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Ability of Extracts of Spirulina for the Removal of Arsenic from Isolated Liver Tissues of Experimental Rat

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Abstract

Background: Spirulina has several effects in the metabolism of the body. **Objective:** The purpose of the present study was to see the ability of extracts of spirulina 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. A set of freshly washed test tubes was taken. All test tubes contained 2ml tyrode solution and twenty small pieces of liver tissue maintaining 00C. 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 370C. Then the tissues were washed properly. The extracts of spirulina was added at 20 µl/ml dose. Second incubation was also for another 45 minutes at 370C. There were duplicates of all test tubes. **Result:** The effects of different extracts of spirulina (E1, E2, E4 and E7) on the removal of arsenic from arsenic loaded tissue were recorded. Amount of accumulated arsenic (mean ± se) in blank was 6.04 ± 3.05 µg/g of protein. After administration of 2.5 µg/ml arsenic trioxide in both incubation, the amount of accumulated arsenic was 245.02 ± 22.37 µg/g of protein. Blank was deducted from the standard and the value was considered as control and it was 238.96 ± 19.32 µg / g of protein. The arsenic loaded tissues were incubated with different extracts (E1, E2, E4 and E7) of spirulina in second incubation for another 45 minutes at 370C and each extract was for 20 µl / ml. After E1 extract of spirulina in second incubation, amount of accumulated arsenic was 136.40 ± 14.23 µg / g. There was 14.81% of removal of arsenic. Second incubation with E2 extract of spirulina (20 µl/ml) on arsenic loaded (2.5 µg/ml) tissue showed the amount of arsenic 242.56 ± 16.50 µg/g of protein (mean ± se). There was 12.59% of removal of arsenic. **Conclusion:** In conclusion extracts of spirulina has a significant ability to remove arsenic from isolated liver tissues of experimental rat. *[Journal of National Institute of Neurosciences Bangladesh, July 2020;6(2): 105-109]*

Keywords: Ability; Extracts of Spirulina; Removal of Arsenic; Isolated Liver Tissues; Experimental Rat

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Introduction

Arsenic exposure leads to high prevalence of cardiovascular disturbance[1]. The most common peripheral vascular disease like Raynauds syndrome, polyneuropathy, peripheral nervous disturbance, black foot disease, carcinogenicity of many internal organs like

genitourinary, respiratory, skin, hepatic, hemopoeitic system can occur due to chronic arsenic exposure[2]. A history of arsenic exposure through exhalation or ingestion is helpful in diagnosis of arsenicosis since skin manifestations cannot be differentiated from normal dark complexioned farmer in tropic who work in field bare bodied under direct sunlight[3].

There are required six things to manage the cases of arsenicosis such as men with knowledge, money, material and method, laboratory, management and continuous medical education[4]. Bangladesh has a poor socioeconomic structure. Rural people are largely has to use ground water for their everyday household use. Thus, especially females are affected more than male due to their poor nutritional status of health and they are in more contact with ground water[5]. Contamination of potable water (well water) with arsenic is a serious problem in Bangladesh. Arsenic contamination in shallow tubewell in Ganges Delta area including Bangladesh has been reported in recent decade[6]. Arsenic contamination in Bangladesh is the severest in the world and it has been estimated that about 35 million people in Bangladesh are exposed to high level of arsenic contamination. There are about 11 million tube-wells in Bangladesh out of which 5 million tube-wells are highly arsenic contaminated[7]. About 75 million people of the affected districts are at risk and total number of patients suffering from arsenicosis are more than 40,000 and out of which about 200 persons already died[7].

Spirulina a blue green algae, belongs to the Oscillatoriaceae family, is Cyanobacteria characterized by spiral shaped chains of cells enclosed in a thin sheath. Spirulina has a very long history of being serving as a source of food for human being. Now a day's spirulina is consumed by millions of people all over the world and they are discovering lots of health benefits apart from its nutritive value[8]. Management protocol for arsenic cases followed the most important step in management of arsenicosis was to stop intake of arsenic contaminated drinking water. Other measures include dietary supplementation, application of keratolylic agent, follow-up and counseling, cryosurgery, symptomatic treatment, antioxidants and appropriate nutrient supplementation[9].

In context of Bangladesh, the affected people neither get effective treatment nor even proper diagnosis[7]. As a result my study was performed to get safe, inexpensive, easily available and effective management which can remove arsenic from accumulated tissues without any hazards. This present study was undertaken to see the ability of extracts of spirulina 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 - 4ºC. 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 different extracts of spirulina. The pieces of liver after washing properly were transferred to the respective test tubes and again incubated for 45 minutes at 37ºC 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. 20µl 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. Both 1st Incubation and 2nd incubation were for 45 minutes at 37°C. None means no arsenic was added. A set of freshly washed test tubes was taken. All test tubes contained 2 ml Tyrode solution and twenty small pieces of liver tissue maintaining 0°C. No arsenic was added in test tube I. 2.5 µl/ml arsenic trioxide was added rest of the test tubes. They all were incubated for 45 minutes of 37°C. Then the tissues were washed properly. In second incubation, different extracts of spirulina (E_1, E_2, E_4 and E_7) were added at 20 µl/ml dose. Second incubation was also for another 45 minutes at 37°C. There were duplicates of all test tubes.

Table 1: Experimental Design

Sample No	Incubation of liver tissues of rat with	
	During 1st Incubation*	During 2nd Incubation*
I	None	None
II	Arsenic 2.5 µg/ml	None
III	Arsenic 2.5 µg/ml	Extract1 of spirulina 20 µl/ml
IV	Arsenic 2.5 µg/ml	Extract2 of spirulina 20 µl/ml
V	Arsenic 2.5 µg/ml	Extract4 of spirulina 20 µl/ml
VI	Arsenic 2.5 µg/ml	Extract7 of spirulina 20 µl/ml

Both 1st Incubation and 2nd incubation were for 45 minutes at 37°C; None means no arsenic was added; 1Hexane extract of garlic = 20 µl / ml; 2Methanol extract of garlic = 20 µl / ml; 3Hexane+ Methanol extract of garlic =(10 µl +10 µl) /ml.

Results

The effects of different extracts of spirulina (E_1, E_2, E_4 and E_7) on the removal of arsenic from arsenic loaded tissue were recorded. Amount of accumulated arsenic (mean ± se) in blank was 6.04 ± 3.05 µg/g of protein. After administration of 2.5 µg/ml arsenic trioxide in both incubation, the amount of accumulated arsenic was 245.02 ± 22.37 µg/g of protein. Blank was deducted from the standard and the value was considered as control and it was 238.96 ± 19.32 µg / g of protein. The arsenic loaded tissues were incubated with different extracts (E_1, E_2, E_4 and E_7) of spirulina in second incubation for another 45 minutes at 37°C and each extract was for 20 µl/ml. After E_1 extract of spirulina in second incubation, amount of accumulated arsenic was 136.40 ± 14.23 µg / g. There was 14.81% of removal of arsenic and after calculation through unpaired 't' test (control was 238.96 ± 19.32) calculated value was not statistically significant.

Table 2: Removal of arsenic by different extracts of spirulina from isolated liver tissues of rat

Incubation of liver tissues of rat with		n	Amount of arsenic µg/g of protein (mean ± se)	% removal of arsenic	P value
1st Incubation (45 minutes at 37°C)	2nd Incubation (45 minutes at 37°C)			-	-
None	None	6	6.04 ± 3.05	-	-
Arsenic 2.5 µg / ml	None	6	245.02 ± 22.37	-	-
Arsenic 2.5 µg / ml	spirulina (E_1)	6	136.40 ± 14.23	14.81	NS1
Arsenic 2.5 µg / ml	spirulina (E_2)	6	242.56 ± 16.50	12.59	NS
Arsenic 2.5 µg / ml	spirulina (E_4)	6	87.26 ± 8.19	66.35	< 0.052
Arsenic 2.5 µg / ml	spirulina (E_7)	6	48.91 ± 10.87	82.10	<0.013

Both 1st and 2nd incubation were 45 minutes at 370C; 1Garlic (Hexane extract) – 20 µl / ml; 2Garlic (Methanol extract) – 20 µl / ml; 3Garlic (Hexane extract 10 µl / ml + Methanol extract– 10 µl / ml); 4<0.001 means highly significant; 5NS = Not Significant

Second incubation with E2 extract of spirulina (20 µl/ml) on arsenic loaded (2.5 µg/ml) tissue showed the amount of arsenic 242.56 ± 16.50 µg/g of protein (mean ± se). There was 12.59% of removal of arsenic. After unpaired 't' test the calculated value was not statistically significant. Similarly E_4 and E_7 extracts of spirulina (each 20 µl/ml) were added to arsenic loaded tissue in second incubation (66.35% and 82.10% removal of arsenic).The amount of arsenic after E_4 and E_7 extracts were 87.26 ± 8.19 and 48.91 ± 10.87 µg / g of protein. Using unpaired 't' test (control value was 238.96 ± 19.32 µg / g of protein) the calculated value was statistically significant.

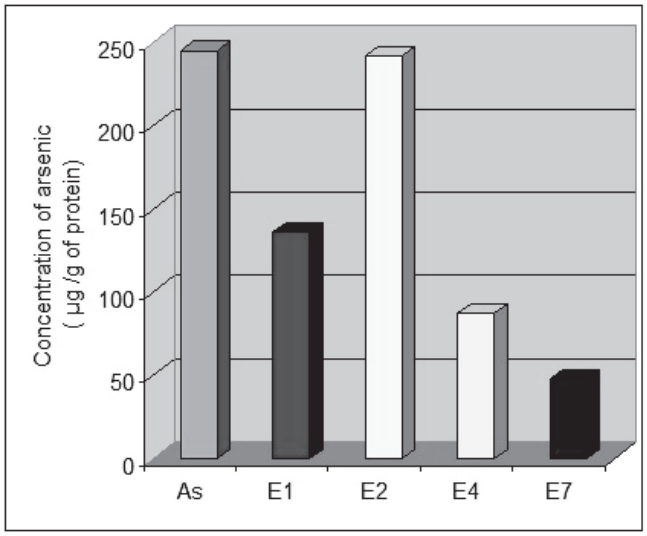

Figure I: Effects of Different Extracts of Spirulina

Discussion

High levels of arsenic in well water are causing widespread poisoning in Bangladesh[4]. In a typical aquifer in southern Bangladesh, chemical data imply that arsenic mobilization is associated recent inflow of carbon. High concentration of radio carbon, young methane indicate that young carbon has driven biochemical process and irrigation pump is sufficient to have drown water to depth where dissolved arsenic is at a maximum[10].

Spirulina is the richest food source of complete protein with more than 60% protein[11]. It is the richest food source of, beta-carotene, which is higher than any other known sources of beta-carotene. Spirulina is the richest food source of vitamin B_{12}, which is more than any other known sources of vitamin B_{12}. It is also richest food source of gamma linolenic acid (GLA), which is precursor to prostaglandin[12]. Spirulina is the richest source of chlorophyll. Spirulina contains a spectrum of natural antioxidants like beta-carotene, vitamin E, vitamin B_1, B_5, & B_6, zinc, manganese, copper and selenium & amino acid methionine[13].

Since the beginning of life on this earth, man is continuously searching for natural resources of foods. Today when man is competing for space with animals and plants; there is need for some alternative sources of food which is 100% natural and could able to grow on arid land and utilize minimum space to produce more high quality food.

Scientist have found Single Cell Protein (SCP) as better alternative to some food sources such as poultry, fishes[6]. SCP is derived from microbial sources like yeasts, bacteria or algae. These SCP sources are extensively researched by scientists. Among these SCP sources, blue green algae have found top place with respect to its cultivation, harvesting and nutritive values[13]. Although spirulina has very old history as food source, only recently it has gained interest to produce for commercial purpose.

This is an animal in vitro study. The present study has been carried out to investigate whether extracts of spirulina (E_1, E_2, E_4, and E_7) 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.

Previous study showed that spirulina was effective in treatment of chronic arsenic poisoning with cutaneous manifestation[14]. Subsequent study in the same laboratory showed that spirulina inhibits the accumulation of arsenic in different tissues of rats following chronic exposure of high concentration of arsenic. In other studies[9,12,] crude spirulina powder was used at a dose of 10g per day orally in three divided doses for four months. In view of patients compliance it was quite large enough. In order to better patients' compliance the dose of spirulina must be reduced and indigenous agents must be selected.

Active compounds of spirulina has been studied in very low dose 20ml/ml in isolated liver tissues rat. The tissues has been incubated with 2.5 mg/ml arsenic trioxide at 37°C for 45 minutes in water bath with shaker in first incubation.

Before the second incubation extracts of spirulina has been added to arsenic loaded tissues at 20 ml/ml dose for 45 minutes at 37°C. The results revealed that E4 and E7 compound of spirulina at 20ml/ml doses reduced accumulated arsenic and caused significant removal of arsenic from isolated liver tissues of rat.

And same type of repetition was performed with extracts of spirulina (E_4 and E_7) compound. The above extracts reduced accumulation of arsenic from liver tissues of rat. So it can be assumed from this observation the effective dose of E_4 and E_7 compound of spirulina is 20ml/ml.

It is quoted in one of the main massages of The World Report 1997 that increased longevity without quality of life is an empty prize that health expectancy is more important than life expectancy[11]. People have understood that it is not just to live longer but now it is more important to stay longer in good health. To maintain good health is no more remained an easy task. In today's world with growing population, competition for comfortable life and today's changed life style people are giving minimum attention on their diet which in fact is more important to keep healthy[14]. Further this problem is more complicated due to the depleted value of food materials and overuse of processed ready to use food. In today's world there is no guarantee of purity of water we drink, food we eat and air we breathe[11].

Spirulina provides almost everything what exactly we expect from food[14]. It is complete food with more than 60% protein higher than any other known sources of protein, almost all vitamins especially rich in vitamin B_{12} which vegetarian lacking in their diet and beta-carotene (Pro vitamin A), minerals in natural form and essential fatty acids like gamma linolenic acid (GLA) which is precursor to prostaglandin[10]. Spirulina is 100% natural and grown in artificial ponds; utilize sunlight and inorganic salts as their nutrients to produce high quality food[11]. Spirulina powder appears green in color due to the presence of chlorophyll and is vegetarian source of food.

Conclusion

In conclusion extracts of spirulina has a significant ability to remove arsenic from isolated liver tissues of experimental rat. The extract of spirulina cannot remove arsenic in a large amount in second incubation, though there is few amount of accumulated arsenic is reduced. 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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