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Original Article

Effect of lead on male gonadal activity in Albino Rats

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Abstract
Lead poisoning often prevails in children and industrial workers. The present study was undertaken to evaluate the effects of lead acetate on steroidogenic functions of testis, serum levels of gonadotrophins and testosterone in albino rats. Testicular steroidogenic activity was evaluated by measuring the activities of two steroidogenic key enzymes, Δ^5–3β hydroxysteroid dehydrogenase (Δ^5–3β–HSD) and 17β–hydroxysteroid dehydrogenase (17β–HSD). Administration of lead acetate at a dose of 8mg/kg body weight for 14 days lowered the weights of testes and accessory sex organs, and decreased testicular Δ^5–3β – HSD and 17β– HSD activities and serum levels of FSH, LH and testosterone but 7 days of lead acetate administration showed no effect on the above parameters. This report is perhaps the first evidence to show that lead exerts some deleterious effects on testicular steroidogenesis indirectly by decreasing serum levels of gonadotropins.

Keywords: Lead, male gouad, gonadotropins, Albino Rat

Lead is a heavy soft metal, occurs in nature as an oxide or salts. Chronic lead poisoning is commonly seen in young children from sucking lead paint or lead toys, in workers engaged in printing, paint and petroleum industries. Effects of chronic lead poisoning are the disorders in gastrointestinal and haemopoietic system, and muscular weakness leading to paralysis.[1]

Literature survey so far available indicates that lead treatment causes anemia.[2] Testicular histochemical changes and spermatogenic inhibition have also been observed after lead administration in rats.[3,4]

Though the lead administration results the fall in the serum level of testosterone in rats,[5,6] the effect of lead salt on testicular steroidogenesis and its mechanism of action on the male gonads have not been studied.

Therefore, the present study has been undertaken on albino rats to investigate the effects of lead acetate on testicular steroidogenic enzymes and serum levels of gonadotropins and testosterone.

Materials and methods

The present study was conducted (with 32 mature male albino rats) in the laboratory of the department of physiology, Calcutta University, Calcutta, India. The animals were of Wistar strain, weighing 150 – 170gm, divided equally into two groups and maintained under standard laboratory conditions (14 h light 10h darkness; at 28 ± 4°C) with standard animal diet and water available adlibitum. Lead acetate was purchased from E, Merck Chemical Company (Bombay, India). It was dissolved in sterile distilled water. Eight animals of one group received 1ml sterile distilled water kg $^{-1}$for 7 days and eight animals of other group received the same vehicle for 14 days. The remaining eight animals of each group were injected intraperitoneally with lead acetate at a dose of 8.0mg (1.0ml distilled water), $^{-1}$ kg day $^{-1}$kg for the same duration as vehicle injected rats. Animals were killed on 8th and 15th day of treatment following protocols and ethical procedure, and their body weights were noted. Blood was collected from dorsal aorta, centrifuged and serum was stored at -20°C for radioimmunoassay. The testes were dissected out and used for enzymatic study.

Measurement of testicular enzymes

One testis from each animal was used for studying the activities of Δ^5–3β–hydroxysteroid dehydrogenase (Δ^5–3β–HSD) and 17β–hydroxysteroid dehydrogenase (17β–HSD). Testicular Δ^5–3β –HSD and 17β–HSD were measured in UV spectrophotometer according to the procedure of Talalay (1962)[7] and Jarabak et al (1962)[8], and subsequently modified by Biswas et al (1983; 2003; 2004)[9–11] . One unit for both Δ^5–3β – and 17β–HSD was defined as the amount causing a change in absorbance of 0.001/min at 340nm.

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Radioimmunoassay of hormones

Serum FSH and LH were measured according to Moudgal and Madhwaraj (1974)[12] by RIA using reagents supplied by the Rat Pituitary Distribution Program and NIDDK (Bethesda, MD, USA). Pure rat FSH (NIDDK-rFSH^{-1-5}were iodinated using the chloramine T (Sigma chemical co., St. Louis, MO, USA) method according to Greenwood et al (1963).[13] NIDDK anti– rat –FSH –S –II and NIDDK–anti–rat–LH–S–5 were used as antisera. Goat anti rabbit y-globulin was used as the second antibody. Serum samples were expressed as ug/L of serum. The intraassay variations were 6% and 5% for FSH and LH respectively.

Serum testosterone was assayed according to the procedure of Auletta et al. (1974).[14] Samples were assayed in duplicate. The antiserum to testosterone was purchased form Endocrine Science (Tarazone, CA, USA) and it had a 44% cross reactivity with 5α - dihydrotestosterone (DHT). The intra assay variation was 6.0%. Reported values are the sum of testosterone and dihydrotestostrone as chromatographic purification of testosterone and dihydrotestosterone are not possible.

Statistical Analysis for statistical analysis of the data, the two- tailed students' t-test was used. Differences were considered significantly when $p<0.05$.

Results

Table 1: Changes in body weight and testicular, prostatic and seminal vesicular weight after lead acetate administration at a dose of 8mg/kg body wt' values are mean ± SEM (n = 8).

		Body weight (g)		Testis	Prostate	Seminal Vesicle
Duration of Treatment	**Condition**	**Initial**	**Final**	**(g/kg body wt)**	**(mg/kg body wt)**	**(mg/kg body wt)**
7 days	Control	150.8± 8.5	161.9±5.7	24.8±0.3	845±47	1235±110
	Lead	153±10.3	160.3±8.5	23.9±0.5	859±52	1147±100
14 days	Control	155.9 ±9.2	163.8±8.2	24.9±0.8	905±40	1285±90
	Lead	156.8±7.5	168.2±10.1	20.3±0.5*	795±0.38*	930±70*

P – Values * <0.05 compared with the corresponding vehicle treated controls.

Table 2: Serum levels of FSH, LH, testosterone and testicular Δ^5–3β – HSD, 17β– HSD activities after lead acetate administration at the dose 8mg/kg body wt. Each value represents mean ± SEM (n=8).

Duration of Treatment	**Condition**	**Serum FSH ug/L**	**Serum LH ug/L**	**Serum Testosterone ug/L**	**Δ^5–3β – HSD (unit/mg tissue per h)**	**17β– HSD (unit/mg tissue per h)**
7 days	Control	195.32± 10.28	38.62±3.39	3.25±0.19	25.12±0.152	26.13±0.35
	Lead	181.49±9.25	35.91±2.83	3.41±0.21	24.41±0.132	25.49±0.41
14 days	Control	186.25 ±8.39	40.52±3.28	3.56±0.20	26.39±0.141	27.82±0.58
	Lead	152.38±10.42*	29.59±2.62*	2.15±0.17*	20.09±0.140*	21.62±0.43*

P. – Values * <0.05 compared with the corresponding vehicle treated controls.

Body and organ weights

Lead acetate administration resulted in no significant effect on body weight. Testicular and accessory sex organ weights were decreased remarkably after 14 days of lead administration, but there was no significant effect on the above reproductive organs after lead acetate administration for 7 days in comparison with control animals (Table 1).

Enzymatic study

Testicular (Δ^5–3β–HSD) and 17β–HSD activities were decreased remarkably after 14 days of lead acetate administration in respect to control. Seven days of lead administration has no effect on the activities of above steroidogenic enzymes (Table 2).

Hormones

Serum levels of FSH, LH & Testosterone were significantly lowered after lead acetate administration for 14 days but administration of lead for 7 days

resulted in no effect on the serum levels of gonadotropins and testosterone (Table 2).

Discussion

The present study demonstrates that 14 days after administration of lead acetate in rats, there is a significant decrease in the activities of steroidogenic enzymes and fall of serum levels of FSH, LH and testosterone. Δ^5–3β –HSD and 17β–HSD, key steroidogenic enzymes in androgen biosynthesis[15, 16] are controlled partly by LH[17] and partly by FSH as FSH plays a role in induction of leydig cell LH receptor.[18,19] Therefore, the decreased level of testosterone and reduction of the activities of testicular steroidogenic enzymes in lead-treated rats is a reflection of reduced pituitary gonadotropins secretion.[20] The mechanism by which the levels of gonadopropins levels decrease in lead treated rat can not be determined from the present experiments. But the effect of lead acetate on serum level of testosterone is consistent with others[5,6], where lead administration results a fall of serum level of testosterone. Moreover testicular size and weight are normally regulated by fluid secretion from Sertoli cells and the production of sperm in the seminiferous tubules.[21,22] Reduction of testicular weight less than control values after 14 days of lead administration is in support of degeneration of germ cells and sertoli cells in lead-treated rats.

In conclusion, our findings suggest that chronic administration of lead to normal mature albino rats can reduce the serum levels of FSH, LH and testosterone which in turn result inhibition of testicular activity and the fall in accessory sex organs weight.

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