A COMPARATIVE STUDY ON PROTECTIVE ROLE OF CHEMICALS AND NATURAL SUPPLEMENTS AGAINST METAL MIXTURE INDUCED HEPATIC DAMAGE 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 The young rats (3 months) were exposed to lead acetate 20mg/kg body weight, Cd^{2+} 20mg/kg body weight and As^{3+}20 mg/kg body weight intraperitoneally daily for a period of 2 weeks. Animals were supplemented with Vit-E 20mg/kg body weight and Ca+Zn+Fe (2mg/kg body weight each) After the period of exposure, the animals were sacrificed and the tissues were collected rats in terms of the data showed that the exposure to heavy metals like $Cd^{2+}As^{3+}$ and Pb^{2+} led to a significant increase in mitochondrial succinate dehydrogenase activity compared to the treated. The increased activity of succinate dehydrogenase activity was observed in animals treated with chemical supplement like Ca^{2+}+Zn^{2+}+Fe^{3+} and Vitamin-E. A significant increase in mitochondrial Isocitrate dehydrogenase activity compared to the treated. The increased activity of Isocitrate dehydrogenase activity was observed in animals treated with chemical supplement like Ca^{2+}+Zn^{2+}+Fe^{3+}. A significant decrease in Cytosolic Glucose-6- Phosphate dehydrogenase activity compared to the treated. The increased activity of Glucose-6-Phosphate dehydrogenase activity was observed in animals treated with chemical supplement like Ca^{2+}+Zn^{2+}+Fe^{3+} and Vitamin-E. A significant decrease in mitochondrial Glutamate dehydrogenase activity compared to the treated. The increased activity of Glutamate dehydrogenase activity was observed in animals treated with chemical supplement like Ca^{2+}+Zn^{2+}+Fe^{3+} and Vitamin-E.

Key words: Ca^{2+}+Zn^{2+}+Fe^{3+} and vitamin E, Lipid peroxidation, Glutathione and peroxidase, Catalase

INTRODUCTION:

Cadmium (Cd^{2+}) is a wide-spread environmental pollutant, characterized by its toxicity to various organs, including kidney, liver, lung, testis, brain, bone, blood system (Gunnarsson *et al.,* 2003). Cd has been demonstrated to stimulate free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals (Shen Y, Sangiah S *et al.,* 1995). In the last two decades, however, occupational exposures have dropped, following the dramatic reduction of exposure limits in most industrialized nations. Most important effects were renal injuries (including tubular and glomerular dysfunctions), immune deficiencies, apathies, bone injuries (osteomalacia and osteoporosis), femoral pain, lumbago and skeleton deformations. Cadmium contaminations were due to the effluents from zinc mine located in the upper reaches of a river and profoundly affected the health of the human population living in that area, At very low (above 1 μM) concentrations, it is hypermutagenic in yeast by inhibiting mutation avoidance rather than by direct DNA damage. It affects genome stability by inducing reactive oxygen species (ROS) in cells but also by inhibiting several DNA repair systems and depleting the activity of cellular antioxidants like glutathione) (Shen Y, Sangiah S *et al.,* 1995).

Cadmium has multiple effects on cells, Cadmium affects cell cycle progression, proliferation, differentiation, DNA replication and repair, as well as apoptotic pathways. It regulates cell cycle progression by activation of some cellular signals, inhibition of DNA methylation and/or interference with E-cadherin mediated cell adhesion. The effects on DNA synthesis and cell proliferation are dose dependent. Cadmium exposure inhibits DNA synthesis and cell division at concentrations above 1 μM (Misra UK *et al.,* 2003).

Approximately 57million people are drinking groundwater with arsenic concentrations above 10 ppb. Arsenic has caused poisonings in Bangladesh, Bengal, Thailand, Finland, Hungary, Chile, Taiwan, Vietnam, Cambodia, Mexico, Argentina, and China, where geological environments are conducive to generate high amounts of arsenic compounds in groundwater (Smith *et al.,* 2006). Furthermore, many states within the United States also have significant concentrations (up to 50 ppm) of arsenic in the groundwater. Chronic exposure to arsenic can cause skin, lung and bladder cancers (Cohen *et al.,* 2000; Smith *et al.,* 2006). A small but measurable increase in the incidence of bladder cancer was associated with exposure to concentration as low as 10ppm of inorganic arsenic (Chu and Crawford-Brown, 2006). Arsenic

exerts its toxicity in part by generation of ROS (Kitchin and Ahmad, 2003; Liu *et al.,* 1992, 2001a,b, 2003 and 2006; Das *et al.,* 2005). Consistent with the role of ROS in arsenic toxicity/carcinogenicity, endogenous sulfhydryl groups and the non-protein sulfhydryl glutathione (GSH) detoxification of arsenic (Duyndam *et al.,* 2001). The exogenous antioxidant N-acetylcysteine is also able to prevent arsenic-induced toxicity (Liu *et al.,* 2003).

Therefore, inorganic, methylated, and dimethylated arsenicals are found in urine of humans exposed to iAs. Arsenic (+3 oxidation state) methyl transferase (As3mt), the prototype As methyltransferase in mammals, was initially identified in Rattus norvegicus (Thomas *et al.,* 2007). It has been long known that arsenic exposure is associated with skin pathology, including hyperpigmentation, hyperkeratosis, and skin cancers. In the majority of cases in which an internal cancer has been ascribed to arsenic exposure, a dermatologic hallmark of arsenic poisoning was also identified (Tsai S-M *et al.,(*1999).

Abnormal liver function, manifested by gastrointestinal symptoms such as abdominal pain, indigestion, loss of appetite and by clinical elevations of serum enzymes, frequently occurs from exposure to arsenic in the drinking water (Mazumder, 2005), or from environmental exposure to arsenic through burning high-arsenic coal in interior stoves (Zhang *et al.,* 2000). Chronic arsenic exposure in animals can also produce liver endothelial cell damage, which subsequently damages parenchymal cells (Straub *et al*., 2007). Arsenic is well absorbed from the gastrointestinal tract, and first reaches the liver. Arsenate is reduced to arsenite in the liver (Gregus and Nemeti, 2002). Because the liver is rich in glutathione, it is a major site of arsenic detoxication, either from glutathione acting as an antioxidant, or by glutathione arsenic conjugation for cellular efflux and biliary excretion (Liu *et al.,* 2001a; NRC, 1999). The liver is also the major site of arsenic methylation, which is catalyzed by arsenic methyl transferase or AS3MT using S-adenosyl methionine (SAM) as the substrate (Thomas, 2007).

Lead (Pb^{2+}) is a ubiquitous, naturally occurring environmental toxicant metal. Pb^{2+}-induced toxic effects can manifest in several organs but the brain and kidney are clearly primary targets (Coon *et al.,* 2006; IARC, 2006; White *et al*., 2007; Wu *et al.,* 2008). Compelling evidence is emerging that Pb exposure, particularly early life exposure, may cause neurodegeneration later in life (White *et al.,* 2007; Wu *et al.,* 2008). For example, life time whole-body occupational Pb^{2+} exposure has been recently shown to be a risk factor for Parkinson's disease (Coon *et al.,* 2006), a neurodegenerative disorder characterized by regional

aggresomal protein inclusions. Similarly, an Alzheimer's disease-like pathology has recently been induced in adult monkeys after early life Pb^{2+} exposure (Wu *et al.,* 2008). An important evolving concept in Pb^{2+} neurotoxicity is that environmental factors can play a role in increasing susceptibility (White *et al.,* 2007).

A remarkable characteristic of Pb^{2+} intoxication is the production of protein-Pb^{2+}complexes which appear in target cells of poisoned humans or animals as inclusion bodies (IBs). After toxic levels of Pb^{2+} exposure, IBs will first form in the cytoplasm, and then migrate to the nucleus (Nolan and Shaikh, 1992). These IBs are common in the kidney but also can be found in cells of the nervous system and other target sites of Pb^{2+} (Goyer and Rhyne, 1973). The origin and nature of the protein component of IBs remains poorly defined, but IBs are clearly protective against acute and chronic Pb^{2+} toxicity (Qu *et al.,* 2002; Waalkes et al., 2004b). IBs bind large amounts of Pb and likely render it toxicologically inert, thus blocking interactions with more critical cellular targets (Fowler, 1998).

Acute exposure to lead is known to cause proximal renal tubular Damage (WHO, 1995). Long-term lead exposure may also give rise to kidney damage and, in a recent study of Egyptian policemen, urinary excretion of NAG was positively correlated with duration of exposure to lead from automobile exhaust, blood lead and nail lead (Mortada WI *et al.,* 2001).

MATERIALS AND METHOD:

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

Lead acetate, Cadmium chloride and sodium arsenite were selected as test chemicals. The chemicals used in this study namely NADPH, INT, ADP, NAD, Sodium arsenite were obtained from Sigma, USA. The remaining chemicals obtained from Qualigens, India.

Animal exposure to Pb, Cd and As:

The young rats (3 months) were exposed to lead acetate 20mg/kg body weight,(Ahmed E et al., 2011) Cd^{2+} 20mg/kg body weight (Subhadip K et al., 2009) and As^{3+}20 mg/kg body weight (Kapil Bhatt and S.J.S Flora, 2009) intraperitoneally daily for a period of 2 weeks. Animals were supplemented with Vit-E 20mg/kg body weight (Mostafa MH et al., 2010) and Ca+Zn+Fe (2mg/kg body weight each) Control rats were not given any test solutions. After the period of exposure, the animals were sacrificed and the tissues were stored at -80^0C for further biochemical analysis.

BIOCHEMICAL STUDIES

Preparation of Liver Mitochondrial Fraction:

Liver mitochondrial fractions were prepared by homogenizing 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 centrifuge at 3 *g* for 10 min at 4°C, and then the supernatant was centrifuged at 15*g* for 40 min. Then the pellet of mitochondrial fraction was suspended in SET buffer. The supernatant was considered as cytosolic fraction.

Estimation of Succinate Dehydrogenase (SDH**)**

SDH activity was estimated by the method of Nachlas *et al.,* (1906) as modified by Prameelamma, (1976). The total reaction mixture contained 100 µmoles of phosphate buffer (pH 7.2), 50 µmoles of sodium succinate and 4.0 µmoles of INT in a final volume of 2.5 ml. the reaction was initiated by addition of mitochondrial fraction. After incubation for 30 min at 37^0 C, the reaction was stopped by addition of 5 ml of glacial acetic acid. The formazan formed was extracted overnight into 5.0 ml of toluene and the colour was measured at 495 nm in spectrophotometer against toluene blank. The enzyme activity was expressed as µmoles of formazan formed/mg of protein/ hr.

Estimation of Glucose-6-Phosphate Dehydrogenase (G-6-PDH**)**

G-6-PDH activity was estimated by the method of Bergmeyer and Bruns, (1965). The assay mixture final volume contain 100 µmoles of phosphate buffer (pH 7.2), 50 µmoles of glucose, 0.1 µmoles of NADPH and 4.0 µmoles of INT. the reaction was initiated by addition of mitochondrial fraction. After incubation for 30 min at 37^0 C, the reaction was stopped by addition of 5 ml of glacial acetic acid. The formazan formed was extracted overnight into 5.0 ml

of toluene and the colour was measured at 495 nm in spectrophotometer against toluene blank. The enzyme activity was expressed as µmoles of formazan formed/mg of protein/ hr.

Estimation of Isocitrate Dehydrogenase Activity: (ICDH**)**

ICDH activity was estimated by the method of Keous DA and Mcalister-henn L (1990). The assay mixture final volume contain 100 µmoles of phosphate buffer (pH 7.2), 20 µ moles of Isocitrate,0.2 µmoles of NAD,10µ moles $Mgcl_2$,0.2µ moles of ADP and 4.0 µmoles of INT. The reaction was initiated by addition of mitochondrial fraction. After incubation for 30 min at 37^0 C, the reaction was stopped by addition of 5 ml of glacial acetic acid. The formazan formed was extracted overnight into 5.0 ml of toluene and the colour was measured at 545 nm in spectrophotometer against toluene blank. The enzyme activity was expressed as µmoles of formazan formed/mg of protein/ hr.

Estimation of Glutamate Dehydrogenase Activity: (GDH)

GDH activity was estimated by the method of Lee and Lardy, (1965). The assay mixture final volume contained 100 µmoles of phosphate buffer (pH 7.2), 40 µ moles of Sodium Glutamate, 0.2 µmoles of NAD and 4.0 µmoles of INT. The reaction was initiated by addition of mitochondrial fraction. After incubation for 30 min at 37^0 C, the reaction was stopped by addition of 5 ml of glacial acetic acid. The formazan formed was extracted overnight into 5.0 ml of toluene and the colour was measured at 545 nm in spectrophotometer against toluene blank. The enzyme activity was expressed as µmoles of formazan formed/mg of protein/ hr.

Estimation of protein content:

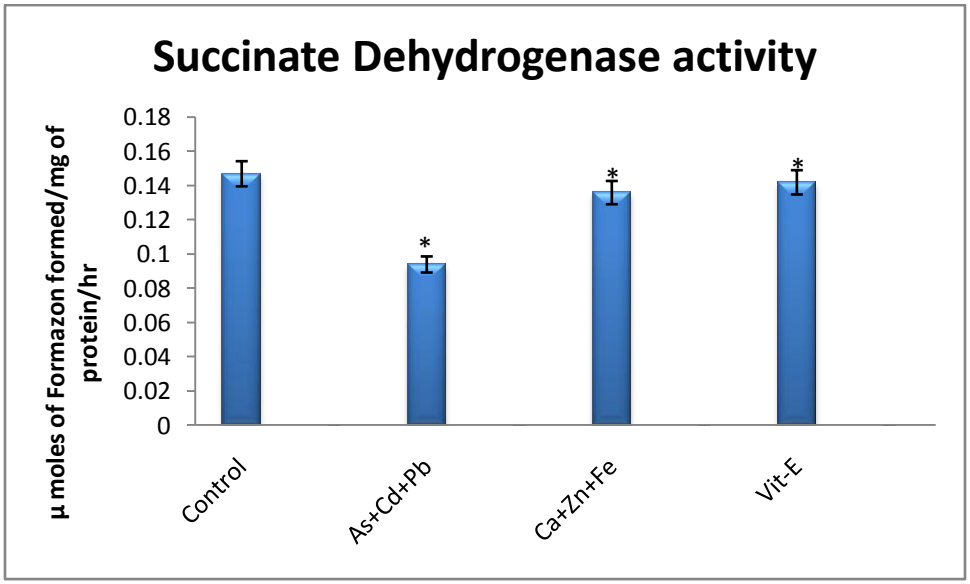
Protein content of the tissues was estimated by the method of Lowry et al. (1951). 1% (W/V) homogenates of the tissues was prepared in 0.25 M ice cold sucrose solution. To 0.5ml of crude homogenate, 1ml of 10% TCA was added and the samples will be centrifuged at 1000 g for 15min. supernatant was discarded and then the residue was dissolved in 0.5ml of 1N NaOH. To this 4ml of alkaline copper reagent was added followed by 0.4ml of folin-phenol reagent (1:1 folin:H_2O). The color was measured at 600 nm in a UV- vis spectrophotometer (Hitachi model U-2000) against blank. The protein standard graph was prepared using Bovine serum albumin. The protein content of the tissues was calculated using the standard graph.

RESULTS AND DISCUSSION:

Succinate Dehydrogenase Activity (SDH)

In the present investigation the data showed that the exposure to heavy metals like Cd^{2+} As^{3+} and Pb^{2+} led to a significant increase in mitochondrial succinate dehydrogenase activity (0.146±0.035) compared to the treated (0.095± 0.053). The increased activity of succinate dehydrogenase activity was observed in animals treated with chemical supplement like Ca^{2+}+Zn^{2+}+Fe^{3+} (0.141±0.058) and Vitamin-E (0.135±0.033). However, among Ca^{2+}+Zn^{2+}+Fe^{3+} and Vit-E showed more inhibitory activity against Heavy metals.

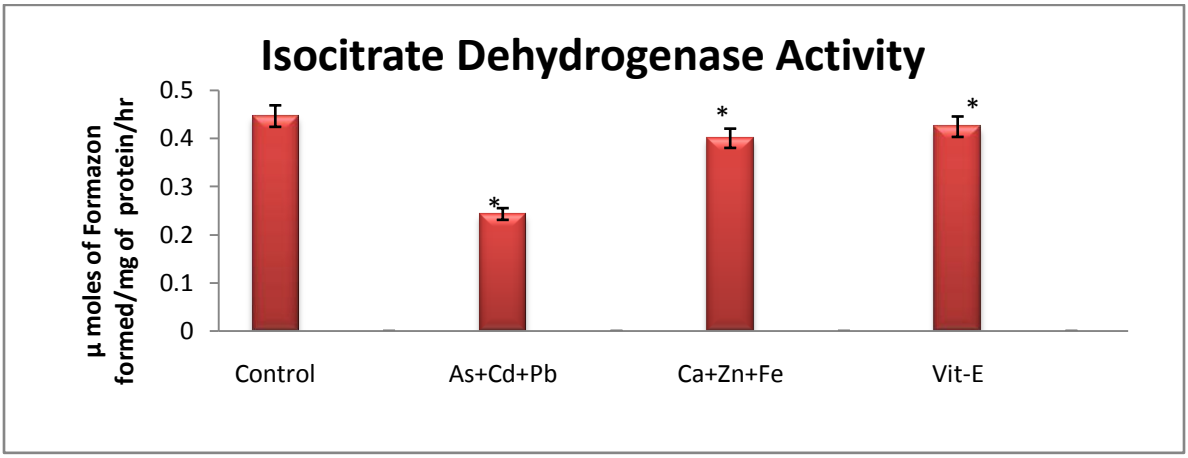
Fig.1. Effect of heavy metals on Succinate Dehydrogenase activity in albino rats.



Isocitrate Dehydrogenase Activity (ICDH)

In the present investigation the data showed that the exposure to heavy metals like Cd^{2+},As^{3+} and Pb^{2+} led to a significant increase in mitochondrial Isocitrate dehydrogenase activity (0.447±0.095) compared to the treated (0.242± 0.082). The increased activity of Isocitrate dehydrogenase activity was observed in animals treated with chemical supplement like Ca^{2+}+Zn^{2+}+Fe^{3+} (0.40±0.052) and Vitamin-E (0.425±0.066). However,among Ca^{2+}+Zn^{2+}+Fe^{3+} and Vit-E showed more inhibitory activity against Heavy metals.

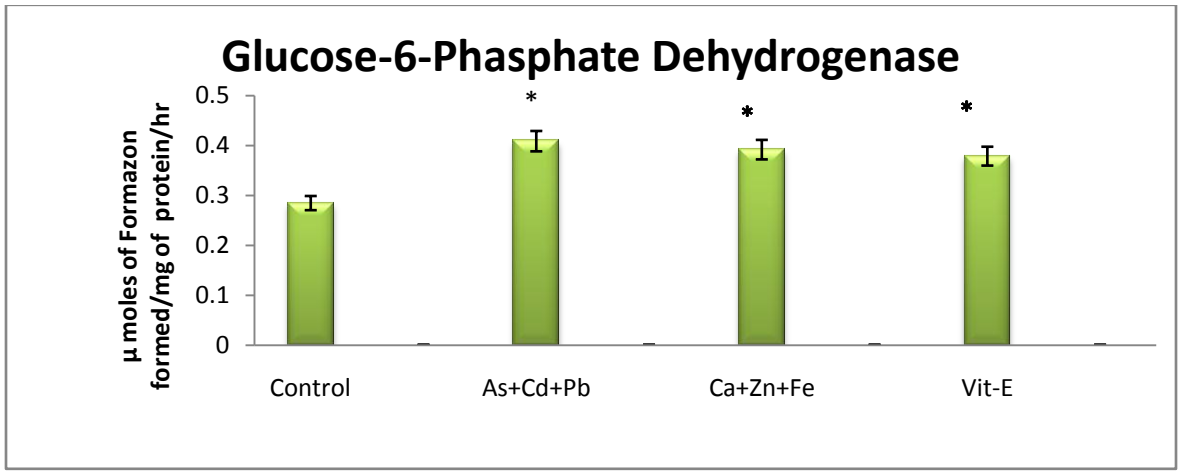
Fig.2. effects on Isocitrate dehydrogenase activity of rats treated with different chemical supplements



Glucose-6-phosphate Dehydrogenase Activity (G-6-PDH)

In the present study the data showed that the exposure to heavy metals like Cd^{2+},As^{3+} and Pb^{2+} led to a significant decrease in Cytosolic Glucose-6- Phosphate dehydrogenase activity (0.283±0.066) compared to the treated (0.407± 0.040). The increased activity of Glucose-6-Phosphate dehydrogenase activity was observed in animals treated with chemical supplement like Ca^{2+}+Zn^{2+}+Fe^{3+} (0.392±0.060) and Vitamin-E (0.377±0.023). However,among Ca^{2+}+Zn^{2+}+Fe^{3+} and Vit-E showed more inhibitory activity against Heavy metals.

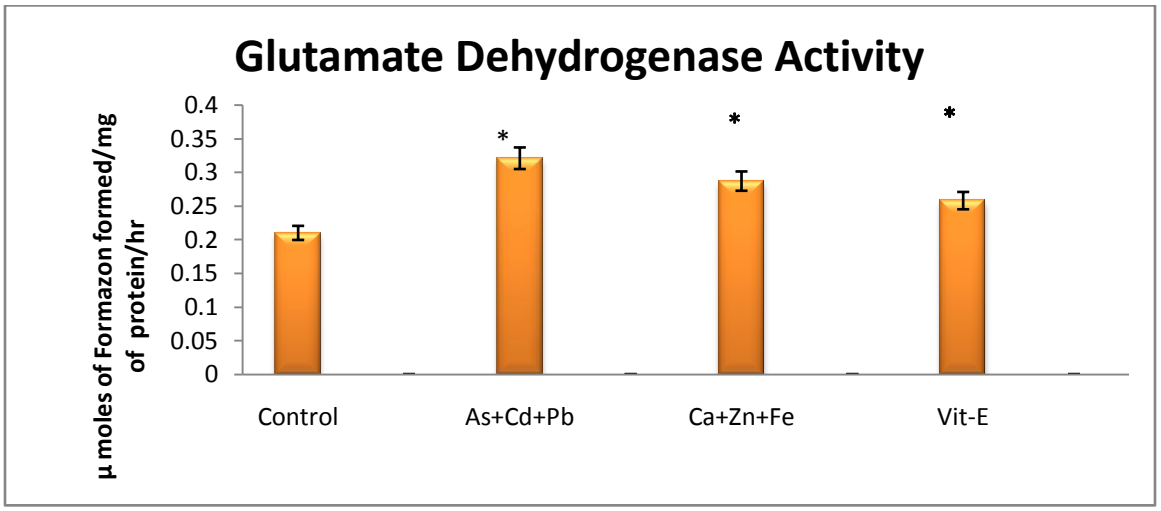
Fig.3. Effect of heavy metals on Glucose-6-Phasphate Dehydrogenase activity in albino rats.



Glutamate Dehydrogenase Activity (GDH)

From the data of present study shows that the heavy metals like Cd^{2+},As^{3+} and Pb^{2+} led to a significant decrease in mitochondrial Glutamate dehydrogenase activity (0.21±0.075) compared to the treated(0.321± 0.059). The increased activity of Glutamate dehydrogenase activity was observed in animals treated with chemical supplement like Ca^{2+}+Zn^{2+}+Fe^{3+} (0.287±0.049) and Vitamin-E (0.258±0.051). However,among Ca^{2+}+Zn^{2+}+Fe^{3+} and Vit-E showed more inhibitory activity against Heavy metals.

Fig:4. Effect of heavy metals on Glutamate Dehydrogenase activity in albino rats.



The liver is a vital organ present in vertebrates and some other animals. It has a wide range of functions, including detoxification, protein synthesis, and production of biochemicals necessary for digestion. The liver is necessary for survival; there is currently no way to compensate for the absence of liver function long term, although liver dialysis can be used short term. This organ plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification.

Heavy metals can induce several cellular dysfunctions including cell death, decreased DNA repair and increased mutagenesis. DNA lesions arising after heavy metal exposure are not due to direct effects. They appear to be mainly mediated by an indirect production of ROS, which at least in part, is due to the inhibition of cellular antioxidants and constitutes oxidative stress. In addition, heavy metal affects several DNA repair systems: MMR, NER and BER.

Several groups have demonstrated that proteins involved in repair are inhibited or have diminished activity after heavy metal intoxication. Heavy metals can interfere with proteins that contain a zinc finger motif, which are implicated in the maintenance of genome stability or in DNA repair and DNA damage signaling (Hartwig *et al.,* 2002). Some heavy metals, like cadmium, lead and arsenic have high affinity for thiol groups present in cysteine residues.

Heavy metals can displace and replace zinc in these motifs and disrupt target proteins as suggested by the fact that addition of zinc, during or after intoxication by heavy metals, can reverse the effects of heavy metal exposure. Another proposed mechanism heavy metals intoxication is a heavy metal-mediated production of ROS, leading to oxidation of cysteine residues of zinc finger domains (A. Witkiewicz-Kucharczyk, W. Bal 2006). Moreover, many effects of heavy metals on mitochondria are associated with the generation of ROS. Apart from mitochondria as principal source of ROS production in cells, there are other extra mitochondrial ROS sources such as cytochrome P450-mediated reactions in several tissues (liver, heart, gastrointestinal tract, kidney and lung).

The present study is designed to study the effect of SDH, ICDH, G-6-PDH and GDH activities in liver tissue to understand the enzyme levels and their role in male albino rats. The results showed a significant decrease in ICDH and SDH levels but in contrast, same treatment caused a significant increase in GDH and G-6-PDH levels.

Most of the dehydrogenases investigated in the present are altered significantly in the rats treated with metal mixture. These findings led us to suggest that the alterations in the activity of liver dehydrogenases might be due to deleterious actions exerted by heavy metals on the cytoplasmic organelles especially the mitochondria. The inhibition if succinate dehydrogenase, isocitrate dehydrogenase which are mitochondrial enzymes and involved in the citric acid cycle might support this suggestion and indicate a reduction in the aerobic metabolic processes especially the electron transport and oxidative phosphorylation of the liver cells due to heavy metal intoxification. In addition, these histochemical alterations let us to conclude that the changes in the activity of dehydrogenases due to heavy metals intoxification might represent responses to the need for an adaptation to the catabolism of the degrading damaged liver cells structures or to partial impairments of their functions.

The results of the present investigation have shown an increase in the activities of glucose-6-phosphate. Glucose-6-phosphate dehydrogenase catalyzes the first step of oxidation in

the hexose monophosphate shunt pathway by which glucose may enter the pentose monophosphate shunt and producing NADPH which is required as hydrogen donor for reactions of various biochemical pathways. This enzyme plays an important role in the regulation of sugar metabolism and determines whether glucose shall undergo glycolysis or be utilized via the pentose phosphate pathway. The increase in the activity of glucose-6-phosphate dehydrogenase due to heavy metal intoxification might indicate an increased demand to generate reducing power in the form of NADPH under the oxidative stress fail induced by lead.

Heavy metal intoxication induces cell death. However, the mechanisms are not yet clear, notably the induction of death receptors, of mitochondrial effectors and of caspase dependent and -independent apoptotic pathways.

The severity of heavy-metal indued cytotoxicity is greatly affected by the cellular sulf-hydryl levels (chan and cherian, 1992). Inactivation of protein thiols by heavy metals may lead to toxicity by disruption cellular redox state.

In conclusion, it appears that heavy metal acts mainly by inducing oxidative cellular stress and by modulating intracellular redox homeostasis. It thus affects several cellular compartments by inducing mitochondrial dysfunction and ROS, oxidatively generated damage to DNA, membranes and proteins and a decrease in cellular antioxidants. Predominant effects of heavy metals are those affecting structure and function of important proteins. Intracellular signaling as well as apoptotic pathways are clearly impaired after heavy metal exposure. Concerning heavy metal induced mutagenesis (and probably carcinogenesis), it appears that heavy metals like Arsenic, Cadmium and Lead acts as a double-edged sword inducing DNA damage and inhibiting its repair. Heavy metals are likely to interfere not only with the repair of oxidatively generated damage but also with the repair of even more genotoxic lesions such as DNA double-strand breaks and DNA inter strand-crosslinks.

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