1.0. INTRODUCTION

Arsenic is an element with both metallic and non-metallic character generally termed as a metalloid which exists in trivalent, pentavalent and elemental form. Arsenic poisoning is most common in mineralized groundwater.

1.1. Different forms of arsenic

Arsenic is present in our environment in different forms. Arsenic poisoning is spread mainly in the compound form such as inorganic arsenic, organic arsenic, and arsine gas (Saric et al., 1986). Arsenic exists in the environment as four oxidation states –3, 0, +3, and +5 (WHO, 2003; IARC, 2004). The inorganic form of arsenic compound (As-III) exhibits a more toxic and more complex than the organic arsenic compound As (V) (Vahter, 2002). Researchers have revealed that the trivalent form of the arsenic interacts with the cellular antioxidant enzymes and proteins results in oxidative stress (Kitchin and Wallace, 2008).

1.2. Source of arsenic

One third of arsenic is coming from the natural sources of atmosphere. Another natural source of arsenic is volcanic explosion (Nriagu and Azcue, 1994). Generally arsenic is present in 200 minerals known as arsenopyrite. Arsenic is found in the rock, soil, sediment, and water (Patel et al., 2005). At high concentrations of arsenic are coming from the groundwater (Ravenscroft et al., 2009). Inorganic form of arsenic contaminates ground water as well as drinking water in numerous parts of the world namely India, Bangladesh, China, and other endemic areas (Garelick et al., 2009). It was proposed that the users of arsenic contaminated drinking water has no option of getting safe drinking water and therefore their bound to take arsenic contaminated water from tube wells (Ahmed, 2004). Irrigation of the agricultural land with arsenic contaminated ground is adequate enough to deposit arsenic in different foods (Roychowdhury et al., 2005).

1.3. Global scenario of Arsenic

The arsenic is the most dangerous water pollutant in several countries (Uddin and Huda, 2011). Long term of arsenic-contaminated drinking has been directly linked to various health disorders (Marshall et al., 2007). India, Thailand, Bangladesh, Taiwan, China, Mexico, Argentina, Finland, and Hungary are also affected by this arsenic-contaminated water pollutant (Chappell et al., 1997).

1.3.1. In India and Bangladesh

In India, hundreds out of thousands people near the Ganges, Brahmaputra bay of Bengal are mostly exposed to arsenic (Chappell et al 1997). A good number of people in Bangladesh are slow poisoned following the utilization of arsenic-contaminated drinking water from hand-pumped wells (Uddin and Huda, 2011). In India, millions of people are at a risk due to arsenic-contaminated drinking water (Chakraborti et al., 2002). The eastern part of the Tripura is also highly exposed to the arsenic-contaminated water (Chappell et al., 1997).

1.3.2. Arsenic contamination in West Bengal State

In West Bengal 8 out of 23 districts are heavily exposed to arsenic. People of 58 blocks and 830 villages are affected by using highly toxic arsenic-contaminated drinking water (Chakraborti et al., 2002). It has been observed that people of West Bengal are affected in highly concentrated arsenic of groundwater (Guha et al., 1998). The level of ground water arsenic in the affected regions of West Bengal varies from 0.02 ppm to 0.96 ppm. Majority of the affected areas contains high level of arsenic with 0.4ppm concentration (Mukherjee and Bhattacharya, 2001).

1.4. Toxic effect of arsenic

1.4.1. Arsenic toxicity in animals

Arsenic behaves as a heavy metal poison for the domestic animals. Trivalent form of arsenic is more toxic for domestic animals (Selby et al., 1977). Epidemiological study showed that

arsenic is a carcinogen for animal beings (WHO, 2003). Trivalent form of arsenic is mainly absorbed in the gastrointestinal tract (GIT) (Freeman et al., 1995). Studies reported that oral administration of methylated arsenic is also absorbed through gastrointestinal tract (GIT) of animals (Ellickson et al., 2001; Rahaman et al., 2012). It was observed that arsenic is obtained in the blood of different mammalian species (rat, rabbit, and mice) in a dose-dependent manner (Naranmandura et al., 2007; Marafante et al., 1985; Martinez et al., 2011). *In vivo* experiments explored same dose of arsenic has shown erythrocytes of rats as compared to the rabbits (Lerman and Clarkson, 1983). The other study revealed that, rats and dogs are also affected at 0.72 to 2.8 mg doses per kg per day of arsenic intoxication (Byron et al., 1967).

1.5. Arsenic induced oxidative stress

Arsenic produces the oxidative stress by the generation of hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot-}$), peroxyle radical ($ROO^{\cdot}$), nitric oxide ($NO^{\cdot}$), singlet oxygen (O_2), and dimethylarsinic peroxyle radicals [$(CH_3)_2AsOO^{\cdot}$]. Increased ROS generation damages different cellular system at various concentration of arsenic (Shi et al., 2004; Pi et al., 2003). Oxidative stress is produced due to the arsenic accumulation in the cells by the depletion of enzymes through the lipid peroxidase (Stohs and Bagchi, 1995). Higher level of ROS generation due to arsenic toxicity further induces cytoxicity (Yedjou and Tchounwou, 2007). ROS formation also promotes the formation of fragile DNA (Pannunzio and Lieber, 2017). ROS modifies DNA via several pathways which is closely associated with the DNA damages (Zhang et al., 2016). It breaks the single and double stranded DNA (Caldecott, 2003). ROS formation can lead to disrupt the component of lipid membrane layer (Girotti, 1989). ROS are projected towards apoptosis the way of appreciably cell death (Veljkovic et al., 2011). Higher concentration of ROS generation is contributed in the inhibition of intracellular enzymes like glutathione peroxidase and also glutathione-*S*-transferase (Waalkes et al., 2004).

1.6. Toxicity of arsenic in different organs

Arsenic affects different mammalian organs. Inorganic arsenic compound accumulates in brain astrocytes (Koehler et al., 2014). Chronic arsenic exposure interrupts the mitosis of granule cells (Htike et al., 2016). Earlier study reported that arsenic decreases the normal development of mice cerebellum (Ding et al., 2013). Kidneys are the major part of the body for the arsenic excretion and are also damaged by arsenic poisoning (Schoolmeester and White, 1980). Arsenic may also act on the thrombocytes function to develop cardiovascular disorder. Higher rate of agglutination of thrombocytes has been found following the exposure of trivalent form of arsenic (Lee et al., 2002). Intake of arsenic exposed drinking water elevates arterial thrombus formation and thereby induces cardiovascular diseases (Lee et al., 2012). It was revealed that intake of higher amount inorganic arsenic compound of few milligrams to grams is responsible for severe cardiovascular manifestations like heart failure and hypotension (Zettel, 1943). Chronic arsenic poisoning increases the peripheral vascular diseases. This report also suggested an increased prevalence of the peripheral vascular diseases due to arsenic (Tseng et al., 1961). Arsenic interferes with the function of central nervous system and endocrine system (Lauretta et al., 2019). It is a causative factor behind the neural tube defects of the developing embryo (Shalat et al., 1996).

1.6.1. Toxic effect of arsenic on metabolic system

Liver is the most targeted organs of arsenic (Liu and Waalkes, 2008). A study reported that a higher concentration of arsenic can easily enter into the liver results in an increased hepatic density (Dick et al., 1990). Particularly both inorganic and organic compound are toxic for mammalian liver. Santra et al 2000 suggested that long time of arsenic exposure contributes hepatic fibrosis in an animal model. They established that arsenic poisoning increases fatty infiltration in liver. Chronic exposure of arsenic is linked with inflammatory responses deteriorating, and also the neoplastic changes of liver (Neiger and Osweiler, 1989). Arsenic

exposure exhibited a significant reduction in hepatic glutathione contents and a marginal increase of serum SGOT and SGPT level (Flora et a., 1995). The chronic arsenic ingestion in mice induces liver fibro genesis and also develops mild fibrosis as a result of collagen deposition in extracellular matrix with a high level of hepatic hydroxyproline which is also allied with the Kupffer cell hyperplasia (Sarin et al., 1999). It was revealed that arsenic poisoning causes cancer in different internal organs (Martinez et al., 2011). Continuous intake of arsenic-contaminated drinking water results in a high rate of cancer in various organs such as liver, lung, kidney, etc (Chen et al., 2004). Inorganic arsenic poisoning cause's appearance of tumours in the fetus of mice also affected different organs (Waalkes et al., 2007).

1.6.2. Arsenic exposure and human carcinogenesis

Millions of people are easily exposed through the arsenic contaminated drinking water (Rahman et al., 2018). Bates et al 1992 reported that long term intake of inorganic arsenic contaminated water is increases the risk of cancer mediated death. This study of Bates estimated to be 21 cancerous deaths out of 1000 affected people. Epidemiological studies established that skin cancer of a man is appeared due to inorganic arsenic (Fowler and Weissburg, 1974). Epidemiological studies also confirmed the existence of cancer among the people of Taiwan (Chen et al., 1988) Bangladesh (Kurokawa et al., 2001) and Argentina (Hopenhayn et al., 1998) consumed higher concentration of arsenicated drinking water (150 μg/L). In addition another epidemiological study reported that comparatively lower concentration of arsenicated (≤100 μg/L) drinking water increases the basal cell carcinoma of the people in Hungary, Romania, and Slovakia (Leonardi et al., 2012). It is also reported that dose dependent arsenic intoxication increases the risk of lungs cancer (Ferreccio et al., 2000; Smith et al., 2006). A higher mortality rate of lungs cancer are found in Taiwan patient, who was consumed inorganic arsenic contaminated drinking water for many year (Chiu et al.,

2004). Ingestion of arsenicated drinking water causes the bladder cancer (Moore et al., 2002). International Agency for Research on Cancer (IARC) 2004 has classified the arsenic mediated carcinogenicity. Particularly methylated form of arsenic produces liver tumours (Nishikawa et al., 2002). Arsenic also causes a placental carcinogenesis (Fei et al., 2013). Waalkes et al 2007 explored that arsenic is a uterine carcinogen in the mice. The toxic effects of arsenic produce solid tumour cells such as prostrate and ovarian carcinoma (Zhang et al., 1999).

1.7. Effect of arsenic in reproductive organs

Literature survey so far covered till date published there is a lack of many information about reproduction toxicity against arsenic. Arsenic exposure disrupts the male and female reproductive systems of mice, and rats (Ahmad et al., 2001; Pant et al., 2004). The experiments on animals studies that the reproductive activity like fertility rate is significantly reduced by the consumption of arsenic (Nie et al., 2006).

1.7.1. Toxic effect of arsenic on Male Reproduction

Various studies indicated that arsenic exposure interferes with the male spermatogenic process (Silva et al., 2017). Das et al 2009 described that arsenic contamination affects the reproductive function in male testicular micro environment and subsequently inhibits semen quality. As an endocrine disruptor arsenic impairs the functions of the hypothalamic-pituitary-testicular axis and alters the normal hormonal levels (Lauretta et al., 2019). This further induces the damage of the testicular architecture that resulted in the alteration of testicular growth and sperm production (Silva et al., 2017; Kim and Kim, 2015). Sodium arsenite treatment at doses of 0.6 and 0.8 ppm per 100 g body weight of Wistar strain rats for 13 and 26 days exhibited significant inhibition of testicular steroidogenic activities: Δ^{5}-3β-HSD and 17β-HSD without showing any significant alterations at a dose of 0.4ppm (Sarkar

et al., 1991). Moreover 0.6 and 0.8ppm of sodium arsenite also resulted in a significant reduction in prostate-somatic and seminal vesicular-somatic indices (Sarkar et al., 1991).

1.7.2. Toxic effect of arsenic on Female Reproduction

It is reported that exposure to arsenic induced the female reproductive malfunction (Dash et al., 2018). Female reproductive functions are generally controlled by ovarian sex hormones estradiol and progesterone (Reed and Carr, 2015). These ovarian sex steroids again controlled by the gonadotrophins: FSH and LH. Chattopadhyay & Ghosh 2010 have been reported that LH, FSH, and estradiol levels are diminished in arsenic ingested rats. Generally estradiol maintains the uterine growth (Patil et al., 1998). The recent study has shown arsenic alters the estrogen-signalling pathway (Chatterjee and Chatterji, 2010). Exposure of arsenic also alters the normal pattern of esterous cyclicity. It is reported that arsenic decreases uterine and ovarian growth (Maity et al., 2018). However, exposure to arsenic affects the circulating of estradiol level and disrupts the uterine architecture (Chatterjee and Chatterji, 2010). The ovarian steroidogenic pathway is important for the normal ovarian processes including follicle growth, oocytes maturation, and ovulation (Raju et al., 2013). Exposure to arsenic interrupts the activity of ovarian steroidogenic enzymes: Δ^5, 3β-HSD and 17β-HSD (Jana et al., 2006). Ghersevich et al 1994 reported these two ovarian steroidogenic enzymes are down regulated by the lower level of plasma gonadotrophins. Arsenic possibly lowers the expression of estrogen receptor and as well as estrogen receptive genes (Chatterjee and Chatterji, 2010). For the duration of pregnancy arsenic is also accumulated in placenta and women suffer from various pregnancy related difficulties (Golub et al., 1998). Intake of high dose of arsenic through drinking water during pregnancy time reduces birth weights with increased fetal loss (Hopenhayn et al., 2003). Hopenhayn et al 2003 also reported that arsenic can disrupt the fetus development. Arsenic exposure is responsible for the loss of fertilized

eggs (Chattopadhyay et al, 2002). The sexual maturation time of female offspring is also reterded by arsenic exposure (Rodriguez et al., 2015).

1.8. Biotransformation of arsenicals

Liver is the most important and targetive organ for higher rate of arsenic methylation (Styblo et al., 2000). When arsenic enters into the body it is easily passed into the liver for the methylation process (Thomas et al., 2004). Arsenic metabolism in the liver appears through the reduction and oxidation of the methylation process (Marafante et al., 1985). The toxicity of MMA and DMA are arsenic metabolic products (Vahter and Concha, 2001). The pentavalent forms of MMA^{v} and DMA^{v} have lower toxicity and it is easily excreted through the urine (Gebel, 2002). Whereas trivalent forms intermediate products MMA^{III} and DMA^{III} exhibit more poisonous effect than that of inorganic arsenic compound (Thomas et al., 2001; Kitchin, 2001). In general As V converted into the As III during the arsenic metabolism in liver known as first methylation process. In secondary methylation MMA is methylated into DMA (Marapakala et al., 2012). MMA also acts as a substrate for the secondary methylation (Li et al., 2011). In mammals the inorganic form of arsenic is methylated via the SAM dependent arsenic methyltransferase (Hamdi et al., 2012). SAM plays a crucial role as a substrate for the arsenic methylation process in liver (Vahter, 1999). The methyltransferase enzyme catalyzes and transfers the methyl group of SAM to trivalent form of arsenic (Ajees et al., 2012). This trivalent form of arsenic has a higher affinity for the sulfhydryl (-SH) (Shen et al., 2013). During this methylation process arsenite binds with the sulfhydryl groups and directly interacts with the enzymes, proteins, and glutathione (Lu et al., 2007). It has stronger protein-binding capacity than the inorganic arsenic (Summers, 2009). After the methylation process arsenic is rapidly excreted through the urine (Chung et al., 2014).

1.9. Background: Therapeutic approach against arsenic toxicity

Chelation therapy is one of the important way for the treatment of arsenic toxicity (Kosnett, 2013). The different chelating drugs DMSA, DMPS and BAL have been used to remove the arsenic toxic through urine (Fournier et al., 1988; Mishra et al., 2008; Gubrelay et al., 1998; Guha et al., 1998). These are also used as a chelating agent. Arsenic could be removed through urine using the chelating agent, BAL when patients were suffering from arsenic exposed dermatitis (Luetscher et al., 1946; Carleton et al., 1948). Rafati-Rahimzadeh et al 2014 confirmed that BAL has a high detoxifying capacity against arsenic from different organs but has medium to moderate side effects. Vasken et al 1984 observed that treatment of BAL in rabbit against radiolabelled arsenite could extensively redistribute its toxic effect to the brain. However, the chelating drugs have many side effects like abdominal pain, rashes, nausea, and hypertension and altered body temperature (Flora, 2009). Few studies have shown the role of antioxidants on arsenic toxicity as well as female reproductive malfunction. It is explored that different plant parts and its antioxidant properties manage the arsenic toxicity against the different heavy metals (Ratnaike, 2003; Bhattacharya and Haldar, 2013). Gurel et al 2005 have observed that vitamin-E has an antioxidant activity which maintains the free radicals mediating membrane damages. It helps to reduce the oxidative stress in male reproductive organs (Kutlubay et al, 2007). Vitamin-E and selenium have the capability to diminish the cardio-toxicity in arsenic ingested rats (Bhattacharjee and Pal, 2014). Sharma et al 2009 reported that Amla has an immune modulatory activity and metal binding properties. It also diminishes the oxidative stress (Poltanov et al., 2009), reduces the hepato-toxicity and diminishes hepatic DNA damages against arsenic toxicity (Maiti et al, 2014). Jana et al 2018 informed that the seed extract of *Moringa oleifera* maintains the arsenic mediated repro-toxicity. Curcumin is the most important part of turmeric. The turmeric is belonging to the family *Zingiberaceae* (Altenburg et al., 2011). The curcumin powder extracted from the rhizome part of turmeric of *Curcuma longa* (Iqbal et al., 2003). Generally curcumin is used as

a spice, herbal medicine and cosmetics ingredient (Ammon and Wahl, 1991). Curcumin has three main components known as "curcuminoids" such as curcumin (77%), demethoxycurcumin (17%), and bisdemethoxycurcumin (3%) (Bharti et al., 2003). Curcumin has numerous excellent properties such as anti-oxidant, anti-cancer, anti-inflammatory, anti-angiogenic, anti-diabetic activities and anti-proliferative role (Aggarwal and Harikumar, 2009). It is used to reduce the arsenic mediated oxidative stress as it has potent antioxidant activity (Mukherjee et al., 2007). Curcumin is also used to diminish the arsenic induced neurotoxicity in rats (Kaushal et al., 2019). It is widely used for detoxification of metal (Chattopadhyay et al., 2004). Curcumin has a vital role in the arsenic mediated liver (Mathews et al., 2012), kidney (Negrette-Guzmán et al., 2015) and uterine-ovarian (Wang et al., 2017) toxicity. Curcumin helps to reduce the ROS generation (Larasati et al., 2018). It is also used to decrease the uterine free radical generation (MDA and CD level) in rat and mice (Perveen et al., 2019; Wang et al., 2017). Ovarian and uterine toxicity is prevented by increasing expression and activities of enzymatic antioxidant SOD, catalase, and GPx. Curcumin has a free radical scavenger activity and closely associated to increase the reducing antioxidant enzymes activity in mouse ovary against arsenic induced toxicity (Wang et al., 2017). Moreover, the proliferation of granular cells in mouse ovaries are also promoted by the application of curcumin (Wang et al., 2017). Curcumin inhibited the DNA repair enzymes due to arsenic toxicity at protein and gene levels It can be able to diminish the DNA damages induced by arsenic exposure (Roy et al., 2011). Curcumin can prevent arsenic mediated neuro-developmental damages. It helps to prevent the apoptotic neuronal stem cells through the diminution of caspase-3 activities (Jahan-Abadet al., 2017). Curcumin maintains the mitochondrial dysfunction against arsenic toxicity by the modulating of ROS generation in apoptosis (Mukherjee et al., 2007). Curcumin plays a critical role to prevent the cancer of rat tissue (Plummer et al., 2001). Curcumin also diminishes the ovarian ischemic damage via

reducing oxidative stress markers (Sak et al., 2013). It has wide pharmacological effectiveness. It is still not used clinically due to its poor solubility in aqueous solution, poor bio-availability and extremely low half life (Dulbecco and Savarino, 2013). Several methods have already been tried to resolve these problems including curcumin incorporation into the liposome and phospholipids vesicles. In addition, polymer based nanoparticles also overcome the poor bio-availability and solubility in an aqueous solution of curcumin (Umerska et al., 2018). Chitosan is a safe polymer which is used to the invention of nanoparticles (Mohammed et al., 2017). Chitosan or related polysaccharides like β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine has chelating property (chitin) towards heavy metal or metalloid (Espinoza, 2005). Chitosan is a mostly second abundant polymer and also found in nature after cellulose and is found in high concentrations in the shells of crustaceans (Ly et al., 2003) and is widely used because of its nontoxic properties (Dai et al., 2011). Chitosan has a potential action to eliminate arsenic from the biological system (Kartal and Imamura, 2005). Chitosan has been shown to be biologically renewable, biodegradable, biocompatible, and non-antigenic and bio functionalities (Shahidi and Synowiecki, 1991). The Chitosan is efficient for heavy metal scavengers due to the presence of hydroxyl and amino groups (Kumar et al., 2004). To overcome these limitations and several studies have been focusing on polymer-based nanoparticles using the Chitosan (Mohammed et al., 2017). It is one of the methods to enhance curcumin solubility and bioavailability (Younes and Rinaudo, 2015). Sankar et al 2015 have reported that nano-formulated of curcumin has unique antioxidant and chelating properties than the free curcumin against arsenic induced liver toxicity in the rat model. *Momordica charantia* also contains numerous phenolic compounds that acts as potential antioxidant which further stimulate the immunelogical system by activating natural killer cells (Budrat and Shotipruk, 2008). The extracted polysaccharide from *Momordica charantia* has various properties such as antioxidant, anti-

inflammatory, anti-tumour, anti-bacterial activities (Panda et al., 2015). This pectic polysaccharide is helpfull in reducing the ROS generation (Perveen et al., 2019). D-galactose and D-methyl galacturonate of polysaccharide from *Momordica charantia* are proved to be explored the potential antioxidant capacity with splenocytes, thymocytes and macrophage activation (Tiwari and Ram, 2009). *M. charantia* polysaccharide prevents the Intra-cerebral hemorrhage mediated free radicals generation by its antioxidant capability through JNK3 signalling pathway (Duan et al., 2015). Polysaccharide (*Momordica charantia*) elevates the intracellular antioxidant enzymes SOD and catalase as well as reduces the MDA level because of its anti-oxidant capacity (Mohammad et al., 2016). It has found that it could be used as a powerful activator of peroxisome proliferators-activated α-receptor which regulates the expression of the genes (Chao et al., 2003). Our previous studies confirmed that the Pectic polysaccharide from *Momordica charantia* has different positive effects on liver against arsenic-induced toxicity *in vitro* (Perveen et al., 2017). Polysaccharide (*Momordica charantia)* also improves the STZ (Streptozotocin) induced kidney and pancreatic dysfunction (Wang et al., 2019). It reveals that this polysaccharide has remarkable hypoglycaemic effects in the alloxan-mediated diabetic mice (Wang et al., 2013). Hence the present proposal develops to mature an easily available, non-invasive highly effective therapeutic agent or nutraceuticals with chelating property along with an effort to understand the underlying mechanism of sodium arsenite induced female reproductive anarchy for the future benefit of arsenic affected vast population and its possible protection at the preliminary level. This study is planned to emphasize how arsenic mediated reproductive toxicity is managed in the way of establishing the therapeutic efficacy of these nutraceuticals.