CHAPTER 1

INTRODUCTION

Arsenic is a naturally occurring metalloid trace element with potent toxic and mutagenic effects (Klein et al., 2007). It is present ubiquitously in the environment and is released from both natural and man-made sources (Erbanova et al., 2008). It is a natural component of the Earth’s crust, generally found in trace quantities in all rock, soil, water and air.

Arsenic can exist in many different chemical forms in combination with other elements. It commonly occurs in pentavalent As (V) and trivalent As (III) forms. As (III) is found to be relatively more toxic when compared to As (V) (Aposhian, 1989). The acute toxicity of arsenic at high concentrations has been known about for centuries. It was only relatively recently that a strong adverse effect on health was discovered to be associated with long-term exposure to very low arsenic concentrations. Drinking water is now recognised as the major source of human intake of arsenic in its most toxic (inorganic) forms. Inorganic arsenic, the form found in soil, water and crops, is classified by the US Environmental Protection Agency (EPA) as a Group A human carcinogen meaning that sufficient knowledge exists to substantiate a causal relationship between human exposure and cancer occurrence (NAS, 1999). Incidents of arsenic contamination in the ground water have been reported from widespread areas throughout the world such as Taiwan, Mexico, Chile, Argentina, Thailand, Bangladesh, Poland, USA, Canada, Hungary, Japan and India.

Humans can be exposed to arsenic through the intake of air, food and water (Tchounwou et al., 1999). Arsenic in ground water is becoming an arising issue in the water supply and health sectors of various parts of India. In rural areas of Assam high
percentage of peoples depends on hand pumped tube wells and wells for drinking purpose. The most common pathway of environmental exposure to inorganic arsenic worldwide is through drinking water. Drinking water poses the greatest threat to public health from arsenic (Hindmarsh and Mc Curdy, 1986; Cui and Liu, 1988). Arsenic is distributed throughout the earth’s crust and is introduced in to water through the dissolution of minerals and ores. Arsenic is known water contaminant raising big health issues in India and world over (ATSDR, 2005).

Hundreds of millions of people, mostly in developing countries, daily use drinking water with arsenic concentrations several times higher than the World Health Organization (WHO, 1996) recommended limit of 10 millionths of a gram per litre of water (10 µg/L). The full extent of the problem and related consequences are at present unclear, given the long time it takes for visible symptoms of arsenic related diseases to develop and the similarity of symptoms with those of other diseases. However, the effects of arsenicism are serious and ultimately life-threatening, especially as the long term ingestion of arsenic in water can lead to several forms of cancer. The most serious damage to health has taken place in Bangladesh and West Bengal, India. In 2000, a WHO report (Smith et al., 2000) described the situation in Bangladesh as: “the largest mass poisoning of a population in history … beyond the accidents at Bhopal, India, in 1984, and Chernobyl, Ukraine, in 1986”. UNICEF estimates that 12 million people in Bangladesh were drinking arsenic contaminated water in 2006, and the number of people showing symptoms of arsenicism was 40,000, but could rise to one million (UNICEF, 2006).

Chronic exposure to arsenic causes numerous toxic effects and is classified as Group I carcinogen in humans (IARC, 1987a). Arsenic compounds during their metabolism in cells generate reactive oxygen species (ROS). These ROS contribute to the pathogenesis of various acute and chronic liver diseases (Sollott et al., 2006) and also cause cellular damage. The greater number of populations who had a history of relatively long periods of arsenic exposure confirmed higher incidences of
Chromosomal aberrations (Ostrosky-Wegman et al., 1991), Sister chromatid exchanges (Hsu et al., 1997). Arsenic can induce DNA damage in multiple test systems (Schaumloeffel et al., 1998). There are reports regarding increases in liver cancer mortality in childrens who had exposure to high concentrations of arsenic in drinking water starting soon after birth (Liaw et al., 2008). Reductions in birth weight have been found in a low-arsenic-exposure study in Chile (<50μg/L) (Hopenhayn et al., 2003). High levels of hydroarsenicism have been associated with cardiovascular diseases, developmental abnormalities, neurologic disorders (Rao and Avani, 2004), diabetes, hearing loss, hematological disorders, Black foot disease and cancers (Florea et al., 2005) including bladder cancer (Bates et al., 1992).

Arsenic genotoxicity has been analysed extensively in a wide range of in vivo and in vitro studies and the overall conclusion is that there is a clear induction of genotoxic effects, including an increase in micronucleus (MN) frequency and a decrease in the proliferation index that reflects its toxic potential (Hayakawa et al., 2005; Mandal et al., 2004). In vitro and in vivo studies on DNA damage induction seem to indicate that arsenic acts indirectly by inhibiting DNA repair (Gebel, 2001 and Kitchin, 2001). After the arsenic calamity came to be exposed the WHO has lowered the upper limit for arsenic content in drinking water from 50 μg/l to 10 μg/l (WHO, 1996). Arsenic-induced genotoxic effects are implicated in carcinogenic outcomes (NRC, 1999). Chronic ingestion of inorganic arsenic has been related to increased incidence of skin, bladder, liver and kidney carcinomas, while inhalation of arsenic causes lung cancer (IARC, 1987b; Abernathy et al., 1999; Goering et al., 1999). The exact mechanism of arsenic-induced carcinogenicity still remains elusive; however, it acts as a clastogen, inducing the formation of chromosomal aberrations and micronuclei in animal and human systems (Basu et al., 2001). The metabolism of arsenic has an important role in its toxic effects. Many, but not all, mammalian species methylate inorganic arsenic (Vahter, 1994).
Antioxidants are known to effectively protect against arsenic-induced oxidative stress and apoptosis (Sarkar et al., 2003; Pal and Chatterjee, 2004). It is also a well known fact that arsenic exposure leads to inhibition of the antioxidant defense mechanism of the body (Zarazua et al., 2006). Reactive oxygen species generated in response to arsenic exposure leads to oxidative stress and accumulation of intracellular hydrogen peroxide (Chen et al., 1998; Bernstam and Nraigu, 2000). The enzymatic and non-enzymatic antioxidants also get reduced as a result of subsequent free radical generation (Flora, 1999; Shila et al., 2005). The free radical species thus generated lead to DNA damage and may act as a tumor promoter or apoptotic inducer depending upon the level of exposure (Waalkes et al., 2003). Arsenic-induced oxidative stress has been studied for different cancers and disorders in humans, but the precise mechanism of arsenic-induced endocrine disruption is yet to be elucidated.

Hence, an increase in the intracellular antioxidant levels may have therapeutic effects on arsenic-induced oxidative genotoxicity (Sinha et al., 2010). Vitamin C is known to be an antioxidant and is able to scavenge free radicals.Vitamin C is the most widely cited water-soluble antioxidant and prevents oxidative damage to the cell membrane induced by radicals in the aqueous environment (Monteiro and Stern, 1996). Vitamin C proved to be as efficient drug against arsenic mediated toxicity (Panneerselvam et al., 2005).

In the light of the above discussion, the proposed study was undertaken with following objectives:

**Objectives:**

1) Collection of ground water sample from tube wells from Cachar, Karimganj and Hailakandi district of Barak valley region of Southern Assam and estimation of arsenic content in ground water.
2) To study the arsenic content in human biological samples i.e. hair and nails from the arsenic exposed individuals and also control individuals.

3) To determine micronuclei (MN) frequencies and other nuclear abnormalities in exfoliated buccal cells from the arsenic exposed individuals through arsenic contaminated water and control populations.

4) To evaluate the genotoxic potential of arsenic trioxide, sodium arsenite and sodium fluoride \textit{in vivo} in murine test system and protective effects of vitamin C.

5) To evaluate the genotoxic potential of arsenic trioxide and sodium arsenite \textit{in vitro} in human lymphocytes and mitigating effects of Vitamin C.

6) Level of glutathione (GSH) content in arsenic trioxide treated mice \textit{in vivo} and protective effects of Vitamin C.

7) Lipid peroxidation level as other toxicological parameter for evaluation of cellular dysfunction due to oxidative stress in arsenic trioxide and sodium fluoride and ameliorative effects of Vitamin C.