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Review Article

HEAVY METAL INTOXICATION, OXIDATIVE STRESS AND ANTIOXIDANTS THERAPY: A REVIEW

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Abstract: The survival of human race depends upon, better environmental management. Therefore, continuous and sincere efforts will have to be carried out by everyone involved in environmental management, protection, monitoring, assessment, research, education, planning, conservation and sustainable development to use the resources. ATSDR toxicological profiles [1-7], published in USA have reported a number of hazardous chemicals and characterized the toxicological and adverse health effects. Nevertheless, these as well as other recent studies are incapable to provide much information about therapeutic data. Therefore, studies on this subject are regularly carried out by several investigators where the animals were intoxicated with hazardous heavy metals, such as mercury, methyl mercury, lead, chromium, cadmium, aluminum, zink, and other toxicants such as floride and arsanic etc. and thereafter, pre or post therapies were provided to eliminate the toxicants, to improve their health and to restore the altered conditions caused thereby. The objective of present contribution is to review the toxic effect of some heavy metals and possible therapy provided with antioxidants.

Key words: Heavy metals, Oxydative stress, Antioxidants

INTRODUCTION

In heavily populated countries, where environment is extremely polluted due to increased industries, profound mining, scarcities of safe drinking water, defective drainage system in villages and poorly developing housing colonies in cities, under nutrition, poor hygienic knowledge, greed and unawareness about the affect of pollutants, have enforced popular news paper Indian Express to write a title" The Everywhere Poison" (June 23, 2015). Though under this title, the author, Thuppil Venkatesh, has taken up the issue of lead poisoning in particular city, but condition is same with other heavy metals too all over country. All heavy metals are equally responsible for ill health in human and animals, but the children and adults differ in the relative risks of sources, metabolism and the ways in which toxicities are expressed. The offspring have a wide spectrum of subclinical and clinical effects for all type of poising.

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The metals with specific gravity greater than about 5 or of high relative atomic weight are defined as a heavy metal. Heavy metals like lead, mercury, manganese, chromium, cadmium, arsenic, Iron, cobalt, copper, manganese, molybdenum, zinc and aluminum, exist in all parts of the environment. Some of these are essential for human and animal body for development and general metabolism in small quantity but their excess is injurious. Iron, copper, manganese, and zinc, chromium etc belong to this category. Some of the heavy metals, such as mercury, arsenic, lead are non essential and injurious for human health even in small quantity. However, these and numbers of other heavy metals are used in modern technology for commercial purposes. Heavy metals can neither be degraded nor destroyed. For whatever purposes these heavy metals or their compound are used, all return to environment polluting water, soil and atmosphere. This is never ending process and all living organisms have to face it. It is also true that young generations are more severely affected than adult. Under such conditions, the major job left for scientists is to search for suitable therapy to treat the affected ones.

Antioxidants are a variety of chemicals with a capacity to scavenge free radicals and other reactive oxygen species. Oxygen is essential for living organisms but can damage the cell by releasing free radicals that are highly reactive chemicals produced in bodies, indiscriminately attack and destroy tissue, according causing aging, inflammation, allergies, disease and disrupt organ and enzyme and hormonal functions. Oxidative stress is the destruction caused by free radical molecules due to an imbalance between reactive oxygen species (ROS) and body antioxidant system. Oxidative stress also results from exposure to toxic chemicals, during periods of exercise, physical and emotional stress. Antioxidants combat free radicals by giving up the electron they would otherwise rob from nearby tissues. The protect and repair cells and neutralize free radicals that otherwise, damage the organs, cells and cell organelles. Dietary antioxidants available in fruits and vegetables are important in modulating oxidative stress.

1. Methylmercury intoxication and ashwagandha, vitamin E and glutathione therapies: Stress is one of the important risk factors that causes ill health and many times forecasts number of diseases. Herbal medicines have been used from the ancient time to control and improve the stress response [1,2]. Heavy metal, methylmercury, is an important stressor [3]. Its elimination from body is difficult and a slow process as compared to its quick adverse action in all body organs, especially in nervous system, where it acts like a neurotoxin. Mercury exists in metallic, inorganic and organic forms and all forms are toxic. Mercury is not known to be essential for any metabolic process, yet it readily accumulates in most body organs. About 80% of mercury released from human activities is elemental mercury [4] Mercury is transformed in the environment by biotic and abiotic oxidation and reduction, bioconversion of inorganic forms and methylation by microorganisms [4] There are evidences that the bacteria in the loop of intestine can methylate inorganic mercury to methylmercury [5], however another report gives no evidence of the synthesis of organomercury compounds in human and mammalian tissues [6].

Methylmercury, whether given orally or by injections, is completely absorbed and bound largely to plasma proteins and thiol compounds, L-cysteine and GSH. It is immediately transported to all body organs, especially the liver where it become highly construe in cell membranes. In most of the body organs methylmercury is converted to inorganic mercury. The conversion of methylmercury to inorganic mercury is considered a key step in the process of excretion of mercury after exposure to methylmercury [7].

Withania somnifera (ashwagandha) is a commonly used herb in Ayurvedic medicine. Rastogi and Mehrotra [8] identified and isolated about 35 chemical constituents in different parts of this herbal plant, which have been applied in a number of therapies including neuroprotection [10]. It is also known to be an antistress component [1] that relieved the animal from depression [2]. The active absolute constituents of *Withania somnifera*, sitoindosides VII-X and withaferin A (glycowithanolides) [9,11].

Since methylmercury is a potent stressor agent [3] and releases free radicals leading to lipid peroxidation which brings oxidative stress to the animals [12]. Since the free radicals are formed in different compartments of cell and antioxidants have different capacities to enter the diverse sections, a single antioxidant may not be sufficient [14], therefore application of mixed therapy may be useful to the toxicated subject. The objective of the present review is to relieve the animals from metal stress by

neutralizing the free radicals by antioxidants. The effectiveness of the mixed therapy of three antioxidants viz., Ashwagandha, vitamin E and GSH has been investigated on MMC induced stressed chicks in our laboratory [14].

Since MMC is an important stress agent [3] and releases free radicals [12] that increase lipid peroxidation in all compartments of the cell [15] leading to cellular damage, it is absolutely necessary to provide immediate therapy to the toxicated subject. Apart from this, methylmercury is known to decrease all macro- and micro-nutrients in experimental animals [16-19]. The metal has high affinity for enzymes, membrane proteins, nucleic acids, lipids, components of immune system, microtubules, hormones and neurotransmitters [20-25]. Thus methylmercury brings histopathological, biochemical, immunological and gerentological changes along with decrease in tissue and body weights.

Out of several phytochemicals existing in ashwagandha (AG), the sitoindosides VII-X and withaferin A (glycowithanolides), have been found to be important antioxidants and free radical scavengers [9,26]. AG also reverses the changes caused by lipid peroxidation that damages the cells [27], though the mechanism of action is not clear. It is one of the most common herb of antistress nature that increase life span and delay ageing [28]. Glycowithanolides found in AG are active principle ingredients act as potent natural antioxidant to increase defense system of body [26]. They also the protects cells from metal toxicity and environmental toxins [26].

Free radicals are molecules containing unpaired electrons. They attack the nearest stable molecules and rob their electrons. When the "attacked" molecule loses its electron and becomes a free radical itself, it is the beginning of a chain reaction. Excessive production of free radicals, or their inadequate neutralization by antioxidants, leads to damage the cellular macromolecules, the most vulnerable being lipids, proteins and nucleic acids. Free radicals are produced continuously in cells either as by-products of metabolism or deliberately as in phagocytosis and continuously eliminated by the body, both by enzymatic (superoxide dismutase, catalase, and glutathione peroxidases) and non-enzymatic (glutathione, tocopherol (Vitamin E), carotenoides, centrophenoxine and ascorbic acid) antioxidants [29].

In most of the earlier studies several therapeutic agents have been tried but in no case an absolute elimination of metals from the body and total reversal of ill effects have not been reported [4]. In those cases, where mixed therapy was tried, better results were obtained [16,17,19]. Therefore, it was desirable to screen various combinations of natural, herbal and synthetic antioxidant therapies. In this respect the combination of curcumin, vitamin B complex and glutathione has been found to be quite successful to eliminate MMC induced stress [30]. Hence, a multiplicity of antioxidants is considered to be beneficial because specific antioxidant molecules can be particularly effective for neutralizing specific ROS or RNS. A multiplicity of antioxidants is also beneficial because different antioxidants tend to locate preferentially in different areas of tissues and cells.

The ranking of antioxidants depends upon their redox potential, or their ability to be oxidized and reduced. As for example, Packer et al. [31] reported redox potential of lipoic acid is approximately negative 325 millivolts, for vitamin C it is approximately 290 millivolts and for glutathione, it is negative 250 millivolts. Moreover solubility of antioxidants (in fat and water) also increase the importance of antioxidants. As for example lipoic acid is both water and fat-soluble, hence more effective than water soluble vitamin C and fat soluble vitamin E [32]. Vitamin E is only effective in lipid-containing areas, whereas glutathione can be found in the "watery" areas. Depending upon the ability to enter diverse cell parts, different antioxidants operate in different cellular compartments as well as within different tissues. Chaudhari [33] also reported that particular antioxidant effect is not only compartmentalised in the cell but also is tissue specific. Overall data clearly advocate mixed antioxidant therapy, which is supported by the present investigation.

The production of malondialdehyde is used as a biomarker to measure the level of oxidative stress in an organism. The increase of malondialdehyde during MMC intoxication [14] is a clear indication of increased oxidative stress during intoxication and release from stress during Ashwagandha, vitamin E and GSH therapies. Further complete control level of this biomolecule during mixed therapy as well as increase

of body weight in these growing chicks [34]. are strong indications of relieving the animals from stress.

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2. Lead intoxication and alpha-lipoic acid and bamboo shoot extract therapies: The role of reactive oxygen and hydroxyl species, with the subsequent oxidative corrosion of biological macromolecules in the toxicities associated with transition metal ions is reviewed [1,2]. The last century has seen both the greatest ever exposure of the general population to lead and a surprising amount of new research on lead exposure and toxicity. In ancient civilizations, uses of lead included the manufacture of kitchen utensils and other decorative articles. However, lead is also toxic to humans, with the most deleterious effects on the hemopoietic, nervous, reproductive systems and the urinary tract.Even very low levels of lead have adverse effects on the brain and behavior in children. The majority of cases of lead poisoning are due to oral ingestion and absorption through the gut. Lead poisoning in adults occurs more frequently during exposure in the workplace [3] and primarily involves the central nervous system. Lead is a ubiquitous environmental toxin that induces a broad range of physiological, biochemical, and behavioral dysfunctions [4]. Its toxicity has been known from ancient times and many studies have explored the mechanisms and symptoms of this toxicity through the years. Despite the knowledge that lead can induce oxidative stress, the usefulness of antioxidants alone

or in conjunction with synthetic chelators has not been thoroughly explored. The toxicity of lead is probably related to its affinity for cell membranes and mitochondria, as a result of which it interferes with mitochondrial oxidative phosphorylation. In their review Kalia and Flora [5] suggested that treatment for lead toxicity involves the utilization of chelating agents, principally calcium disodium EDTA, dimercaprol, penicillamine and succimer.

Bamboo is a natural resource in the world. Bamboo shoots are the young and tender culms of bamboo utilized as one of the food items in many countries. Bamboo shoots are low in fats and cholesterol contents, but very high in potassium, carbohydrates and dietary fibers. Bamboo shoots are generally 8-12 inches long, taper to one end and grow extraordinarily. However, their size and weight depend considerably upon the various environmental and geological factors viz. location, depth and nutrition of the soil, watering and drainage conditions, rainfall, temperature, pH and soil fertility. With different flavones and glycosides, bamboo shoots have excellent anti-microbial qualities. Hypolipidemic effect of bamboo shoot oil has been examined on rats [6]. Boiled bamboo shoots are used as appetizers and the decoction of shoots are used for cleaning wounds and maggot infected sores, ulcers etc; mixed with palm-jaggery, it is known to induce parturition and abortion [7] and its shoots are used in preparation of steroidal drugs [8].

Elemental lead and inorganic lead compounds are absorbed through ingestion or inhalation. Lead is absorbed into blood plasma, where it equilibrates rapidly with extra cellular fluid, crosses membranes (such as the blood-brain barrier and the placenta), and accumulates in soft and hard tissues. Recent studies have shown that lead causes oxidative stress by inducing the generation of reactive oxygen species, reducing the antioxidant defense system of cells via depleting glutathione, inhibiting sulfhydryl-dependent enzymes, interfering with some essential metals needed for antioxidant enzyme activities, and/or increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition. Consequently, it is plausible that impaired oxidant/antioxidant balance can be partially responsible for the toxic effects of lead. Various antioxidants of synthetic and natural origin may help to maintain balance in this oxido-reductase potencial of cell.

Because lead and other metals have a high affinity for sulfhydryl (-SH) groups, mercaptides are formed with the -SH group of cysteine, and less stable complexes with other amino acid side chains [9] Lead is shown to inhibit several enzymes having functional SH groups [9]. ALAD is the most known enzyme that is inhibited by lead via direct binding of lead to the -SH groups that are essential for the catalytic activity of the enzyme [10,11]. Administration of antioxidants may inhibit the binding of lead to thiol groups and compete with lead in this manner. By this mechanism it not only recovered erythrocyte ALAd activity but also restore kidney and brain reduced glutathione content.

GSH is a tripeptide containing cysteine that possesses a reactive -SH group with reductive power. Accordingly, GSH plays a vital role in the cell protection against oxidative impairment. It can act as a nonenzymatic antioxidant by direct interaction of the SH group with ROS, or it can be involved in the enzymatic detoxification reactions for ROS, as a cofactor or a coenzyme [12,13]. It possesses carboxylic acid groups, an amino group, a sulfhydryl group, and two peptide linkages as sites for reactions of metals [14]. Pb12 binds exclusively to the SH group[15] which decreases the GSH levels and can interfere with the antioxidant activity of GSH. On the other hand, GPx and catalase are metallop-roteins and accomplish their antioxidant functions by enzymatically detoxifying peroxides, H_2O_2 and O_2^-, respectively. Since these antioxidant enzymes depend on various essential trace elements for proper molecular structure and enzymatic activity, they are potential targets for lead toxicity [16]. Schrauzer [17] indicated antagonistic effects between lead and selenium, resulting in reduced selenium uptake that may affect GPx activity, which requires selenium as a cofactor, and then may increase the susceptibility of the cell to oxidative damage. The lipoic acid and antioxidants present in BSE may inhibit binding of lead to these enzymes active sites and allow their respective cofactors to bind with them and thus support enzyme to work normally. The combined therapy has shown much more advantage over their effect when used alone.

γ-GGT activity and TBARs level are the marker for tissue damage and they increase in the condition of oxidative stress. γ-GGT activity is also one of the responsible mechanism behind the low reduced GSH content as its increased activity split this molecule.

The increase peroxyl radical damage lipid bilayer of cell membrane and thus peroxidative damage to membrane by free radical occur which in turn increase the cell fragility and disrupt intra and extra cellular oxidative balance. As antioxidats used, restore reduced glutathione activity there is no need for γ-GGT to act rapidly and thus no free radical generated.

A number of studies of bamboo have yielded information about the chemical constituents, but no systematic evaluation has been carried out, so it is difficult to determine which of the identified compounds might be among the primary active constituents [18]. It is conceivable that compounds of similar chemical structure in bamboo may contribute to the effects of the herb and its extracts on brain and kidney function. Further work should be carried out in this direction.

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3. **Lead induced nephritic and neuronal oxidative impairment and bamboo leaves extract and alpha-lipoic acid therapies:** The industri-alization and pollution proceed hand in hand. The best way to balance the two is to reduce the extent of pollution and develop some cure for the effective individuals. Lead, a non-essential heavy metal recognized as a general metabolic poison, is ubiquitously found in the environment as a result of high practice of leaded petrol, paints, water pipes, ceramic products, ammunition and roofing, batteries and soldered food cans, variety of medical, scientific and military equipments [1]. Although usage of lead in manufacturing some of the products have been banned since last few decades, even low level exposure of this metal is also very much harmful to all living organisms.Today lead has become omnipotent as it exist in all components of environment (air, soil and all water bodies), in animal and plants or even mother's milk and sperm [1]. The fruits, vegetables, grains, canned food, soft drinks, cigarettes are good source of lead to which, and man is exposed.

Lead enters in body by breathing, food or by skin. Once lead enters in blood it deposits in soft tissues (liver, kidney, brain, spleen muscles and heart). In adults 94% of lead is stored in bones and teeth. The lead that is not stored in hard tissues leaves the body through urine and feces.

Lead exposure may cause memory loss, weakness, blood pressure, anemia, damage the brain, kidney and testis, causes miscarriage and effect thyroid functions. Along with this lead causes immunological, biochemical, histopathological changes, decrease macro- and micro-nutrients. Unborn children are exposed to lead through mother and newly born through mother's milk [1].

Till date direct mechanism of lead toxicity on organs or systems is unrevealed [2,3] but still reports suggest its contribution in developing various physiological

dysfunctions as a result of production of oxidative stress and impairment in a range of metabolic and biochemical pathway [4]. Studies conducted in vivo on animals and epidemiological studies on human population suggested that lead exerts its effect by disturbing cell's pro-oxidant-antioxidant status which results in the production of free oxygen radicals, a key player which cause cell damage and also damage to cellular organelles [5,6].

Lead interrupt mainly three systems i.e. hematopoietic, renal and nervous system [7, 8]. To overcome this problem of scavenging free radicals produced by heavy metal intoxication, antioxidant therapy is the best suggested therapy. Treatment of antioxidants of different synthetic and natural origin is the best recommended way to conquer the problem of lead toxicity. To our knowledge, very limited work has been done on the use of antioxidants in reduction of oxidative stress induced by lead in the animal model. For this purpose, in the present study we have intended to check the effect of synthetic antioxidant "**α**-lipoic acid (LA)" in combination with a herbal antioxidant i.e. "bamboo leaves methanolic extract (BME)" on lead induced oxidative stress on albino rats. Several reports suggested the use of many thiol containing antioxidants to nullify the effect of metal induced oxidative stress and tissue damage [9,10].

α-lipoic acid, a low molecular weight dithiol that possesses metal chelating capabilities, found in most of the eukaryotic and prokaryotic cells. It can readily be absorbed through gut, crosses blood brain barrier and reaches to brain. **α**-lipoic acid and its reduced form dihydroxylipoic acid both are important cofactors in the mitochondria and strong antioxidants. However, antioxidants derived from plant products (or dietary antioxidants) have been proposed as excellent replacements to compensate the natural antioxidant of body when ever reduced during stressed conditions. As for example bamboo leaves have been used as an herbal remedy from the ancient time in Asia [11-13]. Fu et al. [14] found that antioxidant of bamboo leaves was capable of blocking chain reactions of lipid auto oxidation, chelating metal ions of transient state, scavenging nitrite compounds and blocking the synthetic reaction of nitrosamine.

Since lead is a potent stressor agent, its concentration in all environments is increasing day by day and ultimately effecting the human population either directly or through food chain, it is necessary to search proper and meaningful therapy. For this purpose, mix therapy including herbal, natural and/or synthetic antioxidants is considered to be quite advantageous as in the case of mercury and methylmercury detoxication [15,16] or as protector against lead [17]. Inspired from these investigations therapy to lead intoxicated subjects with **α**-lipoic acid and bamboo leaves extracts, either alone or in combinations has been applied and desired results are obtained.

Out of total circulating lead, 99% is present in the erythrocyte [18] which also interferes in most of the reactions of haem biosynthesis. Due to direct the binding of metal to -SH group, many enzymes containing sulfydryl group at its active site becomes inactive [19]. Out of 99% lead 80% of lead binds only to a sulfydryl containing enzyme **δ**-aminolevulinic acid dehydratase as a result of this its substrate can't be converted to product, porphobilinogen, a precursor of haem synthesis pathway and δ-aminolevulinic acid accumulates in the erythrocytes and oxidized. This oxidized substrate further form free oxy- radicals to cause progressive tissue damage. In our study both the source of antioxidants succeed to prevail over effect of lead to significant extent but their combined therapy is more effective.

GSH, the key player of antioxidative system and an intermediate of **γ**-GGT cycle, is highly susceptible to lead induced oxidative damage because lead have the tendency to bind '–SH' group of protein and make them unavailable for their original functioning of buffering redox status and is also a substrate cofactor for many antioxidative enzymes. The same mechanism is applicable for the membrane bound and unbound total sulfydryl content. The glutathione system also plays role in neutralization of peroxides and maintenance of protein thiols in their reduced state. Any change in GSH or total thiol (decline or incline) is an indicative of antioxidative imbalance. Initially as oxidative stress produced in the cell GSH increases but on the progression of higher oxidative stress the level goes down. Any damage and restoration related to reduced glutathione and total thiol in all the groups receiving different treatments were more in brain than in kidney. A plasma membrane bound enzyme, **γ**-glutamyl transpeptidase (**γ**-GGT) metabolizes extra cellular reduced glutathione. **γ**-GGT break GSH into **γ**-glutamyl and cysteinyl-glycine moieties whose thiol is much more reactive than the original compound. Because later

lacks the **α**-carboxyl group of glutamate, which have been reported to prevent the interaction of cysteine –SH group with transition metal ions [20]. Also later one reacts with iron present freely in the system and gets oxidized. Reduced iron formed during this reaction reacts with molecular oxygen and at last form hydrogen peroxide. In the experimental animals exposed to lead have shown higher **γ**-GGT activity, which was further lowered down in the groups receiving LA or MBE alone and more efficiently by combined therapy. The decrease in GSH content in lead treated group [21] and its recovery in the animals given antioxidant therapy in both the organs might prevent oxidation of protein thiol and imprecise binding of lead to GSH. Also it has been proved that **α**-lipoic acid plays a very important role in replenishing body's other antioxidant elements like ascorbic acid, **α**-tocoferol, reduced glutathione etc and scavenging reactive species [22].

High level of peroxides produced due to action of **γ**-GGT can be removed by GPx and CAT like antioxidative enzymes. The lead exposure has decreased the activities of antioxidative enzymes like glutathione peroxidase (GPx) and catalase (CAT) leads to the increased H_2O_2 production. GPx, a selenium dependent enzyme has shown decreased activity, as lead has the tendency to bind selenium [23,24]. and also due to impairment of functional groups like GSH and NADPH.

Increase in thiobarbituric acid reactive substances (TBARs) level have been reported as a marker of endogenous lipid peroxidative damage and have also been studied extensively as a factor responsible in lead induced toxicity [25]. Lead exerts its effect on cell membrane lipids directly [26]. or as a result of loss in reduced glutathione. High lipid peroxidation of membrane lipids leads to alteration in their integrity and deterioration of erythrocyte [2]. As a result cell membrane disruption occurs and essential components for enzyme activities and other antioxidative molecules of cells leached out. The lead induced lipid peroxidation was observed in both brain and kidney, as an indicative of peroxidative damage, which was almost restored by combined antioxidant therapy and to some extent LA and MBE therapy alone has also been proved beneficial. Sood et al. [27] have observed that both the antioxidants (LA and MBE) were more effective in brain than in kidney and reaches almost near to control group.

It appears that lead acetate causes significant oxidative stress in the kidney and brain tissues as a result of free radicals generated by oxidative stress, which was recognized by increased lipid peroxidation and **α**-GGT activity; decreased level of antioxidants molecules like GSH and TSH and significantly diminished activities of antioxidative enzyme GPx and CAT [27]. The lead exposed groups subjected to antioxidants treatment either alone or in combination has shown the beneficial effects where α-lipoic acid proved it self a better antioxidant than bamboo leaves methanolic extract. Although bamboo leaves methanolic extract as a whole not exerted as good effect as **α**-LA in the present study, it didn't prove it weak antioxidants because extracted material was not 100% pure. It may contain some impurity besides very important chemical constituents, previously isolated and proved beneficial as antioxidants. Still combined therapy showed best results than their therapies given alone.

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4. Inorganic mercury intoxication in fish and monothiole and vitamin B complex therapies

(Proteins, Cholesterol, Triglycerides, acid and alkaline phosphatases): On one hand, whole world is after the pollution control, on the other hand heavy metals are being spread extensively in air, soil and water due to human activities. This condition is not only in developing countries, but developed countries too are equally responsible for damaging the earth environment (Toronto Star April 7, 2010 reported dumping of 9,000 kg mercury in to Wabigoon River). Such reports on different heavy metals, pesticides, insecticides and other harmful substances are routines in daily news and periodicals all over the world.

Recently Guizhou province in China is declared as mercury capital of china as 80% of country mercury deposits are present in this area [1]. In an interesting study, Zhang et al. [2] found high concentration of methylmercury in rice rather in fish [3]. They fond 96% methylmercury in human body through rice consumption. In India the mercury pollution is tremendous and reported time to time in different area of country [4-6]. In USA studies [7] some isolated case are reported in review.

It is usually said prevention is better than cure. In spite of best efforts, when former fail the later becomes important. Therefore, presently therapy has become the key factor for human health along with prevention at all steps.Proteins are highly complex nitrogenous organic compounds of biological systems. They are extremely sensitive to mercury compounds [8-10]. Rao et al [11] showed that B vitamins and monothiols are ideal agents for mercury mobilization [11] it is essential to look the biochemical machinery. These authors have demonstrated a high concentration of mercury in all major tissues (brain, liver, kidney, gills and muscles) of fish *Channa punctatus*.

All mammalian cells are able to synthesize cholesterol. In blood a definite pools of cholesterol is maintained in all animals which fluctuate by several factors like diet, physiological stress, disturbance in cholesterol synthesizing machinery and xenobiotic agents. A limited number of studies are available in the literature indicating the increase of cholesterol in plasma [12,13] and decrease in blood cells, kidney, liver and brain [14-16]. Studies to restore cholesterol level in brain of rat [17] and nervous and non-nervous tissues of chick and mice [16] in methyl mercury intoxicated animals with GSH and vitamin therapy is available in literature.

Triglycerides or neutral fats are the fatty acid ester of glycerols, which is a most common and widespread class of lipids in nature and the fat depots of animals. Triglycerides are abundant under skin, abdominal cavity and mammary glands. They are better adopted than glycogen to serve as a storage form of energy, therefore, provide energy source during hibernation, dormancy or migration. Decrease of triglyceride in nervous and non-nervous tissues of rat and mice during methyl mercury intoxication is reported by Sood et al. [15,16], but no information is available in literature about fish tissues. However, enhanced fatty acids in brain and kidney of guanine pig [14] and reduced phospholipids in mouse brain [18] and galactolipids in rat brain [19] during organic mercury intoxication has been reported.

Amongst the hydrolases, acid phosphatase is one of the most widely studied enzymes due to its ubiquitous presence in lysosomes and its fluctuations during toxicity [17,20]. It is known to be inhibited in animal tissues during mercury intoxication [21-23]. The fluctuations of the enzymes in animal tissues during

mercury toxication create physiological and pathological alteration [24], therefore, it is necessary to maintain its normal level in all the tissues of animals.

There are some isolated studies in literature demonstrating the inhibition of alkaline phosphatase during mercury intoxication [25]. Moreover, it is a zinc and magnesium dependent enzyme [26] and both these metals are significantly removed from tissues by mercury [27], therefore, recovery of such a mercury sensitive enzyme is a good evidence to judge the therapeutic capacity of any antidote. Rao etal. [11,28] studies shows decreased level of proteins, cholesterol and triglycerides in all the fish tissues irrespective of differential mercury content in intoxicated and mercury washed groups. Since the source of raw materials for all these macromolecules is the intestinal food stuff, one of the factor for their low level appears to be deficient intestinal adsorption as mercury retards this function [29,30]. Sastry and his associates [31,32] also reported that heavy metals, including mercury decrease the rate of glucose, fructose and xylose adsorption in the intestine of *Channa punctatus*. Raut et al.[33] also discovered the effect of metabolic inhibitors on the absorption of amino acids and sugars in the fish intestine.

In one study the animals were not fed during the intoxication period which was only 96h [28], therefore, during this period only a little mercury can enters in gastrointestinal tract through food. Hence most of the mercury was absorbed by gills and skin. High concentration of mercury in various tissues of *Channa punctatus* verifies this fact [11]. Disturbance of biochemical machinery in different tissues of animals by mercury is well recognized [34,35]. Nevertheless, the recovery of bimolecules is least known except a few isolated studies [36,37]. Our laboratory study showed recovery of proteins, cholesterol and triglyceride as well as phosphatases during GSH, B vitamins, NAHT and their virious combinations [11]. Interestingly mixed therapy appears to be the best in mercury elimination in fish and restoration of normal conditions of animals.

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5. Methylmercury intoxication, myelin degeneration, CNPase inhibition and vitamin B complex and glutathione therapies: Myelin marker enzyme, 2',3' cyclic nucleotide 3' phosphohydrolase (CNPase), has been found to be inhibited during myelin degeneration in the central nervous system of the mice intoxicated with methylmercury [ ]. However, during B vitamin and glutathione therapies (either alone or in combination), a significant recovery of enzyme is accomplished with myelin regeneration in all parts of brain**.** 2',3' cyclic nucleotide 3' phosphohydrolase (CNPase) is a myelin marker enzyme [1]. Its high concentration has also been demonstrated in the membranes of astroglial and neuronal cells [2], erythrocytes [3] spleen [4], retina [5], liver [6] (Dreiling et al., 1981) as well as in cerebrospinal fluid [7]. It hydrolyses 2',3'-cyclic nucleotide derivatives of adenine, guanine, cytidine and uridine to 2'-nucleotides in a decreasing order [8]. CNPase is known to be severely inhibited during inorganic and organic mercury intoxication [9,10], pathological conditions and demyelination [11]. Recently evidences are pouring in that myelin regenerates in CNS in different experimental and pathological conditions [12-15]. During the course of investigation of suitable therapeutic agent for methylmercury detoxication, authors found a correlation of mercury deposition with CNPase inhibitionand myelin degeneration, which are recovered significantly when metal burden is reduced during therapy (Figs.1,2).

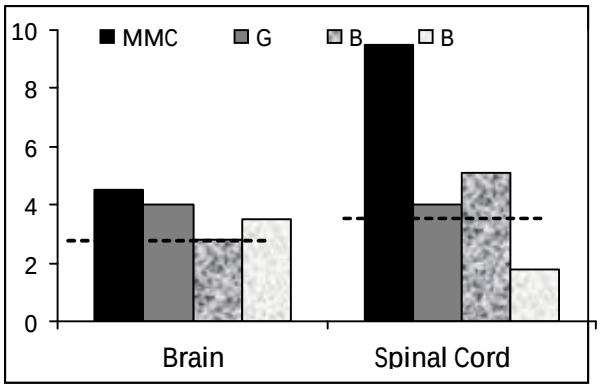

Fig. 1: represent mercury deposition (MMC) and its mobilization with glutathione (G), vitamin B-complex (B) and their combination (B_2) therapies in brain and spinal cord of mice. The broken line represents the least significant dif-ference ($p<5\%$). [16].

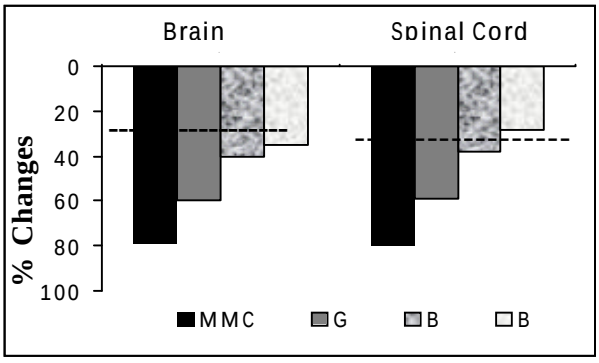

Fig. 2: represents inhibition of CNPase activity and its restoration with glutathione (G), vitamin B-complex (B) and their combination (B_2) therapies in brain and spinal cord of mice. The broken line represents the least signifi-cant difference ($p<5\%$). [16].

The CNPase activity is decreased by 79 to 82 % in brain and spinal cord of MMC intoxicated animals [16]. Banik et al.[17] also reported about 60 % loss of enzyme from myelin during spinal cord injury. The enzyme is known to perform multiple functions. In nervous tissue it is related to myelin protein synthesis [18] and myelin formation [19,20] during development. The enzyme is also supposed to be involved in demyelinating neuropathies in animals and human beings [21,22]. Both mercury and methylmercury inhibit CNPase in isolated myelin as well as in central and peripheral nervous system [9,10]. The high lipid solubility of methylmercuric chloride may also divert organomercurial to the myelin of the nerve, where it very efficiently inhibits neuronal excitability [23]. The enzyme is primarily bound to the membrane, thus related to membrane function [6]. Specific reasons for its inhibition with methyl-mercury is far from clear as neither metal-mercaptide

complex formation nor increased lipid peroxidation, triggered by methylmercury, is supposed to inhibit the enzyme activity [9]. These authors also found restoration of enzyme with thiol-reducing agents and suggested "enzyme sulfhydryl groups are partially oxidized in the native myelin or during isolation procedure" or "methyl radical itself (CH_3), generated in close vicinity of enzyme active center, would play a role in the irreversible enzyme inhibition and methylation of the enzyme active groups, once achieved, would account for the irreversible blocking of the active site".

An acute myelin degeneration has been found in Minamata patient [24] and experimentally MMC intoxicated animals [16,25]. Banik et al. [17] reported that decreased CNPase activity corresponds with the myelin loss and the extent of loss of the enzyme activity depends upon the severity of the injury. Investigations from our laboratory also supports their findings as 78 – 82 % loss of CNPase in MMC intoxicated mice leads to severe myelin degeneration in all brain areas and spinal cord of mice.

Both, GSH and vitamin B complex reduced mercury burden from central nervous system that immediately led to the restoration of CNPase in brain and spinal cord and simultaneously appreciable repairing of myelin sheath [16]. Such observations clearly indicate the loss and recovery of the enzyme is an important factor related to demyelination and repairing respectively. Interestingly enough, myelin and axonal regeneration has recently been discovered in CNS of several experimental animals and pathological conditions [26-30]. Mercury compounds are known to reduce the synthesis and secretion of interlukin I, a cytokines [31] and Nieder et al. [32] reported that administration of cytokines upregulates the myelin synthesis and promote myelin regeneration in adult CNS. Sharief [33] also found that cytokines are responsible for remyelination and repair. Further, vitamins modulate cytokines synthesis [34] and methylmercury decrease most of the vitamins [35,36]. The deficiency of vitamins results the cytoarchi-tectural alteration and decrease myelination in the brain [37] and simultaneously reduction in CNPase [38].

In several studies, the loss of B vitamins in central nervous system of mercury and methylmercury intoxicated animals has been reported [35,36]. Deficiency of these vitamins has been shown to create metabolic defect in CNS. As for example spongy demyelination during vitamin B_{12} deficiency [37], demylination and encephalopathy during vitamin B_2 deficiency [37], and axonal degeneration during vitamins B_1, B_6 and B_{12} deficiencies [38]. Our laboratory studies show that exogenous application of these vitamins to methylmercury intoxicated animals accelerates the metal elimination that may restore B vitamins levels as demonstrated by Sood and Vijayalakshmi [35] that repair of myelin sheath and neurofilaments. Thiamine deficiency also causes the decrease of cholesterol synthesis, which is major constituent of myelin sheath [39]. Low cholesterol level in the brain of MMC intoxicated mice treated under similar experimental conditions and its restoration during vitamin B complex application has been well documented [40]. Kromidas et al. [41] also reported that glutathione and vitamin B complex co-administration prevents the loss of microtubules. GSH, likewise, is an important naturally occurring antioxidant, decreased from brain during MMC intoxication and recovered during its exogenous application [42]. The GSH deficiency increases lipid peroxidation and release free radicals that appear to be a serious cause of myelin degeneration.

From over all data it appears that several factors, such as loss of vitamins, GSH, cytokines, cholesterol and other lipids, increased lipid peroxidation and free radical formation and deficiency of myelin metabolizing enzymes including CNPase, are involved in myelin degeneration. During therapy, mercury burden is reduced, lipids level is restored, vitamins, cytokines and GSH levels are maintained and lipid peroxidation and free radicals are reduced. All these factors together appear to be responsible for repairing myelin sheath.

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6. Inorganic mercury intoxication in fish, glycosidases inhibitin, and GSH, NAHT, vitamin B complex therapies: Mercury is a potent poison and ubiquitously distributed all over the earth. It methylates in nature by several bacterial species and forms organic mercury that enters in food chain [1]. Thus mercury exists in nature as metallic mercury, inorganic mercury and organic mercury and all forms are poisonous. Fish may accumulates mercury either directly from water or through components of food chain. Mercury has long half-life in fish, approximately 2 years [2]. Mercury excretion by fish varies depending upon species, temperature and several other factors. Along with alterations in biochemical machineries of all cells [3-5], it causes weakness, vomiting sensation, headache and loss of memory in humans [6] as well as causes physiological, behavioral, degenerative, teratological and immunological changes in animals and all these together lead to irreparable damage causing ill health and death of affected subject [1].

In the past a number of chelators have been tried to eliminate the metal from animal body as well as to reverts the ill effect of mercury intoxications, and in many cases significant results were obtained by the application of natural and synthetic antioxidant mixed therapy [7- 11]. Takehara et al. used N-acetyl-DL-homocysteine thiolactone (NAHT, a synthetic antioxidant [12]. Along with this, Sood et al. [9] applied glutathione (a natural antioxidant synthesized in animal body) along with B-vitamins which may

behave like adjuvant and assist the antioxidants in the renovation of glycosidases inhibited in toxicated fish.

Sood et al. [10] studied various glycosidases (alpha and beta glucosidase, beta galactosidases and alpha mannosidase) in all major tissues (brain, liver, kidney, gills and muscles) of fish *Channa punctatus*, and found a significant fluctuations of enzymes in all tissues during mercury intoxication that indicates the disturbed metabolism leading to ill health of the animals. When compared with other enzymes in fish tissues [9], glycosidases show different conditions in different tissues. As for example mercury decreases alpha glucosidaes in brain, kidney, liver and gills, but increases significantly in fish muscles; beta glucosidase decreases in muscles and brain and increases in other tissues; beta galactosidases and alpha mannosidase decrease in brain, gills and muscles and increase significantly in kidney and liver. Our earlier investigation showed highest concentration of mercury in fish kidney followed by liver, gills, brain and muscles under similar experimental conditions [13]. Thus mercury quantity in any tissues is insignificantly related to degree of enzymes alteration. Almost similar results are reported in brain, liver and kidney of rat [14], mice [15], chicken [16]. Thus it appears that liver and kidney though restrain a higher load of mercury as compared to other tissues, the enzymatic changes are not concordat with mercury concentration.

Glycosidases are localized mainly in lysosomes [17] though their presence in myelin sheath and microsomes of several animals is also reported [18,19]. In plant cells the enzymes are exclusively reported in lysosomes [20]. Whatsoever may be the locales of the enzymes, the net result is that there is a significant alterations of all the four glycosidases studied in brain, liver, kidney, gills and muscles of fish during mercury intoxication. Further, most of the organs of mercury washed groups showed recovery of the glycosidases, which clearly indicates that mercury in tissues was mainly responsible for the altered condition of the enzymes, though load of mercury in any tissue was not related to the extent of enzymes alterations. Interestingly our earlier study on *Channa punctatus* showed mercury elimination in mercury washed as well as in therapeutic groups under similar experimental conditions [13].Vaidehi et al. [21] also found quick elimination of mercury in methylmercury intoxicated fish (*Rasbora buchanani*) tissues during the application of glutathione, vitamin B complex, and their combination therapy.

Mercury is known to be an important stressor agent [22] that releases free radicals in different cellular compartments and increase reactive oxygen species [23]. Since antidotes have different capacity to penetrate different cellular slots, a particular antidote or antioxidant unquestionably can not be helpful in eliminating the toxicants or repairing the damage caused by toxicant in all cellular compartments. This interpretation is helpful to explain why multiple therapeutic agents to gather revealed best results. Similar observations are made in chicks [24]. Along with potent antioxidants, the application of some helper, for example vitamin B complex in the present case, further assist the antioxidants as B vitamins are known to maintain the adaptive capacity to resist a large number of chemical and physical stressor agents commonly encountered in the community and industrial environment [25]. They preserve tissue glutathione that maintains -SH groups [26]. Further, vitamin B_6 and B_{12} mobilize heavy metal from brain and repair cellular machinery including myelin sheath [27].

Thus it appears that GSH and NAHT along with decreasing the mercury burden from body, compensate the antioxidants and B vitamins take up the repairing work immediately. An important evidence in this respect is that all vitamins including B complex severely decreased during mercury intoxication [28].

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7. Inorganic mercury intoxication in fish, Na^+, K^+, Ca^{++} ATPases inhibitin, and GSH, NAHT Vitamin B complex therapies: Accumulation of pollutants in water bodies is one of the major problems causing health hazards to human being either directly or through food chain. Mercury (both inorganic or organic compounds) is much more dangers than other heavy metals due to several reasons: i) completely non-essential element, ii) enters in food chain, iii) inorganic mercury is easily converted to organic form (which is much more toxic) in water body by bacterial species [1], iv) being lypophilic in nature methylmercury can enter the cell easily, v) methylmercury is a potent neuropoison, vi) after deposition, mercury leaches out slowly from non-nervous tissues and excreted through urine and bile, but during recirculation the metal concentration increases in brain, vii) metal interacts with -SH and –SS- groups of proteins and DNA and disturb metabolic functions, viii) deletes natural antioxidants and cause oxidative stress, ix) creates acute neurodegeneration and paralysis, x) decrease essential macro- and micro-nutrients from the body [2-9], xi) enters in mother milk and sperm effecting the next generation [10], xii), decreases ATP and glucose levels [11], xii) tetragenic in nature, xiii), the long term poisoning creates hypertension [12], xiv) effect the immune system of body [13] and xv) it is an important carcinogen agent [1].

In spite of this baste knowledge, neither the use of mercury has not been stopped or reduced nor much preventative measure have been adopted, specially in third world countries. Therefore, the challenge left for scientists is to search for suitable therapy. A number of chelating agents have been used in past [1,14-16] and in many cases success has been achieved, but a lacuna is left in most of the studies, such as: i) therapy is provided either as pre-treatment or simultaneously (post therapeutic treatments are limited), while we are aware with the fact that therapy is required to toxicated subjects, hence trial should be carried out as post therapy, ii) in most of the studies all of the body organs are not taken in to account which is absolutely necessary for screening the therapeutic capacity of any chelator and finally iii) many time chelators are toxic themselves. In our

laboratory investigations natural antioxidant like GSH, synthetic antioxidant (NAHT) and B vitamins (alone or in various combinations) are used as post therapeutic agents considering that they will not be injurious if used in optimum doses and their exogenous application will replace the lost concentrations of these essentials, should help in mercury elimination from all body organs, and repair histopathological and biochemical lesions. In our previous investigation, we have reported that monothiols and vitamins are ideal therapeutic agents for mercury elimination from fish tissues [17]. It is known that Na^+, K^+ and Ca^{++} transport is also disturbed by mercury [18-20], therefore, present work is restricted to the recovery of these enzymes in nervous and non nervous tissues of fish *Chana punctatus*.

In recent years, increasingly attention has been paid to analyze the possible adverse health effects of mercury and its analogues due to their wide spread distribution in the environment. Recently, some reports have indicated that the mercury-soil pollution (mostly mercuric sulfide) is cumulative as a consequence of the enormous increase in contaminant mercuric compounds in Asia [21]. Metallic and inorganic mercury are released into the environment (primarily into air) from mining, smelting, industrial activities, combustion of fossil fuels, and natural processes [1]. The metallic and inorganic mercury from all sources are deposited to water and soil, where they are transformed by microorganisms into methylmercury.

Monothiols and the vitamins are well recognized metal chelators. Their individual efficiency as well as combination therapy against mercury elimination has already been reported by us [22-26]. A comparison of this study with the previous onces [25,26], it is evident that ATPases fluctuations in any tissues directly depends on the elimination of the metal from the tissues. Thus the tissues from where mercury is eliminated, Na^+, K^+ and Ca^{++} ATPases are also recovered. This may be perhaps due to increased synthesis of the enzymes as a result of repairing of protein synthesizing machinery in mercury stress free cells.

Freitas et al. [27] demonstrated inhibition of Ca^{++} and Ca^{++} ATPase in E/R of mercury stressed animals. They also found that Ca^{++} channels blockers (ruthenium red, procaine, heparin) did not affect the increase in passive Ca^{++} efflux induced by mercury compounds, possibly indicating that Ca^{++} release occurs through Ca^{++} ATPase. Burlando et al. [28] also reported that the effect of Hg^{++} was reduced by the Ca^{++} channel blocker verapamil. Likewise, Chetty et al. [29] found that Mg^{++} dependent ATPase was also inhibited in microsomes and was dependent upon pH, temperature, enzyme and Mg^{++} concentration.

All mercury compounds increase oxidative stress releasing free radicals and cause lipid peroxidation. The most common and fruitful therapeutics discovered till date is the chelation therapy, which is simply defined, as the process by which a molecule encircles and binds to the metal and removes it from tissue. N-acetyl-cysteine, derived from the simple amino acid cysteine, provides significant protection against a broad array of modern toxins. It is a sulphur rich amino acid, helps the body to produce glutathione that has ability to neutralize free radicals. N-acetyl-cysteine is also an antioxidant that protects the liver from potentially adverse effects of exposure to a broad range of toxic chemicals, including those that can poison the body through cumulative use. Glutathione is synthesized in the body from the three amino acids L-glutamic acid, L-cysteine, and glycine. It is one of the body's most important and powerful antioxidants.

The present study shows best protective effect against mercury when natural antioxidant (GSH), synthetic antioxidant (NAHT) and B vitamins were combined. Perhaps the later served as adjutant since B vitamins have repairing capacity in mercury damaged CNS [30]. Interestingly, vitamin C also plays major function to keep glutathione, L-cysteine, and N-acetyl-cysteine in reduced form so that they can continue to have their powerful free radical quenching effects. In the light of these observations the it may be presumed that (i) mercury is a potent toxicant in the biological system, (ii) ATPases are one of the main affected membrane bound enzymes by mercury intoxication, (iii) mode of action of mercury varies from tissues to tissues and also duration dependent, (iv) monothiols and vitamins are potent therapeutics to nab the heinous effect of the metal and their conjugation therapy proves to work better to that of their singular therapy.

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8. Arsenic intoxication and bamboo leaves extract therapies: Arsenic is a metalloid, ubiquitously found in the environment [1]. Inorganic arsenic acts as a tumor promoter, which rapidly induce ROS generation in mammalian cells, leads to production of oxidative stress [2,3] and human cancer [4]. Cellular mechanism of arsenic toxicity is manifested by generation of reactive oxygen species (ROS) like oxygen radical, super oxide radical, nitrite radical and hydroxyl radical. Free radicals are also believed to play major role in arising aging and various detrimental effects resulting in physiological disturbance like diabetes [5], cancer [6], vascular disorders [7] and peripheral neuropathy [8]. Free radicals produced due to imbalance between pro-oxidant and antioxidant homeostasis may damage cellular macro molecules viz. DNA [9], proteins [10] and lipids[11]. Damage to DNA results in carcinogenesis, protein damage affect cellular functions of proteins involving: receptors, signal transduction mechanism, transport system and enzymes and damage to lipid moiety results in loss of cell viability due to loss of cell membrane integrity. Arsenic intoxication also involves a positive correlation among arsenite induced nitric oxide (NO) production and activation of poly [ADP-ribose] polymerase (PARP) due to endothelial dysfunction. Arsenic has also been reported to cause ATP depletion [12]. Furthermore, the antioxidant enzymes like CAT and SOD were shown to effectively diminish the incidence of arsenite induced sister chromatid exchanges in human peripheral lymphocytes and X-ray sensitive cells [13].

Recently, interest has increased considerably in finding naturally occurring antioxidants in food or medicinal flora to replace synthetic antioxidants due to their adverse side effects [14]. Several studies have been to assess the antioxidant properties of natural products. Scientific information on chemical constituent and antioxidant properties of various plants

less widely used in the medicine is still rather scarce. Hence assessment of chemical constituent and such properties remain an interesting and useful task particularly for finding new source of natural antioxidant, functional food and neutraceuticals including polyphenolic such as flavonoids, tannin, and proanthocyanidin [15].

For the purpose of finding such naturally occurring antioxidant we have chosen bamboo leaves because of its remarkable medicinal properties. Antimutagenic activity of bamboo trees and inhibition of hepatic toxicity by bamboo extracts coated rice has been previously studied [16]. Experimental evidences indicated that antioxidant of bamboo leaves was capable of blocking chain reactions of lipid autooxidation, chelating metal ions of transient state, scavenging nitrite compounds and blocking the synthetic reaction of nitrosamine *in vitro*. Bamboo leaf extract has significantly reduce serum cholesterol in mice [17]. Recently, it has been shown that this bamboo extract exhibits antioxidant activity against the 1,1-diphen-yl-2-picrylhydrazyl radical and cytoprotective effects against oxidative damage in HepG2 cells [18].The phenolic compounds isolated from bamboo plant extract exhibited inhibitory effects on the P-glycoprotein in adriamycin-resistant human breast cancer cells [19]. Sasa borealis bamboo extract showed blockade of chronic high glucose induced endothelial apoptosis in human umbilical endothelial cell line [20].

In higher organisms, including mammals, inorganic arsenic is metabolized in the liver [21]. The purpose of present study was to investigate the preventive role of bamboo leaves methanolic extract against arsenic induced hepatic oxidative insult. In previous studies a strong link has been shown between the formation of ROS, RNS and arsenic induced oxidative damage to membrane lipids, intracellular proteins and DNA [22-25]. Despite of these, the biological mechanism of the source of ROS and RNS formation in arsenic intoxication still remains unclear.

Activities of SGOT and SGPT are regarded as the biomarkers for proper functioning of liver [26]. In our experiment increased activities of these enzymes suggested hepatic injury caused by arsenic intoxication in arsenic treated rats. Whereas different bamboo leaves treated groups showed significant decline in SGPT and SGOT level compared to arsenic treated group which indicated protective role of bamboo leaves against hepatotoxicity.

The liver possesses an antioxidant defense system that prevents cellular ingredients from oxidative damage. GSH containing thiol group acts as a catalyst in disulfide exchange reaction as well as it serves as an essential antioxidant molecule responsible for metabolism of xenobiotics [27,28]. *In vitro* trivalent arsenic react with GSH to form $(GS)_3$ As(III) [29]. Vahter [21] has reported that GSH plays a critical role in detoxifying arsenic species. It is reported that hepatic glutathione status was greatly impaired following arsenic intoxication, as marked by significant decrease in the total thiol level [30]. Treatment with bamboo leaves extract to arsenic treated group could prevent the toxin induced alteration probably due to its antioxidative properties by restoring GSH level [30].

In course of excess free radical removal from the system, GPx utilize GSH. Decrease in reduced glutathione level due to arsenic intoxication simultaneously decreased the activities of GPx. But bamboo leaves showed its effectiveness by elevating GPx activities in group treated with different doses of bamboo leaves extract [30].

The loss of protein –SH group provides further confirmation of increased protein oxidation in the hepatic tissues with arsenic exposure. The previous studies revealed that arsenic species can release the redox-active iron from ferritin, which could cause protein oxidation. Thus it can be concluded that iron released from ferritin and iron-dependent ROS generation could be a mechanism of action of protein oxidation induced by arsenic. However, in our study bamboo leave extract couldn't significantly decrease the level of advanced oxidative protein product compare to arsenic exposed group [30].

GSH depletion may result in the accumulation of free radicals that initiate lipid peroxidative damage of membrane lipids. Level of malondialdehyde, a byproduct of lipid peroxidation reaction; continue to be a reliable method to excess the degree of peroxidative damage resulting in hepatocellular injury and a loss of cell viability due to arsenic intoxication [31]. The decreased systemic NO production caused by prolonged exposure to arsenic is associated with increased oxidative stress as evaluated by lipid

peroxidation. In our experiment hepatic tissues of rats exposed to arsenic showed high degree of lipid peroxidation, which again diminished by the treatment of bamboo leaves extract.

SOD and CAT are two basic subcellular defense of antioxidant system that prevents cellular ingredients from oxidative damage by eliminating ROS from the system. Aldehyde (MDA), produced due to peroxidative damage to cell membrane lipids, react with thiol groups of proteins to damage them, thus inhibiting enzymes requiring -SH group for their activities. Kono and Fridovich [32] suggested that CAT activities can also be suppressed by the super oxide radical. Furthermore, CAT required NADPH for its activation from inactivating form. Insufficient supply of NADPH due to arsenic intoxication leads to decrease CAT activity [33]. SOD activity decreased due to the over production of super oxide radical anions in arsenic exposed rats. Arsenic species affect Xanthine oxidase activity, resulting in decreased superoxide production [34] In our experiment bamboo leaves extract help increasing SOD and CAT activity in dose dependent manner to reach almost equal to normal.

As a traditional medicine, bamboo leaves have been clinically used for treating hypertension, arteriosclerosis, cardiovascular diseases and cancer [35]. Due to such conventionally known medical effects, several studies have been carried out to identify the antioxidants from bamboo leaves [17,36] Bamboo is also recognized for its anti-inflammatory, antipyretic and diuretic properties [37-40]. The transcription factor NF-E_2-related factor 2 (Nrf_2) is a fundamental protein that interacts with ARE and regulates transcription of genes encoding antioxidant –proteins [41]. Ju et al. [42] demonstrated induction of Nrf_2 pathway-driven antioxidative response through phosphatidylinositol-3-kinase signaling by isoorientin, which is one of the most abundant flavonoids in the bioactive fraction of n-BuOH extract of bamboo leaves.

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9. Cr (IV) intoxication, lipid peroxidation and ashwagandha, garlic, vitamin E and GSH therapies: Hexavalent chromium [Cr(VI)] is a carcinogenic heavy metal. It exerts its effect on the cellular system by generating free radicals and increasing lipid peroxidation. Therefore, any agent that reverse these phenomena would certainly prove to be an ideal therapeutic agent. Increase of Cr(VI) in the environment is alarming due to its excessive use in industries like: chrome plating, welding, paints and dyes, steel manufacturing, alloy, cast iron and wood treatment etc. A scientific study reveals that everybody is exposed to chromium [1] which enter in the body through diet, water and inhalation. In animal tissues and cells Cr(VI) enters cells via the sulfate anion transporter system and is readily reduced by natural antioxidants to stable form Cr (III) via Cr (V) and Cr (IV) intermediates [2]. During this process free radical generation and lipid peroxidation are most prominent [3,4]. According to O,Brine et al. [5] soluble Cr(VI) oxyanions in the immediate cellular microenvironment traverse the cell membrane by non-specific anionic transporters and reductively metabolized within cells by ascorbic acid, glutathione and cysteine, It is further stated by these authors that Cr(VI) exposure DNA damage response within cells including activation of the p53 signaling pathway and cell cycle arrest or apoptosis [5]. Chromium VI is more toxic than chromium III [6] According to Kimura et al. [7] "metallothionein cannot bind chromium, but by scavenging reactive oxygen species through its cysteine residues, it may act as a protective factor against Cr(VI)-induced DNA lesions by reducing Cr(VI) directly to Cr(III), thereby avoiding the creation of the toxic intermediates." Costa [8] mentioned that "hexavalent Cr causes increased risk of bone, prostate, lymphomas, Hodgkins, leukemia, stomach, genital, renal, and bladder cancer, reflecting the ability of hexavalent chromate to penetrate all tissues in the body and metal responsive element-binding transcription factor-1 (MTF-1) is involved in sensing heavy metal load and the induced transcription of several protective genes, including metallothionein (MT)-I, MT-II, zinc transporter-1, and gamma-glutamylcysteine synthetase. Cr(VI) inhibits zinc-induced MT transcription via modifying transactivation potential of MTF-1."

Literature reveals a number of therapeutic studies that control free radical formation and lipid peroxidation generated by different toxicants [9-14]. However, such studies in chromium detoxification are limited [2,15,16]. A few herbal products like amla [17], seabuckthron [18], *Premna tomentosa* [19], kombucha tea [20] etc. have been found to reduce chromium induced toxicity and oxidative stress in different animals. In our investigation chromium intoxication, its effect on lipid peroxidation and therapeutic effect of two herbal (ashwagandha and garlic) and two physiological (vitamin E and glutathione) antioxidants have been reviewed. Along with these, vitamin B complex, which is proved to be an ideal therapeutic agent in chromium, mercury and methylmercury detoxification [21-25], has been

screened for its therapeutic capacity against lipid peroxidation.

Chromium (VI) intoxication to developing chicks revealed significant alterations of total GSH, -SH group, glutathione peroxidase and malondialdehyde (MDA), which are considered as important indicators for lipid peroxidation [26]. The first three were significantly decreased while MDA level increased tremendously [27]. The increased MDA in tissues and serum of chromium exposed animals and simultaneously increase lipid peroxidation has also been reported by Bagchi et al. [28] and Sinha et al. [29]. The conversion of Cr(VI) to Cr(III) via Cr(V) and Cr(IV) intermediates in cells [2] results in generation of tremendous free radicals [3] that induce lipid peroxidation [29].

A number of studies in literature reveal that chromium impairs cellular glutathione [15,30] and different enzymes of glutathione metabolic pathways [13,14]. De Mattia et al. [31] discovered decrease of both oxidized and reduced GSH in red blood of human beings professionally exposed to chromium. Vijayalakshmi and Sood [32] also reported similar results in methylmercury intoxicated mice.

The decrease of glutathione peroxidase in chromium intoxicated animals [13,22] clearly reveals the disturbance in glutathione metabolism. The alteration of this enzyme has also been reported under the stress of different toxicants [31-33]. The enzyme catalyzes GSH dependent reduction of hydrogen peroxide in number of tissues that coupled with oxidation of glucose-6-phosphate and 6-phosphogluconate that provides NADPH for the reduction of GSSG by GSSG reductase [31].

Chromium has affinity for -SS- and -SH groups [35] and amino acid cysteine is considered as a major binding factors. Kim and Na [36] found decrease of total amino acids within two hours of chromium injection. Sulfhydryl groups are closely associated with number of proteins and enzymes including the enzymes of glutathione metabolism such as glutathione disufphide reductase, γ-glutamyltr-anspeptidase and thiotransferase [37]. Among the three amino acids present in glutathione, the cysteine is the most important one due to the presence of -SH groups and about 90% of soluble -SH groups are associated with glutathione in mammalian cell [38]. The decrease of cysteine during mercury intoxication has been reported to alter the -SH groups and GSH levels in the cells [31]. Chromium intoxication also appears to behave in similar fashion. Thus by blocking or inhibiting -SH groups chromium reduces several cellular activities.

The therapeutic effect of both herbal (ashwagandha and garlic) and physiological (vitamin B-complex, vitamin E and GSH) agents are quite prominent, as all the micro-molecules show significant recovery during their exogenous applications [26] . By comparing the data of these therapeutics, it is evident that body physiological antioxidants like glutathione and vitamin E showed better results than ashwagandha and garlic [23]. In many cases the later did not show desirable results, though it has been reported to be a potent antioxidant [39].

To us it appears that herbal therapy requires a long term treatment, while study conducted by Chundawat et al. [26] were limited to seven days therapy only. Further, it is also reported that chromium elimination is faster during vitamins and GSH therapies [21] as compare to herbal products [40]. Thus it appears that chromium increases lipid peroxidation and ashwagandha, garlic, vitamin E and glutathione therapeutics neutralize ill effects, suggesting their suitability for chromium intoxicated subjects.

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10. Cr (IV) elimination with vitamins and GSH: Chromium is a naturally occurring element found in rocks, animals, plans, soil, volcanic dust and gases. It occurs in various oxidative states from Cr (II) to Cr (VI). A significant amount of chromium enters into the environment form chrome plating, textile dyes and paint industries. It has been known for over 100 years to be a human carcinogen. The greatest risk of cancer from chromium exposure is associated with Cr(VI). [1]

Rajkot is an industrial city and many such factories are located in its vicinity and nearby areas. Electroplating alone is contributing a good level of chromium in the environment, as there are more than 100 units located only in Rajkot. Amongst the population of five countries viz., USA, Canada, Japan, Poland and India, Takagi et al. [2,3] found maximum pollution of chromium in India.

Chromium can enter in body through air, food and water. Most abundant toxic from of this metal in the environment is Cr (VI). In animal body it is quickly converted into Cr (III) through Cr (V) and Cr (IV) intermediates. Chromium (VI) readily enters all type of cells through a general anion channel of plasma membrane and reduced into Cr (III) by various oxidants. This form of chromium is trapped and accumulated within the cell, as it is less permeable through cell membrane than Cr (VI). In cell Cr (V) and Cr (IV) intermediates act as free radicals and increase oxidative stress. Natural antioxidants vitamin E, ascorbic acid, riboflavin, cytochrome P-450

reductase, glutathione reductase etc. are free radical scavengers and quickly convert the highly toxic form of Cr (VI) to Cr (III) in the tissues which does not readily leave the cell [4]. The metal is slowly released through kidney and bile [5]. However, during its stay, Cr (III) forms stable complexes with legands of protein, DNA and GSH and causes all kind of metabolic, genetically, immunological, developmental and carcinogenic changes [4,6]. Therefore, a quick removal of the metal from the body is necessary. The objective of current review is to acceleration of chromium elimination with exogenous application of GSH and vitamins (B complex and E) and to check the reversal of toxicity by biochemical parameters.

Chelation therapy is the only treatment of choice. However, many times the chelators themselves are toxic. Levine [7] claimed the following criteria to consider any compound as an antidote: 1). antidote complexes with the poison rendering it inert, 2) the treatment should start before chronic poisoning, 3) antidote accelerates metabolic conversion of toxic to non-toxic product, 4) antidote blocks metabolic formation of toxicant from less toxic precursor, 5) antidote specifically accelerates the excretion of toxicant, 6) antidote competes with the toxicant for essential receptors, 7) antidote blocks receptors that are responsible for toxic effects, 8) antidote restores normal functions by repairing or bypassing the effects of toxicants. However, it is difficult to get an antidote, which fulfill all the seven criteria mentioned above except the natural detoxifying system exists in the animal body. The GSH is one of the cell generated xenobiotic, which plays such role of detoxification. In earlier investigations we have been able to accelerate the elimination of mercury and methylmercury from all the tissues (including brain) and reverts almost all toxic effect by using natural physiological components like vitamins and GSH [8-15]. It is worth studying other metals too, under similar experimental conditions.

Chundawat [16] selected newly hatched chick as an experimental animal and the first symptom of toxic effect of chromium VI, observed was the decrease in food intake. He intoxicated the animals with 10 mg/kg daily dose of potassium dichromate and noticed the toxic effect on the third day of intoxication when the animal become sluggish, lost interest in routine activities and stood idle in the corner of cage. The process continued and after seven days of intoxication the food intake is reduced significantly. Nevertheless when these animals were kept without further treatment the food intake was slowly resumed. The difference in food intake is clearly evident in animal weight, as the intoxicated animals showed about 30 % reduction of body weight [16].

In toxicated animals the tissues weight was also reduced significantly [16]. Thus, chromium causes deficient food intake that influence the development of the organs in growing chicks leading to reduce body weight. The decrease of body weight during chromium intoxication is also reported in mice, rat and human [17-19]. Identical results were obtained in mice [20], fish [10] and chick [14,21] during mercury and methylmercury intoxication under similar experimental conditions.

Several studies show significant decrease of protein content in different tissues and serum which is certainly an important factor for weight loss in tissues and animal as a whole. Kim and Na [22] also found decrease in total amino acid within two hours of chromium injection. Chromium binds to active site of proteins and/or DNA to form complexes that results DNA-protein and DNA-amino acid cross links, DNA replication, DNA damage, gene mutation and gene expression [23-26] The interaction of chromium with GSH also modifies DNA and gene expression. Chromium has affinity for –SS and –SH group [27] and the amino acids such as cysteine is considered major binding factor. Cysteine of GSH also binds to DNA and reduce this important antioxidant in the tissues. Voitkun et al. [28] found free amino acids (cysteine, glutamic acid, histidine, theronine and tyrosine) are known to cross-link by chromium to DNA *in vitro*. All these factors appear to be responsible for reduced protein content in various tissues.

Chromium also decreases lipids significantly in liver, kidney, muscles and serum of chick [16]. Literature review reveals conflicting reports on chromium induced fluctuation of lipids. Deliconstantinos et al. [29] demonstrated biphasic effect of chromium in rat liver microsomes cholesterol biosynthesis. With low dose rate of biosynthesis rises, while at higher dose of intoxication the cholesterol biosynthesis was decreased by 50 %. Decrease in total cholesterol, LDL cholesterol and other lipids and increase in HDL cholesterol have also found in different animal tissues during chromium intoxication [30,31]. Mercury and methylmercury intoxicated chick, fish and mice also

reveal the loss of most of the lipids [10,14,20,21]. Along with other macronutrients, the sugars level is also significantly low in different tissues of chromium intoxicated developing chick. The same trend is seen in chick during mercury and methylmercury intoxication [14,21].

Chromium intoxication causes behavior alteration leading to dizziness, indigestion, diarrhea, abdominal pain, vomiting, gastritis and weakness [4] that may stop the animals to take food. This also effects the digestion indicating that complex food stuffs (proteins, carbohydrates and fats) are not broken down to final products. Further, the decrease in intestinal brush border enzymes like, alkaline phosphatase, maltase and leucine amino peptidase during chromium intoxication [32] certainly reduces absorption and cellular transport of end products. Sastry and Tyagi [33] also demonstrated that enzymes involved in active transport of nutrients in intestine are reduced and absorption rate of fructose and tryptophan are severely affected. The reduction of all major macronutrients (proteins, carbohydrates, lipids) in different tissues appears to be non-availability of raw materials needed for their synthesis due to reduced digestion and intestine absorption of end products. Thus the decrease of chromium burden from body by supplementation of GSH and vitamins (B_1, B_6, B_{12} and E) restrain all major macronutrients. Dey et al. [34] also found alpha-tocopherol supplementation on chromium toxicity restrain the membrane cholesterol and phospholipids levels. It is concluded from overall evidences that chromium elimination from body restore the food and water intake, tissue and animal weight leading to normal growth of the developing animals.

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11. Comparison of herbal and inherent antioxidants in heavy metal detoxification: Several studies in literature have argued that herbal antioxidants are quite useful in heavy metal detoxication and serve as antistressor agents [1-3]. But simultaneously it isalso claimed that many medicinal herbs and pharmaceutical drugs are therapeutic at one dose and toxic at another [4]. To verify this view, we selected six well known herbal (ashwagandha, brahmi, garlic, amla, moringa oil and C-phycocyanin) products, two inherent antioxidants (glutathione, vitamin E) and one vitamin, associated with general cell metabolism, vitamin B complex, were used to eliminate hexagonal chromium and release the animals from stress [5] and showed that antioxidants application is useful in metal elimination up to some extent as metal enters in all compartments of cell and antioxidant have different power of penetration. On account of this a mixed therapy containing herbal, natural and synthetic antioxidants which can reach all cellular compartments can give desired results.

ATSDR toxicological profiles [6-13] published in USA, have reported a number of hazardous chemicals and characterized the toxicological and adverse health effects. Nevertheless, desirable therapeutic data is still not available. On account of this several studies have been carried out by investigators where the animals were intoxicated with hazardous heavy metals, such as mercury, methylmercury, lead, chromium, cadmium, aluminium, zink, floride and arsenic. All heavy metals are toxic and create oxidative stress that primarily cause hepatotoxicity, neurotoxicity, genotoxicity and nephrotoxicity in animals and humans [14], thereafter, therapy was provided to eliminate the toxicants, to improve their health and to restore the altered conditions caused in the body [5].

Though chelation therapy is the only treatment of choice, but many times the chelators themselves are toxic. Levine [15] claimed the following criteria to consider any compound as an antidote: 1). Antidote complexes with the poison rendering it inert, 2). The treatment should start before chronic poisoning, 3). Antidote accelerates metabolic conversion of toxic to a non-toxic product, 4). Antidote blocks metabolic formation of toxicant from less toxic precursor, 5). Antidote specifically accelerates the excretion of toxicants, 6). Antidote competes with the toxicant for essential receptors, 7). Antidote blocks receptors that are responsible for toxic effects and 8). Antidote restores normal functions by repairing or bypassing the effects of toxicants. Nevertheless, it is difficult to get an antidote, which fulfills all the eight criteria mentioned above except the natural detoxifying system exists in the animal body. The GSH is one of such cell-generated xenobiotic, which plays such role of detoxification.

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation [16].
Antioxidants protect the body from oxidative damage induced by free radicals and reactive oxygen species by (i) suppressing their formation; (ii) acting as scavengers and (iii) acting as their substrate. Antioxidants boost immunity system also by playing other important roles such as in cellular metabolism, signal transduction, gene activation, and transcription.

For the last three decades we are working on potential health effects of heavy metals to evaluate the therapeutic potencies of various chemical compounds against their poisoning in experimental animals. Our findings indicate that various organs have differential capacities to absorb, store, metabolize and excrete metals depending upon dose and duration of intoxication [17,18].

We tried several well known therapeutic agents, used by earlier workers, for heavy metals elimination [19-22]. Most of these agents are able to remove mercury from non-nervous tissues but least from brain. Discouraged from these findings, body's physiological agents like glutathione and vitamins were tried. Results were quite exciting as they eliminated mercury both from nervous and non-nervous tissues [23-25]. Encouraged from these findings we also used glutathione, N-acetyl-DL- homocysteine thiolactone and vitamins (B-complex and E) for mercury elimination from the central nervous system. It was

noticed that along with other tissues mercury was also partly eliminated from brain in rat, mice, chick and fish. These agents were also found to restore biochemical lesions and histopathological changes especially in central nervous system caused by mercury and methylmercury intoxication [26-31]. Nevertheless, in all above studies investigators never come across any incident where heavy metals were completely eliminated from the animal body. Chundawat et al. [32] applied GSH and vitamins (B and E) to eliminate hexagonal chromium from chick tissue including brain. However, they could reduce the burden of metal up to some extent only. Therefore, it was decided to use herbal, natural and synthetic antioxidants, either alone or in various combinations.

Sood et al. [5] employed nine herbal antioxidants: (Ashwagandha, brahmi, garlic, amla, moringa oil and C-phycocyanin), two natural antioxidants (glutathione, vitamin E) as well as vitamin B complex to eliminate Cr_6 from different tissues of experimental animals. Study shows diverse effect of different herbal and natural antioxidants on different tissues. As for example in liver C-phycocyanin, in kidney brahmi, in muscles vitamin E, in brain amla, in blood cells vitamin B complex and in serum moringa oil and vitamin B complex demonstrated the best results [5].

Under above conditions it is difficult it assume which antioxidant is better and should be recommended for therapy. Simultaneously it is also true that all of them have been suggested as potent antioxidants in one or another study. Though the applications of these herbal and inherent antioxidants are able to reduce metal burden, significantly from one or another tissues, but neither a complete elimination was possible nor any antioxidant could successfully eliminate the toxicant from all tissues together. To us it appears a long term therapy is required to eliminate the metal in individuals intoxicated for short term. In such cases metal will slowly leaches out through urine. But if the subject is continuously intoxicated, as for example with metal contaminated vegetables, or mercury or other heavy metal intoxicated fish, or other edibles, it will certainly lead to serious conditions. Under such conditions mix therapy is recommended as antioxidant have different power of penetration in cellular compartments and long and continuously therapy is required.

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12. Vitamins deficiencies during Cr (IV) intoxication and recovery with vitamins and GSH: Vitamins (one of the six nutrients class) are the low molecular weight organic substances needed in small amounts in the diet to all animals for normal growth, health, reproduction and regulation of various metabolic functions. They are present in minute quantity in most of the natural food stuffs. Vitamins are not broken down in the digestive processes and are absorbed eventually as they occur [1]. The vitamins participate as components of catalysts, which enable the more inert chemical substances and serve as raw materials for the fabrication of living tissues to undergo the changes in metabolism [2-4]. They have essential functions; hence their deficiency creates many diseases in animals [5]. Most of the vitamins cannot be manufactured by the body, and therefore, must be supplied in food [6].

Amongst important valence states of chromium, the hexavalent form (Cr(IV)) is one of the most toxic one produced from anthropogenic activities. Due to its widespread use in the industries (dyes and pigments, metal finishing, wood preservatives etc.), the concentration of Cr(IV) is increasing in environment. The general population in exposed to chromium by food supplements, drinking water and inhaling air that contain chromium [7]. In animal body, Cr(IV) is quickly convert to stable form Cr(III) by natural defense mechanism. A number of body antioxidants, like vitamins and GSH have been demonstrated to reduce Cr(IV) to Cr(III) [7,8]. However, once in the cell, the elimination of Cr(III) becomes difficult due to its membrane impermeability. It forms DNA-protein crosslinks and causes a severe damage to DNA strands [9,10].

Literature reveals a few isolated therapeutic effect of vitamins. as for example chromium in low dose enhances the level of vitamins but at higher dose tissues become exhausted [11] and decreases the ability of the cell to reduce Cr(IV) [7]. An increase in GOT and GPT were found by Kaufman et al. [12] at lower concentration of chromium. Sugiyama [8] also demonstrated the role of vitamins (E and C) in Cr(IV)-induced damage and the reduction of Cr(IV) to Cr(III). However, in none of the earlier studies, status of vitamins in Cr(IV) intoxicated developing animals has been reported. Sood and Chundawat [13] demonstrated the influence of hexavalent chromium on the fluctuation of vitamins (vitamins B_1, B_2, B_6 and E) in different tissues (liver, kindly, muscles) and serum of developing chick. They also attempted to restore the vitamins level in different tissues of intoxicated animals by exogenous application of vitamins (B and E) and glutathione.
Karimov [14] studied chromium induced vitamin deficiency in blood of chrome industry workers and discovered deficient levels of vitamins (A, E, C and B_2). The decrease in activity of GOT and GPT representing the Vitamin B6 was demonstrated in chromium intoxicated rabbits by Anjum and Shakoori [15]. The B vitamins are water-soluble vitamins required as coenzymes for enzymes essential for cell function.

Since, vitamins are dietary constituents their fluctuation mostly depends upon dietary intake. Reduced food intake during chromium intoxication [16], decreased intestinal absorption of nutrients during Cr(IV) poisoning [17] and indigestion and diarrhea [7] are major factors collectively give the impression to be responsible for the decreased vitamins level during chromium intoxication. Chundawat et al. [18] (2001), also reported that when chromium supply in ceased in intoxicated chick the

burden of metal is slightly reduced, the food intake is also gradually restored [16]. Interestingly enough, during vitamins (B complex and E) and glutathione therapy chromium is eliminated quickly and the food intake also restored simultaneously in detoxifying animals [16,18] We have also demonstrated a quick recovery of various macromolecules (proteins, carbohydrates and fats) under similar experimental conditions [16,19-21]. The exogenous application of vitamins and glutathione quickly reduce metal burden and increased food intake also compensate their deficient levels [13].

Sugiyama [22] found that vitamin B_2 and vitamin E were able to alter the toxic effect of Cr(IV) through their abilities to modify levels of chromium (V) in cell. The author further claimed that protective effect of vitamin E and enhancing effect of vitamin B_2 on chromate-induced DNA breaks might be due to the formation of Cr(V) in cell. The conversion of Cr(IV) to Cr(III) in tissues releases free radicals that amplify lipid peroxidation [23]. Geetha et al. [24] reported that chromium decreases GSH and increases lipid peroxidation. Being an antioxidant and free radical scavenger, vitamin E quickly eliminates them. Bagchi et al. [25] also reported that vitamin E attenuate chromium-induce toxicity. Vitamin E plays several role in the cell viz. decreases cytotoxicity, increase glutathione reductase activity which is suppressed by Cr(IV), reduce oxidative stress, protect the enzymes containing –SH goups and act as membrane stabilizer, etc. B vitamins also play important role in the maintenance of human adaptive capacity to resist a large number of chemical and physical stressor agents commonly encountered in the community and industrial environments [26].

Various B vitamins have been used in past for heavy metal detoxication [27]. B vitamins are also known to preserve glutathione and maintain thiol compounds in the cell [28,29]. In number of studies, the effects of chromium have been examined in different tissues pretreated with vitamins. Susa et al. [30] demonstrated that pretreatment of cultures of rat hepatocytes with vitamin E for 20h prior to exposure to potassium dichromate resulted in marked decrease of Cr(IV)-induced cytotoxicity. They also found pretreatment raised glutathione and vitamin C levels in the tissue. Thus, physiological antioxidants including active oxygen scavengers (glutathione, Vitamins B_2, E and C) modify the toxic effect of chromate by accelerating chromium elimination and decreasing free radicals and lipid peroxidation.

Literature reveals that (i) hexacalent chromium reduces vitamins levels in all the tissues and serum, (ii) their exogenous application as therapeutics reduces chromium toxicity and reinstates vitamins level in body. The exogenous application of therapeutic agents (Vitamins and GSH) to methylmercury-intoxicated mice and chick also reveals similar results [28,31-33] and confirm earlier investigations. Panel Atef, and Al-Attar [34] reported the protective influences of vitamin E on a mixture of some heavy metals (Pb, Hg, Cd and Cu) on renal and testicular injuries in male mice. Likewise, Flora et al. [35] also reported that vitamins C, E and A are quite satiable for the treatment of arsenic, lead, mercury and cadmium intoxications. In the Unites States, approximately 40% of the population consumes vitamins [36]. Depeint et al. [37] reported that various B vitamins (B1,B2,B3,B5,B6,B7,B12) have essential role in mitochondrial function and toxicity. Dietary pretreatment of Cr(VI)-intoxicated rats with ascorbic acid or α-tocopherol normalized vitamin C levels in lungs but not in kidneys [38]. Raynolds and Zhitkovich discovered that cellular vitamin C increases chromate toxicity and this vitamin plays a dual role in Cr(VI) toxicity protective outside and potentiating inside the cell [39]. All above data together definitely prove that an exogenous application vitamins is quite advantageous to relieve the animals including human from heavy metal toxicity. Of course simultaneous mineral therapy will be much beneficial.

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