

# Hepatotropic effects of acute heavy metal poisoning in rats

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## Abstract

**Introduction:** Increasing technologic use of metals has posed hazards to health of humans as well as animals. Excessive concentration of metals occurs in water, air and soil as results of natural deposits as well as technologic use of non biodegradable materials. Further metals released into the environment may be biocentrated and thus enter the food chain. In Industrial Toxicology, Occupational exposure to Lead, Cadmium fume, Chromic acid and Chromates is significant. **Aims and Objectives:** To study the Hepototropic effects of acute heavy metal poisoning in rats **Materials and Methods:** In the present study 24 rats were divided in four groups (Cadmium group, Chromium, Lead and Control group) containing 6 rats each. The rats were exposed to respective heavy metal salt by two daily injections. The acute doses have been chosen based on previous studies in literature. On the third day the entire animals were weighed and sodium pentobarbitone 30 mg/kg body weight was administrated intraperitoneally. The time off administration of drug, the time of loss of righting reflex and the recovery of the animals were noted. 1ml blood sample was withdrawn by retro-orbital puncture from all the animals for the blood assay. **Results:** Onset of sleeping time and duration of sleeping was increased in all the group but the difference was not statistically significant. The difference in SGOT levels of control and lead group was statistically insignificant. The SGPT levels were increased with statistically significant difference in cadmium group as compared to control group. The rise in alkaline phosphatase was statistically significant in cadmium and lead group when compared with control group. Serum proteins were also increased with statistical significance in cadmium and lead group. **Conclusion:** It may be stated that acute heavy metal exposure is associated with some changes in enzymic activity of liver and significant decrease in serum proteins.

**Keywords:** Hepototropic, acute heavy metal poisoning.

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## INTRODUCTION

Many different definitions for heavy metals have been proposed, some based on density, some on atomic number or atomic weight, and some on chemical properties or toxicity. The group of heavy metals can include elements lighter than carbon and can exclude some of the heaviest metals. One of such definitions entails that heavy metals are those inorganic elements

which have five times the specific gravity of water. Among the heavy metals having serious health implications are arsenic, lead, cadmium, and mercury<sup>1</sup>. Increasing technologic use of metals has posed hazards to health of humans as well as animals. Excessive concentration of metals occurs in water, air and soil as results of natural deposits as well as technologic use of non biodegradable materials. Further metals released into the environment may be biocentrated and thus enter the food chain. In Industrial Toxicology, Occupational exposure to Lead, Cadmium fume, Chromic acid and Chromates is significant. In modern time Lead (Pb) is ubiquitous in the environment as a result of its natural occurrence and industrial use<sup>2</sup>. To quote a few sources of environmental lead it is discerned in drinking water supplied through lead pipes – soft water has been reported to dissolve lead much more rapidly than hard than hard water<sup>3</sup> in automobile exhaust tetraethyl lead is used as an antiknock compound and canned food. Overall, human exposure to Pb is primarily from food<sup>2</sup>. Cadmium (Cd)

occurs in nature in association with Zinc and Lead and extraction and processing of these metals lead to environmental contamination with Cadmium and their combustion releases cadmium into the environment. Cadmium poisoning in human population is due to exposure from contamination of food. Drinking water does not contribute significantly to Cadmium intake but cigarette smoking does one cigarette contain 1-2ug of cadmium with 10% pulmonary absorption. Cadmium has strong affinity for liver and kidney over a wide range of exposure levels. Cadmium is highly cumulative and is suggested by human autopsy showing peak in kidney at the age of 50. Cadmium is widely distributed in the body of both man and animal have been isolated from kidney, liver, spleen, intestine, heart, brain, lung and skin. Cadmium can induce tumor in prostate.<sup>4</sup> Acute and chronic toxic effects of Chromium are mainly caused hexavalent compounds. Aqueous solution of potassium dichromate locally applied to skin result in local inflammation. Chromates cause renal damage mainly involving the convoluted portion of the proximal tubule with associated lysozymuria and proteinuria. Hepatotoxic effects of chromium (both trivalent and hexavalent) after parenteral administration have been described with hexavalent Chromium these can be thickening of the liver capsule congestion in central vein and adjacent semisolid and coagulation necrosis of hepatic cells throughout the parenchyma. The liver, via the portal vein, is the first organ exposed to enterally absorbed nutrients and other xenobiotics. The liver is composed of highly active metabolic tissue containing a huge complement of detoxification machinery referred to as phase I and phase II enzyme systems that ideally serve to guard other physiological systems from the toxic effects of xenobiotic compounds. Earlier studies on potential hepatotoxicity of Pb in experimental animal systems used relatively high doses of inorganic Pb salts. These studies reported alterations in hepatic xenobiotic metabolism, cholesterol metabolism, liver cell proliferation, and DNA synthesis indicative of Pb induced hepatic hyperplasia<sup>5</sup>. Thus the present study was conducted to study the effect of acute poisoning lead, chromium and cadmium on liver enzymes and proteins.

### AIMS AND OBJECTIVES

To study the Hepatotropic effects of acute heavy metal poisoning in rats.

### MATERIALS AND METHODS

The present study was conducted to assess the effect of acute heavy metal poisoning on liver function in rats. For this purpose twenty four adult albino rates (12 female and 12 male) each weighing 180 to 120 gms were obtained from King Institute, Guindy, Chennai. The animals were caged individually in a room with constant temperature and relative humidity (56-70%). A 12 hour light and dark cycle was maintained. A standard rat diet (pellets) was provided ad libitum. Tap water was supplied ad libitum in polythene bottles which were filled daily with fresh water and cleaned weekly. The animals were acclimatized for a week in the Institute animal house. The animals were fasted overnight prior to drug administration. For the purpose of study four groups were formed containing six animals each.

- Control group
- Cadmium group
- Chromium group
- Lead group

On the day of the experiment, initially the animals were individually weighed and the weights were recorded. For the purpose of acute poisoning a study conducted by Adler and Adler<sup>6</sup> in 1977 was used as guide line. The rats were exposed to heavy metal salt by two daily injections. The acute doses have been chosen based on previous studies in literature. The calculated amount of Cadmium was 2.5mg/kg body weight; Chromium was 10mg/kg body weight and that of Lead was 50mg/kg body weight. The metal salts were injected intraperitoneally using 24 G needle and syringe for two subsequent days. On the third day the entire animals were weighed and sodium pentobarbitone 30 mg/kg body weight was administered intraperitoneally. The time of administration of drug, the time of loss of righting reflex and the recovery of the animals were noted. 1ml blood sample was withdrawn for the blood assay after two doses of acute lead, cadmium and chromium administration. Blood samples were collected by retro-orbital puncture from all the animals. Serum was immediately separated after centrifuging at 2500rpm for 20min and kept at minus 20°C until assayed. Assay of various liver enzymes and serum proteins was done and levels were recorded. The data pertaining to various experimental groups were compared with those of the respective controls employing student's test for unpaired data. The minimum level of significance for the observed differences was kept at  $p < 0.05$  level.

## RESULTS

**Table 1:** Mean onset and duration of pentobarbital sleeping time in heavy metal pretreated rat

Treatment	Period of onset of sleep (sec)	Duration of sleep (min)
Control (n=6)	420±73	111.38±25.89
Cadmium (n=6)	439.8±117	119.83±49.20
Chromium(n=6)	499.8±170	110.17±29.61
<b>Lead (n=6)</b>	<b>439.8±176</b>	<b>219.17±53.60*</b>

\* Significant.

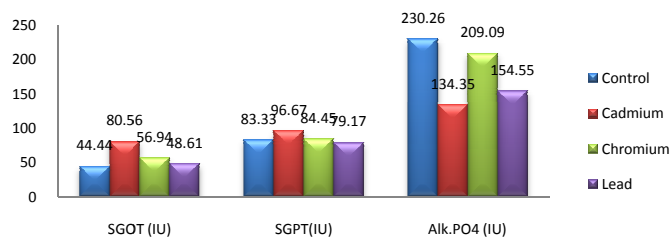
It was observed that time for onset of sleeping was increased in all the three study group as compared to the control group. For the purpose of statistical calculation control group was compared with the study group separately. The difference in onset of sleeping observed in all the three study group was statistically insignificant. The duration of sleep was almost same in control,

cadmium and chromium group whereas it was increased in lead group. The difference observed in sleeping duration in cadmium and chromium group as compared to control group was statistically significant. Whereas the difference in sleeping duration in lead and control group was statistically significant.

**Table 2:** Serum Enzymic Activities and Protein levels after administration of Pentobarbital in heavy metal pretreated rats

Treatment	Enzymes			Protein gms/100ml
	SGOT (IU)	SGPT(IU)	Alk.PO <sub>4</sub> (IU)	
Control	44.44±4.65	83.33±7.40	230.26±51.13	4.15±0.23
Cadmium	80.56±17.03*	96.67±6.53*	134.35±19.88*	3.50±0.20*
Chromium	56.94±6.94*	84.45±7.92	209.09±43.75	3.42±0.10*
Lead	48.61±11.06	79.17±8.84	154.55±19.47*	3.34±0.13*

**Serum Enzymic activity after administration of Pentobarbital in heavy metal pretreated rats**



It was observed that SGOT levels were increased in all the three groups as compared to control group. When the levels of SGOT of cadmium and chromium groups were compared with control group the difference was statistically significant. However the difference in SGOT levels of control and lead group was statistically insignificant. The SGPT levels were increased with statistically significant difference in cadmium group as compared to control group whereas the increase in other groups was not statistically significant. The rise in alkaline phosphatase was statistically significant in cadmium and lead group when compared with control group. Serum proteins were also increased with statistical significance in cadmium and lead group.

## DISCUSSION

The effect of heavy metals on pentobarbitone sleeping time was investigated with the objective of finding out any possible influences of the former on activity of hepatic drug metabolizing enzymes. The premise was that in the presence of such an effect, there would be corresponding changes in the time of onset and duration of pentobarbitone induced sleep. As the present result indicate the latency period for the onset of sleep was not significantly different between control and heavy metal treated animals did show any appreciable difference. The duration of sleeping also did not show appreciable difference except the lead group. In which a longer sleeping time (219.17 + 53.60) was noticed with statically significant difference. Serum enzyme and protein levels were recorded after heavy metal treatment and pentobarbital. It was observed that SGOT activity in

heavy metal treated animals was significantly different in cadmium and chromium group from control animals. The value recorded was in the range of  $44.44 \pm 4.65$  to  $80.56 \pm 17.03$  IU. The pyruvate transaminase activity in the heavy metal treated animals was similar to that of control group except cadmium group where the difference was statically significant. Alkaline phosphatase Activity recorded in the control as well as heavy metal treated groups were within normal limits and the range being  $134.35 \pm 19.88$  to  $230.26 \pm 51.13$  IU. The activity of heavy treated animals and control group did not differ significantly except lead group. It is quite possible that at least with regard to lead treatment if the sample size of experimental animals was greater, the chances of a significant prolongation of pentobarbitone sleeping time were quite possible. While the control group had a mean protein level of  $4.15 \pm 23$  gms/100ml. Heavy metal treated group showed a decrease in serum protein content, the value being  $3.50 \pm 0.20$ ,  $3.42 \pm 0.10$  and  $3.34 \pm 0.13$  in cadmium, chromium and lead treated groups respectively. The decreases in values recorded in chromium and lead treated groups were statistically significant ( $p < 0.05$ ) when compared with control group. In brief the acute heavy metal exposure and the present experimental condition did not alter the activity of the enzyme study. However, the fall in serum protein in chromium and lead treated groups probably reflect a diminution in the rate of protein synthesis. At this junction, it may be speculated that the changes in enzymatic activity might have appeared if the serum samples had been collected later than on the third day as done in the present study. The effect of several cations on drug metabolizing enzymes found in hepatic microsomes has been previously studied in experimental animals. Some of these cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{SR}^{2+}$ ) stimulate while certain other ( $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$ ) inhibit drug metabolism<sup>7</sup>. The stimulating cation also increases NADPH cytochrome P-450 activity<sup>8</sup>. Rebeiro *et al* has shown that Beryllium, Mercury and Pb inhibit hexobarbital metabolism in vivo. On the other hand, through in vitro studies, he had shown arsenic, Beryllium and Pb (upto  $10^3$  m) to be devoid of any effect on hexobarbital metabolism while mercury was inhibited. Hepatic microsomal preparation of male rates, after treatment with cerium chloride, has shown to have a significantly inhibited hexobarbital and N-methylaniline metabolism by Arvela and Karke *et al*<sup>9</sup>. Through studies on the effect of cadmium acetate on drug metabolism, Hedly *et al*<sup>10</sup> observed that hexobarbital sleeping time was potentiated about 9-12 hours following the administration of a single dose of cadmium acetate administrated to male sprague – Dawley rates 3 days, prior to sacrifice produced a significant (50-80%) inhibition of metabolism of hexobarbital, P-nitroanisole,

aminopyrine, and zoxazolamine, but not progesterone. There was also a significant reduction in the hepatic microsomes in these Cadmium treated rates. Hadley *et al*<sup>10</sup> also demonstrated in an in vitro, inhibition of the same substrates by adding Cadmium acetates ( $5 \times 10^{-4}$  -  $5 \times 10^{-7}$  M) directly to incubate isolated hepatic microsomes of untreated rates. It is known that single toxic does of some heavy metals such as Cd, Co and Ni inhibit the activity of the rat liver microsomal mixed function oxidases.<sup>8,4,14,25</sup> As regards Cadmium, it has been reported to activate or inhibit several liver enzymes.<sup>8</sup> Single i.p injections of cadmium chloride at doses of 2.5 or 3.75 mg/kg (equivalent to 1.5 or 2.3 mg of Cadmium respectively) into male wister rates produced significant inhibition (25-60%) of aniline hydroxylase and nitroreductase activity and also lowered the microsomal cytochrome p-450 content to 50% of its control value. While the higher doses of this toxic metal salt significantly reduced the activities of the pentobarbital – induced hepatic microsomal drugs =metabolizing enzymes and of cytochrome p-450 the lower does was without significant effects on this induction, with the one exception, of aniline hydroxylase when assessed in a Tris-buffer system. Given repeatedly in subtoxic doses in the drinking water for 30 days, the effect of Co ( $\text{No}_3$ )<sub>2</sub>,  $\text{CDSO}_4$ ,  $\text{NiSO}_4$ ,  $\text{ZnSO}_4$ ,  $\text{HgCl}_4$ , on rat liver monooxygenases was studied experimentally on male wister rats. The salts of Co., cd and Zn increased the activity of benzphetamine - N - demthylese and the content of cytochrome p-450 and microsomal heme. These finding suggested on enzyme inducing effect of these salts on the hepatic monooxygenases. The metal salts also increased the activity of S-aminoceruleic acid (ALA) synthetase and decreased that of heme oxygenase. Stoyteher and Kadiiska *et al*<sup>11</sup> has shown previously that these salts shortened the hexobarbital sleeping time and increased ethylmorphine - N - demthylese activity. The above reports in previous literature have clearly shown that the heavymetal cation are capable of modulating the activity of drug metabolizing enzymes present in the liver, and thus could induce significant changes in sleeping time and need for Barbiturates administrated after heavy metal exposure.

## CONCLUSION

It may be stated that acute heavy metal exposure is associated with some changes in enzymic activity of liver and significant decrease in serum proteins. And thus either increase in the dose or frequency of heavy metal administration or allowing a longer period of heavy metal exposure will cause major hepatic damage and will bring some appreciable changes in these parameters.

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