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Adverse health effects due to arsenic exposure: Modification by dietary supplementation of jaggery in mice

Nrashant Singh [a,c], D. Kumar [a], Kewal Lal [b], S. Raisuddin [c], Anand P. Sahu [a,*]

[a] Preventive Toxicology Division, Indian Institute of Toxicology Research, M. G. Marg, P.O. Box No. 80, Lucknow 226001, India
[b] Inhalation Toxicology Division, Indian Institute of Toxicology Research, M. G. Marg, P.O. Box No. 80, Lucknow 226001, India
[c] Department of Medical Elementology and Toxicology, Jamia Hamdard (Hamdard University), New Delhi 110062, India

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ABSTRACT

Populations of villages of eastern India and Bangladesh and many other parts of the world are exposed to arsenic mainly through drinking water. Due to non-availability of safe drinking water they are compelled to depend on arsenic-contaminated water. Generally, poverty level is high in those areas and situation is compounded by the lack of proper nutrition. The hypothesis that the deleterious health effects of arsenic can be prevented by modification of dietary factors with the availability of an affordable and indigenous functional food jaggery (sugarcane juice) has been tested in the present study. Jaggery contains polyphenols, vitamin C, carotene and other biologically active components.

Arsenic as sodium-m-arsenite at low (0.05 ppm) and high (5 ppm) doses was orally administered to Swiss male albino mice, alone and in combination with jaggery feeding (250 mg/mice), consecutively for 180 days. The serum levels of total antioxidant, glutathione peroxidase and glutathione reductase were substantially reduced in arsenic-exposed groups, while supplementation of jaggery enhanced their levels in combined treatment groups. The serum levels of interleukin-1β, interleukin-6 and TNF-α were significantly increased in arsenic-exposed groups, while in the arsenic-exposed and jaggery supplemented groups their levels were normal. The comet assay in bone marrow cells showed the genotoxic effects of arsenic, whereas combination with jaggery feeding lessened the DNA damage. Histopathologically, the lung of arsenic-exposed mice showed the necrosis and degenerative changes in bronchiolar epithelium with emphysema and thickening of alveolar septa which was effectively antagonized by jaggery feeding. These results demonstrate that jaggery, a natural functional food, effectively antagonizes many of the adverse effects of arsenic.

Introduction

The most essential component of life, water, contaminated with arsenic is a global human health hazard. Millions of the populations worldwide are exposed to arsenic-contaminated drinking water. Arsenic is widely distributed in nature and released into the environment through natural sources, industrial processes and agriculture usage (Nordstrom, 2002). Arsenic pollution is a serious problem in the developing countries, West Bengal (India), Bangladesh, and Red River delta of Vietnam, and is also chronic problem in China and Thailand (EPA, 2001; Singh et al., 2007; Baastrup et al., 2008; Lindberg et al., 2008). In Asia, the arsenic problems are amplified by the additional arsenic consumed in rice, the primary food source, cooked in arsenic-contaminated water. Arsenic also has been accumulating in paddy soil, resulting in direct contamination of rice grain. Rice contributes to an estimated 30–60% of the dietary intake of arsenic in polluted regions (Rahman et al., 2008).

Continued ingestion of arsenic for a long period leads to inflammatory, neo-plastic and degenerative changes of skin, respiratory, nervous, cardiovascular and reproductive system (IARC, 2004). Skin manifestations are the major negative health effect of chronic arsenic poisoning (Singh et al., 2007). Respiratory complications are also found with lung cancer as a critical endpoint of chronic arsenic toxicity. Numerous epidemiological studies reported chronic cough, chronic obstructive pulmonary disease, and interstitial lung disease as common respiratory complications among the affected population (Mazumdar et al., 2000; Milton and Raham, 2002; Smith et al., 2006; Parvez et al., 2008). Experimental studies document that arsenic affects the normal functioning of alveolar macrophages resulting in the pulmonary oxidative stress and production of pro-inflammatory cytokines (Lantz et al., 1995; Islam et al., 2007; Palmieri et al., 2007).

The seriousness of the problem of arsenicosis is because of the large population (up to 100 millions of people) exposed and the absence of effective treatments. Thus nutritional supplementation approaches to this problem is an attentive possibility (WHO, 2004; Singh et al., 2007). Low socioeconomic status and malnourishment enhance the disease conditions because such population has no alternative but to drink the available arsenic-contaminated water (McCarty et al., 2006;

* Corresponding author. Preventive Toxicology Division, Gheru Campus IITR, Sarojini Nagar Industrial Area, Kanpur Road, Lucknow 226008, UP, India. Fax: +91 522 2628227.
E-mail address: preventive.tox@gmail.com (A.P. Sahu).

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Zablotska et al., 2008). Nutritional factors can modify the host response to environment toxicants. Nutritious diet may be able to inhibit and/or reverse the toxic mechanism of arsenic, whereas a deficient diet can increase the susceptibility to adverse effects of arsenic in drinking water (Vahter, 2007; Lindberg et al., 2008). Several epidemiological and experimental studies suggested that nutritious diet reduces the arsenic toxicity by increasing methylation of arsenic (Mitra et al., 2004; Gamble et al., 2005; McCarty et al., 2006).

The aim of the present study was to examine a dietary strategic prevention plan against arsenic poisoning. We examined jaggery as a dietary supplement of antioxidants. Jaggery is a natural sweetener made from sugarcane juice (*Saccharum officinarum*) without the use of any chemicals/synthetic additives or preservatives. Jaggery contains an enormous wealth of protein, carbohydrate, vitamins and minerals and has been found to have great nutritive and medicinal value. Jaggery is included in the Indian system of medicine Ayurveda (Table 1). Previous studies documented that jaggery has a remarkable beneficial effect on pulmonary system against environmental toxicants (Sahu and Saxena, 1994; Sahu and Paul, 1998) and that jaggery can counter the genotoxic effects induced by arsenic *in vivo* (Singh et al., 2008).

Materials and methods

Chemicals and reagents. Sodium-m-arsenite was procured from Sigma. The kits for estimating total antioxidant status, glutathione peroxidase and glutathione reductase were purchased from Randox Laboratories, UK. The immunological analysis of IL-1β, IL-6 and TNF-α were performed by the kits purchased from R&D Systems. All other chemicals used in the experimental studies were of highest analytical grade and commercially available.

Experimental animals. Laboratory bred Swiss albino male mice with an average weight 30 ± 3 g were used in the present study. The mice, obtained from Indian Institute of Toxicology Research (Formerly ITRC), Lucknow animal house were housed 5 per cage in plastic cages (28 × 22 × 14 cm) and given pellet diet and water *ad libitum*. The pellet diet provided to the experimental animals is manufactured by Hindustan Lever Ltd., Mumbai, India under the trade name "Gold Mohar Mice Feed." The feed contained 22–24% protein, 4–5% fat, 45–55% nitrogen free extract and 4% crude fiber with adequate minerals and vitamin contents. The pellet diet does not contain any added vitamin C or phenolics, but the diet is likely to contain a nonzero amount of vitamin C that comes from either animal or plant sources. It does contain 0.2 ppm carotene and 0.12 g/kg vitamin E. The housing facility was maintained under good laboratory practice conditions at a temperature of 22 ± 2 °C and 12/12-h light/dark cycle and relative humidity of 50 ± 15%.

Table 1
General composition of Indian jaggery.

Content	Value, range
Carbohydrate %	83.5–95.0
Sucrose	72.8–80.3
Reducing sugar	6.8–14.2
Minerals %	0.6–2.6
Calcium	0.2–0.36
Chloride	0.2–0.34
Phosphorus	0.03–0.22
Potassium	0.10–0.16
Sodium	0.006–0.025
Iron	0.005–0.020
Magnesium	0.008–0.105
Copper	0.007–0.010
Cobalt, nickel and molybdenum	0.001–0.008
Protein %	0.35–0.40
Nonprotein nitrogen (mg/100 g)	19.6–42.9
Protein nitrogen (mg/100 g)	13.7–17.6
Vitamins, mg/100 g	
Thiamin	0.018–0.030
Riboflavin	0.042–0.046
Nicotinic acid	3.92–4.50
Vitamin C	5.20– 30.00
Carotene, mg/100 g	155.0–168.0
Phenolics, mg/100g	280.0–320.0
Fat, wax pectin and organic acid, %	0.10–0.60
Moisture, %	3.9–7.2

The table was adopted from the published work of Sahu and Saxena (1994).

Experimental protocol. Animals were randomized and divided in the following five groups. Each group comprised 10 animals. Arsenic (as sodium-m-arsenite) was dissolved in distilled water and administered by gavage. Jaggery was also suspended in distilled water and given by gavage. The molar ratio of arsenic (as sodium-m-arsenite at 0.05 ppm and 5 ppm) to jaggery (using the molecular weight of sucrose) is about 10^7 and 10^5, respectively. Jaggery provided about 4% of total calories of pellet diet. When both the treatments (i.e., arsenic and jaggery) were given concurrently (group IV and V) there was a gap of 6 h in between the first arsenic treatment and second jaggery treatment. Treatment was given for 180 days.

Group I: Control
Group II: Arsenic (0.05 ppm)
Group III: Arsenic (5 ppm)
Group IV: Arsenic (0.05 ppm) along with jaggery (250 mg/kg)
Group V: Arsenic (5 ppm) along with jaggery (250 mg/kg)

Food, water intake and body weight of the animals were monitored throughout the study. On completion of the experimental period, blood samples were drawn from retro-orbital plexus for biochemical and immunological parameters. Animals were euthanized and necropsied for gross morphological observations. Lungs and femurs were surgically excised for histopathological examination and bone marrow preparation for comet assay, respectively.

Biochemical investigation

Estimation of total antioxidant status (TAS). Total antioxidant quantification in serum was carried out by using $ABTS^+$ (2,2′-azidodi 3-ethylbenzothiazolin sulphonate) radical formation kinetics (Kit from Randox Laboratories, UK). The presence of antioxidants in plasma suppresses the bluish-green staining of the $ABTS^+$ cation, which is proportional to the antioxidant concentration level. The intensity of colour is measured at 600 nm, normal range between 1.30 and 1.77 mM. Low antioxidant levels were considered to be ≤1.29 mM.

Glutathione peroxidase (GPx). Glutathione peroxidase levels in heparinised blood were quantified spectrophotometrically at 340 nm by using the GPx kit obtained from Randox Laboratories, UK. The assay principle is based on Paglia and Valentine (1967). GPx catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted back to the reduced form with a concomitant oxidation of NADPH to $NADP^+$ that is measured as decreased absorbance at 340 nm.

Glutathione reductase (GR). Glutathione reductase was assayed in serum by commercially available kit (Randox Laboratories, UK). In brief, glutathione reductase catalyses the reduction of oxidized form of glutathione (GSSG) in the presence of NADPH which is oxidized to $NADP^+$. The resultant decrease in absorbance at 340 nm is measured in a UV double beam spectrophotometer.

Immunological analysis

Tumor necrosis factor-alpha (TNF-α). The Quantikine mouse TNF-α immunoassay (R&D Systems) is used to measure TNF-α level in