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EFFECTS OF CALCIUM CARBONATE, POTASSIUM IODIDE AND ZINC SULPHATE IN LEAD INDUCED TOXICITIES IN RAT MODEL

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ABSTRACT

A study was carried out to find out the effects of calcium carbonate, potassium iodide and zinc sulphate in lead induced toxicities in rat with the observation of toxic signs, postmortem changes and determination of lead quantity in different organs of the body. Twenty-five long Evans rats weighing between 202-305g were randomly divided into five groups keeping group A as untreated control. Rest four groups (B, C, D & E) were treated with lead acetate @ 20mg/kg body weight in addition, rats of groups C, D & E were given Calcium carbonate (50mg/kg body weight), potassium iodide (20mg/kg bwt) and zinc sulphate (10mg/kg bwt.) respectively. Treatments were continued for 42 consecutive days. Rats of group B showed reduction in fecal consumption, anxiety, indigestion, fatigue, muscle tremor, paralysis and ruffled hair coat. However, rats of group C were apparently normal but rats of group D and E showed mild toxic signs of similar nature. On postmortem examination, severe congestion and blackish discoloration with enlargement of the liver, kidney, spleen and inflammation of the gastric mucosa were observed in rats of group B. However, these changes were less severe in other groups. The significant reduction of lead in blood, liver, kidney and brain was observed in rats of group C, D and E in comparison to group B. In addition, significant reduction of lead content was observed in femur of group C only. The present findings revealed that during lead exposure administration of calcium carbonate may be effective in modifying and preventing lead deposition in tissues followed by zinc sulphate and potassium iodide in rat.

Key words: Toxicity, lead, calcium carbonate, potassium iodide, zinc sulphate, rat

INTRODUCTION

The detrimental effects of lead poisoning have been well known since ancient times but some of the most severe consequence exposure to this metal has only been described recently. Lead affects the higher function of the central nervous system and undermines brain growth, preventing the correct development of cognitive and behavioral function (Marchettic, 2003). Acute and chronic exposure to lead would predominantly affect two specific protein complexes; protein kinase C and the N-methyl –D-aspartate subtype of glutamate receptor (Marchettic, 2003). Some researchers have been reported progressive deterioration in mental capacity of workers with many years of excessive industrial lead exposure (Marchettic, C 2003). Lead inhibits haeme synthesis. It does this primarily by inhibiting ALAD, although more enzymes in the biosynthetic pathway are also inhibited (Roels *et al.*, 1977). The salt with 1% may remove lead and prevent lead damage of kidney. (Hunan *et al.*, 1999). The protective role of combined zinc and vitamin C supplementation was observed against chronic lead toxicity on the liver function (Zayat *et al.*, 1996). Vitamins were effective in mobilizing lead from blood, liver, and kidney into urine and or faces and in restoring partially blood zinc protoporphyrin level (Tandon and Singh, 2000). Calcium supplementation during lead exposure reduced tissue lead but had no effect when applied after lead exposure (Marija *et al.*, 2004). In the present study an attempt has been undertaken to study the effect of calcium carbonate, potassium iodide and zinc sulfate in lead induced toxicities in rats.

MATERIALS AND METHODS

The experiment was carried out in the experimental Pharmacology and Toxicology laboratory, Department of Pharmacology, Bangladesh Agricultural University to observe the effect of calcium carbonate, potassium iodide, and zinc sulphate in respective group of lead in toxicities on long Evans rats.

Grouping and treatment of animal

All the rats were randomly divided into five (05) equal groups (5×5) comprised of five rats and were marked as group A, B, C, D and E. Rats were subjected to be treated with the help of a measuring dropper once daily for a period of six weeks. Group A was untreated and was given normal feed and water as per requirement; where as Lead acetate 20 mg/kg bwt, Lead acetate 20 mg/kg b.wt plus calcium carbonate 50 mg/kg b.wt., Lead acetate 20 mg/kg bwt plus potassium iodide 20 mg/kg bwt, Lead acetate 20 mg/kg b.wt plus zinc sulfate 10 mg/kg bwt in 4ml distilled water for group B, C, D and E respectively.

Toxic signs and body weight

After feeding of lead acetate alone and with calcium carbonate, potassium iodide and zinc sulfate to 4 groups of rats separately for 42 consecutive days all the control and treated rats were observed carefully for appearance of any toxic signs. Body weight of the control and treated rats were taken before starting of experiment and at 14 days interval during 42 days of feeding.

Hematological parameter

For determination of hematological parameter blood samples were collected just before treatment on day 14, day 28 and day 42 of treatment from tail and heart of the control and treated diethyl-anesthetized rats. Immediately after collection of blood, blood was transferred to sterile test tube containing anticoagulant at a ratio of 1: 10. The collected blood was used for different hematological parameters within two hours of collection. The hematological parameters were determined as per method cited by Lamberg and Rothstein (1977).

Gross pathological changes

At the end of the experimental period i.e. 42 days of treatment period, all the rats were sacrificed and were dissected for observing the postmortem changes.

Determination of lead acetate in different organs of the body

The different tissues and organs (blood, liver, kidney, brain and femur) were collected and stored for detection of lead in the different tissues and organs as Semi quantitative lead determination by “Merck Lead Test” kit.

Preparation of tissue homogenates and blood sample

From each group of rats, individual organs were taken aseptically and were grinded with the help of mortar and pestle in normal saline and then the tissue materials (homogenates) were taken in different test tubes. Normal saline was added for the preparation of this homogenate at the rate of 5 ml per grams of tissues. For blood samples, normal saline was also added at the same rate.

Statistical analysis

Data obtained from the experiment on body weight, hematological parameters such as TEC, Hb was analyzed statistically using students T-test following the standard methods by Khan (1989).

RESULTS AND DISCUSSION

Toxic signs

Rats of group A (control) were normal during the total experimental period. Group B and group D were also apparently normal up to 1st week of feeding whereas rats of group C were apparently normal without any possible toxic signs up to 3rd weeks of feeding and up to 4th weeks for group E (Table 1). However, the above toxic signs might be appear due to the inhibition of amino levulinic acid dehydrates (ALAD), inhibition of hame synthesis, and inhibition of transport protein (transferrin) synthesis and penetration of blood brain-barrier. (Korsrud and Meldrum, 1988; Batra *et al*., 1998; Hunan *et al*., 1999).

Table 1. Effect of calcium carbonate, potassium iodide and zinc sulfate in lead induced toxicities on toxic signs in rats

Groups	1st week	2nd week and upwards
A	Normal	Normal
B	Apparently normal	Reduced feed intake, anxiety, indigestion, loss of muscle coordination, tremor, dizziness, fatigue, posterior paralysis, ruffled, hair coat and weight loss markedly
C	Apparently normal	Mild toxic signs i.e. anorexia, idle sitting, salivation and ruffled hair coat
D	Apparently normal	Loss of appetite, ruffled hair coat, salivation, ataxia and in coordination
E	Apparently normal	Feed consumption was slightly reduced

Effect on body weight

The reduction of mean body weight in group B was statistically significant ($p<0.01$). In group D and E, the body weight of rats were slightly decreased ($p<0.05$), however, they were also statistically significant (Table 2). In group C the body weight was gradually decreased but not statistically significant upto 42nd day of treatment. The reduction of body weight might be due to the interruption in absorption and metabolism of feed nutrients essential for health. Similar findings were also observed in lead toxicity (Marija *et al*., 2004).

Table 2. Effect of calcium carbonate, potassium iodide and zinc sulfate in lead induced toxicities on mean body weight (gm) in rats

Parameters	Duration	Group A	Group B	Group C	Group D	Group E
Body weight (gm)	Day 0	265.6 ± 0.01	275.8 ± 0.04	248.5 ± 0.01	244.0 ± 0.01	240.0 ± 0.2
	Day 14	270.05±0.04	270.04 ± 0.02	250.5 ± 0.02	245.6 ± 0.01	242.8 ± 0.01
	Day 28	277.5 ± 0.80	265.0 ± 0.03	253.5 ± 0.03	251.2 ± 0.07	246.25±0.05
	Day 42	310 ± 0.90	258.5* ± 0.03	260.5**±0.01	253.0 ± 0.02	255.5**± 0.01
TEC (million/l)	Day 0	9.02 ± 0.03	8.5 ± 0.02	8.6 ± 0.01	8.2 ± 0.05	8.5 ± 0.01
	Day 14	9.82 ±0.01	7.46 ± 0.02	8.13 ± 0.01	9.91 ± 0.01	7.80 ± 0.01
	Day 28	9.85 ± 0.02	7.36 ± 0.04	8.12 ± 0.01	7.6 ± 0.01	7.6 ± 0.01
	Day 42	10.51±0.04	7.01* ± 0.03	8.01 ± 0.02	7.29 ± 0.02	7.04 ± 0.01
Hemoglobin Content (gm %)	Day 0	11.02 ± 0.51	11.8 ± 0.01	12.05 ± 0.02	12.60 ± 0.04	11.86 ± 0.01
	Day 14	11.3 ± 0.04	11.0 ± 0.05	11.9 ± 0.02	11.5 ± 0.06	11.3 ± 0.46
	Day 28	12.4 ± 0.06	10.2 ± 0.02	12.0 ± 0.01	11.8 ± 0.05	11.5 ± 0.04
	Day 42	12.3 ± 0.07	09.08* ± 0.08	11.8 ± 0.04	11.4 ± 0.05	11.02 ± 0.06

*Significantly decreased, **Significantly increased.

Total erythrocyte count (TEC)

In group B total erythrocyte count was significantly reduced ($p<0.01$) on 28 and 42 day of treatment as well as in group D and group E TEC were also significantly reduced ($p<0.01$) but in group C TEC reduction was gradually decreased which is not significant (Table 2). The reason why the reduction of total erythrocyte counts might be due to the effects of lead acetate on the hematopoietic organs of the body or might distroy the RBC in the body during continuous administration of lead acctate orally (Purser *et al*., 1983; Wilson *et al*., 1979).

Hemoglobin content

Hemoglobin content in group B was significantly reduced ($p<0.01$) on day 28 and day 42 of treatment (Table 2). On the contrary, In group D and group E, hemoglobin content was also reduced but it was not statistically significant compared to group B. In group C hemoglobin content was gradually decreased but not significant. The decreased value of Hb content may be due to decrease in the value of total erythrocyte count. The possible cause of this result might be due to adverse effect of lead acetate on hematopoietic system and on the absorption of essential vitamins and minerals from the gut and the destruction of the RBC. The present study is in agreement with the earlier reports. (Hu *et al.*, 1999; Georeg and Duncan, 1981; Marchetti, 2003).

Gross pathological changes in some organs

There are non specific observations observed in all groups. It is difficult to distinguish the different groups by the observation of gross pathological changes (Table 3). However this finding is supported by Marija *et al.* (2004).

Table 3. Effect of calcium carbonate, potassium iodide and zinc sulfate in lead induced toxicities on gross pathological changes in rats

Group	Liver	Spleen, Heart and kidney	Stomach	Intestine
A	All the vital organs were apparently normal			
B	Heavily congested, necrotic and become blackish in color and slightly enlarged	Congested and blackish in color	Rose-red inflammation found throughout the stomach mucosa	Severe-hemorrhagic enteritis were observed
C	Pin point hemorrhage were found throughout the liver	Slightly congested	Slightly congested	Slightly hemorrhages were found
D	Slightly hemorrhage and congested	Slightly congested, congestion was found around the coronary band	Slightly congested	Hemorrhagic enteritis was found
E	Pin point hemorrhage were found throughout the liver	Slight hemorrhages were found	Slightly congested Stomach	Hemorrhagic enteritis was found

Semi-quantitative estimation of lead in the tissues samples

The significant reduction of lead in blood, liver and kidney was observed (Group C<E<D) in comparison to group B (Table 4). The lead content of brain was slightly decreased and in femur was slightly increased in Group E. Lead may be rapidly absorbed and reached considerable amount in the blood. Biotransformation occurs in the liver. So, the lead treated groups showed highest level of lead in the blood and liver. In other groups the amount was less then that of group B. The reason behind this may be calcium carbonate; potassium iodide and zinc sulfate reduce lead absorption, deposition of lead in different organs and enhance excretion of lead by the kidney. These findings are supported by (Batra *et al*., 1998; Hunan *et al*., 1999 and Marija *et al.*, 2004).

Table 4. Mean concentration of lead in different tissues of rat following treatment with calcium carbonate, potassium iodide and zinc sulfate

Group	Blood (mg/l)	Liver (mg/kg)	Kidney (mg/kg)	Brain (mg/kg)	Femur (mg/kg)
A	0	0	0	0	0
B	550.0 ± 5.12	440 ± 3.24	330 ± 3.02	262.5 ± 2.15	122.4 ± 1.25
C	220.0 ± 2.25	55.0 ± 1.21	44.0 ± 0.84*	63.0 ± 0.63	88.0 ± 0.89
D	330.0 ± 3.20	88.0 ± 0.82	77.0 ± 0.78	88.0 ± 0.85	132.0 ± 1.45**
E	275.0 ± 2.50	77.0 ± 0.72	66.6 ± 0.65	77.0 ± 0.75	110.0 ± 1.56

Values above represent the mean ± SE of 5 rats, *Significantly decreased, **Significantly increased.

The present findings reveal that during lead exposure administration of calcium carbonate may be effective in modifying and preventing lead deposition in tissues followed by zinc sulphate and potassium iodide in rat.

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