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Assessments of Possible Gonadotoxic Effect of Lead on Experimental Male Rabbits

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Abstract: The present study investigated the toxic effects of lead (Pb) on the male gonads of experimental rabbits (weight ranging from 1.2-1.42 kg). The experimental animals were exposed to doses of lead in form of lead nitrate (Pb $(NO_3)_2$) in varying concentrations of 0,100 and 200 mg/L via oral exposure for five weeks. At the end of the fifth week, the animals were sacrificed and the blood collected and analyzed for luteinizing hormone (LH) and testosterone using standard procedures. The testis were collected and studied for histopathology. Data collected were represented in tables and plates. Results showed high serum LH concentration in control animals and low concentration in test animals. The testosterone concentrations also follow similar result. Histological examination of testis showed deformities in morphology of testis in test animals with gross damage within the somniferous tubules. A strong correlation was established between LH and testosterone (r =0.873) suggesting that both biomarkers were synthesized at the same site. Measurement of serum LH and testosterone provide important indices for both hypothalamic pituitary testicular functions. It was concluded that lead is a gonadotoxic with tendency of suppressing LH and testosterone levels of animals.

Key words: Gonads · Luiteinizing hormone · Testosterone · Serum · Histology

INTRODUCTION

Luiteinizing hormone (LH) is regulated in mammals through the release of gonadotropin-releasing hormone (GnRH) and also through the feedback effects of steroids [1-4]. Testosterone is responsible for growth and development of male sex organs (gonads) as well as secondary sexual characteristics. Many authors have reviewed hormonal regulation of testicular functions [5, 6]. Mammalian reproductive process is complex and could be disrupted by exogenous agents. These agents (radiation, drugs, heat, metals, chemicals, pesticides etc) may involve in gametogenesis resulting into reduced fertility [7]. Exposure to these agents could affect male gonads at the developmental stage due to exposure by mother during pregnancy and during the quiescent pre-pubertal period before the commencement of active spermatogenesis and during post-pubertal period of activity of spermatogenesis [8]. Some toxins act indirectly by inhibiting enzymes in steroid synthesis or by interfering with neuronal control of hormone levels in sexual functions. Gonad toxicity has been reported by Barlow and Sullivan [9] and Bataineh *et al.* [10]. Effects of testosterone or LH response to GnRH have been demonstrated in mouse by Huang *et al*. [5] and Biswas and Ghosh [1]. Aribarg and Sukchareon [11] observed that lead caused alteration in plasma LH and testosterone and non-significant effects on hypothalamic pituitary testicular axis. Lead was also found to affect sexual behaviors, territorial aggression, fertility and reproductive systems failure [10, 12]. Occupational exposure to lead showed hypogonadism and decreased serum testosterone, with a reproductive and endocrine impact in hypothalamic-pituitary-testuicular axis [13]. The main objective of this study was to assess the possible gonadotoxic effect of lead on experimental animals (male rabbits).

MATERIALS AND METHODS

The experiment involved the use of nine experimental male rabbit within the age bracket 4-6 months.

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The animals were grouped into three blocks consisting of 3 rabbits in each group. Lead nitrate prepared as 0 mg/L Pb, 100 mg/L Pb and 200 mg/L Pb were administered orally. Exposure of the animals to lead lasted for 5 weeks and the animal were sacrificed and the blood samples were collected with the aid of syringe into hyparinized bottles. The blood samples were centrifuged for one minute and the serum was stored at -20°C and transferred to University Teaching Hospital (UCH), Ibadan for LH and testosterone analysis by double antibody radio immunoassay [14, 15]. A detail of the validity of assay has been described by Schanbacher and D'occhio [15]. Functional sensitivity for testosterone and LH was 0.1 ng/mL and intra-assay and inter-assay coefficient of variation (CV) were 6 and 8 % respectively. Histological examination of scrotum was also carried out by removing the animal scrotum tissues, which was also sent to UCH for staining and photomicrograph.

RESULTS

Table 1 shows the mean values of serum luiteinizing hormone and testosterone as well as the animal weights. The mean weight of the experimental animals rose from 1.42-1.60 kg, 1.20-1.59 kg and 1.42-1.69 kg in treatment 1 (control), treatment 2 (100 mg/L Pb) and treatment 3 (200 mg/L Pb) respectively.. Histological examinations of the testis were presented in plates 1-3.

DISCUSSIONS

It was observed from this study that LH and testosterone levels in the animals were greatly reduced in the experimental animals, but not significant (Table 2). This was similar to the study of Ait *et al*. [16], Mukherjee and Mukhopadhyay [17] and Sokol *et al*. [18] who found that LH level of male rats exposed to lead was lower than the control. However, this contradicted the study of Rodamilans *et al*. [19] who observed a non-progressive increase in LH of peopled exposed to lead. Rodamilans *et al.* [19] also demonstrated that there's a reduction in testosterone of individuals exposed to lead for less that 12 months. Reduction in LH at increase dose of lead concentration from 100 mg/L to 200 mg/L was almost half in this study. Wadi and Ahmad [20] observed that the target of lead in animals is spermatogenesis and sperms within the epididymus by producing reproductive toxicity rather than acting at sites within the hypothalamic-pituitary testicular axis.

Reduction of LH and testosterone levels in animals exposed to lead indicates enzyme inhibition in the biosynthesis (steroid genetic) pathway by lead [21]. A significant positive correlation was established between testosterone and LH at $p<0.05$ ($r=0.873$) (Table 3), this is also an indication that both hormones are synthesized at similar site. Negative correlation observed between LH, testosterone and treatments (Table 3) suggested that as the dose of treatment increases the levels of both LH and testosterone decreases. There was also a reduction in weight initially as the doses were administered to the animals, later the animals were able to overcome the stress and gained more weight as they grow. There was a significance ($p<0.05$) between the initial and final weight of the animals in control. This may not suggest possible lead toxicity because the animals exposed to 100 mg/L lead gained more weight (32.5 %) when compared to control.

Table 1: Mean weight, luteinizing and testosterone of the Pb administered animals and control.

	Experimental Animals (Mean±SD)		
	1	2	3
Parameters	0 mg/L (control)	100 mg/L	200 mg/L
Weight (kg) initial final	1.42±0.16	1.20±0.18	1.42±0.16
	1.60±0.15	1.59±0.09	1.69±0.08
LH (ng/ml)	116.7±51.33	72.4±47.8	32.7±14.92
Testosterone (ng/ml)	369.5±150.6	107.5±45.96	92.0±5.66

LH- luteinizing hormone, SD-standard deviation

Table 2: Anova table for Weight, testosterone and LH between control and lead treated animals

Parameters	F	Sig.
Weight initial	94.33	0.002*
Weight final	106.17	0.002*
Testosterone	5.88	0.092
LH	2.06	0.273

* significant ($P<0.05$)

Table 3: Correlation coefficient between parameters

	Treatments	Weight initial	Weight final	Testosterone	LH
Treatments	1				
Weight initial	-0.019	1			
Weight final	0.984**	0.120	1		
Testosterone	-0.794	0.440	-0.699	1	
LH	-0.761	0.089	-.0688	0.873*	1

* significant at p<0.05, ** significant at p<0.0, LH- luteinizing hormone,

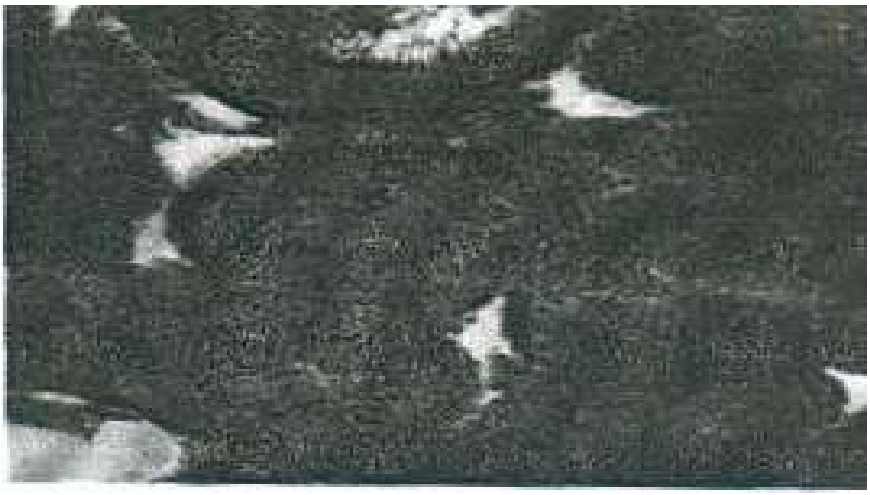

Plate 1: Testicular tissue of experimental animal that was not exposed to lead toxicity (Control) (x250)

Plate 2: Effect of low dose lead concentration (100 mg/L) on testicular tissue of the experimental animal (x250)

Histological examination of the testis of the animal given a dose of 200 mg/L Pb showed deformities in the testis architecture with a serious damage within the somniferous tubules (Plate 3) (white portion). This was also noted in the animal given a dose of 100 mg/L (Plate 2), but not as serious as that of the animals given a dose of 200 mg/L. The control histological microphotography was normal (Plate 1). The damage in the testicular tissues did not affect the germinal epithelium but showed toxic effects on the sperm.

In conclusion, this study has shown gonadotoxic effects of lead on the intra-testicular sites with minimal effects on hormonal levels and no effect on extra testicular sites. The data suggested that lead has gonadotoxic suppression on LH and testosterone levels. Lead also has effect on the morphology of the sperm by destroying the somniferous tubules.

Plate 3: Effect of high dose lead concentration (200 mg/L) on testicular tissue of the experimental animal

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