DETRMINATION OF HEAVY METALS ALTERATIONS ON STRUCTURE AND FUNCTION OF IMPORTANT PROTEINS AND HEAVY METAL INDUCED MUTAGENESIS IN ALBINO RATS

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Abstract:

Heavy metal toxicity is a serious worldwide problem, which adversely affects the growth, health, reproductive performance, and life span of all living organisms. Metals play an important role in human biology, and trace amounts of some metals such as manganese, for example are essential to life. At higher concentrations, however, the same metals are toxic. In addition, some metals, lead, for example do not occur naturally in the body, and its presence, usually as a result of occupational or pollution-related exposure and are detrimental to health. In the present investigation is aimed to study the metal mixture induced toxicity on protein expression in rats. The exposed liver of treated rats showed a significant decrease in protein expression compared to control rats. An increase in the band intensity was observed when the animals were supplemented with Ca^{2+}+Fe^{3+}+Zn^{2+} and Vit-E.

Keywords: Heavy metals, Rats, SDS PAGE, Ca^{2+}+Fe^{3+}+Zn^{2+} and Vit-E.

Introduction:

Cadmium is a non-essential trace element, which is toxic to many plants and animals. Cadmium is an industrially used substance with negative long time effects on human health. Acute toxicity may result from the ingestion of cadmium through contaminated foods and beverages. Mammalian organisms are exposed to cadmium by its technological utilization and the epidemiological studies shows that cadmium is one of the most toxic of the heavy metals to humans. Exposure to sublethal concentration of cadmium to the fishes leads to haemolysis. Cadmium produces toxic lesions in various tissues. It causes morphological and functional changes in the liver. Cadmium ingestion causes testicular abnormalities in early sperm development in mice. Cadmium inhibits the activity of enzymes by binding to their sulfhydryl groups (SH), consequently causing the peroxidative destruction of cell membranes. Cadmium is also known to alter several physiological activities such as alteration in carbohydrate metabolism in rat, mice and rabbit. Cadmium is known to be embryotoxic in animal models and to cause brain, limb and craniofacial malformations. Among numerous mechanisms proposed for cadmium toxicity are oxidative stress and lipid peroxidation.

Lead is known to be toxic when present in traces and enters human body as a result of environmental pollution (Kanwar KC *et al.,* 1987). Occupational hazards due to lead exposure produce reversible changes in mood and personality as fatigue, irritability, depression, deficits in vascular motor functioning, memory, and verbal ability (Foulkes EC *et al.,* 1990). Children exposed to lead are reported to have adverse effects on central nervous system and kidneys (Sangha JK *et al.,* 2001). Maternal blood lead level as an environmental factor is an apparent predictor of low birth weight and child body mass ratio (Odland JO *et al.,*1999) and low to moderate environmental exposure increases the risk for spontaneous abortion (Borja-Abuto VH *et al.,*1999). Anemia, which is frequently observed in lead poisoning, was a result of decreasing lifetime of erythrocytes and synthesis of heme (Terayama K *et al.,* 1988). In Ludhiana (Punjab, India), the analysis of water samples of Budha Nallah after the input of effluents by dying industries and pesticide manufacturing units indicates that the concentration of lead has increased manifold (Mittal SK *et al.,*1992) and the mean daily intake of lead was 162.32±19.1 μg/day (Sangha JK *et al.,*2001).

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, 2003). 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 (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).

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).

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

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.

SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS:

Polyacrylamide gel electrophoresis (PAGE) was followed for separation of protein fractions. SDS-PAGE system was employed for protein studies.

1. Composition of different solutions used in the preparation of slab gels are furnished below

A. Acrylamide solutions

Acrylamide	:	30.0 g
N, N' methylene bisacrylamide	:	0.8 g

Dissolved in 80 ml of distilled water and volume made upto 100 ml. The solution was filtered and stored at 4°C in an amber coloured bottle.

B. Resolving gel buffer: (1.5 M Tris - HCl, pH 8.8)

Tris : 18.15 g

Dissolved in 80 ml of distilled water and pH was adjusted to 8.8.

C. Stacking gel buffer (0.5 M Tris - HCl, pH 6.8)

Tris : 6 g

Dissolved in 80 ml of distilled water and pH was adjusted to 6.8.

D. 10% sodium dodecyl sulphate

SDS : 1 g

Distilled water : 10 ml

E. Polymerizing agent

Ammonium per sulphate (10%) : Catalyst

(0.1 g/ml distilled water freshly prepared)

N,N,N',N', tetramethyl ethylenediamine (TEMED) - chain initiator.

F. Electrode buffer

i. For proteins

Tris : 1.8 g

Glycine : 8.64 g

SDS : 0.6 g

Distilled water to make 600 ml

2. Gel composition

Stock solution	**12% resolving gel (30ml)**	**4% stacking gel (5 ml)**
30% acrylamide	8.0 ml	2.7 ml
Resolving gel buffer	6.0 ml	-
Stacking gel buffer	-	2.0 ml
10% SDS*	0.3 ml	0.05 ml
Distilled water	16 ml	1.5 ml
10% Ammonium per sulphate**	0.2 ml	0.2 ml
TEMED	0.05	0.05

* For proteins only

** Freshly prepared before use

3. Preparation of gels

Gel plates were washed thoroughly with cleaning solution followed by distilled water and dried. Appropriate spacers were placed between the glass plates on sides. Then these glass plates were sealed using a special adhesive tape in order to prevent leakage of gel solution. Resolving gel solution was prepared by mixing the stock solution in quantities given in the above table. The solution was poured into the sandwich to a level of 2 cm from the top. Distilled water was added gently along the wall of the sandwich to form uniform gel surface and allowed for polymerization. After polymerization, the water on the resolving gel was poured off and wiped off with filter paper. Stacking gel was prepared and overlayed on the resolving gel. The comb was inserted into the stacking gel and allowed to polymerize.

4. Preparation of samples

Proteins samples of control and 0.2 % Pb treated of both 21 and 28 days were prepared by using sample buffer.

A. Sample buffer for proteins (8.2 ml)

0.1 M Tris HCl	:	1 ml
Glycerol	:	0.8 ml
10% SDS (w/v)	:	1.6 ml
Mercaptoethanol	:	0.4 ml
Bromophenol blue (w/v)	:	0.2 ml
Distilled water	:	4 ml

Fifty microlitres of protein extraction was mixed with an equal volume of sample buffer in an eppendorf tube, boiled for 5 minutes in a water bath and cooled rapidly by keeping on ice.

5. Loading of samples

The inserted comb was gently removed from the gel after polymerization. The air bubbles, if any, were removed by rinsing with distilled water. The lower and upper chambers of electrophoretic apparatus were filled with electrode buffer. About 70 µl of sample was loaded in each well.

6. Running of gel

The electrophoretic unit was connected to power pack and a regulated electric power supply of 60V, slowly raised to 100V was supplied for the separation of proteins. The electrophoresis was run till the dye front reached the bottom of the gel, which took 4 h. Then the power supply was switched off. The gel was carefully dismantled after electrophoresis and incubated in respective staining solution.

7. Staining of the gels

A. Staining solution for proteins

Coomassie brilliant blue R-250	:	0.1 g
Methanol	:	4.0 ml
Acetic acid	:	10 ml
Distilled water	:	50 ml

B. Destaining solution for proteins

Methanol	:	40 ml
Acetic acid	:	10 ml
Distilled water	:	40 ml

The gel was incubated in staining solution for 12 h and then gel was taken out and properly destained by keeping it in destaining solution. The destaining solution was changed every four hours and destaining was hastened by gently rotating the trays.

Results and Discussion:

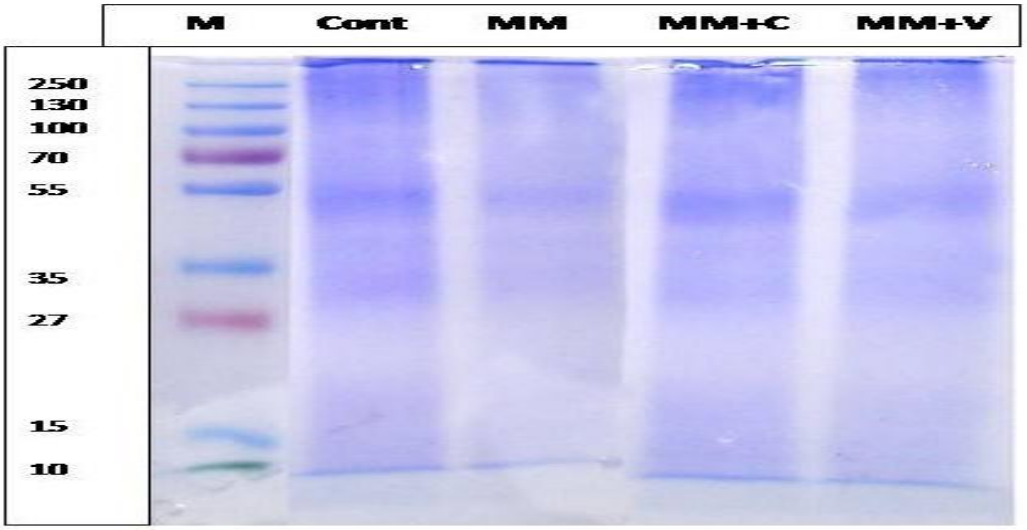

Fig: 1: Expression Profile of Proteins by SDS-PAGE in rats

The metal mixture exposed liver of treated rats showed a significant decrease in protein expression compared to control rats. An increase in the band intensity was observed when the animals were supplemented with Ca^{2+}+Fe^{3+}+Zn^{2+} and Vit-E. However, among them both, the intensity was more pronounced in chemical supplemented than vitamin-E supplemented rats.

A band of higher intensity at 55 kDa was observed in control. And a same band of lower intensity was observed in the group treated with metal mixture. The 55kDa band expressed with high intensity in the groups supplemented with Ca^{2+}+Fe^{3+}+Zn^{2+} and Vit-E. However, no significant change was observed in the intensity or thickness of the bands at other regions like 250 kDa, 130 kDa, 100 kDa (Fig. 1.)

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. 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 is 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.

Proteins are important structure builders of food system and would be expected that any alterations in the conformational state would change their functional properties. Treatement with metal mixture resulted in altered structure and associated changes in functional properties that have direct consequences on conformation which finally resulte in the variations in the expression profiles of SDS-PAGE.

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