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Changes in the amount of Glutathione in Arsenic Loaded Tissue reflecting the effectiveness of arsenic removal from Isolated Liver Tissues in Experimental Rat

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Abstract

Background: Removal of glutathione is essential during metabolism of the body. **Objective:** The purpose of the present study was to see the ability of spirulina, Allium sativum (Garlic), Ipomoea aquatic (Water Spinach) for the prevention of depletion of intracellular glutathione in arsenic loaded isolated liver tissues of experimental 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. **Results:** The concentration of glutathione was 434.02 µg/g of protein. With 2.5 µg/ml arsenic trioxide, the amount of gluthathione was depleted to 344.33 µg/g of protein. There was 79.26% of glutathione in that tissues comparing with control. The amount of glutathione was 20.74% less than control. About 2.5 µg/ml arsenic trioxide added tissues were subsequently incubated with SI extract of spirulina and the amount of glutathione was 351.66 µg/g of protein. There was 80.87% glutathione when compared with control and there was 19.13% less than control. There was 4.84% less of amount of glutathione. Both G1 and G2 (hexane and methanol) extract of garlic added tissues showed amount of glutathione 346.85 peg of protein. After comparing with control there was 82.25% glutathione. So there was 17.75% depletion of glutathione. **Conclusion:** In conclusion ability of extract of spirulina, Allium sativum (Garlic), Ipomoea aquatic (Water Spinach) have the role in the prevention of depletion of intracellular glutathione in arsenic loaded isolated liver tissues of experimental rat. *[Journal of National Institute of Neurosciences Bangladesh, January 2022;8(1):46-51]*

Keywords: Ipomoea aquatic; Allium sativum; water Spinach; arsenic; Glutathione; liver tissues; experimental rat

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Introduction

High levels of arsenic in well water are causing widespread poisoning in Bangladesh[1]. In a typical aquifer in southern Bangladesh, chemical data imply that arsenic mobilization is associated with inflow of carbon. High concentration of radio carbon, young methane indicate

that young carbon has driven recent biochemical process and irrigation pump is sufficient to have drawn water to depth where dissolved arsenic is at a maximum[2]. Low dose arsenic trioxide can induce complete remission in patient with acute promyelocytic leukemia who have relapsed[3]. However, at very high level of arsenic in drinking water, symptoms may occur long before appearance of skin lesions. The most common early symptoms are gastrointestinal, diarrhoea and abdominal pain; furthermore, such non-specific sign-symptoms in a patient living in an arsenic region should alert physician or nurse to investigate the source of drinking water[4].

About 60.0% of patients continue to pass arsenic in urine even after stop drinking arsenic contaminated water[5]. Ingestion of seafood may result in total urinary arsenic level of more than 10 µmoll while persons without such exposure to arsenic usually have urinary level in range 0.1 to 0.7 µmoll[6]. Measurement of inorganic arsenic in urine should therefore give a better estimate of exposure to inorganic arsenic than total urinary arsenic[7]. In Bangladesh a paper has been presented on `management on arsenicosis and it has been identified that six things are required to manage the cases of arsenicosis such as men with knowledge, money, material and method, laboratory, management and continuous medical education[8].

In the context of Bangladesh, the affected people neither get effective treatment nor even proper diagnosis[9]. Moreover, they also become socially deprived due to their illness and lack of knowledge about arsenicosis. It is as such very important on the part of the patients that they are diagnosed properly and treated with due care. The awareness of the mass people about arsenicosis is also very important for prevention of any further exposure[5]. In an effort to find out scientific basis for a more cost-effective approach to management of arsenicosis, the present study was undertaken to see the ability of spirulina, *Allium sativum* (Garlic), *Ipomoea aquatic* (Water Spinach) for the removal of glutathione in arsenic loaded isolated liver tissues of experimental rat.

Methodology

The experimental animal study was conducted in the Department of Pharmacology at Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh from January 2004 to December 2005 for a period of two years. This experiment was carried out on isolated liver tissues of Long Evans Norwegian adult healthy male rats. The rats were 3 to 6 months of age weighing 160 to 200 gram and was obtained from animal house of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. These were housed in standard plastic cages with a light or dark cycle of 12 hours at room temperature in a well-ventilated room. Extracts of spirulina, Allium sativum (Garlic), Ipomoea aquatic (Water Spinach) were supplied from the Department of Pharmacology at Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.

Extracts of Spirulina: Extracts of spirulina were supplied by Mr. Nazrul Islam of the Department of Pharmacology, BSMMU. These extracts were prepared and separated by Column chromatography. As a result, S1, S2, S4 and S7 extracts were studied on arsenic loaded liver tissues of rat. S3, S5 and S6 extracts could not be studied due to their small amount.

Extracts of Garlic: Extracts of garlic were supplied from the Department of Pharmacology, BSMMU. Hexane and methanol (G1 and G2) extracts of garlic were studied.

Extracts of Water Spinach: Extracts of water spinach were prepared and supplied from the Department of Pharmacology, BSMMU. They were named as W1 and W2 and were studied on isolated liver tissues of rat.

Preparation of Stock Solution: In order to prepare stock solution of arsenic trioxide (As203) -2 mg/ml, 132 mg of As203 was taken in a 50 ml volumetric flask. Then the ingredients of Tyrode solution were added and 5N NaOH was added up to the 50 ml mark. Finally, the stock solution was preserved in a refrigerator and labeled as stock solution (2 mg/ml).

Sacrifice of Animal: The rats (fasting overnight) were sacrificed under chloroform anesthesia and then decapitation was done.

Collection of Liver: By giving a midline incision, the abdomen was opened and the liver was taken out and it was immediately immerged into Tyrode solution. The beaker containing Tyrode solution was always kept in ice bath and the temperature of Tyrode solution was maintained at 0 - 4°C.

Determination of Glutathione Concentration

Principle: This spectrophotometer method based on the method of Ellman (1959), who reported that the disulfide chromogen, 5.5 dithiol 2 nitrobenzoic acid (DTNB) was readily reduced by -SH groups and produced one mole of 2-nitro -5- mercapto benzoic acid anion had an intense yellow colour and can be used to measure -SH. groups. Ultraviolet Spectrophotometer measured the absorbances of the reduced chromogen at 412 nm and it was directly

proportional to the GSH concentration. Amount of glutathione was expressed as µg/g of protein.

Procedure for Measurement of Glutathione Level: 1 ml from each homogenates was properly preserved. Each 1 ml homogenate was added to 5 ml of 5% TCA solution. The solution was shaken properly and then centrifuged for 5 minutes at the rate of 4,000 rotations per minute. Clear solution was separated to another test tube (Supernatant). Supernatant (250 µl) was added to the mixture of DTNB and Na2HPO4 and the absorbance were measured at 412 nm after 4 minutes of addition. After subtracting the initial absorbance from the final absorbance, the calculation was done.

Analytical Method Applied in Testing Significance of Data: For calculation amount of arsenic and glutathione were converted into pg/g of protein. Homogenates were made upto 5 ml. From the homogenates 1 ml for glutathione and 20 µl were separated for protein estimation. Proteins and glutathiones were measured by Ultraviolet Spectrophotometer. Rests of the homogenates were digested through acid method. From the digest arsenic was estimated by Atomic Absorption Spectrophotometer with Hydride Generator. By unpaired `t' test (or student `t' test) the values were verified whether they were statistically significant or not.P values were significant when $P<0.05$ and very significant <0.01.

Results

The isolated liver tissues of rat could not be weighed accurately because always they were immerged into Tyrode solution. The wet tissues could yield wrong measurement. For accuracy, all samples' protein content was calculated from absorbance value of Ultraviolet Spectrophotometer. Then all values were multiplied by 5 as initially the homogenates were 5 ml. From the raw data, Sample I (test tube 1 and 2) was considered as blank (none) because there were only tissues and no arsenic was added. Sample II (test tube 3 and 4) was considered as standard. Tissues were incubated with 2.5 µg/ml arsenic in first incubation and second time with nothing. Standard minus blank (none) value was taken as control. Rests of the test tubes were named as samples. All samples were incubated with 2.5 µg/ml arsenic trioxide in first incubation. Different extracts were added to them before second incubation. Blank (none) value was deducted from all sample values. Each experiment took at least three days and after calculation one set of raw datum was collected.

Amount of Glutathione in Different Samples: The amounts of glutathione concentrations in different samples were recorded. None was considered as control. No arsenic was added in both incubations and there were only tissues. The concentration of glutathione was 434.02 µg/g of protein and that value was converted into percentage. With 2.5 µg/ml arsenic trioxide, the amount of gluthathione was depleted to 344.33 µg/g of protein. There was 79.26% of glutathione in that tissues comparing with control. The amount of glutathione was 20.74% less than control. About 2.5 µg/ml arsenic trioxide added tissues were subsequently incubated with SI extract of spirulina and the amount of glutathione was 351.66 µg/g of protein. There was 80.87% glutathione when compared with control and there was 19.13% less than control. S2 extract of spirulina gave the result 385.78 µg/g of protein and it was 88.70% of glutathione comparing with control. There was 11.30% depletion of glutathione. S4 extract of spirulina was added before second incubation to arsenic loaded tissues and the amount of glutathione was 401.03 µg/g of protein. In comparison to control there was 92.39 % of glutathione. So there was 7.61 less than control. S7 extract of spirulina was added to arsenic loaded tissues. The concentration of glutathione was 413.40 µg/g of protein. The amount of glutathione was 95.16 % comparing with control. So there was 4.84 % less of amount of glutathione. Gl extract of garlic was added to arsenic loaded tissues (hexane extract of garlic) and the amount of glutathione was 421.07 µg/g of protein. Comparing with control there was 97 % of glutathione. So there was only 3 less of glutathione. G2 extract (methanol) of garlic was added to arsenic loaded tissues and the amount of glutathione was 346.85 µg/g of protein. There was 79.72 % of glutathione and there was 20.28 % less than control. Both G1 and G2 (hexane and methanol) extract of garlic added tissues showed amount of glutathione 346.85 peg of protein. After comparing with control there was 82.25 % glutathione. So there was 17.75 % depletion of glutathione. W1 extract of water spinach was added to arsenic incubated tissues in second incubation. Similarly, W2 extract of water spinach added and the amounts of glutathione were 359.46 and 364.23 µg/g of protein. There were 82.71 and 83.87 % of glutathione so 17.29 and 16.13 % of depletion in amount of glutathione. Control (none) = In 2 ml Tyrode solution only tissues. The amount of glutathione was 434. 02 µg/g of protein in control and the value was converted into 100%. Standard (arsenic 2.5 µg/ml) and sample values were converted into percentage and compared

Table 1: Amount of glutathione in different sample

Sample		Amount of glutathione (µl/g of protein)	Decrease of glutathione from control (µg/g of protein)	Amount of glutathione (in percentage)	Depletion of Glutathione from control (in percentage)
None (Control)		434.02		100	
Standard	6	344.33	89.69	79.26	20.74
Arsenic 2.5 µg/ml + Spirulina Sl		351.66	82.36 48.24	80.87	19.13
Arsenic 2.5 µg/ml + Spirulina S2		385.78	32.99	88.70	11.30
Arsenic 2.5 µg/ml + Spirulina S4		401.03	20.62	92.39	7.61
Arsenic 2.5 µg/ml + Spirulina S7		413.40	12.95	95.16	4.84
Arsenic 2.5 µg/ml + G1 (extract of garlic)		421.07	87.17	97	3.00
Arsenic 2.5 µg/ml + G2 (extract of garlic)		346.85	76.79	79.72	20.28
Arsenic 2.5 µg/ml + G1 + G2 (extracts of garlic)		357.23	74.56	82.25	17.75
Arsenic 2.5 µg/ml + Wl extract of W.s.		359.46	69.79	82,71	17.29
Arsenic 2.5 µg/ml + W2 extract of W.s.		364.23		83.87	16.13

with control value (Table 1).

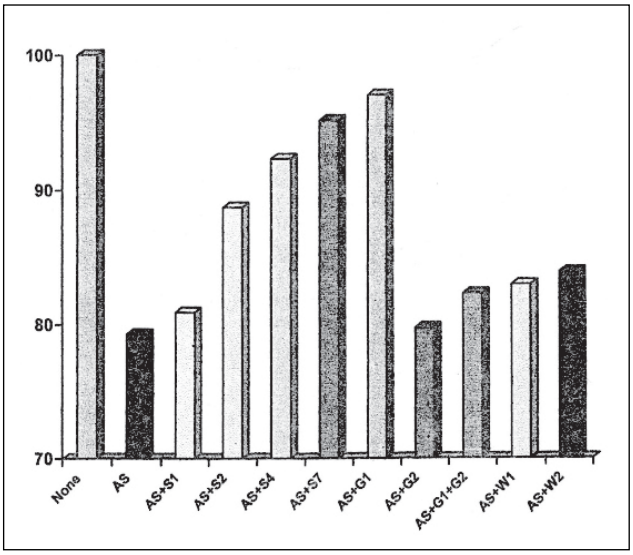

Figure I: Amount of glutathione in different sample (None = No Arsenic was added; As = Arsenic trioxide 2.5 µg/ml; S 1= Extract of spirulina (20 µl/m1); S2 = Extract of spirulina (20µl/m1); S4 = Extract of spirulina (20 µl/ml); S7 = Extract of spirulina (20 µl/m1); W1 =Extract -I of water spinach; W2 = Extract- 2 of water spinach; G1= Hexane extract of garlic (20 µl/m1); G2 = Methanol extract of garlic (20 µl/m1)

Discussion

The present study was carried out to experiment whether the hexane and methanol extracts of garlic, extracts of spirulina or extracts of water spinach could be prevented depletion of intracellular glutathione. This work is very important in present perspective of Bangladesh when arsenicosis has been reported as the largest environmental health hazard in the world and there is no specific treatment. Before the second incubation extracts of spirulina, garlic and of water spinach were added to arsenic loaded tissues at 20 µ1/ml doses for 45 minutes at 37°C except none (blank) and another two test tubes (standard). None (blank) value was deducted from all sample and standard values. Standard minus none value was considered as control. Sample values were compared with control value. All values were expressed as µg/g of protein.

It is thought that the cytotoxic action of arsenic is mediated through the generation of free radicals induced by the element[10]. The efficiency of antioxidant system is also important for detoxification of free radicals. It was suggested that arsenic could suppress the activities of antioxidants in the liver of rats[11]. Spirulina contains antioxidants (3-carotene, Vitamin-E), antioxidant element like selenium and

antioxidant enzymes SOD (super oxide dismutase). Selenium, an essential element is needed in trace amount for the biosynthesis can antagonize arsenic from tissues[12]. However, provision of arsenic free drinking waters must be ensured otherwise antagonism may be reversed into synergistic toxicity.

Garlic (Allicin, diallyl thio sulfmate) is moderately soluble in hexane and non-polar7. The curative action of garlic has been shown for a long time. It was previously shown the chemoprotective role of diallyl disulfide (DADS), a naturally occurring anticancer agent in garlic. They also increase the amount of glutathione in the liver and for stomach tissue of mice treated with diallyl monosulfide to diallyl disulfide. It was found that the sulfur compound found in garlic reacts with cysteine, which involves the thiol disulphide exchange and oxidation of garlic sulfur compounds, and cysteine of the animal tissue thereby brings about some changes in quantities of glycogen, lipid and protein etc. It is suggested that synthesis of protein is increased by garlic[13].

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[14]. A number of intracellular reducing agents, such as ascorbate, vitamin- E and beta-carotene are able to reduce and thus detoxify oxygen intermediates in cells. Consumption of foods rich in these antioxidant compounds has been correlated with a reduced risk of certain types of cancer as well as decreased frequency of other chronic health problems[7].

Reduced glutathione, (y-glutamyl-cysteinyl-glycine), present in most cells, 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[15]. Reduced glutathione, a thiol containing tripeptide, plays a key role in cell protection against radiation, reactive oxygen species and other toxic compounds. There is abundant evidence that GSH depletion is involved in the initiation and progression of a wide variety of cancer. It has been proposed that GSH could be of therapeutic value in prevention and treatment of cancer. GSH exhibits anti-tumoral activity in vitro as well as in vivo models. There are abundant data demonstrating that glutathione-derivatives are effective apoptosis inducer in human leukemia cells[16].

In the present study, S_4 and S_7 extracts of spirulina and hexane extracts of garlic tried to recover the depletion of glutathione from arsenic loaded tissues, that can be important to detoxify arsenic trioxide[17]. Methylation is considered the detoxification pathway of inorganic arsenic and it occurs mainly in liver. The source of methyl group for arsenic methylation is S-adenosyl methionine, sulfur-containing amino acids like methionine, cysteine and protein deficiency are considered to decrease the cofactors necessary to synthesize S-adenosylmethionine[7]. 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. The mechanisms by which spirulina, hexane extracts of garlic or water spinach caused removal of arsenic from liver tissues and recovered depletion 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.

Glutathione may enhance elimination of arsenic by producing metabolites which are mainly excreted by kidneys[11]. It was found that garlic increased the amount of glutathione in the liver of mice. Among other functions, glutathione participates in reductive processes that are essential for the synthesis and degradation of proteins and in the protection of cells against reactive oxygen compounds and free radicals. It can also act as a coenzyme for several enzymatic reactions and transport form of cysteine[9]. A decrease in the glutathione level of hepatocyte rat primary culture has been studied. The recovery of the normal levels of this thiol and its stabilization can be obtained by addition of methionine. The link between methionine metabolism and glutathione synthesis is established through cysteine. The amino acid can be obtained from the diet or it can be synthesized from methionine through the transsulfuration pathway in the liver.

Conclusion

In conclusion arsenic is metabolized by living system using oxidation, reduction and methylation reactions. Reduced glutathione has been shown to be important in that metabolism. Though here specific studies will be needed to find out the mechanisms of action, the present study indicates that hexane extract of garlic and extracts of spirulina (S_4 and SO) may be of value in the removal of arsenic.

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