Chapter III

Effect of lead and cadmium either alone or in combination on hepatic, hypothalamic and pituitary steroid metabolism in non pregnant rats

- Introduction
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Introduction

Toxic effect of lead and cadmium on female reproduction due to environmental pollution is well known. Both lead and cadmium have been reported to disrupt reproductive physiology and behavior at different levels along the hypothalamic-pituitary-gonadal axis as discussed earlier. Hydroxysteroid dehydrogenases catalyze the interconversion of hydroxyl and carboxyl groups of steroids. Both hypothalamic and pituitary 3α-hydroxysteroid dehydrogenase (3α-HSDH) catalyzes the oxido-reduction at carbon 3 of steroid hormones and is postulated to initiate the complete mineralization of the steroid nucleus to CO_2 and H_2O.

In addition to metabolizing toxins, the liver plays a key role in hormone homeostasis, as it metabolizes both peptide and steroid hormones. Hepatic metabolism of many steroids and toxins can occur in a sexually dimorphic pattern and thus can serve as a biomarker for exposure to both naturally occurring and synthetic hormones. The control of steroid hormone profiles involves several variables, including rate of hormone synthesis. interactions among hormones, and rates of secretion, transport, biotransformation and elimination. One route through which normal control could be disrupted is xenobiotic induction of sex steroid metabolizing cytochrome P-450 enzymes. Inducing or blocking these enzymes conceivably alters the natural balance of circulating sex steroids. Several mechanisms are used for hormone biotransformation in the liver. Direct conjugation. in which the steroid is conjugated to glucuronic acid or sulphate. produces a more water-soluble product that can then be excreted in urine (de Bethizy and Hayes. 1994). Steroid hydroxylation accomplishes the same goal by stereo-selectively and regio-specifically attaching hydroxyl groups to a steroid (Wilson et al., 1998). Oxido-reduction of estradiol

to estriol is another hepatic biotransformation pathway that influences circulating concentrations of estradiol.

Eventhough the general toxicity of lead and cadmium are very well known. only a few reports are available on the effect of these metals on hepatic steroid metabolic enzymes (Murashow. 1966; Neshkow, 1971; Odenbro et al.. 1982) with no reports on hypothalamic-pituitary enzymes. Moreover, most of the studies on these two metals has dealt with each in isolation that too with higher doses. On the other hand, populations in real life always have simultaneous multiple exposures, indicating the need for experimental work with combination of substances. In the present study an attempt has been made to evaluate the effect of lead and cadmium either alone or in combination on neuroendocrine and hepatic steroid metabolism in non-pregnant rats.

Experimental design

The experiment was carried out in two ways i.e., dose dependent study and time dependent study.

Dose dependent studies:

There were four groups of animals in the study. control (sodium acetate), lead acetate. cadmium acetate and lead acetate and cadmium acetate in combination. The animals received different dosage of metal solutions (0.025, 0.05 or 0.10 mg/kg body wt./day) for 15 days. The combined treated group was also exposed to same dose by taking half concentration of each metal. Animals of all groups were sacrificed after 15 days of metal exposure in proestrous stage or estrous stage (checked by vaginal cytology). After the metal exposure blood was collected, serum samples obtained after centrifugation at 2500 *g* for 10 min were used for various toxicity parameters such as serum glutamate pyruvate

transaminase (Reitman and Frankel, 1957), alkaline phosphatase (Bowers & McComb, 1975) and creatinine (Bonsnes and Taussky, 1945). The liver, hypothalamus and pituitary were immediately removed and processed for steroid metabolizing enzyme assays like 17β hydroxy steroid oxidoreductase (17β HSOD; Shivanandappa and Venkatesh, 1997), UDP Glucoronyl Transferase (UDPGT; Gorski and Kasper, 1977) and 3α hydroxy steroid dehydrogenase (3α HSD; Shivanandappa and Venkatesh, 1997). Activity of acid phosphatase (Bowers & McComb, 1975) and biochemical parameters such as metallothionein (Bayne et. al., 1985), Cytochrome P450 (Omura and Sato, 1964), cholesterol (Leffler et al., 1963), DNA (Burton, 1956) and RNA (Schneider, 1957) were determined in the liver samples. Both tissue and blood were analyzed for lead and cadmium levels by GBC 902 Atomic Absorption Spectrophotometer. Histology of liver was done using standard histological techniques.

Time dependent studies:

From the dose dependent studies, 0.05 mg/kg body wt has been selected as the optimum dose and further experiments were carried out for 30 and 45 days with four groups of animals in the similar manner, control (sodium acetate), lead acetate, cadmium acetate and lead acetate and cadmium acetate in combination. Animals of all groups were sacrificed after the metal exposure in proestrous stage, blood was collected and serum samples obtained after centrifugation at 2500 ***g*** for 10 min were used for various toxicity parameters. The liver, hypothalamus and pituitary were immediately removed and processed for steroid metabolizing enzyme assays. Metallothionein content was determined in the liver samples. Both tissue and blood were analyzed for lead and cadmium levels. Liver was used for histological observations.

Results

Dose dependent studies

The metal exposed animals were healthy during the treatment period. The body weight were unaffected by the metal exposure (Table 1). Activity of hypothalamic 3α-HSDH was most affected in cadmium treated group in a dose dependent manner (Fig 1). The 0.025 mg/kg body wt. dose was not showing effect on the enzyme activity in any of the metal treated groups. Both in 0.05 and 0.1 mg/kg body wt. dose, all metal exposed animals were showing significant decrease in the enzyme activity with cadmium showing maximum inhibition.

In case of pituitary 3α-HSDH, the enzyme activity was not affected in any of the metal exposed groups in the 0.025 mg/kg body wt. dose (Fig 2). In the 0.05 and 0.1 dose treated groups the enzyme activity was significantly inhibited in all metal exposed groups, where as cadmium was showing maximum effect and combined exposed group showing intermediate results.

Alteration of relative liver weights in the metal treated groups, compared to control, were mostly insignificant. Activities of liver 17β HSOR and UDPGT after the metal treatment is shown in Fig. 3 and 4. The enzyme activity was most affected in cadmium treated group. Lead treated group was showing minimum effect and the combined treatment group showed intermediate results. 0.025 mg/kg body wt. dose of metal treatment, both alone and in combination, failed to cause inhibition on the UDPGT activity. These results indicated that lead and cadmium treatment affected the hepatic estradiol metabolism in a dose dependent manner.

Table 1: Dose dependent effect of lead and cadmium alone and in combination on body weight (gm).

Groups	**0.025** mg/kg body wt.		**0.05** mg/kg body wt.		**0.1** mg/kg body wt.	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
Control	180.2±7.9	202.2±4.8	184.5±6.33	195.6±4.5	186.2±2.79	198±4.79
Pb	182.2±6.98	198.8±6.99	187.4±6.5	200±5.88	195±4.89	200.4±4.39
Cd	184.6± 4.2	195.6±5.55	191.4± 8.2	213.6±9.3	189±5.4	200±6.59
Pb+Cd	187.1±4.94	199.6±4.9	190±3.8	205.6±7.8	180.4±5.71	201±3.77

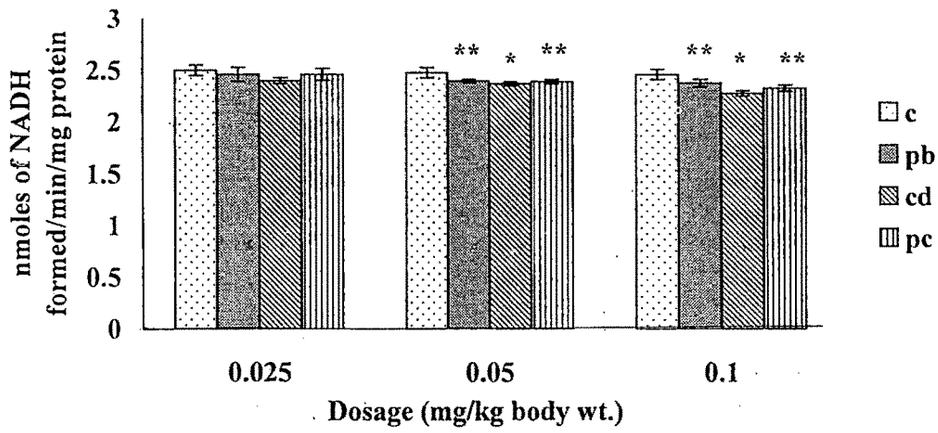
Table 2: Dose dependent effect of lead and cadmium alone and in combination on hepatic lead and cadmium content (μg/g).

Groups	0.025		0.05		0.1	
	Pb	Cd	Pb	Cd	Pb	Cd
Control	1.0±0.1	0.273±0.027	1.1±0.1	0.296±0.027	0.9±0.02	0.3±0.009
Pb	1.2±0.01	0.28±0.017	$1.63\pm0.14^{a*}$	0.28±0.017	$2.0\pm0.2^{a*}$	0.32±0.011
Cd	1.0±0.1	1.0 ± 0.098^{12}	1.13 ± 0.06^{b}	$2.0\pm0.09^{45**}$	1.1 ± 0.1^{b}	$2.4\pm0.098^{45***}$
Pb+Cd	1.13±0.06	0.3 ± 0.02^{3}	$1.46\pm0.06^{ac*}$	$1.9\pm0.16^{45***}$	1.7 ± 0.2^{ac}	$2.3\pm0.033^{45***}$

[1]P<0.01; [4]P<0.001 compared to the identical dose of control: [2]P<0.01; [5]P<0.001 compared to the identical dose of lead acetate group: and [3]<0.01 compared to the identical dose of cadmium acetate group.

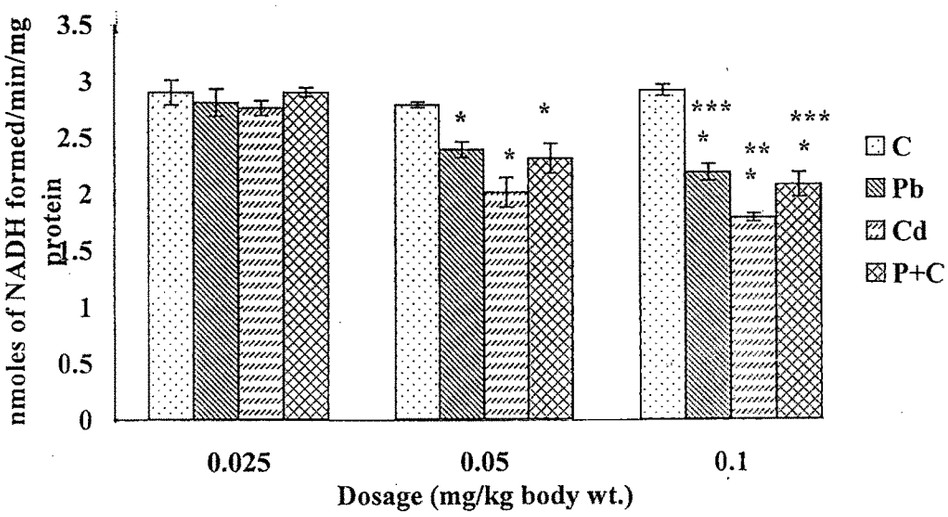
*P<0.05; **P<0.01; ***P<0.001 within same metal treatment compared to the lowest dose. Superscripts [a b] and [c] indicate P<0.05 in comparison to control, lead acetate and cadmium acetate groups of identical dose respectively. (n = 3-4)

Fig 1: Dose dependent effect of lead and cadmium alone and in combination on hypothalamic 3α– hydroxy steroid dehydrogenase activity



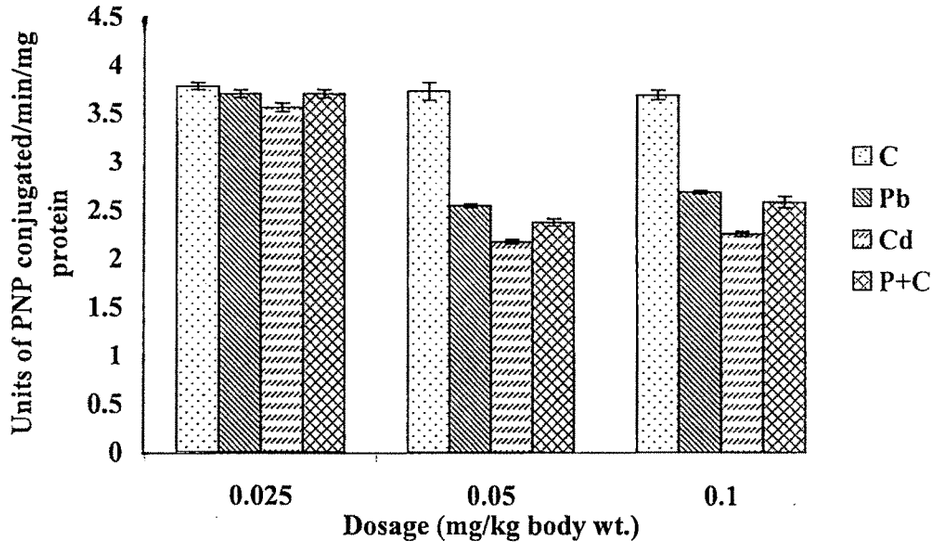
*P<0.001; **P<0.01 vs. control (n = 5 or 6)

Fig 2: Dose dependent effect of lead and cadmium alone and in combination on pituitary 3α– hydroxy steroid dehydrogenase activity



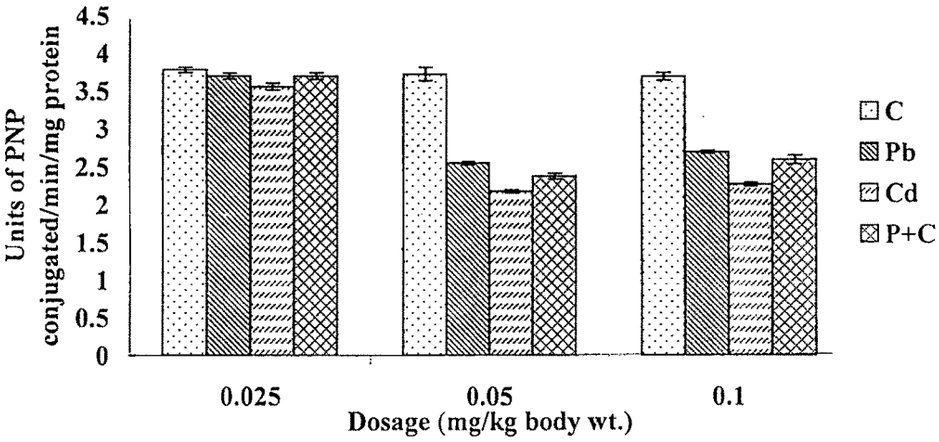
*P<0.001 vs. control; **P<0.001 vs. lead and ***P<0.001 vs. cadmium group. (n = 5-6)

Fig 3: Dose dependent effect of lead and cadmium alone and in combination on hepatic 17β– hydroxy steroid oxidoreductase activity



*P<0.001 vs. control; **P<0.001 vs. lead and ***P<0.001 vs. cadmium group. (n= 5-6)

Fig 4: Dose dependent effect of lead and cadmium alone and in combination on hepatic UDPG Transferase activity



*P<0.001 vs. control; **P<0.001 vs. lead and ***P<0.001 vs. cadmium group. (n = 5-6)

Histological observation of the liver after various doses of lead and cadmium treatment showed marked alterations (Fig. 5). The metal treatment caused marked changes in liver such as massive fatty degeneration in the hepatocytes and large vacuoles in cytoplasm. Staining affinity of nucleus was comparatively poor and was pycnotic. in appearance. Focal necrotic regions could be seen scattered in combined metal treated group in 0.05 mg/kg body wt. dose (Fig. 5F). Higher dose of combination treatment led to infiltration of lymphocytes in liver. Cytoplasm of hepatocytes showed vacuoles and nuclei were pycnotic (Fig. 5G), but significant histological changes were observed only in combined metal treated groups in 0.05 and 0.10 mg/kg body wt. doses. Significant histological changes such as degenerative damage, focal inflammatory changes and hepatic necrosis were observed in the cadmium and combined metal exposed animals in 30 and 45 days metal exposure studies. Tables 2 and 3 summarize the results on metal content in liver and blood respectively. There was progressive increase in both hepatic and blood content of lead and cadmium after the treatment.

To understand further, some more parameters were analyzed by treating the animals with lead and cadmium at a dose concentration of 0.05 mg/kg body wt. for 15 days as the optimum dose. In the case of CYP450, maximum reduction in the content was observed in cadmium treated group (Table 4). The combined treatment again showed intermediate results. It is well known that the metal exposure induces the synthesis of metallothionein (MT), which show metal scavenging effect. Therefore, it was of interest to see whether the given amount of metal treatments were able to cause displacement of essential metal bound to metallothionein or not. For this the concentration of Zn, the native essential metal bound to MT was measured in the fractions corresponding to MT or MT

Fig. 5: Histological observation of the liver after various doses of lead and cadmium treatment.

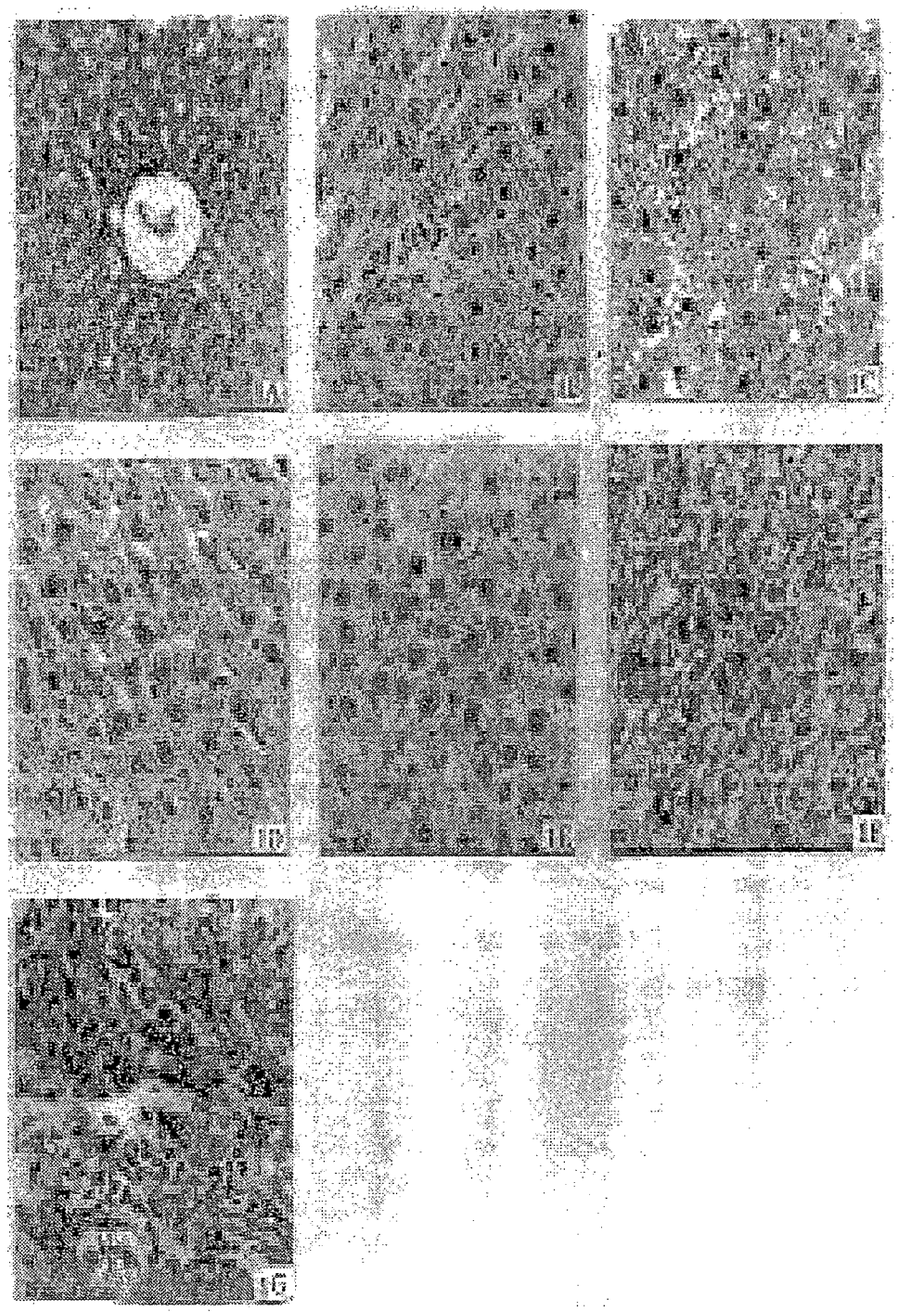


Table 3: Dose dependent effect of lead and cadmium alone and in combination on lead and cadmium levels in blood (μg/ml).

Groups	0.025		0.05		0.1	
	Pb	Cd	Pb	Cd	Pb	Cd
Control	N.D	N.D	N.D	N.D	N.D	N.D
Pb	1.83±0.102*	N.D	3.92±0.063* #	N.D	5.8±0.270* # ##	N.D
Cd	N.D	0.076±0.011*	N.D	0.205±0.035*#	N.D	0.299±0. 049* #
Pb+Cd	1.21±0.390*	0.055±0.012*	1.54±0.092* **	0.133±0.036* #	3.2±0.273* ** # ##	0.159±0.081* *** #

*P <0.001 vs. control; **P<0.001 vs. lead and ***P<0.001 vs. cadmium of identical dose; #P<0.001 and ##P<0.001 within same metal treatment compared to 0.025 and 0.05 dose respectively. (n = 3 or 4)

Table 4: Effect of lead and cadmium alone and in combination on hepatic acid phosphatase activity and cytochrome P450 content (Dosage: 0.05 mg/kg body wt./day).

Groups	Acid phosphatase@	CytochromeP450$
Control	1.067± 0.06	1.027±0.013
Pb	1.34±0.01*	0.842±0.032* ***
Cd	1.39±0.005* **	0.736±0.018* **
Pb+Cd	1.39±0.004* ***	0.82±0.012* ***

@ uM of PNP formed/30 min/mg protein; $ (nmols/mg protein) (n = 5-6)

* P<0.001 vs. control: ** P<0.001 vs. lead and ***P<0.001 vs. cadmium.

like proteins. Level of zinc in the fraction corresponding to MT of cadmium treated animals was found to be least among the different treatment groups. It is clear from Fig. 10 that both lead and cadmium can displace the zinc from the metallothionein fractions.

The results indicate that none of the heavy metals in above experimental groups, caused change in acid phosphatase activity at the level of 0.025 mg/kg b.wt. dose (Table 4). Significant increase in enzyme activity was observed in all metal treated groups at 0.05 and 0.1 mg/kg b.wt. doses compared to control. No change in hemoglobin levels was found after the metal exposure. Liver function tests like serum GPT and serum ALP and kidney function test like serum creatinine were done to evaluate the toxic effect of the administered dose of metal solution in rats (Table 5). The metal exposure resulted a dose dependent decrease in ALP with increase in SGPT in all groups. Serum creatinine levels were increased in both individually and combined metal treated groups, but results were within the normal range. Table 6 summarises the data on hepatic DNA, RNA and microsomal cholesterol. Hepatic DNA content was decreased in cadmium and combined metal exposed groups; with no change in RNA content. Metals alone and in combination caused a decrease in the hepatic cholesterol level also.

Time dependent studies

Metal exposure to animals for different time period has not affected their body weight (Table 7). Exposure of animals to metals for different time period showed that hypothalamic 3α-HSDH activity was inhibited further with increase in the exposure time (Fig 6). It is interesting to note that there was a significant decrease in the enzyme activity from 15 days to 30 days, but such a significant change was not observed from 30 days to 45 days. Similar kind of result was also observed in pituitary 3 α-HSDH (Fig 7). In both

Table 5: Dose dependent effect of lead and cadmium alone and in combination on safety parameters.

Dosage	Groups	Hemoglobin (g%)	SGPT (IU/dl)	ALP (IU/dl)	Serum creatinine (mg%)
0.025	control	14.32±0.81	23.4±2.06	101.6±6.7	0.84±0.05
	Pb	14.72±0.24	26.2±1.7	79±2.54	0.86±0.09
	Cd	14.87±0.73	30.4±1.4	60.5±1.81	1±0.06
	Pb+Cd	15.0±0.3	23.3±1.3	70.1±2.3	1±0.063
0.05	control	15.62±1.4	25.6±1.85	102.8±9.1	0.79±0.09
	Pb	15.2±0.47	29.4±0.9	69.8±0.8	0.83±0.11
	Cd	14.9±0.53	34.6±2.0	59.7±1.57	0.96±0.12
	Pb+Cd	14.9±0.24	29.8±1.4	66.4±2.2	1.0±0.07
0.1	control	15.62±0.54	24.7±1.6	101.0±5.2	0.89±0.08
	Pb	15.3±0.27	29.5±1.4	61.3± 2.3	1.02±0.08
	Cd	15.1±0.27	41.5± 2.7	50.6±1.97	1.4±0.134
	Pb+Cd	15.2±0.0.31	39.8±1.8	61.9±2.15	1.2±0.108

ALP: IU-μ mols of PNP formed/min., (n = 5-6)

GPT: IU-μ mols of pyruvate formed/min., (n = 5-6)

Table 6: Effect of lead and cadmium alone and combination on hepatic DNA, RNA and microsomal cholesterol (Dosage: 0.05 mg/kg body wt./day).

Groups	DNA (ug/g tissue)	RNA (ug/g tissue)	Cholesterol (ug/mg microsomal protein)
Control	2.58±0.04	10.11±0.93	33.79±0.29
Lead	2.46±0.021*	9.07±0.66	36.58±0.56*
Cadmium	2.29±0.015* **	8.56±0.81	41.45±0.63* **
Lead+cadmium	2.33±0.012* **	8.80±1.1	38.03±0.41*

* P<0.001 vs. control and ** P<0.001 vs. lead group. (n = 5 or 6)

Table 7: Time dependent effect of lead and cadmium alone and in combination on body weight (gm). (Dosage: 0.05 mg/kg body wt./day).

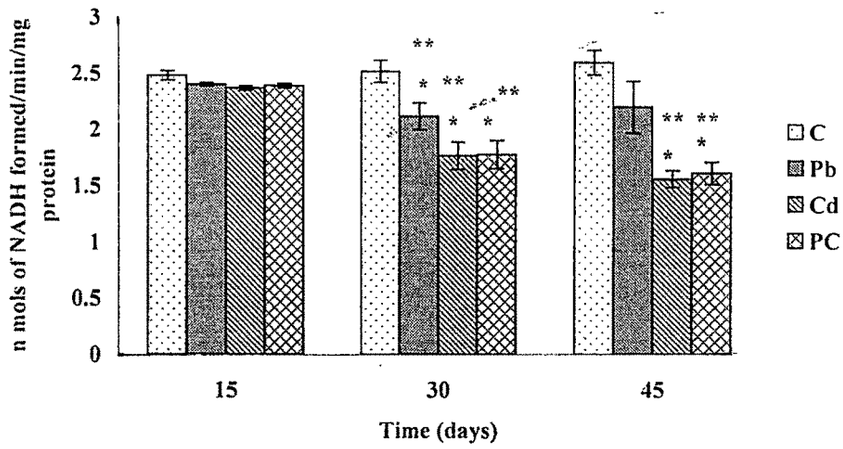
Groups	15		30		45	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
Control	180.2±7.9	202.2±4.8	182.4±3.3	201.2± 2.2	184.6±4.34	208±5.12
Pb	182.2±6.98	198.8±6.99	182±4.9	203.2±5.2	191.2±3.3	210±5.54
Cd	184.6±4.2	195.6±5.55	189.4±4.5	200.8±5.4	184.4±3.99	198.4±3.7
Pb+Cd	187.1±4.94	199.6±4.9	187.2±3.1	199.4±3.63	186±7.6	211±6.98

Table 8: Time dependent effect of lead and cadmium alone and combination on hepatic cytochrome P450 content (Dosage: 0.05 mg/kg body wt./day).

Groups	Days		
	15	30	45
Control	1.027±0.013	0.961± 0.06	0.932 ±0.017
Lead	0.842±0.032****	0.78 ±0.011$^{****\#}$	0.76±0.048$^{****\#}$
Cadmium	0.736±0.018***	0.724±0.016***	0.69±0.009$^{***\#}$
Lead+cadmium	0.82±0.012***	0.73±0.008$^{***\#}$	0.70±0.019$^{***\#}$

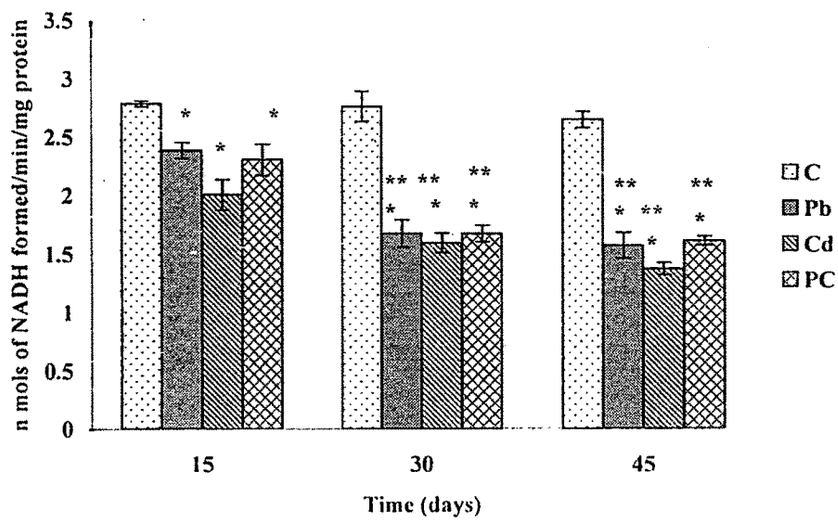
* P<0.001 vs. control; ** P<0.001 vs. lead; ***P<0.001 vs. cadmium and $^{\#}$P<0.001 vs. identical group in 15 days treatment. (n = 5 or 6)

Fig. 6: Time dependent effect of lead and cadmium alone and in combination on hypothalamic 3α hydroxy steroid dehydrogenase activity



*P<0.001 vs. control and **P<0.001 vs. lead group. (n = 5 or 6)

Fig. 7: Time dependent effect of lead and cadmium alone and in combination on pituitary 3α hydroxy steroid dehydrogenase activity



*P<0.001 vs. control and **P<0.001 vs. lead group. (n = 5 or 6)

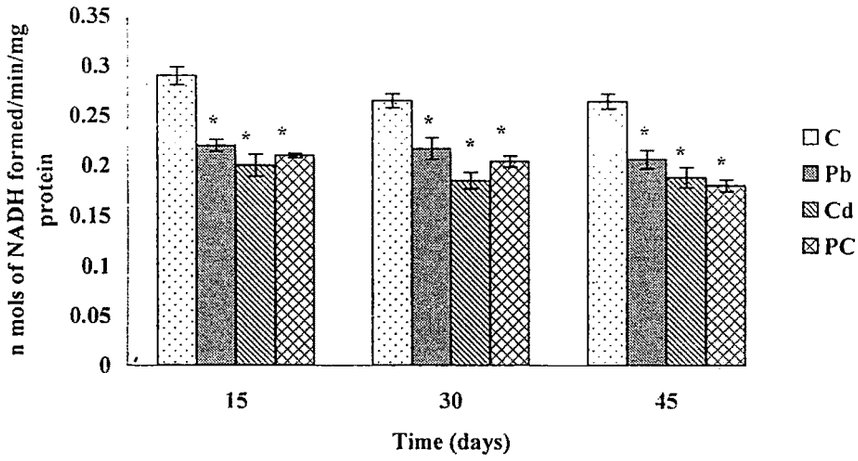
cases, the cadmium exposed animals showed maximum inhibition in the enzyme activity with the combined exposure resulting in intermediate effects. There was a time dependent decrease in the hepatic 17β HSOR and UDPGT activities, which was maximum in cadmium treated group (Fig 8 and 9). There was no significant change in the activity between 30 days and 45 days exposure groups. Cytochrome P450 content was decreased further in all metal exposed groups with increase in time of exposure (Table 8). The Zn concentration in metallothionein fraction was further decreased in cadmium and combined metal exposed groups (Fig 10 a,b,c and d).

Tables 9 and 10 summarize the results on metal content in liver and blood respectively, of animals exposed to metals for different time period. There was progressive increase in both hepatic and blood content of lead and cadmium with increase in time of exposure. The hemoglobin levels were unaffected by the metal exposure. Although there was a time dependent increase in SGPT in all metal exposed groups, the values were within normal range. Similarly no significant change was observed in serum creatinine levels in the metal exposed animals compared to control group. Serum ALP was also found decreased after the metal exposure (Table 11).

Discussion

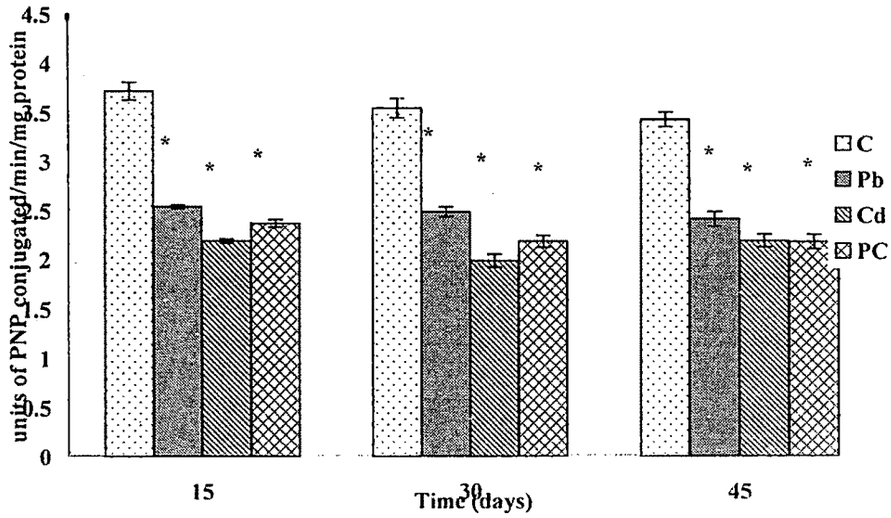
The results indicate that both lead and cadmium inhibit the activity of the enzyme 3α- HSDH in dose and time dependent manner. The chemical mechanism indicates that the enzyme operates by a "push-pull mechanism" involving three amino acids in the active site, tyrosine, histidine and lysine (Schlegel et al., 1998). Therefore, the decrease in the activity of the enzyme observed could be due to the known general mechanism of toxicity for these metals i.e., binding to the –SH groups present in the above amino acids. But both

Fig 8: Time dependent effect of lead and cadmium alone and in combination on hepatic 17β hydroxy steroid oxidoreductase activity



*P<0.001 vs. control (n = 5-6)

Fig 9: Time dependent effect of lead and cadmium alone and in combination on hepatic UDPG Transferase activity



*P<0.001 vs.control (n = 5-6)

Table 9: Time dependent effect of lead and cadmium alone and combination on hepatic lead and cadmium content (µg/g). (Dosage: 0.05 mg/kg body wt./day)

Groups	15		30		45	
	Pb	Cd	Pb	Cd	Pb	Cd
Control	1.1±0.1	0.296±0.027	1.16±0.12	0.28±0.04	1.28±0.12	0.34±0.02
Pb	1.63±0.14*	0.28±0.017	7.57±0.37***	0.32±0.04	8.23±0.62***	0.36±0.07
Cd	1.13±0.06	2.0±0.09*	1.2±0.11	2.4±0.43*	1.03±0.19	3.32±0.25*=
Pb+Cd	1.46±0.06*	1.9±0.16*	4.0±0.15***	2.12±0.29*	3.6±0.27*	2.27±0.14*=

* P<0.001 vs. control; ** P<0.001 vs. identical group in 15 days treatment: =P<0.001 vs. identical group in 30 days treatment. (n = 3 or 4)

Table 10: Time dependent effect of lead and cadmium alone and combination on lead and cadmium levels in blood (µg/ml). (Dosage: 0.05 mg/kg body wt./day).

Groups	15		30		45	
	Pb	Cd	Pb	Cd	Pb	Cd
Control	N.D	N.D	N.D	N.D	N.D	N.D
Pb	3.92 ±0.063*	N.D	4.26±0.54*	N.D	5.73±0.44* ** # ##	N.D
Cd	N.D	0.205±0.035*	0.81±0.19	0.234±0.013*	1.1±0.072	0.4±0.033*# ##
Pb+Cd	1.54 ±0.092*	0.133±0.036*	1.62±0.13* **	0.19±0.014*	1.7±0.16* **	0.34±0.035*# ##

*P <0.001 vs. control; **P<0.001 vs. lead and ***P<0.001 vs. cadmium of identical dose. #P<0.001 and ##P<0.001 within same metal treatment compared to 15 and 30 days respectively. (n = 3 or 4)

Fig. 10a: Time dependent effect of lead and cadmium either alone or in combination on Zn level in hepatic metallothionein fraction in control group

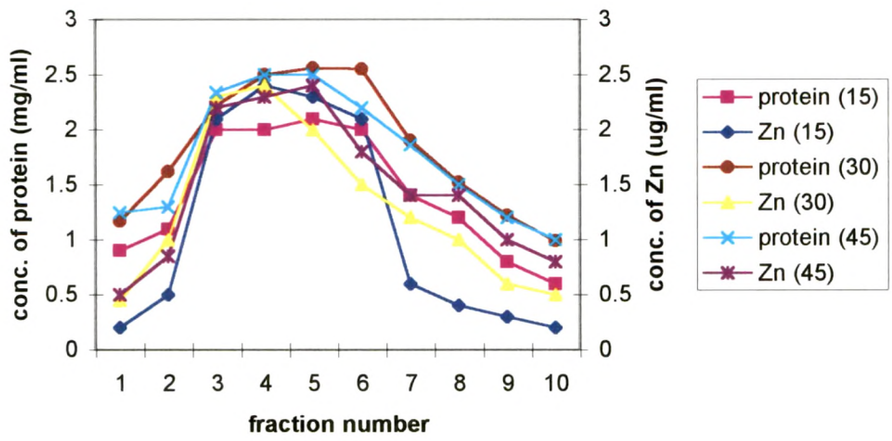


Fig. 10b: Time dependent effect of lead and cadmium either alone or in combination on Zn level in hepatic metallothionein fraction in lead group

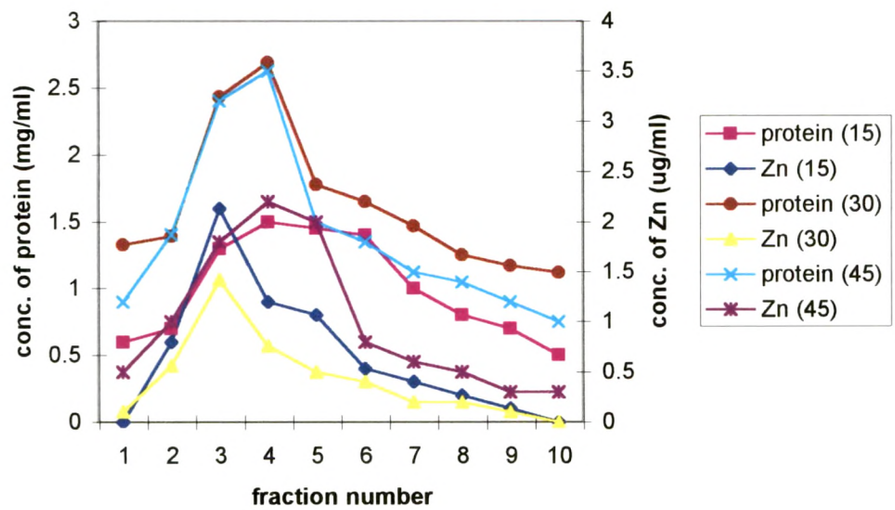


Table 11: Time dependent effect of lead and cadmium alone and in combination on safety parameters. (Dosage: 0.05 mg/kg body wt./day).

Days	Groups	Hemoglobin (g%)	SGPT (IU/dl)	ALP (IU/dl)	Serum creatinine (mg%)
15	Control	15.62 ±1.4	25.6± 1.85	102.8±9.1	0.79± 0.09
	Pb	15.2±0.47	29.4± 0.9	69.8± 0.8	0.83± 0.11
	Cd	14.9±0.53	34.6± 2.0	59.7±1.57	0. 96± 0.12
	Pb+Cd	14.9±0.24	29.8± 1.4	66.4± 2.2	1.0± 0.07
30	Control	15.12±0.67	23.7± 0.93	100.8±5.1	0.93±0.08
	Pb	13.62±0.43	29.7±0.93	71.3± 5.2	1.5± 0.13
	Cd	14.2±0.42	35.4±1.6	55.6± 3.8	2.06±0.14
	Pb+Cd	13.3±0.5	29.2±1.36	63.6± 4.5	1.5± 0.09
45	Control	15.2±0.72	26.3±0.79	100.48±3.4	0. 9± 0.21
	Pb	14.1±1.2	32.3±1.2	66.7± 5.2	2.1± 0.11
	Cd	14.1±0.52	34.1±0.8	53.7± 6.7	2.54±0.22
	Pb+Cd	13.2±0.32	31.7±0.92	67.7± 6.9	2.5±0.163

ALP: IU-u mols of PNP formed/min., (n = 5 or 6)

GPT: IU-u mols of pyruvate formed/min., (n = 5 or 6)

hypothalamic and pituitary enzyme activity was not showing any further inhibition when the time of exposure was increased from 30 to 45 days. This suggests two possible reasons, either saturation at the active site of the enzyme with the metals or protection of the enzyme by some metal binding protein, which may be induced with long time of exposure. With respect to the second reason, metallothionein present in hypothalamus and pituitary might be playing an important role as the protective mechanism against heavy metal toxicity. MT inducibility by cadmium has also been reported in pituitary cells (Henkle et al., 1987). Recently metallothioneins were identified both in the neuro and adenohypophysis of the bovine pituitary gland (Zatt et al., 2001).

Studies on distribution of lead and cadmium showed that the metal content in liver and blood was significantly increased by the treatment. Dose dependent increase in the tissue metal content underlined the observed decrease in metabolic enzyme activities after the treatment. It is known that regardless of the route of administration, the organs, which accumulate larger quantity of cadmium, are liver and kidney (Berlin and Ulberg, 1963). The histological studies showed that lead+cadmium treatment caused more cytotoxic effect compared to Cd and Pb individually. As both the metals are known to produce alterations in the hepatic tissue, when they are present together the effect (damage) will be more than the individual metal effect.

Lead and cadmium treatment decreased the activities of enzymes metabolizing estradiol, but among the two metals cadmium showed more inhibitory effect than lead due to the fact that cadmium is more hepatotoxic than lead (Cook et al., 1974), also the amount of cadmium acetate absorbed more in the hepatic tissue than lead acetate. Since the active site of 17β HSOR contains lysine and tyrosine residues the changes observed in the

activity of this enzyme can be due to the binding of the divalent metal ions to these amino acid residues. On the basis of the subunit mol. wt of the UDPG-Transferase there are approximately 531 aminoacid residues of which 48 mol% are polar residues and 52 mol% nonpolar. According to Gorski and Kasper (1977), UDPGT has methionine (Met) at its active site. Since Met contains –SH group, both lead and cadmium is able to bind to –SH groups resulting in a decrease of enzyme activity. Intermediate effects have been observed in the combined treated group, it can be proposed that two metals compete for a single binding site.

Maximum reduction in CYP P450 content in cadmium treated animals was also in accordance with catabolic enzymes. This indicated that conversion of 17β estradiol into its metabolites was apparently most affected by cadmium compared to other two treatments. The CYP P450 content was decreased further as the time of exposure of rats to metals is increased.

When excess toxic metals penetrate into the cell, they displace the loosely bound essential metals from the thioneins normally present in the cytosol, as evidenced by the reduction in the concentration of Zn bound to the metallothionein or metallothionein like proteins. Detoxification of a trace metal involves a combination of displacement of the nontoxic metal already bound to the protein by the toxic one and also denovo synthesis of the protein. Displacement of Zn from the metallothionein fraction was increased with increase in time of metal exposure. Both cadmium and combined groups were showing significant decrease in Zn level as compared to the other two groups. However the decrease in the concentration of Zn was not equal to the increase the Cd level in liver. If these results are to be consistent with the spill over hypothesis, then binding of Cd by MT

or MT like proteins should protect other proteins from the inhibitory effects which however is not true in the present study. An established deficiency of essential metal in the enzyme pool and the increased ability of toxic metals to compete for the binding sites of the high molecular weight proteins may explain the occurrence of high levels of toxic metal in the enzyme pool and thus causing intermediate inhibitory effects on the enzyme activities in the combined metal treated group.

Acid phosphatase (ACP) is a marker enzyme for lysosomal permeability. In order to evaluate whether the effect of heavy metals are due to a general increase in membrane leakiness or not, the permeability of the lysosomal membrane was evaluated by measuring ACP as indicator of lysosomal permeability. An increase in leakiness would have been associated with an increase in measurable activity of the enzyme. The results indicate that none of the heavy metals tested change the apparent permeability at the level of 0.025 mg/kg b.wt. dose. Significant increase in enzyme activity was observed in all metal treated groups at 0.05 and 0.1 mg/kg b.wt. doses. This indicates that both lead and cadmium can cause leakage in the lysosomal membrane, which results in the release of ACP from lysosomes. Lysosomal labilisation by these metals might be resulted from their high affinity binding with thiol groups, which are important functional groups for the integrity of lysosomal membrane.

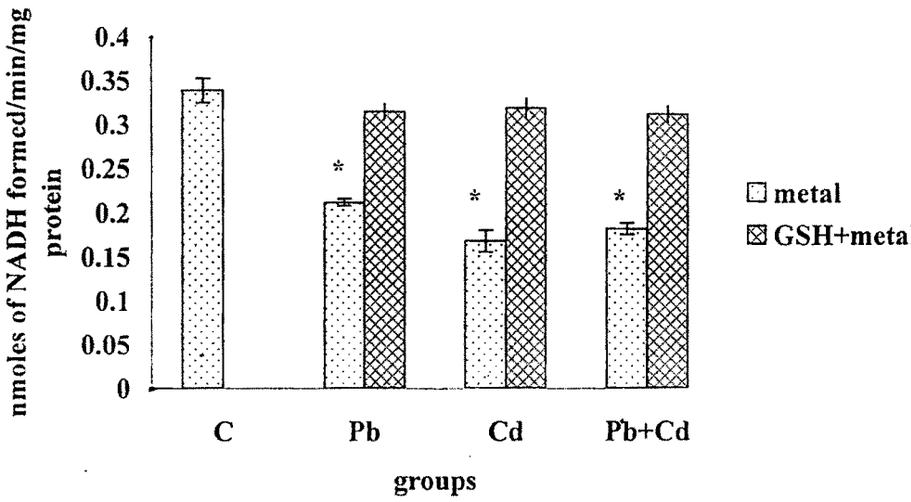
No change in hemoglobin levels was found after the metal exposure. The decrease in alkaline phosphatase (ALP) levels observed in the metal exposed animals could be due to the replacement of Zn, the metal ion present at the active site of ALP by the heavy metals. The statistically significant decrease of DNA content in liver suggests that liver acted as the main target organ, absorbing most of Cd overload. This is in agreement with

the observation of Dudley et al., 1985 that chronic exposure to Cd in rats resulted firstly in liver injury, even prior to renal damage. Lead toxicity appears to manifest itself in ways other than direct DNA damage, whereas Cd caused direct DNA damage.

To understand whether effect on metabolizing enzymes are direct or due to general hepatotoxicity, *in vitro* experiments with metal treatment with concentration equivalent to concentration reaching the tissue were performed. These results showed same order of inhibition, which suggests direct effect of metals; these effects can be protected by pretreatment with GSH (Fig 11). The steroid metabolizing enzyme activities were monitored in estrous stage also. But there was no significant difference observed between proestrous and estrous results.

From the present study it was seen that the combined metal exposed animals were showing intermediate effects on the enzymes, but the histological studies showed more cytotoxic effect in combined metal treatment group. Also the *in vitro* studies carried out with these metals having a concentration equivalent to their concentration in liver after the treatment have shown same order of inhibition in the activity of 17β HSOR. This implied that the changes in the metabolizing enzyme activities produced by the heavy metals were due to their direct inhibitory action rather than general hepatotoxicity. Thus it can be concluded that lead and cadmium, which are known endocrine disrupters not only act at the gonadal level but also play significant role in the neuroendocrine and hepatic estradiol metabolism. As estradiol can bind to their receptors present in hypothalamus and thereby regulate GnRH and gonadotropin release, the inhibitory effects on estradiol metabolizing enzymes observed in the present study directly implicate their role in regulation of hormonal milieu. Also, it might have long-term effect on estrous cyclicity and reproductive

Fig 11: Effect of lead and cadmium alone and in combination on hepatic 17β hydroxy steroid oxidoreductase activity (*in vitro* study) - role of GSH



*P<0.001 vs. control group.(n= 5-6)

behavior.

Summary

Adult synchronized female rats were treated intraperitonially with lead acetate and cadmium acetate separately and in combination in dose dependent manner (0.025, 0.05 and 0.1 mg/ kg body wt/day for 15 days) and time dependent manner (15, 30 and 45 days with 0.05 mg/ kg body wt/day). The hypothalamic and pituitary steroid metabolizing enzyme (3α-hydroxy steroid dehydrogenase) activity was inhibited. The cadmium treated group showed significant decrease in enzyme activity at 0.05 and 0.10 mg/kg body weight doses compared to other groups. In 0.025 mg/kg body weight dose there was no change in the enzyme activity in any of the treatment groups. The hepatic steroid metabolizing enzymes (17β-hydroxy steroid oxidoreductase and UDP glucoronyl transferase) activities decreased with increasing dose showing significant change compared to control. From the dose dependent studies, 0.05 mg/kg body weight was selected as the optimum dose. Significant decrease in cytochrome P450 (CYP450) content was found after the treatment. The enzyme activities were decreased with increase in time of exposure. Displacement of zinc bound to metallothionein was more in cadmium treated rats compared to other groups. Hepatic lysosomal membrane permeability was affected at 0.05 and 0.10 mg/kg body weight doses. There was a time and dose dependent decrease in serum ALP with slight elevation in SGPT in all metal exposed groups. No significant change was observed in hemoglobin and serum creatinine levels. There was a progressive increase in the hepatic content of lead and cadmium with increase dose and time of exposure.

The protein, DNA and RNA content were found to be decreased in all treated groups compared to control. The microsomal cholesterol levels were found increased after

the metal treatment in a dose dependent manner. In all these parameters, treatment in combination of lead and cadmium showed intermediate results indicating some kind of competition between the two metals. But the histological studies showed that combined treatment causes more cytotoxic effect than cadmium and lead alone. These results indicated that metal cations tested did have a direct inhibitory effect on the metabolizing enzyme activities. There was no significant change observed between estrous and proestrous results.