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Article

Effect of lead acetate alone and in combination with whole milk (Star ship®) on body growth and liver functions in an experimentally induced lead toxicity in rat

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Abstract: The Effect of lead acetate alone and in combination with whole milk on body weight gain and some biochemical parameters were carried out on a total of 15 (15 days old) male weaning Long- Evans strain rats. The rats were randomly divided into 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 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 day 56 while in group C, body weight was reduced significantly ($P< 0.05$) on day 56. The reducing body weight gain was less in group C than group B. A most significantly ($P< 0.01$) increased SGOT and SGPT values were observed in Group B but in group C, those count increased significantly ($P< 0.05$) on day 56 of experiment. From the study it was concluded that treatment with lead acetate at low doses has adverse effects on body growth and liver functions in experimental animals.

Keywords: lead acetate; whole milk; body weight; liver function; rat

1. Introduction

Lead is one of the major environmental pollutants (toxin) in the modern world whose higher concentrations particularly in industrial zone; adversely affect the vitality and production performance of domestic animals (Kwatra *et al.*, 1986). Lead usually induce adverse effects on the central nervous system as irritating, immunosuppressive, genotoxic, teratogenic, nephrotoxic and other toxic effects on the haematopoietic system. Lead is also known to modify the metabolism of trace elements and nutrients (Levander, 1979). Also, lead administration decreased liver copper level whereas additional dietary copper increased the liver lead level (Bafundo *et al.*, 1984). It was postulated that lead interferes with copper and iron metabolism (Klauder and petering, 1977). Lead is considered as pathogenic factor of atherosclerosis, arterial hypertension and may cause an anemia. Belacy *et al.*, (1996) reported that lead induce inhibition of renal and hepatic transaminase and alkaline phosphates. Several studies have led to reports that lead has effects on glucose utilization at low levels

resulting in disturbed acetylcholine synthesis and energy metabolism (Yun and Hoger, 2000). On the other hand Gupta *et al.,* 1995 stated that the body attempts to regulate the Pb toxicity by promoting self defense by enhanced production of thiol compounds such as glutathione. The concentration of lead residues in tissues of farm animals depend upon the rout of entering and period of exposure. Most orally ingested lead is deposited in the skeleton (National Academy of Science, 1972). Initially, lead deposited in bone until a possible threshold is reached then it deposited in other tissues especially in the liver and kidneys. The lead particles which ingested or inhalated pass to the blood stream and 82.0% was excreted in faeces and urine. Only 0.5% was excreted with the milk and the rest 17.95% remains and restored in tissues and body organs (Baars *et al*., 1988). Frangenberg (1986) found that administration of very high dose of lead to animals resulted in highest lead accumulation in the kidney then liver, bone marrow, brain and finally in the heart muscles. The aim of the present study is to show the effect of lead on body growth & some biochemical parameters related to liver functions in rat and investigate the protective role of whole milk against the adverse effects of lead toxicity.

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2. **Materials and Methods**

2.1. 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 aluminium 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.

2.2. 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.

2.3. 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, biochemical parameters eg. SGOT and SGPT) were recorded at specific day intervals.

2.4. Reagents for liver function test

2.4.1. Determination of SGOT

a. Buffer substrate: (i) Tris buffer (84mmol/L P^H7.5) (ii) L-aspartate (260nmol/L)
b. Enzyme/ co-enzyme/α-oxoglutarate: (i) α-oxoglutarate (12 mmol/L) (ii) LD ≥1.2 U/ml (iii) NADH 0.18 mmol/L (iv) MDH ≥ 420U/L

2.4.2. Determination of SGPT

a. Buffer substrate: (i) Tris buffer (100mmol/L P^H7.5) (ii) L-alanine (0.6 mol/L)
b. Enzyme/ co-enzyme/α-oxoglutarate: (i) α-oxoglutarate (15 mmol/L) (ii) LD ≥1.2 U/ml (iii) NADH 0.18 mmol/L

2.5. Measurement of body weight

The body weight of each rat was measured just before starting of treatment and body weight gain or loss was recorded in each 7 days interval up to sacrificing of the animals.

2.6. Procedures for the collection of blood sample for serum separation

Blood was collected just before treatment i.e. day 0 and day 56 of treatment directly from tip of the tail of ether-anesthetized rat. Immediately after collection blood was transferred to sterile tube containing anticoagulant (4% sodium citrate solution) at a ratio of 1:10 and used for different hematological parameters immediately after collection.

2.7. Biochemical study related to liver functions

Two widely used biochemical test such as SGOT and SGPT were determined by UV method using IFCC used Humalyzer 2000, Human type Germany.

2.8. Determination of SGOT & SGPT

0.1 ml of serum was mixed with 1.0 ml kit solution 2 enzyme/coenzyme/α-oxoglutarate AL 1205 including buffer substrate with L-aspartate for SGOT and buffer substrate with L-alanine for SGPT determination. The wave length was set at 340nm Hg, 1 cm light path cuvette was used and analysis was done at 37^0C. After mixing the cuvette was placed in the Humalyzer 2000. Initial absorbance was read after 1 minute. The final record was made at 1, 2, 3 minutes after initial reading. Absorbance was recorded each time (0.11 and 0.16 at 340nm/Hg 340nm). First two values for the first 2 minutes were used for the calculation.
Calculation: SGOT concentration: 1746 x absorbance U/1
Calculation: SGPT concentration: 1746 x absorbance U//1

2.9. Statistical analysis

The data of the body weight, SGOT and SGPT were analyzed statistically using T- test.

3. Results and Discussion

3.1. 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 was in accordance with the result of studies which reported that lead caused reduction in growth rate in experimental animals when fed lead (Ali *et al.,* 2010; Seddik *et al.,* 2010). It has been observed reduction of body weight in lead induced toxicities in rats (Aseth *et al.,* 1995; Teijon *et al.,* 2006). The body weight gain was decreased after treatment with lead in a dose of 400 mg/kg of the fodder (Szymezak *et al.,* 1983). The body weight loss might be resulting from the interruption of lead acetate in absorption and metabolism of feed nutrients essential for health (Marija *et al.,* 2004). Similar findings were also reported by Ibrahim *et al*., (2012).

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	Pretreat-ment	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.

3.2. Effect on biochemical parameters related to liver functions

The activities of SGOT and SGPT were significantly elevated in all treated groups than control. In group B (only lead acetate @ 6mg/ml drinking water) the elevation of SGOT and SGPT were much more higher ($P<0.01$) than group C ($P<0.05$) (lead acetate @ 6mg/ml plus whole milk (Star ship®) 150 mg/ml drinking water) (Table-2).

Table 2. Effect of oral administration of lead acetate alone and in combination with whole milk in drinking water on some biochemical parameters such as SGOT/ AST and SGPT/ ALT (U/L at 37^0C) in rats

Parameters	Group	Chemicals with dose	Pre-treatment	Post treatment
			Day 0	Day 56
SGOT/ AST	A	Control	216 ±0.12	216± 0.25
	B	lead acetate @6mg/mldrinking water	214± 0.93	234± 0.15**
	C	lead acetate @ 6mg/ml plus whole milk (star ship) 150 mg/ml of drinking water	215± 0.52	218± 0.83*
SGPT/ ALT	A	Control	101 ±0.14	104± 0.16
	B	lead acetate @6mg/mldrinking water	102± 0.85	121± 0.15**
	C	lead acetate @ 6mg/ml plus whole milk (star ship) 150 mg/ml of drinking water	103± 0.87	107± 0.81*

Values above represent the mean ± SE of 5 rats
* Indicates significant values ($P<0.05$)
** Indicates highly significant values ($P<0.01$)

The activities of two enzyme namely serum glutamate pyruvate transaminase (SGPT); recently called as alanine transaminase (ALT) and serum glutamate oxaloacetate transaminase (SGOT); recently called aspartate transaminase (AST) have been widely used to assess the liver functions. ALT was a cytoplasmic enzyme while AST was found in both cytoplasm and mitochondria. SGPT or ALT was found to increase in acute hepatitis (viral or toxic), jaundice, liver cirrhosis. SGOT or AST was found to increase in myocardial infarction and different liver disorders. In the present study the values of SGOT and SGPT were significantly increased at day 56 in all treated groups (B & C) than in control group A. The present results were agreed by the results of Khan *et al.,* (2008) who reported that the activities of serum AST and ALT were significantly increased in lead exposed rats. Activities of ALT, AST and ALP were significantly increased in rats given daily lead acetate in diet as 500 mg/kg after 2, 4 and 6 weeks of treatment (Dioka *et al.*, 2004; Othman *et al.*, 2004; Shalan *et al.*, 2005; Al-Wabel *et al*., 2007; Herman *et al*., 2009; Mehana *et al*., 2010; Lynda *et al*., 2011; Nabil *et al*., 2012). Increasing of the serum activities of AST and ALT was most likely a consequence of the hepatotoxic effect of lead i.e the occurrence of toxic hepatitis. The lead entering the body by ingestion was delivered to the liver through the portal blood circulation and smaller part of the lead "break the liver barrier" and enters the body circulation. The accumulated lead in the liver can act by directly damaging the hepatocytes primarily by destroying the permeability of the cell membrane, with resultant release of cellular enzymes leading to increase their serum values (Todorvic *et al*., 2005). Durgut *et al*. (2008) found that, the increasing serum activities of AST and ALT were associated with liver damage and or cardiac or skeletal muscle damage. Furthermore Nabil *et al*. (2012) reported that the high plasma AST and ALT activities was accompanied by high liver microsomal membrane fluidity, free radical generation and alteration in the liver tissue. On the other hand, the present results were disagreed by the results of Singh *et al.* (1994). A possible explanation for such differerence results were the quite different in view of the experimental design and the applied doses of lead, duration of exposure and the way how lead got into the organism. The result of this study was in agreement with the findings found in a study conducted by Hanan *et al*., (2012) on rats, at a dose of 0.5 g/100 ml drinking water for 2 months, whose dose and the duration of the exposure were almost similar to the present study. The increase was less in group C than B, the reason was not clear but this might be due to the positive effect of whole milk (star ship) supplementation.

4. Conclusions

Treatment with lead acetate at low doses has adverse effects on body growth and liver functions in experimental animals. Therefore, whole milk (star ship) might be helpful to reduce the body burden of lead toxicities.

Conflict of interest
None to declare.

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