Modulatory effect of administration of Jaggery and Choline on arsenic-induced toxicity in mice
Millions of people world over are forced to consume arsenic contaminated water above the safety guideline value (10 ppb) set by WHO and US EPA (EPA, 2001; Nordstrom, 2002; WHO, 2004). As is widely distributed in environment especially in parts of China (Inner Mongolia), South-East Asia, Taiwan and United State (Alaska, California, Nevada and Utah), but the scenario with regard to the increased population and water resources is worst in West Bengal (India) and Bangladesh (Chen et al., 2005; Ehrenstein et al., 2005). Across the Gangetic plains in India, and neighboring Nepal is also recently reported as the area affected from As poisoning (Maharjan et al., 2005; 2007).

Prolonged consumption of contaminated drinking water with As has been results in multifactorial disorders. The clinical manifestations of chronic exposure are dermatosis, respiratory system complications and cancers of skin, lungs, bladder and liver are also frequently occurred (Ahsan et al., 2006; Singh et al., 2007). IARC (2002) report As as “Group I” human carcinogen. As is well-known mutagen and causing the genotoxicity by inhibiting the enzyme system responsible for DNA damage (Gebel, 2001). The functioning of antioxidant defense system is decreased by chronic As toxicity results as oxidative damage to cellular molecules and causing apoptosis (Hei and Filipic, 2004; Chang et al., 2007). Despite skin disorders, respiratory system is also the target organ of chronic toxicity. As causes the adverse affects on lungs results in reduced lung functions, chronic bronchiectasis and lung cancer (Mazumdar et al., 2000; Milton and Raham, 2002; Ehrenstein et al., 2005).

Defilement of As in groundwater is alarming public health problem and an immediate action is essential for prevention of adverse health effects. Early intervention approaches included alternations in water sources, filtration system, rainwater harvesting, removal technologies and bioremediation, but these remedial
efforts are not sufficient to provide the relief to the large affected population (Mitra et al., 2004; Parvez et al., 2006; Maharjan et al., 2007). Chelation therapy is effective and applicable only in the acute toxicity but the supplementation of the balanced diet along with the nutritive food can be effective and considered as the alternative remedial action (Gupta and Flora, 2005a; 2005b). Some research investigations suggested that nutrition can play an important role in As toxicity and good nutrition can modulate the delayed effects of As in drinking water. The individual with well-nourished food are less susceptible to chronic As poisoning (Gamble et al., 2005; Steinmaus et al., 2005; McCarty et al., 2006).

The present study, aimed to find out the reduction of the problems with respect to the effective and affordable nutrient because the affected individuals mainly belong to the categories of below the poverty line. Experimental studies were undertaken with jaggery and choline. Choline is an essential nutrient, important component of cell membrane and plays a key role to maintain structure integrity and signaling functions of cells (Sahu et al., 1985; Sahu, 1989; Zeisel, 2000). It is the precursor to neurotransmitter, acetylcholine and has been playing a critical role during the developmental period (Zeisel, 2004). Choline serves as methyl donor involved in DNA methylation, altered gene expression and cell proliferation (Zeisel, 2006). Thus, choline deficiency may reduce the As methylation and leads to the negative health effects. Jaggery as functional food, has specific nutrients and food components and positively affects the target functions in the body. Jaggery is a natural sweetener made from sugarcane juice without the use of any chemicals/synthetic additives or preservatives. It contains an enormous wealth of minerals, protein and vitamins (Table 1). More importantly, jaggery has great nutritive and medicinal value as reported in Indian system of
medicine -Ayurveda and has the property of antitoxic and anticarcinogenic activity (Sahu and Saxena, 1994; Sahu and Paul, 1998).

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**: Arsenic (As) as arsenic trioxide (12.9 mg/kg body weight) subcutaneously weekly

**Group III**: Animals treated with As along with choline as choline chloride (16 mg/kg body weight) intraperitoneally weekly and Jaggery (250 mg/mice) by gavaging daily

**Group IV**: Mice received Jaggery and choline up to experimental period

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 evaluation of toxic effects of As and the protective effects of jaggery along with choline, 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

BALF cells of control mice showing normal alveolar macrophages with clear cytoplasm. Alveolar macrophages from As treated mice showing apoptosis and micronuclei in the cytoplasm. As+ CC + Jaggery treated mice showing alveolar macrophages with clear cytoplasm (Fig. 11). The cell, cytoplasm and nucleus area of As treated alveolar macrophages were significantly (P<0.05) increased as compared to control. Whereas decrement in cell, cytoplasm and nucleus area were found in preventive group in comparison to As treated (Fig. 12). Morphometric calculation of the As-treated alveolar macrophages showed significant (P<0.05) increment in percentage cytoplasm area and significantly (P<0.05) decrement in percent nucleus area as compare to control. The percentage cytoplasm area is decreased in As + CC + jaggery (Group III) treated animals, while percent nucleus area was increased in As + CC + jaggery (Group IV) treated animals as compared to As alone (Fig. 13).

Histopathological observations

Histopathological changes were clearly shown in all treated groups after 30 days of exposure. As appears very toxic to respiratory system due to the degeneration of cells. In As treated group, hyperplasia in atypical bronchiolar epithelium with detachment of cells from epithelium are shown in figure 13 a. The collection of nodular hyperplastic bronchoepithelial cells in the lung parenchyma (Fig. 13 b) and thickened alveolar septum with occasional collection of cellular debris, cells in alveoli (Fig. 13 c) were found in As treated group. While, in preventive group the picture is near to normal and shows the encountered effect of CC with jaggery against the As induced toxicity (Fig. 13 d, e & f).
Discussion

Around the world, millions of the population with the devasting disorders and the peril of death due to the consumption of drinking water contaminated with As, are basically from rural areas with lower economic status. As a single substance, it is responsible to the multisystem adverse health effects including common cutaneous manifestations to the cancers of skin, lung, liver and bladder (Parvez et al., 2006; Singh et al., 2007). Despite the prevalence of lung cancer and other malignant disorders to the respiratory system including decrement of the lung function, chronic bronchiectasis, irritation of nasal mucosa, bronchiectasis, tracheobronchitis and pulmonary insufficiency (WHO, 1981, Milton and Raham, 2002; Ehrenstein et al., 2005).

In the As detoxification, nutritional substances can play an important role. The diet deficient in protein can enhance the toxicity because the low protein diet decreases the excretion of As (Vahter and Marafante, 1987). As disrupt enzyme system by combining with the sulphahydryl groups which leads to toxicity. Another reason for the aggravation of toxicity with reference to the low protein diet is the reduction of the free thiol groups, which play an important role in the As metabolism (Mckinney, 1992). Several other studies suggested that diet deficient of protein lead to the enhancement of the developmental toxicity (Lammon and Hood, 2004) and inhibiting the response of the cellular protection component (Maiti and Chatterjee, 2000).

Choline, a dietary component is essential for the normal functioning of the cell. Choline is an important source and donor of methyl-groups. A metabolites of choline participated in the methylation of homocysteine to methionine, which plays a key role in the As methylation (Zeisel and Blusztajn, 1994; Zeisel, 2004). Vahter and Marafante (1987) reported that diet-deficient of choline deplete the
Ticylation capacity of As. Tice et al. (1997) suggested that choline-deficient diet ceased the methyl-group donor capacity result in DNA damage. Supplementation of choline normalized the QT interval abnormality and modulates prolonged action potential duration induced by As exposure (Sun et al., 2006). An epidemiological study suggested that diet may also be important determinant of methylation. Dietary intake of choline along with cysteine, methionine and racin modulate the negative health effects due to As in drinking water (Heck et al., 2007).

Several studies found a positive correlation between the dietary supplementation of naturally occurring nutrients and the intervention of the toxicity of environmental toxicant including the heavy metals (Nandi et al., 1997). Towards the mitigation of As toxicity, naturally occurring Aloe Vera and Moringa oleifera were administered against As induced oxidative stress and the ameliorative effects were observed (Gupta and Flora, 2005a; Gupta et al., 2007).

An ancient Indian medicinal system "Ayurveda" deals with the medicinal properties of the naturally occurring plants and plants product. These plants have indigenous active ingredient, which are used for the remediation of the disorders. International recognition of the functional food has suggested that the functional foods are the food that provides health benefits beyond the basic nutrition to improve the physiological function of the body system (Arai et al., 2002). The functional food – jaggery is a natural product of sugarcane, which is used in Ayurvedic medicine as well as a part of daily food. More importantly, it has the efficiency to improve the pulmonary defense system countered by particulate matter and smoke (Sahu and Saxena, 1994).

BALF analysis is a method to investigate respiratory damage in toxicological studies. BALF analysis is also an early indicator of cytological and
biochemical cellular response of lungs (Henderson, 2005). A morphometric analysis of the AM showed significant changes in cytoplasm area and nucleus area in As treatment group in the comparison to the control. Whereas simultaneous supplementation of jaggery and choline, depleting the ratio of nucleus and cytoplasm area. The histopathological observations suggested that subcutaneous injection of As is potentially effective for the enhancement of the malformation of the normal architecture of lung. As exposure shows hyperplasia in atypical bronchiolar epithelium with characteristic of epithelial cells detaching from the epithelium and the parenchyma showing thickened alveolar septum with large number of alveolar cells. While, administration of jaggery along with choline shows reduction in hyperplasia in bronchiolar epithelium with less number of cells and near to normal lung parenchyma. These ameliorating effects of jaggery against As toxicity might be due to its beneficial contents. Earlier, it was suggested that a normal choline diet (not in excess) and regular consumption of whole sugar jaggery may help in the prevention of occupational respiratory disorders (Sahu and Paul, 1998).

The mitigation of toxicity due to contamination of As in drinking water is the primary aim, because millions of population especially persons belong to low-socioeconomic status at risk of various adverse health effects including cancers and have not alternative safe water resources and remedial actions. The result showed that As seems to be very toxic by destroying the normal structure of lung, while supplementation of jaggery and choline significantly recover the effects of As-toxicity. Constituents of jaggery which help to recover the adverse effects by inhibiting the toxic mechanism of As. The continued consumption of jaggery can be beneficial and have preventive measures against As induced toxicity to the effected population.

600 x; Giemsa Stain.
Figure 12. Morphometric analysis of the BLAF cells- alveolar macrophages showing the changes in the cell area, cytoplasm area and nucleus 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 13. 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 14. Mice exposed to Arsenic (Arsenic trioxide); Lungs at 30 days showing hyperplasia in atypical bronchiolar epithelium with characteristic of epithelial cells detaching from the epithelium (a); Collection of hyperplastic broncho epithelial cell in nodular form (b); the lung parenchyma showing thickened alveolar septum with large number of alveolar cells and occasional collection of cellular debris, cells in alveoli (c); Mice exposed to Arsenic + Choline Chloride + Jaggery; Lungs at 30 days showing reduction in hyperplasia in bronchiolar epithelium (d) and the cellular debris are shown in the blood vessels of the lung parenchyma (e); near normal lung parenchyma showing prevention due to Choline Chloride and Jaggery (f). H&E 40x.