Journal of Cell and Tissue Research Vol. 18(2) 6473-6484 (2018)
(Available online at www.tcrjournals.com)
ISSN: 0973-0028; E-ISSN: 0974-0910

Review Article

EFFECT OF HEAVY METALS IN PHYSIOLOGICAL ALTERATIONS AND RECOVERIES DURING NATURAL AND HERBAL ANTIOXIDANTS THERAPIES: A REVIEW

SOOD, P. P.[1] AND CHUNDAWAT, R. S.[2]

[1]Emeritus Medical Scientist, ICMR , New Delhi (Rtd), Emeritus UGC Scientist, New Delhi (Rtd), Professor and Head, Department of Biosciences, Saurshtra University, Rajkot 360 005 (Rtd), [2] Department of Bioscience, School of Science, Mody University of Science and Technology, Lakshmangarh,Sikar 332311.
E. mail: prempsood@yahoo.com, Cell: 9427270210

Received: June 5, 2018; Accepted: July 12, 2018

Abstract: Considering that none of the therapies used so far has completely recovered the toxic effect of most of the heavy metals, as evident in several biochemical, histopathological or ultrastructural studies, the present review summarizes varies studies dealing with the combined effect of herbal and inherent antioxidant therapies used in chromium (VI) and methylmercury intoxications.

Key words: Heavy metals, Herbal antioxidant, Natural antioxidant

1. Instability and reinstallation of carbohydrates during chromium (VI) intoxication and therapy: Chromium (VI) is being released into the environment from chrome plating, stainless steel welding, chromate production, battery, candle, dye, rubber, printing and dyeing and cements manufacturing industries. In animal cell Cr(VI) is reduced to Cr(III) thorough Cr(V) and Cr(IV) intermediates [1]. Both the transitional forms act as free radicals and increase oxidative stress in the cell. Natural antioxidants (vitamin E, ascorbic acid, riboflavin, cytochrome P450 reductase, glutathione reductase etc.) are free radical scavengers and quickly convert the highly toxic Cr(VI) to a stable form Cr(III) in the tissues [1-3]. Cr(III) in trace amount is thought to be essential for the function of insulin controlling carbohydrate metabolism [4]. The human requirement is 50 to 200 µg per day for adults [3].Cr(III) forms stable complexes with ligands of protein,DNA and glutathione (GSH) and released slow ly through kidney and bile [5]. It causes neurological, immunological, developmental, reproductive, genotoxic and carcinogenic effects [3,7]. Chromium increases blood glucose level [7] and depicts glycogen content [8,9]. Merkur'eva et al. [10] reported alteration in carbohydrate metabolism during chromium intoxication. Carbohydrate metabolism involves a series of enzymes to catalyse their individual steps, therefore, any effect of chromium on carbohydrate metabolizing enzymes can alter their integrity. Kim and Na [8] showed that chromium toxicity impair the primary energy producing pathways.

Anjum et al. [11] illustrated the effect of Cr (VI) on various drug-metabolising enzymes. Inhibition of glucose-6-phosphatase [12], cytochromes P450 3A1 and/or 3A2 and 2C11 [13], LDH, SDH, PDH [14] alkaline phophatase [15], phosphotyrosine phosphatase [16], glutathione reductase, glutathione peroxidase, superoxide dismutase and alkaline. Sood and Chundawat [17] showed decrease of all major

carbohydrates (total sugar, reducing sugar, non-reducing sugar and glycogen) and metabolizing enzymes (glucose-6-phosphatase, glucose-6-phosphatase dehydrogenase, succinic dehydrogenase and lactic dehydrogenase) in liver, kidney, muscles and serum in chromium intoxicated animals. They also claimed that during vitamins (B and E) and GSH post therapies a significant recovery of all these components during post therapy was possible.

Carbohydrates are major source of energy. They are stored in glycogen and can be converted into fats (triglycerides). Boge et al. [18] found that chromium intoxicated trout stopped feeding and simultaneously there was a decrease in intestinal brush border enzymes (alkaline phosphatase, maltase and leucine amino peptidase) as well as decrease in the intestinal weight. Reduced food intake and decrease in tissue and body weights is also recorded in chick during Cr (VI) intoxication by authors [19]. Further, the indigestion and diarrhea caused by chromium [20] also suggests that complex carbohydrates are not broken down to monosaccharides.

Sastry and Tyagi [21] also demonstrated that enzymes involved in active transport of nutrients in intestine are reduced and absorption rate of fructose and tryptophan are severely affected. Thus, the low carbohydrate content in the tissues of intoxicated animals appears to be due to the indigestion, reduced food intake and absorption of end products. Chromium also interferes in cellular transport due to the inhibition of membrane transport enzymes [18]. Nath and Kumar [7] demonstrated a significant depletion of glycogen in fish liver but the blood glucose level was increased significantly, perhaps due to alteration in glycogenolysis.

Chromium has been demonstrated to increase the catecholamine secretion [22], increase c-AMP [23] that inhibits glycogen synthesis leading to accumulation of glucose in the circulation (hyperglycemia). Hence, glycogen level in all the tissues is decreased. Several studies have demonstrated decreased level of glycogen in Cr(VI) intoxication animals [7-9,14].

Cr(VI) in animal system is readily transformed to Cr(III) through Cr(V) and Cr(IV) and thereby increases production of reactive oxygen species and lipid peroxidation, leading to severe histopathological, biochemical and carcinogenic affects in the cell. Cr(VI) is also known to cause DNA damage and modulation of apoptotic regulatory gene P53 [24,25], calcium metabolism, energy metabolism, protein synthesis and cell cycle regulation [26]. Cr (VI) deplicts the content of intracellular GSH, glutathione reductase, glutathione transferase and lactic dehydrogenase [27]. Pourahmad and O'Brien [28] also reported decrease in mitochondrial membrane potential and increase lysosomal membrane rupture during Cr(VI) intoxication.

The inhibition of carbohydrate metabolizing enzymes is an important factor for reduced carbohydrate level in different tissues. Present study shows inhibition of glucose-6-phosphatase, glucose-6-phosphat dehydrogenase, succinic dehydrogenase and lactic dehydrogenase in all the tissues and serum of developing chick. In pervious investigations from this laboratory, Patney et al. [29] and Vijayalakshmi [30] also demonstrated the inhibition of these enzymes during mercury and methylmercury intoxication. A few isolated studies also exist in literature demonstrating the status of carbohydrate metabolizing enzymes during chromium (VI) intoxication. The inhibition of succinic dehydrogenase and lactic dehydrogenase [14,31,32], aminotransferase, pyruvate dehydrogenase, Na+K+ ATPase [33,34], glucose-6-phasphat dehydrogenase [35], isocytrate dehydrogenase [30] and P450 reductase has been demonstrated during chromium intoxication. Fernandes et al.[36] observed that in rat liver mitochondria of NADH-ubiquinone oxidoreductase (complex I) and succinic dehydrogenase (complex II) were inhibited, while cytochrome oxidase (complex IV) was not affected and ATPase (complex V) activity was stimulated. Thus it is evident from overall data that Cr(VI) interferes in energy dependent biochemical processes.

Literature also reveals a few isolated therapeutic studies, where a number of natural and synthetic products such as melatonin, ascorbate, vitamins C, B_2 and E, salicylate deferoxamine, N-ethylmaleimide, manntol, butylated hydroxyl anisole, butylated hydroxyl toluene, cytochrome P 450 inhibitor, C/P 2E1, kombucha tea, applejuice, amla, deferoxamine, diethyldithio-carbamate and sulfoethylglucan [15,30,37-45] etc have been used in past. Nevertheless, the studied are restricted to one or the other organs or therapeutic agents are themselves injurious or the results are never further confirmed. Along with this, either the pre- or simultaneous

therapy is provided in most of the cases, while toxicated subject always needs post therapy.

Sood and Chundawat [17] administered B and E vitamins and GSH as therapeutic agents during post therapy, and demonstrated that these therapeutic agents are able to increase chromium elimination from chick tissues [46]. Further increase in food intake leads to normal development of animal [60], restores E and B vitamins level [47] and various lipids [48]. Vitamins and monothiols have also been found to restore mercury depleted foresaid macro- and micro nutrients in developing chick and mice [30,49]. Vitamin E plays several roles in cell. It increases GSH, reduces toxicity stress, protects the enzymes containing –SH groups and act as membrane stabilizer. B vitamins on the other hand play important role in the maintenance of adaptive capacity to resist large number of chemical and physical stressor agents commonly encountered in community and industrial environment [50], B vitamins are also known to preserve GSH and maintain thiol compound in the cell. Vitamin B_2 and vitamin E are able to alter Cr VI) induced toxicity [51,52].. GSH is an important cellular reducing agent, detoxifying agent, antioxidant, co-enzyme, substrate and co-substrate. It plays role in destruction of free radicals and maintainance of –SH groups. According to Hiaishi et al. [53] "extra cellular GSH protects cultured gastric cells from H_2O_2 damage by accelerating intracellular GSH synthesis; this is mediated by membrane-bound gamma-glutamyl transpeptidase acting on extracellular GSH (which supplies cysteine to these cells) and then by intracellular gamma-glutamylcysteine synthetase".

2. Instability and reinstallation of circulating thyroid hormones during chromium (vi) intoxication and therapy: Developing animals are most vulnerable to environmental contaminants. The problem becomes more severe when they disturb the endocrine hormones and particularly growth hormones in developing animals. Assessment of the hormones related to thyroid can be a good indicator of physiological functioning in the developing animals as thyroid hormones play important role in cellular metabolism, growth, maintenance of body temperature, protein and fat distribution and Ca ion maintenance. All the thyroid secretion follows a definite trend in their concentration in the circulating body fluid at various stages of their life with respect to the environmental conditions. There are a number of metals (lead, lithium, cadmium, chromium, manganese, mercury) that have been proved to be hazardous to the circulating endocrine secretion [54,55]. Donmez et al. [56] found that the optimal concentration of the zinc triggers the thyroid secretion (T3, T4) in the body fluid, while the higher concentration is toxic and reduce the follicular cell of the thyroid. Changes in morphology and function of thyroid gland were also reported in rats fed with chromium in diet [57]. Literature review reveals a few isolated studies on the effect of chromium on thyroid hormones but therapeutic data, in relation to metal toxicity, is scanty. Sood et al. [58] discovered alteration and recovery of TSH, T3 and T4 simultaneously using potent natural (vitamin E, GSH) and herbal (ashwagandha (*Withania somnifera*), garlic (*Allium sativum*)) antioxidants. They also used vitamin B complex (B1, B6, and B12) as therapeutic agent as some of the B vitamins have been shown to be protective in nature [59,60].

According to Cherel et al. [61] decrease in food intake decreases the thyroid hormone level and the growth of the animal is retarded. Any toxicant which reduces food intake also retards its growth as well as disturbs all metabolic functions. In earlier investigations from this laboratory, it was observed that Cr (VI) intoxication to developing chicks remarkably reduce the body weight and growth [62] and simultaneously decrease proteins, carbohydrates, lipids and vitamins [63,64]. Almost similar results are found during methylmercury intoxication [65]. Chromium fed rats also show changes in morphology and function of thyroid gland [57]. Since thyroid growth and functions are controlled by TSH secreted by anterior pituitary, we observed its circulatory level in toxicated animals. Study shows a significant decrease in the hormonal level in serum [Sood et al., 58] which clearly indicates disturbances in thyroid gland function.

Thyroid hormones regulate the metabolism of every cell of the body and their deficiency can affect virtually all body functions [66]. Both T3, T4 are the products of thyroid gland. T4 is the major hormone secreted by thyroid. T3 is more active than T4. The bulk of T3 is derived by deionization of T4 in the peripheral tissue by the enzyme thyroid peroxidase. Iodine is responsible for maintaining thyroid hormone level in blood. Thyroid hormones may be altered due to iodine deficiency [67]. The status of thyroid hormones is also influenced by growth hormones [68]. Dietary iodine has been shown to be important in the induction of the thyroiditis in susceptible chicken

strains although the underlying mechanism remains unknown [69]. [19]. Though there is no direct evidence that Cr VI interferes in iodine absorption, but the metal certainly retards absorption of essential nutrients [70]. In such case, deficiency of iodine may occur in circulation leading to thyroid disfunction. When T3 and T4 level fall due to any defect in thyroid, the TRH gene expression in periventricular nucleus of hypothalamus increases, causing more TSH production, which serves to derive the failing thyroid level and has been considered as more sensitive test for thyroid failure [71].

Hexavalent chromium has also been found to decrease reduced glutathione (GSH) and increased malondialdehyde and creatine phosphokinae levels, and also enhances glutamate oxaloacetate transferase and glutamate pyruvate transferase levels in serum [72]. Increased lipid peroxidation and free radicals formation during chromium intoxication has also been reported by Pourahmad and O'Brien [73]. Free radicals reduce thyroid hormone and sensitivity of the thyroid and pituitary gland receptors by influencing 52 monodeiodinase activity [74], thus synchronization of the endocrine system and feedback mechanism breaks, leading to thyroid disfunction.

A possible reason for the unusual functioning of the either the receptors of the thyroid gland, pituitary, hypothalamus as well as the enzyme responsible for the conversion of the T4 to T3 (i.e. thyroid peroxidase) may be the intoxication. Deficiency of thyroid hormone may rise due to lack of stimulation by the pituitary gland, defective hormone synthesis or impaired conversion of T4 to T3. Mercury toxicity has often been related for impaired cellular conversion of T4 to T3 inhibiting the enzyme thyroid peroxidase [75]. Sood et. al. [58] reported TSH, T3 and T4 are reducedin chromium intoxicated chick. TSH reduction in serum indicates chromium affects on the hypothalamus or anterior pituitary functions. Direct action of chromium on hypothalamus and pituitary has not been worked out. However, low TSH level in circulation is certainly due to chromium action which simultaneously reduce T3, T4 levels in circulation. It has been claimed that mercury and other heavy metal toxicity decrease the sensitivity of the pituitary gland to recognize thyroid hormone deficiency in blood [75].

Sood et al. [58] demonstrated quick elevation of all the three hormones during vitamin B complex therapy. Amongst B vitamins riboflavin (B_2), niacin (B_3), and pyrididoxine (B_6) as well as vitamin C are necessary for normal thyroid hormone manufacture [75]. A recent study from our laboratory demonstrated decrease in vitamin B_1, B_2 and B_6 levels in serum of chromium intoxicated chick [76]. B vitamins are commonly used to treat iodine deficiency mediated hyperthyroidism [77]. They are potent iodine coupler in the thyroid gland and play important role in trapping iodine from the blood stream and thus can raise T3, T4 levels [75]. Cashin et al. [78] also reported that vitamin B_3 (Niacin) administration decreases globulin and T4 level but increases the T3 uptake ratio.

Vitamin E application too, showed recovery of thyroid hormones. Vitamin E is a potent antioxidant [79]. Zinc, vitamin E and vitamin A function together in many body processes including the manufacture of thyroid hormone [75]. Engelmann et al. [80] reported that moderate dose of vitamin E triggers thyroid hormone, while higher doses reduce it. As mentioned earlier, generation of free radicals cause damage and malfunctions of the endocrine glands. Being free radical scavenger, vitamin E decreases lipid peroxidation [81]. Glutathione is the other most effective antioxidant that reduces free radicals generated by heavy metals [82] and thereby regulates circulating thyroid hormone. It is known that Cr(VI) concentration in serum and various organs is reduced by GSH application [83] and three molecules of GSH are required per molecule of Cr(VI) [84].

Ashwagandha and garlic application to Cr(VI) intoxicated chick also restored the lost thyroid hormone. Both these herbal products are well known antioxidants and free radical scavengers [85-88]. Garlic exerts its protective effect against lipid peroxidation generated free radical toxicity by modulating lipid peroxidation and enhancing levels of GSH and GSH dependent enzymes [89]. Panda and Kar [90] has already reported the efficiency of ashwagandha in raising the thyroid hormone level.

It is concluded from overall data that (i) hexavalent chromium reduces the dietary absorption of iodine causing its deficiency in the circulation. This view is supported by the fact that elimination of the metal from body during therapy increase food intake [91] that maintains iodine level and restore the thyroid functions, (ii) vitamins, GSH, ashwagandha and garlic decrease oxidative stress thereby reduce free radicals

and restore thyroid functions. The raised level of TSH, T3 and T4, as evident in the present study, is a good evidence to support this contention.

3. Instability and reinstallation of neurotransmitters during methylmercury intoxication and therapy: Neurotransmitters are the chemicals secreted by nerve endings that transfer the chemical signal from one nerve cell to another or to the target cell. They are the substances of diverse chemical nature involved in a variety of physiological and behavioral regulation in animals. Literature reveal variable effect of mercury and methylmercury on neurotransmitters [92-94]. Since methylmercury is a typical neuropoison and accumulates preferably in brain, it causes acute degenerative changes including synapses both in central and peripheral nervous system in humans [95,96] and experimental animals [97-99]. Chang and Annu [100] noticed an abnormal synapse formation in the cerebellum of methylmercury intoxicated animals. A number of studies have been carried out in different laboratories to analyze the impact of methylmercury on the biosynthesis and release of acetylcholine [101,102], dopamine [102-104], norepinephrine [105], gamma-aminobutyric acid and glutamate [106], taurine, glycene and aspertate[107] during inorganic and organic mercury intoxication under different experimental conditions. Since the neurotransmitters are the important signal inducing chemicals, their alterations with methylmercury may cause serious changes in the brain function, therefore, maintenance of normal levels of these chemicals is absolutely necessary in the toxicated subject. However, such studies are quite scanty. Sood et al. [108] studied 5 neurotransmitters viz., 5HT, dopamine, epinephrine, norepinephrine, acetylcholine and their metabolites (5-HIAA, DOPAC) in mice during methylmercury intoxication. They also screened their recoveries during vitamins and monothiols therapies and obtained significant results.

In our earlier investigations it was found that the vitamins and monothioles used either alone or in various combinations are able to eliminate mercury both from brain and spinal cord and restore animal's behavior, body weight, proteins, triglycerides, cholesterol, carbohydrates, vitamins, essential elements, supfhydryl group, glutathione and the enzymes of important metabolic pathways [97,109-115]. Along with this, the restoration of histopath-ological lesions in brain of mice during the application of vitamin B complex, GSH and their combination, under identical experimental conditions, have also been recorded in this laboratory by Bapu and Sood [116].

Literature reveals both increased release of dopamine [102,117,118]. and 5-HT as well as decreased release of these neurotransmitters [104,119] during MMC intoxication. Sood et al. [108] found insignificant alteration of these neurotransmitters in seven days MMC intoxicated mice but the decreased are significant. when these animals were kept without intoxication for another seven days indicating that decrease is duration dependent.

There may be several reasons for the decreased dopamine and 5-HT in withdrawal groups (mice intoxicated for 7 days and kept for another 7 days without intoxication) [108]. During this period there is about 89% further increase of mercury concentration in brain of mice as compared to seven days intoxicated animals [120], which perhaps responsible for decreased level of these neurotransmitters. Likewise, vitamin B complex and vitamin E treated animals exhibit 50-60% elimination of mercury [120] and simultaneously a significant recovery of these neurotransmitters is reported [108]. Ken-Ichi et al. [119] reported that accumulation of mercury in specific neurons or certain nuclei (substantia nigra, striatum, pons, medulla and cerebral cortex which synthesized and store dopa-mine, 5-HT, acetylcholine and norepinephrine) altered neurotransmitters levels. However, it is not always true as mercury concentration is found to be increased in NAHT and its mixed therapy with vitamins [120] , yet report is available revealing a significant recovery of dopamine and 5-HT [108]. This clearly indicates that recovery of the neurotransmitters is not only due to mercury elimination but they are also re-synthesis due to the repair of cellular machinery and stability of membrane structure as vitamin E is potent membrane stabilizer [121] and vitamin B complex components are involved in the synthesis and maintenance of neurotransmitters [122,123] and repairing the cellular machinery [116].

The synthesis of dopamine and 5-HT are dependent on the availability of the amino acids tyrosine and tryptophan respectively. Since these amino acids are also decreased in this group of animals [124], one may assume that sufficient raw material is not available for the synthesis of dopamine and 5-HT.

Along with this, decreased food intake [124] could be another factor for the non-availability of precursor substance. Further, the 5-HT also appears to be converted into 5-HIAA under the influence of intoxication as the later is enhanced in brain.

Sood et al. [108] reported in 7 day MMC intoxicated mice the neurotransmitters are either significantly (epinephrine and norepinephrine) or insignificantly (acetylcholine) enhanced. However, when these animals are kept for another 7 day with out further intoxication, the former two are recovered, while the later is enhanced, but still all of them show significantly high level. Increase [101,102,104] or decrease [119,125] of these neurotrasmitters have been reported by number of studies during mercury intoxication under different experimental conditions, which has been explained on the basis of increased release [102,126], decreased release [127,128], increased uptake [129], decreased uptake (see Komulainen and Tuomisto [130] for review), increased synthesis [104] or decreased synthesis [131].

There is regular synthesis and breakdown of acetylcholine associated with choline acetyltransferase and acetylcholinesterase. The alterations of any of these enzymes have direct effect on the level of the acetylcholine. Tsuzuko [132]. and Sood et al. [133] reported a significant decrease of acetylcholinesterase in the central nervous system of methylmercury intoxicated rats. A number of investigators have reported either negligible effect [106,134] or decrease [133,135] of cholin acetyltransferase in mercury intoxicated animals along with impaired supply of acetyl coenzyme A from mitochondria [93] [2] and cholin uptake [106] and decrease release of ACh. Therefore, one may assume that the neurotransmitter is not enhanced due to increased synthesis but, due to less utilization caused by low level of degrading enzyme, acetylcholinesterase.

4. Instability and reinstallation of glutathione metabolism during methylmercury intoxication and therapy: There are number of toxicants that enter the body easily but their elimination is complicated as during their short or long term stay they create so much havoc in the cell, tissue and organs, they are not repairable and cause serious disease. All heavy metals can be included in this category and among these methylmercury chloride (MMC) is the most dangerous one as: i) mercury and methylmercury are widely used in various industries and agriculture; ii) there is continuous methylation of inorganic mercury in the environment by several bacterial species to methylmercury which is a potent neurotoxin; iii) being lipophilic in nature MMC cross BBB and nerve cell membrane, causing damage to all cell organelles, myelin sheath and axons, iv) there is clear selectivity of MMC for specific cell types and brain structures, which is not yet fully understood, v) the high thiol reactivity of MMC has been suggested to be the basis of its harmful biological effects. vi), the main mechanisms involved are inhibition of protein synthesis, microtubule disruption, increase of intracellular calcium and disturbance of neurotransmitter function; vii) causes genotoxicity, oxidative stress and triggering of excitotoxicity mechanisms.

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 functions. Oxidative stress is the destruction caused by free radical molecules. 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 near by tissues. Antioxidants 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.

Since Minamata episode in Japan in 1953, where a number of persons died due to methylmercury poisoning, the scientists throughout the world started searching for suitable therapy [136]. Literature is full of such articles, but in a nut shell, till today desired therapeutic agents are not available that can eliminate the toxicant from the body and repair the damaged biochemical machinery and histological structures [137-151]. .

Methylmercury also causes oxidative stress [152] leading to lipid peroxidation and release of free radicals [153]. . During intoxication of environmental

pollutants, that behave like slow poison, the natural antioxidants immediately take up their work. However, there is a limited supply; hence exogenous application of antioxidants is required. For this purpose the application of herbal products rich in antioxidants like ashwagandha, curcumin, ambla, green tea etc will certainly be helpful. Considering these aspects, the present study was designed to nullify the oxidative stress by using herbal antioxidants curcumin, inherent antioxidant glutathione either alone or in various combinations along with vitamin B complex as the latter has cell repairing property [154].

The antioxidant activity of curcumin was reported as early as 1975 [155]. It acts as a scavenger of oxygen free radicals [156,157] It can protect haemoglobin from oxidation [158]. *In vitro*, curcumin can significantly inhibit the generation of reactive oxygen species (ROS) like superoxide anions, H_2O_2 and nitrite radical generation. Curcumin also lowers the production of ROS *in vivo* [159]. It exerts powerful inhibitory effect against H_2O_2-induced damage in human keratinocytes and fibroblasts [160], and decreases lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates [161]. This is brought about by maintaining the activities of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase [162].

The stress induced by heavy metals also releases free radicals that lead to lipid oxidation. Antioxidants (herbal, natural and synthetic) work in three ways: i) reduce the energy of the free radicals, ii) stop the free radicals from stealing ions from cell components in the first place and iii) interrupt the oxidizing chain reactions to minimize the damage caused by free radicals. The reduction of free radicals is the primary job of natural and dietary antioxidants. The antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase and catalase destroy several harmful free radicals. Likewise, a lot of vitamins and minerals such as vitamin C, vitamin E, beta- carotene, lutein, lycopene, vitamin B_2, coenzyme Q10, and cysteine act as natural antioxidants. All earlier studies revealed different effects of metal in different tissues and same is also true with most of the chelating agents [149-151]. It may be due to the fact that different organs have different capacity to absorb and store the toxicants [136]. Therefore, individual application of therapeutic agents may not be so effective. Some helper, adjutant or agent, which exerts action similar to main chelator, may accelerate the function.

It has been observed by earlier workers that a particular chelator may eliminate the metal or recover the biochemical lesion from one tissue successfully, but may not show a similar effect on other tissues [139,140,147]. Sinha [163], Bapu [164], Rao [165], Patney [166] and Chundawat [167] clearly demonstrated that mercury, methylmercury and chromium are eliminated from different tissues of experimental animals at different rates during mono-thiol, dithiol, vitamin and herbal antioxidant therapy.

Antioxidants play an important role against free radical mediated oxidative stress. Curcumin is a potent herbal antioxidant [168] Its dietary application is quite advantageous when the natural antioxidants like, vitamin C, vitamin E, **β**-carotene, glutathione, coenzyme Q10, **α**-lipoic acid, are insufficient during acute intoxication. Decrease of body's natural antioxidants, release of free radicals generated in biological systems that cause oxidative stress in tissues, resulting in lipid peroxidation have been demonstrated by numerous studies. Once natural antioxidants defense system becomes weak immediately there is biochemical lesion in many metabolic pathways accompanied by structural damage of tissue [169] Disturbances in glutathione metabolism [170] enzymes of carbohydrate metabolism [171] energy yielding system and electron transport [172] general cell metabolism [173] membrane transport [174] etc leading to decrease of all the macro- and micronutrients [150,151] in all major tissues that ultimately cause histopathological changes [169], have been noticed in our laboratory during MMC intoxication.

Sood et al. [175] found a significant reduction of glutathione and its metabolizing enzymes in all the tissues of chick during MMC intoxication. Similar trend was also recorded in mice [176]. These authors showed significant, but incomplete, recovery of GSH, during vitamin B complex and GSH therapy [176]. Contrary to this, a mixed therapy including vitamin B complex and GSH along with curcumin ameliorated the functions of each other as their individual applications are also able to recover all the foresaid components, of course to a lesser degree.

Malondialdehyde is the end product of lipid peroxidation. Since lipid is an important constituent of all biological membranes, the integrity of membrane is

definitely disturbed during lipid peroxidation. MMC is known to disturb membrane structure [169,174]. Increase of lipid peroxidation in all the tissues of chick during MMC intoxication is a definite clue of free radicals generation and oxidative stress. MMC is also known to increase ROS [177] which include superoxide radicals, hydrogen peroxide, hydroxyl radicals hypoclorous acid and peroxy nitrite [177,178] The free radicals are generated in different areas or compartments of the cell, therefore, antioxidants which cover all the cell compartments are needed. This can not be a function of one antioxidant as they vary in their ability to penetrate these compartments; hence a collection of antioxidants is required to keep free radicals in check as well as a lipid peroxidation free environment. However, curcumin alone application to MMC intoxicated animals relieved the animals from stress up to some extent as indicated by decrease of lipid peroxidation byproduct, malondialdehyde and significant recovery of GSH metabolizing enzymes (glutathione reductase and glutathione peroxidase) as well as total glutathione. Study further shows that supplementation of vitamin B complex along with curcumin helps the latter to act more powerfully and recovered the total GSH and enzymes better than curcumin alone application. The antioxidative capacity is further increased when dose of GSH was also included along with curcumin and vitamin B complex. Thus mixed therapy almost completely recovered the biomolecules and brought normal conditions to the animals. The improvement of animal growth, as judged by the progress of their body and tissue weights also supports this contention.

.

Acknowledgements: The work presented in this review is the contribution by many scholars and students worked with senior scientist in his laboratory for last several years. We acknowledge and respect all of them.

REFERENCES

[1] Ma, X.Y., Su, Y.B., Zhang, F.R. and Li, J.F.: J. Environ.Pathol. Toxicol. Oncol., 15(1): 51-53 (1996).
[2] Wenbo, Q., Russel, J.R., Dun-Sian, T., Joaquin, J.G., Lucien, C.M., Malgorzata, K. and Juan, R.C.: Environ. Health Perspect., 92: 63-70 (2000).
[3] ATSDR: *Toxicological profile for chromium* (update). U.S. Department of Health and Human Service, Public Health Services. (1999).
[4] Rabinowitz, M.B., Gonick, H.C., Levin, S.R. and Davidson, M.B.: Diab. Care, 6(4): 319-327 (1983).
[5] Yamaguchi, S., Sano, K. and Shimojo, N.: Ind. Health,21: 25-34 (1983).
[6] Singh, J., Carlisle, D.L., Pritchard, D.E. and Paterno, S.R.: Oncol. Rep., 5: 1307-1318 (1998).
[7] Nath, K. and Kumar, N.: Sco. Total. Environ., 15: 175-181 (1988).
[8] Kim, E. and Na, K.J.: Arch. Toxicol., 64: 644-649 (1990).
[9] Joshi, S.N. and Patil, H.S.: Proc. Environ. Poll. Prev. Control Healthy Living, New Delhi, 21st Nov. (2000).
[10] Merkur'eva, R.V., Koganova, Z.I., Gabdullina, M.K., et al.: Gig. Sanit., 8: 75-76 (1982).
[11] Anjum, F., Shakoori, A.R. and Gorrod, J.W.: J. Environ.Pathol. Oncol., 15: 41-40 (1996).
[12] Kumar, A., Rana, S.V.S. and Prakash, R.: Int. J. Tissue React., 7: 333-338 (1985).
[13] Witmer, C., Faria, E., Park, H.S., Sadrieh, N., Yurkow, E., O'Connell, S., Sirak, A. and Schleyer, H.: Environ. Health Perspect., 102(3): 169-176 (1994).
[14] Sastry, K.V. and Tyagi, S.: Toxicol. Lett., 11: 17-21 (1982).
[15] Chorvatovicova, D., Kovacikova, Z., Sandula, J. and Navarova, J.: Mutat Res., 2(4): 207-211 (1993).
[16] Davis, C.M., Sumrall, K.H. and Vincent, J.B.: Biochemistry, 35: 1296-1299 (1996).
[17] Sood, P.P. and Chundawat, R.S.: J. Cell Tissue Res., 5(1): 313-321 (2005)
[18]Boge, G., N'Diaya, P., Roche, H. and Peres, G.: J. Physiol., 83: 57-63 (1988-89).
[19] Chundawat, R.S. and Sood, P.P.: J. Tissue Res., 4(2): 231-237 (2004).
[20] Partingron, C.N.: Br. Med. J., 2: 1097-1098 (1950).
[21]Sastry, K.V. and Tyagi, S.: Nat. Acad. Sci. Letters, 7: 131-134 (1984).
[22]Larsson, A.: Gen. Comp. Endorinol., 20: 155 (1973).
[23]Lehninger, A.L.: *Principles of Biochemistry*. (Lehninger, A.L., Nelson, D.L. and Cox, M.M. eds). CBS Publishers and Distributers, New Delhi (1993).
[24]Bagchi, D., Stohs, S.J., Downs, V.W., Bagchi, M. and Preuss, H.G.: Toxicology, 186(1-2): 171-173 (2003).
[25]Hayashi, Y., Kondo, T., Zhao, Q.L., Ogawa, R., Cui, Z.G., Feril, L.B.Jr., Teranishi and H., Kasuya, M.: Toxicol. Appl. Pharmacol., 197(2): 96-106. (2004).
[26]Ye, J. and Shi, X.: Mol. Cell Biochem., 222 (1-2): 189-197 (2001).
[27]Ueno, S., Susa, N., Furukawa, Y., Aikawa, K. and Itagaki, I.: Nippon Juigaku Zasshi., 51(1): 137-145 (1989).
[28]Pourahmad, J. and O'Brien, P.J.: Adv. Exp. Med. Biol., 500: 203-207 (2001).
[29]Patney, V., Chundawat, R.S. and Sood, P.P.: Poll. Res., 21(4): 483-493 (2002).
[30]Vijayalakshmi, K.: *Vitamins and Thiols in Methylmercury Detoxication: A Biochemical Study*. PhD Thesis, Saurashtra University, Rajkot. 1-192 (1994).
[31]Anjum, F. and Shakoori, A.R.: J. Environ. Pathol.Toxicol. Onchol., 16: 51-59 (1997).
[32]Bag, S., Vora, T., Ghatak, R., Nilufer, I., D'mello, D., Pereira, L., Pereira, J., Cutinho, C. and Rao, V.: Ecotoxicol. Environ. Saf., 42: 163-170 (1999).

[33]Dey, S.K., Nayak, P. and Roy, S.: J. Environ. Sci., 13: 272-275 (2001).
[34]Thaker, J., Chhaya, J., Mittal, R., Nuzhat, S. and Kundu, R.: Ind. J. Marine Sci., 28: 45-49 (1999).
[35]Lee, D.N., Yen, H.T., Shen, T.F. and Chen, B.J.: Biol. Trace Elem. Res., 77(1): 53-64 (2000).
[36]Fernandes, M.A., Santos, M.S., Alpoim, M.C., Madeira, V.M. and Vicente, J.A.: J. Biochem. Mol. Toxicol., 16(2): 53-63 (2002).
[37]Chorvatovicova, D., Ginter, E., Kosinova, A. and Zloch, Z.: Mutat. Res., 262: 41-46 (1991).
[38]Fernandes, M..A., Geraldes, C.F., Oliveira, C.R. and Alpoim, M.C.: Toxicol. Lett., 114(1-3): 237-243 (2000).
[39]Pandima, D.K., Sia Ram, M., Shripriya, M., Ilavazhagan, G. and Devaki, T.: Biomed. Pharmacother., 57 (2): 105-108 (2003).
[40]Qi, W., Reiter, R.J., Tan, D.X., Garcia, J.J., Manchester, L.C., Karbownik, M. and Calvo, J.R.: Environ. Health Perspect., 108(5): 399-402 (2001).
[41]Sai Ram, M. Anju, B., Pauline, T., Dipti, P., Kain, A.K., Mongia, S.S., Sharma, S.K., Singh, B., Singh, R., Ilavazhagan, G., Kumar, D. and Selvamurthy, W.: J. Ethonopharmacol., 71(1-2): 235-240 (2000).
[42] Sai Ram, M., Neetu, D., Deepti, P., Vandana, M., Havazhagan, G., Kumar, D. and Selvamuthy, W.: Phytother. Res., 17(4): 430-433 (2003).
[43] Shi, D. and Jiang, B.H.: J. Environ. Pathol. Toxicol.Oncol., 21(3): 233-242 (2002).
[44] Sugiyama, M.: Biol. Trace. Elem. Res., 21: 399-404 (1989).
[45] Susa, N., Ueo, S., Furukawa, Y., Ueda, J. and Sugiyama, M.: Toxicol. Appl. Pharmacol., 144: 377-384 (1997).
[46] Chundawat, R.S., Ansari, N.H. and Sood, P.P.: Res. Com. Pharmacol. Toxicol., 6(1&2): 35-45 (2001).
[47] Chundawat, R.S. and Sood. P.P.: Biometals (In press) (2004).
[48] Chundawat, R.S. and Sood, P.P.: J. Tissue Res., 3(1): 64-70 (2003).
[49] Sood, P.P., Ansari, N.H. and Sinha, N.: Res. Com. Pharmacol. Toxicol., 1&2: 3-22 (2002).
[50] Calabrese E.J.: *The influence of nutritional status on pollutant toxicity and carcinogenecity*. In: *Nutrition and Environmental Health.* Vol 1, *The Vitamins.* New York (1980).
[51] Bagchi, D., Hassoun, E.A., Bagchi, M. and Stohs, S.J.: Comp. Biochem. Physiol., 110: 117-187 (1995).
[52] Sugiyama, M.: Environ. Health. Perspect., 92: 63-70 (1991).
[53] Hiraishi, H., Terano, A., Ota, S., Mutoh, H., Sugimoto, T., Harada, T., Razandi, M. and Ivey, K.J.: Gastroenterology, 106(5): 1199-1207 (1994).
[54]Fernicola, C., Govoni, S., Coniglio, L. and Trabucchi, M.: G. Ital. Med. Lav. (Pavia), 7(5-6): 175-180 (1985).
[55]Gaberscek, S., Kalisnik, M., Pezdirc, M., Pavlin, K. and Hojker, S.: Folia Biol. (Praha), 48(5): 200-204 (2002).
[56]Donmez, H.H., Karsl, M.A., Mera, I., Donmez, N. and Simsek, N.: Dtsch. Tierarztl. Wochenschr (Hannover), 109(10): 438-442 (2002).
[57] Goncharov, A.T. and Ametov, A.S.: Probl. Endokrinol. (Mosk), 23(6): 59-61 (1977).
[58] Sood, P.P. Misra, S., Lavekar, G.S. and Chundawat, R.S.: J. Cell Tissue Res., 7(2): 1047-1052 (2007)
[59]Rose, D.P.: Vitam. Horm., 36: 53-99 (1978).
[60] Stokstad, E.L. and Nair, C.P.: J. Nut., 118(12): 1495-1501 (1988).
[61]Cherel, Y., Durant, J.M. and Lacroix, A.: Gen. Comp. Endocrinol., 136(3): 398-405 (2004).
[62]Chundawat, R.S.: J. Tissue Res., 4(1): 185 (2004).
[63]Chundawat, R.S. and Sood, P.P.: Toxicology, 211(1-2): 124-131 (2005).
[64] Sood, P.P. and Chundawat, R.S.: J. Cell Tissue Res., 5(1): 313-321 (2005).
[65]Sood, P.P. and Vijayalakshmi, K.: J. Nut. Environ. Med., 5: 15: 133-141 (1995).
[66] Huszenicza, G., Kulcsar, M. and Rudas, P.: Vet. Med. (Czech), 47(7): 199-210 (2002).
[67] Awadeh, F.T., Kincaid, R.L. and Johnson, K.A.: J. Animal Sci., 76: 1204-1215 (1998).
[68] Svanberg, E., Healey, J. and Mascarenhas, D.: Eur. J.Clin. Invest., 31: 329-336 (2001).
[69] Bagchi, N., Brown, T.R., Herdegen, D.M., Dhar, A. and Sundick, R.S.: Endocrinology, 127(4): 1590-1595 (1990).
[70] ATSDR: Toxicological Profile for Chromium (update). U.S. Department of Health and Human Service, Public Health Services (1999).
[71] Flier, J.F., Harris, M. and Hollenberg, A.N.: J. Clin. Invest., 105: 859-861 (2000).
[72] Geetha, S., Sai Ram, M., Mongia, S.S., Singh, V., Ilavazhagan, G. and Sawhney, R.C.: J. Ethnopharmacol, 87(2- 3): 247-251 (2003).
[73] Pourahmad, J. and O'Brien, P.J.: Adv. Exp. Med. Biol., 500: 203-207 (2001).
[74]Brzezinska, S.E.: J. Physiol. Pharmacol., 52(2): 275-284 (2001).
[75] http.//Tuberose.com/thyroid.html.
[76] Chundawat, R.S. and Sood, P.P.: Toxicology, 211(1-2): 124-131 (2005).
[77] Rajatanavin, R., Safran, M., Stoller, W.A., Mordes, J.P. and Braverman, L.E.: Am. J. Med., 77(2): 378-384 (1984).
[78] Cashin-Hemphill, L., Spencer, C.A., Nicoloff, J.T., Blankenhorn, D.H., Nessim, S.A., Chin, H.P. and Lee, N.A.: Ann. Int. Med., 107(3): 324-329 (1987).
[79] Preuss, H.G., Montamarry, S., Echard, B.,Scheckenbach, R. and Bagchi, D.: Mol. Cell Biochem., 223(1-2): 95-102 (2001).
[80] Engelmann, D., Flachowsky, G., Halle, I. and Sallmann, H.P.: J. Exp. Zool., 290(1): 41-48(2001).
[81] Dean, R.T. and Cheeseman, K.H.: Biochem. Biophys. Res. Comm., 50: 310-315 (1987).
[82] Vijayalakshmi, K. and Sood, P.P.: Cell. Mol. Biol. 40: 211-224 (1994).
[83] Chundawat, R.S., Ansari, N.H. and Sood, P.P.: Res.

Comm. Pharmacol. Toxicol., 6(1&2): 35-45 (2001).
[84] Aaseth, J., Alexander, J. and Norseth, T.: Acta Pharmacol. Toxicol. (Copenh), 50: 310-315 (1982).
[85] Dhuley, J.N.: J. Enthnopharmacol., 60: 173-178 (1998).
[86] Chaudhary, A., Sharma, R.K. and Saini, K.: J. Tissue Res., 4(1): 113- 115 (2004).
[87] Bhatnagar, M., Saxena, A. and Bhatnagar, R.: J. Tissue Res., 4(2): 227-230 (2004).
[88] Tang, Z.G., Xu, X.P. and Shen, Z.H.: Hunan Yi Ke Da Xue Xue Bao, 25(1): 27-29 (2000).
[89] Arivazhagan, S., Balasenthil, S. and Nagini, S.: Phytother. Res., 14(4): 291-293 (2000).
[90] Panda, S. and Kar, A.: J. Pharm. Pharmacol., 50(9):1065-1068 (1998).
[91] Chundawat, R.S.: J. Tissue Res., 4(1): 185 (2004).
[92] Maekawa, M., Satoh, S., Murayama, T. and Nomura Y.: Biochem. Pharmacol., 59(7): 839-845 (2000).
[93] Hrdina, P.D., Peters, D.A.V. and Singhal, R.L.: Res. Commun. Chem. Pathol. Pharmacol., 15: 483-494 (1976).
[94] Gasso, S. Sunol, C., Sanfeliu, C., Rodriguez-Farre, E. and Cristofol, R.M.: Life Sci., 67(10):1219-1231(2000).
[95] O'Kusky, J.: Exp. Neurol., 89: 32-47 (1985).
[96] Tekeuchi, T.: In: *Minamata Disease* (Katsuma, M. ed), Kumamoto University, Tokyo, Japan, pp 229-252 (1968).
[97] Sood, P.P., Vijyalakshimi, K., Bapu, C., Chundawat, R.S. and Tyagi, S.: J. Tissue Res., 4: 75-81 (2004).
[98] Hargreaves, R.J., Foster, J.R., Pelling, D., Moorhouse, S.R., Gangolli, S.D. and Rowland, I.R.: Neuropath. Appl. Neurobiol., 11: 383-401 (1985).
[99] Vinay, S.D., Raghu, K.G. and Sood, P.P.: Cell. Mol. Biol., 36: 487-501 (1990).
[100] Chang, L.W. and Annau, Z.: The neurochemistry of vitamin deficiencies. In: *Hand book of Neurochemistry.* Vol. X (Lajtha, A. ed), Plenum Press, New York, pp. 757-777 (1985).
[101] Faro, L.R., do Nescient, J.L., San Jose, J.M., Alfonso, M. and Duran, R.: Neuroses. Res., 25(2): 225-229 (2000)
[102] Minnema, D.J., Cooper, G.P. and Greenland, R.D.: Toxicol. Appl. Pharmacol., 99: 510-521 (1989).
[103] Hare, M.F., Minnema, D.J., Cooper, G.P. and Michaelson, I.A.: Toxicol. Appl. Pharmacol., 99: 266-275 (1989).
[104] Kirubagaran, R. and Joy, K.P.: Bull. Environ. Contam.Toxicol., 45: 88-93 (1990).
[105] Slotkin, T.A. and Bartolome, J.: Neurotoxicology, 8: 65-84 (1987).
[106] O'Kusky, J.R. and MeGeer, E.G.: J. Neurochem., 53: 999-1006 (1989).
[107] Kimiko, H., Inouye, M. and Fujisaki, T.: Arch. Toxicol.,53: 35-40 (1985).
[108] Sood, P.P. Vijyalakshmi, K. and Chundawat, R.S.: J. Cell Tissue Res., 5(2): 425-430 (2005).
[109] Bapu, C. Purohit, R.C. and Sood, P.P.: Human Exp.Toxicol., 13: 815-823 (1994).
[110] Sood, P.P. and Vijyalakshmi, K.: J. Nut. Environ. Med., 5: 133-141 (1995).
[111] Sood, P.P., Raghu, K.G., Vijyalakshimi, K. and Bapu, C.: J. Environ. Pathol. Toxicol. Oncol., 12: 149-154 (1993).
[112] Sood, P.P. and Patney, V.: J. Anim. Morphol. Physiol., 48: 113-120 (2001).
[113] Vijyalakshmi, K. and Sood, P.P.: Cell. Mol. Biol., 40: 211-224 (1994).
[114] Vijyalakshmi, K. Bapu, C. and Sood, P.P.: Bull. Environ.Contam. Toxicol., 49: 71-77 (1992).
[115] Bapu, C., Sood, P.P. and Nivsarkar, M.: BioMetals, 16: 279-284 (2003).
[116] Bapu, C. and Sood, P.P.: Protective effect of vitamin B comlex and glutathione against methylmercury induced histopathological alteration in mice brain. In: *Heavy Metal Pollution Toxication and Chelation* (Sood, P.P. and Prakash, R. ed), M.D. Publication Pvt Ltd., New Delhi, pp. 359-379 (1998).
[117] McKay, S.J., Reynolds, J.N. and Racz, W.J.: Can. J.Physiol. Pharmacol., 64: 1507-1514 (1986).
[118] Kiyofumi, S., Inove, Y. and Sumino, K.: Jpn. J. Hyg.,42: 887-893 (1987).
[119] Ken-Ichi, S., Shimoji, N., Suzuki, M. and Yamaguchi, S.: Jpn. J. Hyg., 42: 821-826 (1987).
[120] Sood, P.P., Vijayalakshmi, K. and Bapu, C.: Cell. Mol.Biol., 39: 213-220 (1993
[121] Lucy, J.A.: *Functional and Structural Aspects of Bio-logical Membrane: A Suggested Structural role for Vitamin E in the Control of Membrane Permeability and Stability.* Ann. N.Y. Acad. Sci., 203: 4-11 (1972).
[122] Drefyus, P.M.: The neurochemistry of vitamin deficiencies. In: *Handbook of Neurochemistry.* (Lajtha, A. ed) Plenum Press, New York, pp. 757-777 (1985).
[123] McDowell, L.R.: Vitamins in animals nutrition.Comparative aspects to human nutrition. In: *Animal Feeding and Nutrition* (Cunha, T.J. ed) Academic Press, New York, pp. 7-473 (1989).
[124] Vijayalakshmi, K.: *Vitamins and thiols in methylmercury detoxication: A biochemical study.* Ph.D. Thesis, Saurashtra University, Rajkot, India, pp. 1-192 (1994).
[125] Mirzoian, A. and Luetje, C.W.: J Pharmacol. Exp. Ther., 302(2): 560-567 (2002).
[126] Komulainen, H. and Tuomisto, J.: Acta. Pharmacol.Toxicol., 48: 199-204 (1981).
[127] Atchison, W.D., Joshi, U. and Thornburg, J.E.: J.Pharmacol. Exp. Ther., 238: 618-624 (1986).
[128] Faro, L.R., do Nascimento, J.L., Alfonso, M. and Duran, R.: Ecotoxicol. Environ. Saf., 48(3): 263-267 (2001).
[129] Bartolome, J., Whitmore, W.L., Seidlier, F.J. and Slotkin, T.A.: Life Sci., 35: 657-670 (1984).
[130] Komulainen, H. and Tuomisto, J.: The neurochemical effect of methylmercury in the brain. In: *Toxicity of Methylmerury* (Eccles, U.C. and Annau, Z. ed) The John Hopkin Univ. Press, Baltimore, pp. 172-188 (1987).

[131]Kobayashi, H., Yuyama, A., Matsusaka, N., Takeno and Yanagiya, I.: Toxicol. Appl. Pharmacol., 54: 1-8 (1980).

[132]Tsuzuki, Y.: Toxicol. Appl. Pharmacol., 60: 379-381 (1981).

[133]. Sood, P.P., Raghu, K.G., Bapu, C. and Vijayalakshmi, K.: J. Envirom. Pathol. Toxicol. Oncol., 12: 149-154 (1993).

[134] Mei-Ping, K., Kostyniak, P.J., Olson, J.R., Sansone, F.M., Nikerson, P.A., Malone, M.A., Ziembiec, N. and Roth, J.A.: Neurotoxicology, 10: 41-52 (1989).

[135] Omata, S., Hirakawa, E., Daimon, Y., Uchiyama, M., Narashita, H., Horigome, T., Sugano, I. and Sugano, H.: Arch. Toxicol., 51: 285-294 (1982).

[136] ATSDR: *Toxicological profile for mercury* (update). US Department of Health and Human Services. Public Health Service, Atlanta, Georgia, USA, pp 1-617 (1999).

[137] Sood, P.P., Dass, V.S. and Raghu, K.G.*: Heavy Metal Pollution, Toxication and Chelation*. M. D. Publications Pvt. Ltd., New Delhi, pp. 1-387 (1998).

[138]Sood, P.P. and Vijayalakshmi, K.: J. Nut. Environ. Med., 5: 133-141 (1995).

[139] Sood, P.P., Bapu, C., Sinha, N. and Rao, A.P.: J. Nut. Environ. Med., 7: 155-162 (1997).

[140]Sinha, N., Chundawat, R.S. and. Sood, P.P.: Ad. Pharmacol. Toxicol. 1: 29-51 (2000).

[141]Sood P. P. and Patney, V.: J. Anim. Morphol. Physiol., 6: 113-120 (2001).

[142] Sood, P.P., Ansari, N.H. and Sinha, N.: Res. Com.Pharmacol. Toxicol., 7: 3-22 (2002).

[143]Patney, V., Chundawat, R.S. and Sood, P.P.: Poll. Res. 49: 491-501 (2002).

[144]Bapu, C., Sood, P.P. and Nivasarkar, M.: BioMetals 16, 279-284 (2003).

[145]Sood, P.P. Vijayalakshmi, K., Bapu, C., Chundawat, R.S. and Tyagi, S.: J. Cell Tissue Res., 4(1): 75-81 (2004).

[146] Sood, P.P., Raghu, K.G., Vijayalakshmi, K. and Bapu, C.: J. Environ. Pathol. Toxicol. Oncol., 12: 149-154 (1993).

[147] Sood, P.P., Bapu, C. and Vijayalakshmi, K.: Pathol.Toxicol. Oncol., 14: 101-105 1995).

[148]Sood, P.P. Vijayalakshmi, K. and Chundawat, R.S.: J. Cell Tissue Res. 5(2): 425-430 (2005).

[149] Vinay, S.D., Raghu, K.G. and Sood, P.P.: Bull. Environ. Contam. Toxicol., 49: 78-84 ((1992).

[150] Sinha, N.: *Therapeutic Effects of Vitamins and Monothiols in Methylmercury Intoxicated Postnatally Developing Chick*: An analysis of Nutritional value. Ph. D thesis, Saurashtra University, Rajkot (2000).

[151] Bapu, C.: *Protective Effect of Monothiols and Vitamins During Methylmercury Toxication: A Biochemical and Histopathological Study.* Ph. D thesis, Saurashtra University (1994).

152]Woods, J.S. Ellis, M.E.: Biochemical Pharmacol., 50(10): 1719-1724 (1995).

[153]Zahir, F., Rizvi, S.J., Haq, S.J. and Khan, R.H.: Ind. J.Clin. Biochem. 21(2): 149-152 (2006).

[154] Calabrese, E.: Nutrition and environmental Health. The influence of nutritional status on pollutant toxicity and carcinogenecity. In: *The Vitamin*, vol. 1, New York (1980).

[155]Sharma, O.P., Biochem. Pharmacol., 25: 1811-1812 (1976).

[156] Ruby, A.J., Kuttan, G., Dinesh Babu, K., Rajasekharn, K. N. and Kuttan, R.: Cancer Lett., 94: 79-83 (1995).

[157]Subramanian, M., Sreejayan Rao, M.N.A., Devasagayam, T.P.A. and Singh, B.B.: Mutat. Res.,22: 249-255 (1994).

[158]Unnikrishnan, M.K. and Rao, M.N.: Pharmazie, 50: 490-492 (1995).

[159] Phan, T.T., See, P., Lee, S.T. and Chan, S.YJ.: Trauma, 60: 927-931 (2001).

[160]Lim, G.P., Chu, T., Yang, F., Beech, W., Frantschy, S. A. and Cole, G.M.J.: Neurosci., 21: 8370-8377 (2001).

[161]Reddy, P. ACh., P. and Lokesh, B.R.: Mol. Cell.Biochem., 111: 117-124 (1992).

[162] Reddy, P., ACh., P. and Lokesh, B.R.: Food Chem. Toxicol., 32: 279-283 (1994).

[163] Sinha, N.: *Therapeutic Effects of Vitamins and Monothiols in Methylmercury Intoxicated Postnatally Developing Chick*: An analysis of Nutritional value. Ph. D thesis, Saurashtra University, Rajkot (2000).

[164] Bapu, C.: *Protective Effect of Monothiols and Vitamins During Methylmercury Toxication: A Biochemical and Histopathological Study.* Ph.D thesis, Saurashtra University (1994).

[165] Rao, A.P.: Analytical and biochemical alterations in nervous and non-nervous tissues of *Channa punctatus* during mercury intoxication and detoxication. Ph.D. thesis, Saurashtra University, Rajkot (2002).

[166] Patney, V.: Protective effects of glutathione and vitamins against inorganic mercury toxicity. Ph.D. thesis, Saurashtra University, Rajkot (2002).

[167] Chundawat, R.S.: Protection against hexavelant chromium toxicity in developing chick: An analytical and biochemical study. Ph.D. thesis, Saurashtra University, Rajkot (2002).1

[168]Lukita-Atmadja, W., Ito, Y., Baker, G.L. and McCuskey, R.S.: Shock, 17: 399-403 (2002).

[169]Sood, P.P. Vijayalakshmi, K., Bapu, C., Chundawat, R.S. and Tyagi, S.: J. Tissue Res., 4 (1): 75-81 (2004).

[170] Vijayalakshmi, K. and Sood, P.P.: Cell. Mol. Biol.., 40: 211-224 (1994).

[171]Sood P.P. and Patney, V.: J. Anim. Morphol. Physiol., 48: 113-120 (2001).

[172] Raghu, K.G.: Effect of organomercurials pollution in animals. 1. Evaluation of biochemical changes in the central nervous system of rat with MMC and chelators. Ph.D. thesis, Saurashtra Univ., Rajkot (1992).

[173] Sood, P.P., Teraiya, R., Kodi, R.B., Vijayalakshmi, K. and Bapu, C.: Asian J. Exp. Sci., 7: 1-9 (1993).
[174] Sood, P.P. and Unnikumar, K.R.: J. Pathol. Toxicol. Oncl., 7: 21-28 (1987).
[175] Sood, P. P.,Joshi, R., Gupte, K., Vekariya, V., Vekaria, P., Delvadiya, C., Ankola, P., Barchha, S., Manvar, R., Kundu, R. S., Joshi, K. K. And Shah, A.: J. Cell Tissue Res., 9(2) 1803-1810 (2009)
[176] Vijayalakshmi, K. and Sood, P.P.: Cell. Mol. Biol.., 40: 211-224 (1994).
[177] Okada, F.: Int. J. Cancer, 121: 2364-2372 (2007).
[178] Balakrishnan, S., Manoharan, S., Linsamary, A., Vellaichamy, L. and Menon, V.P.: J. Cell Tissue Res., 8(3): 1525-1530 (2008).