Chapter 3 Effect of lead and cadmium co-exposure on hypothalamus, pituitary, testicular and hepatic steroid metabolism in adult rats

3.1 Introduction

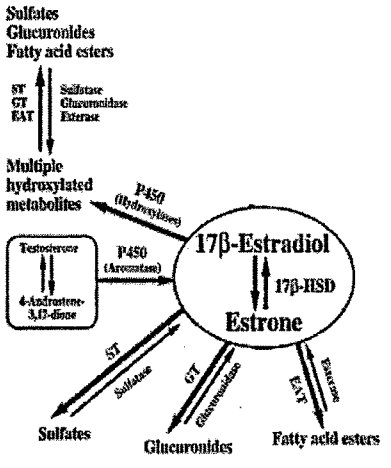
Male reproductive system is considered to be the main target for toxic environmental pollutants (Sokol, 1997). Steroid biosynthesis is as per the pathway depicted in figure 4 of chapter 1, where cholesterol is precursor. Both 3β-HSD and 17-β-HSD enzyme activities are the key enzymes of testicular steroidogenesis. These key enzymes belong to the class of hydroxysteroid dehydrogenases that catalyze the interconversion of hydroxyl and carboxyl groups of steroids. Apart from steroidogenesis, steroid metabolism is also important for the regulation of their cellular and physiological actions. 3β-HSD catalyzes the conversion of pregnenolone to progesterone and further dehydroepiendrosterone to androstenedione. Similarlly, 17β-HSD catalyzes the conversion of estrone to estradiol and androstenedione to testosterone. 3β-HSD and 17β-HSD are widely distributed in large number of peripheral tissue (Marte et al., 1992) skin, adipose tissue and liver etc (Bleau et al., 1974; Weisntein et al., 1968; Kaufmann et al., 1995).

Apart from steroid synthesizing tissue, regulatory organs - hypothalamus and pituitary also has a role in metabolism of steroids. Both hypothalamic and pituitary 3α-hydroxysteroid dehydrogenase (3α-HSD) catalyzes the oxido-reduction at carbon 3 of steroid hormones. It converts 5 alpha-pregnane-3,20-dione (DHP) into 5 alpha-pregnane-3 alpha-ol-20-one (3αHP). The purified enzyme displays one-sixtieth of the specific activity of the rat liver enzyme. No peptide sequence information exists on the purified rat brain enzyme. In rat brain, the regional distribution of enzyme activity shows that the highest levels are found in the olfactory bulb and olfactory tubercle, with lower amounts residing in the hypothalamus (Kreiger, 1984). In all brain regions studied, 3a-HSD activity was at least 100-fold higher than 5α-reductase activity, implying that in vivo, the formation of neuroactive tetrahydrosteroids is favored. (Kreiger, 1983). In the brain, 3α-HSD regulates the formation of the allosteric effectors, tetrahydroprogesterone and tetrahydrocorticosterone, that bind to the GABA receptor (Majewski et al., 1986; Majewski, 1992; Lambert et al., 1995). In this manner 3α-HSD can control Cl^- conductance and regulate the anxiolytic and

anesthetic actions of 3a-hydroxysteroids. Thus, 3α-HSD regulates the amount of hormone that binds to a receptor, whether it is a member of the nuclear receptor superfamily or a membranebound, ion-gated channel. (Trevor et al., 1996). The gonadal steroid 3αHP is a neuroactive steroid with anxiolytic and analgesic actions. In addition, 3αHP has been shown to inhibit GnRH activity on gonadotropes and selectively suppress FSH release from pituitary cells, without an effect on LH (Gao et al., 2002).

Liver is the main organ where metabolism of hormones as well as toxic elements occurs. Regulation of steroid is also governed by its catabolism. One route through which normal hormonal homeostasis could be disrupted is through xenobiotic induction of sex steroid metabolizing cytochrome P-450 enzymes. Inducing or blocking these liver metabolizing enzymes conceivably alters the natural balance of circulating sex steroids. Several mechanisms are used for hormone biotransformation in the liver. Major pathway of biotransformation includes 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). Oxido-reduction of estradiol to estriol is another route of hepatic biotransformation pathway that influences circulating concentrations of estradiol.

Figure 1. Estrogen synthesis and metabolism



Estradiol is synthesized from testosterone by a cytochrome P450 aromatase. Once formed, estradiol can be metabolized to multiple hydroxylated products by enzymes of the cytochrome P450 family. Estradiol and its hydroxylated metabolites can also be esterified to fatty acid esters or conjugated by glucuronidation or sulfonation which in turn may undergo de-esterification by esterase or deconjugation by b-glucuronidase or sulfatase to release active estrogens either in the liver or directly in target cells. This complexity of estrogen synthesis and metabolism (illustrated in Figure 1) provides many potential sites for the regulation of steroid action.

Both lead and cadmium have been reported to disrupt reproductive physiology and steroid metabolism at different levels, along the hypothalamic-pituitary-gonadal-hepatic axis (Sokol, 1997). Lead and cadmium, divalent environment toxicant is a potent disruptor of the male endocrine system (Ng and Liu, 1990; Singhal et al., 1985). All the above stated data have illustrated effects of single metal exposure. Also the metal exposure has been used in high concentration but in environment, population receives simultaneous multiple exposures, at low dose indicating the need for experimental work with combinations of toxicants. In this direction, our laboratory has earlier demonstrated potent effects of these metal ions in combination on female HPGH axis at various tissue levels (Pillai at al., 2002; Pillai et al., 2003; Laxmipriya et al., 2004). Eventhough the general toxicity of lead and cadmium in isolation 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.

In view of this, present study was performed to understand the effect of low level exposure of lead and cadmium both in isolation as well as in combination on hypothalamic, pituitary, testicular and hepatic steroid metabolism in adult male rats.

3.2 Experimental design

The experiment was carried out in two ways i.e., *in vivo* study and *in vitro* study.

3.2.1 In vivo studies:

Adult charles foster male rats weighing 200-220 g of body weight were maintained under controlled conditions (12 h light and 12 h dark period) and temperature (24 ± 2°C), having free access of diet and drinking water. The animals were divided into 4 groups containing 6-8 animals per groups. Group 1 received sodium acetate, Group 2 animals were exposed to lead acetate, Group 3 animals received cadmium acetate and Group 4 animals were exposed to both lead acetate and cadmium acetate in combination, intraperitoneally in a dose of 0.025 mg/kg body weight per day for 15 days. The combined exposure contains half of lead and cadmium for a total dose of 0.025 mg/kg. The dose was selected on the basis of previous studies of our laboratory on the effect of simultaneous exposure of lead and cadmium on hepatic estradiol metabolism (Pillai and Gupta, 2005). The animals were sacrificed by decapitation; the procedure was completed within 5 to 10 sec to avoid stressors. After 15 days of metal exposure, blood was collected from the orbital sinuses and serum was separated by centrifuging at 3000 rpm for 15 min at RT. The hypothalamus, pituitary, testis and liver were immediately excised out and processed for steroid metabolizing enzyme and assayed for activities as per method of Shivanandappa and Venkatesh, 1997. 3α hydroxy steroid dehydrogenase (3α HSD) activity was also assayed from hypothalamus and pituitary (Shivanandappa and Venkatesh, 1997). Testis was used for the mesurment of enzyme activities like 3β hydroxy steroid dehydrogenase (3β HSD) and 17β hydroxy steroid dehydrogenase (17β HSD). Activity of 17β hydroxy steroid oxidoreductase (17β HSOR) was assayed from liver. Also the xenobiotic metabolizing enzyme like UDP Glucoronyl Transferase (UDPGT; Gorski and Kasper, 1977) activity was carried out from liver. Biochemical parameters such as DNA (Burton, 1956) and RNA (Schneider, 1957) were also determined in the liver samples. Liver was used for estimation of various toxicity parameters such as serum glutamate pyruvate transaminase

(SGPT; Reitman and Frankel, 1957), alkaline phosphatase (ALP; Bowers & McComb, 1975). Both testis and liver were analyzed for lead and cadmium levels by GBC 902 Atomic Absorption Spectrophotometer (AAS). Histology of testis was done using standard histological techniques. Details of each method have been already described in chapter 2.

Serum and intra testicular concentrations of testosterone was measured with commercially available kit (Immunotech, France), following the radioimmunoassay (RIA) with a testosterone I^{125}. Radio activity was counted in gamma counter. Sample preparation was carried out using the method described by Tohda et al., 2001.

3.2.2 *In vitro* studies:

In order to understand direct toxic effect of metal, *In vitro* experiments were carried out; with the concentration of lead and cadmium reaching the tissues after *in vivo* exposure for 15 days. Testicular fraction obtained were incubated at 37°C for 1hour with 3 µM lead acetate, 0.5µM cadmium acetate and co-exposure of lead acetate (2µM) and cadmium acetate (0.015µM) in a mixture containing 0.175M KCl, 25mM Tris-HCl at pH 7.4 in a total volume of 1 ml. The reaction was stopped on ice. Aliquots were used to determine 3β-hydroxy steroid dehydrogenase and 17β-hydroxy steroid dehydrogenase enzyme activities (Shivanandappa and Venkatesh, 1997).

3.3 Results

3.3.1 *In Vivo* experiments:

The animals remained healthy and showed no signs of clinical toxicity throughout the exposure period. There were no significant change in the body weight of rats exposed to lead and cadmium in isolation and combination as compared to controls (Table 1). Tissue weights of hypothalamus, pituitary, testis, prostate and liver also remained unchanged in all exposed groups as compared with the corresponding group of control animals (Table 1).

Table 1. Effect of lead and cadmium alone and in co-exposure for 15 days (0.025 mg/kg body weight) on (A) Body weight gain and (B) Relative liver weights of hypothalamus, pituitary, testis, prostate and liver (g).

Parameters	Groups			
	Control	Pb	Cd	Pb + Cd
(A) Body weight gain(g)	53.2±1.0	54.9±1.83	48.8±2.8	51±1.8
(B) Hypothalamus(mg)	10 ± 0.4	9 ± 0.5	10 ± 1	8 ± 1
Pituitary(mg)	3.56 ± 0.2	3.3 ± 0.2	4.2 ± 0.3	3.7 ± 0.2
Testis(g)	0.49±0.01	0.49±0.03	0.59±0.02	0.51±0.04
Liver(g)	3.49±0.19	3.45±0.19	3.99±0.25	3.36±0.23

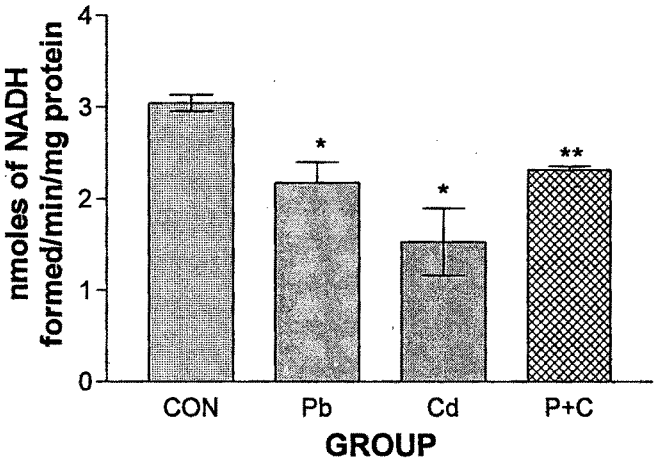
Values are expressed as mean ± SEM (n= 8-10 in each group).

Table 2. Effect of lead and cadmium in isolation and co-exposure on hepatic DNA, RNA content and alanine transaminase activity of adult male rats.

Group	DNA (µg/g)	RNA (µg/g)	ALT (IU)	ALP (IU)
Control	322 ± 37	0.076 ± 0.007	32.55 ± 3.15	2.06 ± 0.30
Lead	214 ± 11*	0.065 ± 0.001	31.94 ± 5.08	0.77 ± 0.12**
Cadmium	182 ± 24**	0.063 ± 0.002	35.13 ± 1.51	0.73 ± 0.14**
Lead + cadmium	277 ± 37	0.071 ± 0.005	34.90 ± 3.76	1.78 ± 0.31#$

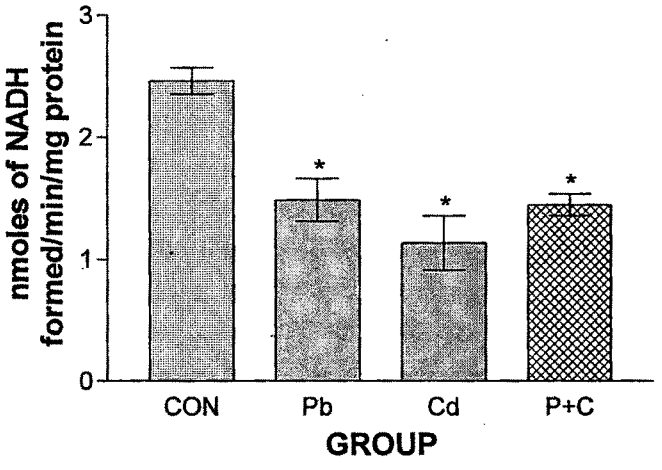
Values are expressed as mean ± SEM (n = 4-6 in each group). *P<0.05, **P<0.01 vs. control; #P<0.05 vs Pb; $P<0.05 vs Cd group.

Figure 2. Effect of lead and cadmium alone and co-exposure on hypothalamic 3α-hydroxy steroid dehydrogenase activity in adult male rats treated with a dose of 0.025 mg/kg body weight daily for 15 days.



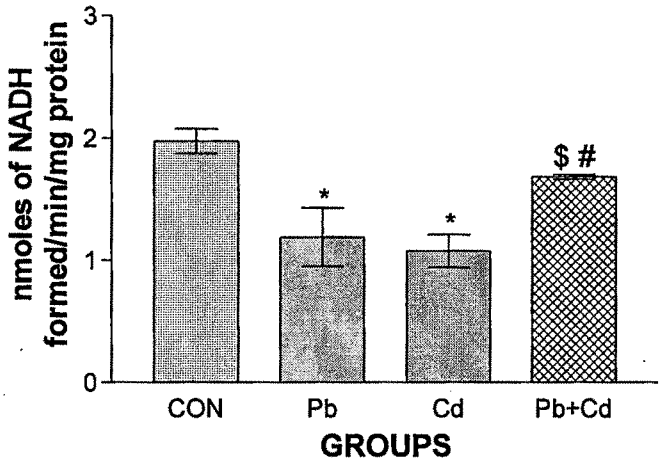
Values are expressed as mean ± SEM (n=5 in each group).
*p<0.01, **p<0.001 vs. control group.

Figure 3. Effect of lead and cadmium alone and co-exposure on pituitary 3α-hydroxy steroid dehydrogenase activity in adult male rats treated with a dose of 0.025 mg/kg body weight daily for 15 days.



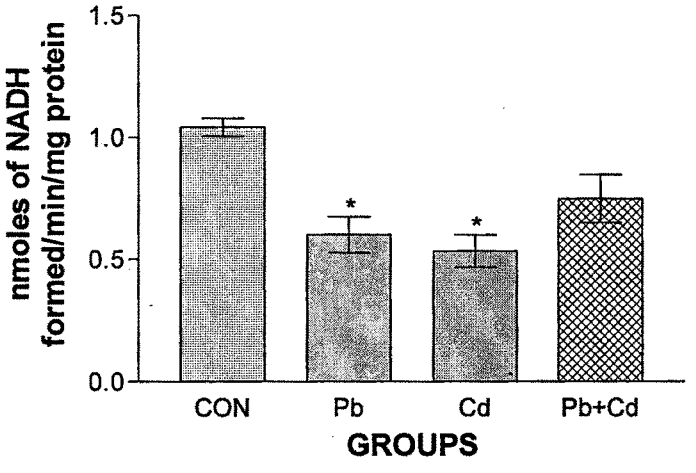
Values are expressed as mean ± SEM (n=5 in each group).
*p<0.001 vs. control group.

Figure 4. Effect of lead and cadmium alone and co-exposure on testicular 3β-hydroxy steroid dehydrogenase activity in adult male rats treated with a dose of 0.025 mg/kg body weight daily for 15 days.



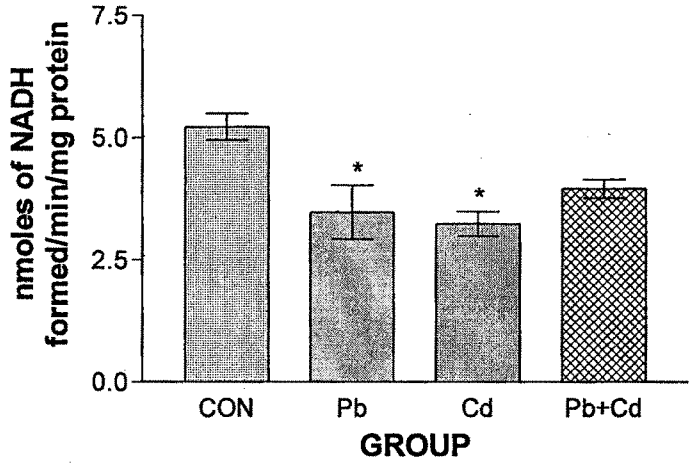
Values are expressed as mean ± SEM (n=5 in each group). *p<0.01 vs. control; #P<0.05 vs. lead and $P<0.05 vs. cadmium group

Figure 5. Effect of lead and cadmium alone and co-exposure on testicular 17β-hydroxy steroid dehydrogenase activity in adult male rats treated with a dose of 0.025 mg/kg body weight daily for 15 days.



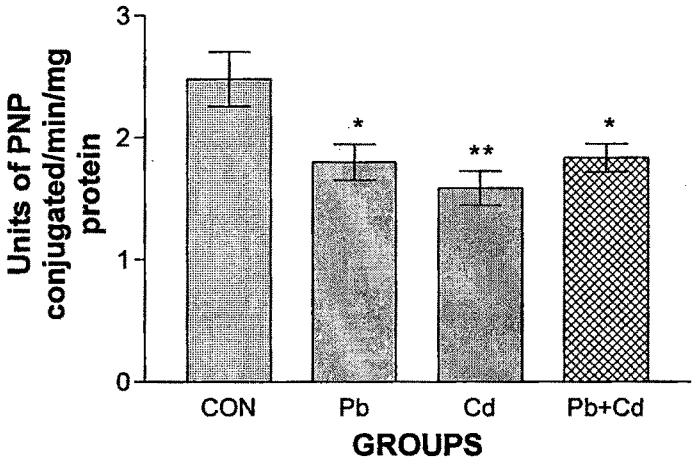
Values are expressed as mean ± SEM (n=5 in each group).
*p<0.01 vs. control

Figure 6. Effect of lead and cadmium alone and in co-exposure on liver 17β-hydroxy steroid oxidoreductase activity in adult male rats treated with a dose of 0.025 mg/kg body weight daily for 15 days.



Values are expressed as mean ± SEM (n=5 in each group).
*p<0.01 vs. control group.

Figure 7. Effect of lead and cadmium alone and in co-exposure on liver UDPGT activity in adult male rats treated with a dose of 0.025 mg/kg body weight daily for 15 days.



Values are expressed as mean ± SEM (n=5 in each group).
*p<0.05, **p<0.01 vs. control group.

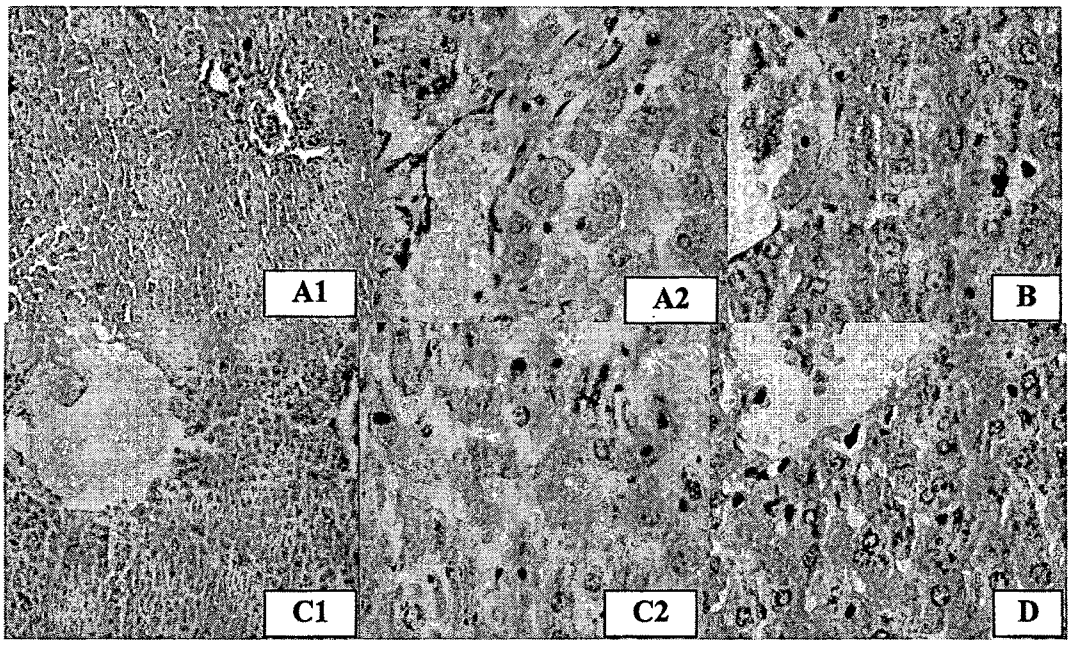
The activities of 3α-HSD were significantly affected in hypothalamus (Figure 2) and pituitary (Figure 3) in all metal exposed groups as compared to control groups. However, no significant change was observed in 3α-HSD enzyme activity within the groups both in hypothalamus and pituitary. Hypothalamic 3α-HSD activity was significantly ($p < 0.01$) reduced to 29%, 50% and 24% of the controls on treatment with Pb, Cd and co-exposure (Pb and Cd) respectively. Compared to the control, activity of pituitary 3α-HSD was markedly reduced to 39% (Pb exposure), 44% (Cd exposure) and 41% (co-exposure of Pb and Cd) ($p < 0.01$). The pattern of inhibition for 3α-HSD activity in both the tissues were similar, where in cadmium exposed animals showed maximum inhibition. Figures 4 and 5 explain the effect of lead and cadmium in isolation and in combination on testicular 3β-HSD and 17β-HSD activities. Both enzyme activities were significantly decreased in all metal exposed groups compared to control. Cadmium exposed group show highest reduction (33%) in 3β-HSD enzyme activities followed by lead (47%), while co-exposure (26%) showed antagonist effect, hence least toxic. Similarly, 17β-HSD activity was decreased up to 37%, 50% and 14% in Pb, Cd and coexposed groups respectively.

Activities of liver 17β-HSOR and UDPGT after the metal exposure are shown in Figure 6 and 7. The 17β HSOR enzyme activity was most affected in cadmium exposed group (38%). Lead exposed group demonstrated a intermediate effect (34%) while the combined exposure group was least effective (24%). Similar pattern was also observed in UDPGT activity. These results indicated that lead and cadmium exposure affected the hepatic steroid catabolism and their biotransformation.

General toxicity parameters in liver are represented in Table 2. Liver function tests like serum GPT and serum ALP were done to evaluate the toxic effect of administered dose of metal. All metal exposed groups demonstrated a decrease in serum ALP activity while, SGPT activity exhibited no change and within the normal range. Table 2 also summaries the data on hepatic DNA and RNA content. Hepatic DNA content was significantly decreased in lead and cadmium

exposed groups compared to control. Cadmium exposed group demonstrated highest reduction in both levels followed by lead, while co-exposure exhibited antagonist effect, hence least toxic. Metal alone and combination does not exhibit any change with respect to RNA content. Histological observations of testis after lead and cadmium exposure are shown in Figure 8. The lead and co-exposed groups did not reveal any alteration. Cadmium treatment caused marked changes in liver such as massive fatty degeneration in hepatocytes. Nucleus was pycnotic in appearance and necrotic regions could be seen.

Figure 8. Histological observation of the liver after lead and cadmium exposure.



(A) Liver of control, (A1=10 X, A2=40 X) **(B)** Pb treated group (40 X) and **(D)** Pb+Cd treated (40X) group rat showing normal hepatocytes. **(C)** Hepatocytes showing pycnotic nuclei with moderate degenerative changes and large vacuoles after exposure to 0.025 mg/kg body wt. dose of cadmium for 15 days (C1=10 X, C2=40 X).

The testicular and hepatic concentration of lead and cadmium in the adult male rats are shown in Table 3 and Table 4 respectively. Both metals were accumulated in the testis and liver in amounts significantly higher than in control group after exposure of 0.025 mg/kg body weight for 15 days. Testicular lead concentration was higher in lead (80% increase; $P<0.05$) and combined (26% increase; $P<0.05$) exposed groups and cadmium concentration was higher in cadmium (688% increase; $P<0.01$) and combined (129% increase; $P<0.01$) exposed groups, compared to control group. Similarly in liver, the concentration of lead in lead and combined metal exposed groups was significantly higher (99 and 26% increase respectively). Hepatic cadmium concentration was higher in cadmium (1270% increase) and combined (87% increase) exposed groups than in control. When lead and cadmium present together, accumulations of both metals in testis as well as liver were decrease compared to individual exposed groups.

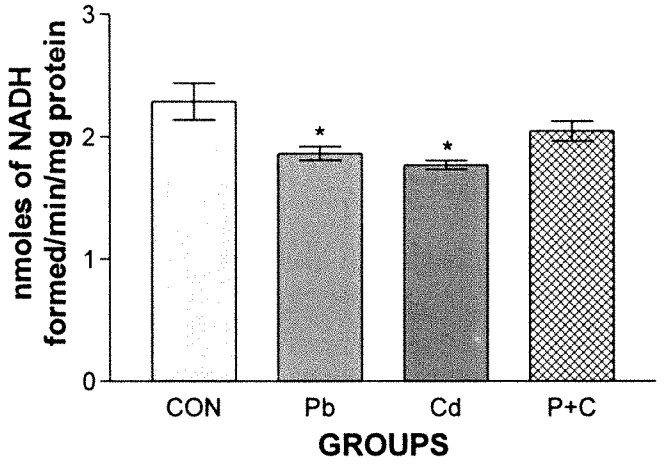
3.3.2 *In Vitro* experiments:

To evaluate the direct effects on steroidogenesis, we carried out in vitro experiments and measured the enzyme activities of both 3β-HSD (Figure 9) and 17β-HSD (Figure 10). 3β-HSD activities were reduced with cadmium (23%) followed by lead (19%) and co-exposed groups (11%). 17β-HSD activity was reduced to 7%, 25% and 10% of the controls on treatment with Pb, Cd and co-exposure (Pb and Cd) respectively. Extent of inhibition in enzyme activity was lower in *in vitro* studies than *in vivo* experiment.

3.4 Discussion

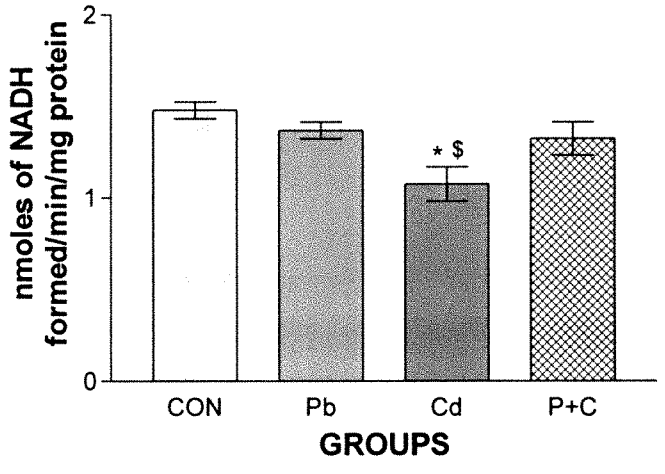
The results from the present study indicate that exposure to lead and cadmium (0.025 mg/kg body weight daily for 15 days, intraperitoneally), resulted in altered hypothalamic, pituitary, testicular and hepatic steroid metabolism.

Figure 9. 3β-hydroxy steroid dehydrogenase activity in testicular fraction after treatment with lead and cadmium alone and in co-exposure *in vitro*.



The values are expressed as mean ± SEM (n=4 in each group).
*p<0.05 vs control group.

Figure 10. 17β-hydroxy steroid dehydrogenase activity in testicular fraction after treatment with lead and cadmium alone and in co-exposure *in vitro*.



The values are expressed as mean ± SEM (n=4 in each group).
*P<0.05 vs control; P<0.05 vs lead group.

Table 3. Lead and cadmium levels in the testis of male rats exposed to lead acetate and cadmium acetate alone and co-exposure for 15 days (0.025 mg/kg body weight per day).

Group	Lead (μg/g)	Cadmium(μg/g)
Control	0.602 ± 0.03	0.017 ± 0.003
Lead	1.085 ± 0.07**	0.013 ± 0.002
Cadmium	0.517 ± 0.04$^{\#\#}$	0.134 ± 0.01$^{**\#\#}$
Lead + cadmium	0.757 ± 0.03$^{*\#\Psi}$	0.039 ± 0.004$^{*\#\#\Psi\Psi}$

Values are expressed as mean+ SEM (n=5 in each group).
*P<0.01, **P<0.001 vs. control;
#P<0.01, ##P<0.001 vs. lead and ΨP<0.01, ΨΨP<0.001 vs. cadmium group.

Table 4. Lead and cadmium levels in the liver of male rats exposed to lead acetate and cadmium acetate alone and co-exposure for 15 days (0.025 mg/kg body weight per day).

Group	Lead (μg/g)	Cadmium (μg/g)
Control	0.4546 ± 0.072	0.07425 ± 0.00725
Lead	0.9128 ± 0.0788*	0.006833 ± 0.00296**
Cadmium	0.1991 ± 0.0311$^{**\#\#\#}$	1.017 ± 0.1329$^{**\#\#}$
Lead + cadmium	0.5738 ± 0.0376$^{\#\Psi\Psi}$	0.1388 ± 0.04609$^{\#\Psi}$

Values are expressed as mean+ SEM (n=5 in each group).
*P<0.05, *P<0.01, **P<0.001 vs. control;
#P<0.05, ##P<0.01, ###P<0.001 vs. lead and ΨP<0.01, ΨΨP<0.001 vs. cadmium group.

Both lead and cadmium inhibit the activity of the enzyme 3α-HSD in hypothalamus and pituitary. 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). Also, Cys-242, and Cys-217 play an important role in substrate binding affinity and alteration in structure can increase Km values for NADH (Pawlowski and Penning, 1994). Thus, the decrease in enzyme activities observed, could be due to the known general mechanism of toxicity for these metals i.e., binding to the -SH groups present in the cysteine residues. We also assayed 3β-HSD (Figure 1) and 17β-HSD (Figure 2) enzyme activities as the markers of steroidogenesis in testis. Both the key enzymes belong to the class of short chain alcohol dehydrogenases, which contain the Tyr-X-X-X-Lys at its active site (Persson et al., 1991). The metal ions lead and cadmium can interact with these amino acid residues or get bound to the -SH groups of cysteine residues present at the NAD binding domain (Persson et al., 1991) and alter the structure so that the substrate binding and function gets significantly affected.

The present study demonstrates that exposure to lead and cadmium either alone or in combination decreases the activities of steroids catabolising enzymes, 17-βHSOR and UDP glucoronyl transferase. Since the active site of 17-βHSOR contains lysine and tyrosine residues the changes observed in the enzyme activities can be due to the binding of the divalent metal ions to these amino acid residues. The inhibition in the activity of UDP glucoronyl transferase can be explained by a similar mechanism as HSD inhibition since the enzyme has methionine (Met) at its active site. Lead and cadmium are known as sulfhydryl reactive metals, thus can interact with the -SH group of Met resulting in decrease enzyme activity. Also our earlier work on female rats showed similar kind of inhibition (Pillai et al., 2002). Between the two metals used, cadmium showed more inhibitory effect than lead. This could be due to the fact that cadmium is more hepatotoxic than lead (Cook et al., 1974) and the amount of admiumacetate retained is more than lead acetate in all tissues.

Amongst all tissue, testis exhibited the highest reduction in steroidogenic enzyme activities with metal exposure. This could be due to the inability of metals to induce metallothionein (MT) protein in interstitial cells of testis (Ren et al., 2003b). *In vitro* exposure of metals demonstrated lower extent of inhibition on 3β-HSD and 17-βHSD enzyme activities, compared to *in vivo* exposure. This suggests the important role of hypothalamus and pituitary in regulation of gonadal steroidogenesis.

The lowest effects observed in the co-exposed group might be due to the competition between the two metals for a single binding site. Our results show that testicular lead content was decreased by 14% in cadmium exposed rats, while cadmium content was decreased by 23% when lead is given. While in liver accumulation of lead content was decreased by 33% in cadmium exposed rats, and cadmium content was decreased by 91% when lead is given. Thus, suggesting higher elimination of cadmium by lead in testis as well as liver compared to control. Also the testicular accumulation of lead and cadmium in lead and cadmium exposed groups are 80% and 688% higher respectively compared to control. When both metals were given together, accumulation of lead declined from 80% to 26% (3 fold decrease), while cadmium content declined from 688% to 129% (5 fold decrease). Similarly, liver co-exposed group showed a ~4 fold and ~13 fold decline in lead and cadmium content respectively. This suggests that higher percentage elimination of more toxic metal, i.e. cadmium could be one of the reasons for co-exposed group being least affected. Hypothalamic and pituitary metal content could not be estimated due to insufficient amount of tissue.

The greater degree of toxicity exhibited by cadmium would be also correlated to metallothionein (MT) status of tissue. Metallothionein, a sulfahydryl rich protein, binds with heavy metal like cadmium and reduces the bioavailability and plays an important role in Cd^{2+} homeostasis. Lead also binds metallothionein, but with lesser affinity as compared to Zn/Cd and does not appear to displace cadmium or zinc. Previous studies demonstrated that metallothionein (MT) gene expression appears to be not only tissue specific but

also cell specific (Vasconcelos et al., 2002; Ren et al., 2003a). In rodents, the testis has been shown to have a higher sensitivity to cadmium induced toxicity than many other tissues (Abe et al., 2000). Recent study also reported that cadmium induces MT mRNA level without increases in MT protein in interstitial cells of the testis. The inability to induce metal-detoxicating MT-protein, in response to cadmium, might account for the higher susceptibility of testis to metal toxicity, while in liver both mRNA and protein increases in response to metal induction (Ren et al., 2003b).

Histological studies showed that only hepatic cadmium treatment caused cytotoxic effect compared to control. As cadmium is more hepatotoxic and significantly accumulates in liver it could be one of the reason for marked alteration in histological observations.

The decreased ALP activity could be due to replacement of the zinc (Zn) from the active site of ALP molecule by lead and cadmium. The specific activity of alanine transaminase ALT, a marker enzyme for hepatic injury remained unchanged indicating absence of tissue injury in lead and cadmium in isolation and combination on exposed rats. The statistically significant decrease of DNA content in liver suggests that liver absorbs most of cadmium overload and act as a target organ for heavy metal toxicity. This is in agreement with the observation that chronic exposure to cadmium in rats resulted firstly in liver injury, even prior to renal damage (Dudley et al., 1985). Lead toxicity appears to manifest itself in ways other than direct DNA damage, whereas cadmium caused direct DNA damage. In conclusion, the results of present study indicated that even sub-clinical exposure of metals can affect steroid metabolism both by direct and indirect effects.

Overall inhibitory effect of metals on steroidogenesis, the peripheral conversion of the steroids in hypothalamus, pituitary and its biotransformation in the liver thus, results in disrupted homeostasis of the hormones. As reported earlier, inhibition of 3α-HSD activity in pituitary and hypothalamus will decrease local concentration of tetrahydroprogesterone and 3α HP, which are known to regulate GnRH action on gonadotrophs (Gao et al., 2002). Similarly,

decreased 3β and 17β-HSD activity in testis will lead to decreased testosterone production causing altered feed back inhibition on pituitary thus causing disruption of the HPG axis. Inhibitory effect on biotransformation enzymes will cause decreased elimination thereby causing overall disturbance in gonadal steroid milieu.

3.5 References

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