Chapter 1

Introduction

1.0. INTRODUCTION

Arsenic (As) is familiarly well known as the "Poison of Kings and the King of Poisons" (Vahidnia et al., 2007). Arsenic is placed in Group 15 with atomic number 33 and atomic mass 74.92 of the periodic table. Arsenic chemically termed as a metalloid has a unique property of both metals and nonmetals. Arsenic draws the worldwide attention from researchers because of its adverse impact on the human biological system.

1.1. Different forms of arsenic:

The oxidation states of arsenic are (-3), (0), (+3), and (+5). The predominant oxidation states of arsenic are arsenite (As^{III}) and arsenate (As^{V}) in reducing and oxygenated conditions (WHO, 2001; IARC, 2004). Arsine gas, organic, and inorganic arsenic compounds are the major source of arsenic in the environment. Arsenic in its inorganic state exists in trivalent arsenite and pentavalent arsenate form. Arsenobetaine acid, arsenilic acid, and methyl arsenic acid, dimethyl arsenic are the common forms of organic arsenic (WHO, 2000). Inorganic trivalent arsenic has a more toxic effect than that of inorganic pentavalent arsenic and organic arsenic (Andrewes et al., 2004, Yousef et al., 2008). The reduced form of pentavalent arsenic (arsenate) produces trivalent arsenic (arsenite) in the presence of methyltransferase enzyme and co-factors S-adenosylmethionine (SAM) and glutathione (GSH) and thereby promotes the production of monomethylarsonous (MMA) and dimethylarsinous (DMA) as of end metabolites (Mandal and Suzuki, 2002). Arsenic and its compounds cannot be ruined because of their portable properties in the environment. The different forms of arsenic can be transformed or separated by the interaction with oxygen or other molecules present in nature and lived bacteria (Fergusson, 1990).

1.2. Sources of arsenic:

Arsenic is liberated from mines, smelters, agricultural sources (pesticides or fertilizers), etc. to contaminate the drinking water (Matschullat, 2000). It is also commonly found in soil, water, and food (Nuntharatanapong et al., 2005). Natural calamities like rock erosion, volcanic explosion, and forest fires also release this metalloid to the environment. Three twenty minerals or more are enriched with arsenic. Arsenopyrite (FeAsS), Orpiment (As_2S_3), Realgar (As_2S_2), Pyrite (FeS_2) are the most common minerals where arsenic present in solid solution (Nickson et al., 2000; Kumaresan and Riyazuddin, 2001). The minerals that contain arsenic are an important source of arsenic present in natural water (Korte, 1991).

1.3. The concentration of arsenic in different areas:

Extremely rural areas contribute 0.02 $ng.m^{-3}$ to 4 $ng.m^{-3}$ average concentration of arsenic. The ambient concentration of arsenic in urban areas shown to have a range of 3 $ng.m^{-3}$ to 200 $ng.m^{-3}$ of arsenic. Industrial value very often goes up to 1000 $ng.m^{-3}$. Ocean water arsenic ranges between 1 $\mu g.l^{-1}$ to 2 $\mu g.l^{-1}$. In the river and lakes, the concentration of arsenic is below 10 $\mu g.l^{-1}$ but near anthropogenic sources, the concentration in water is increased up to 5 $mg.l^{-1}$ (IPCS, 2001). The acceptable limit of arsenic in drinking water is 0.01- 0.05 ppm and as high as 68 ppm arsenic in groundwater declared by World Health Organization (WHO) (Hundal et al., 2007; Bhatt, 2012). In rice, the limit of arsenic is 0.08-0.2 ppm (Zavala and Duxbury, 2008). Trioxide form of arsenic is used in food preservatives (Baxley et al., 1981).

1.4. Worldwide distribution of arsenic:

The arsenic-contaminated groundwater can cause several health hazards of people throughout the world. Taiwan (1961-1985) is well known for the contamination of arsenic in well water. Consumption of artesian-well water containing 0.10-1.8 ppm of arsenic caused arsenism and

black-foot disease in 100,000 population of Taiwan (Lu, 1990). From the year 1959 to 1983, a large number of population in Antofagasta, Chile, and Northern Mexico were affected by higher arsenic ingestion up to the range of 0.41 ppm-0.8 ppm arsenicated drinking water (Borgono and Greiber, 1971; Cebrian et al., 1983). The 200000 people in Mexico were chronically exposed to higher than 0.05 ppm of arsenic. The impression of chronic arsenic poisoning in the skin was shown in 21.61% population of these exposed inhabitants (Cebrian et al., 1983). In northern Argentina, arsenic toxicity leads to arsenical skin disease and cancer via arsenicated drinking water of 0.1 mg l^{-1} concentration. The incidence in that region is known as "the illness of Bell Ville (Cordoba)" (Astolfi et al., 1981). Groundwater trifling in Minnesota, USA (Feinglass, 1973), Ontario, Canada (Wyllie, 1937), Nova Scotia, Canada (Grantham and Jones, 1977), New Zealand (Ritchie, 1961) and Nakajo, Japan (Terade et al., 1960) also caused arsenic poisoning among the small group of population.

1.5. Arsenic contamination in India:

In Asia, arsenic affected nations are Bangladesh, India, China (Xia and Liu, 2004). Many areas located in the Ganges plain of Bihar, Jharkhand, Uttar Pradesh, and West Bengal are affected by arsenic with thousands of suffering people along with millions of people at the risk zone (Chakraborti et al., 2004). Arsenic can cover West Bengal's deltaic plain from the source of arsenic-rich sediments in Chotonagpur Rajmahal Highlands (Acharya et al., 2000). The Ganga Brahmaputra delta basin of West Bengal has an increased concentration of arsenic in groundwater (Mukherjee and Bhattacharya, 2001). In West Bengal South and North 24- Pargana, Murshidabad, Malda, and Nadia districts are mostly affected by groundwater arsenic. The arsenic affected zones are present inside the upper plain of delta and southeastern part of delta in

the mouth at shallow depth made up of sediments deposited by meandering streams and levees (Talukdar et al., 2009).

1.6. Arsenic toxicity in plants:

The pentavalent form of arsenic (As^V) is taken by the plants. In plants, significant stress, retarded growth (Stoeva and Bineva, 2003), physiological dysfunction (Stoeva et al., 2005a), and ultimate death occur due to As^V exposure. Arsenic uses phosphate as an analog and enters the phosphate transport system to cross the plasma membrane (Stoeva and Bineva, 2003), and finally interfering the metabolic processes of the plants. Cytoplasmic As^V gives effective toxicity to the plants. In cytoplasm, As^V is reduced to As^{III} (Meharg and Hartley-Whitaker, 2002; Stoeva and Bineva, 2003). There is a crucial difference in cell types of plants named as hyperaccumulators and non-accumulators in response to the exposure at a higher levels of specific As species. The non-accumulators hold arsenic in root cells and shoot cells with lesser concentration where the hyperaccumulators contain arsenic at higher concentrations in the aerial tissues and root. (Zhao et al., 2009, 2010; Zhu and Rosen, 2009; Mendoza-Cózatl et al., 2011).

1.7. Arsenic toxicity on animals:

Arsenic compounds develop higher toxicity in rats than that of mice (Harrison et al., 1958). Turkeys and dogs have shown more toxicity in response to 3-nitro-4-hydroxyphenylarsonic acid than that of chickens and rats (Kerr et al., 1963). Variable strains of the mice have different abilities to develop toxicity according to the difference in the nature of arsenic compounds. Testing arsenic toxicity in different species and strains of laboratory animals as an analytical model has imperative implications for a human response (Harrison et al., 1958). The undissolved compound of arsenic was less toxic than the dissolved solution of arsenic trioxide (Schwartze, 1922). The livestock in arsenic-contaminated areas is exposed to arsenic by

drinking water vegetables, rice plants, and feed materials. These animals retained a higher concentration of arsenic in blood, urine, stool, hair, tissues of animals. Environmental pollution of arsenic also causes by the use of cow dung for domestic and agricultural purposes (Pal et al., 2007).

1.8. Arsenic exposure and carcinogenesis:

Inorganic arsenic at 50 ppb in water is co-related with the risk of cancerous death up to 21/1000 population (Bates et al., 1992). There is sufficient evidence found in human carcinogenicity that arsenic is considered as a class I human carcinogen (International Agency for Research on Cancer, 2004). Ingestion of arsenic leads to cancer of different organs and a system like skin, viscera, lung, liver, kidney, bladder, and prostate (Marshall et al., 2007). Frequent skin neoplasia is associated with arsenic intoxication along with fatal lung cancer (Smith et al., 1992). A higher concentration of arsenic (>250μg/L) is associated with liver cancer (Wang et al., 2014). Inorganic arsenic intoxication in the fetus of mice can produce tumors in the liver and other organs in their adulthood (Waalkes et al., 2007). Liver tumors are potentiated by the methylated form of arsenic (Wanibuchi et al., 2004). In mice, a variety of internal uterine tumors can be produced by ingestion of chronic arsenic (Waalkes et al., 2014). Arsenic polluted drinking water could interfere with the DNA repair system about carcinogenesis in humans (Andrew et al., 2006). The DNA repair system is blocked by the carcinogenesis of arsenic via epigenetic modification (Tokar et al., 2010; Salazar et al., 2010). Studies report that arsenic encourages the mutation of tumor suppressor gene P_{53} (TP53) resulting in the increased risk of bladder cancer (Kelsey et al., 2005).

1.9. Toxicity of arsenic in different organs:

Due to its protoplastic poison nature, arsenic affects cells' sulphydryl group and interferes with the enzymes of cells, cell respiration, and mitosis (Gordon and Quastel, 1948). In humans, long term oral inorganic arsenic ingestion at the doses of 0.05-0.1 mg/kg bw/day caused neurological and haematological toxicity, but rats, dogs, and monkeys had shown no effect following exposure of arsenite or arsenate at the doses of 0.72 to 2.8 mg/kg/day (Byron et al., 1967). Mucous membrane irritation, laryngitis, bronchitis, rhinitis, and tracheobronchitis are found in affected people due to the inhalation of arsenic dust or fume in humans. This produces sore throat, stuffy nose, hoarseness, and chronic cough, etc (Dekundt et al., 1986). Blood vessels or the heart are damaged by a long time arsenic exposure. Arsenic at the dose of 0.6 mg/l, when taken by children, cause myocardial infarction and arterial thickening (Zaldivar, 1974). The intoxication of short-term and long-term arsenic resulted in cardiac arrhythmias with myocardial depolarization as the causative factors of heart failure (Goldsmith and From, 1986). Acute voluntary huge arsenic exposure is the cause of hyper-contracted fibres in muscles dominated by mitochondrial abnormalities and myofibrillar disruption (Fernandez-Sola et al., 1991). Gastrointestinal irritation is characterized by several abdominal ailments starting from thirst, nausea, and painful swallowing due to acute arsenic ingestion (Campbell and Alvares, 1989; Goebel et al., 1990). Arsine at the dose of 10 ppm promotes death within hours due to hemolytic incidence (Sittig, 1985). This type of effect occurs in a few weeks after the ingestion of low levels of arsenic at the dose of 0.5-5.0 ppm (ACGIH, 1986). In humans, bone marrow depression takes place from high doses of arsenic (EPA, 1984). Acute and chronic oral arsenic intoxication introduced haemolysis, anemia, and leucopenia (Glazener et al., 1968, Lerman et al., 1980) and erythropoietic suppression (Kyle and Pearse, 1965). In humans, liver disease is common due to

arsenic ingestion. Chronic exposures of arsenic over months or years accumulate arsenic significantly in the liver (Clarkson, 1991). The clinical symptoms of the hepatic problem were bleeding of esophageal varices, ascites, jaundice, or engorged liver tenderness. Following the intake of Fowler's solution (arsenic-containing medicines) hepatic injury was also introduced. The liver was puffy and tender (Mazumdar et al., 1988), and the level of hepatic enzymes increased after arsenic intoxication (Franzblau and Lilis, 1989). Mitochondrial injury and impaired mitochondrial functions may be affected by arsenic, and it may affect porphyrin metabolism. Pentavalent form of arsenic converts into a trivalent form or more toxic form in the kidney, which is the most important route of arsenic excretion. Capillaries, tubules, and glomeruli of the kidney are injured by arsenic (Winship, 1984). Broken cells of proximal tubule introduced proteinuria in the urine. Oliguria and mitochondrial damage of tubular cells are produced from arsenic intoxication, but when arsenic poisoning becomes mild to severe; shock and dehydration occur and ultimately develop renal failure. Overcoming this difficulty, dialysis is inevitable (Giberson et al., 1976). Arsenic exerts its effect on the skin such as hyperkeratosis, hyper-pigmented corns on the surface of the soles and palms along with hypo-pigmentation of back, face, and neck (Bickley and Papa, 1989). Winship et al., 1984 reported in their study that arsenic has an adverse effect on the function of the central and peripheral components of the nervous system (Winship, 1984). In humans, neurological damage can be induced by the intoxication of arsenic. This type of damage can be associated with the peripheral neuropathy of motor and sensory neurons with the sign of loss of reflexes, muscle weakness, and numbness, (Feldman et al., 1979). Arsenic at the dose of 1.0 mg/kg/day or more is the reason for encephalopathy that shows clinical symptoms such as hallucination, seizures, lethargy headache, and mental confusion (Dannan et al., 1984).

1.10. Effect of arsenic in reproductive organs:

Arsenic is responsible for introducing severe reproductive health hazards (Pott et al., 2001). Male reproductive toxicity is directed towards testicular degeneration, testicular steroidogenic inhibition with spermatogenic arrestation in response to chronic exposure of arsenic (Sarkar et al., 2003). It is the cause of the loss of mass of testis (Ahmad et al., 2008), necrotic changes occurring in testicular tissue (Mukherjee and Mukhopadhyay, 2009), enormous changes of germ cells (Sanghamitra et al., 2008), atrophy of Leydig cells (Sanghamitra et al., 2008) and diminution of the protein level of the testis (Chinoy et al., 2004). In copper smelting industries, arsenic-induced malfunction of the male reproductive system was found in male workers (Ahmad et al., 2001). Reducing the spermatogenetic enzymes in the male reproductive system, arsenic alters the spermatogenesis process. It can change the spermatogenic meiosis and the post-meiotic stages (Sarkar et al., 2008). Alteration of the testicular function is the outcome of arsenic poisoning and oxidative stress (Shi et al., 2004) with free oxygen radicals (Manna et al., 2008b). Declining the synthesis of testosterone, apoptosis, and necrosis, arsenic introduces the male gonad dysfunction (Shen et al., 2013).

The women affected by arsenic suffer from various pregnancy-associated problems like (Ahmad et al., 2001; Sen and Chaudhuri, 2008), spontaneous abortion, stillbirth, babies are born with low weight, and lactation insufficiency (Ahmad et al., 2001; Milton et al., 2005). Arsenic ingestion in rats caused up-regulation of adrenocortical steroidogenesis followed by down-regulation of ovarian steroidogenesis (Ghosh et al., 1999). Arsenic can induce toxicity of the brain or pituitary or directly on the germ cells. Arsenic has several effects on the female reproductive system, including ovarian follicle degeneration, uterine cell degeneration, ovarian steroidogenesis, continuous diestrous, reduced level of plasma estradiol, and progesterone (Zhang et al., 2000).

After entering into the developing fetus via the placenta, arsenic exerts its toxicity on the development of the fetal brain and postnatal behaviors in developmental biology (Wang et al., 2006). Ovarian sex steroids, estradiol (E_2), and progesterone manage the function of the reproductive tract in a female that is again maintained by gonadotrophins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Ovarian hazards are seen following the intoxication of arsenic (Chatterjee and Chatterji, 2010; Chattopadhyay and Ghosh, 2010). In arsenicated rats, the levels of E_2, LH, and FSH are reduced (Chattopadhyay and Ghosh, 2010). The alteration of uterine histology is found due to the disrupted circulating level of E_2 (Chatterjee and Chatterji, 2010). The severe problems in sustaining normal reproductive function and pregnancy come from these effects. Nevertheless, the accurate mechanism remains unknown. Exposure of chronic arsenic causes increased preterm birth, fetal mortality. Continuous arsenic exposure can be able to change the release of urine and distribution of metabolites, which have effects on fetus development (Hopenhayn et al., 2003). Arsenic is responsible for the apoptosis, necrosis, and loss of fertilized eggs (Chattopadhyay et al., 2002). Downregulation of Dvr1 (decapentaplegic VG related 1) expression is found in arsenic-induced embryotoxicity in zebrafish model. The asymmetric left-right embryo is produced due to arsenic (Li et al., 2012).

1.11. Biotransformation of arsenicals:

After ingestion of arsenic in the body, it is readily absorbed from the gastrointestinal tract to the liver. As^V is reduced to As^{III} in the liver (Gregus and Nemeti, 2002). Then the arsenite is methylated to MMA^V. After methylation MMA^V is reduced to MMA^{III}. MMA^{III} is further transferred to DMA^V and then converted to DMA^{III} (Melak et al., 2014). Using SAM as substrate arsenic methyltransferase (AS_3MT) enzyme contributes a critical role in the methylation of

arsenic in the liver (Thomas, 2007). As^{III} has a high affinity to bind with sulfhydryl (thiols) groups within proteins and non-protein molecules such as glutathione. During this process, arsenic inactivates the varieties of enzymes (Shen et al., 2013). As^{III} is more toxic than As^{V} because of its tendency to hinder the varieties of enzymatic reactions and higher affinity to sulfhydryl (-SH) or hydroxyl (-OH) groups. After arsenic ingestion, some of the inorganic arsenic (8-16%) is released through urine from the body initially. The methylated form of arsenic is excreted as MMA (5-6%) and DMA (79-85%) (Rasheed et al., 2016).

1.12. Arsenic toxicity at the genetic level:

As aneugen lower dose of As^{III} contributes spindle function interference with micronuclei formation along with centromeres and as clastogen at higher doses produces micronuclei without centromeres in human fibroblasts (Yih and Lee, 1999). The high concentration of inorganic arsenic is the cause of rapid micronuclei formation in buccal mucosa cells, exfoliated bladder epithelial cells, and lymphocytes (Basu et al., 2001). Injurious effects of arsenic are seen in animal and human cells including abnormality of chromosome (Rossman et al., 2001), aneuploidy and formation of micronuclei (Wang and Huang, 1994), cross-linking of DNA-protein and sister chromatin replacement (Dong and Luo, 1993), suppression of DNA repair system and amplification of gene (Lee et al., 1988). By suppressing the DNA ligase, arsenite can suppress the DNA repair system (Lynn et al., 1997). Aneuploidy is associated with the disruption of spindle tubulin (Ramírez et al., 2007). Arsenite reduces the activity of DNA ligase in nuclear extracts (Li and Rossman, 1989) where the level of enzyme is not directly affected (Li and Rossman, 1989; Hu et al., 1998). Moreover, arsenite indirectly inhibits the activity of DNA ligase by changing the cellular redox state and influencing signal-transduction as well as protein phosphorylation associated with DNA ligase activity (Hu et al., 1998). By the activation of

NADH oxidase, arsenite produces $O2^{\cdot—}$ radicals. In mammalian cells, arsenite is the reason for DNA strand breaks and huge deletion mutations because of the production of $O2^{\cdot—}$ and its secondary radicals (Lynn et al., 2000). Trivalent forms of arsenic and MMA^{III} is responsible for histone modification during the process of DNA methylation (Zhou et al., 2008).

1.13. Arsenic induced oxidative damage:

During cellular metabolism of arsenic, ROS is generated and damages the cells (Barchowsky et al., 1999). Producing reactive oxygen species (ROS) indirectly or reacting with –SH groups directly arsenic trioxide exerts its toxicity (Chen et al., 1998). ATP and DNA synthesis are hampered by the replacement of phosphate groups following As^{V} treatment. Arsenic produced ROS and free radicals comprising of hydrogen peroxide (H_2O_2) (Chen et al., 1998), hydroxyl radicals species (HO-), nitric oxide (NO-) (Gurr et al., 1998), superoxide anion (O2·—) (Lynn et al., 2000), dimethyl arsinic peroxyl radical [$(CH_3)_2$ AsOO-] and dimethyl arsenic radical [$(CH_3)_2$As-] (Yamanaka et al., 1997, 2001). The breaking of the DNA single-strand is introduced due to these free radicals (Kitchin, 2001). At the initiation and promotion stages of carcinogenesis, ROS may play an important driving factor in cancer development (Shackelford et al., 2000). The gene transcription profiles of human hyperkeratosis are modified by oxidative stress induced by arsenic, which can influence the cancer-relevant pathways including calcium and Wnt/β-catenin signaling pathways (Korswagen, 2006). Exposure of arsenic is associated with oxidative damage (measured as guanine oxidation), which induces skin tumors (An et al., 2004). Exposure of arsenic is associated with oxidative damage that induces skin tumors (Kligerman et al., 2010). Inducing the modification of DNA, protein, and lipid peroxidation ROS can damage the cells. It alters the structure of DNA by accelerating base-pair mutations, rearrangement, deletions, insertions, and sequence amplifications without point mutations

(Rossman et al., 1980). For the beginning of cancer, DNA damage and mutagenesis are responsible due to ROS. Among the ROS, hydroxyl radicals species ($OH^{\cdot -}$) are reacted, but $O2^{\cdot -}$ and H_2O_2 are not reacted with DNA base pairs (Wiseman et al., 1996). Affecting the cytoplasmic and nuclear transduction pathways, ROS can alter the cell signaling response and regulate the gene expression (Lander, 1997). Elevating the transcription of the nuclear factor kappa B (NFκB) and the activator protein 1 (AP-1) (Wijeweera et al., 2001) arsenic induces ROS generation and alters cell signaling and transcription factor binding to DNA (Germolec et al., 1996).

1.14. Therapeutic approach against arsenic toxicity:

Arsenic toxicity treatment and management is a bigger challenge because of the availability of very few chelators-based therapeutic strategies to overcome the global burden of disease caused due to heavy metals or metalloids (e.g., mercury, cadmium, arsenic, lead, etc.) poisoning in patients. These chelation therapies include the use of 2,3- dimercaptopropane sulfonic acid (DMPS) or unithiol or meso 2,3-dimercaptosuccinic acid (DMSA) and 2,3-dimercaprol (British Anti Lewsite). Earlier BAL has been extensively used for arsenic removal through urine in patients suffering from arsenical dermatitis (Luetscher et al., 1946; Carleton et al., 1948). Although intramuscular injection of BAL has proven its arsenic detoxifying efficacy by its high excretory capability of arsenic, it may also cause life-threatening adverse effects (Rafati-Rahimzadeh et al., 2014). A study with radiolabelled arsenite in rabbits has been shown that BAL could redistribute this toxic metalloid to the brain (Aposhian et al., 1984). Unlike BAL, DMSA and DMPS have comparatively milder toxicity with higher therapeutic index and can also be administered orally, though these are most usually delivered through invasive treatment (Aposhian et al., 1984; Kosnett, 2013). Although chelation therapy in chronic exposure to

inorganic arsenic undoubtedly diminishes metal load in organs, there is a paucity of information regarding the prospective and curative effectiveness of these chelating agents in limiting morbidity and mortality rate of arsenic affected the population. Moreover, these chelators are associated with moderate to severe side effects, e.g., the appearance of rashes; nausea, anorexia, leucopenia, and diarrhea (Rooney, 2007; Rafati-Rahimzadeh et al., 2014). Very often painful intramuscular injections, with such side effects are the major constraints to continue this prolonged treatment (Flora et al., 2008).

Treating arsenic toxicity with different antioxidants and herbal products showed effective results in a studied experimental model. *Emblica officinalis* (amla) can diminish the arsenic-induced DNA and hepatic damage (Maiti et al., 2013). Alpha-tocopherol (Vitamin E), an antioxidant has the capability to avert free radicals induced membrane damage mediated (Gurel et al., 2005) and diminish the testicular oxidative stress (Kutlubay et al., 2007). In arsenic exposed mature Wistar rat L-ascorbate (vitamin C) has an important role in maintaining the normal activities of ovary and brain monoamines (Chattopadhyay et al., 2001). Sodium selenite can ameliorate arsenic-induced female reproductive toxicity (Chattopadhyay et al., 2003). Selenium and vitamin E can reduce the cardiotoxicity in arsenic exposed rats (Bhattacharjee and Pal, 2014a). The body burden of arsenic is diminished by spirulina in arsenicosis patients (Momotaj and Hussain, 2001; Islam et al., 2009). Managing the arsenic toxicity in the male reproductive system, the mixture of prebiotics and probiotics is helpful (Mona et al., 2014). *Moringa oleifera* seed extract can decrease arsenic-induced hepatocellular toxicity (Chattopadhyay et al., 2010).

For the management of myocardial illness, hematopoietic disorder, hypertension, and dyslipidemia arjunolic acid, a triterpenoid saponin of *Terminalia arjuna* is useful because of its effective free radical scavenging as well as antioxidative property. Metal chelating property of

antioxidant arjunolic acid protects the organs from metal intoxication (Brahmachari, 2015). Arjunolic acid has shown to be established its cardio-protective effect (Manna et al., 2008a). Elevating the antioxidative defense activities arjunolic acid proved its protective role against arsenic-induced renal toxicity (Sinha et al., 2008a; Khan et al., 2015). Arjunolic acid protects the experimental model animals by reducing oxidative stress through the improvement of the antioxidant level (Hemalatha et al., 2010). By activating the free radical scavenging activity arjunolic acid guards the kidney tissue from arsenic-induced oxidative stress (Vasanthi and Parameswari, 2012; Khan et al., 2015). Arsenic induced oxidative injuries are shown to be protected by arjunolic acid in the murine brain and liver (Sinha et al., 2008b; Manna et al., 2007). Arjunic acid and arjunolic acid have an advantageous role against cancerous growth on the liver and ovarian cells *in vitro* (Saxena et al., 2007). The intrinsic antioxidant property of arjunolic acid has shown to be an established chemo-preventive role at the time of reducing male reproductive toxicity induced by arsenic (Manna et al., 2008b).

Cyanocobalamin (vitamin B_{12}) is antioxidant and water-soluble that helps the brain cells and nervous system for performing normal function and work for the formation of blood. It also helps in the metabolism of every cell of the human body, fatty acid synthesis, DNA synthesis, and production of energy (Ashok, 2014). Vitamin B_{12} and folic acid can maintain the level of lipid profile and metabolic functions in cardiac tissue. These have been shown to develop a cardioprotective role (Bhattacharjee and Pal, 2014b) and oxidative damage in cardiac tissue (Bhattacharjee et al., 2013). Vitamin B_{12} can alleviate arsenic-mediated hepatic DNA fragmentation (Chattopadhyay et al., 2012). Vitamin B_{12} has an imperative function to maintain the methylation process which controls the biotransformation of arsenic as a methionine synthase enzyme (Refsum, 2001) and repairs DNA, and prevents cancer (Fenech, 1999; Wu et al., 1999).

As it is a water-soluble vitamin, the risk of toxicity is very low and is removed from the body via urine regularly (Hathcock, 1997). The risk of cardiovascular disease is associated with an elevated level of plasma homocysteine. By lowering the level of homocysteine vitamin B_{12} protects the body from atherosclerosis and other cardiovascular diseases (Weir and Scott, 1999; Quinlivan et al., 2002). Neural tube defects are allied with the deficiency of maternal vitamin B_{12} (Molloy et al., 2009). For the synthesis of methionine synthase, vitamin B_{12} is the main cofactor and $B_{12,}$ along with folate work jointly for the remethylation of Hcy (Mills et al., 1995). The activity of methionine synthase enzyme is increased by improving the level of vitamin B_{12} intake that converts homocysteine to methionine (Refsum, 2001). Vitamin B_{12} and folic acid alone or jointly have the ability to reduce the hepatic damage (Chattopadhyay et al., 2012), hepatic mitochondrial oxidative stress and dysfunction (Majumdar et al., 2012), pancreatic oxidative damage (Mukherjee et al., 2006) as well as systemic and islet cell mitochondrial dysfunction and DNA damage (Majumdar et al., 2009) in arsenic intoxicated ones. Detoxifying arsenic, vitamin $B_{12,}$ and folic acid can diminish the accumulation of arsenic in the tissue of the testis and decrease the alteration of the antioxidant defense system in the testis of the rat. Vitamin B_{12} and folic acid can reduce the testicular oxidative stress in arsenic ingested rats (Sarkar et al., 2013). In replication, B_{12} plays an important role. Low sperm count is introduced by the deficiency of B_{12}. Henceforth, pernicious anemia occurs and favoring infertility. In the conception of women, supplements of B_{12} have an essential role (Singh and Sachan, 2011).

Considering the above aspects, the present experiments are intended for the management of the arsenic-induced ovarian and uterine disorder with the help of arjunolic acid and vitamin B_{12}. Due to the several disadvantages of the conventional painful, invasive chelating therapies, this study emphasizes the new insights into the development of new strategies which may bring

about value addition to the management and therapeutic interventions for the disease. We have chosen arjunolic acid and vitamin B_{12} both as therapeutic agents against arsenic induced ovarian and uterine toxicity in rats because we were expecting that arjunolic acid may promote its action as antioxidant to nullify arsenic mediated toxicity where as on the other hand vitamin B_{12} may help in the elimination of arsenic by promoting its methylation via S-adenosine methionine pool.