Chapter V

Effect of lead and cadmium either alone or in combination on pregnant rats

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Introduction

Lead and cadmium are known to produce various adverse effects on reproduction. The toxic effects of lead and cadmium are exerted mostly through direct permeation into the cell, resulting in suppression of the activity of sulfhydryl enzymes. Pregnancy causes many physiological and biochemical changes associated with the metabolism of trace elements in the dam. Therefore it is suspected that pregnancy may also affect mobilization of the tissue level of lead and cadmium and enhance the toxicity of these metals. Metabolic changes in fetus, resulting in reduced haematocrit values (Prigge, 1978) and inhibition of zinc dependent enzymes (Samawickrama and Webb, 1981), have also been reported, but teratogenic effects have not been frequently described (Pond and Walker, 1975; Prigge, 1978) on cadmium exposure. During pregnancy cadmium is retained in the placenta, which thus acts as an important, but not complete, barrier to protect the fetus from cadmium exposure. This has been shown in rodents (Webster, 1988), cattle (Smith et al., 1991) as well as in humans (Korpela et al., 1986). Lead poisoning during pregnancy, its teratogenicity and fetal toxicity have also been extensively studied (Zhang et al., 1999; Han et al., 2000; Dearth et al., 2002).

In view of the effect of lead and cadmium exposure causing inhibitory effect on steroid metabolizing enzymes, gonadotropin and neurotransmitter levels in non pregnant rats as reported in earlier chapters it is worthwhile to study their effect during pregnancy. Thus the purpose of the present investigation was to study the effect of lead and cadmium either alone or in combination in pregnant rats with special attention on their effects on reproductive performance, hypothalamic neurotransmitter levels and neuroendocrine and hepatic steroid metabolism.

Experimental design

Adult virgin female rats (200-220 g) were divided into four groups each consisting of 13-14 animals. The animals of group 1 were given subcutaneous injection of sodium acetate as control. The 2 and 3 groups were treated with lead acetate and cadmium acetate respectively. The group 4 animals were treated with combined dose of lead acetate and cadmium acetate. The dosage was 0.05 mg/kg b. wt./day subcutaneously. On the fifth day of treatment animals in estrous were mated with males. The treatment was continued till sacrifice on day 21 after delivery. Pregnant rats were housed individually in macrolon cages under standard laboratory conditions.

On day 20 post conception four animals from each group were sacrificed. Fetal and maternal liver were removed, weighed and stored at -20^0 C. The other dams were allowed to deliver. Litter size, number of dead pups and total litter weights were recorded. Eight pups of each litter were randomly assigned to stay with the mother. Liver from other pups were removed, weighed and stored at -20^0 C. Pups that stayed with their mother were checked for body weight 7, 14 and 21 days after birth. The pups were weaned at an age of 21 days. The pituitary, hypothalamic and hepatic steroid metabolizing enzymes were determined (17β hydroxy steroid oxidoreductase - Shivanandappa and Venkatesh, 1997; UDP Glucoronyl Transferase - Gorski and Kasper, 1977 and 3α hydroxy steroid dehydrogenase - Shivanandappa and Venkatesh, 1997) in pregnant (day 20 post conception), fetal (day 20 post conception), neonatal (day 21 after birth) and lactating (day 21 after delivery) animals. The hypothalamic content of serotonin (5-HT), dopamine (DA) and norepinephrine (NE) were analyzed (flourimetric method of Shellenberger and Gordon, 1971) in pregnant and lactating animals. Hepatic metallothionein (Bayne et. al.,

1985) and cytochrome P450 content (Omura and Sato, 1964) were determined in pregnant and lactating animals. Biochemical parameters such as DNA (Burton, 1956), RNA (Schneider, 1957) and glycogen content (Seifter et al., 1950) were determined in liver from lactating and neonatal animals. Both tissue and blood were analyzed for lead, cadmium and zinc levels in pregnant, fetal, neonatal and lactating animals using GBC-902 Atomic Absorption Spectrophotometer.

Results

Metal exposure either alone or in combination has no significant effect on reproductive performance (Table 1). Data on maternal, fetal and neonatal body weights, liver weights and litter sizes are presented on Table 2. The liver weights, pup weights or pup liver weights did not differ significantly.

The hepatic concentration of lead, cadmium and zinc in the maternal and lactating animals are shown in Table 3. Both lead and cadmium accumulated in the maternal liver after exposure. Zn concentration was increased in cadmium and combined metal exposed pregnant and lactating rats. There was relatively little change on the Zn concentration from day 20 post conception to day 21 after delivery in control, lead and combined metal exposed groups. But significant decrease in the Zn concentration was observed in cadmium exposed animals.

In fetal and neonatal liver, concentration of lead and cadmium were detectable only in the metal exposed groups (Table 4). Zn concentration in both fetal and neonatal liver was decreased in all metal exposed groups. There was a significant decrease in Zn concentration from day 20 post conception to day 21 post natal in the cadmium exposed group. Metal analysis in blood samples show that both lead and cadmium are significantly

Table 1: Numbers of successful pregnancies and their occurrence in the different exposure groups.

	Control	Pb	Cd	Pb+Cd
Mated	14	14	14	13
Pregnant	12	11	12	12
Sacrificed 20 days p.c.	4	4	4	4
Allowed to deliver	8	7	8	8

Table 2: Maternal, fetal and neonatal weights, litter sizes, relative liver weights in control rats and in rats exposed to lead/and cadmium. p.c.: post-conception, p.n.: post-natal

	Control	**Pb**	**Cd**	**Pb+Cd**
Maternal weight (g)				
at conception	192±5.11 (12)	202±8.47 (11)	188.6±8.1 (12)	185±7.92(12)
20 days p.c.	274.5±7.97 (12)	264.45±9.93(11)	251.66±5.7 (12)	258.7±6.35(12)
Rel. wt. gain(%)	42.23±6.9 (12)	29.77±8.9 (11)	39.55±10 (12)	45.7±7.9(12)
after delivery	208.5±13.34(8)	219.4±10.13 (7)	210.5±16.74(8)	214.9±10.39(8)
Rel. wt. gain(%)	8.62±1.21 (8)	7.62±0.89 (7)	11.76±1.56(8)	17±2.6 (8)
Litter size	8.0±0.5 (8)	10.77±0.52 (7)	8.78±0.5 (8)	8.3±0.54 (8)
No. of dead pups	1	0	1	1
Total litter weight (g)	63±9.75(8)	55.83±15.94 (7)	59±10.84 (8)	46±8.22 (8)
Pup weights (g)				
20 days p.c.(fetus)	4.52±0.25(24)	5.05±0.63(20)	3.56±0.47 (21)	3.99±0.27 (19)
7 days p.n.	13.2±2.16(23)	13±2.45 (20)	10.74±2.04(20)	14.27±2.87(18)
14 days p.n.	23.5±2.64(21)	22.7±3.56(20)	18.75±4.33(20)	21.22±2.59(17)
25 days p.n.	38.5±4.51(12)	45.25±7.63(15)	41.88±6.9 (19)	34.16±5.3 (17)
Rel. liver weights (mg/g)				
6-8 hr. p.n.	3.46±0.38(24)	2.56±0.54(20)	2.37±0.35(21)	1.5±0.38(19)
25 days p.n.	42.2±4.78(12)	44.64±4.31(15)	47.8±9.36(19)	41.46±5.07(17)

Table 3: Effect of lead and cadmium either alone or in combination on hepatic lead, cadmium and zinc levels (ug/g) in pregnant and lactating animals.

Groups	Pregnant rats			lactating rats		
	Pb	Cd	Zn	Pb	Cd	Zn
Control	1.2±0.035	0.3±.0.04	19.15±0.87	0.95±0.064	0.175±0.025	14.7±0.73
Pb	1.7±0.07*	0.25±0.029	19.5±0.645	1.92±0.103*	0.21±0.044	15.85±1.1
Cd	1.1±0.071	0.72±0.075*	35±2.85***	0.825±0.1	0.95±0.12*	28.45±1.74***
Pb+Cd	1.15±0.126	0.55±0.05*	24±1.51***	1.8±0.108*	0.77±0.063*	20±1.25***

* P<0.001 vs. control; ** P<0.001 vs. lead and *** P<0.001 vs. cadmium exposed group

Table 4: Effect of lead and cadmium either alone or in combination on hepatic lead, cadmium and zinc levels (µg/g) in fetus and neonatal animals.

Groups	Fetal liver			Postnatal 21 days liver		
	Pb	Cd	Zn	Pb	Cd	Zn
Control	N.D	N.D	44.37±2.06	N.D	N.D	35.5±4.12
Pb	0.122±0.011*	N.D	39.12±2.91*	0.1±0.005*	N.D	33.21±3.26
Cd	N.D	0.026±0.002*	28±3.6**	N.D	0.024±0.002*	19.6±2.7**
Pb+Cd	0.112±0.007*	0.023±0.002*	28.28±3.12**	0.063±0.003*	N.D	24.64±2.3**

* P<0.001 vs. control and ** P<0.001 vs. lead exposed group

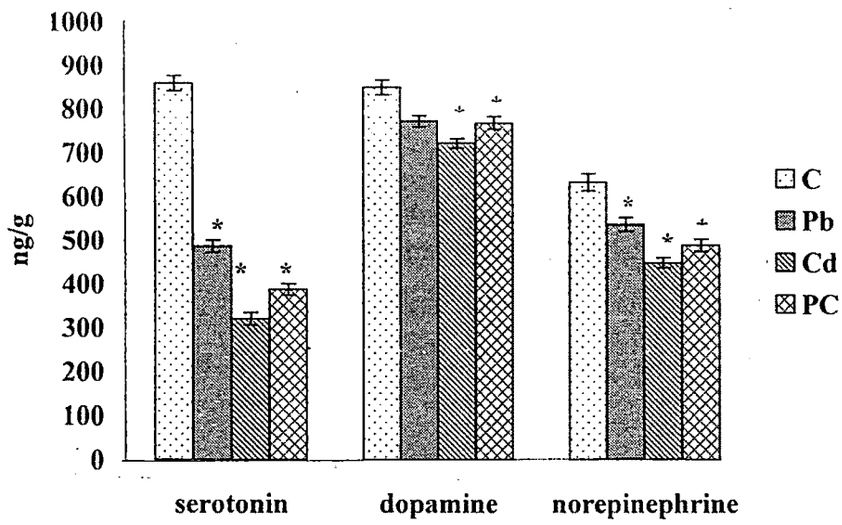
accumulated in the isolated and combined metal exposure groups (Table 5). Similar results were observed in pituitary and hypothalamic tissues also.

Fig. 1 and 2 show the estimated concentration of hypothalamic serotonin, norepinephrine and dopamine in various groups of pregnant and lactating animals. The serotonin content was decreased in all metal treated pregnant and lactating animals with cadmium showing maximum reduction. The norepinephrine (NE) content was decreased in both isolated and combined treatment groups. Among the three groups cadmium exposure was showing maximum reduction in NE content. The dopamine content in pregnant animals was decreased in the cadmium and combined metal exposed animals where as in lactating animals dopamine content was decreased in all metal exposed groups. Among the three neurotransmitters studied, maximum decrease with metal exposure has been observed in serotonin levels.

Activity of hypothalamic 3α HSDH was decreased in cadmium and combined metal exposed pregnant animals (Fig 3). The enzyme activity was inhibited in all metal exposed groups in lactating animals. Pituitary 3α-HSDH activity was decreased in all metal exposed groups in pregnant animals with cadmium showing maximum effect (Fig 4). In lactating animals no change was observed in lead exposed animals whereas cadmium and combined metal exposed animals were showing inhibitory effects.

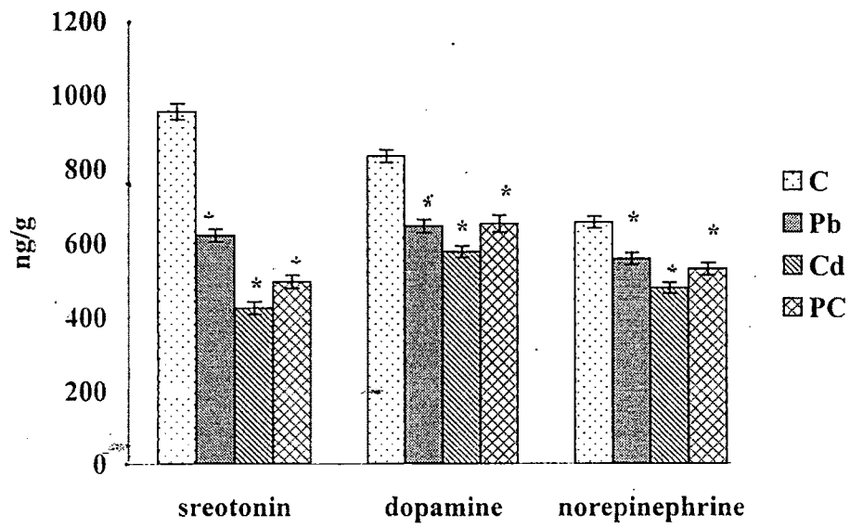
Activity of hepatic 17β HSOR was inhibited in cadmium and combined metal exposed pregnant animals whereas the activity was inhibited in all metal exposed groups in lactating animals (Fig. 5). Hepatic UDPGT activity (Fig. 6) and cytochrome P450 (Table 6) content were significantly decreased in all metal exposed groups in both pregnant and lactating animals. Activity of fetal hepatic 17β HSOR was inhibited in all metal exposed

Fig 1: Effect of lead and cadmium alone and in combination on hypothalamic serotonin, dopamine and norepinephrine content in pregnant animals.



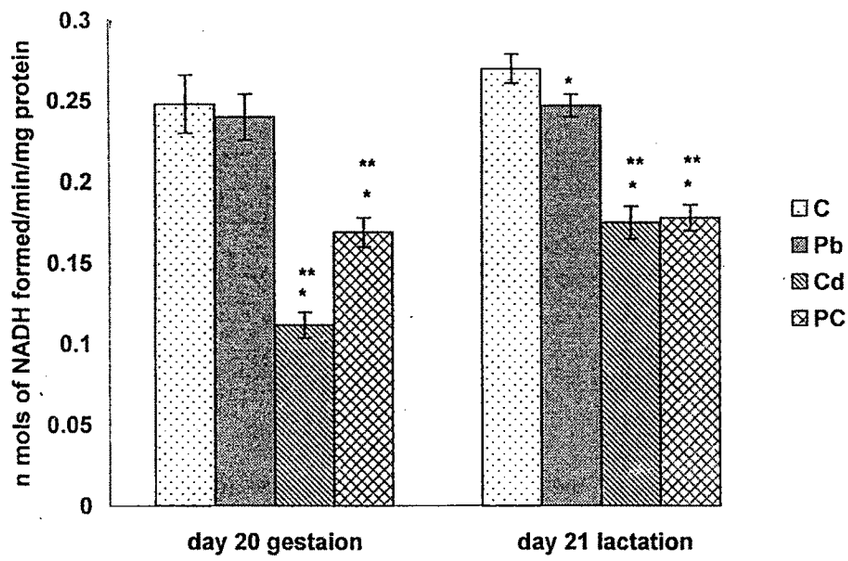
*P<0.001vs. control group (n = 4-5)

Fig 2: Effect of lead and cadmium alone and in combination on hypothalamic serotonin, dopamine and norepinephrine content in lactating animals.



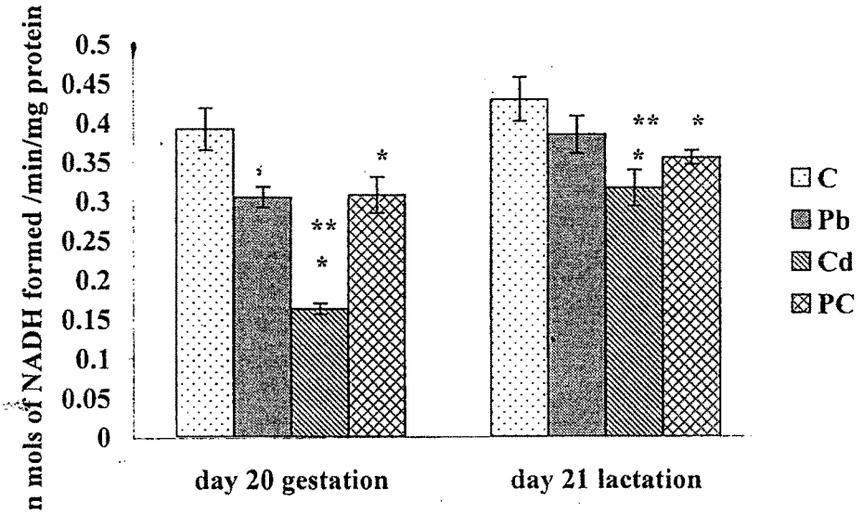
*P<0.001vs. control group (n = 4-5)

Fig 3: Effect of lead and cadmium alone and in combination on hypothalamic 3α hydroxy steroid dehydrogenase activity in pregnant and lactating animals.



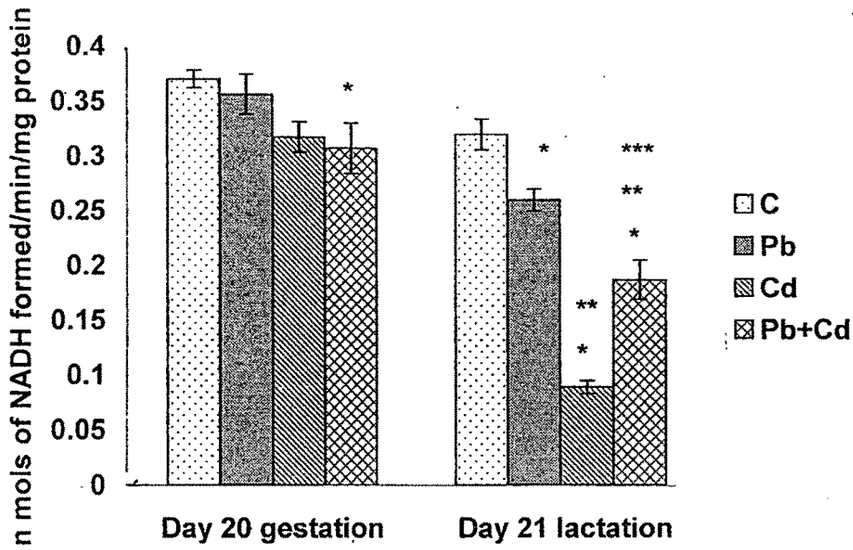
*P<0.001vs. control and **P<0.001 vs. lead group (n = 5-6)

Fig. 4: Effect of lead and cadmium alone and in combination on pituitary 3α hydrox steroid dehydrogenase activity in pregnant and lactating animals.



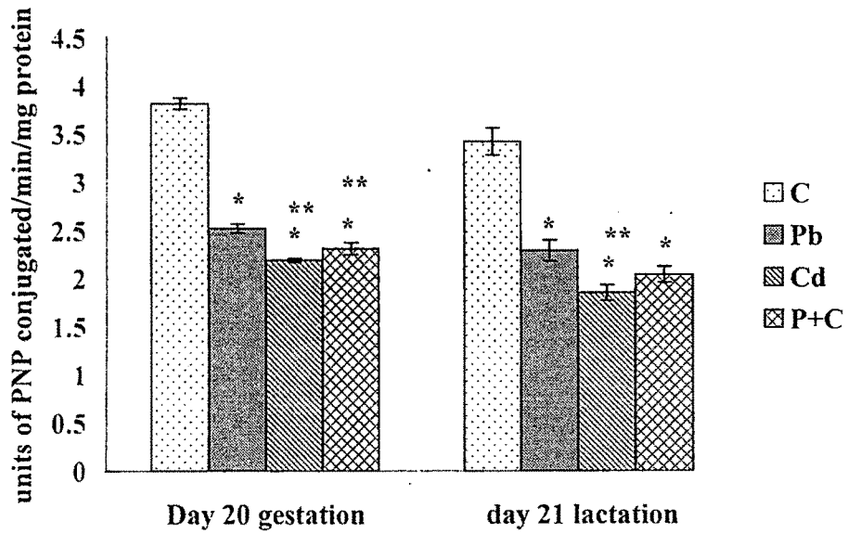
*P<0.001vs. control and **P<0.001 vs. lead group (n = 5-6)

Fig. 5: Effect of lead and cadmium alone and in combination on hepatic 17β hydroxy steroid oxidoreductase activity in pregnant and lactating animals.



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

Fig 6: Effect of lead and cadmium alone and in combination on hepatic UDPG Transferase activity in pregnant and lactating animals.



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

Table 5: Effect of lead and cadmium either alone or in combination on lead and cadmium levels in blood, hypothalamus and pituitary of pregnant animals.

Groups	Blood (μg/ml)		Hypothalamus (μg/g)		Pituitary (μg/g)	
	Pb	Cd	Pb	Cd	Pb	Cd
Control	0.6±0.008	0.433±0.088	3.1±0.088	0.606±0.036	5.93±0.11	0.81±0.098
Pb	2.49±0.27*	0.45±0.07	3.86±0.18*	0.57±0.108	10.11±0.38*	0.84±0.102
Cd	0.733±0.062	1.36±0.1*	2.89±0.06	1.22±0.61*	6.2±0.06	1.77±0.11*
Pb+Cd	1.25±0.096*	0.875±0.048*	3.47±0.21	0.9±0.088*	6.88±0.36	1.31±0.348

* P<0.001 vs. control and ** P<0.001 vs. lead exposed group.

Table 6: Effect of lead and cadmium either alone or in combination on hepatic cytochrome P450 content in pregnant and lactating animals.

Groups	Pregnant mother	Lactating mother
Control	1.47±0.055	1.458±0.029
Pb	1.29±0.083*	1.254±0.049*
Cd	1.059±0.059**	1.015±0.057**
Pb+Cd	1.081±0.078**	0.986±0.064**

* P<0.001 vs. control and ** P<0.001 vs. lead exposed group.

groups whereas the neonatal enzyme activity was significantly decreased only in cadmium and combined metal exposed groups (Fig. 7). In case of UDPGT, the activity was inhibited both in fetal and neonatal liver samples from isolated and combined metal exposed animals (Fig. 8).

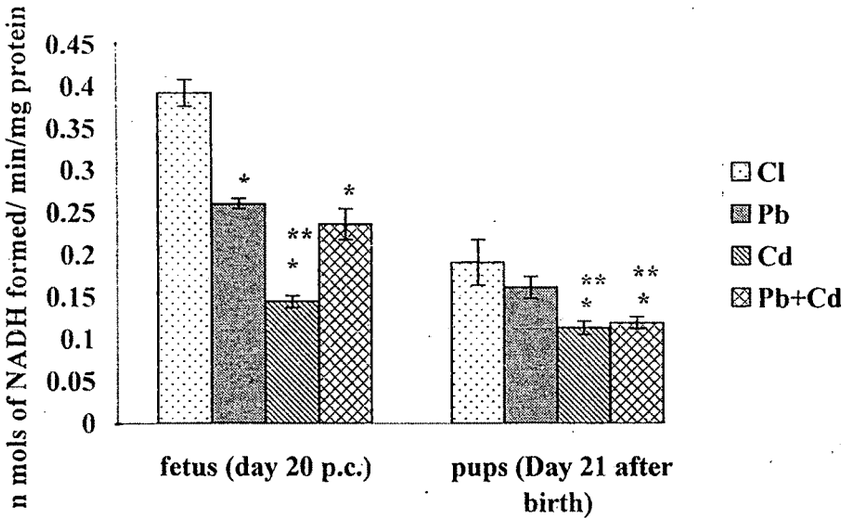
There was little change in hepatic metallothionein (MT) concentration as determined from the displacement of Zn from MT fraction, in pregnant and lactating control animals (Fig 9a and b). Table 7 summarises the results on the effect of lead and cadmium on hepatic glycogen, DNA and RNA content in lactating and neonatal animals. The hepatic glycogen content was decreased significantly in cadmium and combined exposed groups in lactating animals. In neonatal animals both individual and combined treatment groups showed decrease in hepatic glycogen content. Hepatic DNA content in both lactating and neonatal animals was decreased in cadmium and combined exposed groups.

Discussion

There was no change in the reproductive cyclicity in any of the exposure groups. Frequency of pregnancy was equally distributed over all metal exposed groups and no effect was observed on reproductive performance. The litter size, placental weights, pup weights, pup liver weights, maternal weights or maternal liver weights did not differ significantly.

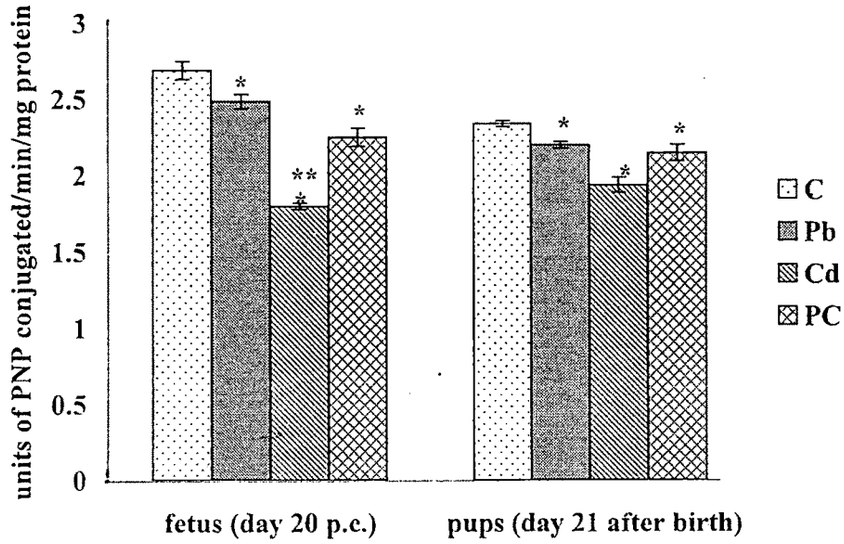
The administration of lead and cadmium either alone or in combination appeared to have no effect on the percentage of successful pregnancy, litter size, number of resorption, and number of dead pups or birth weights. The absence of effects of these metals on litter size and birth weights indicates that lead and cadmium when administered from the day of conception onwards is less harmful than when administered only during organogenesis or

Fig 7: Effect of lead and cadmium alone and in combination on hepatic 17β hydroxysteroid oxidoreductase activity in feus and neonatal animals.



*P<0.001vs. control and **P<0.001 vs. lead group (n = 5-6); p.c.-post conception

Fig 8: Effect of lead and cadmium alone and in combination on hepatic UDPG Transferase activity in fetus and neonating animals.



*P<0.001vs. control and **P<0.001 vs. lead group (n = 5-6); p.c.-post conception

Fig. 9a: Effect of lead and cadmium either alone or in combination on Zn level in hepatic metallothionein fraction from pregnant animals (day 20 gestation)

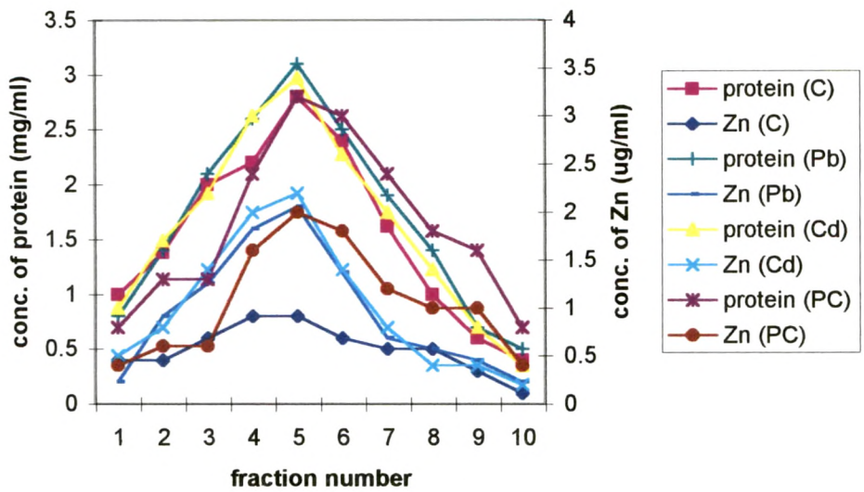


Fig. 9b: Effect of lead and cadmium either alone or in combination on Zn level in hepatic metallothionein fraction from lactating animals (day 21 lactation)

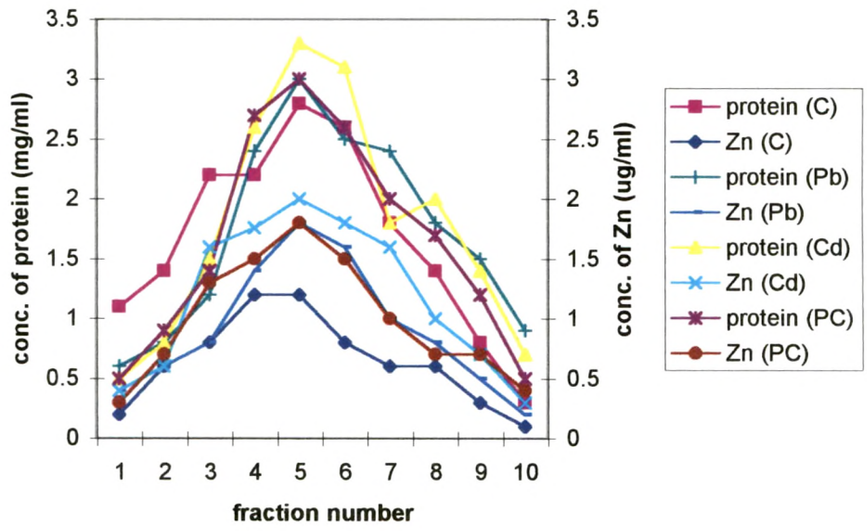


Table 7: Effect of lead and cadmium either alone or in combination on hepatic DNA, RNA and glycogen content in neonatal and lactating animals.

Groups	lactating mother (Day 20 after delivery)			pups (Day 21 after birth)		
	DNA (µg/g tissue)	RNA (µg/g tissue)	Glycogen (mg/g tissue)	DNA (µg/g tissue)	RNA (µg/g tissue)	Glycogen (mg/g tissue)
Control	3.028±0.144	10.61±0.59	124.81±10.87	2.8±0.117	11.99±0.3	138.75±7.66
Pb	3.14±0.312	10.24±0.51	110.01±6.3	2.7±0.22	10.29±0.5	118±6.51
Cd	2.32±0.12***	9.39±0.96	52.41±3.64**	1.99±0.08***	9.01±0.5	74.32±2.49***
Pb+Cd	2.37±0.17***	9.906±0.37	102.3±4.03****	2.154±0.11*	9.39±0.59	110.66±5.29****

* P<0.001 vs. control; ** P<0.001 vs. lead and ***P<0.001 vs. cadmium exposed group

in late gestation. These metals are able to inhibit ovulation and decrease fertility rate in rats when given during the cycle. Though biological half-life of cadmium is rather long (>20 years in humans), when given prior to mating it does not seem to influence pregnancy (Chiquoine, 1965). Maternal and fetal tolerance to cadmium can partly explained by its kinetics. It is postulated that following subcutaneous injection superinduction of hepatic metallothionein (MT) mRNA occurs because of the continued influx of cadmium over 5-10 days (Hazelhoff Roelfzema et al., 1988). MT having a high affinity for divalent cations reduces the bioavailability of highly toxic free Cd^{2+}. By the time of the already sensitive early postimplantation phase of pregnancy started, the major part of circulating cadmium will have been stored in the target organ, in the proximal cells of the kidney. Maternal liver and fetal portion of placenta accumulate cadmium more rapidly than other organs (Levin and Miller, 1981). Lead also binds metallothionein, but does not appear to displace cadmium or zinc. Metallothionein is apparently not induced by lead, although metallothionein sequesters lead in the cell (ATSDR, 1999).

Interactions between cadmium and zinc (Zn) are known to occur during pregnancy. Fetal and neonatal rats accumulate a high hepatic level of essential metals such as Zn as MT during development (Webb, 1987). In order to meet this requirement, Zn as well as Cu must be mobilized from the maternal tissues and transferred to the fetuses during gestation. The mobilization of these essential metals could result from the hormonal changes during pregnancy. The elevated levels of female sex hormones during gestation may also contribute to tolerance against Cd as synthesis of MT has been described to be induced by estradiol in the liver and kidney of rats (Chiu et al., 1988) and by progesterone in humans (Blazka and Shaikh, 1991). Significant decrease in hepatic Zn concentration in cadmium

injected rats has been reported during pregnancy and lactation (Chan and Cherian, 1993). In the present study also, a significant decrease in hepatic Zn concentration has been observed in cadmium exposed animals from day 20 post conception to day 21 after the delivery. As lead does not displace Zn, the observed changes in the combined metal exposed animals could be due to the inhibitory effects produced by cadmium.

The decrease in Zn concentration in fetal liver by lead and cadmium exposure could be due to the inhibition of Zn transporting proteins in the placenta. In the control rats, very little Zn in the liver was bound to MT, whereas a much higher proportion of the hepatic Zn in the cadmium exposed rats was bound to MT in the form of Cd, Zn-MT (Onosaka and Cherian, 1981). Therefore in our studies, when Zn was mobilized from the liver of the cadmium exposed rats during pregnancy and lactation, both Zn and Cd may be released to the blood in the form of Cd, Zn-MT. Studies have shown that following Cd exposure during pregnancy, most of the Cd will be present in rat placenta associated with a MT- like protein, which may prevent the metal from migrating to the fetus (Lucis et al., 1972; Arizono et al., 1981). The pups from metal exposed animals in the present study contained very small amounts of cadmium in the liver, showing that there was a placental barrier for transport of Cd to the fetus. Lead is known to cross the placental barrier and is not easily excreted, so it will continue to accumulate in fetal tissues throughout gestation, mostly accumulating in fetal brain. Although lactational transfer of cadmium is low, mammary gland is the dominating transfer route from dams to pups when rodents are exposed during the lactational period (Tanaka et al., 1972; Whelton et al., 1993).

The changes observed in the activity of steroid metabolizing enzymes could be due to the binding of the divalent metal ions to –SH groups present at the active site. The

changes in hypothalamic neurotransmitter content on metal exposure were similar to the observed changes in the non-pregnant rats except that the dopamine content in non-pregnant and pregnant animals was decreased in the cadmium and combined metal exposed animals where as in lactating animals dopamine content was decreased in all metal treated groups. Cortical levels of serotonin were significantly reduced in rats exposed to cadmium (5 ppm in drinking water to dams) during lactation as well as in rats exposed to cadmium during both lactation and postweaning (Andersson et al., 1997). Antonio et al., 1999 have reported a significant increase of indoleamine content in different brain areas in the cadmium-lead group and so the dopamine and its metabolite in mesencephalon, whereas dopamine levels in metencephalon decreased significantly on gestational and early lactational exposure to low dose of cadmium (10 mg/l) and lead (300 mg/l). The discrepancies with other studies could be due to differences in the age of animals, mode of treatment, duration and brain areas analyzed. Dearth et al., 2002 reported that peripherally derived IGF-1 acts at the hypothalamic level to facilitate LH release at puberty. They have observed delay in the timing of puberty associated with suppressed serum levels of insulin-like growth factor-1, luteinizing hormone, and estradiol on lead exposure (12 mg/ml) during gestation and lactation.

Toxic metals such as lead and cadmium appear to use the transport pathways that exist for biologically essential metals. The transport of Cd in hepatocytes does not require energy, therefore is not an active process. It occurs by temperature-sensitive processes, i.e. ion channels and carriers that involve interaction with sulfhydryl groups. These processes apparently exist for the transport of essential metals such as calcium, copper, and zinc (Blazka and Shaikh, 1991; Shaikh et al., 1995). Once inside the cell, Cd ions can interfere

with the cell metabolism mostly by mimicking the action of other divalent cations (especially of calcium) that are employed in activating or inhibiting the action of various enzymes. It has been reported that many women of child-bearing age were exposed to lead as children, and this lead has accumulated in their bones, threatening the health of their babies many years later (Gonzalez-Cossio et al., 1997). During pregnancy and breast-feeding, calcium is released from the mother's bones to meet the needs of the growing fetus. Lead stored in bone can be released with the calcium into the mother's blood and cross the placenta. As a result, infants may be exposed to the lead that accumulated in the bones of their mothers years before pregnancy.

Changes in gluconeogenisis are important to metabolic adaptations in human beings and other mammals, particularly in response to changing physiologic and pathologic conditions. In the present study we have observed a significant decrease in hepatic glycogen content in lactating mother and pups. Lead intoxication of mothers in gestation and lactation (300 mg/L) resulted decrease in hepatic glycogen content at days 12 and 21 PN, with a higher level of glucose in the blood (Corpas et al., 1996). Prolonged Cd treatment was found to lower the levels of hepatic glycogen through stimulation of the glycogenolytic enzyme, phosphorylase a. Hart and Borowitz, 1974 reported that Cd intoxication induces an increase in catecholamine production and discharge consequently promoting the conversion of glycogen stores into glucose. The decrease of DNA content in liver suggests that liver acted as the main target organ, absorbing most of Cd overload. These results indicate that long-term exposure of pregnant rats to moderate levels of lead and cadmium, when started before implantation, appears to have only minimal consequences on reproduction.

Thus animals getting pregnant despite the heavy metal exposure proved to be fairly tolerant against effects during pregnancy concerning maternal effects, fetal outcomes and postnatal developments though there were alterations in the steroid metabolism and neurotransmitter levels. Although in the present study exposure to heavy metals both in isolation and combination only showed subclinical toxicity. It is possible that subclinical toxicity might result in frank clinical toxicity affecting reproduction, gestational and lactational performance of mother as well as developmental effects in fetus with compromised nutritional status. Such a situation very often exists in general population with low socio-economic background or with high physical and mental stress.

Summary

Adult synchronized female rats were pretreated subcutaneously (0.05 mg/ kg body wt/day) with lead acetate and cadmium acetate separately and in combination during gestational and lactational period with a pretreatment for 5 days before mating. There was no change in the reproductive cyclicity in any of the exposure groups. Frequency of pregnancy was equally distributed over all metal exposure groups and no effect was observed on reproductive performance. The litter size, placental weights, pup weights, pup liver weights, maternal weights or maternal liver weights did not differ significantly.

Hypothalamic serotonin and norepinephrine levels decreased in individually and combined metal treated groups whereas dopamine levels were decreased only in cadmium exposed group. The accumulation of both metals increased in hypothalamus and pituitary after the treatment. Activity of 3α-hydroxy steroid dehydrogenase in hypothalamus and pituitary was decreased after the metal treatment with cadmium showing maximum inhibition. Hepatic steroid metabolizing enzymes (17β-hydroxy steroid oxidoreductase and

UDP glucoronyl transferase) were inhibited by the metal exposure. Both maternal and pups catabolizing enzymes showed decrease in activity after the exposure. Also, significant decrease in cytochrome P450 (CYP450) content was found after the treatment. Displacement of zinc bound to metallothionein was more in cadmium treated rats compared to other groups. Hepatic DNA and glycogen content were decreased in cadmium and combined treated groups in both lactating mother and 20 p.n. pups. There was accumulation of lead and cadmium in the treated groups as compared to control. The accumulation of metals was also observed in fetal and day 20 p.n. pups. Zinc content was increased in cadmium and combined treated groups in pregnant mother whereas fetal liver showed decrease in the metal level in the above groups as compared to control.