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

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A B S T R A C T

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 (3 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.

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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; Bastrup 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 arsenicism 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;
Zablotska et al., 2008). Nutritional factors can modify the host response to environmental 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 (Valter, 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%.

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 107 and 105, 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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
</tr>
<tr>
<td>II</td>
<td>Arsenic (0.05 ppm)</td>
</tr>
<tr>
<td>III</td>
<td>Arsenic (5 ppm)</td>
</tr>
<tr>
<td>IV</td>
<td>Arsenic (0.05 ppm) along with jaggery (250 mg/kg)</td>
</tr>
<tr>
<td>V</td>
<td>Arsenic (5 ppm) along with jaggery (250 mg/kg)</td>
</tr>
</tbody>
</table>

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

Table 1
General composition of Indian jaggery.

<table>
<thead>
<tr>
<th>Content</th>
<th>Value, range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate %</td>
<td>83.5–95.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>72.8–80.3</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>6.8–14.2</td>
</tr>
<tr>
<td>Minerals %</td>
<td>0.6–2.6</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.2–0.36</td>
</tr>
<tr>
<td>Chloride</td>
<td>0.2–0.34</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.03–0.22</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.10–0.16</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.006–0.025</td>
</tr>
<tr>
<td>Iron</td>
<td>0.005–0.020</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.008–0.105</td>
</tr>
<tr>
<td>Copper</td>
<td>0.007–0.010</td>
</tr>
<tr>
<td>Cobalt, nickel and molybdenum</td>
<td>0.001–0.008</td>
</tr>
<tr>
<td>Protein S</td>
<td>0.35–0.40</td>
</tr>
<tr>
<td>Nonprotein nitrogen (mg/100 g)</td>
<td>19.6–42.9</td>
</tr>
<tr>
<td>Protein nitrogen (mg/100 g)</td>
<td>13.7–17.6</td>
</tr>
<tr>
<td>Vitamins, mg/100 g</td>
<td></td>
</tr>
<tr>
<td>Thiamin</td>
<td>0.018–0.030</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.042–0.046</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>1.92–4.50</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>5.20–30.00</td>
</tr>
<tr>
<td>Carotene, mg/100 g</td>
<td>155.0–168.0</td>
</tr>
<tr>
<td>Phenolics, mg/100g</td>
<td>280.0–320.0</td>
</tr>
<tr>
<td>Fat, wax pectin and organic acid, %</td>
<td>0.10–0.60</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>3.9±7.2</td>
</tr>
</tbody>
</table>

The table was adopted from the published work of Sahu and Saxena (1994).
serum by using the solid phase ELISA. This assay employs the quantitative sandwich enzyme immunoassay technique. The 96-well pre-coated plate with polyclonal antibody specific for mouse TNF-α were used. The intensity of the color measured was in proportion to the amount of mouse TNF-α bound to the sample. Then TNF-α levels were calculated by using a standard curve.

**Interleukin-1 beta (IL-1β).** The IL-1β levels in serum of different experimental groups were estimated by using the Quantikine mouse IL-1β kit obtained from R&D Systems. This assay employs the quantitative sandwich enzyme immunoassay technique. An affinity purified polyclonal antibody specific for mouse IL-1β pre-coated plates was used. The intensity of the color measured was in proportion to the amount of mouse IL-1β bound to the sample.

**Interleukin-6 (IL-6).** The levels of IL-6 in the serum of control and all treated groups were quantified by using an enzyme linked immunosorbent assay (ELISA). The Quantikine mouse IL-6 kit (R&D Systems) was used for quantification. The intensity of the color measured was in proportion to the amount of mouse IL-6 bound to the sample. The sample levels are then read off the standard curves.

**Comet assay.** Single cell gel electrophoresis or comet assay was done as per the protocol of Singh et al. (1988). Briefly, 20 μl of bone marrow cell suspension from control or treated mice was mixed with 200 μl of 0.75% low melting agarose in PBS at 37 °C and quickly put onto a microscope slide which already had a dried layer of 1% agarose. A cover glass was put slowly on the slide to make a uniform layer of agarose above the layer containing the cells. Afterwards the slide was re-photographed. The status of total serum antioxidant in control and exposed animals was microscopically compared for alteration from normal tissue structure.

**Histopathological investigation.** After sacrificing the animals, lungs were dissected out and washed in normal saline and fixed in 10% buffered formaldehyde solution. Fixed tissue was washed in slow running tap water for 6 h, dehydrated in graded alcohols, cleaned in xylene, and embedded in paraffin wax and 5-mm-thick sections were cut and stained with haematoxylin and eosin (H&E). Slides prepared from normal and exposed mice were microscopically compared for alteration from normal tissue structure.

**Statistical analysis.** Group values are expressed as mean ± SE. Significance of the difference between mean values was determined by one way analysis of variance (ANOVA) followed by Student’s t test. P < 0.05 was considered significant.

### Results

On completion of the experimental period, the body weight of arsenic-exposed groups (groups II and III) and combined treatment groups (groups IV and V) showed significant (P < 0.05) changes in comparison to controls. Combined treatments groups also showed significant (P < 0.05) change in body weight in comparison to arsenic-alone exposed groups (Table 2).

### Total antioxidant status (TAS) activities

The status of total serum antioxidant in control and exposed animals is presented in Fig. 1. Arsenic treatment at dose 0.05 ppm and 5 ppm caused significant (P < 0.05) depletion in TAS activities compared to the control group. Administration of jaggery along with arsenic 0.05 ppm in Figs. 1 and 2.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Arsenic (0.05 ppm)</th>
<th>Arsenic (5 ppm)</th>
<th>Arsenic + jaggery (0.05 ppm)</th>
<th>Arsenic + jaggery (5 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BW</td>
<td>30.15 ± 1.07</td>
<td>32.05 ± 1.36**</td>
<td>31.59 ± 1.64**</td>
<td>30.68 ± 1.58</td>
<td>30.31 ± 1.41</td>
</tr>
<tr>
<td>Final BW</td>
<td>36.03 ± 1.61</td>
<td>28.76 ± 1.38**</td>
<td>27.04 ± 1.39**</td>
<td>32.62 ± 0.63**b,c</td>
<td>31.43 ± 2.70**c</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. Asterisk (*) indicates significant (P < 0.05) difference of data between control and treated animals: a, as compared with control; b, as compared with arsenic (0.05 ppm); c, as compared with arsenic (5 ppm).
Arsenic in group IV and V antagonized the toxic effects of arsenic and significantly (P<0.05) increased the TAS activities compared to the arsenic alone exposed groups.

Glutathione peroxidase (GPx) activities

The activities of GPx in control and experimental groups are shown in Fig. 2. Significant (P<0.05) arsenic-induced depletion of GR activity in groups II and III was found compared to controls. Simultaneous supplementation of jaggery along with arsenic in groups IV and V significantly (P<0.05) elevated the levels of GPx compared to the arsenic alone-exposed groups.

Glutathione reductase (GR) activities

Fig. 3 depicts the effects of jaggery and arsenic on GR activities in control and treated groups. Activities of GR were significantly (P<0.05) lowered in groups II and III due to the arsenic exposure compared to the control group. Combining jaggery with arsenic treatment significantly (P<0.05) increased the activities of GR in groups IV and V in comparison to the arsenic alone groups.

Tumor necrosis factor-alpha (TNF-α)

Fig. 4 describes the effects of jaggery on TNF-α level in the serum in arsenic treated and control animals. TNF-α level in 0.05 ppm and 5 ppm arsenic treated mice was significantly (P<0.05) higher than that of control animals. Supplementation of jaggery in groups IV and V significantly (P<0.05) decreased the TNF-α level compared to the arsenic alone-exposed groups.

Interleukin-1 beta (IL-1β)

Fig. 5 describes the results of the IL-1β activities in control and exposed mice. Arsenic treatment in groups II and III significantly (P<0.05) increased the IL-1β levels compared to control. However, jaggery feeding along with arsenic in groups IV and V significantly (P<0.05) reduced the IL-1β level in comparison to the arsenic-exposed groups.

Interleukin-6 (IL-6)

Significantly (P<0.05) increased secretion of IL-6 in the serum was observed in arsenic treated animals in groups II and III in the comparison to control animals (Fig. 6). Simultaneous jaggery feeding with arsenic in groups IV and V significantly (P<0.05) decreased the IL-6 level in the serum compared to the arsenic alone treated groups.

Comet assay

The comet assay was carried out to measure the single-strands DNA breaks in bone marrow cells of control, arsenic treatment and arsenic along with jaggery treated mice. DNA breaks are seen with...
arsenic treatment at both doses (Figs. 7B and C). Morphometric analysis of tail migration (DNA strand breaks) was 2-fold increased in arsenic treated mice ($P<0.05$) at both doses compared to the control animals. Simultaneous supplementation of jaggery antagonizes the toxic effects of arsenic resulting in the lower DNA damage in both combined treatment groups (Figs. 7D and E). In the combined treatment groups, administration of jaggery along with arsenic significantly ($P<0.05$) reduced the DNA damage when compared to arsenic alone at different doses (Fig. 8).

**Histopathological observations**

The structural observations suggested that the arsenic damaged the normal architecture of the lung. Fig. 9A shows the normal structure of the lung. Severely necrotic bronchial epithelium lining cells with inflammatory infiltrate in the alveolar sacs and thickened alveolar septa were observed in arsenic (0.05 ppm) treated mice (Fig. 9B). Arsenic (5 ppm) treatment showed increased severity of these changes with emphysema (Fig. 9C). Mice treated with jaggery along with arsenic (0.05 ppm) showed slightly thickened alveolar septa and bronchiolar epithelium (Fig. 9D). Mice given jaggery and arsenic (5 ppm) treatment showed slight thickening of alveolar septa and bronchiolar epithelium (Fig. 9E).

**Fig. 7.** Bone marrow cell comet assay results of all experimental groups after 180 days of exposure. (A) Control group. (B) Arsenic (0.05 ppm). (C) Arsenic (5 ppm). (D) Arsenic (0.05 ppm) + jaggery (250 mg/mouse). (E) Arsenic (5 ppm) + jaggery (250 mg/mouse).

**Fig. 8.** Effect of jaggery and arsenic exposure on DNA tail moment in bone marrow cells of all experimental groups. Values are expressed as mean±SE. Asterisk (*) indicates significant ($P<0.05$) difference of data between control and treated animals: a, as compared with control; b, as compared with arsenic (0.05 ppm); c, as compared with arsenic (5 ppm).
arsenic (5 ppm) showed thickened alveolar septa and bronchiolar epithelium lining cells with less inflammatory infiltrate (Fig. 9E).

**Discussion**

The pulmonary system is a major concern regarding chronic arsenic poisoning. Numerous epidemiological as well as experimental studies have demonstrated that prolonged consumption of drinking water contaminated with arsenic causes obstructive and restrictive disorders to pulmonary system (Mazumdar et al., 2000; Lantz et al., 1995; Palmieri et al., 2007; Parvez et al., 2008; Smith and Steinmaus, 2009). The present study was performed to determine the inflammation-mediated changes in respiratory system due to the arsenic exposure and mitigative efforts by dietary modulation with jaggery. The results of present work suggested that arsenic exposure was immunosuppressant and affected the antioxidant defence system leading to the molecular and cellular damage to the pulmonary system.

Antioxidants both enzymatic and non-enzymatic are the first line of defence against free radical induced toxicity. A redox balance between pro-oxidants and antioxidants is essential for the normal cellular functioning (Nordberg and Arner, 2001). Impairment in the ratio of oxidants and antioxidants initiates the pathophysiological events that culminate in molecular and cellular damage to macromolecules and vital organs (Valko et al., 2004). The pulmonary system may be at great risk of oxidant-mediated cellular injury by direct tissue oxidation because it exists in oxygen rich environment. An increase in the generation of free radicals or suppression in antioxidant defence mechanism leads to the pulmonary dysfunction (Hays et al., 2006). The results of our study showed that arsenic exposure impaired the defence mechanism of antioxidants leading to the oxidative stress by significantly depleted total plasma antioxidant status at both low and high arsenic doses. Supplementation of jaggery along with arsenic at both doses antagonized or reversed arsenic-induced chain of events possibly via better maintaining the redox equilibrium within the body.

Glutathione (GSH) is the main detoxifying antioxidant and the first line of defence against the oxidative stress induced by various exogenous and endogenous compounds (Meister and Anderson, 2004).
The glutathione related enzymes GPx and GR play a key role in the protection of cells from free radical generated toxicity. GPx and GR participating in normalizing the cellular ratio of reduced glutathione and oxidized glutathione above 300:1 (Alpert and Gilbert, 1985). Inhibition of GPx and GR activity diminishes the intracellular glutathione level and leads to the increased concentration of free radicals resulting in the oxidative stress. Thus, co-ordinated activity of GPx and GR maintains the intracellular redox status (Ketterer, 1986). GSH together with the GPx can detoxify free radicals into non-toxic products (Sinha et al., 2008a). Several experimental studies demonstrated that arsenical compounds are potent inhibitors of both GPx and GR (Styblo et al., 1997; Avani and Rao, 2008). GPx is a selenoenzyme and the inhibition of GPx may be due to the interaction between trivalent arsenicals and selenium (Miyazaki et al., 2005).

Numerous studies reported that some natural products can restore the activities of GPx and GR affected by arsenic exposure through various biological mechanisms (Sharma et al., 2007; Flora and Gupta, 2007; Sinha et al., 2008b). In the present study, arsenic treatment at low and high doses decreased the activities of GPx and GR and leads to the cellular damage of pulmonary system. Simultaneous administration of the natural product jaggery improved the antioxidant defence system by increasing GPx and GR activity.

Macrophages produce pro-inflammatory cytokines during the normal course of cellular functions. TNF-α, IL-1β and IL-6 the major pro-inflammatory cytokines and increased secretion due to the environmental toxicants culminates the acute phase inflammatory responses (Duramad et al., 2007). These inflammatory mediators are involved in various biological and cellular responses including tumor progression, growth factor, transcription factor and activation of proapoptotic proteins (Sugarman et al., 1985; Manna et al., 2000). Besides the inflammatory responses, these inflammatory cytokines are also involved in the generation of free radicals via mitochondrial respiratory chain reaction (Volk et al., 2000). Several studies reported that impairment in macrophages leads to the increased secretion of inflammatory mediators especially TNF-α and IL-1β which are associated with the development of pulmonary inflammation and fibrosis (Le and Vilcek, 1987; Driscoll et al., 1990; Piguet et al., 1990). Numerous epidemiological as well as experimental studies documented that arsenic works as an immunosuppressant and causes the immunologically mediated disorders at molecular and cellular levels (Islam et al., 2007; Biswas et al., 2008).

Exposure to arsenic through intra-tracheal installation inhibits the pulmonary macrophage derived cellular functions resulting in increased production and secretion of TNF-α and IL-1β that leads to respiratory complications (Huaux et al., 1995). A recent field study in West Bengal (India) reported that prolonged ingestion of arsenic-contaminated drinking water acted as immunosuppressor and affected the secretion of variety of cytokines in a susceptible population (Biswas et al., 2008). Supplementation of dietary antioxidants can reverse the arsenic-induced immunotoxicity (Bongiovanni et al., 2007). Studies suggested that increased secretion of inflammatory mediators due to arsenic exposure was significantly antagonized by the administration of antioxidants (Ramanathan et al., 2005; Mukherjee et al., 2006). The results of our study suggested that prolonged arsenic exposure caused immunotoxicity by inhibiting the pro-inflammatory cytokine activity and showed the dose-dependent toxicity of arsenic. The increased secretion of inflammatory mediators may cause cellular damage to respiratory system via development of inflammatory reactions. Simultaneous administration of jaggery along with arsenic in preventive groups significantly reduced the levels of inflammatory cytokines.

Weak antioxidant defence system with increased ROS would leave cells vulnerable to oxidative DNA damage (Ray and Husain, 2002). Arsenic is well known genotoxic and mutagen (IARC, 2004). Several experimental studies reported that DNA single-strand breaks mainly occurs either due to increased ROS or inhibition of DNA repair enzymes after arsenic exposure (Andrew et al., 2006; Wang et al., 2006; Kadirvel et al., 2007). Our results showed that after 180 days of exposure, single cell gel electrophoresis (comet assay) showed single-strand breaks of DNA in the bone marrow cells both at low and high doses of arsenic. DNA tail formation can be considered as the biochemical indicator of apoptotic cell death. The tail moment was increased at low and high dose treatment of arsenic, while jaggery reduced the tail moment. Thus, we interpret that jaggery feeding can antagonize the arsenic-induced DNA damage.

After continued arsenic exposure the histopathological observations revealed that low dose arsenic treatment caused severely necrotic lesions in bronchial epithelium lining cells with inflammation in the alveolar sacs. Exposure to high arsenic doses showed severely necrotic and degenerative changes of bronchial epithelium lining cells with emphysema and thickening of alveolar septa. Combined treatment groups showed slight inflammatory changes in bronchial epithelium and alveolar sacs had lower number of necrotic cells.

Several field studies suggested that ingestion of arsenic-contaminated drinking water is responsible for the various malignant and non-malignant respiratory diseases. The toxicopathogenesis of respiratory system due to arsenic exposure remains unclear, but numerous studies suggest that deposition of arsenic in lung was the cause of pulmonary dysfunction. Moreover, studies showed that deposition of arsenic may increase inflammation and cause fibrosis that culminates in pulmonary cellular dysfunction (Gerhardsson et al., 1988; Saady et al., 1989; Nemery, 1990). The results of our study suggest that oxidant-mediated pathogenesis may be the cause of disorders to normal respiratory functions by disequilibrium in the oxidant and antioxidant ratio. This could happen either by impairment in antioxidant defence system or increased generation of free radicals due to arsenic exposure leading to the direct oxidation of lung tissue (Romieu and Trenga, 2001). A number of studies documented that arsenic is well known to impair the antioxidant defence system leading to the oxidative stress resulting in the molecular and cellular damage (Lantz and Hays, 2006; Chang et al., 2007; Flora and Gupta, 2007; Sinha et al., 2008b). Therefore, the oxidant-mediated pathogenesis may be the toxicological mechanism of arsenic-induced dysfunction to pulmonary system.

Some natural products have indigenous active ingredients, which have pharmacological efficiency to remediate the disease conditions. Arai and co-workers (2002) suggested that functional foods provide health benefits beyond the basic nutrition to improve the physiological function of the body system. Jaggery is commonly known as Gur in India and Panela in South America. Previous studies on jaggery reported that supplementation of jaggery improves the pulmonary defence mechanisms against respiratory disorders induced by various occupational environmental toxicants including coal and particulate matter (Sahu and Saxena, 1994; Sahu and Paul, 1998). An important ingredient of jaggery is vitamin C, a non-enzymatic antioxidant (not synthesized within the human body) which is essential for the normal lung function. The extracellular fluids lining the lung contain vitamin C which decreases the susceptibility to airway inflammation and oxidant attack (Ghio et al., 1998; Romieu and Trenga, 2001). Our earlier study found that simultaneous supplementation of jaggery antagonized the genotoxic effects (chromosomal aberration) induced by arsenic exposure (Singh et al., 2008). Recently, Nayaka and co-workers (2009) reported that jaggery (3837 μg GAE [gallic acid equivalent]/g) has approximately 150-, 100- and 10-fold total phenolic content in comparison to refined (26.5 μg GAE/g), white (31.5 μg GAE/g) and brown (372 μg GAE/g) sugar, respectively. They suggested that continued consumption of jaggery has efficacy in minimizing the negative effects induced by environmental toxicants. Our earlier studies on jaggery showed that jaggery feeding helps to improve the body defence mechanism and does not appear to have any negative toxicological effects.

We conclude that prolonged exposure to arsenic induced the dose-dependent toxicity via oxidative stress with immunotoxicity and...
pathomorphological lesions to the respiratory system, jaggery feeding antagonized these arsenic-induced negative effects. The mechanism of prevention is not known with certainty but the active components present in jaggery may play an important role in preventing the development of arsenicism in healthy and susceptible subpopulations. The vitamins, minerals, carotene and polyphenols present in jaggery may help to maintain the redox equilibrium within the body to minimize the molecular and cellular oxidative damage caused by arsenic. Dietary antioxidants can mobilize and upregulate the antioxidant capacity of cells to neutralize excessive free radicals.

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References


