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Metal Poisoning: Threat and Management

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Abstract: Exposure to toxic metals remains a wide spread occupational and environmental problems in world. Due to their widespread use in human activities such as industry, agriculture and even as medicine numerous health risks may be associated with exposure to these substances. Lead, arsenic and cadmium generally interferes with a number of body functions such as the haematopoietic system, central nervous system (CNS), liver and kidneys. Over the past few decades there have been growing awareness and concern that the toxic biochemical and functional effects are occurring at lower level of metal exposure than those that produce overt clinical and pathological signs and symptoms. Despite many years of research we are still far from an effective treatment of chronic heavy metal poisoning. The main therapeutic option for chronic metal poisoning relies in chelation therapy. Chelating agents are capable of linking together metal ions to form complex structures which can be easily excreted from the body. They have been used clinically as antidotes for acute and chronic poisoning. 2, 3-dimercaprol (BAL) has long been the mainstay of chelation therapy of lead or arsenic poisoning. Meso 2, 3, -dimercaptosuccinic acid (DMSA) has been tried successfully in animals as well as in few cases of human lead or arsenic poisoning. However, one of the major disadvantages of chelation with DMSA has been its inability to remove heavy metal from the intracellular sites because of its lipophobic nature. Further, it does not provide protection in terms of clinical/ biochemical recovery. A new trend in chelation therapy has emerged to use combined treatment. This includes use of structurally different chelating agents or a combination of an antioxidant and a chelator to provide better clinical/biochemical recovery in addition to mobilization of heavy metal form intracellular sites. The present review article attempts to provide update information about the current strategies being adopted for a safe, effective and specific treatment for toxic metals/ metalloid (lead, arsenic and cadmium).

Key words: lead toxicity, oxidative stress, gossypin, rats

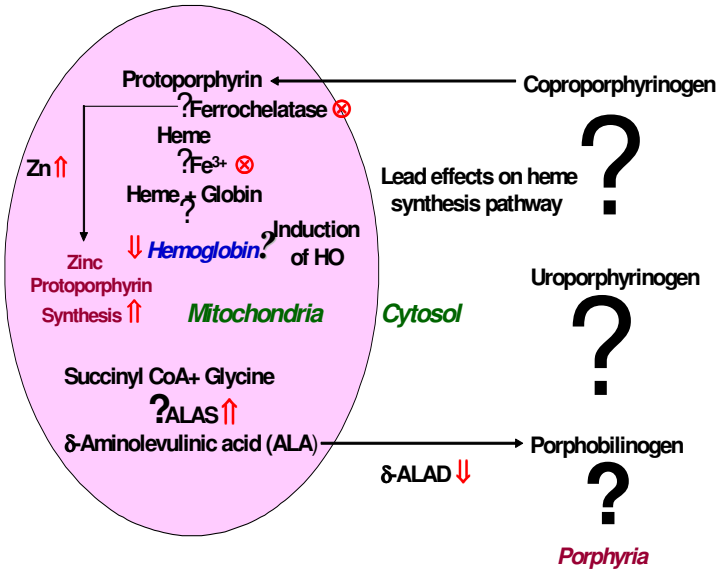
Introduction:

Metals and metal compounds are natural constituents of all ecosystems, moving between atmosphere, hydrosphere, lithosphere, and biosphere. Metal compounds are increasingly introduced in the environment and could finally accumulate in a/biotic systems. Exposure to heavy metals is potentially harmful especially for those metal-compounds, which do not have any physiological role in the metabolism of cells. A **heavy metal** is a member of an ill-defined subset of elements that exhibit metallic properties, which would mainly include the transition metals, some metalliods, lanthanides, and actinides. Heavy metals have a high atomic weight and a density much greater (at least 5 times) than water. In modern times, anthropogenic sources of heavy metals, i.e. pollution, have been introduced to the ecosystem. Waste-derived fuels are especially prone to contain heavy metals so they should be a central concern in a consideration of their use. There are more than 20 heavy metals, but lead (Pb), cadmium (Cd), and inorganic arsenic (As) are of special concern. According to the

U.S. Agency for Toxic Substances and Disease Registry (ATSDR) these three heavy metals are in the top six hazards present in toxic waste sites. They are highly toxic and can cause damaging effects even at very low concentrations. They tend to accumulate in the food chain and in the body and can be stored in soft (e.g., liver, kidney) and hard tissues (e.g., bone). Being metals, they often exist in a positively-charged form and can bind on to negatively-charged organic molecules to form complexes. If heavy metals enter and accumulate in body tissue faster than the body's detoxification pathways can dispose of them, a gradual buildup of these toxins will occur. Human exposure to heavy metals has risen dramatically in the last 50 years as a result of an exponential increase in their use in industrial processes and products [1]. In today's industrial society, there is no escaping exposure to toxic chemicals and metals. In many countries tons of toxic industrial waste are mixed with liquid agricultural fertilizers and dispersed across farmlands. Acute heavy metal toxicity is rare; however, chronic low-grade toxicity may be more damaging, contributing to chronic illness. Heavy metals have specific neurotoxic, nephrotoxic, hepatotoxic, fetotoxic and teratogenic effects. They can directly influence behavior by impairing mental and neurological function, influencing neurotransmitter production and utilization, and altering numerous metabolic body processes [2]. Systems in which toxic metal elements can induce impairment and dysfunction include the blood and cardiovascular, eliminative pathways (colon, liver, kidneys, skin), endocrine (hormonal), energy production pathways, enzymatic, gastrointestinal, immune, nervous (central and peripheral), reproductive, and urinary. Toxic heavy metals target sites such as membrane or structural proteins, enzymes, or DNA molecules [3]. Once at the target site, they can displace an important mineral from its binding site and pretend to be this mineral however; they cannot perform the mineral's function and so inhibit any activity at the binding site, affecting cellular function. This review paper provides a comprehensive account of environmental exposure to arsenic, lead and cadmium, their biochemical and toxic effects and recent development in the preventive and therapeutic measures in terms of reducing the concentration of toxic metal and achieves physiological recoveries.

Lead : Lead is a metal of antiquity and is detectable in practically all phases of the inert environment and in all biological systems, having widespread industrial applications. Lead's atomic number may not be 1 (it is 82) but it ranks near the top when comes to industrial uses. The dangers of lead toxicity, the clinical manifestations of which are termed 'plumbism', have been known since ancient times. Through human activities such as mining, smelting, refining, manufacturing, and recycling, lead finds its way into the air, water, and surface soil. Lead-containing manufactured products (gasoline, paint, printing inks, lead water pipes, lead-glazed pottery, lead-soldered cans, battery casings, etc.) also contribute to the lead burden. Lead in contaminated soil and dust can find its way into the food and water supply. The biochemical basis for lead toxicity is its ability to bind the biologically-important molecules, thereby interfering with their function by a number of mechanisms. At the sub-cellular level, the mitochondrion appears to be the main target organelle for toxic effects of lead in many tissues [4-5].

Toxicological effects of lead: Lead (Pb) binds to sulfhydryl and amide groups, frequent components of enzymes, altering their configuration and diminishing their activities. It may also compete with essential metallic cations for binding sites, inhibiting enzyme activity, or altering the transport of essential cations such as calcium [6]. Children are more vulnerable to lead exposure than adults because of the frequency of hand-to-mouth activity (pica), and a higher rate of intestinal absorption and retention. Lead has been reported to impair normal metabolic pathways in children at very low blood levels [7]. Centers for Disease Control and Prevention (CDC) determined that primary prevention activities in children should begin at blood lead levels (BLLs) > 10 μg/dL [8]. Numerous epidemiologic studies over the past three decades have shown no evidence of a threshold for such effects, and, indeed, the indications are that the slope of the dose–response curve steepens as it approaches zero [9]. A recent risk assessment by the California Environmental Protection Agency calculated that a 1-μg/dL change in BLL in the range of 1–10 μg/dL results in a population-level decrement of one IQ point [10]. Even a 1-point change in Full Scale IQ score, although within the standard error of an individual's single measurement, is still highly significant on a population basis [11]. Lead crosses the placenta from the maternal to the fetal circulation without impediment, and BLLs in mother and fetus are virtually identical. Developing fetuses and young children absorb Pb more readily than adults, and Pb enters the brain quite freely. Some of the neurobehavioral effects related to Pb exposure during fetal neurodevelopment appear to be permanent and persist into childhood [12-13]. Now-a-days lead paint and dust account for up to 70% of elevated BLLs in children. The contribution of dust and soil is most critical for children 1-3 years of age, typically the age with the highest BLLs and greatest hand-to-mouth behaviors [14]. Lead produces a range of effects, primarily on the haematopoietic system, the nervous system, and the kidneys. Lead inhibits many stages in the pathway of haem synthesis which are presented in fig.1. δ-aminolevulinic acid dehydratase (ALAD), which, catalyses the formation of porphobilinogn from δ-aminolevulinic acid (ALA) and ferrochelatase, which incorporates iron into protoporphyrin [15]. It is suggested that the inhibition of ALAD can occur at blood lead as low as 5 μg/dL. ALA in urine has been used for many years as indicator of exposure, inhibition of haematopoiesis among industrial workers, and the diagnosis of lead poisoning. A significant correlation coefficient between lead in blood (and lead in urine) and ALA-U or ALAD has been suggested [15-16]. Ferrochelatase catalyzes the incorporation of iron into the porpohyrin ring. As a result of lead toxicity, the enzyme is inhibited and zinc is substituted for iron, and zinc protoporphyrin concentration is increased [17]. The major consequences of this effect, which have been evaluated in both adults and children, are reduction of haemoglobin and the inhibition of cytochrome P 450-dependent phase-I metabolism. Lead inhibits normal haemoprotein function in both respects, which results in basophilic stippling of erythrocytes related to clustering of ribosome and microcytosis. The threshold for this effect in children is approximately 15 μg/dL [9].



Abbreviations: ALAS- Aminolevulinic acid synthase; ALAD- Aminolevulinic acid dehydratase; HO- Heme oxygenase

Fig. 1: Effects of lead on haem-synthesis pathway

(Source: Kelada et al. [18])

The symptoms resulting from lead poisoning are subtle, and often the patients remain asymptomatic until significant reductions of renal function have occurred. Because of the rich blood supply in the kidney in relation to its mass, this organ in particularly liable to damage from toxic substances. Overt effects of lead on the kidney in man and experimental animals, particularly the rat and mouse, begin with acute morphological changes consisting of nuclear inclusion bodies or lead protein complexes and ultra structural changes in organelles, particularly mitochondria [19]. Lead-induced chronic renal insufficiency may result in gout. A direct effect on the kidney of long-term lead exposure is nephropathy. Dysfunction of proximal renal tubules (Fanconi syndrome) as manifested by glycosuria, aminoaciduria and hyperphosphaturia in the presence of hypophosphataemia and rickets was first noted in acute lead poisoning [20]. The most vulnerable target of lead poisoning is the nervous system. The generally recognized effect of lead on the CNS is encephalopathy while, headache, poor attention irritability, memory loss, and dullness are the early symptom. The developing nervous system of the child is more sensitive to lead-induced impairment. Lead encephalopathy rarely occurs at blood lead below100 µg/dL. A chronic form of encephalopathy has also been described in which progressive mental retardation, loss of motor skills, and behavioural disorders occur rather than the more precipitous symptoms seen in acute encephalopathy [20-22]. One common feature of lead neuropathy and one of the characteristic features of

this disorder in adults is wrist drop due to paralysis of the distal, upper extensor muscles, which are innervated by the radial nerve. Foetal brain may have greater sensitivity to lead than the mature brain [23]. One of the important mechanisms known for lead induced neurotoxicity is the disruption of calcium metabolism by lead. Lead may act as a surrogate for calcium, resulting in subtle disruptions of essential functions. Calcium is important in the release of neurotransmitters, regulation of some rate-limiting enzymes of neurotransmitter synthesis, storage of transmitters in presynaptic vesicular compartments, and regulation of hormone-sensitive cyclases. Several recent investigations have suggested that Ca^{2+}/calmodulin and cyclic nucleotide mediated synaptic events influence long term changes in the nervous system and play an important role in the process of memory and learning [24]. Studies have also suggested that learning selectively affects the phosphorylation of synaptic proteins, that are crucially involved in the process of memory and learning [25]. Eventually, Ca^{2+}/calmodulin and cAMP carry out these functions via the action of protein kinases [24]. The calmodulin and cAMP dependent protein kinases regulate the phosphorylation of a number of synaptic vesicle proteins of which synapsin-I is the major one. Phosphorylation of synapsin-I and other synaptic vesicle proteins, facilitates neurotransmitter release from the nerve terminals and plays an important role in neurotransmission [26]. Therefore, any alternations in phosphorylation state proteins may play a central role in permanent changes in neurons and may affect processes of memory and learning [25]. Lead can activate calmodulin and can displace calcium. Therefore, lead can interfere with calmodulin present on the surface of synaptic vesicles affecting protein phosphorylation and hence neurobehavior.

Arsenic : Arsenic is listed as the highest priority contaminant on the ATSDR/EPA priority list of hazardous substances at Superfund sites [27]. Major anthropogenic sources of arsenic in the environment include smelting operations and chromated copper arsenate (CCA), a variety of pesticide used in pressure treating wood for construction purposes. Arsenic can be transmitted not just by drinking water, but also by direct exposure to skin and hair. It can also be transmitted through food grains and the possible transmit of arsenic through summer (Boro) rice grown in the Bengal basin is an issue of debate [28]. High levels of arsenic have been found in 10 developing countries, including India, Taiwan, China, Bangladesh, Mexico, Argentina, Chile, and Romania and approximately 45 million individuals are exposed to arsenic by drinking water [29-30]. In Bangladesh, 57.5% of the studied population had skin lesions caused by arsenic poisoning (n = 1,630 adults) [31]. In India, many areas from West Bengal have shown to be affected, whereas Bihar is an emerging area with high arsenic contamination [32]. Newer areas are suspected to be Assam, Arunachal Pradesh, Bihar, Manipur, Meghalaya, Nagaland, Uttar Pradesh and Tripura.

Toxicological effects of arsenic: Arsenic toxicity is associated with various hepatic, renal, neurological and skin disorders. At chronic exposure it is known to produce carcinogenic effects also. Arsenic is rapidly and extensively accumulated in the liver, where it inhibits NAD-linked oxidation of pyruvate or α-ketoglutarate. This occurs

by complexation of trivalent arsenic with vicinal thiols necessary for the oxidation of this substrate [33]. The kidneys are the main routes of excretion for arsenic compounds. Sub-lethal arsenic poisoning has resulted in renal necrosis and insufficiency [34]. Arsenic is considered a major risk factor for an endemic peripheral artery disease characterized by severe arteriosclerosis. Accumulating evidence demonstrates that arsenic exposure modulates the coagulation status and enhances the aggregation activities of platelets [35]. Arsenite exposure has been recently related to an impaired NO homeostasis. Arsenic inhibited acetylcholine induced relaxation of aortic rings and reduced the levels of guanosine 3,5-cyclic monophosphate (cGMP), a surrogate for NO [36]. Arsenic-mediated impaired vasomotor tone, as a result of reduced NO bioavailability, may contribute to arsenic-related atherosclerosis and hypertension. Dermatological changes following chronic arsenic intoxication are common features and the initial clinical diagnosis is often based on hyper pigmentation, palmar and solar keratosis. Toxic effects of arsenic also include changes in behavior, confusion, and memory loss. Exposure to arsenic in drinking water has been associated with a decline in intellectual function in children. This association has been established recently on the basis of a cross-sectional study of 201 ten-year-old children in Bangladesh [37]. Itoh et al. [38] reported changes in brain monoamine metabolism and locomotor activity in mice. They found that arsenic passes into the brain in extremely small amounts exerting an influence on metabolism. Flora et al. [39] also reported arsenic induced alterations in brain biogenic amines level. Reproductive and developmental effects of inorganic arsenic on humans and animals species have also been reported [40]. Arsenic is classified as a group 1 carcinogen to humans based on strong epidemiological evidence [41]. Areas in Bangladesh and India with arsenicosis showed high incidences of tumors in local residents [42]. The mechanisms by which arsenic causes human cancers are not well understood. Recent *in vivo* studies indicate that methylated forms of arsenic may serve as co-carcinogens or tumour promoters [43-44]. One of the important mechanisms of arsenic induced disorders is its ability to bind with sulfydryl group (-SH) containing molecules. Trivalent inorganic arsenicals, such as arsenite, readily react with reduced glutathione (GSH) and cysteine and decrease their bio-availability [45]. Generally, there are three pathways that arsenic can decrease cellular levels of GSH. In the first pathway GSH possibly acts as an electron donor for the reduction of pentavalent to trivalent arsenicals. Secondly, arsenite has high affinity to GSH. The third pathway involves oxidation of GSH by arsenic-induced generation of free radicals. Taken together, exposure to arsenite is likely to cause depletion of GSH level. Therefore, arsenic blocks the Krebs cycle and interrupts oxidative phosphorylation, resulting in a marked depletion of cellular ATP and eventually death of the metabolising cell [45]. Pyruvate dehydrogenase (PDH) is a multi sub-unit complex that requires the cofactor lipoic acid, a dithiol, in gluconeogenesis reaction. Arsenite inhibits PDH activity [46], perhaps by binding to the lipoic acid moiety. There are sulfhydryl groups on dihydrolipoate, part of the pyruvate dehydrogenase complex, and arsenic binds to these and prevents the oxidation of dihydrolipoate to lipoate. Lipoate is needed in the formation of acetyl-CoA from

pyruvate and in the formation of succinyl-CoA from alpha-ketoglutarate. Inhibition of PDH ultimately leads to decreased production of ATP. Inhibition of PDH may also explain in part the depletion of carbohydrates observed in rats administered arsenite [47]. Pal and Chatterjee, [48] also demonstrated hypoglycemia associated with glycogenolysis and other associated changes are supposed to be involved in arsenic-induced alternation of glucose homeostasis. Methylated trivalent arsenicals such as MMA^{III} are potent inhibitors of GSH reductase [49] and thioredoxin reductase [50]. The inhibition may be due to the interaction of trivalent arsenic with critical thiol groups in these molecules. The activity of methylated arsenicals may alter cellular redox status and eventually lead to cytotoxicity.

Cadmium: Cadmium (Cd) is one of the most toxic metal ions of our environment bound in the air, food and water [51]. This heavy metal is non-biodegradable and the environmental levels of Cd are increasing due to industrial practices [52]. About 13,000 tons of cadmium is produced yearly worldwide, mainly for nickel-cadmium batteries, pigments, chemical stabilizers, metal coatings and alloys. Uptake of Cd is known to interfere with the utilization of essential metals. After ingestion, Cd ions are absorbed by most tissues of the body and become concentrated mainly in the liver and kidney and has a long biological half-life of 17 to 30 years in humans [53]. Cadmium is listed by the US Environmental Protection Agency as one of 126 priority pollutants. The most dangerous characteristic of cadmium is that it accumulates throughout a lifetime.

Toxicological effects of cadmium: Because of its carcinogenic properties, cadmium has been classified as a #1 category human carcinogen by the International Agency for Research on Cancer of USA [54]. Cadmium is a potent human carcinogen and has been associated with cancers of the lung, prostate, pancreas, and kidney. Cadmium can cause osteoporosis, anemia, non-hypertrophic emphysema, irreversible renal tubular injury, eosinophilia, anosmia and chronic rhinitis. Cadmium intoxication is responsible for alterations in various metabolic processes [55] and the inhibition of nucleic acid and protein synthesis [56]. Moreover, this metal has been extensively reported as being carcinogenic, mutagenic and teratogenic under experimental conditions. While the latter action has sometimes been attributed to placental or yolk sac damage in rodents [57], it has also been reported that cadmium is found in early organogenesis-stage embryonic tissues [58], indicating that embryonic cells may be the direct targets of cadmium action. A variety of experiments have suggested that cadmium causes oxidative damage to cells. In V79 cells (Chinese hamster lung fibroblasts), 50 mM cadmium caused cellular toxicity and a depletion of reduced glutathione (GSH), which could be inhibited by the radical scavenger, butylated hydroxytoluene [59]. Casalino et al. [60] proposed that cadmium binds to the imidazole group of the His-74 in SOD which is vital for the breakdown of hydrogen peroxide, thus causing its toxic effects. Cadmium inhibition of liver mitochondrial MnSOD activity was completely removed by Mn(II) ions, suggesting that the reduced effectiveness of this enzyme is probably due to the substitution of cadmium for manganese. These authors also observed antioxidant capacity of Mn(II) ions, since they were able to normalize the increased TBARS

levels occurring when liver mitochondria were exposed to cadmium. Cd-induced nephrotoxicity is clearly the most important and the most frequently occurring ailment in humans as a result of chronic exposure to the metal [61]. The most sensitive cellular targets of cadmium seem to be ion transport and signal transduction [62]. These include intracellular mobilization of second messengers such as inositol triphosphate and calcium [62], inhibition of plasma membrane calcium channels [63], and inhibition of Ca^{2+}-ATPases of the sarcoplasmic reticulum [64]. At the gene level, cadmium induces the expression of metallothionein and inhibits the repair of DNA damage [65]. Large numbers of enzymatic activities are influenced by cadmium and the mechanisms of these effects have been hypothesized to be due to, either displacement of a beneficial metal from the active site or through binding to the active site in the enzyme itself. The various toxic effects induced by cadmium and other heavy metals in biological systems might be due to alterations in the antioxidant defense system [66]. This includes reduced glutathione (GSH), glutathione peroxidase, thioredoxin reductase (TrxR), and selenium. Cadmium is thought to induce lipid per oxidation and this has often been considered to be the main cause of its deleterious influence on membrane-dependent function [67-68]. Cadmium is also known to cause its deleterious effect by deactivating DNA repair activity [69]. Although, there are a number of mechanism that exists to prevent DNA mismatch like direct damage reversal, base excision repair, nucleotide excision repair, double stand break repair and mismatch repair (MMR) but cadmium inhibits only MMR mode of repair.

*Metal toxicity and oxidative stress :*Oxidative stress, a condition describing the production of oxygen radicals beyond a threshold for proper antioxidant neutralization, has been implicated as a pathologic condition in several cellular disorders. Vast experimental evidence has demonstrated that many metals produce ROS through different mechanisms. Arsenic is one of the most extensively studied metals that induce ROS generation and result in oxidative stress. Experimental results show that superoxide radical ion and H_2O_2 are produced after exposure to arsenite in various cellular systems [70-72]. Shi et al. [73] provided evidence that arsenic generates free radicals that leading to cell damage and death through the activation of oxidative sensitive signaling pathways. ROS play a significant role in altering the signal transduction pathway and transcription factor regulation. Numerous reports have indicated that arsenic affects transcriptional factors either by activation or inactivation of various signal transduction cascades. Arsenic is known not only to produce ROS but also, nitric oxide (NO•) dimethylarsinic peroxyl radicals $(CH_3)_2AsOO•$ and also the dimethylarsinic radical $(CH_3)_2As•$ [74-75]. It has been shown that arsenite (AsIII) enhances the production of heme oxygenase, an indicator of oxidative stress, in a variety of human and mammalian cell types, [76] and generates free radicals in livers of mice [77] and in human keratinocytes [73]. The generation of ROS by various arsenic metabolites was confirmed by cell cultures [78] and animal experiments [79]. Oxidative DNA lesions induced by arsenic were observed both in vivo [80] and in vitro [81-82] studies. In a study by Schiller et al. [83] it was shown that arsenite can inhibit pyruvate dehydrogenase (PDH) activity

through binding to vicinal dithiols in both the pure enzyme and tissue extract. The mechanism of arsenite toxicity was reported owing to its effects on the generation of the hydroxyl radical [84]. The time-evolution of the formation of the hydroxyl radical in the striatum of both female and male rats who underwent a direct infusion of different concentrations of arsenite was investigated. The treatment with arsenite induced significant increase of hydroxyl radical formation. These results support the participation of hydroxyl radicals in arsenic induced disturbances in the central nervous system. Studies have shown that lead causes oxidative stress by inducing the generation of reactive oxygen species (ROS) and weakening the antioxidant defence system of cells [85-86]. Depletion of cells' major sulfhydryl reserves seems to be an important indirect mechanism for oxidative stress that is induced by redox-inactive metals [87-88]. When GSH is reduced by lead, GSH synthesizing systems start making more GSH from cysteine via the γ-glutamyl cycle. Several enzymes in antioxidant defense system may protect this imbalance but they also get inactive due to direct binding of lead to the enzymes' active sites, if the sites contain sulfhydryl group e.g. δ-aminolevulinic acid dehydratase (ALAD). Further, zinc which usually serves as a cofactor of many enzymes could be replaced by lead, thereby making the enzyme inactive. The increased lipid peroxidation and inhibition of enzymes responsible to prevent such oxidative damage have demonstrated lead induced oxidative injury [89]. Lead induced disruption of the pro-oxidant/ antioxidant balance could induce injury via oxidative damage to critical biomolecules. A significant decrease in the activity of tissue superoxide dismutase (SOD), a free radical scavenger and metalloenzyme (zinc/copper) on lead exposure have been reported [90]. Catalase is an efficient decomposer of H_2O_2 and known to be susceptible to lead toxicity [85]. Lead induced decrease in brain GPx activity may arise as a consequence of impaired functional groups such as GSH and NADPH or selenium mediated detoxification of toxic metals [91]. Antioxidant enzyme glutathione S-transferase (GST) is known to provide protection against oxidative stress and the inhibition of this enzyme on lead exposure might be due to the depletion in the status of tissue thiol moiety. These enzymes are important for maintaining critical balance in the glutathione redox state. Malondialdehyde (MDA) levels were strongly correlated with lead concentration in the tissues of lead exposed rats [92]. The concentration of TBARS, which is a reflection of endogenous lipid oxidation level, gets increased on lead exposure. The interaction of lead with oxyhaemoglobin (oxyHb) has been suggested as an important source of superoxide radical formation in RBCs. Ercal et al. [88] postulated that antioxidant enzymes inhibited haemoglobin auto-oxidation by lead, suggesting O_2^{o-} and H_2O_2 were involved in this process. ALAD is a sulfhydryl-containing enzyme that catalyzes the asymmetric condensation of two δ-aminolevulinic acid (ALA) molecules yielding porphobilinogen, a haem precursor. Consequently, ALAD inhibition can impair haem biosynthesis and can result in the accumulation of ALA, which may disturb the aerobic metabolism and also have some pro-oxidant activity. It has also been suggested that accumulation of ALA, resulting from inhibited ALAD activity, may undergo metal catalyzed auto-oxidization, resulting in the conversion of

oxyhaemoglobin to methaemoglobin in a process that appears to involve the formation of reactive oxygen species such as superoxide and hydroperoxides [93]. Cadmium, unlike other heavy metals is unable to generate free radicals by itself, however, reports have indicated superoxide radical, hydroxyl radical and nitric oxide radicals could be generated indirectly [94]. Watanabe et al [95] showed generation of non-radical hydrogen peroxide which by itself became a significant source of free radicals via the Fenton chemistry. Cadmium could replace iron and copper from a number of cytoplasmic and membrane proteins like ferritin, which in turn would release and increase the concentration of unbound iron or copper ions. These free ions participate in causing oxidative stress via the Fenton reactions [60, 96]. Recently, Watjen and Beyersmann [97] showed evidence in support of the proposed mechanism. They showed that copper and iron ions displaced by cadmium, were able to catalyze the breakdown of hydrogen peroxide via the Fenton reaction [97]. Acute intoxication of animals with cadmium has shown increased activity of antioxidant defense enzymes like copper-zinc containing superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase [98].

*Preventive measures for metal toxicity :*One of the best measures to minimize metal exposure is by maintaining nutritional health. Absorption of heavy metal is increased in subjects with deficiencies in iron, zinc, vitamins (like thiamine); thus maintaining good nutrition minimizes their dietary absorption. The best non chelation therapy is the removal of subject from the source of metal exposure.

Role of vitamins: Vitamins (particularly vitamin B, C and E) are expected to play a major role in preventing the toxicity of heavy metals. Exposure to lead has been found to decrease vitamin contents of different tissue and blood [99]. Fisher et al. [100] using mammalian cell culture reported the, usefulness of vitamins in modifying the uptake, cytotoxicity and release of lead from these cultured cells. Vitamin E is an important antioxidant, which is suggested to play a physicochemical role in the stabilisation of bio-membrane by virtue of lipid-lipid interaction between the vitamin and the unsaturated fatty acids [101]. Ascorbic acid (vitamin C) is known to have number of beneficial effects against metal toxicity. Simon and Hudges, [102] reported a population-based study that indicates an inverse relation between serum ascorbic acid and blood lead levels. The potential of ascorbic acid to counter lead intoxication may also be attributed to its ability to *in vivo* reduce the disulphides to thiol containing compounds required for the stimulation of lead inhibited haem synthetase activity. Supplementation of ascorbic acid and α-tocopherol has been known to alter the extent of DNA damage by reducing TNF-α level and inhibiting the activation of caspase cascade in arsenic intoxicated animals [103]. Our group has also reported beneficial effects of vitamins supplementation during arsenic intoxication [70]. *In vivo* and *in vitro* antioxidant effect of vitamin-E on the oxidative effects of lead intoxication in rat erythrocytes suggests that simultaneous supplementation of vitamin-E to lead treated erythrocytes prevent the inhibition of δ-aminolevulinic dehydratase activity and lipid oxidation [104].

Role of antioxidants: Metal induced disruption of the pro-oxidant/antioxidant balance contributes to tissue injury via oxidative damage to critical biochemical like lipids,

proteins and DNA. Gurer and Ercal, [105] summarized how ALA is involved in ROS generation. Studies have also reported alterations in antioxidant enzymes activities such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) and changes in the concentrations of some antioxidant molecules such as glutathione (GSH) in lead and arsenic exposed animals and humans [70, 106]. Gurer and Ercal, [105] were the first to start exploring the beneficial role of n-acetylcysteine (NAC) in both *in vitro* and *in vivo* models. They investigated the lead extent of oxidative injuries and the benefits of administering NAC orally to rats**.** They indicated that the antioxidant action of NAC could provide some beneficial effects in lead poisoning treatment independent of chelation [107]. A study conducted by Santra et al [108] showed that treatment with NAC in arsenic intoxicated mice could deplete cellular stores of the GSH and is an effective intervention against oxidative stress developed due to arsenic exposure. Efficacy of NAC as a potent antioxidant has also been reported in cadmium intoxication and it has been reported that simultaneous supplementation of NAC could protect Cd-induced nephrotoxicity and it can also act as a therapeutic agent against Cd intoxication [109]. One of the first reports by Pande et al [110] suggested that NAC could be used both as preventive and therapeutic agent along with MiADMSA or DMSA in the prevention and treatment of lead poisoning. Combined administration of NAC along with DMSA post arsenic exposure lead to a significant turnover in variables indicative of oxidative stress and removal of arsenic from soft organs [33]. α-Lipoic Acid (LA) is an endogenous thiol antioxidant, which possesses powerful potential to quench reactive oxygen species, regenerate GSH and to chelate metals such as iron, copper, mercury and cadmium. The ability of LA to cross blood brain barrier is an extra advantage. Beneficial effects of LA are independent of its ability to chelate lead but are associated with LA's potential for bolstering thiol antioxidant capacity. Inside cells and tissues, lipoic acid is reduced to dihydrolipoic acid which is more potent antioxidant and its co-administration with succimer has been known to reduce lead induced toxic effects [111]. *In vitro* studies revealed that, among the mono and dithiols (glutathione, cysteine, dithiothreitol, and lipoic acid), lipoic acid was the most potent scavenger of free radicals produced during cadmium-induced hepatotoxicity [112]. Taurine, a semi essential amino acid has been shown to have a role in maintaining calcium homeostasis, osmoregulation, removal of hypochlorous acid and stabilizing the membranes [113]. 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 [114]. In the studies conducted by Gurer and Ercal [105], taurine was shown to have beneficial effects in lead induced oxidative stress in Chinese Hamster Ovary (CHO) cells and F344 rats. Recently Flora et al. [115] suggested beneficial role of taurine against arsenic induced toxicity.

Therapeutic measures for metal toxicity:

Chelation treatment: 'Chelation' is the formation of a metal ion complex in which the metal ion is associated with a charged or uncharged electron donor referred to as

ligand. The ligand may be monodenate, bidenate or multidenate, that is, it may attach or co-ordinate using one or two or more donor atoms. Bidenate ligands form ring structures that include the metal ion and the two-ligand atoms attached to the metal [116]. Their efficacy depends not solely on their affinity for the metal of interest but also on their affinity for endogenous metals, mainly calcium. An ideal chelator should have high solubility in water, resistance to biotransformation, ability to reach site of metal storage, ability to retain chelating ability at the pH of body fluid and property of forming metal complexes that are less toxic than the free metal ion.

Calcium disodium ethylene diamine tetraacetic acid ($CaNa_2EDTA$): The most commonly used chelating agents that have been the forerunners in chelation therapy belong to the polyaminocarboxylic groups. Calcium disodium ethylene diamine tetra acetic acid ($CaNa_2EDTA$) is a derivative of ethylene diamine tetra acetic acid (EDTA); a synthetic polyamino-polycarboxylic acid and since 1950s has been one of the main stays for the treatment of childhood lead poisoning [117]. $CaNa_2EDTA$ is mainly distributed extra-cellularly. One of the major drawbacks with $CaNa_2EDTA$ appears to cause redistribution of lead to the brain [118]. Treatment with $CaNa_2EDTA$ resulted in rapid decrease in plasma zinc concentrations. Administering the sodium salt of EDTA *in vivo* will result in the formation of the calcium salt, which will be excreted. This can result in an increase in the urinary excretion of calcium and hypocalcaemia with the risk of tetany. To overcome this hazard, $CaNa_2EDTA$ was introduced for the treatment of lead poisoning. In this case, the lead-EDTA chelate has the higher stability constant. Thus $CaNa_2EDTA$ chelates the lead in the body fluids, $PbNa_2EDTA$, which is excreted leaving Ca behind.

D-penicillamine: D-Penicillamine (DPA) is β-β-dimethylcysteine or 3-mercapto-D-valine, a sulfhydryl containing amino acid, is as an antidote for low or mild lead poisoning [119]. It can penetrate cell membranes and then get metabolized. It can be absorbed through the gastro intestinal tract and thus can be administered orally. Prolonged treatment with DPA may lead to anorexia, nausea, vomiting in human. Apart from this, DPA is also a well recognized teratogen and lathyrogen that causes skeletal, cutaneous and pulmonary abnormalities [119].

British Anti Lewisite (BAL): 2, 3-dimercaprol (BAL) is a traditional chelating agent that has been used clinically in arsenic poisoning since 1949. It is an oily, clear, colorless liquid with a pungent, unpleasant smell typical of mercaptans and having short half life. In humans and experimental models, the antidotal efficacy of BAL has been shown to be most effective when administered immediately after the exposure. Beside rapid mobilization of arsenic from the body, it causes a significant increase in brain arsenic [120]. Due to its oily nature, administration of BAL requires deep intra-muscular injection that is extremely painful and allergic.

Meso 2, 3-dimercaptosuccinic acid (DMSA): It is a chemical derivative of dimercaprol and contains two vicinal sulfhydryl (-SH) groups. It has been shown to be an effective chelator of lead and arsenic. Few major advantages of DMSA include its low toxicity, oral administration and no redistribution of metal from one organ to another [121]. DMSA has been tried successfully in animal as well as in cases of human arsenic poisoning [122]. In an interesting perspective, double blind,

randomised controlled trial study conducted on few selected patients from arsenic affected West Bengal (India) regions with oral administration of DMSA suggested that it was not effective in producing any clinical or biochemical benefits [123]. Animal studies suggest that DMSA is an effective chelator of soft tissue but it is unable to chelate lead from bones [121]. DMSA for being an antioxidant and a strong lead chelator has been shown to deplete significantly lead from hippocampus leading to recovery in the oxidative stress and apoptosis induced by lead [124]. One of the major drawback with the use of DMSA is that it is basically a soft tissue lead and arsenic mobilizer and thus unable to remove these metals from hard tissues and intracellular sites.

Sodium 2, 3 dimercaptopropane-l-sulphonate (DMPS): Sodium 2, 3-dimercaptopropane sulfonate (DMPS) is another analogue of BAL and is mainly distributed in extra cellular space and it may enter cells by specific transport mechanism. DMPS is rapidly eliminated from the body through the kidneys. No major adverse effects following DMPS administration in humans or animals have been reported [125]. A pilot study of DMPS in lead poisoned children by Gersl et al. [126] indicates less efficiency than $CaNa_2EDTA$ and DMSA. DMPS appears to be bio-transformed in humans to acyclic and cyclic disulphides. DMPS is distributed both in an extra-cellular and to a small extent an intracellular manner [116]. DMPS is not the appropriate drug as far as lead is concerned. Oral administration of DMPS also did not adversely affects late gestation, parturition or lactation in mature mice and fetal and neonatal development do not appear to be adversely affected. DMPS although known for its antidotal efficacy against mercury and has also been reported to be an effective drug for treating lead and arsenic poisoning (71, 118). It is thus clear from above that most of the conventional chelators are compromised with many side effects and drawbacks and there is no safe and effective treatment available for metal poisoning. Structures of common chelators are presented in fig. 2

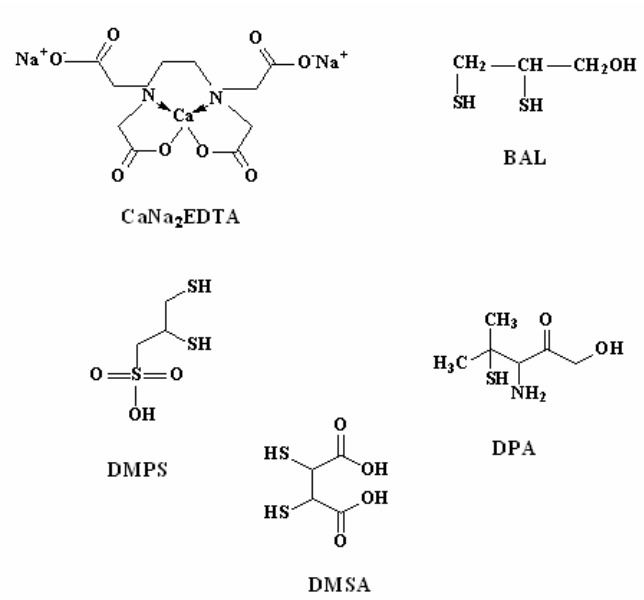


Fig. 2: Common metal chelators and their chemical structures

Synthesis of new chelating agents: A large number of esters of DMSA have been synthesized for achieving optimal effects of chelation compared to DMSA (Fig. 3). 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. 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 has also been reported that these mono and diesters have a better potential in mobilizing arsenic and lead from the soft tissues [127].

Monoisoamyl DMSA (MiADMSA): Among the new chelators, monoisoamyl ester of DMSA (MiADMSA; a C_5 branched chain alkyl monoester of DMSA) has been found to be the most effective [128-129]. 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, [130] 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. Mehta et al. [131] reported the toxicological data of MiADMSA when administered in male and female rats through the oral as well as the intra-peritoneal route (25, 50 and 100 mg/kg for 3 weeks). Mehta et al. [132] have suggested that MiADMSA had no effect on length of gestation, litter-size, sex ratio, viability and lactation. Results suggested that MiADMSA administration increased in activity of ALAD in all the age groups and increased blood GSH levels in young rats [132]. MiADMSA also potentate the synthesis of MT in liver and kidneys 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.

Monomethyl DMSA (MmDMSA) and monocyclohexyl DMSA (MchDMSA): MmDMSA has a straight and branched chain methyl group while MchDMSA has a cyclic carbon chain. Thus they can have better lipophilicity characteristic and might penetrate cells more readily that extracellularly acting chelating agent like DMSA. Both these chelating agents are orally active. Jones et al. [133] in their *in vivo* study on male albino mice exposed to cadmium for seven days observed that administration of MmDMSA and MchDMSA produced significant reductions in whole body cadmium levels. Further, no redistribution of cadmium in brain was observed. The *in vivo* evaluation of these monoesters derived from higher alcohols (C_3 – C_6 monoesters) proved to have better efficacy as compared to the monoesters derived from lower ones (C1 – C2 monoesters) [133]. Their ability to be administered orally suggests that they may possess considerable advantages in the clinical treatment of lead toxicity however, extensive studies are further required to reach a final conclusion.

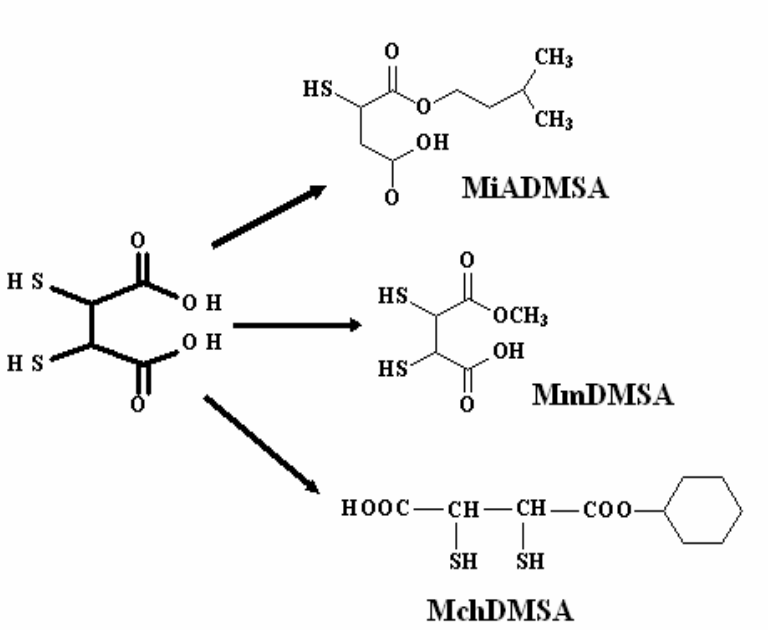


Fig. 3: Newly synthesized monoesters of DMSA

Combination therapy: This is a new trend in chelation therapy to use two structurally different chelators. The idea of using combined treatment is based on the assumption that various chelating agents are likely to mobilise toxic metals from different tissue compartments and therefore better results could be expected [118, 134]. Flora et al. [118] observed that combined administration of DMSA and $CaNa_2EDTA$ against chronic lead poisoning lead to a more pronounced elimination of lead and better recoveries in altered lead sensitive biochemical variables beside no redistribution of lead to any other organ was noticed. Co-administration of DMSA and MiADMSA at lower dose (0.15 mmol/kg) was most effective not only in reducing arsenic-induced oxidative stress but also in depleting arsenic from blood and soft tissues compared to other treatments. This combination was also able to repair DNA damage caused following arsenic exposure. We thus recommend combined administration of DMSA and MiADMSA for achieving optimum effects of chelation therapy [135]. Beside the use of the two different chelators for the combined therapy, number of studies have been reported where a co-administration of a dietary nutrients like a vitamins e.g. thiamine [136], an essential metal viz. zinc [137-138] or an amino acid like methionine [139] with a chelating agent lead to many beneficial effects like providing better clinical recoveries as well as mobilization of heavy metal.

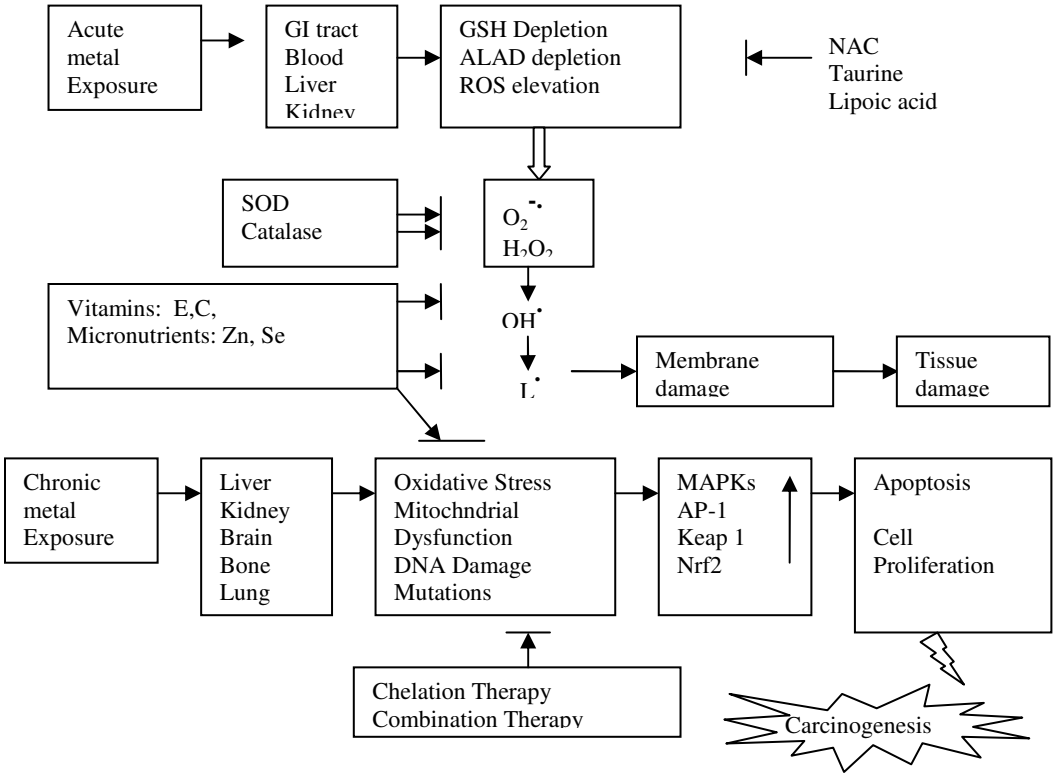


Fig. 4: Acute and chronic symptoms of metal toxicity and possible preventive and therapeutic measure

Combined administration of vitamin C with DMSA and vitamin E with MiADMSA was found to have more pronounced depletion of brain arsenic and useful in the restoration of altered biochemical variables particularly the effects on heme biosynthesis and oxidative injury [140]. Vitamin E administration with MiADMSA was found to be beneficial in reducing body lead burden whereas co-administration of vitamin C was beneficial in reducing oxidative stress condition [140-141]. Figure 4 describes effects of acute and chronic heavy metal poisoning and role of different preventive and therapeutic measures to against them. Although, there are number of chelating drugs which have been tried as treatment for metal poisoning but they are known to be compromised with side effects particularly their binding to essential metals within the system which significantly reduce their efficacy. These facts led to few novel strategies/approaches for treating cases of metal poisoning like including administration of antioxidants, either individually or in combination with chelating agents. Thus combination therapy could be a better treatment regimen to treat cases of chronic heavy metal poisoning.

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