In vivo Study of the Ameliorative Effect of beta-carotene on the Liver Toxicity of Arsenite in Swiss Albino Mice

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ABSTRACT

In recent times Arsenic toxicity in the groundwater of southern West Bengal (India), and southern and eastern parts of Bangladesh, has become an alarming environmental problem. Over 3 to 4 million people in six districts in southern West Bengal, located to the east of the Hooghly River, are found to be affected by Arsenic poisoning resulting in skin and various internal organ cancers. This study aims at finding a novel and indigenous way of checking the menace of arsenic toxicity. Carotenoids are the principal pigments responsible for the red, orange, yellow and green colors of vegetables and fruits. Beta-carotene is responsible for the colour of carrots. Beta-carotene is a principal dietary carotenoid with antioxidant activity. It has been demonstrated to quench singlet oxygen, scavenge peroxyl radicals and inhibit lipid peroxidation. In the present study, Swiss albino mice (Mus musculus) were administered Sodium Arsenite injections (i.p.) in sub-lethal doses over different exposure periods. The group with the highest toxic response was given oral supplementations of beta-carotene. Arsenic-induced hepatotoxicity and its protective action by beta-carotene was studied by various cytogenetic and biochemical parameters. This study shows a new direction in the search for a safe and preventive measure against arsenic toxicity.

Keywords: Sodium Arsenite, Beta-carotene, Hepatotoxicity, Swiss albino mice (Mus musculus)

1. INTRODUCTION

Arsenic toxicity is still growing at an alarming rate not only in India but also all over the world. It is considered as one of the worst mass poisoning cases in the history of environmental pollution. It is expanding in a manner similar to that of global food trade exposing millions and millions of people to a lethal risk [1]. With the passage of time arsenic has changed its place from the medicine cabinet (as used to treat leprosy in the fifth century AD) to ground water imperil [2]. Arsenic

pollution via groundwater is a tremor in the whole of Asia, especially: Bangladesh (most districts including Chandpur, Noakhali, etc), India (Nadia district of West Bengal, Bihar), China (Inner Mongolia, Xinjiang, Shanxi Province), Nepal (Terai Region) [3]. Recent advancement in research has yielded new reports on traces of arsenic being found in South-east Asia, Mexico and Slovakia.

The metalloid arsenic has been proved to be one of the most hepatotoxic and carcinogenic of all the natural ground water contaminants. (IARC Class I Carcinogen). The exposure to arsenic contaminated drinking water of more than 50µg/l is corroborated as a significant cancer risk especially in India and Bangladesh where the problem is growing by leaps and bounds daily [4,5].

Table 1. Details of Occurrence of High Arsenic in the Ground waters of India [6]

NA=not available

State	Districts having As > 50 µg/L	Number of people affected
Assam	Dhemaji	5,71,994
Bihar	Begusarai, Bhagalpur, Bhojpur, Buxar, Darbhanga, Katihar, Khagaria, Kishanganj, Lakhisarai, Munger, Patna, Purnea, Samastipur, Saran, and Vaishali	1,04,71,869
Chattisgarah	Rajnandgaon	NA
Jharkhand	Sahibganj	NA
Manipur	-	NA
Uttar Pradesh	Agra, Aligarh, Balia, Balrampur, Gonda, Gorakhpur, Lakhimpur Kheri Mathura, and Muradabad	60,00,000
West Bengal	Bardhaman, Hooghly, Howrah, Malda, Murshidabad, Nadia, North 24 Pragannas, South 24 Pragannas, and South Calcutta	2,60,00,000

Chronic exposure to arsenic has been known to be the underlying cause of skin cancer and also has a carcinogenic effect on diverse organs that include lung, bladder, liver, prostrate glands and kidney in both humans and animals [7-9]. In the present study, these adverse effects of trivalent Arsenic were treated for the amelioration effect with the most potential carotenoid, the most abundant form of provitamin A: Beta carotene. Epidemiological studies substantiate the fact that people who were treated with high dietary or high blood levels of this nutrient have shown minimized risk of various diseases like cancer, heart diseases and hepatotoxic ailments [10].

Carcinogens are especially known to inhibit gap junction communications which can be normalized using the antioxidant property of the chemical effects of Beta carotene. This terpenoid acts as an herbal remedy to quench a single molecule of oxygen to inhibit peroxyl free-radical reactions. Henceforth, it's well established that, the chemical abilities of beta-carotene allow it to be one of the hottest topics of research in the field of medical ailment especially, where clinical trials are concerned [11-15].

Beta-Carotene, an important pro-vitamin A, is considered an efficient antioxidant acting as a quencher of singlet oxygen and free radicals. Experiments in animals have also suggested that beta-carotene is able to reduce DNA and chromosomal damage induced by alkylating agents such as ethylnitrosourea and methylmethanesulfonate, and also by pro-carcinogens as benzo[α]pyrene and cyclophosphamide [16]. Beta-carotene has been extensively used in cancer chemopreventive studies, in which it displayed suppressing activity against oral and colon tumors. This study aims at using beta-carotene as a possible protective agent caused by arsenic-induced hepatotoxicity.

2. MATERIALS AND METHODS

Arsenic and Beta-carotene treatment groups. Healthy female Swiss Albino mice (*Mus musculus* Linn.), about 3 to 4 months old, and weighing between 20 and 25 gm were procured from M/s Scientific Concern, Kolkata, India, and reared in animal cages. Four mice were used for each treatment group. Two groups were intraperitoneally injected with two doses of Sodium Arsenite (Merck, USA) @ 300 µl/kg body wt. and 600 µl/kg body wt.

The dose with higher toxicity (300 µl/kg body wt.) was co-treated with two doses of oral supplementations of beta-carotene @ 90 µg/kg body wt. and 180 µg/kg body wt. All the mice were sacrificed after 7 days of exposure.

Control groups. The vehicle control group of mice was injected (i.p.) with distilled water @ 1ml/100gm body weight. This group served as the negative control as arsenic was dissolved in distilled water.

Another group of mice were fed only with beta-carotene @ 90 µg/kg body wt. to check for any toxicity caused by beta-carotene itself.

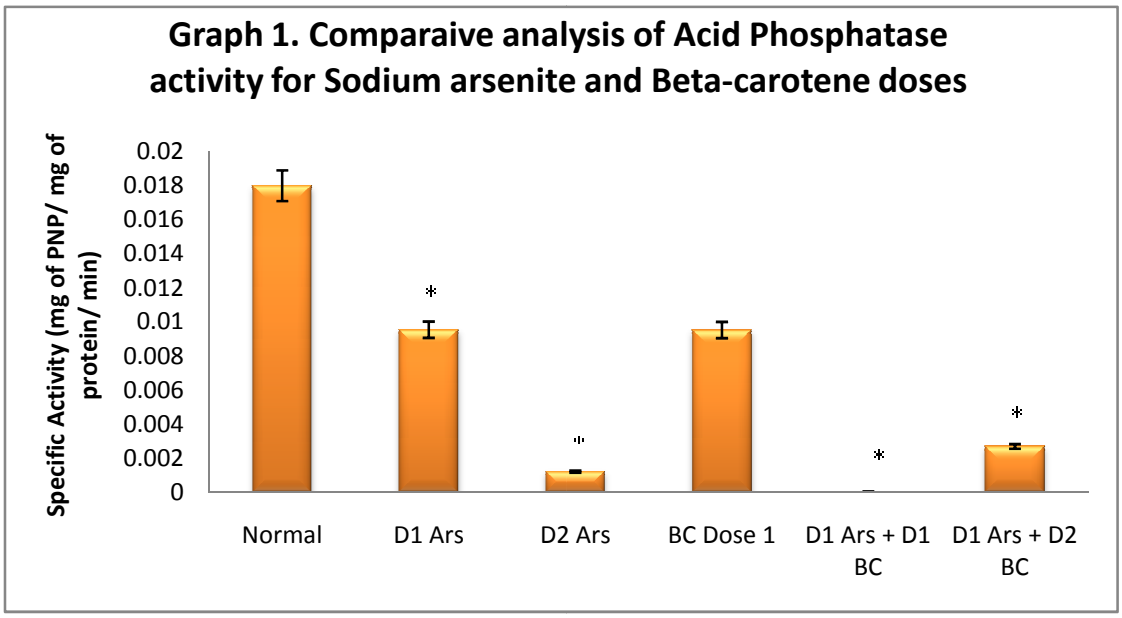
Parameters studied. Specific enzyme activity of Acid phosphatase, Alkaline phosphatase and Glutathione were studied.

Assay protocols. For estimation of specific activity of phosphatase enzymes, the method of Bergmeyer and Brent [17] was followed with some minor modifications. The specific activity was expressed as mg of PNP produced per mg of protein per minute. [18].

The reduced Glutathione level (GSH) was determined by the method Ellman *et al* [19]. Enzyme activity was expressed as microgram per unit tissue.

Statistical analysis. All the Mean ± S.E. treatment values were checked for statistical significance by Student's t-test compared with the control values. P value of 0.05 was considered as significant.

3. RESULTS



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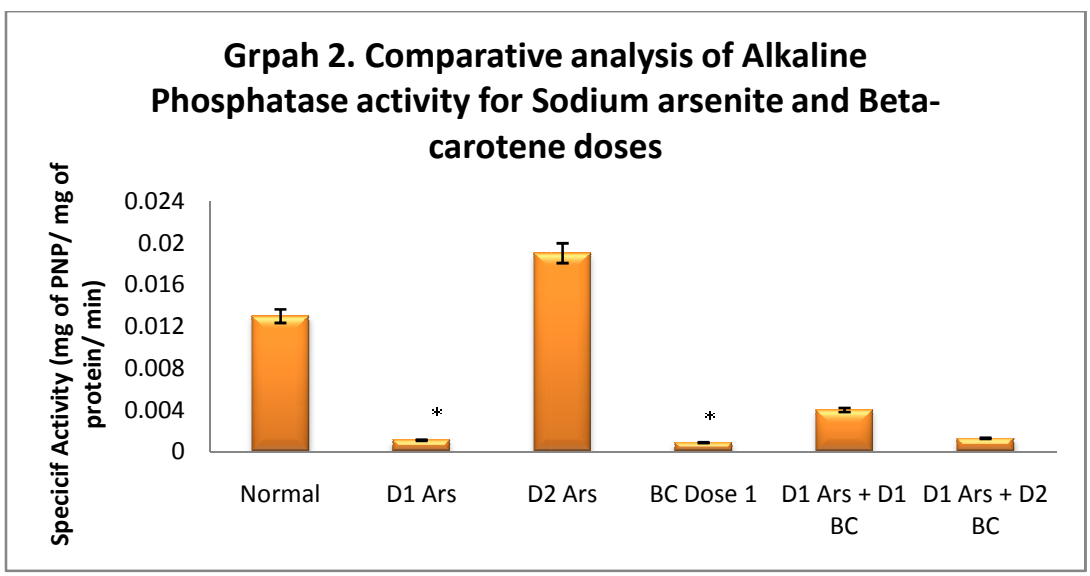

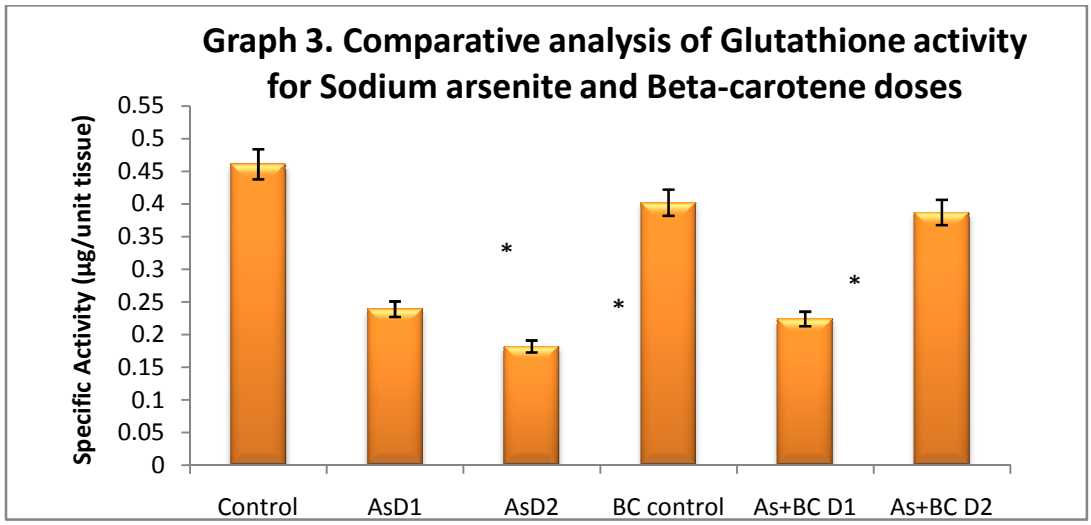

**** indicates p ≥ 0.05***

Sodium arsenite exposure to the cells resulted in impairment of the normal biochemical activities in the liver, which is evident from the lowering of the specific activities of the acid phosphatase and glutathione, with the exception of alkaline phosphatase. Increase in dose of arsenic injection lowered the activity further to a statistically significant level compared to the control series. The activity was regained significantly in the groups supplemented with beta-carotene orally. Increase in dose of beta-carotene increased the activities to a considerable extent, although it was not significantly different from the control series. Vehicle control and beta-carotene control did not show any significant toxicity.

4. DISCUSSION

This study has shown that beta-carotene can check the hepatic stress and toxicity to a certain extent caused by arsenic. This change is evident from the biochemical parameters studied from the liver. Phosphatases are enzymes that catalyze the splitting off of phosphoric acids from certain monophosphoric esters, a reaction of considerable importance in several body processes including neoplastic growth. Acid and alkaline phosphatases have been directly implicated in the extent of cellular damage and toxicity, particularly of liver tissue. The levels of both the enzymes are seen to decrease with increase in arsenic dose; the level decreases mainly as a result of liver, bile duct or gall bladder dysfunction. It is also possible that to meet the ever-increasing demand of inorganic phosphate for intracellular utilization that is affected by chronic arsenic toxicity, the terminal phosphate groups from phosphate compounds are continuously being cleaved by the action of phosphatases, thereby hindering their high activity.

Stress condition reactions can deplete the cellular glutathione. Also, the reactive metabolites can oxidize glutathione and other thiol groups and thereby cause a change in thiol status. When the rate of oxidation of glutathione exceeds the capacity of gluathione reductase then oxidized gluathione (GSSG) is actively transported out of the cell and thereby lost. Thus, after exposure of cells to arsenic, which causes oxidative stress, glutathione readily conjugates to reduce the cellular GSH level.

Beta-carotene protects cells from oxidative stress by quenching free radicals capable of causing cellular damage. Unsaturated lipids in cell membranes are prime targets for free radical reactions [16]. This is also evident in our study. Beta-carotene, being a potent anti-oxidant and chemopreventive agent has very successfully checked the toxic effects of arsenic by activation of DNA repair pathways and trapping of free oxygen radicals.

5. CONCLUSION

Beta-carotene might be used as novel and indigenous supplement against patients suffering from arsenic-induced liver injury and carcinogenesis.

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