Chapter 5

Prevention of arsenic-induced toxicity by Jaggery administration along with vitamins in mice
In past few decades, individuals of developing countries face a new crisis of drinking water defilement with arsenic. As is abundantly found in earth-crust, rocks, sediments and metal ores and is released into the work environment via natural, industrial and agriculture process (Nordstrom, 2002; Singh et al., 2007). As contamination in drinking water is the global problem (EPA, 2001; WHO 2004). The millions of population suffering from this epidemic problem are the resident mostly of developing countries included Argentina, Chile, Hungary, Inner-Mongolia, Italy, Romania, Taiwan and Thailand but the situation is serious in West Bengal (India) and Bangladesh (Lindberg et al., 2008; Ungaro et al., 2008). Despite the drinking water, food especially rice grown in As contaminated area is also a major source of exposure because of about 30 to 60 percent of population of transition countries depend on it (Saha and Ali, 2007; Rahman et al., 2008).

Epidemiological studies mentioned that chronic poisoning is more dangerous than acute toxicity because there is no specific remedial treatment of it. Ingestion of As contaminated drinking water results in multiorgan dysfunctions including common cutaneous manifestation to cancer of skin, liver, bladder and pulmonary system (Ahsan et al., 2006; Singh et al., 2007). As caused cytotoxicity by disturbing the enzymes associated with nucleic acid. It is not clear but seems to be the monomethylarsonous acid (MMA$^{iii}$), an intermediate compound in As biotransformation (methylation) process is responsible for As induced toxicity (Vahter, 1999; Gebel, 2001, Thomas, 2007). Biotransformation of As seems to be detoxification process. The methylation capacity is varying from individual to individual depending upon its socio-economic status and nutrition. Methylation, a key of detoxification of As toxicity is correlated with the well-nourished diet (Vahter, 2002; Steinmaus et al., 2005; McCarty et al., 2006; Xu et al., 2008). The diet rich in protein, vitamins and minerals enhance the methylation capacity; resulting in
decrease of the susceptibility to diseased conditions. Malnutrition, age, genetics and sex are correlated with As poisoning (Mitra et al., 2004; Lindberg et al., 2008). Mal-nutrient diet may not protect the individual from diseased condition caused by drinking water with low As, but high concentration of As in drinking water may not cause the disorders to the persons with well-nourished food (Chowdhury et al., 2000). Good nutrition and safe drinking water can remediate the dysfunction induced by As in drinking water (Gamble et al., 2005; Maharjan et al., 2007).

The present research work aimed to observe the mitigation of dysfunction induced by As intoxication on respiratory system by feeding jaggery along with vitamin C and vitamin E. Jaggery-functional food, a well-recognized therapeutic agent in Indian medicine-Ayurveda. Jaggery has enormous wealth of protein, carbohydrate, vitamins and minerals especially iron, which have a great efficiency to reduce the affectivity of As (Table 1).

**Experimental Protocol**

Laboratory bred Swiss albino mice with an average weight 25 ± 3 g were used in this study. The 40 mice were divided into four groups:

**Group I**: Control

**Group II**: Mice received arsenic (As) as sodium-m-arsenate at a concentration of 0.16 mg/kg body weight corresponding to the 1/50 of LD$_{50}$ once in a week Intraperitoneally

**Group III**: Animals treated with As along with Jaggery (250 mg/ mice), ascorbic acid (200 mg/kg body weight) and alpha tocopherol (400 mg/kg body weight) by oral gavage daily
Group IV: Mice were given Jaggery (250 mg/mice), ascorbic acid (200 mg/kg body weight) and alpha tocopherol (400 mg/kg body weight) by oral gavage daily.

Food, water intake and body weight of the animals were monitored throughout the 30 days of experimental period. On completion of the experimental period animals were sacrificed.

For the determination of the toxic effects of As and the modulating effects of jaggery along with vitamins, following parameters were performed:— BALF analysis, histopathology. The details of all the procedures are described in materials and methods section in Chapter 3.

Statistical analysis

Value is expressed as mean ± S. E. and significance of the difference between mean values were determined by one way of variance ANOVA followed by students - t test and P<0.05, P<0.01 and P<0.001 values were considered significant.
Results

Bronchoalveolar Lavage Fluid analysis

The cytological studies of alveolar macrophages derived from BALF shows hyperchromatic cytoplasm with eosinophils and monocytes in As exposed mice (Group-II) (Fig. 7b). The cell area, cytoplasm area and nucleus area of alveolar macrophages in As-treated animals (Group II) were significantly (P<0.05) decreased in comparison to control mice (Group I). The As + Jaggery + Vitamins treated group showed increment in cell area, cytoplasm area and significant elevation (P<0.05) in nucleus area of alveolar macrophages in comparison to As treated animals while there were slight decrement in comparison to control animals (Fig. 8). The jaggery fed animals shown significant increment (P<0.05) in cell area, cytoplasm area and nucleus area in comparison to preventive group whereas non-significant decrement as compare to control. On the basis of morphometric calculation the percent cytoplasm area of the macrophages reduced in all three groups. The percent nucleus area were increased in As-treated and As + jaggery + vitamins animals in comparison to control (Fig. 9).

Histopathological Observations

After the 30 days exposure pathological changes were determined in all treated groups. Lungs of control animals show normal structure, while As-treatment showed destruction of the normal architecture of bronchioles, parenchyma and alveolar septa. Degenerative changes in bronchiolar epithelium with flattened cuboidal structure appeared in As-treated animals (Fig. 10a). In bronchioles, heavy collections of small cells with pre-neoplastic cells were shown in Group-II (Fig. 10b). Lung parenchyma of As-treated mice showing slight thickening of alveolar septa with collection of cells in the alveoli (Fig. 10c).
However, simultaneous administration of Jaggery and Vitamins reduced the cuboidal nature of bronchiolar epithelium and showing the ameliorating effect against As toxicity (Fig. 10 d & e). Moreover, jaggery and vitamins reduced the thickness of alveolar septa and shows normal structure (Fig. 10 f).

Discussion

In the present study, we examined the adverse health effects induced by As as well as mitigative effects of jaggery along with vitamins on respiratory system. In the histopathological observation the lung showed the toxic effects of As and reduction in the negative effects by jaggery feeding.

Oxidative stress seems to be the major cause of toxicity of As. Antioxidant enzymes especially vitamins play a vital role in the intervention of As-induced toxicity. Several experimental studies proved that co-administration of vitamins such as vitamin C and E can enhanced the modulating capacity by reducing reactive oxygen species and intervent the toxic health effects of As (Ramanathan et al., 2005; Wei et al., 2005; Chang et al., 2007). Besides, the antioxidant properties of vitamin C and E, vitamin A was also positively administered to suppress the oxidative stress induced by As. In As induced genotoxicity, co-administration of vitamin A can significantly encounter the genotoxic endpoints (Avani and Rao, 2007).

Naturally occurring dietary supplements have the pharmacological activity to suppress the negative effects induced by heavy metals or environmental pollutants (Nandi et al., 1997). A positive correlation between naturally occurring nutrients and mitigation of heavy metals or environmental pollutants toxicity was reported by several experimental studies (Odunola, 2003; Chowdhury et al., 2008; Manna et al., 2008a; Sinha et al., 2008a). Jaggery, a natural product of sugarcane
has the great nutritive and also medicinal values as reported in Indian system of medicine - Ayurveda. It contains the great amount of carbohydrate, proteins, vitamins especially vitamin C and various micronutrients. Jaggery has specific nutrients and food components and positively affecting the target functions in the body. The previous experimental studies reported that jaggery modified the coal-induced lesions in rats (Sahu et al., 1988a; 1988b), encountered the toxic effects of lead (Flora et al., 1988); improved the pulmonary defense system countered by particulate matter (Sahu and Saxena, 1994) and also lowered the incidence of occupational respiratory disease (Sahu and Paul, 1998).

In our previous experiment, we examined the genotoxic effects of As as chromosomal aberration and its amelioration by co-administration of jaggery. The results of the study reported that As seems to very toxic by inducing and increasing the clastogenic frequencies, while co-administration of jaggery significantly decreases the clastogenicity. Constituents of Jaggery help to reduce the frequency of chromosomal aberrations by inhibiting the toxic mechanism of As (Singh et al., 2008). In this direction, the preventive efforts of the present study showed the toxic effects of As exposure on lung and the mitigation of negative effects by supplementation of jaggery. The cytological studies of alveolar macrophages of As exposed mice shows hyperchromatic cytoplasm with eosinophils and monocytes; whereas, preventive group alveolar macrophages appears near to normal. The histopathological observations of lung showed that As destroyed the normal architecture of bronchioles and alveolar septa. Degenerative changes in bronchiolar epithelium and flattened cuboidal structure with heavy collections of small cells with pre-neoplastic cells appears in As treated animals. However, simultaneous administration of jaggery and vitamins reduced
the cuboidal nature of bronchiolar epithelium and showing the ameliorating effect against As toxicity.

The mechanism of prevention of toxicity is not well known but, it is supposed that the active ingredients of jaggery especially carbohydrate as well as vitamins play a provital role to reduced the toxic effects of As. The results of this study suggest that nutritive diet can remediate the toxic effects due to the drinking of As contaminated water.
Figure 7. Bronchoalveolar lavage fluid cells of control mice - [A] showing normal macrophages with clear cytoplasm.
BALF from arsenic treated – [B] showing macrophages with darkly stained cytoplasm (single head arrow), eosinophil (double head arrow), and monocytes.
BALF from Arsenic + Jaggery + Vitamins treated mice– [C] macrophages with stained cytoplasm
BALF from Jaggery + Vitamins treated mice [D] showing macrophages with small monocytes.
600 x; Giemsa Stain. Insets are with high magnifications.
Figure 8. Morphometric analysis of bronchoalveolar lavage fluid cells- alveolar macrophages of experimental groups showing the changes in the cell area, nucleus area and cytoplasm area. Value is expressed as mean ± S. E.. Asterisks (*) indicate significant (P<0.05) difference of data between control and treated animals, as compared with control.

Figure 9. Morphometric analysis of the BLAF cells- alveolar macrophages of treated groups showing the changes in the percent nucleus area and percent cytoplasm area. Value is expressed as mean ± S. E.. Asterisks (*) indicate significant (P<0.05) difference of data between control and treated animals, as compared with control.
Figure 10. Mice exposed to Arsenic (Sodium-m-arsenite); Lungs at 30 days, the bronchiolar epithelium showing flattened cuboidal structure with heavy collection of small cells around bronchioles and blood vessels (a); bronchiolar epithelium at places have a collection of pre-neoplastic cells (b); lung parenchyma showing slight thickening of alveolar septa with collection of cells in the alveoli (c). The bronchiolar epithelium of As + Jaggery treated mice showing reduction in the cuboidal nature of bronchiolar epithelium (d); around bronchioles, the dilated lymphatic channel which helps and demonstrated the preventive effect in the lung parenchyma (e); near normal alveolar structure with occasional collection of cells in alveoli (f). H&E 40x.