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Effect of Lead on Fetal Development in Rats Fed with 8% Casein Diet

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Lead is an ubiquitous environmental pollutant and it's effects on nervous, haemopoietic and renal systems are well documented (Passow et al. 1961). There are enough epidemiological evidences to indicate that women exposed to lead, are more susceptible to abortions or decreased fertility (Nordstrom et al. 1978; Rom 1976). High incidence of sterility, miscarriage and still births have been reported in women employed in British Pottery Factories (Hamilton and Hardy 1974). Besides, a variety of teratogenic effects following lead administration to pregnant rats have also been reported (McClain and Becker 1970).

Nutritional status is known to affect the susceptibility of humans and animals to chemical insult. Prevalence of protein malnutrition in developing countries and increasing reports of exposure to lead through environmental pollution (Chandra 1980) have led us to investigate the embryotoxic and teratogenic effects of lead in pregnant rats maintained on low protein diet so as to assess the developmental toxicity of lead in protein malnourished state.

MATERIALS AND METHODS

ITRC bred female albino rats (170 ± 20 g body weight) of proven fertility were mated with males and the presence of sperms in vaginal smears was designated as the zero day of pregnancy. They were housed individually in plastic cages in an airconditioned room where regular alternate cycle of 12 hr light and darkness (lights on 06.00 hrs) was maintained. The dams were routinely observed for weight, water intake and physical signs of toxicity following treatment. The pregnant females were randomly grouped and assigned to various treatment schedules as shown in Table 1.

The synthetic diet containing 21% casein (normal protein) and 8% casein (low-protein diet) was prepared according to Wetherholtz et al. (1969). In low-protein diet, starch replaced casein. The dose of lead acetate was selected on the basis of earlier report by Kennedy et al. (1975) who did not observe any kind of marked embryotoxic or teratogenic effects in rats. The animals were maintained on the above

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Table 1. Treatment schedule for control and experimental groups

Experimental group	Treatment (from '0' day till 20th day of gestation)
I	21% casein diet + drinking water
II	21% casein diet + lead acetate (1 mg/ml) in drinking water
III	8% casein diet + drinking water
IV	8% casein diet + lead acetate (1 mg/ml) in drinking water

Daily lead intake as calculated by the water consumed was 17.48 ± 3.27 mg/rat/day (approximately 102.82 ± 19.23 mg Pb/kg body weight orally).

dietary and lead exposure schedule from 0 day till 20th day of gestation. Pregnant rats were anesthetized by ether on day 20, the uterine horns and the ovaries of both sides were removed by caesarian section. The foetuses were taken out, examined for external malformations and viability and weighed. Total ovarian and uterine weight, individual pup weight, number of live and dead foetuses, implantation and resorption sites, sex-ratio, crown-rump length, number of corpora lutea, pre and post-implantation losses were recorded on day 20. Pre and post-implantation losses were calculated by the method as described by Palmer et al. (1978). Live foetuses were fixed in 95% ethanol for skeletal abnormalities using alizarin Red S Technique (Staples and Schnell 1964) and examined under dissecting microscope.

Estimation of lead in the blood of pregnant rats on day 20 was done as described by Singh et al. (1976) using a Perkin-Elmer model 5000 Atomic Absorption Spectrophotometer.

Differences between the foetuses of control and treated rats were evaluated using one of the two methods, (a) Yates Chi-Square test was used to calculate the significance of the difference in embryonic deaths (resorptions) between control and treated groups, (b) Student's 't' test was used to see the difference in gestation weight gained, fetal weight and crown-rump length between control and experimental groups. The 5% level of significance was chosen for all studies.

RESULTS AND DISCUSSION

No change in general appearance could be seen in mothers during the course of the treatment. Water consumption in control and experimental animals was 32 ± 6 ml/rat/day. Except the rats of group I (+9.04%), the rest groups registered a decrease in weight (-11.3%, -13.4% and -21.19%, respectively). Significant loss of weight was noticed in group IV compared to group III. The consumption of food was almost the same in all the groups. One and two rats from group III and IV, respectively, died during the course of the experiment. Their autopsy could not ascertain the cause of death.

Though no marked difference was observed in the number of dead and live foetuses, number of corpora lutea and sex-ratio amongst all the groups,

Table 2. Embryo and fetotoxic effects of lead in rats

Parameters	Gr. I (21% casein diet)	Gr. II (21% casein diet + lead)	Gr. III (8% casein diet)	Gr. IV (8% casein diet + lead)
No. of dams used (litters)	10 (9)	12 (10)	10 (9)	12 (11)
Maternal food consumption (g)	24.63±1.28	20.09±0.99	23.00±0.85	21.78±1.16
Gestation weight gained (%)	+9.04	-11.29	-13.38	-21.19
Blood lead level (μg/ml)	0.75±0.06	0.93±0.08 $p < 0.01^{\bullet}$	0.77±0.08	2.19±0.16 $p < 0.001^{\bullet\bullet}$
Litter size	10 + 4	8 ± 4	7 ± 3	6 ± 3
Sex ratio per mother (M/F)	1.14/3.71	3.14/4.71	1.75/5.0	2.33/5.00
No. of resorption sites*	1.24±0.06	1.57±0.04	1.83±0.04	6.37±0.9 $p < 0.001^{\bullet\bullet}$
Crown-rump length (cm)	2.90±0.11	2.62±0.07	2.90±0.15	2.53±0.05 $p < 0.05^{\bullet\bullet}$
No. of corpora lutea	8.85±0.67	8.33±0.68	8.66±0.71	9.87±0.58
Weight of foetuses (g)	1.66±0.09	1.36±0.08	1.58±0.05	1.23±0.08 $p < 0.01^{\bullet\bullet}$
Pre-implantation loss (%)	28.76±2.75	12.50±2.23	29.44±3.22	27.27±3.25
Post-implantation loss (%)	12.07±1.68	14.12±1.87	22.34±3.15	52.08±5.49 $p < 0.01^{\bullet\bullet}$

$^{\bullet}$Compared with group I; $^{\bullet\bullet}$Compared with group III; *Yates Chi-Square Test; M/F = Males/Females

the intra-uterine growth retardation was found to be significantly high in group IV (-20%; $p < 0.01$; average pup weight decreased from 1.66 gm in control to 1.23 g in group IV), crown-rump length reduction found to be significantly high in group IV (-14%; $p < 0.05$) compared to their counter part controls (Group III). Markedly significant incidences of resorption sites were also observed in rats of group IV (average number 6.37 compared to 1.84 in group III; $p < 0.001$). Mean litter size was reduced in group IV compared to group III, the difference was, however, not statistically significant.

Though no difference could be observed in pre-implantation loss between all the groups, significantly high incidence of post-implantation losses were observed in lead treated mothers maintained on 8% protein diet ($p < 0.01$; +133%) compared to group III control (Table 2).

The incidences of reduced cranial ossification, wavy ribs, delayed and variant ossification of cervical centra, ribs and caudal vertebra and bent ribs were evident in foetuses of all the groups (Table 3). There were, however, no gross deformities of skeletal structures amongst all the groups.

Table 3. Skeletal deformities (number of incidences in pups born to lead treated mothers).

Parameters	Gr. I	Gr. II	Gr. III	Gr. IV
Number of pups observed	65	50	46	37
Reduced cranial ossification	2	7	5	9
Lumber centra absent	2	1	1	3
Wavy ribs	1	1	2	5
Bent ribs	3	1	3	2
Agenesis of sacrum	6	9	5	6
Pubis unossified	0	1	0	1
Reduced limb ossification	1	0	3	1
Sacral vertebrae unossified	0	2	6	6
Missing tail vertebrae	2	1	0	2

As shown in Table 2, blood lead level showed an increase in rats of group II and IV, the lead levels in group IV were significantly higher compared to group II ($p < 0.001$).

Lead poisoning is reported to affect the fetal development (Zegarska et al. 1974) as well to produce teratogenic changes in rats (Hackett et al. 1982). A variety of teratogenic effects, including non-ossification of cervical centra were reported after the administration of a single intravenous dose of Pb (NO_3) to pregnant rats at various stages of gestation (McClain and Becker 1970). Reduced number of pups, their weight and survival rate is reported in rats given lead acetate in the diet to mothers before and during pregnancy (Stowe and Goyer 1971). However, in the present study no such changes were noted in lead treated females maintained on 21% casein diet alone. Our observations are in accordance with the results of Kimmel et al. (1976) who administered lead acetate to pregnant rats through drinking water and found no significant resorption site and teratogenic effects. The lack of any ill-effects on developing foetuses

in such cases was attributed to slow absorption of lead after oral administration (McClain and Becker 1975).

Exposure of lead to pregnant rats, maintained on low-protein diet, resulted in significantly high incidences of resorption sites, intra-uterine growth retardation and post-implantation loss. Presumably it may be due to increased lead absorption in protein deficient host as is evident from increased blood lead level in these rats. Der et al. (1974) have also reported higher blood lead concentration in rats fed 4% protein diet compared to rats fed 20% protein diet.

Exposure to lead may produce effect on the developing foetuses either (1) directly by interfering with the normal development of the embryo, or (2) indirectly by affecting the maternal metabolism. Direct interference of lead on the developing foetus may possibly be due to the lead transportation through the placenta in rats. Placental transfer of lead has been reported by McClain and Becker (1975). In the present study significantly reduced maternal weight during pregnancy in group IV rats also reflects the possibility of toxic action of lead on the mother. Lead exposure in pregnant females may modify the metabolism of hormones needed for the implantation process. An indication of this can be derived from the studies of Leonard et al. (1983) where interrupted implantation process was noted due to hormonal imbalance in lead intoxicated pregnant females.

Although the exact mechanism of intra-uterine deformities due to lead exposure is not known, the present study reveals that exposure of lead to pregnant mother maintained on 8% casein diet produce more embryo and fetotoxic effects than mother fed on 21% casein diet.

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