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ORIGINAL ARTICLE

Effect of Lead Acetate Alone and in Combination with Whole Milk (Star Ship) on Body Growth and Fertility in Male Rat

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Abstract
The Effect of lead acetate alone and in combination with whole milk on body weight gain and fertility in male rat including testicular weight gain and sperm motility were carried out on a total of 15(15 days old) male weaning Long-Evans (ICDDRB strain) rats. These15 rats were randomly divided in to three equal groups, each consisting of five rats. Rats of group- A were kept as control (without giving any treatment), group-B received lead acetate alone @ 6mg/ml drinking water and group-C received lead acetate @ 6mg/ml plus whole milk (star ship) 150 mg/ml of drinking water. The result showed that body weight gain of control group per week per rat was found to increase but in treated group-B, the body weight gain was found to decrease most significantly (P< 0.01) on 56th day while in group-C, body weight was reduced significantly (P< 0.05) on 56th day. The reducing body weight gain was less in group C than group B. The significant (P< 0.05) reduction of testicular weight gain was noticed in left testis of group B (only lead acetate). The significant (P< 0.05) reduction of sperm motility was found in group C (lead acetate plus whole milk) and motility percentage of spermatozoa was found highly significant (P< 0.01) in group B (only lead acetate). From this study, it is concluded that, treatment with lead acetate at low doses has harmful effects on experimental animals including fertility in male rate.

Key words: *Lead acetate, Whole milk, Body weight gain, Testis, Sperm, Fertility, Rat*

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INTRODUCTION

Environmental pollution by lead is a wide spread problem attributed to various sources. Inorganic emission from the combustion of leaded gasoline contributed a majority (61%) of the total emission of lead in to the environment from anthropogenic sources in 1974-75 [17]. Other sources for the dispersion of lead compounds include exhausting of workroom atmosphere; abrasive action of automobile traffic on lead painted lines on streets and highways; resuspension of lead by high speed cars; welding of lead painted structural or other steels and the weathering of painted surfaces with the resultant flaking and distribution of lead bearing dust particles in to the atmospheres. The incineration of leaded plastics and secondary smelting of old battery cases, lead pipes and sheet also result in lead air pollution [8]. Lead exposure to animals and humans is unavoidable as it occurs through many routes including contaminated air, water, soil, food, and consumer products. The safe threshold for lead exposure has not been identified, as there is no accurate amount specified for lead toxicity. Recently, scientific research has acquired knowledge that Pb concentration in the blood (PbB) in the range 20–50 µg/dl can cause adverse effects [6]. The organs or systems which may be affected at such exposures can be the haemopoietic, nervous, cardiovascular, reproductive, and immune systems [18, 3]. It is known that Pb influences biological enzyme systems and it can be assumed that numerous mechanisms of interaction are yet to be elucidated.

Furthermore, the regulatory mechanisms of the male reproductive system are very complex and also not completely understood. It is likely that Pb interacts with one or more of those mechanisms: it could be on reproductive organs at different levels, or on endocrine control of reproduction, or both. In Bangladesh there is no available data in this context, so this research work has been carried out to study the effect of lead acetate alone and in combination with whole milk (Star ship) on body growth and fertility in male rat.

MATERIALS AND METHODS

Materials

Experimental Animals

Fifteen days old male weaning Long Evans rat (*Rattus norvegicus*) weighing between 182-294 g were purchased from ICDDRB, Dhaka and brought to the Experimental Pharmacology and Toxicology laboratory at Bangladesh Agricultural University (BAU) for the present study. They were housed throughout the entire period of study in Perspex cages with aluminum grid on the bottom fixed on inch a part to facilitate fecal materials and urine in a room maintaining 23 $\pm 1^0$C. After 06 days of acclimatization animals were segregated on the basis of their age and body weight without significant differences. The rats were fed on standard rat chow (15 g/rat/day) for 56 days formulated by ICDDRB, Dhaka and supplied fresh water.

Experimental chemicals

Lead acetate 500mg (BDH co.) from Hatkhola market, Dhaka and Whole milk (Starship) from local market were purchased and brought to the laboratory for this study.

Experimental design

A total of 15 (15 days old) male weaning Long Evans rats were used. These rats were randomly divided in to 3 equal groups, and numbered them as group A, B and C. Out of 3 groups, rats of group A was kept as control without giving any treatment, rats of group B received lead acetate alone @ 6mg/ml drinking water and group C received lead acetate @ 6mg/ml plus whole milk (Star ship®) 150 mg/ml of drinking water. Prior to segregation, initial body weight of each rat was recorded and kept group wise in cages. After administration of lead acetate with drinking water all the rats were kept under close observation for a whole period of study and all the parameters (body weight gain or loss, testicular weight and sperm motility) was recorded at seven days intervals.

Methods

Measurement of body weight

The body weight of each rat was measured with the help of balance on the zero day and sequentially 7 days interval up to sacrificing of the animals.

Procedure for the evaluation of testis and semen

Testicular weight

Immediately after sacrificing the rats, the testis and epididymis were removed and kept in to clean, sterile petridish. After removing the fat, the testes were detached from epididymis and weight, length, breadth and gross alteration were recorded for each testis and epididymis.

Semen Examination

Epididymal sperm suspensions were prepared by releasing the contents of each caudal epididymal from mature male rats in to a PBS medium (which was suspended with bovine serum albumin). In order to examine the acrosome, mid piece and tail of spermatozoa semen samples were fixed in buffered normal-saline. Buffered normal saline was prepared by dissolving disodium hydrogen phosphate with 2 molecule water (34.7 m mol), potassium dihydrogen phosphate (18.7m mol), sodium chloride (92.6 m mol) and formaldehyde (1.54 mol) in distilled water (1 L). The semen and normal saline fixed spermatozoa at phase contrast microscopy were classified as proximal and distal cytoplasmic droplets, abnormal piece, abnormal acrosome, detached head, bent tail, coiled tail and double folded tail categories. At least 200 spermatozoa from individual replicates were examined at a magnification of 1000X. The proportion of normal spermatozoa in normal saline preparation included only those which had no abnormalities in the acrosome, midpiece and or tail. The morphology of sperm head was evaluated in thin smear stained with William's technique. Williams stain was prepared according to [23] and [13]. The formulate Williams stain, stock solution I was prepared by dissolving 10 g of basic fuchsine in 100 mml of 95% alcohol. Stock solution –II was a saturated solution of bluish eosin in 90% alcohol. Stock solution III was prepared by 10 ml of stock solution I with 170 ml of 5% phenol. The final stain contained 25 ml of stock solution-II and 50 ml of stock solution III. The stain was left at least 14 days and filtered before use. A thin smear of undiluted semen was prepared on a clean slide, dried in air, fixed on flame, rinsed in acetic acid and methanol (1:3), treated with absolute alcohol for 3-4 minutes and dried off. Then the smear were treated

with 0.5% chloramin for 1 to 2 minutes, washed in distilled water followed by rinsing in 95% alcohol and finally stained with carbol fuchsin for 8 to 10 minutes. After staining the slides were washed in tap water dried off and examined (1000X). At least 500 spermatozoa were examined from individual smears.

Statistical Analysis

The data of the body weight, testis weight and sperm motility were analyzed statistically using T- test.

RESULTS AND DISCUSSION

Effect on body weight

The body weight of rats of control group was found to increase but in treated group B the body weight was found to decrease (-9.99%) most significantly (P<0.01) on day 56 while in group C body weight was reduced (-5.95%) significantly (P<0.05) on day 56 (Table 1). The reducing body weight was less in group C (lead acetate plus whole milk) than in group B (only lead acetate) most probably due to the positive effect of whole milk supplementation. These observations are in accordance with the result of studies which reported that lead caused reduction in growth rate in experimental animals when fed lead [2, 19]. It has been observed reduction of body weight in lead induced toxicities in rats [5, 22]. The body weight gain was decreased after treatment with lead in a dose of 400 mg/kg of the fodder [21]. The body weight loss might be resulting from the interruption of lead acetate in absorption and metabolism of feed nutrients essential for health [15]. Similar findings were also reported [12].

Table 1**:** Effects of oral administration of lead acetate alone and in combination with whole milk in drinking water on body weight in rats

Gr.	**Chemicals with dose**	**Pretreatment**	**Post treatment**								
		Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**	**Day 42**	**Day 49**	**Day 56**	**% increased/ decreased**
A	Control	254.60± 5.80	259.20± 5.66	262.80± 7.17	268.20 ±6.79	272.00 ±7.14	277.00 ±7.24	278.20 ±7.29	280.00 ±7.23	285.80 ±6.87	+12.25%
B	lead acetate @6mg/ml drinking water	240.66± 9.74	242.80± 11.67	245.60± 6.65	252.40± 16.53	253.20± 14.39	245.20± 9.23	240.40± 11.95	230.20± 0.80	218.80± 0.40*	- 9.99%
C	lead acetate @ 6mg/ml plus whole milk (star ship) 150 mg/ml of drinking water	243.80 ±0.85	247.60± 0.95	228.40± 6.31	273.00± 6.22	245.60± 9.51	236.20± 7.26	235.0± 9.19	232.60 ±8.70*	230.10± 0.11**	- 5.95%

Values above represent the mean ± SE of 5 rats

* Indicates significant values

** Indicates highly significant values

\+ indicates % increased – indicates % decreased.

Effect on testis weight

The significant (P <0.05) weight reduction was noticed in left testis of group B(only lead acetate).No remarkable weight variation of right and left testis of group C supplemented with whole milk@150

mg/ml of drinking water was observed (Table-2). Similar findings were found in another study [14, 5]. The differences between the findings in group B and C might be due to the positive effect of whole milk.

Table-2: Effect of oral administration of lead acetate alone and in combination with whole milk in drinking water on testis weight (gm) in rats.

Group	Chemicals with dose	Day 60	
		Right testis	Left testis
A	Control	2.67 ±0.20	2.40 ±0.02
B	lead acetate @6mg/ml drinking water	2.52± 0.05	2.25± 0.02**
C	lead acetate @ 6mg/ml plus whole milk (star ship) 150 mg/ml of drinking water	2.61± 0.30	2.34± 0.20*

Values above represent the mean ± SE of 5 rats
* Indicates significant values
** Indicates highly significant values

Effect on Sperm motility

The motility percentage of spermatozoa of rats was counted in group A, B and C. The significant ($P < 0.05$) reduction of sperm motility was found in group C (lead acetate plus whole milk) and motility percentage of spermatozoa was found highly significant ($P < 0.01$) in group B (only lead acetate) (Table-3). These findings are similar to the observations stated by [1] who observed that the concentrations of 2.5fg Pb/ml significantly reduced sperm motility but 0.25 fg Pb/ml had no effect and concluded that presence of lead in seminal plasma would not have a significant biological effect on sperm function.

Table-3: Effect of oral administration of lead acetate alone and in combination with whole milk in drinking water on sperm motility (%)

Group	Chemicals with dose	Day 60	
		Right testis	Left testis
A	Control	90 ±1.75	85 ±4.5
B	lead acetate @6mg/ml drinking water	52± 0.42	50± 0.18**
C	lead acetate @ 6mg/ml plus whole milk (star ship) 150 mg/ml of drinking water	75± 0.15	74± 0.30*

Values above represent the mean ± SE of 5 rats
* Indicates significant values
** Indicates highly significant values

Der *et al.,*1976 stated that Pb concentrations >30–40µg/ dl during at least 30 days are associated with impairment of spermatogenesis and reduced concentrations of circulating androgens in other rat strains and other rodents. In the animal experiments, several factors can interfere with the results: type of Pb compound, presence of systemic intoxication, age at start of the experiment, duration of exposure, variability between and within species, and biological variation in hormone concentrations. Age and maturity of the animal may have bearings for the results in several ways. It has been shown that prepubertal rats are less sensitive to the toxic effects of Pb on testosterone and sperm production than animals with exposure to Pb beginning after puberty has been initiated [20]. The distribution patterns of Pb in tissues may differ significantly when lead exposure occurs during the later stages of life [7]. Similarly, Momcilovic and Kostial found marked differences in Pb distribution in suckling rats compared with adult rats [16]. Thus, the same exposures can cause different tissue concentrations at different ages. Therefore it can assume that in rats different organs can be at risk at various ages. At 104 weeks 20% of rats develop atrophy of the seminiferous epithelium [10]. In rat studies it is therefore important to specify the age at the start of the experiment. Der *et al.,* (1976) suggested that gene differences might be an important factor of difference in response to foreign compounds, when they compared their own findings in Sprague-Dawley rats with those of Sesco rats [11]. The Sprague-Dawley rat strain seemed to be more resistant to the toxicity of Pb than the Sesco strain. The significant differences in vulnerability to Pb toxicity between strains could be due to a different toxicokinetics of Pb accumulation in the testes or to a different functioning of the blood-testis barrier. In particular, Sprague-Dawley rats and NMRI mice seem to be rather resistant to the reproductive effects of Pb. In other strains, exposure to Pb in sexually mature animals caused minor to major signs of impaired spermatogenesis or of a disturbed endocrinogy

at Pb concentrations ranging from 30–187µg/dl [6]. In this study, though young aged rat at prepubertal stage (Long Evans rat -15 days old) used as experimental animals but the highly significant reduction in sperm motility percentage was observed which is dissimilar to the findings stated by [20]. The reasons behind this are not clear because the blood Pb concentration level were not estimated in this study due to some limitations. The reasons might be due to elapsing time between collection of the sample and observation period of the sample or the dosage of the chemicals used or duration of the exposure of chemicals. Methods of administration of Pb to the experimental rats was with drinking water orally so some variation may occur in daily water intake per rat due to individual factor and this might be another reason. Unfortunately, the great lack of uniformity, whether it concerns age of the animal, duration of exposure, assessment methods for reproductive end points, or internal doses, makes it impossible to drawn any strong conclusions on dose-response relations. However, there could be a relations among testicular Pb content, histopathological changes and sperm motility.

CONCLUSION

Treatment with lead acetate at low doses has harmful effects on experimental animals including fertility in male rate. Therefore, whole milk (star ship) might be helpful to reduce the body burden of lead toxicities.

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