. It does not grow well when the temperatures are below 23.9 degree Celsius[6].

In the management of patient suffering from chronic arsenic poisoning, the first step is to stop the consumption of arsenic contaminated water[2]. Other measures include, consumption of arsenic free drinking water, protein and vitamin rich food, anti- oxidant vitamins and chelation therapy[5]. Zinc and Iron supplementation can reduce arsenic toxicity[9]. The objective of the chelation therapy is to provide the patient a chemical, which has the property of strongly attracting arsenic and it is then excreted in the urine. This present study was undertaken to see the ability of Kalmishak for the removal of arsenic from isolated liver tissues of rat.

Methodology

This animal study was carried out on isolated liver tissues of Long Evans Norwegian adult healthy male rats weighing 160 to 200 g. The rats were 3 to 6 months of age obtained from animal house of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh from 2004 to 2005. The rats were housed in standard plastic cages with a light/dark cycle 12/12 hours at room temperature in a well-ventilated room. This experiment was an in vitro study. Measurements and all tasks were performed in a very careful manner. The rats were sacrificed by inhalation anesthesia. Inhalation anesthesia is very effective in rats. Liver tissues of rat was extracted with help of a forceps and a pair of scissors and placed into Tyrode solution. The temperature of Tyrode solution was tried to be maintained at O^o - 4^o C. Two freshly washed test tubes containing 2 ml Tyrode solution were separated and kept into ice pieces. These would be blank as they contain no arsenic.

Arsenic was added to Tyrode solution contained in a beaker from stock solution (stock solution was 2 mg/ml) to make 2.5 µg/ml concentration of arsenic. Then 2 ml of Tyrode solution of 2.5 µg/ml of arsenic concentration was transferred to each test tube from the beaker with the help of automatic micro pipette with blue tip except two test tubes which were considered as blank. All the test tubes were kept into ice pieces. Small and approximately equal pieces of liver tissues were put into the individual test tubes with the help of forceps. Approximately 20 small pieces of liver were given in each test tube.

Two test tubes contained 2.5 µg/ml concentration of arsenic with no extract and these were called standard. Rest of the test tubes contained 2.5 µg/ml

concentration of arsenic in first incubation and different types of extract were added in second incubation and these all were samples. Then all test tubes were incubated for the first time at 37°C for 45 minutes in water bath with shaker. After first incubation all test tubes were taken outside and again placed under ice pieces. All the open ends of the test tubes were covered with parafilm and washed with Tyrode solution properly. The test tubes were taken & shaken at a time. The tissues were washed for two times to remove loosely bound arsenic externally. Another sets of test tubes were taken containing 2 ml of Tyrode solution along with kalmishak.

The pieces of liver after washing properly were transferred to the respective test tubes and again incubated for 45 minutes at 37°C in water bath with shaker. The tissues were again properly washed as before after second incubation. Then all tissues were homogenated individually by a hand tissue homogenizer. The homogenate was made up to 5 ml by adding Tyrode solution. All the time homogenizer was washed properly with deionized water. 20 µl of homogenate was separated from each test tube and kept in refrigerator with parafilm coverage for protein estimation. Rest of the homogenates were transferred to the previously marked conical flasks and digested through acid method.

Then the conical flasks were left for 5 minutes to become cool and all fumes to exhaust away. The exhaust fans were always working in the laboratory to eliminate all the fumes. There were 5 tripod gas burner by which conical flasks were boiled. There was a sliding glass made wall, which separated all gas and fumes from the laboratory workers. The conical flasks were left to become cool after boiling. If the content of any flask was not clear then 2 ml perchloric acid was added and again boiled and left to be cool. 50 µl potassium iodide (KI-10%) was added to each conical flask to make all pentavalent arsenic to trivalent arsenic as the

Atomic Absorption Spectrophotometer with Hydride Generator would show only trivalent arsenic. Then the clear digested solutions were diluted up to 10 ml with de-ionized water. From the diluted solutions, 1 ml was taken to another sets of test tubes as marked before. These diluted samples were run through Atomic Absorption Spectrophotometer with Hydride Generator.

Table 1: Experimental Design

Sample No	**Type of Sample**	
	1st Incubation*	**2nd Incubation***
I	None	None
III	Arsenic 2.5µg / ml	None
V	Arsenic 2.5µg / ml	+ compound- 1[1]
VII	Arsenic 2.5µg / ml	+compound- 2[2]

Both first and second incubation for 45 minutes at 37 ^{0}C; None means no arsenic was added; [1]Compound 1 = 20 µl/ml extract of kalmishak; [2]Compound 2 = 20 µl/ml extract of kalmishak

A set of freshly washed test tubes was taken. All test tubes contained 2ml tyrode solution and twenty small pieces of liver tissue maintaining 0^{0}C. No arsenic was added in test tube I. 2.5 µg/ml arsenic trioxide was added rest of the test tubes. They all were incubated for 45 minutes at 37^{0}C. Then the tissues were washed properly. In second incubation, different extracts of kalmishak (C 1and C 2) were added at 20 µl/ml dose. Second incubation was also for another 45 minutes at 37^{0}C. There were duplicates of all test tubes.

Table 2: Removal of Arsenic from Isolated Liver Tissues of Rat by Kalmishak (C-1 and C-2)

Incubation of liver tissues of rat with		n	**Amount Of arsenic µg / g of protein (mean ± se)**	**Percent removal of arsenic**	**P value**
1st Incubation	**2nd Incubation**				
None	-None-	6	6.62 ± 3.40	-	-
Arsenic 2.5µg/ml	-None-	6	98.32 ± 36.10	-	-
Arsenic 2.5µg/ml	+Kolmishak(C-1)[1]	6	69.57 ± 7.60	13	NS[3]
Arsenic 2.5µg/ml	+Kolmishak(C-2)[2]	6	43.11 ± 3.40	29	NS

Both 1st and 2nd incubation for 45 minutes at 37^{0}C; [1]Kalmishak (C 1) – 20 µl / ml; [2]Kalmishak (C 2) – 20 µl / ml; [3]NS = Not Significant.

Result

When the tissues were incubated with no arsenic in both 1st and 2nd incubation the amount of arsenic was found 6.62 ± 3.40 µg / g (mean ± s.e) of protein and it was considered as blank. The liver tissues of rat loaded with 2.5 µg / ml arsenic were incubated for 45 minutes at 37°C with no extract and the amount of arsenic was 98.32 ± 36.10 µg / g (mean ± s.e) of protein and the value was considered as standard.

The blank value was then deducted from the standard and the derived value was considered as control. After exposure with Compound -1 (20 µl /ml) in second incubation for 45 minutes at 37°C, the amount of arsenic was 69.57 ± 7.60 µg / g (mean ± s.e) of protein. That is there was 13.0% removal of arsenic. With the help of unpaired 't' test (student's 't' test) the calculated value was not statistically significant.

The same type of experiment was done with Compound-2 (20 µl / ml) and the amount of arsenic was 43.11 ± 3.40 µg / g (mean ± s.e) of protein. That is there was 29% removal of arsenic. After unpaired 't' test (student's 't' test) the calculated value was not statistically significant.

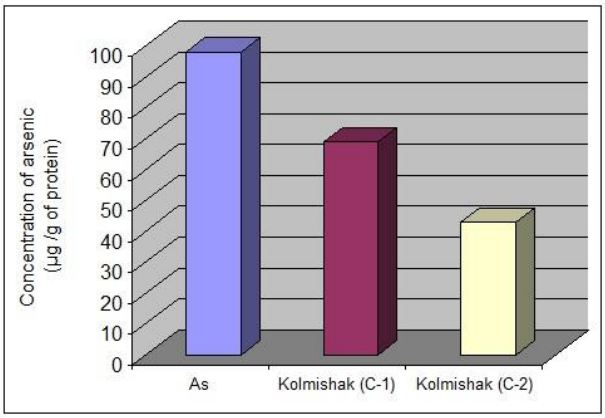

Figure I: Effects of kolmishak (C-1) and (C-2) on arsenic loaded (2.5µg/ml)

Discussion

Ipomoea Aquatica is relatively rich is S-methyl methionine and traditionally to treat gastric and intestinal disorders[11]. It is commonly used as food plant because the plant is rich in iron content. Furthermore it has been found to have insulin like properties acting as an anti-hyperglycaemic agent[12].

Previous phytochemical study revealed that the plant Ipomoea aquatica contains flavonoides and antioxidant activity and various compounds such as alkaloids myricetin, quercetin, kaempferol, luteolin and pigment[13]. The plant is also rich in protein, amino acid, oxalic acid and tetrasaccharide. The root and leaf of ipomoea aquatica contains phenol which is absent in stem. Owing to its high iron content it is often fed to anemic patient. It is beneficial for nervous and general debility in females. Juice is given to liver complaints as emetic, purgative[14]. Buds are used in treatment of ringworm and also useful in leprosy, leukoderma and fever[15]. Ipomoea aquatica is as effective as Tolbutamide in reducing blood glucose level of glucose challenged Wister rats[11].

The present study was carried out to investigate whether the extracts of kalmishak (water convovulus) could remove the accumulated arsenic from isolated liver tissues of rat. This work is very important in present perspective of Bangladesh when chronic arsenicosis has been reported as the largest environmental health hazard in the world and there is no specific treatment of the disease.

Active compounds of kalmishak (water convolvulus) has been studied in very low dose 20µl/ml in isolated liver tissues rat. The tissues has been incubated with 2.5 µg / ml arsenic trioxide at 37^0C for 45 minutes in water bath with shaker in first incubation.

Before the second incubation extracts of compound of kalmishak (water convolvulus) has been added to arsenic loaded tissues at 20 µl / ml dose for 45 minutes at 37^0C. The results revealed that the extract of garlic at 20 µl/ml reduced accumulated arsenic from isolated liver tissues of rat and values were highly significant. E_4 and E_7 compound of spirulina at 20µl/ml doses reduced accumulated arsenic and caused significant removal of arsenic from isolated liver tissues of rat.

Methylation is considered the detoxification pathway of inorganic arsenic and it occurs mainly in liver[16]. The source of methyl group for arsenic methylation is 5-adenosylmethionine, sulfur-containing amino acids like methionine, cysteine and protein deficiency are considered to decrease the co-factors necessary to synthesize 5-adenosylmethionine[10].

Population thriving on diets low in methionine is likely to suffer more from arsenic toxicity due to decreased methylation and increased accumulation of inorganic arsenic[9]. The mechanisms by which spirulina, hexane extracts of garlic or kalmishak (water convolvulus) caused removal of arsenic from liver tissues and prevented the reduction of glutathione is not known but several points may be pointed here spirulina may enhance the removal of arsenic from liver by increasing the methylation of

inorganic arsenic[11]. Arsenic is an important toxicant which has both natural and industrial sources. Arsenic predominantly exists in two oxidation states As (v) and As(III) and each species hypothesized to act through different mechanisms[12].

The most important mechanism for As(III) toxicity is postulated to be through binding to sulfhydryl-containing enzymes[7]. Reduced glutathione, a tripeptide thiol, (γ-glutamylcysteinylglycine), present in most cell, can chemically detoxify hydrogen peroxide.hydrogen peroxide continuously is formed as by product of aerobic metabolism and through reactions with drugs and environmental toxins. This hydrogen peroxide can cause serious chemical damage to DNA, proteins and unsaturated lipids[11].

Conclusion

In conclusion Kalmishak has the ability to remove of arsenic from isolated liver tissues of rat. After exposure with Kalmishak in second incubation, there is a removal of arsenic. However, the calculated value is not statistically significant. Therefore the amount of arsenic has been removed from the liver tissues of rat which is not statistically significant. Further study should be carried out in multicenter.

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