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Review Paper

Arsenic induced oxidative stress and the role of antioxidant supplementation during chelation: A review

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Abstract: *Arsenic is a naturally occurring metalloid, ubiquitously present in the environment in both organic and inorganic forms. Arsenic contamination of groundwater in the West Bengal basin in India is unfolding as one of the worst natural geoenvironmental disaster to date. Chronic exposure of humans to high concentration of arsenic in drinking water is associated with skin lesions, peripheral vascular disease, hypertension, Blackfoot disease and high risk of cancer. The underlying mechanism of toxicity includes the interaction with the sulphydryl groups and the generation of reactive oxygen species leading to oxidative stress. Chelation therapy with chelating agents like British Anti Lewisite (BAL), sodium 2,3- dimercaptopropane 1-sulfonate (DMPS), meso 2,3 dimercaptosuccinic acid (DMSA) etc., is considered to be the best known treatment against arsenic poisoning. The treatment with these chelating agents however is compromised with certain serious drawbacks/ side effects. The studies show that supplementation of antioxidants along with a chelating agent prove to be a better treatment regimen. This review attempts to provide the readers with a comprehensive account of recent developments in the research on arsenic poisoning particularly the role of oxidative stress/ free radicals in the toxic manifestation, an update about the recent strategies for the treatment with chelating agents and a possible beneficial role of antioxidants supplementation to achieve the optimum effects.*

Key words: *Arsenic poisoning, Oxidative stress, Chelation therapy, Antioxidants*

Introduction

Arsenic has been recognized as a human carcinogen. Current uses of arsenic compounds are in the glass industry as a clarifier, as a wood preservative (copper arsenite), in the production of semiconductor (gallium arsenide) as a desiccant and defoliant in agriculture and as a byproduct of the smelting of non ferrous metals, particularly gold and copper. Human may encounter arsenic in water from wells drilled into arsenic rich ground strata or in water contaminated by industrial or agro chemical waste. Exposure *via* drinking water has been associated with cancer of the skin and various internal organs as well as hyperkeratosis, pigmentation changes and effects on the circulatory and nervous system. Chronic arsenic toxicity due to drinking of arsenic contaminated water has been reported from many countries. Recently, large population in West Bengal in India and Bangladesh has reported to be affected with arsenic (Smith *et al.*, 2000; Guha Mazumder *et al.,* 1998). Cutaneous and hepatic manifestation of arsenic contaminated ground water is reported among a large number of inhabitants of various districts of West Bengal (Guha Mazumder *et al.,* 1988). An important feature of chronic arsenic toxicity in West Bengal is a form of hepatic fibrosis and causes hypertension but does not progress to cirrohosis (Guha Mazumder *et al.*, 1998, 1997; Santra *et al.*, 1999). The problem is of major concern in the USA for example, the arsenic content of drinking water from public and private sources in Millard country ranges from 14 parts per billion (ppb) to 166 ppb (Lewis *et al.,* 1999). Exposure to arsenic via drinking water is correlated with a significantly elevated risk of skin and bladder cancer (Rossman, 1998). About 60-90% of soluble arsenic compounds are absorbed from the gastro intestinal tract following ingestion; inhalation exposure may be similar (ATSDR, 1990). Absorption through intact skin is negligible. Absorbed pentavalent arsenic is converted to more toxic and carcinogenic trivalent form (Bertolero *et al.*, 1987; Hall, 2002). Once absorbed arsenic is stored in liver, kidney, heart and lung while lower amount were present in muscle and neural tissues. Two to four weeks after ingestion, it is incorporated into the nails, hair and skin by binding to keratin sulfhydryl groups. The first sign of poisoning which may emerge as long as 10 years after someone starts drinking arsenic laden water, is appearance of black spots on the upper chest, back and arms, a condition termed melanosis in medical parlance. Palms of the hands or soles becomes hard and loose sensation (keratosis). A patient may also suffer from conjuctivitis, bronchitis, diarrhoea and abdominal pain. In the second stage, white spots appear mixed with the black ones, legs swell and the palms and soles crack and sometimes bleed. These sores can become infected and make working and walking very painful. In addition, neural problems appear in the arms and legs, and the kidneys and liver start to malfunction. In the third stage, the sores turn gangrenous, kidneys and liver may give up and in around 20 years, cancer shows up. Transverse white striae (Mee's lines) in nails are indicative of arsenic exposure. The characteristic Mee's line appear as single, solid, transverse white band of about 1 or 2 mm in width completely crossing the nail of all fingers at the same relative distance from the base. In humans, absorbed inorganic

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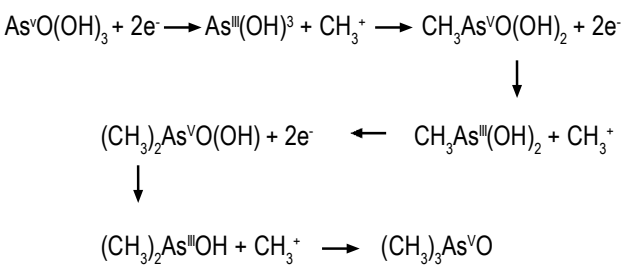
pentavalent arsenic is biotransformed to trivalent arsenic. Trivalent form of arsenic undergoes methylation to form less toxic compounds that are excreted in urine but some inorganic arsenic is excreted in the urine unchanged (Hall, 2002; Hopenhayn Rich *et al.,* 1993). They can inhibit various enzymatic including glycolysis and TCA cycle by binding to sulfhydryl groups of enzymes. Pentavalent arsenic compound can uncouple mitochondrial oxidative phosphorylation. Inhalation exposure to arsenic is associated with an increased risk of lung cancer. Absorption and toxicity of arsenic depends on the form in which it is ingested. Arsenic predominantly exists in two oxidation states arsenic (III) and arsenic (V). Soluble inorganic species are most readily absorbed from the gastrointestinal tract with typical absorption rate being 40-100% of the ingested amount (Pontius *et al*., 1994). Methylation of arsenic has long been regarded as a detoxification process because the pentavalent methylated arsenic metabolites, monomethylarsonic acid (MMAsV) and dimethylarsinic acid (DMAsV) are much less toxic and excreted more readily than As (III). Inorganic arsenic is metabolized by a sequential process and described by Vahter (1994) as below:



The toxicity of arsenic compounds highly depends on the oxidation state and chemical composition of the arsenicals. Traditionally, inorganic arsenicals have been considered more toxic than organoarsenicals. Gallium arsenide (GaAs) is another inorganic arsenic compound of potential human health concern due to its widespread use in the microelectronic industry. Available information suggests that GaAs is poorly soluble, it undergoes slow dissolution and oxidation to form gallium trioxide and arsenite (Flora, 2000; Flora *et al*., 2002a; Flora *et al*., 1999; Webb *et al*., 1984). Therefore, its toxic effects are attributable to arsenite plus the additional effect of gallium.

Chronic arsenic poisoning is much more insidious in nature, often involving multiple hospital admission before the correct diagnosis is made (Saha *et al*., 1999). The bone marrow, skin and peripheral may become involved after chronic exposure.

Mechanism of arsenic toxicity and oxidative injury: One of the major effects of chronic arsenic toxicity in humans from oral exposure is skin lesions, which are characterized by hyperpigmentation, hyperkeratosis and hypopigmentation (Cebrian *et al*., 1988). In Taiwan, Blackfoot disease, a vasoocculusive disease that leads to gangrene of the extremities, is also observed in individuals chronically exposed to arsenic in drinking water. Although arsenic and its mode of action has been the subject of reviews and symposia, little data exist regarding specific mechanism(s) differentiating its action as a carcinogen to cause cancer and as a chemotherapeutic agent used in the treatment of cancer. The molecular mechanisms of arsenic toxicity are still unknown mainly for the reason that (i) its predilection to undergo a variety of complicated metabolic conversion *in vivo* (ii) the complex interactions between arsenic metabolites and intra and extracellular macromolecules, (iii) the influence of concomitant exposure to other toxic agents and (iv) the lack of appropriate animal models for most of the pathologies associated with inorganic arsenic exposure (Wildfang *et al.,* 1998). Although, high concentration of arsenic (V) has been shown to substitute for phosphate in enzyme catalyzed reactions, arsenic toxicity is postulated to be primarily due to the binding of arsenic (III) to sulfhydryl group containing enzymes. Glutathione (GSH) plays a critical role in both the enzymatic and nonenzymatic reduction of pentavalent arsenicals to trivalent and in the complexation of arsenicals to form arsenicothiols during methylation process (Scott *et al.,* 1993). The interaction of arsenic with glutathione and its related enzymes by changing their redox status and this may lead to the alterations of their biological function. Inactivation of GSH related enzymes could have deleterious effects on the detoxification processes and other critical cellular processes involving GSH mediated redox regulation.

Oxidative stress is a relatively new theory of arsenic toxicity (Flora *et al.,* 2005; Kitchin, 2001; Flora, 1999). Since about 1990, additional data supporting this theory and scientific acceptance of this mode of action have continued to occur. The first oxidative theory of arsenic carcinogenesis that includes a detailed metabolic pathway was presented by Yamanaka *et al*. (1990). Dimethylarsine (a trivalent arsenic form) is a minor *in vivo* metabolite of DMA (a pentavalent arsenic form) produced by a process of reduction *in vivo* (Yamanake and Okada, 1994). Dimethylarsine can react with molecular oxygen form a $(CH_3)_2As^{\cdot}$ radicals and superoxide anions. This $(CH_3)_2As^{\cdot}$ can add another molecule of molecular oxygen and form the $(CH_3)_2AsO^{-}$ radical. Hydroxyl radical may be produced *via* cellular iron and other transition metals. Exposure to these free radicals can lead to DNA damage (single strand breaks), (Kitchin, 2001).

Oxidative stress theory for arsenic carcinogenicity can be partially explained by its ability to cause cancer at high rates in the lung, bladder and skin. Human lung may be an organ responsive to arsenic carcinogenesis because of high partial pressure of oxygen and the fact that dimethylarsine, a gas is excreted *via* the lungs (Yamanaka and Okada, 1994). Human bladder may be another organ responsive to arsenic carcinogenesis because of high concentration of DMA and MMA that is stored in the lumen of the bladder. A large amount of DMA (III), dimethylarsenine or MMA (III) might be generated by reductive processes of DMA (V) in the liver and the kidney is exposed to high concentration of DMA as it filters DMA into the urine.

Reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anion, singlet oxygen and hydroxyl radical can directly or indirectly damage cellular DNA and protein. Among these ROS, hydroxyl radical is generally assumed to be the critical species that directly attacks DNA. For hydroxyl radical to be involved in arsenic carcinogenesis a free transition metal (such as iron) is normally thought to be required for the Haber Weiss type processes to cause DNA damage. When tested as releasers of iron from ferritin, (a) methylated arsenic form were found to be more active than arsenate or arsenite, (b) the trivalent arsenic forms were more active than the pentavalent arsenic forms and (c) DMA (III) was by far the most active releaser of iron from ferritin (Ahmad *et al*., 2000).

A combined *in vitro* exposure to both ascorbic acid (a well known iron releaser) and DMA (III) resulted in a large synergistic (greater than additive) increase in iron released from ferritin (under either aerobic or anaerobic conditions) and also in large synergistic increase in DNA damage (Ahmad *et al*., 2000). Arsenite administration induces hepatic and renal heme oxygenase isoform 1 in rats (Kitchin *et al*., 1999). Heme oxygenase induction results in the production of carbon monoxide, biliverdin, and free iron.

8-Hydroxy-2'-deoxyguanosine (8-OhdG) is one of the major ROS induced DNA damage products and used as biomarker of oxidative stress to DNA. In mice gavaged with 720 mg/kg of DMA, urinary 8-OhdG increased to about 3, 6 and 8 times compared to control levels at 3, 6 and 9 hr after treatment respectively (Yamanaka *et al*., 2001). In a long term carcinogenesis study in rats, hepatic 8-OhdG levels increased in DMA treated rats, suggesting that DMA elevates rate of free radical attack on DNA (Wanibuchi *et al*., 1997). Barchowsky *et al.* (1999) demonstrated that free radicals are produced in mice after acute exposure to inorganic arsenic. In culture human lymphocytes exposed to arsenite, increased sister chromatid exchange frequency was antagonized by the addition of superoxide dismutase and catalase (Nordenson and Beckman, 1991). Induction of micronuclei in CHO-K1 cells 20 μm arsenite was antagonized by either nitric oxide syntheses inhibitors superoxide dismutase or uric acid (Gurr *et al*., 1998). These results suggest that some clastogenic effects of arsenic are mediated *via* free radicals.(*e.g*., peroxynitrite, superoxide, hydrogen peroxide and possible free iron).

Trivalent arsenic toxicity could be carried out either directly by attacking –SH groups, or indirectly through generation of reactive oxygen species (ROS) (Chen *et al*., 1998). The toxicity of iAs^{v} appears to be mediated through its ability to substitute phosphate groups, affecting enzymes that depend on this group for their activity (*e.g.*, interfering in the synthesis of ATP and DNA). Arsenic generates ROS and free radicals like hydrogen peroxide (H_2O_2) (Wang *et al*., 1996; Barchowsky *et al*., 1996; Chen *et al*., 1998), hydroxyl radicals species ($HO^{\cdot}$), nitric oxide ($NO^{\cdot}$), (Gurr *et al*., 1998) or superoxide anion ($O_2^{\cdot-}$) (Lynn *et al*., 2000), dimethyl arsinic peroxyl radical [$(CH_3)_2 AsOO^{\cdot}$] and dimethyl arsenic radical [$(CH_3)_2As^{\cdot}$] (Yamanaka *et al*., 1997, 2001). There is the proposal that all of these reactive species are responsible for the stress response elicited by arsenicals. However, the mechanism for the production of this reactive intermediate is still not fully understood, although Yamanaka *et al*. (1997) proposed the formation of intermediary arsenic species (Yamanaka *et al*., 1991, 1997, 2001). Other possibilities for the formation of ROS by arsenic lay on the oxidation of iAs^{III} to iAs^{V} that under physiological condition, will produce H_2O_2 as follows :

$$H_3AsO_3 + 2H_2O + O_2 \rightarrow H_3AsO_4 + H_2O_2$$

Hydroxyl radicals were previously proposed as initiation of lipid peroxidation (LPO) through iron catalyzed fenton reaction in membranes (Halliwell and Gutteridge, 1986). Erythrocytes may be susceptible to oxidative damage due to the presence of haem iron, polyunsaturated fatty acid (PUFA) and oxygen, which may initiate the reactions that induce oxidative changes in RBC. The enzymatic antioxidants in erythrocyte may counteract oxidative stress. For instance, superoxide dismutase (SOD) catalyses the conversion of superoxide radical ($O_2^{\cdot}$) to hydrogen peroxide (H_2O_2) while catalase (CAT) or glutathione peroxidase (GSH-Px) converts H_2O_2 to H_2O. These antioxidant enzymes can therefore, alleviate the toxic effects of ROS. The cell has several ways to alleviate the effects of oxidative stress, either by repairing the damage or by directly diminishing the occurrence of oxidative damage by means of enzymatic and non enzymatic antioxidants. Enzymatic and non enzymatic antioxidants have also been shown to scavenge free radicals and ROS. Non-enzymatic antioxidant such as vitamin E, vitamin C can also act to overcome the oxidative stress. Arsenic induced oxidants, such the superoxide anions and hydroxyl peroxide, have been implicated in *in vitro* studies (Wang and Huang, 1994; Lee and Ho, 1994). The oxidants are suggested to damage macromolecules in cells or to act as second messengers, leading to alteration of the gene expression profile in cells and subsequent enhancement of cell proliferation (Farber, 1994) (Fig. 1).

Arsenic also reduces antioxidant levels in plasma, which may accelerate disease development at target site. This is true with the recent studies shown that the levels of β-carotene were lower in patients with arsenic induced skin cancers as well as in patients with ischemic heart disease than healthy controls (Hsueh *et al*., 1997, 1998). Arsenic has been shown to induce sister chromatid exchanges, micronuclei and chromosomal aberrations (Wang and Huang, 1994; Ho and Lee, 2002) but not mutation in several kinds of gene assays in mammalian cells (Lee *et al*., 1985; Jacobson Krom and Montalbano, 1985), although it was weakly mutagenic in bacteria (Flessel, 1977) and yeast (Kharab and Singh, 1985).

Recently Li and Chou (1992) showed that people with normal glutathione would be expected to have same risk of arsenic induced mutation and consequent development of cancer as people with reduced levels of antioxidants.

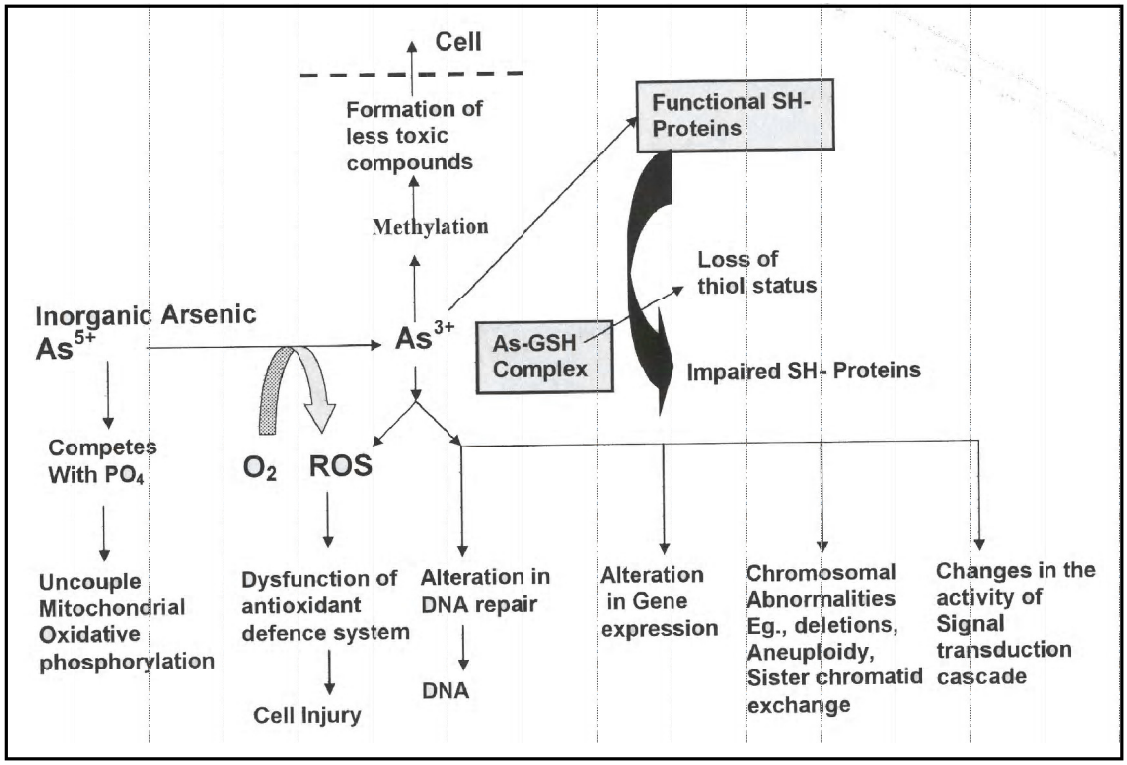


Fig. 1: Mechanism of arsenic poisoning (Vahter, 1994)

In liver and possible other organs, inorganic arsenic is metabolized to methylated compounds (monomethyl arsonic acid and dimethylarsinic acid). Although, methylation has been believed to decrease arsenic biological activity in recent years and this interpretation has been questioned (Murai *et al*., 1993). Recent data from free radical, biochemical and carcinogenic studies of dimethylarsinic acid (DMA) suggest that methylation of arsenic may instead be of a toxification pathway. DMA has been identified as both a complete carcinogens (in rat urinary bladder cancer and in mouse fibrosarcomas) (USEPA, 1994) and as a promoter of carcinogenesis in mouse lung (Yamanaka *et al*., 1996) and in rat liver, kidney, urinary bladder and thyroid gland (Yamamoto, 1995).

Ahmad *et al*. (1999) observed a significant decrease in hepatic GSH content in DMA treated mice. Depletion of hepatic GSH content was also reported in MMA and DMA treated rats (Brown *et al*., 1997). Some researchers have suggested that GSH might be involved in clastogenic action of DMA (Oya-ohta *et al*., 1996) and in apoptosis in HL60 cells exposed to DMA (Ochi *et al*., 1996). Delnomdedieu *et al*. (1994 a,b) have demonstrated the formation of glutathione dimethylarsinite complex $(CH_3)_2$AsSG. However, *in vivo* formation of such complex during arsenic exposure has not been described in the literature. Sakurai *et al*. (1998) suggested that the interaction of DMA with GSH is an important factor potentiating the cytotoxic effect of DMA.

Recent studies have also indicated that arsenic exerts toxicity by generating reactive oxygen species, but the mechanism is still unclear (Ito *et al.,* 1998; Flora, 1999). These reports suggested that intracellular peroxide level is correlated with arsenic induced cellular apoptosis. An important protective role of glutathione (GSH) against arsenic induced oxidative damage has been reported (Ito *et al*., 1998). Ramos *et al.* (1995) also described lipid peroxidation as one of the mechanisms of arsenic toxicity in female rats together with a concomitant decrease in cellular GSH concentration, which is inversely correlated with lipid peroxidation in the liver but not in other tissues (Santra *et al*., 1999). Glutathione related enzymes such as glutathione peroxidase (GPx) and glutathione related reductase function either directly or indirectly as antioxidant and glutathione-S-transferase (GST) plays an important role in metabolic detoxification. Reactive oxygen species have been implicated in the pathogenesis of cancer. Oxidative stress can be involved in initiation, promotion, or progression (Guyton and Kensler, 1993; Kaidobad *et al*., 1997). Several other studies suggest that the genotoxicity of arsenic may be mediated by ROS. Although, the lung is one of the major organs that is affected by arsenic, little information is presently available for the production of ROS in arsenic exposed lung cells. Kitchin (2001), provided an interesting explanation for arsenic's ability to cause human cancer at high rates in the lungs. High partial pressures of oxygen are found in the lungs. Human lungs may be an organ responsive to arsenic

carcinogenesis because of the high partial pressure of oxygen and the fact that dimethylarsine, a gas, is excreted *via* the lungs (Yamanaka and Okada, 1994). Human bladder may be an organ responsive to arsenic carcinogenesis because of the high concentration of DMA and MMA that is stored in the lumen of the bladder and the amount of DMA (III), dimethylarsine or MMA (III) that might be generated by reductive processes (Kitchen, 2001).

As has been suggested above that the trivalent intermediates in the formation of MMA and DMA may have a role in arsenic toxicity as they are known to react with sulfhydryl groups and are highly toxic (Styblo *et al.,* 1997). Although, MMA (III) has been detected in bile and urine following exposure to inorganic arsenic, the extent to which MMA (III) is released from the site of the methylation reaction and transported to the tissues is not known. *In vitro* studies have shown that MMA (III) and DMA (III), like As (III) can form GSH complexes and that these are atleast as toxic as As (III) (Vahter and Concha, 2001; Styblo *et al.*, 1997). Buchet and Lauwerys (1987) have suggested that GSH is required for the monomethylation of arsenite, but not the dimethylation step.

Trivalent arsenic also inhibits pyruvate dehydrogenase (PDH), a multi sub unit complex that needed lipoic acid as a cofactor for enzymatic activity. It has also been reported recently that MMA (III) is more potent inhibitor of PDH than arsenite. PDH oxidizes pyruvate to acetyl CoA, a precursor to intermediates of the citric acid cycle. The citric acid cycle degrades the intermediate and this provides reducing equivalents to the electron transport system for ATP production. Inhibition of PDH may ultimately lead to decreased production of ATP. Also intermediate of the citric acid cycle can be used in glucogenesis (Hughes, 2002). Inhibition of PDH may explain in part the depletion of carbohydrate observed in rats administered arsenic (Reichl *et al.*, 1988; Hughes, 2002).

The haem synthesis pathway plays an important role in all nucleated cells to provide chlorophyll and related structure (Kitchin *et al.*, 1999; Moore *et al.*, 2002). In mammalian and avian tissues the principal product of this pathway is haem ferro-protoporphyrin IX, an essential component of various biological functions including oxygen transport systems, mixed function oxidative reactions and other oxidative metabolic processes. This pathway is known to be highly susceptible to alterations induced by environmental pollutants offering the opportunity to use these changes as indicators of damage caused by many metals or metalloids. About 85% of haem are synthesized in bone marrow where it is required for hemoglobin formation. Remaining 15% is synthesized in liver and other organs where it is required for hemoprotein synthesis. All eight steps of the haem synthesis are catalyzed by enzymes, which require functional sufhydryl (-SH) group for optimal catalytic activity (Bhadauria and Flora, 2004). Since most metals have strong affinity for nucleophilic ligands, each step of the haem biosynthesis pathway is potentially susceptible to direct inhibition as a result of metal mercaptide bond formation with the functional sulfhydryl groups. Arsenic exposure is shown to produce dose related increase in urinary excretion in uroporphyrin and coproporphyrin (Woods and Fowler, 1978). Arsenic exposure has also been known to influence the activity of several enzymes of haem biosynthesis (Albores, 1989). It has been reported that arsenic exposure produces a decrease in ferrochelatase and decrease in COPRO-OX and increase in hepatic 5 aminolevulinic acid synthetase activity (Cebrian *et al.,* 1988; Woods and Southern, 1989). Sub chronic exposure to arsenic has been reported to produce alterations in the hepatic and renal activities of porphobilinogen deaminase (PBG-D), uroporphyrinogen III synthetase (UROIII-S), uroporphyrinogen decarboxylase (URO D) and COPRO-PX in rodents (Martinez *et al.,* 1983). In addition arsenite (III) administration to rats decreases the free heme pool (Cebrian *et al.*, 1988) and increases bilirubin excretion resulting from the degradation of recently synthesized heme (Albores *et al.*, 1989). Sub chronic exposure to arsenic has also been reported to inhibit ALA-S and ferrochelatase activities, which catalyze limiting steps in the heme synthesis pathway, leading to increases uroporphyrin (URO) and coproporphyrin (Woods and Fowler, 1978) and COPRO urinary excretion (Martinez *et al.*, 1983). In chronically exposed humans, arsenic alters heme metabolism as shown by an inversion of the urinary COPRO/URO ratio (Garcia Vargas *et al.,* 1996). Few recent studies also suggested a significant inhibition of blood δ-aminolevulinic acid dehydratase (ALAD) after sub chronic and chronic arsenic exposure (Flora *et al.*, 2002b; Flora, 1999; Kannan *et al.*, 2001). There have been number of recent animal studies which report the effects of GaAs on porphyrin metabolism. Goering *et al.* (1988) first reported that GaAs after single intratracheal instillation produced a dose dependent inhibition of ALAD. They reported that the activity decreases to 5% of the control. Urinary ALA excretion was also maximum 3 to 6 days post exposure. These data were later supported by number of studies from our group. We reported that single or repeated oral ingestion of GaAs produced a dose dependent inhibition of blood ALAD accompanied by an increase in blood zinc protoporphyrin and urinary ALA excretion (Flora *et al.,* 1998, 1999, 2002a, b). Martinez *et al.* (1983) reported that chronic exposure to arsenic alters human haem metabolism since it increases PBG-D and URO-D activities producing uroporphyrinuria III and coprophyrinuria. However, they suggested that severity of the effects appears to depend on characteristics of exposure not yet fully characterized. Although, anemia is often noted in humans exposed to arsenic, red and white blood cell counts are usually normal in workers exposed to inorganic arsenicals by inhalation (Morton and Caron, 1989). Anemia and leucopenia are common effects of poisoning and have been reported from acute, intermediate and chronic exposure. These effects may be due to a direct hemolytic or cyto toxic effect on the blood cells and a suppression of erythropoiesis. Keeping in view the above there was a proposal that the profile of urinary porphyrins could be used as early biomarkers for arsenic toxicity in humans chronically exposed to arsenic via drinking water.

Biological indicators: Biological indicators of arsenic exposure are blood, urine, and hair although blood arsenic is only reliable within few days of acute exposure. In case of chronic exposure, urinary arsenic is the best indicator of current or recent exposure. Hair or fingernail concentration of arsenic may be useful in evaluating past exposure. Most investigators have used hair rather than nails arsenic because the former is easier to obtain in sufficient quantities. The diagnosis of chronic arsenic poisoning must rely on the characteristic, clinical features of the typical skin lesions, debility, weight loss and neuropathy, and hair arsenic levels are only supportive of the diagnosis.

There are no specific biochemical parameters that reflect arsenic toxicity, but evaluation of clinical effects must be interpreted with knowledge of exposure history. A number of studies have demonstrated that porphyrins and other constituents of the haem synthesis pathway might serve as sensitive and specific biomarkers of toxic metal exposure in humans. All eight steps of the haem synthesis pathway are catalyzed by enzymes, which require functional sulfhydryl (-SH) groups for optimal catalytic activity, either as part of the active site configuration or to maintain their structural integrity. Since most metals have a strong affinity for nucleophilic ligands, each steps of the haem biosynthesis pathway is therefore potentially susceptible to direct inhibition as a result of metal-mercaptide bond formation with the functional sulfhydryl groups. δ-aminolevulinic acid dehydratase (ALAD) is a sulfhydryl containing enzymes that catalyzes the asymmetric condensation of two molecules of ALA to porphobilinogen. This reaction is fundamental in the biosynthesis of tetrapyrroles (such as heme), the prosthetic group of various proteins. Due to its sulfhydryl nature, ALAD activity is highly sensitive to the presence of metals, which possess a high affinity for sulfhydryl group. Chronic exposure to arsenic has recently been shown to inhibit ALAD in blood (Kannan *et al.*, 2001; Flora, 1999). Cebrian *et al.* (1988) found an increase in hepatic δ-aminolevulinic acid synthetase (ALAS) activity in rats exposed for 4 weeks to arsenite and interpreted this to be due to reduction in hepatic free haem pool due to an induction of haem oxygenase.

It is advisable that proper investigation should be carried out to define the various manifestations in chronic arsenicosis and these include routine hematological variable like hemoglobin, total and differential count, RBC morphology, urine and stool examination, chest X-ray, electrocardiogram determination of blood sugar, urea and creatinine. The chronic arsenicosis produces protean manifestation which is evident from the report of the clinical features in 156 cases that were drinking arsenic contaminated water in West Bengal, in India (Guha Mazumder *et al.*, 1998). Menzel *et al.* (1998) although proposed the use of human lymphocytes heme oxygenase as biomarker of response to environmental arsenic exposure.

Chelation treatment: British anti-lewisite (BAL) was one of the first chelating agents to be developed as an antidote for war gas, dichlorovinyl arsine (Lewisite) during the second world war (Peter *et al.*, 1945). In the early eighties it was shown that some newer complexing agents like 2, 3-dimercaptopropane 1-sulfonate (DMPS) and meso 2, 3-dimercaptosuccinic acid (DMSA) were effective against arsenic poisoning. When compared to BAL these newer chelating agents were of significant lower toxicity and moreover they could be administered orally or intravenously (Aaseth, 1983). In addition to their heavy metal chelating properties, these agents have a dithiol group that may act as an oxygen radical scavenger and thus inhibit lipid peroxidation (Gersl *et al.*, 1997; Benov *et al.*, 1990, 1992).

DMPS was first introduced in Soviet Union in the 1950s as 'Unithiol'. DMPS is mainly distributed in the extra cellular space; it may enter cells by specific transport mechanism. After intra potential (I.P.) injection of lethal doses the animals were highly irritable for some minutes before they became apathetic and breathing ceased (Klaassen, 1990, Planas Bohme *et al.*, 1980). DMPS is rapidly eliminated from the body through the kidneys.

DMSA has been tried successfully in animal as well as in few cases of human arsenic poisoning (Flora and Tripathi, 1998). DMSA has been shown to protect mice due to lethal effects of arsenic. A subcutaneous (SC) injection of DMSA provided 80-100% survival of mice injected with SC sodium arsenite (Ding and Liang, 1991). Flora and Tripathi (1998) also reported a significant depletion of arsenic and a significant recovery in the altered biochemical variables of chronically arsenic exposed rats. This drug can be effective if given by either oral or intra potential (I.P.) route. Patients treated with 30 mg/kg DMSA per day for 5 days showed significant increase in arsenic excretion and a marked clinical improvement. In a case of attempted suicide by ingesting 2g of arsenic, the patient was given a course of 300 mg DMSA orally every 6 hours for 3 days with good results (Aposhian, 1983). It has been recommended that for treating mild arsenic poisoning, an oral dose of 10 mg/kg DMSA thrice a day for 5-7 days may be given followed by two daily doses of 10 mg/kg for another 10-14 days. While for severe arsenic poisoning, an oral dose of 18 mg/kg thrice a day for first 5-7 days followed by 2 doses of same strength for next 10-14 days are recommended. Number of other studies appeared in the recent past have recommended that DMSA could be safe and effective for treating arsenic poisoning. However, in a double blind, randomized controlled trial study conducted on few selected patients from arsenic affected West Bengal (India) regions with oral administration of DMSA suggested that DMSA was not effective in producing any clinical or biochemical benefits or any histopathological improvements of skin lesions (Guha Mazumder *et al.*, 1998). In an experimental study recently conducted, provided an *in vivo* evidence of arsenic induced oxidative stress in number of major organs of arsenic exposed rats and that these effects can be mitigated by pharmacological intervention that encompasses combined treatment with N-acetylcysteine and DMSA (Flora, 1999).

Esters of succimer (DMSA): A large number of esters of DMSA have been synthesized for achieving optimal chelation as compared to DMSA. These esters are mainly the mono and dimethyl esters of DMSA that have been studied experimentally with the aim of enhancing tissue uptake of chelating agents (Aposhian *et al.*, 1992). In order to make the compounds more lipophillic the carbon chain length of the parent DMSA was increased by controlled esterification with the corresponding alcohol (methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, isopentyl and hexyl). It was observed that all the monoesters, MiADMSA (mono- isoamyl), MnDMSA (mono n-amyl), MnBDMSA (mono n-butyl) and MiBDMSA (mono i-butyl) markedly decreased the arsenic content in most of the organs as soon as 1.5 hr after administration. They found that MiADMSA and MnADMSA were the most effective in increasing the survival of mice (Kreppel *et al.*, 1995). Similar studies were also performed by Flora *et al.* (1997) where they investigated the effect of DMDMSA, DEDMSA DiPDMSA and diidoamyl DMSA (DiADMSA) on sub chronically arsenic treated rats. The results suggested that the diesters reduced the arsenic burden in blood and soft tissue but were only moderately effective in reversing the biochemical recoveries when compared to DMSA (Flora *et al.*, 1997).

Few recent published studies clearly point to the fact that the analogues of DMSA were capable of crossing the membranes and were more effective in reducing the toxic metal burden in acute and sub-chronic metal intoxication (Flora *et al.*, 2004a, b, c, 2003; Flora and Sekhar, 2004; Saxena and Flora, 2004, 2006). Most of these conclusions are based on experimentally induced metal intoxication and no clinical data is available so far.

Monoisoamyl DMSA (MiADMSA): Among these new chelators, monoisoamyl ester of DMSA (MiADMSA; a C_5 branched chain alkyl monoester of DMSA) has been found to be the more effective than DMSA in reducing cadmium and mercury burden (Xu *et al.*, 1995; Gale *et al.*, 1993). It is reported that the toxicity of DMSA with LD_{50} of 16 mmol/kg is much lower than the toxicity of MiADMSA with LD_{50} of 3 mmol/kg but lesser than BAL (1.1 mmole/kg). The interaction of MiADMSA and DMSA with essential metals is same. Mehta and Flora (2001) reported for the first time the comparison of different chelating agents (3 amino and 4 thiol chelators) on their role on metal redistribution, hepatotoxicity and oxidative stress in chelating agents induced metallothionein in rats. We suggested that out of all the 7 chelators, MiADMSA and DMSA produced the least oxidative stress and toxicity as compared to all other 5 chelators (Mehta and Flora, 2001). However, no reports are available about the toxicity of this metal complexing agent except for its developmental toxicity. No observed adverse effect levels (NOAELs) for maternal and developmental toxicity of MiADMSA were 47.5 mg/kg and 95 mg/kg/day respectively indicating that MiADMSA would not produce developmental toxicity in mice in the absence of maternal toxicity (Blanusa *et al.*, 1997). Bosque *et al.* (1994) reported that administration of MiADMSA through the parenteral route to pregnant mice during organogenesis produced maternal toxicity at a dose of 95 and 195 mg/kg with a significant decrease in the body weight and an increase in the liver weights. They also reported that MiADMSA caused embryo/fetotoxicity at a dose of 190 mg/kg by significantly increasing the embryo lethality and non-significant increase in the skeletal defects. Taubeneck *et al.* (1992) showed that the developmental toxicity of DMSA is mediated mainly through disturbed copper metabolism and this may also be true for MiADMSA. Recently, our group was the first to report the toxicological data of MiADMSA when administered in male and female rats (Mehta *et al.*, 2002; Flora and Mehta, 2003) through the oral as well as the intraperitoneal route (25, 50 and 100 mg/kg/3 weeks). We observed that there was no major alterations in the heme biosynthesis pathway except for a slight rise in the zinc protoporphyrin levels suggesting mild anemia at the highest dose. The oral route of administration was also seen to be better when compared to the ip route based on the histopathological studies of the liver and kidney tissues. MiADMSA was seen to be slightly more toxic in terms of copper loss and some biochemical variable in the hepatic tissue in females as compared to male rats. The studies concluded that the administration of MiADMSA in female rats is confounded with side effects and may require caution during its use (Mehta *et al.*, 2002; Flora and Mehta, 2003). Since administration of a chelating agent during pregnancy is always with caution, we studied the effects of MiADMSA administration from day 14 of gestation to day 21 of lactation at different doses through oral and ip routes to examine the maternal and developmental toxicity in the pups (Mehta *et al.*, 2006). Results suggested that MiADMSA had no effect on length of gestation, litter-size, sex ratio, viability and lactation. No skeletal defects too were observed following the administration of the chelator. However, MiADMSA administration produced some marginal maternal oxidative stress at the higher doses (100 mg/kg and 200 mg/kg) based on thiobarbituric acid reactive substances (TBARS) in RBCs and decrease in the δ-aminolevulinic acid dehydratase (ALAD) activity. MiADMSA administration too caused some changes in the essential metal concentration in the soft tissues especially the copper loss in lactating mothers and pups, which would be of some concern. Apart from copper, changes too were observed in the zinc concentrations in mothers and pups following administration of MiADMSA. The study further suggested that the chelator could be administered during pregnancy as it does not cause any major alteration in the mothers and the developing pups (Mehta *et al.*, 2006). Results suggested that MiADMSA administration increased in activity of ALAD in all the age groups and increased blood GSH levels in young rats. MiADMSA also potentiated the synthesis of MT in liver and kidney and GSH levels in liver and brain. Apart from this it also significantly reduced the GSSG levels in tissues. MiADMSA was found to be safe in adult rats followed by young and old rats.

Flora *et al.* (2002b) reported the effect of MiADMSA on the reversal of gallium arsenide (GaAs) induced changes in the hepatic tissue. Rats were exposed for 24 weeks with 10 mg/kg

GaAs, orally, once daily and treated with 0.3 mmol/kg of MiADMSA or DMSA for two courses. They observed that MiADMSA was better than DMSA in mobilizing arsenic and in the turnover of the GaAs sensitive biochemical variables. Histopathological lesions, also responded more favorably to chelation therapy with MiADMSA. In another study, dose dependent therapeutic potential of MiADMSA was compared with monomethyl ester and DMSA in sub chronically GaAs treated rats and it was found that MiADMSA was highly effective in the reversal of altered biochemical variables and in the mobilization of arsenic (Flora *et al.,* 2002b).

Dose and route dependent efficacy of MiADMSA against chronic arsenic poisoning has also suggested that the chelator is highly effective through oral route in reversing the arsenic induced changes in the variables indicative of oxidative stress in major organs as well as in mobilization of arsenic (Flora *et al.*, 2002c, 2004c). Kreppel *et al.* (1995) reported that MiADMSA was effective in increasing the survival of arsenic exposed mice when compared to its parent DMSA.

Despite a few drawbacks/side effects associated with MiADMSA, the above results suggest that MiADMSA may be a future drug of choice owing to its lipophilic character and the absence of any metal redistribution. However, significant copper loss requires further studies. Moderate toxicity after repeated administration of MiADMSA may be reversible after the withdrawal of the chelating agent.

Role of antioxidant during chelation of arsenic (Combination therapy): Induction of reactive oxygen species by arsenic and subsequent depletion of antioxidant cell defense can result in disruption of the pro oxidant / antioxidant balance in mammalian tissues. In the event that oxidative stress can be partially implicated in arsenic toxicity, a therapeutic strategy to increase the antioxidant capacity of cells may fortify the long term effective treatment of arsenic poisoning. This may be accomplished by either reducing the possibility of metal interacting with critical biomolecules and inducing oxidative damage or by bolstering the cells antioxidant defenses through endogenous supplementation of antioxidant molecules. Although many investigators have confirmed arsenic induced oxidative stress, the usefulness of antioxidants along or in conjunction with chelation therapy has not been extensively investigated yet. In the following paragraphs we have tried to provide details about some of the forerunners in the list of antioxidants that have been tried in the treatment of metal poisoning in general or could be useful against arsenic in particular (Fig. 2).

N-Acetyl cysteine (NAC): NAC is a thiol, a mucolytic agent and a precursor of L-cysteine and reduced glutathione. NAC is a source of sulphydryl containing antioxidant that has been used to mitigate various conditions of oxidative stress. Its antioxidant action is believed to originate from its ability to stimulate GSH synthesis, therefore maintaining intracellular GSH levels and scavenging reactive oxygen species (ROS) (Aruoma *et al.*, 1989; Gurer and Ercal, 2000). NAC is also known to have metal

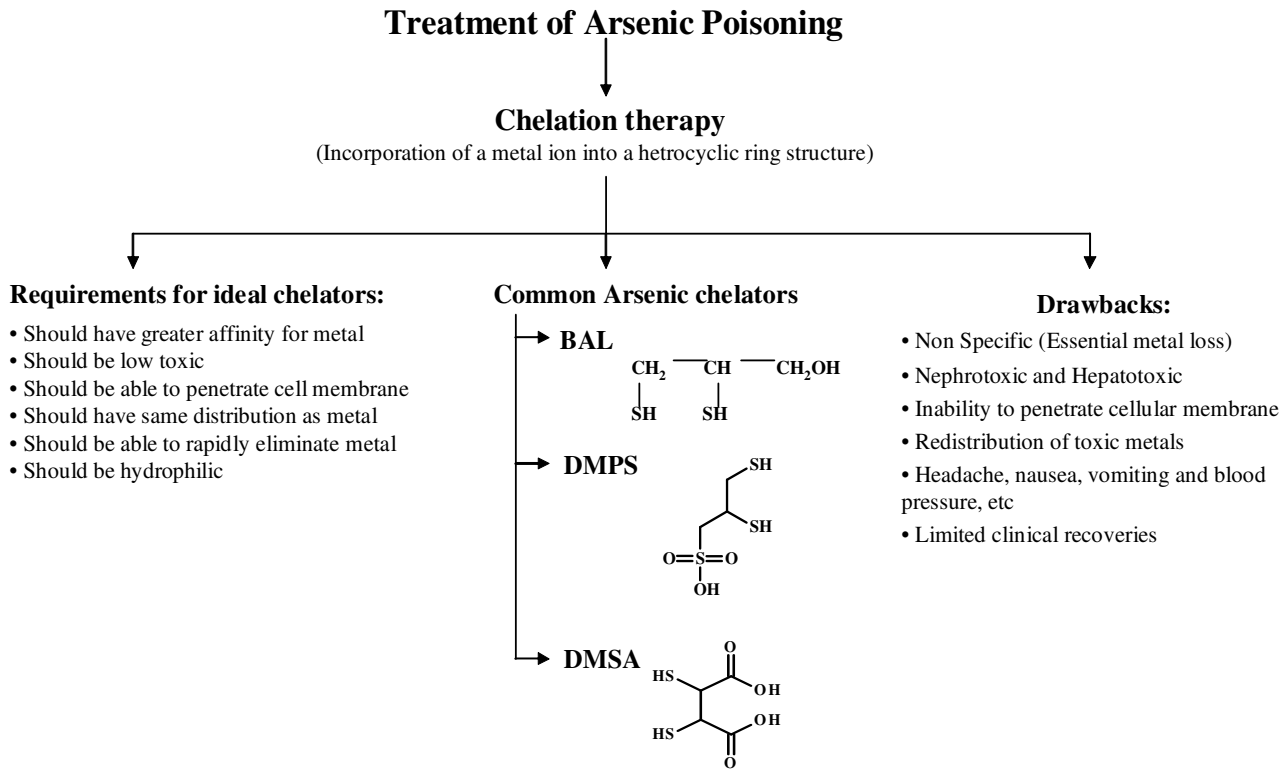


Fig. 2: Treatment of arsenic poisoning

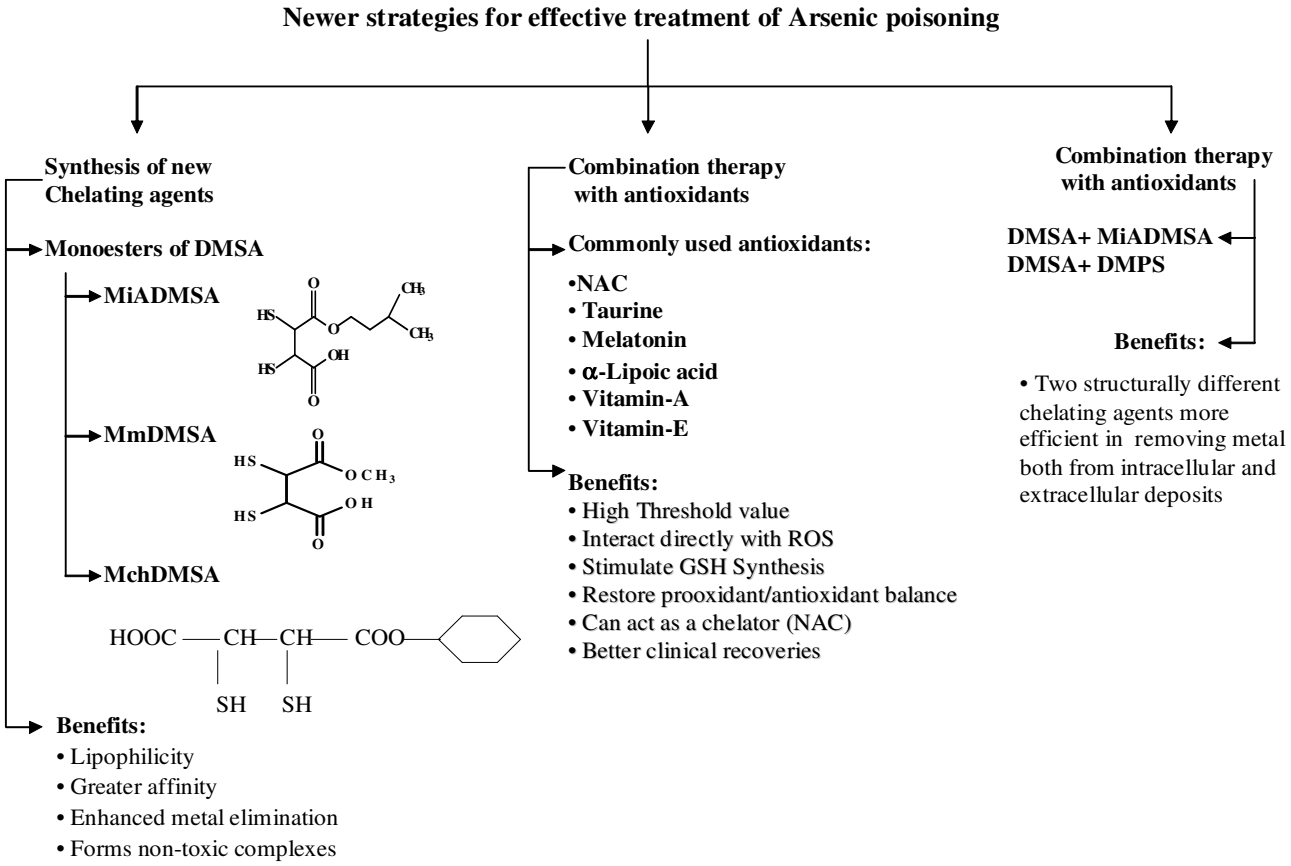


Fig. 3: Newer strategies for treatment of arsenic poisoning

chelating properties (Banner *et al.,* 1986) and has been used in several clinical conditions (Ziment, 1986). Combined administration of NAC and succimer post arsenic exposure led to a significant turnover in variables indicative of oxidative stress and removal of arsenic from soft organs (Flora, 1999; Kannan and Flora, 2006).

Melatonin: Melatonin, N-acetyl-5-methoxy tryptamine, is a hormonal product of the pineal gland that plays many roles within the body including control of reproductive functions, modulation of immune system activity, and limitation of tumorogenesis and effective inhibition of oxidative stress (Hardeland *et al.*, 1993; Reiter, 1993). One major function of melatonin is to scavenge radicals formed in oxygen metabolism (Reiter, 1996; Hardeland *et al.*, 1993), thereby potentially protecting against free radical induced damage to DNA, proteins and membranes (Reiter *et al.*, 1994, 1997; Tan *et al.*, 1993). It has been shown that melatonin stimulates the antioxidative enzyme GPx in the brain, thus providing indirect protection against free radical attack (Barlow Walden *et al.*, 1995). In animal experiments, melatonin prevented the induction of free radical damage by a variety of conditions including ingestion of toxins, ionizing radiation, ischaemia, reperfusion and excessive exercise (Sewerynek *et al.*, 1995; Melchiorri *et al.*, 1994; Blinkenstaff *et al.*, 1994; Sewerynek *et al.*, 1996; Reiter, 1996). Melatonin has a molecular weight of 232 and is both lipid (Costa *et al.*, 1977, 1995) and water soluble (Shida *et al.*, 1994), although its solubility in lipid is clearly greater.

α-Lipoic acid (LA): α-lipoic acid (LA) is a naturally occurring antioxidant and it functions as a cofactor in several multienzyme complexes (Reed, 1974). Its reduced form, dihydrolipoic acid (DHLA), has two free sulfhydryl groups and the two forms LA/DHLA possess a great antioxidant potential (Carreau, 1979). Both LA and DHLA (i) have the ability to scavenge some reactive species (ii) can regenerate other antioxidants (*i.e.* vitamin E and C and GSH) from their radical or inactive forms and (iii) have metal chelating activity. The function of lipoic acid (6,8-thioctic acid) as a prosthetic group in the oxidative decarboxylation of the α-keto acids pyruvate and α-ketoglutarate in mitochondria is well. Lipoic acid has been successfully used as an antidote in intoxication with arsenicals and mercurials (Hatch *et al.*, 1978; Grunert, 1960). Lipoic acid also have an advantage over NAC in opposing GSH loss, since LA is effective in a micromolar range while millimolar NAC is needed for a similar effect (Packer *et al.*, 1995). Coadministration of LA with DMSA has been shown to be of significant value in experimental lead intoxication and there is no reason why such effects could not be possible against arsenic poisoning.

Vitamin E (α-tocopherol) and vitamin C: Various vitamins have been found to reduce the toxic manifestation of heavy metals (Flora, 2002). Vitamin E in the generic term used to describe atleast eight natural occurring compounds that possess the biological activity of α-tocopherol. The group is comprised of α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ-tocotrienol. Vitamin E, which is a low molecular mass antioxidant, interacts directly with the oxidizing radicals (Burton and Ingold, 1986; Jones *et al.*, 1995) and protects the cells from reactive oxygen species (Halliwell, 1994). The lipid soluble, non enzymatic antioxidant, α-tocopherol checks the lipid peroxidation through limiting the propagation of chain reaction of lipid peroxidation (Bueter, 1993). Some of the protective effects of vitamin E also emerge directly from its antioxidant property and some through its influence on the drug metabolising enzyme system (Weber *et al.,* 1997; Machlin *et al,.* 1980; Shukla *et al.,* 1988). Vitamin E has been reported to protect arsenic (Welsh and Sores, 1978). It is believed that vitamin E, as a scavenger of free radicals, might be reacting with methyl radicals that might be formed in the breakdown to provide protection. Additionaly vitamin E may also alleviate arsenic toxicity. It was observed that vitamin E prevented the arsenite induced killing of human fibroblast (Lee and Ho, 1994). The protective mechanism of vitamin E could be attributed to its antioxidant property or its location in the cell membrane and its ability to stabilize membrane by interacting with unsaturated fatty acid chain (Ganther, 1978; Chang *et al.,* 1978). Vitamin C is a low molecular mass antioxidant that interacts directly with the oxidizing radicals (Burton and Ingold, 1986; Jones *et al*., 1995) and protects the cells from reactive oxygen species (Halliwell, 1994). It is well known that ascorbic acid enhances the absorption of dietary iron by increasing the solubility of iron at the alkaline pH of the intestine and by maintaining the ferrous iron in its reduced oxidation state (Cook, 1977). Vitamin C scavenges the aqueous reactive oxygen species (ROS) by very rapid electron transfer that thus inhibits lipid peroxidation (Halliwell, 2002). It acts mainly as an antioxidant molecule and its beneficial effects could be attributed to its ability to complex with heavy metal. Kannan and Flora (2004), reported that administration of vitamin C or vitamin E when given in combination with succimer or its monoisoamyl derivative (MiADMSA) produced profound recoveries in sub chronically arsenic exposed rats. Although the results suggest that vitamin C was better in providing clinical recoveries and vitamin E was equally efficient in decreasing the arsenic burden from the tissues.

Taurine: Taurine, a sulfur containing β-amino acid is found in millimolar concentration, especially in tissues that are excitable, rich in membranes, and generate oxidants (Jacobsen and Smith, 1968; Wright *et al.,* 1986). This semi essential amino acid has been shown to have a role in maintaining calcium homeostasis, osmoregulation, removal of hypochlorous acid and stabilizing the membranes (Wright *et al*., 1986; Huxtable, 1992). The highest concentrations of taurine occur in developing brain, at which time the concentrations of other free amino acids tend to be low (Huxtable, 1992). With development, taurine concentrations fall, with levels in adults being about one third those of neonates. Some of the recent data indicate that taurine can act as the direct antioxidant by scavenging ROS and/or as an indirect antioxidant by preventing changes in membrane permeability due to oxidant injury (Wright *et al.*, 1986). The zwitterionic nature of taurine gives it high water solubility and low lipophilicity. Consequently compared with carboxylic amino acids, diffusion through lipophillic membranes is slow for taurine (Huxtable, 1992). An antioxidant mechanisms rather than a chelating activity, seems to underlie this observed effects of taurine against arsenic induced oxidative stress (Flora *et al*., 2004a). The mechanism proposed for the antioxidant effects of taurine are:

1) As a direct antioxidant, taurine would quench and detoxify some reactive intermediates such hypochlorous acid generated by myeloperoxidase (Wright *et al.,* 1986; Huxtable, 1992; Timbrell *et al.,* 1995), nitric oxide (Redmond *et al.,* 1996), and H_2O_2 (Cozzi *et al.,* 1995).

2) As an indirect antioxidant, taurine may protect cells via intercalating into the membrane and stabilizing it (Nakashima *et al.,* 1982; Gordon *et al.,* 1992; Timbrell *et al.,* 1995). The membrane protective effect of taurine is suggested to be related to an action on permeability to ions and water (Wright *et al.,* 1986; Timbrell *et al.,* 1995).

Essential metals: The defense of biological system against damage caused by activated oxygen involves a battery of interrelated protective agencies, the micronutrients, which have come to be regarded as antioxidant nutrients. Deficiency of several essential elements has been shown to exacerbate the toxic effects of toxic metals, and supplementation of such micronutrients/essential metals ameliorates the toxicity. In addition to the role of micronutrients in modifying metal toxicity, these nutritional components can also act as complimentary chelating agents (adjuvant) increasing the efficacy of a known chelator or by acting independently (Halliwell, 1994). Zinc is one of the essential trace metals which has been studied for its protective value against arsenic. It is known that arsenic is capable of inducing metallothionein suggesting that this cysteine rich low molecular weight protein might play a role in arsenic detoxification (Maitani *et al*., 1987). Based on this theory few studies were planned but provided contradictory data to the above. Kreppel *et al.* (1994) reported that zinc induced increase in MT do not seems to be responsible for the protective role of pre administered zinc against arsenic induced lethality. On the other hand there have been reports suggesting that zinc pre treatment afforded an increase in arsenic elimination. We also recently reported that iron or zinc either alone or in combination with monoisoamyl dimercaptosuccinic acid (DMSA) during and post arsenic exposure provided more pronounced elimination of arsenic in male mice (Modi *et al*., 2006, 2005). It is clear from the above that not much work has been done about the role of zinc against

arsenic. Studies with variable doses of zinc administered during chelation of arsenic particularly against chronic arsenic poisoning are recommended. Another essential trace metals which has received considerable attention is selenium. Selenium is known to promote the biliary excretion of exogenous selenium and selenite also augments the excretion of arsenic into bile (Flora *et al*., 1999; Gregus *et al.,* 1998). These studies suggested that arsenic augmented the hepatobiliary transport of selenium and facilitated accumulation of selenium in red blood cells. Selenium in turn facilitated the biliary excretion of arsenic. Glattre *et al.* (1995) studied the distribution and interaction of arsenic and selenium in rat thyroid and suggested that both arsenic and selenium accumulate in thyroid tissue.

Competition between selenium and arsenic for binding with the functional proteins and bioligand or active tissue sites or to the formation of a reversible compound, metal selenide thus reducing the availability of free concentration of toxic metals ions in the body might be the possible mechanism for the observed antagonism between arsenic and selenium (Gregus *et al.*, 1998).

Plant products: It is clear from the above that dietary intervention or supplementation of naturally occurring dietary nutrients in preventing arsenic induced oxidative injury is of interest. Number of vegetables and plant parts has been reported to reduce toxic effects of heavy metals. Concomitant administration of few plant extract like *Hippophae rhamnoides, Aloe Vera barbadensis* and *Centella asiatica* either during exposure or during chelation treatment on the arsenic induced hematological, renal and hepatic disorders in experimental animals have recently been reported to be of some benefits. Another important plant product has been, Seabuckthorn (*Hippophae rhamnoides*, Elaegnaceae), which is a thorny nitrogen fixing deciduous shrub, native to Europe and Asia and is a rich source of a large number of bioactive substances like vitamin A, C, E, carotenoids and organic acids and its beneficial effects have been attributed to the high contents of antioxidant substances present in this plant. We recently reported that coadministration of *Hippophae rhamnoides* with arsenic is more beneficial in reducing arsenic induced tissue oxidative stress than post arsenic exposure treatment (Gupta and Flora, 2005b, 2006). There is a need to have more detailed studies with Seabuckthorn against arsenic toxicity.

Aloe vera (*Aloe barbadensis*) has been used in the traditional medicine but has been tried in a few limited studies against heavy metals/ metalloid particularly in reducing alteration in biochemical and physiological. The results however, suggest that it has got limited protective value against arsenic induced oxidative stress (Gupta and Flora, 2005a). In the Indian system of medicine Ayurveda, *Centella asiatica* (Umbelliferae) *syn Hydrocotyl asiatica* has been used in various parts of India for different ailments. The whole plant of *C. asiatica* has been shown to be beneficial in improving alteration in arsenic induced oxidative stress besides it is also beneficial in depleting tissue arsenic concentration in limited way from the arsenic exposed animals (Saxena and Flora, 2006; Gupta and Flora, 2006). *Morringa oleifera* (particularly the seed) is another plant product which have recently reported to exhibit significant protection to the altered biochemical variables besides being able to reduce liver and blood arsenic burden from exposed animals (Gupta *et al*., 2005). *Moringa oleifera* (English: Horse radish tree, Drumstick tree, Sanskrit: Shigru) belongs to a monogeric family of shrubs and trees, moringaceae. The mechanism of such protection however is still not clear but it could be due to interaction between cysteine and methionine rich proteins which are present in high amount in the *M. oleifera* seed powder and responsible for removal of arsenic from *in vivo* site. Beside this other antioxidants such as vitamin C, vitamin E, b-carotene *etc.* may be also helpful to combat arsenic induced toxicity in animals.

It is thus clear from the above that we are still far away from having a safe, specific and effective chelating agent for the treatment of metal poisoning. Apart from this, still further knowledge is needed in several basic research areas within the field of *in vivo* chelation of metals and call for studies on (a) Molecular mechanism of action of clinically important chelators, (b) Intracellular and extra cellular chelation in relation to mobilization of aged metal deposits and the possible redistribution of toxic metal to sensitive organs as the brain, (c) Effect of metal chelators on biokinetics during continued exposure to metal, especially possible enhancement or reduction of intestinal metal uptake, (d) Combined chelation with lipophilic and hydrophilic chelators, which presently has a minimal clinical role, (e) Use of antioxidants, micronutrients or vitamins as complimentary agents or antagonists (f) Minimization of the mobilization of essential trace elements during long-term chelation, and (f) Fetotoxic and teratogenic effects of chelators (Fig. 3, 4).

It is clear from number of published reports now that arsenic induces cellular toxicity by damaging body's oxidative defense mechanism which can be prevented by treatment with potential natural antioxidants such as vitamins and essential metals. These antioxidants may be supplemented during chelation therapy with a thiol chelator preferably a lipophilic chelator like monoisoamyl dimercaptosuccinic acid (MiADMSA). Although these recommendations are based on experimental evidence, it strongly support the theory that they have a major role to play in future approach towards finding a safe, suitable and an effective treatment for arsenic poisoning. Although, in these studies synthetic antioxidants like n-acetylcysteine were tried to get optimum therapeutic effects and fewer side effects.

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