ARSENIC INDUCED NEPHROTOXICITY PROTECTIVE ROLE OF ESSENTIAL NUTRIENT SUPPLEMENTATION WITH SPECIALREFERENCE TO SOME SELECTED ENZYMES IN ALBINO RATS

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Abstract:

Studies were conducted to examine the effect of the rats of 90 days old were exposed to As, Ca, Zn and Vit-E intraperitoneally daily for a period of 2 weeks. Control rats received only deionized distilled water injections intraperitoneally daily up to 2 weeks. After the period of exposure, the animals were sacrificed and the tissues were collected rats in terms of lipid peroxidation (LPO), glutathione peroxidase (GPx), catalase (CAT) and data was showed that the exposure to As $^{3+}$lead to a significant decrease in mitochondrial GPx activity compared to the control. The increase in the activity of GPx was observed in animals treated with Ca $^{2+}$ +Zn^{2+} and Vit-E. However, among Vit-E and Ca+Zn supplementation, Vit-E showed more inhibitory activity against As than Ca $^{2+}$+Zn $^{2+}$. A significant increase in mitochondrial Lipid peroxidation activity compared to the control. The decreased activity of Lipid peroxidation was observed in animals treated with Ca^{2+} +Zn $^{2+}$ and Vit-E and significant decrease in mitochondrial Catalase activity compared to the control. The increased activity of Catalase was observed in animals treated with Ca+Zn and Vit-E.

Key words: As, Ca, Zn and vitamin E, Lipid peroxidation, Glutathione and peroxidase, Catalase

Introduction:

Arsenic is the chemical element that has the symbol As^{3+}, atomic number 33 and relative atomic mass 74.92. Arsenic occurs in many minerals, mainly combined with sulfur and metals, and also naturally in the native (elemental) state. It was first documented by Albertus Magnus in 1250 (Emsley and John, 2001). Arsenic is a metalloid. It can exist in various allotropes, although only the grey form is industrially important. The main use of metallic arsenic is for strengthening alloys of copper and especially leads (for example, in automotive batteries).Arsenic is a common n-type dopant in semiconductor electronic devices, and the optoelectronic compound gallium arsenide is the most common semiconductor in use after doped silicon.

A few species of bacteria are able to use arsenic compounds as respiratory metabolites, and are arsenic-tolerant. However, arsenic is notoriously poisonous to multicellular life, due to the interaction of arsenic ions with protein thiols. Arsenic and its compounds, especially the trioxide, are used in the production of pesticides (treated wood products), herbicides and insecticides. These applications are declining, however, as many of these compounds are in the process of being banned. (Sabina C *et al.*, 2005), Meanwhile, arsenic poisoning as a result of the natural occurrence of arsenic compounds in drinking water remains a problem for many parts of the world including the United States.

Minerals with the formula MAsS and MAs_2 (M = Fe, Ni, Co) are the dominant commercial sources of arsenic, together with realgar (an arsenic sulfide mineral) and native arsenic. An illustrative mineral is arsenopyrite (Fe As S), which is structurally related to iron pyrite. Many minor As^{3+}containing minerals are known. In addition to the inorganic forms mentioned above, arsenic also occurs in various organic forms in the environment. Other naturally occurring pathways of exposure include volcanic ash, weathering of the arsenic-containing mineral and ores as well as groundwater. It is also found in food, water, soil and air. The most common pathway of exposure for humans is ingestion, and the predominant source of arsenic in our diet is through seafood. An additional route of exposure is through inhalation.

Arsenic is still added to animal food, particularly in the U.S. as a method of disease prevention and growth stimulation (Nachman, *et al*., 2005). One example is roxarsone which is used as a broiler starter by about 70% of the broiler growers since 1995. The Poison-Free

Poultry Act of 2009 proposes to ban the use of roxarsone in industrial swine and poultry production. (Bottemiller and Helena, 2009). Arsenic trioxide has been used in a variety of ways over the past 500 years, but most commonly in the treatment of cancer. The US Food and Drug Administration in 2000 approved this compound for the treatment of patients with acute promyelocytic leukemia that is resistant to ATRA (Antman and Karen, 2001).

The main use of metallic arsenic is for alloying with copper and especially lead. Lead components in automotive batteries are strengthened by the presence of a few percent of arsenic. Gallium arsenide is an important semiconductor material, used in integrated circuits. It is fabricated by chemical vapor deposition. Circuits made from GaAs are much faster (but also much more expensive) than those made in silicon. Unlike silicon it is direct bandgap, and so can be used in laser diodes and LEDs to directly convert electricity into light. (Sabina *et al.*, 2005). Copper acetoarsenite was used as a green pigment known under many names, including 'Paris Green' and 'Emerald Green'. It caused numerous arsenic poisonings. Scheele's Green, a copper arsenate, was used in the 19th century as a colouring agent in sweets (Timbrell and John 2005). Arsenic is added in small quantities to alpha-brass to make it dezincification resistant. This grade of brass is used to make plumbing fittings or other items which are in constant contact with water. (Davis and Joseph 2001). Arsenic is also used for taxonomic sample preservation.

Some species of bacteria obtain their energy by oxidizing various fuels while reducing arsenate to arsenite. Under oxidative environmental conditions some bacteria use arsenite, which is oxidized to arsenate as fuel for their metabolism. (Stolz *et al.*, 2006). In 2008, bacteria were discovered that employ a version of photosynthesis in the absence of oxygen with arsenites as electron donors, producing arsenates (just like ordinary photosynthesis uses water as electron donor, producing molecular oxygen). Researchers conjecture that historically these photosynthesizing organisms produced the arsenates that allowed the arsenate-reducing bacteria to thrive. One strain PHS-1 has been isolated and is related to the γ-Proteobacterium *Ectothiorhodospira shaposhnikovii*. The mechanism is unknown, but an encoded Arr enzyme may function in reverse to its known homologues.(Kulp, *et al.*, 2008).

Arsenic has been linked to epigenetic changes which are heritable changes in gene expression that occur without changes in DNA sequence and include DNA methylation, histone modification and RNA interference. Toxic levels of arsenic cause significant DNA hypermethylation of tumour suppressor genes p16 and p53 thus increasing risk of

carcinogenesis. These epigenetic events have been observed in *in vitro* studies with human kidney cells and *in vivo* tests with rat liver cells and peripheral blood leukocytes in humans. (Baccarelli and Bollati 2009). Inductive coupled plasma mass spectrometry (ICP-MS) is used to detect precise levels of intracellular arsenic and its other bases involved in epigenetic modification of DNA. (Nicholis, *et al*., 2009). The high affinity of arsenic(III) oxides for thiols is usually assigned as the cause of the high toxicity. Thiols, in the form of cysteine residues, are situated at the active sites of many importantenzymes. (Sabina C. *et al.*, 2005).

Symptoms of acute intoxication usually occur within 30 minutes of ingestion but may be delayed if arsenic is taken with the food. Initially, a patient may have a metallic taste or notice a slight garlicky odor to the breath associated with a dry mouth and difficulty in swallowing. Early clinical symptoms at acute arsenic intoxication may be muscular pain, weakness with flusking skin. Severe nausea and vomiting, colicky abdominal pain, and profuse diarrhoea with rice-water stools abruptly ensure. Capillary damage leads to generalized vasodilation, transudation of plasma, and vasagenice shock. Arsenic's effect on the mucosal vascular supply, not a direct corrosive action, leads to transudation of fluid in the bowel lumen, mucosal vesical formation, and sloughing of tissue fragments. The patient may complain of muscle cramps, numbness in hands and feet, reddish rashes in the body and intense thirst. In severe poisoning, the skin becomes cold and clammy, and some degree of circulatory collapse usually occurs along with kidney damage and decreased urine output. Drowsiness and confusion are often seen along with the development of a psychosis associated with paranoid delusions, hallucinations, and delirium. Finally, seizures, coma, and death, usually due to shock, may ensue. One of us (KCS) found mostly squamous cell carcinoma and Bowen's disease both monocentric and multicentric but Basal cell carcinoma was not found in skin out of 222 malignancies in arseni).Bowen's disease, a rare precancerous skin lesion, is associated cosis. (Saha, 1995). with both arsenic and human papilloma virus (HPV).

Most laboratory animals appear to be substantially less susceptible to arsenic than humans. It has been reported that chronic oral exposure to inorganic arsenic (0.05-0.1 mg/kg/day) causes neurological and haematological toxicity in humans but not in monkeys, dogs, and rats exposed to arsenite or arsenate at doses of 0.72 to 2.8 mg/kg/day (Byron, et al., 1967). Clinical signs of gastrointestinal irritation from acute arsenic poisoning include burning lips, painful swallowing, thirst, nausea and several abdominal colic (Campbell, *et al.*, 1989). The

haematopoietic system is also affected by both short-and long-term arsenic exposures. Anemia and leukopenia are common effects of poisoning and have been reported as resulting from acute Armstrong, (Stroube, *et al.,* 1984).

It is not well established whether ingestion of inorganic arsenic can cause developmental abnormalities in humans. The incidence of spontaneous abortion in women who lived near a copper smelter in Sweden tended to decrease as a function of distance (Norstrom, S., *et al.,* 1979). A couple of studies reported an increased number of miscarriages among women who worked in the semiconductor industry, which cause arsine. (Calabrese, *et al.,* 1983). Hardly any published information exists regarding reproductive effects in humans and animals after inhalation exposure to arsenic or organoarsenicals. The same is true for human oral exposure to these compounds.

Inhalation exposure to arsenic trioxide increased the frequency of chromosomal aberrations in the perpheral lymphocytes of smelter workers (Beckman, et al., 1978). Land in fatal mouse livers of mothers exposed to 22 mg As/m3 during the gestation period (days 9-12) (Nagymajtenyi, *et al*., 1985). Enzymes such as superoxide disumutese and catalase that scavenge for Oxygen free radicals seem to provide protection against arsenic induced DNA damage, indicating a possible basis for the genotoxic effect of arsenic. (Nordenson, *et al.,* 1991).

Materials and methods:

Procurement and maintenance of experimental animals

Young albino rats of were purchased and maintained in the animal house of Dept. of LPM College of Veterinary Science, Tirupati. The animals were housed in clear plastic cages with hardwood bedding in a room maintained at $28^{o} \pm 2^{o}$ C and relative humidity 60 ± 10% with a 12 hour light/day cycle. The animals were fed in the laboratory with standard pellet diet supplied by SKM feed from chittoor and, water *ad libitum*

Chemicals

Sodium arsenite (As), calcium chloride (Ca), zinc chloride (Zn) and vitamin E were selected as test chemicals. The chemicals used in this study namely Thiobarbituric acid, epinephrine were obtained from Sigma, USA. Other chemicals obtained from Qualigens, India.

Animal exposure to As, Ca, Zn and vitamin E

The rats 90 days were exposed to As, Ca, Zn and Vit-E intraperitoneally daily for a period of 2 weeks. Control rats received only deionized distilled water injections intraperitoneally daily up to 2 weeks. After the period of exposure, the animals were sacrificed and the tissues were collected and stored at -80^{0}C for further biochemical analysis.

BIOCHEMICAL STUDIES

Preparation of Kidney Mitochondrial Fraction

Kidney mitochondrial fractions were prepared by following the method of Lai and Clark (1979). Briefly, the tissues were homogenized in 5 volumes (w/v) of SET buffer (0.25 M sucrose, 10 mM Tris-HCl, and 1 mM EDTA, pH 7.4). The homogenate was first centrifuged at 800 *g* for 10 min at 4°C, and then the supernatant was centrifuged at 10,000*g* for 20 min. Then the pellet of mitochondrial fraction was suspended in SET buffer

.**Catalase (E.C. 1.11.1.6) assay:**

Catalase activity in the hepatic mitochondrial fraction was assayed by following the method of Chance and Machly (1955).

The reaction mixture in a final volume of 2.5 ml contained: 0.05 M phosphate buffer (pH 7.0) and appropriate amount of enzyme protein. The reaction was initiated by the addition of 19 mM hydrogen peroxide (H_2O_2). The decomposition of H_2O_2 was followed directly by measuring the decrease in absorbance at 240 nm, at 10 sec intervals for 1 min in a spectrophotometer (Hitachi model, U-2001). The catalase activity was expressed as n moles of H_2O_2 metabolized/mg protein/min

Glutathione peroxidase (GPx) (EC 1.11.1.9) activity:

GPx activity in the hepatic mitochondrial fraction was assayed as described by Rotruck *et al*., (1973).The reaction mixture contained 0.2 ml of EDTA, 0.2ml of sodium azide, 0.2ml of glutathione reduced, 0.2ml of H_2O_2, 0.4 ml of buffer, 01 ml of enzyme source The reaction mixture was incubated at 37º C for 10 min. The reaction was arrested by adding of 0.5 ml TCA. Than centrifuged at 2000 rpm for 10 min. To 0.5 ml of supernatant, 3.0 ml of disodium hydrogen phosphate and 1.0 ml of DTNB were added. The reaction was read at 412 nm in spectrophotometer.

Superoxide dismutase (SOD) (E.C. 1.15.1.1) activity:

SOD activity was determined by using the epinephrine assay of Misra and Fridovich (1972). At alkaline pH, superoxide anion O^-_2 causes the autooxidation of epinephrine to

adenochrome; while completing this reaction, SOD decreased the adenochrome formation. One unit of SOD is defined as the amount of extract that inhibits the rate of adenochrome formation by 50%.

The reaction mixture in a final volume of 2.0 ml contained 0.05 M carbonate buffer (pH 10.2), 30 mM epinephrine (freshly prepared) and the enzyme extract. Changes in absorbance was recorded at 480 nm, measured at 10 sec intervals for 1 min in a spectrophotometer. The enzyme activity was expressed as Units/mg protein.

Lipid peroxidation:

The level of lipid peroxidation in the tissues was measured in terms of malondialdehyde (DMA; a product of lipid peroxidation) content and determined by using the thiobarbituric acid (TBA) reagent. The reactivity of TBA is determined with minor modifications of the method adopted by Hiroshi *et al* (1979).

To 2.5 ml of homogenate, 0.5 ml of saline (0.9% sodium chloride), 1.0 ml of (20% w/v) trichloroacetic acid (TCA) were added. The contents were centrifuged for 20 minutes on a refrigerated centrifuge at 4000 x g. To 1.0 ml of supernatant, 0.25 ml of TBA reagent was added and the contents were incubated at 95˚c for 1 hr. One ml of n-butanol was added to it. After thorough mixing, the contents were centrifuged for 15 minutes at 4000 x g in a refrigerated centrifuge. The organic layer was transferred into a clear tube and its absorbance was measured at 532 nm. The rate of lipid peroxidation was expressed as µ moles of malondialdehyde formed/g wet wt. of tissue.

Statistical treatment of the data:

The mean and standard deviation (SD), analysis of variance (ANOVA) and test of significance or students 't' test was calculated using standard statistical software package.

Results and discussion:

Glutathione peroxidase activity:

From the data was showed that the exposure to As $^{3+}$lead to a significant decrease in mitochondrial GPx activity (0.013 ±0.0006) compared to the control (0.027±0.002). The increase in the activity of GPx was observed in animals treated with Ca $^{2+}$ +Zn^{2+} (0.021±0.006) and Vit-E (0.024±0.001). However, among Vit-E and Ca+Zn supplementation, Vit-E showed more inhibitory activity against As than Ca $^{2+}$+Zn $^{2+}$. (Fig.1.)

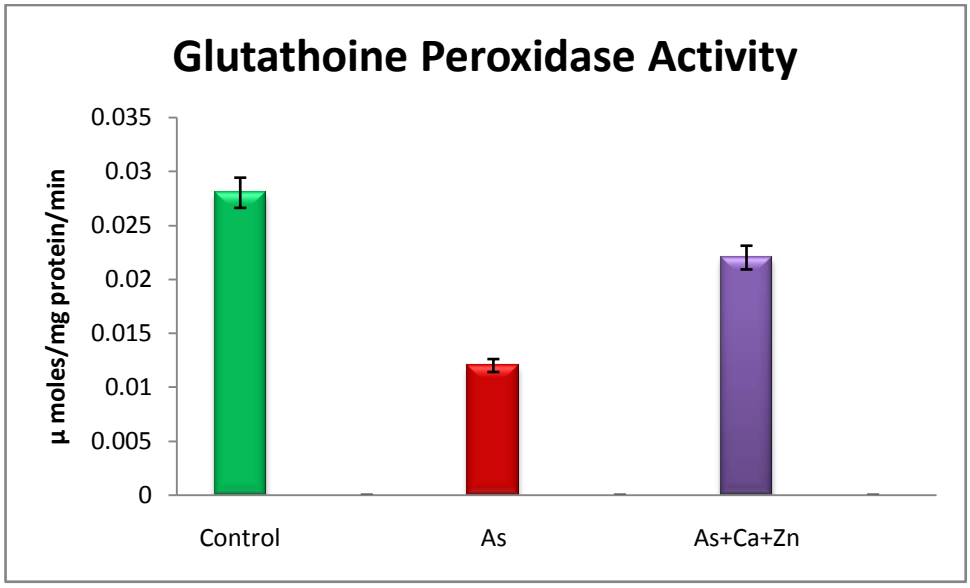
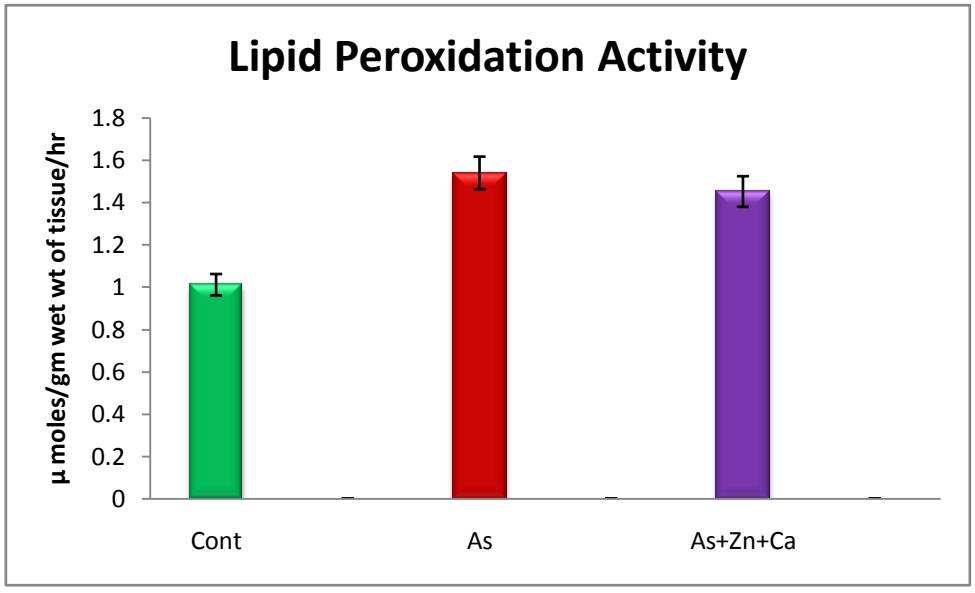


Fig: 1. Effect of As and As+Ca+Zn on Glutathione Peroxidase Activity in Rats

Lipid peroxidation:

From the data presented that the exposure to Arsenic led to a significant increase in mitochondrial Lipid peroxidation activity (1.540 ±0.031) compared to the control (1.01 ±0.0045). The decreased activity of Lipid peroxidation was observed in animals treated with Ca^{2+} +Zn $^{2+}$ (1.44 ±0.031) and Vit-E (1.402 ±0.022). However, among ViEandCa+Zn supplementation, Vit-E showed more inhibitory activity against As than Ca^{2+} + Zn^{2+} supplementation.

Fig.2. Effect of As and As+Ca+Zn on Lipid Peroxidation Activity in Rats



Catalase Activity:

From the data presented in table.5 showed that the exposure to Arsenic led to a significant decrease in mitochondrial Catalase activity (0.020 ±0.006) compared to the control (0.034 ±0.001).The increased activity of Catalase was observed in animals treated with Ca+Zn (0.022 ±0.001)and Vit-E (0.04 ±0.001). However among Vit-E and Ca^{2+} +Zn^{2+} showed more inhibitory activity against As than Ca^{2+} +Zn^{2+} supplement

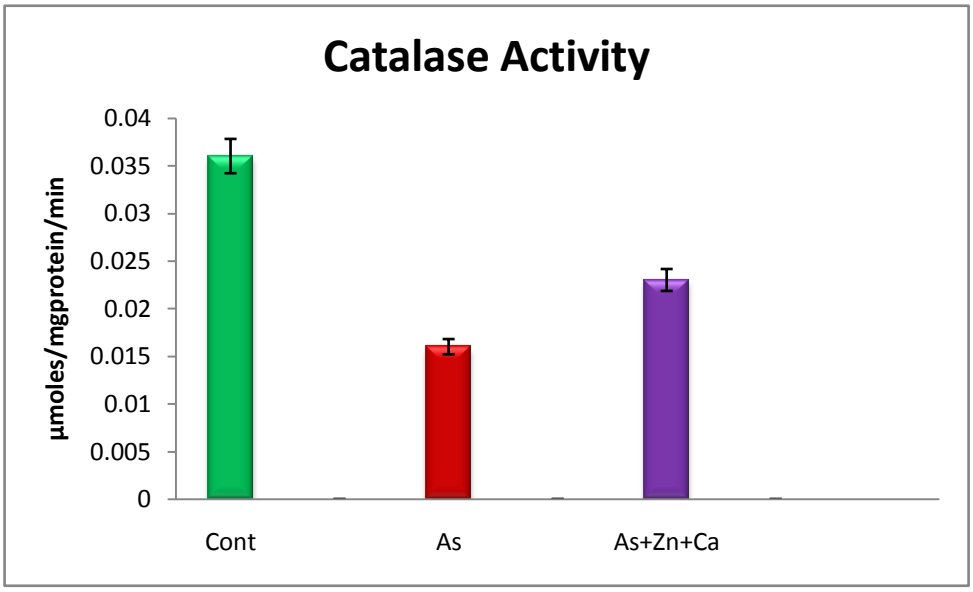


Fig: 3. Effect of As and As+Ca+Zn on Catalase Activity in Rats

Arsenic is a naturally occurring metal that is present in food, soil and water. It is released in the environment from both natural and man-made sources, Global natural emission of arsenic and arsenic compounds has been estimated to be 8000 tons per year whereas emission from man-made sources is about three times higher at 23,600 tons per year. Acute arsenic exposure produces toxicity of liver, kidney, intestine and brain (Liu ,*et al*.,2000). Chronic exposure to arsenic-contaminated water and food causes cancer of skin, liver, lung and bladder (Chiou *et al*. 2001). Toxicity of arsenic may be mediated by its methylated products (Hughes *et al*. 2000). Arsenic is also said to exert its toxicity through oxidative stress by generating reactive oxygen species. Free radicals have been detected in some cells treated with arsenite (Liu *et al*. 2001). Treatment with arsenite has been shown to induce significant increases of hydroxyl radical

formation in brain. It has further been demonstrated that reactive oxygen species are directly involved in oxidative damage to lipids, proteins and DNA in cells exposed to arsenic, which can ultimately leads to cell death.

GPX and SOD and CAT are the main antioxidative enzymes in mammals, and these enzymes could reduce hydrogen peroxide (H_2O_2) and organic hydroperoxides (Wang, 1994). Their activities are commonly used to assess body antioxidative status. Oxidative stress due to enhanced production of free radicals has been incriminated as one of the several mechanisms involved in arsenic-induced toxic effects in different organs.

Arsenic exposure in this experiment resulted in a significant reduction in the level of the GSH and associated with increases in lipid peroxidation in comparison to control group. Arsenic content causes extensive oxidation of intra-mitochondrial NADPH by inhibition of α-ketoacid dehydrogenase. The shortage of NADPH production during arsenic exposure would suppress the reduction of GSSG subsequently decrease the GSH content. The increase in the levels of MDA was due to the increased release of iron that was believed to be involved in the Fenton type of reaction. Arsenic is shown to stimulate the release of iron from ferritin and through the activation of heme oxygenease the rate-limiting enzyme in heme degradation. The free iron is considered as a potent enhancer of ROS formation, as exemplified by the reduction of H_2O_2 with the generation of highly aggressive hydroxyl radical. Therefore, this may be the possible reason for elevation of the levels of MDA with a concomitant fall in the GSH content. The inverse relationship between lipid peroxidation and GSH levels in the liver on arsenic exposure was suggested by (Sabina *et al.*,2005).

The increase in lipid peroxidation was accompanied by a concomitant decrease in the antioxidant enzymes such as CAT, Mn^{2+}-SOD, $Cu^{1+/}Zn$-$^{2+}$SOD and GPx. GPx, a Se-containing enzyme detoxifies H_2O_2 to H_2O through the oxidation of GSH. Depression of GPx activity was observed in the liver of As-exposed animals. Decrease in the activity of GPx during As treatment indicates the reduction in the levels of GSH and increase in the levels of peroxides. The decreased activity of GPx after As intoxication may arise because Se is a cofactor for GPx and As interacts with the essential selenocysteine moiety of the enzyme. involved in the direct elimination of ROS, SOD and catalase are considered primary enzymes since they are involved in the direct elimination of ROS. SOD is an important defence enzyme, which catalyzes the dismutation of superoxide radicals (O_2. - to H_2O_2). Catalase is a heme protein, which catalyzes

the reduction of hydrogen peroxides (converts H_2O_2 to oxygen and water) and protects tissues from highly reactive hydroxyl radicals. In the present study, catalase activity was reduced significantly in the liver and kidneys of As-exposed rats which could be due to the accumulation of superoxide anion radicals and hydrogen peroxide.

In the present study, the activities of superoxide dismutase and catalase in liver of rats were decreased. This may be due to an enhanced superoxide production during arsenic metabolism.Kidney mitochondrial fractions were prepared by following the method of Lai and Clark (1979). Then using mitochondrial fraction the oxidative stress markers (Catalase, Glutathoine Peroxidase, Lipid peroxidation and Superoxide dismutase) activities were observed. GPx and SOD and CAT are the main antioxidative enzymes in mammals, and these enzymes could reduce hydrogen peroxide (H_2O_2) and organic hydroperoxides. Their activities are commonly used to assess body antioxidative status. Oxidative stress due to enhanced production of free radicals has been incriminated as one of the several mechanisms involved in arsenic-induced toxic effects in different organs.

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