Chapter 4 Effect of lead and cadmium co-exposure on hypothalamic-pituitary-testicular axis function

4.1 Introduction

The gonad is considered the main target for environmental toxins (Sokol et al., 1977). Lead and cadmium, among them, are very dangerous to testicular function (Garside and Harvey, 1992; Laskey and Phelps, 1991). Exposure to lead and cadmium is associated with changes in the activity of endocrine system in male and female animals. It was shown that metals can affect the activity of hypothalamus-pituitary-testicular axis by acting at the hypothalamus (Anderson et al., 1997; Antonio et al., 1999; Das et al., 1993), the pituitary (Lafuente et al., 1999; Ronis et al., 1998), the testis (Lafuente et al., 2000) and/or the accessory organs (Klinefelter and Hiss, 1998).

Different studies have shown that cadmium affects plasma gonadotropin levels (Paksy et al., 1989; Lafuente *et al.*, 1999) and the content of several neurotransmitters in discrete areas of the brain (Hrdina et al., 1974). Changes in dopamine, serotonin, and norepinephrine contents in brains after metal exposure were also reported (Chandra et al., 1985; Shukla et al., 1984). Pb and Cd alone or in combination were also caused modifications in serotonin, 5-hydroxyindolacetic acid, dopamine, and norepinephrine contents in the cortex, striatum, and hippocampus of rats (Nation *et al.* 1989). Low-level Cd exposure results in increased catecholamine neurotransmission (Arito et al., 1981; Cooper and Manalis, 1983; Nation et al., 1989). Also, lead exposure can cause changes in catecholaminergic functions (Cooper and Manalis, 1983; Nation et al., 1989; Winder and Kitchen, 1984). Shukla et al., and Das et al., measured the contents of serotonin in the whole hypothalamus, because this area is involved in pituitary hormone release. Catecholamines and indoleamines are other neurotransmitters of the central nervous system which modulate pituitary hormone secretion (Drouva and Gallo, 1976; Lopez et al., 1989).

Most of the above cited studies were carried out with single metal. Available data on combined exposure of lead and cadmium shows that these metals show additive effect on acetyl choline release at the frog neuromuscular junction (Cooper and Manalis, 1984) whereas Nation et al., 1989 observed lesser effects of combined exposure than that in isolation.

Heavy metals like lead and cadmium are among the main endocrine disruptors that can cause male infertility by different fertile stage (Burdorf et al., 2006). Gonadal dysfunction and congenital malformation are the main alterations caused by these substances in the male reproductive system (Burdorf et al., 2006). The diminution of semen quality due to occupational exposure of heavy metals is a major health concern in the globe. (Apostoli et al., 1998; Alexander et al., 1996) Lead exposure and moderate lead absorption produces alteration in fertility with decreased production in spermatozoa in the battery factory workers probably due to the direct toxic effect of lead on germinal epithelium of testis during spermatogenesis (Lancranjan et al., 1975; Bonde et al., 1997). Studies also indicate that, spermatogenesis was reduced in workers exposed to metals (smith et al., 1998) and cause male infertility (Susan et al., 2003). Blood lead level was also inversely correlated with sperm count and viability (Rosa et al., 2003). Reduction in sperm motility, count, density, and low antioxidant profile along with increase incidence of sperm abnormality and sperm membrane lipid peroxidation was prevalent after occupational lead exposure (Rhemrev et al., 2001; Jiun et al., 1994). The positive correlation between heavy metal lead and cadmium in the seminal plasma of oligo, astheno, teratospermia group was found by Kasperczyk et al (Kasperczyk et al., 2002). The gradual decline of semen cholesterol with decreasing sperm count was also evidenced from the earlier study (Das et al., 1974). Interference of inorganic lead to the hypothalamic-pituitary-gonadal axis, semen characteristics, and accessory gland function was prevailed among the working population (Ng TP et al., 1991; Winder et al., 1993). However, very little information is available on sperm motility, activity, and maturation in case of lead and cadmium co-exposure.

In realty Pb and cd coexist in atmosphere. Therefore the effect that is manifested is usually the interaction of these metal toxicants. Therefore it would be worthwhile to study the role of Pb and Cd, both in isolation as well as in combination on male reproductive system. Earlier study in our laboratory in this direction, have shown that co-exposure of Pb and Cd affects Hypothalamic-

Pituitary-Ovarian axis (Pillai et al., 2003; Nampoothiri et al., 2004, 2007). However, the effect of low level co-exposure of Pb and Cd on H-P-T axix has not been clearly demonstrated.

In earlier chapter it has been shown that steroid metabolizing enzymes are inhibited by lead and cadmium both in hypothalamus and pituitary. Also the key enzymes responsible for testosterone production were found to be inhibited by Pb and Cd exposure (Chapter 3). To understand the hypothalamic-pituitary-testicular axis function, it was essential to know if the alterations in testosterone by metal exposure are mediated by changes in the neurotransmitter levels apart from enzymatic inhibition. Therefore, in present study an attempt has been made to evaluate the effect of lead and cadmium either alone or in combination on hypothalamic neurotransmitter content and serum and testicular levels of testosterone.

4.2 Experimental design

There were four groups of 6 animals each in the study. Group 1- animals treated with sodium acetate as control, Group 2- lead acetate, Group 3- cadmium acetate and Group 4- received lead acetate and cadmium acetate in combination. The route of treatment is intraperitoneal with 0.025 mg/kg body weight of metal, per day for 15 days. The combined exposure consists half of lead and cadmium for a total dose of 0.025 mg/kg. The animals were sacrificed by decapitation; the procedure was completed within 25 sec to avoid stressors and different parameters were estimated from experimental animals.

The hypothalamus was immediately removed and processed for further assays. Dopamine and norepinephrine were estimated in the hypothalamus samples by the flourimetric method of Shellenberger and Gordon, 1971.

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 determined by gamma scintillation counter. Sample preparation was carried out using the method described by Tohda et al., 2001.

Testicular and epididymis sperm count and epididymis sperm motility was evaluated using methods described by Eliasson et al., 1977. The epididymis and prostatic fructose content was estimated by Motoshima and Settlage, 1978. Histology of testis was done using standard histological techniques.

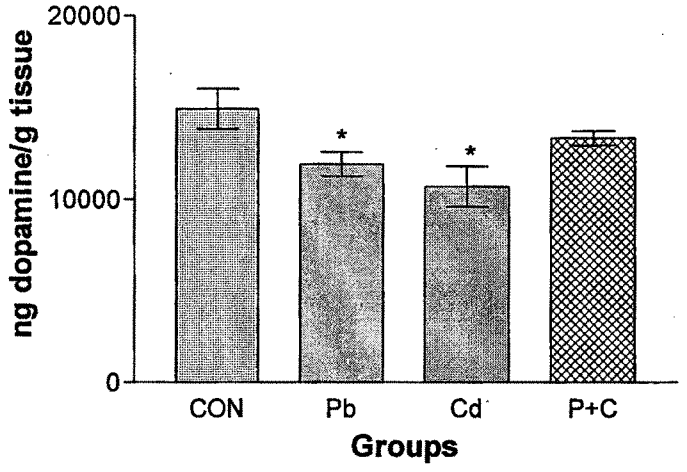
4.3 Results

Figure 1 and 2 shows the estimated concentration of hypothalamic norepinephrine and dopamine in various groups. The norepinephrine content was decreased in all metal treated groups with cadmium showing maximum reduction. The combined treatment with lead and cadmium was associated with changes in norepinephrine relatively similar to those of control group. The dopamine content was also decreased in both isolated and combined treated groups. Among the three groups cadmium exposure was showing maximum reduction in dopamine content.

The animals of lead and cadmium in isolation and combination treated groups showed significant decrease in the serum (Figure 3) and intra testicular (Figure 4) testosterone levels as compared to the control group. Amongst all the treated groups, cadmium again show maximum decrease while co-exposure group exhibited minimum reduction in both serum and intra testicular testosterone levels compared to control.

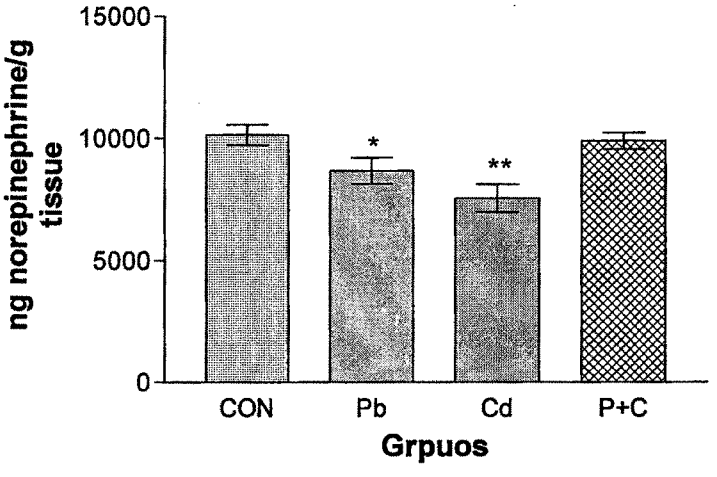
Table 1 shows the effect of lead and cadmium exposure on testicular and epididymal sperm count as well as epididymal sperm motility. Experimental groups showed significantly lower testicular and epididymal sperm count as compared to controls. Epididymal sperm motility was also significantly lower as compared to control groups. Cadmium group exhibited maximum effect and co-exposure showed lowest reduction in sperm count and motility. Figures 5 and 6 summarize the effect of lead and cadmium in isolation and in combination on epididymal and prostate fructose content. Epididymal fructose content was significantly decreased in cadmium exposed animals compared to control, lead and co-exposed groups whereas cadmium and combined metal exposed groups showed significant increase in prostatic fructose content.

Figure 1. Effect of lead and cadmium in isolation and co-exposure on hypothalamic dopamine content.



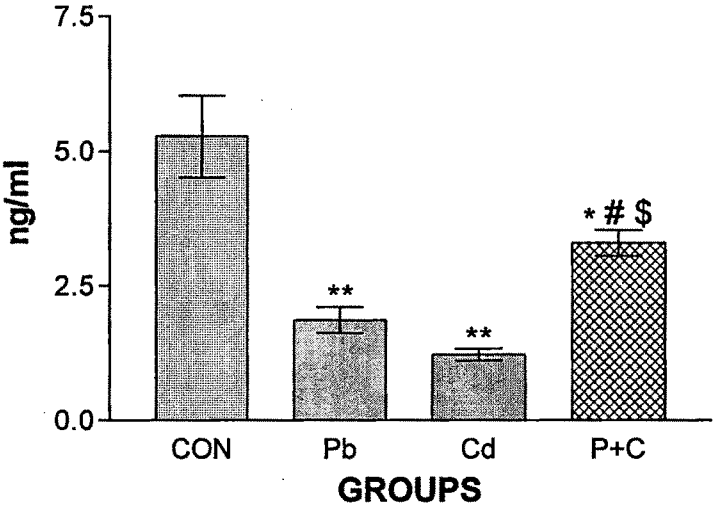
Values are expressed as mean ± SEM (n=4 in each group).
*P<0.05 vs. control group.

Figure 2. Effect of lead and cadmium in isolation and co-exposure on hypothalamic norepinephrine content.



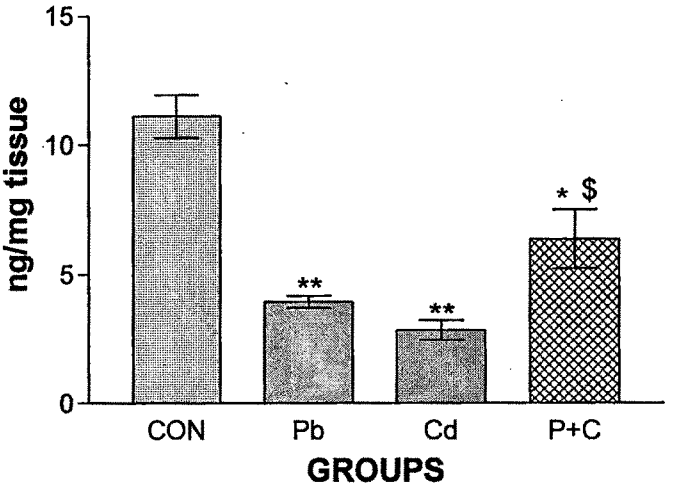
Values are expressed as mean ± SEM (n=4 in each group).]
*P<0.05, ** P<0.01 vs. control group.

Figure 3. Effect of lead and cadmium alone and in co-exposure on serum testosterone levels.



Values are expressed as mean ± SEM (n=4 in each group).
*p<0.05, **p<0.01 vs. control; #P<0.05 vs. lead and $P<0.01 vs. cadmium group.

Figure 4. Effect of lead and cadmium alone and in co-exposure on intra testicular testosterone levels.



Values are expressed as mean ± SEM (n=4 in each group).
*p<0.05, **p<0.01 vs. control and $P<0.01 vs. cadmium group.

Table 1. Effect of lead and cadmium in isolation and co-exposure on testicular, cauda epididymal sperm count levels and cauda epididymal sperm motility.

Groups	Testicular Sperm count (x 10^6)	Cauda Epdidiymal sperm count (x 10^6)	Cauda Epdidiymal % sperm motility
Control	119.6 ± 1.01	20.8± 2.4	73 ± 5.2
Lead	99.2 ± 3.5**	12.2± 1.5*	51 ± 2.3*
Cadmium	85.4 ± 6.1**	8.4 ± 2.8**	34 ± 2.3**$$
Lead+Cadmium	109.6 ± 3.6* #	13.5 ± 2.3*##	58.5 ± 4.9#

Values are expressed as mean ± SEM (n=6 in each group).
*P<0.05, ** P<0.01 vs. control;
$ P<0.05, $$ P<0.01 vs. lead and # P<0.05, ## p<0.01 vs. cadmium group

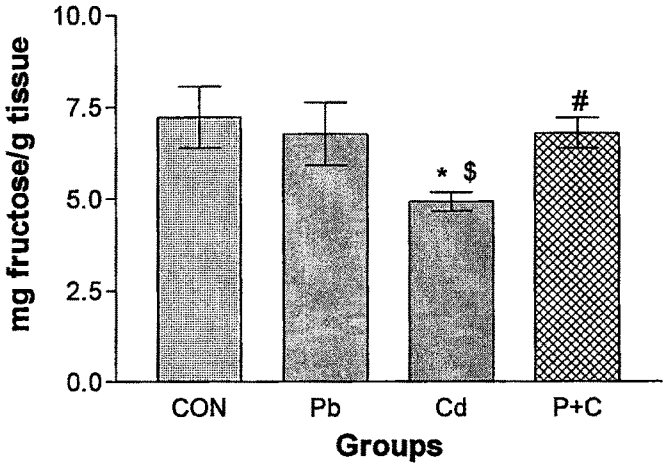
Histological observation of testis was carried out simultaneously. There is and no significant change was observed in testis histology (Figure 7) with lead and cadmium exposed groups compared to control group.

4.4 Discussion

Decrease in norepinephrine content due to the inhibitory effect of cadmium observed in the present study agrees with the results found by Lafuente et al., 2000, where they have shown the effect of cadmium in different hypothalamic regions. Our results indicate that the decrease in norepinephrine content was higher in cadmium exposed group than in the animals receiving lead alone and lead and cadmium in combination. Norepinephrine plays an important role in modulating luteinizing hormone releasing hormone (LHRH) neurons that are involved in the regulation of gonadotropin secretion by the anterior pituitary, which is further essential for testosterone synthesis at testis. It has been reported that lead can act at the hypothalamic level to alter LHRH secretion in the rat (Bratton et al., 1994). Lead blocks the NE induced release of PGE_2 resulting in diminished LHRH secretion. This decrease could be due to the altered Ca^{2+} mobilisation, a limiting step in PGE_2 formation and subsequent

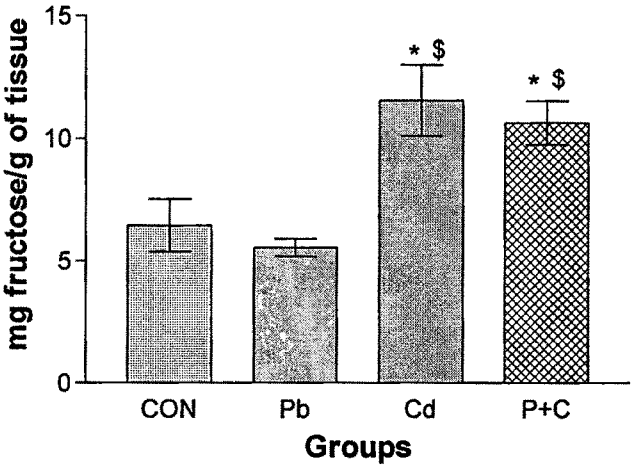
LHRH release. Dopamine (DA) content was not affected by lead alone or by simultaneous exposure of lead and cadmium whereas cadmium alone was showing significant decrease in the DA content. Various rodent studies on the subchronic exposure of lead resulted in significant reduction in DA and its metabolites 3,4 dihydroxyphenylaceticacid and homovanillic acid in the nucleus (Jadhav and Kala, 1994; Kala et al., 1994; Kala and Jadhav, 1995). The discrepancies with other studies could be due to differences in the age of the animals, mode of treatment, dose, duration and brain areas analyzed. Lead has been reported to alter calcium homeostasis by affecting both voltage dependent and receptor operated calcium channels (Audesirk, 1993; Bressler and Goldstein, 1991; Oortgiesen et al., 1993) whereas the invitro studies have shown that lead enhances calcium activated release in brain transmitters (Minnema et al., 1988). Cadmium is shown to inhibit calcium entry and the attendent release of peripheral catecholamines (Hirning et al., 1988). The lowest change observed in the cotreatment group might be due to the fact that lead and cadmium compete with calcium for entry through terminal membrane channels (Cooper and Manalis, 1984) and thus affect neurotransmitter release.

Figure 5. Effect of lead and cadmium in isolation and co-exposure on epididymal fructose content.



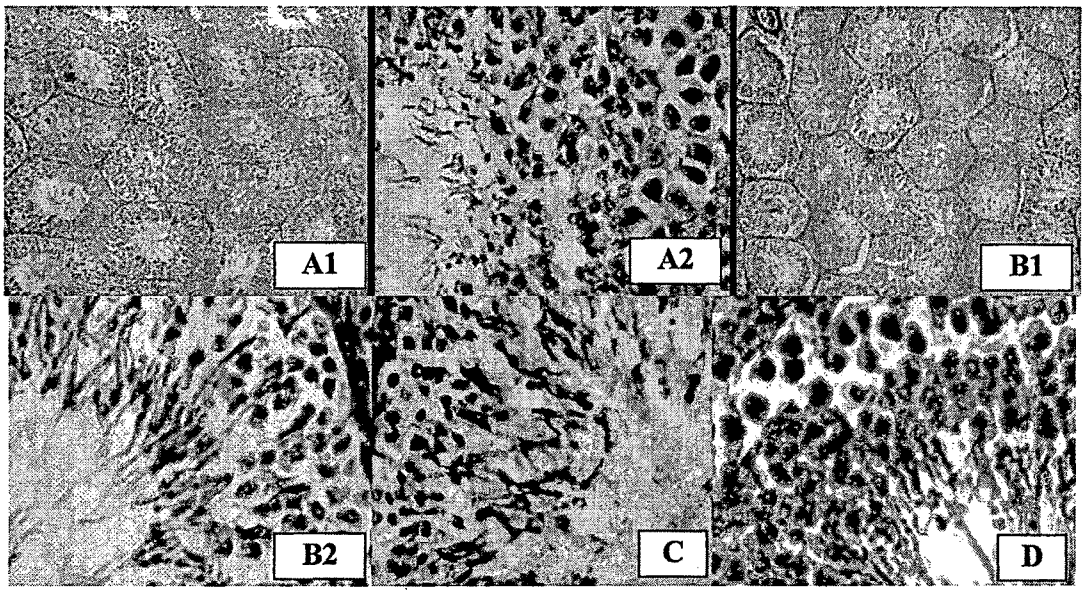
Values are expressed as mean ± SEM (n=6 in each group).
*P<0.05 vs. control; $ P<0.05 vs. lead and # P<0.01 vs. cadmium group

Figure 6. Effect of lead and cadmium in isolation and co-exposure on prostatic fructose content.



Values are expressed as mean ± SEM (n=6 in each group).
*P<0.05 vs. control; $ P<0.01 vs. lead and # P<0.01 vs. cadmium group

Figure 7. Histological observation of the testis after lead and cadmium exposure.



(A) Testis of control, (A1=10 X, A2=60 X) **(B)** Pb treated group (B1=10 X, B2=60 X) **(C)** Cd treated (60X) group and **(D)** Pb+Cd treated (60X) group after exposure to 0.025 mg/kg body wt. dose of cadmium for 15 days.

Serum and testicular testosterone level was significantly decreased in all metal exposed groups. Reduction in testosterone level could be due to decrease in gonadotopin levels (Paksy et al., 1989; Varga and Paksy, 1991), which is urther controlled by neurotransmitter from hypothalamus. Also, the decrease in testosterone level may reflect the direct effect of metal on testis due to metal accumulation and decreased 3β HSD and 17β HSD enzyme activities as discussed in Chapter 3.

Impact of alteration in hypothalamic-pituitary-testicular axis was further evaluated by semen analysis. The decrease in sperm count is correlated with testosterone and increase in ROS. The consequences of such oxidative damage could include loss of motility due to lipid peroxidation indicated by increased MDA levels in the testis and epididymis (Oehninger et al., 1995; Sikka, 2001; Aitken and Sawyer, 2003), Increased MDA level was also observed in present study (Chapter 5). Decrease in epididymal fructose content could be due to the decrease in testosterone level. It has been shown that the synthesis and secretion of fructose in ventral prostate is under the control of androgens and estrogens (Grayhack, 1965). Significant higher fructose content in prostate with cadmium and co-exposed metal groups could be due to the estrogenic and androgenic properties of cadmium.

The present investigation found that combined exposure to lead and cadmium showed toxic effect on whole axis studied. The competition of metals after combined exposure could be a reason for the observed antagonistic effects. From the above results it is clear that cadmium exerts more disruptions in the hypothalamic-pituitary-testicular axis function. This alteration in H-P-T axis function could lead to male infertility.

In light of increase lead and cadmium level in the environment and food, human race is highly vulnerable to such changes demonstrated at HPG axis even at low level of exposure and this in turn suggest the mechanism of increasing evidence of impotency and loss of libido. Situation can be graver when such expression is in association with other life style disease like, diabetes, cardiovascular disorder, hypertension etc.

4.5 References

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