CHAPTER-1
INTRODUCTION

1.0. Introduction

Arsenic is often called a heavy metalloid toxicant, ubiquitous element and chemically it is also known as a metalloid. This element is widely scattered in earth's crust as oxides and causes many health hazards.

1.1. Properties of arsenic

Arsenic exists chemically as an intermediate of metal and non-metal (semi-metal) and its atomic mass is 74.92 along with consisting atomic number 33. It presents in diverse groups in various conditions e.g. natural, inorganic with arsine gas. Among trivalent inorganic structures, the most well-known forms are arsenic trichloride, sodium arsenite plus arsenic trioxide, though; arsenates and arsenic acid are generally common among pentavalent forms. The common types of organic compounds are methylarsonic acid, arsanilic acid, arsenobetaine, and dimethylarsinic acid (cacodylic acid) (WHO, 2000). The different oxidative forms are –3, 0, +3, and +5 (WHO, 2001; IARC, 2004). The inorganic trivalent (As^{III}) type of arsenic is furthermore deadly around 10 folds than arsenate (As^{V}) (Chaineau et al., 1990).

1.2. Application of arsenic

Since hundreds of years arsenic and their compounds have been built and utilized commercially for numerous reasons and these are mentioned herewith.

1.2.1. Medicinal application

Till 1970s, arsenicals have been used for the administration and treatment of numerous sicknesses, for example, bronchial asthma, protozoal malady, psoriasis, and leukemia. (ATSDR, 2007).

1.2.2. Agricultural application

Arsenic was utilized like a poison, pesticides and insecticides inside agricultural field. Some of these forms are less toxic which includes lead hydrogen arsenate (Peryea, 1998), disodium methyl arsenate (DSMA) and monosodium methyl arsenate (MSMA).

1.2.3. Industrial application

In trade sectors elemental arsenic is principally utilized for the creation of composite or alloy especially with copper and lead for making of acid batteries. Gallium arsenide, an alloy utilized as wave gadgets, opto-electronic sources, power microwave, and so on. (IARC, 2006).

1.3. Occurrence of arsenic

1.3.1. Natural

Arsenic is a ubiquitous element found naturally, widely distributed and causes various deleterious effects in both humans and animals (Wang et al., 2012). Worldwide arsenic naturally exists in ground water at high concentration and therefore a huge number of people get exposed to this element via consuming a large arsenic contaminated drinking water regularly (Chappell et al., 1997). In environment, it is a mobile constituent that penetrates into the arsenic cycle via reallocation of rock arsenic sulfides into the most dissolved type of arsenic trioxide within ground water (Mandal and Suzuki, 2002). Almost many countries in the globe arsenic present in air, rocks, soil, sediments and metal ores (Aronson, 1994) very often as arsenopyrite form. Another activity like volcanic eruption causes accumulation of arsenic into the surroundings (Wakao et al., 1988).

1.3.2. Anthropogenic

There are lots of manmade activities such as processing of woods, electronic industry, glassmaking industry, chemical weapons, gold mines, pesticides, pharmaceutical processing; all facilitates to the entrance of arsenic within environment (Han et al., 2003) and contributes to contamination of its in high amount. Activities including smelting of ores, industrial wastes materials, mining of minerals predispose the mobilization of arsenic by nature.

1.4. Exposure of arsenic

Generally people get exposed through the consumption of arsenic polluted food plus drinking water (Nordstrom, 2002; Smith and Steinmaus, 2011) or inhalation of arsine gas. After inhalation or intake from diverse sources it is then absorbed and attributed in different organs from blood stream (Akinrinde et al., 2015).

1.5. Worldwide overview of arsenic distribution

Globally millions of citizens get affected with arsenic driven health hazards through the polluted consumable water. Population of several countries in this world including United States, Taiwan, India, Europe, Bangladesh and China rendered to arsenic at mild, moderate or higher concentration from numerous sources (Mahmudur Rahman et al., 2005). Arsenic content in the earth water plus drinking water varies enormously all through the planet. The major affected areas have been ascertained in South America plus South Asia (Hashim et al., 2019). Epidemiological survey revealed that a very higher intensity of arsenic about 750 to 800 μg/l, was instituted in eating water in Chile (Smith et al, 2000).

All over the world, the most affected area of arsenicism is India plus Bangladesh (Bindal and Singh, 2019). About 35 million of citizens in Bangladesh are severely affected with arsenic contained drinking water (Kinniburgh and Smedley, 2001). The potable water contains exceeding arsenic level as per WHO limit in 61 district of Bangladesh (Huq et al., 2020). In India plus Bangladesh, a mass of population around the Ganges-Brahmaputra Delta introduced to arsenic linked health issues mainly from polluted tube well-water. An investigation reported by van Geen et al., 2002, proposed that the well water contains variable limits of arsenic < 5 to 860 µg/L in a specific region (Araihazar) of Bangladesh (van Geen et al., 2002). Ground water containing arsenic ranging from 3700 ppb to 4700 ppb found in various subcontinent areas of India (Chakraborti et al., 2002). In 2012, almost millions of residents in Bangladesh get contaminated with arsenicism via consumable water wherein arsenic remains at advanced level than WHO prescribed limits (UNICEF, 2015). Approximately, 20% deaths in Bangladesh were allied with arsenic driven cancer.

People of many districts inside West Bengal are vastly affected by arsenicism through the polluted drinking water, thereby showed many warning health sign (Guha Mazumder et al., 1998). Around 40 millions of population inside West Bengal enormously exaggerated with arsenic where drinking water restrains of 60 to 3700 µg/l (Acharyya, 2002). Approximately 16.66 million of residents from 8 districts are vulnerable in West Bengal (Das, 2019). In many states of India like middle Ganga Plain, Bihar are suggesting the elevated intensity of arsenic within tube wells and it varies from 50 µg/l upto 300 µg/l and causes diverse health issues among the population (Acharyya et al., 1999; Chakraborti et al., 2003). The areas of eastern part of India like Burdwan and Murshidabad, residents are at high risk with

arsenic polluted drinking water of 50 to 137 µg/l (Nag et al., 1996). Similar statement also illustrated by another author where Burdwan region has the topmost concentration in groundwater and afterwards Kolkata remains in the second position (Sen and Sarkar, 2019).

1.6. Toxicity of arsenic exposure

Arsenic is familiarly known a group-1 carcinogen by the International Agency for Research on Cancer (IARC) (Wei et al., 2016). According to environment protection agency (EPA) and WHO, the admissible limit of arsenic in consumable water is 10 µg/l (EPA, 2001; WHO, 1992) and when this standard boundary is exceeded it becomes injurious to the inhabitants. Actually drinking water mixed with arsenic becomes a key route of exposure for human population. Both organic along with inorganic types are poisonous but prolonged consumption of inorganic variation causes multiple undesirable effects than organic form.

1.6.1. Acute toxicity

The dosage of arsenic (lethal dose) required for acute toxicity may be ranges from 100 mg-300 mg (Schoolmeester and White, 1980). The clinical symptoms of acute toxicity are characterised by the episode of abdominal pain that very often severe (Mueller and Benowitz, 1989), nausea, vomiting and frequent watery diarrhoea. Some other features include haematological abnormalities (Lerman et al., 1980), peripheral neuropathy (Ghariani et al., 1991), acute psychosis, toxic cardiomyopathy along with seizures (Campbell and Alvarez, 1989).

1.6.2. Chronic toxicity

Long-standing or chronic arsenic exposure is more hazardous in nature and consequently leads to multisystem damage and further malignancy. Actually, the toxic property of arsenic is predominantly dependent upon the chemical constituents and oxidation status of that compound. The symptoms of chronic arsenicosis are insidious and mostly associated with the extent of dose and length of its introduction.

1.6.2.1. General manifestation

Prolonged arsenic toxicity has been strongly allied with the incidence of skin lesions with visible hyperpigmentation including uneven distribution or melanosis in the body and often hypopigmented spots (leukoderma) are found (Guha Mazumder et al., 1998). Arsenic induced hyperkeratosis appears in the extremities and noted in the trunk area as well (Mazumder, 2015). Indeed, another type of sin lesion like Bowen's disease is also noticed which has polycyclic and uneven lenticular appearance. This sort of lesion is manifested as pigmented, keratotic and rough in nature. It is supposed that such skin disease probably an intradermal carcinoma (Abernathy et al., 1999).

1.6.2.2. Systemic manifestation

Chronic arsenication not only produces general manifestations, but also causes many systemic problems and leading to the malfunction in the liver, bladder, GI tract, lung and skin (Aposhian et al., 2003). Although diarrhoea is coupled with acute arsenication but in chronic state repeated diarrhoea along with vomiting are noticed (Poklis and Saady, 1990). Assembly of arsenic within the body may cause

neurobehavioral abnormalities include amnesia, puzzlement, changes in behaviour among adolescence (Tsai et al., 2003). A controversy is there between the episode of type II diabetes and contact with inorganic arsenicals. Tseng et al, proposed that the incidence frequency for diabetes is 2-5 fold higher among individual where drinking water contains high concentration of arsenic than the unexposed (Tseng et al., 2002). However, another study revealed no link with arsenic intoxication and diabetes (Nizam et al., 2013). Ingestion of trivalent arsenite for long duration induced cardiovascular disease through thrombocytic augmentation (Lee et al., 2002). It has been accounted by Rahman, that well water containing arsenic enhances the threat of hypertension (Rahman et al., 1999). However, chronic toxicity is directly linked with myocardial injury and cardiomyopathy (Benowitz, 1992).

1.7. Arsenic and oxidative stress

Arsenic toxicity predominantly linked with oxidative stress which is generated by the imbalance of antioxidant and pro-oxidant homeostasis (Shila et al., 2005). This kind of distorted redox balance results in excessive generation of reactive oxygen species (ROS) such as singlet oxygen ($O_2^{\bullet -}$), superoxide ($O_{2.}$), hydrogen peroxide (H_2O_2), peroxyl radical ($ROO^{\bullet}$), dimethyl arsinic peroxyl radicals [$(CH_3)_2AsOO^{\bullet}$] and nitric oxide ($NO^{\bullet}$). These are accounted for lipid peroxidation and cellular oxidative damage (Gong et al., 2015). Among these ROS; it is assumed that hydroxyl radical is critical that directly attacks DNA and proteins to cause injury. Arsenic induced influx of ROS formation occurs especially when hydroxyl radicals form DNA adducts by binding with DNA bases and contributes to strand breakage and ultimate damage (Kumar et al., 2014). Another study by Yamanaka et al, reported that ROS attributed DNA damage is accomplished by the creation of 8-

Hydroxy-2'-deoxyguanosine (Yamanaka et al., 2001). There are innate intracellular enzymes that protect the biological system against oxidative stress following the production of ROS. Among the antioxidant enzymes, superoxide dismutase (SOD) is functional on superoxide radicals ($O_{2.}$) and is transformed to hydrogen peroxide (H_2O_2) (Wang et al., 2013). However, arsenic driven excessive ROS leads to the accumulation of hydrogen peroxide within the cell and thereby the ROS scavenging capability of antioxidant enzymes are reduced followed by lipid peroxidation and damage of cellular macromolecules (Flora, 1999).

1.8. Arsenic mediated carcinogenicity

There are many mechanisms of arsenic that induce carcinogenicity like oxidative stress, hindrance of DNA re-structure, genotoxic injury, signal transduction and epigenetic outcomes. According to International Agency for Research on Cancer (IARC) arsenic is a renowned carcinogenic component and serves as cancer initiator in various organs when exposed through gastrointestinal and respiratory route (IARC, 1980). Literature survey reported that its trivalent type is strongly associated with skin cancer (Rossman et al., 2004) and keratinization. Besides, lung cancer is also caused by arsenic intake. Moreover, epidemiological studies have been revealed the dose-dependent response of this constituent with lung cancer (Ferreccio et al., 2000; Smith et al., 2006). A study carried out in Taiwan and Bangladesh has shown that intake of arsenic containing drinking water is further accompanied with bladder cancer (Chiang et al., 1993). Indeed, cancer found in liver has been robustly connected with arsenic exposure as liver is the main metabolic dock of arsenic (Perveen et al., 2017). Earlier study by Lin et al, observed that in case of both genderwise exposures to arsenic more than 0.64 mg/L induced hepatic malignancy

in Taiwan (Lin et al., 2013). A study in Native Americans found that even very minimum to medium dose of its inorganic variety increased the frequency of mortality resulted from prostate cancer (Garcia-Esquinas et al., 2013). A high incidence rate of leukemia among pregnant women was observed as a consequence of arsenic invasion which was confirmed through a study in California (Heck et al., 2014). A study by Maiti et al, explored that arsenic directed the development of palmoplantar hyperkeratosis, one type of brownish-black patch noted all over the body which perhaps led to carcinoma formation in the villagers of Estern India (Maiti et al., 2012). Nevertheless, arsenic is regarded as an efficient carcinogenic agent and instigates tumor formation (Liu and Waalkes, 2008). *In vitro* study affirmed the apoptotic cell death where cytotoxicity was aggravated in mouse sertoli cell with arsenic (Kim et al., 2011).

1.9. Arsenic induced inflammatory response and cellular apoptosis

In normal cells, arsenic can stimulate the activity of NF-κB (Liu et al., 2014). Arsenic inducible oxidative stress following the generation of ROS leads to DNA hypomethylation and DNA damage as we previously discussed. Actually, NF-κB is ascertained as stress receptive transcription factors which become activated in the existence of DNA damage. Consequently it translocates from cytoplasm into the nucleus and activates several downstream genes which in succession initiate inflammatory responses (Jing and Lee, 2014). Arsenic initiated activation of NF-κB further stimulates the expressional activity of other inflammation triggered genes like TNF-α, IL-6, iNOS etc (Sun et al., 2017). Moreover, arsenic sensitizes ROS to be focused it as cellular apoptotic factor. Under oxidative stress condition mitochondria acquires stimulation by ROS which further triggers apoptosis due to

the over expression of Bax (pro-apoptotic protein). In due course of arsenic aggravation, the ratio of Bax/Bcl-2 is being altered and favouring the apoptosis process. Actually, arsenic up-regulates the manifestation of Bax while down-regulates the Bcl-2 (anti-apoptotic protein) (Sun et al., 2018).

1.10. Arsenic and reproductive hazards

Various epidemiological studies disclosed that arsenic potentiates developmental and reproductive hazards suggesting the threat to inappropriate development of foetus. Male and female reproductive functional properties are suppressed by the higher propensity of arsenic for both human beings and non-humans (Kim and Kim, 2015; Mondal et al., 2013).

1.10.1. Arsenic and male reproductive hazards

Previous study documented that introduction to arsenic causes dysfunction of male reproduction significantly including erectile dysfunction, poor sperm quality and infertility (Meeker et al., 2010; Hsieh et al., 2008). Earlier report by Chang et al, described that the weight of testes and epididymal sperm count were significantly diminished when male mice was arsenicated via containing consumable water at the dose of 20 or 40 mg/L for 5 weeks (Im Chang et al., 2007). Another investigation proposed that sodium arsenite at the magnitude of 5 mg/L for 4 weeks results in the reduction of testicular plus accessory sex organs' weight and destruction of germ cells and blocks spermatogenic cycle in male rats (Jana et al., 2006). In mice, arsenic treatment interrupts the development of mature sperm that leading to trouble in spermatogenesis (Sanghamitra et al., 2008). Furthermore, in male rat and mice arsenic lowers down the circulatory levels of gonadotropin and testosterone (Pant et

al., 2004; Zubair et al., 2017). The LH along with FSH signaling are decreased and are attributable to the inhibitory role of arsenic on hypothalamic-hypophyseal axis, resulting impaired function of leydig cell and testosterone synthesis (Kim and Kim, 2015). Furthermore, the action of 17β-hydroxysteroid dehydrogenase (HSD) and 3β-HSD were reduced when male mice accommodated with arsenic trioxide at the dose of 0.5 mg/kg orally for 30 days. Actually, these are the key enzymes for sustaining testosterone level (Kabbaj et al., 2003). The spermatogenesis process is inhibited following the exposure of low dose of arsenic while high dose accomplished with the apoptosis in germ cell in response to arsenic driven oxidative stress (Celino et al., 2009). Moreover, arsenic plus SO_2 influenced sperm malformation, interrupted sperm count, and enhanced testicular pathogenesis (Li et al., 2018). In diabetic animals, sodium arsenate suppressed testosterone level which was further hastened decreased quantity of testicular spermatozoa, reduced sperm motility with impaired acrosomal integrity (Souza et al., 2019).

1.10.2. Arsenic and female reproductive hazards

There are lots of theory and evidence about arsenic poisoning and its unfavourable effect on female reproductive functional stability. Both humans plus animals are suffering from arsenic mediated reproductive anomalies. It has been recommended that assemble of arsenic in uterine cells enhanced the possible abnormalities of growing foetus (Vahter, 2009). Amplification with arsenic at high concentration increases the frequency of infant mortality, labour defects and birth defects (Milton et al., 2005; Rahman et al., 2007). Literature survey suggested that spontaneous consumption of arsenicated drinking water with enormous amount during pregnancy causes abnormal pregnancy outcome, pre-mature delivery, inadvertent abortion,

even death of foetus (Chakraborti et al., 2003) whereas, low dose of arsenic was correlated with unusual growth of placenta, uterus leading to low birth weight baby (Rahman et al., 2009). Epidemiological studies revealed that arsenic is acknowledged as a popular developmental toxicant in many animals resulting deformities and growth retardation in foetus which ultimately causes end of foetal life (Golub et al., 1998). It has been substantiated that inorganic arsenic increases the tendency of foetal malformation at early gestation period when given through intravenous (i.v.) along with intraperitoneal (i.p.) route (DeSesso, 2001; Stump et al., 1999). Earlier study by Chernoff et al, proposed that when pregnant mice was exposed to dimethylarsinic acid (DMA^{5+}) orally enhanced the threat of developmental toxicity (Chernoff et al., 1990). Moreover, arsenic treatment with drinking water on pregnant and lactating animals is coupled with the deviation of developmental changes in postnatal period which also affects the offspring (Rodriguez et al., 2002). The functional aspects of female reproduction is regulated by the two important sex hormones namely estrogen and progesterone whereas; the usefulness of these steroids is under the management of follicle stimulating hormone (FSH) along with leutinizing hormone (LH). Arsenic intoxication abrogates the signaling pathway for estrogen in the ovary (Chatterjee and Chatterji, 2010) leading to intermittent ovarian steroidogenesis and also altered the normal histo-architecture of uterus. Disruption of steroidogenic pathway favours the arrestation of follicular maturation in connection with arsenic accounted diminution of oestrogen (Gore-Langton and Daniel, 1990). Arsenic provoked lower level of estrogen possibly and that is attributable to the binding of arsenic with estrogen receptor causing attenuation of estrogen functionality (Du et al., 2012).

1.11. Biomethylation of arsenic

As previously mentioned organic categories of arsenic proposes as little toxic over inorganic ones especially the trivalent ($InAs^{3+}$) form. Deposition of arsenicals in diverse organs implicated several health issues; therefore exclusion of this element has been regarded as a foremost relieving tool. The growing literature study demonstrated that biomethylation or biotransformation is regarded as the best way for urinary abolition of this toxic metalloid (Buchet et al., 1981). This bio-conversion of inorganic type was principally sited in hepatocyte even though other organs have this activity to some extent (Vahter, 2002). Bio-conversion of inorganic variety involves S-adenosyl methionione (SAM) well-known as a universal methyl provider and is catalyzed by the enzyme namely arsenic methyltransferase (Drobna et al., 2005; Lin et al., 2002). In this course inorganic arsenic (InAs) is converted into less toxic metabolites like monmethylarsonic acid (MMA) plus dimethylarsinic acid (DMA) and these are readily excreted through urine (Akter et al., 2005). Generally two imperative steps are there in biotransformation pathway. In the beginning inorganic As^{V} (arsenate) is reduced to As^{III} (arsenite) with the help of enzyme arsenate reductase where GSH acts like reducing mediator (Kobayashi et al., 2005) and form arsenotriglutathione (As^{III}(GS)3) which remain bound with the thiol element of GSH (Thomas et al., 2001). In the next step methylation of arsenite (As^{III}) was catalyzed by arsenite methyltransferase and requires SAM to form MMA^{V}. This trivalent type of arsenic revealed the great affinity towards sulfhydryl group (-SH) of cysteine residue of proteins and peptides (Thompson, 1993). After that MMA^{V} is being reduced and methylated into monomethylarsonous acid (MMA^{III}), afterwards oxidized into dimethylarsinic acid (DMA^{V}) (Le et al., 2000;

Thomas et al., 2001). Next completing this methylation process, arsenic is readily eliminated through urinary excretion.

1.12. Therapeutic management against arsenic intoxication

Managing arsenic intoxication and its associated health implications has become now an urgency all through the world. Most of the residents exposed to arsenication belong from poor socioeconomic position; hence it is quite troublesome to offer the access of arsenic free drinking water to all. Therefore, it is being advised to avoid consumption of arsenicated drinking water, else providing drinking water where arsenic is present below the WHO recommendation as the crucial prevention (Singh et al., 2007). Recently chelation therapy has turn into a fruitful way to have relieves of poisonous heavy metalloid arsenic outside the body and this could be the targets on the way of drug development (Flora and Pachauri, 2010). A metal chelating agent has the capability to solubilise within water and prevent the biotransformation by forming a metal complex which develops into a less toxic component. Among the chelating drugs, British anti-lewisite (BAL) is also known as dimercaprol and is being applied against arsenical dermatitis and this finally enhances the release of arsenic during micturation (Luetscher et al., 1946). Some other chelating agents called meso-2,3-dimercaptosuccinic acid (DMSA), 2,3-dimercaptopropane-1-sulphonic acid (DMPS), derivative of BAL have been very often used for the chelation of arsenic (Kosnett, 2013; Aaseth et al., 2015). However, there were significant drawbacks of utilizing these metal chelating agents. Administration of BAL linked to be neurotoxic and allergic and necessitates painful intra-muscular injection with multiple doses (Andersen, 2004). In contrary DMSA is less toxic than BAL though it encloses the major limitation of incapability to cross the cell covering, thereby unable to chelate arsenic within the cell (Flora, 2009). The

usability of DMPS had no such severe side effects, only some minor things were observed including hypersensitivity, gastrointestinal discomfort, taste impairment, etc (Aaseth et al., 2015). Therefore, treatment of arsenic intoxication with antioxidant and natural plant based product having low or no adverse effects may be an acceptable approach. Likewise, this management strategy has gained attention as a proficient therapy to counteract arsenic intoxication and related significances. Earlier investigation highlighted that extraction obtained from garlic has arsenic chelating characters (Das and Chaudhuri, 2014). Desai et al, verified the fruit extract of *Ananas cosmosus* rich in polyphenols has been endorsed to the arsenic chelating effect (Desai et al 2012). The phytomolecule originated in turmeric plus ginger has antioxidant capability that ameliorates the arsenic exposed undesirable effects and increased the possible exclusion of arsenic from the body in calves (Biswas et al., 2017). In recent times it has been accounted that the seed extracts of *Moringa oleifera* significantly arrested sodium arsenite induced imbalance of ovarian steroidogenesis and also lipid peroxidation accompanied with oxidative stress in Wistar rats (Jana et al., 2018). Another study of Chattopadhyay et al, provided information about the significance of *Moringa oleifera* against hepatocellular toxicity development from sodium arsenite (Chattopadhyay et al., 2011). Prabu and Muthumani, observed that peanuts and soy have been employed successfully as a scavenger of free radical in Sprague Dawley rats when arsenic at a dose of 40mg/kg/day was given. These edible parts are attributable of having isoflavone and biochanin respectively those possess the antioxidant characters and thereby neutralize free radicals (Prabu and Muthumani, 2012). Acharyya et al, signified that vitamin B_{12} plus folic acid has outstanding role in omitting arsenic propagated hepatic DNA breakage, improved antioxidant level in hepatocyte and also

accelerated arsenic removal by their catalytic action (Acharyya et al., 2015). Another observation by Deb et al, has pointed the same output wherein vitamin B_9 and B_{12} both were capable of suppress the sodium arsenite responded uterine anarchy (Deb et al., 2018). The polyphenolic compounds like epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG) of green tea has the capacity to restore uterine redox imbalance in arsenicated rats. The low oxidative enzymes actions in arsenicated rats could be recovered by the oral application with green tea (Dey et al., 2018). Maity et al, have reported that arjunolic acid obtained by *Terminalia arjuna* suppressed the female reproductive tissue damage developed by arsenic when given orally at the quantity of 10mg/kg body weight of Wistar rats (Maity et al., 2018). Perveen et al, proposed that arsenic driven uterine damage and the manifestation of different inflammatory markers where she found raised level of significant markers were significantly corrected by curcumin. Curcumin itself as an antioxidant serves as a safeguard against free radicals during arsenication (Perveen et al., 2019). Different bioactive components like antioxidants, phenolic compound present in *Momordica charantia* from bitter gourd could alleviate infertility following the ingestion with sodium arsenite. Indeed, arsenic provoked activation of apoptosis pathway which was counteracted by peptic polysaccharide (CCPS) extracted from bitter gourd (Perveen et al., 2019). Maiti et al, reported that *Emblica officinalis* (Amla) has the potentiality to dismiss the action endorsed by sodium arsenite on liver. It also diminishes arsenic induced DNA strand breakage and hepatocellular toxicity (Maiti et al., 2014).

1.13. Significance of NAC

N-acetyl cysteine (NAC) is the preacetylated form of amino acid L-cysteine. It also possesses an antioxidant character of glutathione (GSH) and treated as the chief

source of reduced GSH (Pieralisi et al., 2016). This molecule is principally abundant in the onion (*Allium cepa*), the plant of *Allium* species and the estimated amount is 45 mg of NAC/kg (Diniz et al., 2006; Campos et al., 2003). Since 1960 NAC has been considered as a drug thus, it is in the position of 40 in the list of fundamental medicine proposed by WHO (WHO, 2017). It is quickly absorbed when applied orally or via inhalation or through intravenous route and is comparatively less toxic with very low adverse effects (Atkuri et al., 2007). The configuration of NAC is shown below:

Figure 1.0

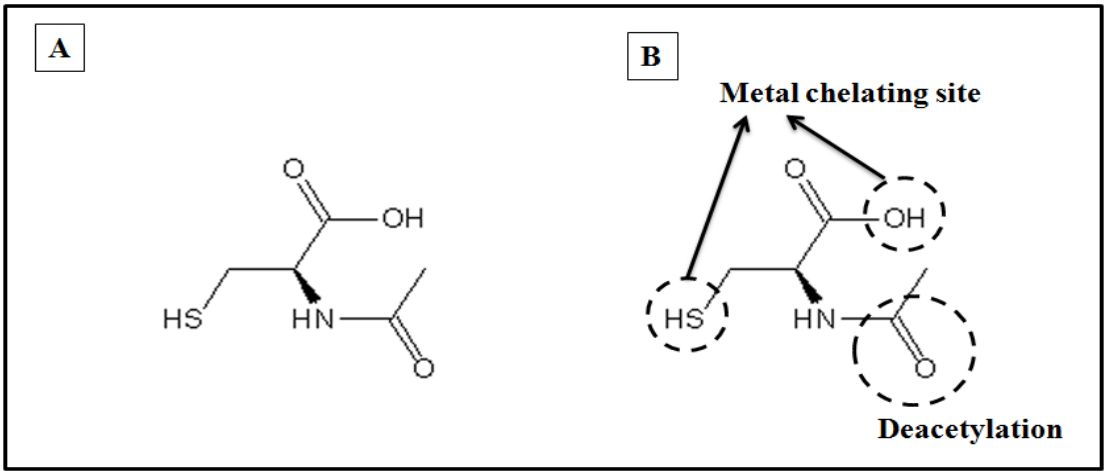

Fig. 1.0: Represented (A) the structural configuration of NAC and (B) two metal chelating site (hydroxyl plus thiol) and deacetylation site accountable for antioxidant potency.

1.13.1. Clinical significance of NAC

1.13.1.1. NAC on COPD (chronic obstructive pulmonary disease)

An encouraging outcome was perceived with NAC in clinical trial of COPD (Dekhuijzen, 2004). Since the patient exposed with NAC for 2 months the cough severity was shown to be reduced by 74% and the expectoration frequency was

upgraded to 71% (Tattersall et al., 1983). Oral NAC is subjected to be a mucolytic agent in severe bronchitis (Jackson et al., 1984).

1.13.1.2. NAC on influenza

One study advocated with affirmative significance of NAC in curtailing warning signs of influenza infection (De Flora et al., 1997). This research protocol was pursued on 262 subjects wherein NAC was served orally at a dose of 600 mg for two times per day or placebo. The episodes of influenza observed to be less severe and less frequent with NAC application. A continuous up-surge of cellular immunity was notable with NAC treatment whereas placebo group did not illustrate any response (De Flora et al., 1997).

1.13.1.3. NAC on polycystic ovary syndrome (PCOS)

NAC clinically improved the ovulation rate and pregnancy outcome in women with PCOS when they were supplied at a dose of 1200 mg with clomiphene regimen (Rizk et al., 2005).

1.13.1.4. NAC on cancer

NAC treatment might be precious in managing some kind of cancer. NAC influenced protection of cells found in bone marrow from the unsafe action of chloramphenicol plus thiamphenicol (Yunis et al., 1986). NAC constituted anti-mutagenic affectivity against genotoxic component and experimentally curtailed tumour growth in intestine (Wilpart et al., 1986). NAC discouraged tumour growth against few carcinogens (De Flora et al., 1986). It was advocated that NAC prevented cancer proliferation by inducing apoptosis and minimizing cancer aggressiveness (Martinez-Outschoorn et al., 2017; Agarwal et al., 2004).

1.13.1.5. NAC on heart disease

NAC is manifested to have optimistic efficacy to protect the cardiac cells from ischemic plus reperfusion damage in myocardial infarction. This condition was resulted with oxidative hazards and declined SH level. NAC infusion for 1 hour upgraded GSH concentration about 38% in this circumstance (Ceconi et al., 1988). Besides, NAC accounted to be lowering homocysteine gathering inside cell when cells were cultured (Hultberg et al., 1997).

1.13.1.6. NAC on degenerative disease

A potential effectiveness was viewed by NAC in neurodegenerative trouble owing to ageing (Tardiolo et al., 2018). NAC certainly protected cadmium propagated neurodegenerative apoptosis by defending ROS challenged Akt/mTOR activation (Chen et al., 2014). Early meta-analysis disseminated the certain positive feedback of NAC on cognition development in both healthy plus mentally sick personnel (Skvarc et al., 2017).

1.13.1.7. NAC on acetaminophen overdose

Overdosing of acetaminophen is a sort of poisoning and damage several major organs. After few hours of consuming this component creates hepatic injury because of producing oxidized metabolites and reduces hepatic GSH reserves. In such cases NAC was applied as an antidote (Smilkstein et al., 1988).

1.13.1.8. NAC on heavy metals

NAC was proven to be clinically useful to chelate some heavy metals. It is much more efficient than other chelating element to excrete boron and chromium via urinary emission. It can efficiently form chelate compound with mercury, gold plus silver (Lorber et al., 1973).

1.13.1.9. NAC against arsenic

The abundance of sulfhydryl group (-SH) in NAC is accountable for upholding the thiol pool and facilitates the creation of intracellular GSH that serves the defence machinery acting as an antioxidant (Sies, 1999; Dickinson et al., 2003). Besides, the amino group substituted with acetyl subunit makes the molecule more stable against oxidation (Bonanomi and Gazzaniga, 1980). The thiol side chain of NAC provides chelating site for many transitional metals and heavy metals just like arsenic and generate a composite excretable form for its emission out of the body. NAC significantly increase the reproductive organ's weight in arsenicated rats. It also reduces arsenic derived oxidative stress generation and reverts arsenic related unfavourable outcomes and thereby manages repro-toxicity in mice. The hindrance of steroidogenic process by arsenic intoxication has been markedly diminished when male mice was introduced with NAC by intra-peritoneal route. Therefore, suppressed reproduction by arsenic was reversed back (Reddy et al., 2011). *In vivo* experimentation recommended that NAC supplementation improved the workability of glucose-6-phosphate in kidney and liver suggesting maintenance of carbohydrate exhaustion in male rats with arsenication (Pal and Chatterjee, 2005). NAC is capable of attenuate arsenic assisted oxidative stress generation in male reproductive organs. Site by site, the impaired redox status corroborated with arsenic in experimental model like male mice has been reversed back by the application of NAC through its stimulation to GSH synthesis (Da Silva et al., 2016). Arsenic promoted hepatocellular injury is coupled with oxidative stress induction followed by mitochondrial redox imbalance and apoptosis which could be counteracted by the pretreatment of antioxidant NAC (Santra et al., 2007). NAC was proved to be protective in functionality of many enzymes concerned with replication and DNA

structure renovation and was also defensive against DNA adduct formation (Vermeulen et al., 1998). However, NAC regulates the cell cycle enabling protection against apoptosis by up-regulating intracellular antioxidant enzymes like superoxide dismutase (SOD), catalase and glutathione peroxidase (Zaragoza et al., 2000; Oh and Lim, 2006). The expressional activity of nuclear factor kappa B (NF-κB) could be suppressed by the action of NAC that resulting simultaneous inhibition in making of consequent cytokines (Kim et al., 2000). Furthermore, NAC amends the over-expression of various kinds of genes, cellular proliferation and apoptosis and also controls different intracellular signaling pathway (Poljsak, 2011). Moreover, NAC has antimutagenic effect against many genotoxic molecules (Wilpart et al., 1986). NAC provides a protective shield in opposition to ROS incorporated female reproductive organs damage and enhances as well as sustains the excellence of oocytes' and follicles maturation in the ovary (Liu and Keefe, 2002). In addition, arsenic integrated diminution of estrogen level has been successfully abrogated by the application of NAC. Moreover, NAC mitigates the arsenic persuaded genotoxicity and utero-ovarian structural abnormalities and restores their morphology towards normalcy (Dash et al., 2018).

Therefore, the present investigation was concentrating on developing easily acceptable, non-invasive, painless and extremely proficient therapeutic cum nutraceutical biomolecule along with metal chelating property to oppose arsenic mediated female reproductive hazards. This also highlights the future usability of this agent in arsenic affected huge population for their further assistance. This thesis experimental work was conducted for understanding the method of action of NAC in the way of managing arsenic driven utero-ovarian abnormalities in animal model and the remedial merit of this bioactive component, NAC.