Original Article

Journal of Practical Biochemistry and Biophysics
Volume 1 Number 2, July - December 2016

Effect of Thiamine Hydrochloride on the Lead Induced Acute Toxicity in Albino Rats

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Abstract

The efficacy of thiamine hydrochloride against lead induced acute toxicity was studied in the experimental rats. Hematological parameters such as RBC Count and Heamoglobin content were determined and was found to be decreased throughout the period of study in the lead induced animals. Biochemical parameters such as protein, urea, uric acid levels increased throughout the study when treated with lead acetate. Triglyceride content was also found to be decreased throughout the period of study, in lead induced animals. Biochemical alterations were reversed on treatment with thiamine hydrochloride due to its antagonistic role in lead induced rats.

Keywords: Lead Acetate; Thiamine; Protein; Urea; Uric Acid and RBC Count; Triglyceride.

Introduction

The term heavy metal refers to any metallic element that has a relatively high density and is toxic or poisonous at low concentration. Examples of heavy metals include, cadmium, mercury, arsenic, chromium, titanium and lead. Heavy metals are natural components of the earth crust. They cannot be degraded or destroyed. To a small extent they enter our body via food, drinking water and ambient air. As trace element, some heavy metal (Copper, selenium, zinc) are essential to maintain the metabolism of human body. However at high concentrations they can lead to poisoning. Heavy metal poisoning could result for instance in drinking water emission or intake via food chain.

They are dangerous and tend to bioaccumulate, bioaccumulate means the concentration of metal in the biological system is much higher than normal. Heavy metals can enter a water supply by industrial or consumer waste or even from acidic rain, breaking down soils and release heavy metals into streams or lakes or rivers or ground water. Lead ranks close to cadmium and mercury as a metal of current toxicological concern. Lead is natural element and widespread in the environment. It is an inorganic toxicant of great environment and occupational concern which was classified as a human carcinogen. The concentration of lead in the earth's crust has been estimated at 12.5 ppm, ranking it as the 36th element in abundance (Abbasi & Soni) Lead is a soft, heavy, toxic, malleable and poor metal .

Lead is emitted into the environment through a large number of natural and anthropogenic sources (Soni). Lead acetate is prepared by and treating litharge (Lead monoxide with acetic acid). Gasoline additives, mainly tetraethyl lead, the principal anthropogenic source of lead in the environment, enter the atmosphere as unburned lead alkyl vapours and as lead halides.

This heavy metal is still and added to many products including paints, eye cosmetics, gasoline, water pipes and health care supplies. Among the

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natural sources, the major contribution comes from wind blown dusts, forest fires, volcanic emission and sea salt sprays (Nriagu). Industrial effluents arising from plating units, and from paper, rayon, dye and pigment, chemical fertilizers, Ghee and battery industries and mine drainage (Soni) are the important sources for lead. Thiamine is absorbed from the small intestine, capacity of human intestine to absorb this vitamin is limited to about mg per day. Thiamine undergoes change in skeletal muscle, heart, liver, kidney, and brain. Excess of thiamine is excreted in urine. Closely associated with functioning of nervous system and body musculation, helps in carbohydrate metabolism, production of hydrohloride that facilitates digeston in the stomach, flow of electrolytes in and out nerve and muscle cells and boosting of enzyme activity for proper digestion, absorption of food and nutrients. Thiamine has antioxidant activity, erythrhropoietic property, mood modulating and glucose regulating activities.

Thiamine Tri Phosphate is necessary for the action of pyruvate dehydrogenase and alpha kG in carbohydrate metabolism and for the action of transketolase in HMP shunt. Thiamine protects against lead toxicity by inhibiting lead induced lipid peroxidation (Mamta Dhawan) Thiamine reverses the lead induced inhibition of the activity of blood , 5 –aminolevulinic acid dehydratase. It helps to maintain normal RBC count, improves circulation.

The heavy metal lead in the form of lead acetate is a neurotoxin and a cumulative poison, hence it is used for the present study. Lead is one of the natural components of the environment and it affects metabolic activities of the body. Extrapolation of the rat to human beings is done for risk assessment.

The Vitamin B1 (Thiamine) in the form of thiamine hydrochloride is given as an antidote to the lead intoxicity in albino rats. Thiamine pyrophosphate, the cofactor form of thiamine, plays a significant role in carbohydrate metabolism, lipid peroxidation and has an impact on other metabolic actions on the human body. Vitamin B1 (Thiamine) has got antagonistic effect towards lead intoxication and hence it is used as an antidote for the present study.

Materials and Methods

Adult male Albino rats (weighing 100-160 g), were procured from Agricultural University Extension Centre, Kattupakkam, Chennai, were kept at room temperature (32 ± 2°C) at L:D (12:12) cycles. All studies were conducted in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" [National institute of health 1]. Animals were randomized and separated into four groups (Group I – control), Group II – lead acetate-treated, Group III – lead acetate- and thiamine hydrochloride treated, Group IV – thiamine hydrochloride treated; n = 6 in each group).

The animals were acclimatized to laboratory conditions prior to the experiment following the procedure of Behringer and NIH. Animals were caged in polypropylene cages and bed was prepared for rats with husk and it was replaced alternatively. [CPCSEA No – IAEC 1/2008/02]. Thiamine hydrochloride was purchased from Sisco Research Laboratories Private Limited, Mumbai India. Lead acetate and all other chemicals used in this study were of analytical grade. Group I animals served as controls. Group II animals were administered with lead acetate intraperitoneally (ip) (100 mg/kg) every day for 14 days [Cory et al]. Group III animals were treated with lead acetate as Group II animals along with Thiamine hydrochloride (150 mg/kg) (ip) [Morrison et al]. Group IV animals received Thiamine hydrochloride (150mg/kg) (ip) for 14 days.

Biochemical determinations were performed after14 days of lead acetate and/or thiamine hydrochloride administration. At the end of experimental period (14 days) animals from all the groups were sacrificed by cervical dislocation. Blood samples were collected from each group of rats. Biochemical analyses were performed in blood. Red blood cell count and Haemoglobin content [Drabkin] content was determined in blood. Uric acid [Caraway], protein [Lowry et al], Urea [Marsh], Triglyceride content [Fletcher] and Alkaline phosphatase (Bower) were also determined. Analysis of variance followed by Least Significant Difference test was carried out to detect the significant differences between control and experimental groups.

Results

Total Count of RBC

Red blood cell count in Group I was 6.5 ± 0.18 million. After the administration of lead acetate, the count in Group II animals was decreased to 3.5± 0.02 million on 7th day, which is then followed by a subsequent decrease on 14 th day. After the administration of thiamine hydrochloride to Group

III animal, the count was around 5.0 ± 1.87 million on 7th and 14th day. In the Group IV animals, the RBC count showed a decrease on 14th day when compared to Group I animal, but the count showed an elevation of 4.6 ± 0.11 million when compared with GroupII animal **(Table1 & Figure 1)**.

Hemoglobin Content

Hemoglobin Content count in Group I was 18.5 ± 0.23 gm%. After the administration of lead acetate, the count in Group II animals was decreased to 9.76± 0.04 gm% on 7th day, which is then followed by a decrease till 14 th day. After the administration of thiamine hydrochloride to Group III animal, the hemoglobin content was around 17.5 ± 0.026 gm% on 7th and 14th day. The haemoglobin content in Group IV animals, showed a decrease on 7th day, 15.0±0.018 gm% and a slight increase on 14th day, the value was 16.35± 0.0312 gm% **(Table 2 & Figure 2)**.

Estimation of Urea

The amount of urea in group I was 23.5±2.5 mg/ dl. After the administration of lead acetate, urea level in Group II animal, showed an elevation on 7th day (34.75 ± 1.09 mg), which is followed by a subsequent elevation on 14th day (40.5±1.32). After the administration of thiamine hydrochloride to Group III animal, the amount of urea was (26.2 ± 1.39 mg) on 7th and 14th day. In the Group IV animals, the amount of urea showed a decrease on 7th day (20.5± 1.11mg) and an increase to (30.7 ± 2.86mg) on 14th day **(Table 3 & Figure 3)**.

Estimation of Urea

The amount of urea present in GroupI was 7.5 ± 0.070g/dl .After the administration of lead acetate in Group II animals, the value of urea showed an increase to 10.25 ± 0.1118 on 7th day, and a decrease to 9.7 0.14g on 14th day. In Group III animals, after the administration of thiamine hydrochloride , the value of urea decreased to 8.5 ± 0.19 g on 7th day which is followed by a slight decrease to 7.75± 0.05g on 14th day. and 14th day. In the Group IV animals, the amount of urea showed a decrease on 7th day(20.5± 1.11mg) and an increase to (30.7 ± 2.86mg) on 14th day **(Table 3 & Figure 3)**.

Estimation of Protein

The amount of protein present in GroupI was 7.5 ± 0.070g/dl. After the administration of lead acetate in Group II animals , the value of protein showed an increase to 10.25 ± 0.1118 on 7th day, and a decrease to 9.7 0.14g on 14th day. In Group III animals, after the administration of thiamine hydrochloride , the protein value decreased to 8.5 ± 0.19 g on 7th day which is followed by a slight decrease to 7.75± 0.05g on 14th day. In Group IV animals, the protein content showed a decrease to 6.75 ± 0.070g on 7th day, which is followed by an increase to 7.57±0.072g on 14th day **(Table 4 & Figure 4)**.

Estimation of Uric Acid

The amount of uric acid in group I was 6.0 ± 0.070 mg. After the administration of lead acetate, uric acid level in Group II animal was increased to 6.5 ± 0.25mg on 7th day, which is followed by an increase to 7.1 ± 0.58 on 14th day, elevation on 14th day. In Group III animals, there was a decrease in uric acid level to 5.2 ± 0.2 mg on 7th day and a slight increase to 5.35 ± 0.12 mg on 14th day. In Group IV animals, there was a decrease in uric acid level on 7th day (5.5 ± 0.30 mg) and a moderate increase on 14th day to 6.07± 0.122mg **(Table5 & Figure 5)**.

Estimation of Triglyceride

The amount of Triglyceride in Group I was 11.31 ± 0.07 mg. After the administration of lead acetate, the value of triglyceride in Group II animals decreased to 31± 2.5 mg on 7th day, which is followed by a decrease to 30.7 ±1.45 on 14th day. After the administration of thiamine hydrochloride to Group III animal, the value of triglyceride was 40 ± 1.41mg on 7th day which is followed by a slight increase to 42.2 ± 2.16 mg on 14th day. In Group IV animal, the value showed 43.25 ± 2.0mg on 7th day, and then a significant decrease to 31.5 ± 1.11 mg on 14th day **(Table 6 & Figure 6)**.

Alkaline Phosphatase

The alkaline phosphatase level in GroupI was 11.31 ± 0.07 IU/L. After the administration of lead acetate in Group II animals, the value was 16.15 ± 0.07 IU/L on 7th day, which is then followed by an increase to 20.11 ± 0.007 IU/L on 14th day. In group III animal, the value was to 24.29± 0.25 IU/l on 7th day and a slight increase to 26.2 ± 0.07 IU/L on 14th day. In Group IV animals, the alkaline phosphatase level was 10.07 ± 0.09IU/L on 7th day, which is followed by an decrease to 8.06±0.01 IU/L on 14th day **(Table 7 & Figure 7)**.

Table 1: Total RBC Count of control and experimental rat (rattus norvegicus) exposed to lead acetate and thiamine hydrochloride

Parameter	Experiment (No. of days)	Control	Lead acetate	Thiamine Hydrochloride	Lead acetate+ Thiamine Hydrochloride
Total RBC count in million/cu.mm.	7	6.52 ± 0.180	3.55 ± 0.022	5.0 ± 1.87	4.62 ± 0.111
	14	6.50 ± 0.132	3.67 ± 0.273	4.87 ± 0.273	4.1 ± 0.180

Values are expressed as Mean ± SD.
Students 't' test
P<0.05, 0.01-significant in all experimental groups.

Table 2: Haemoglobin content of control and experimental animal exposed to lead acetate and thiamine hydrochloride

Parameter	Experimental (No. of days)	Control	Lead acetate	Thiamine Hydrochloride	Lead acetate+ Thiamine Hydrochloride
Haemoglobin content in g%	7	18.55 ± 0.234	9.76 ± 0.040	17.65 ± 0.026	15.02 ± 0.018
	14	18.7 ± 0.187	0.02 ± 0.092	16.56 ± 0.041	16.35 ± 0.0312

Values are expressed as Mean ± SD
Students 't' test
P<0.05, 0.01-significant in all experimental groups.

Table 3: Estimation of uric acid of control and experimental rat exposed to lead acetate and thiamine hydrochloride

Parameter	Experiment (No. of days)	Control	Lead acetate	Thiamine Hydrochloride	Lead acetate + Thiamine Hydrochloride
Uric Acid (mg/dl)	7	6.0 ± 0.070	6.52 ± 0.254	5.22 ± 0.15	5.5 ± 0.308
	14	6.15 ± 0.234	3.67 ± 0.273	5.35 ± 0.122	6.07 ± 0.122

Values are expressed as Mean ± SD
Students 't' test
P<0.05, 0.01-significant in all experimental groups.

Table 4: Estimation of protein of control and experimental rat exposed to lead acetate and thiamine hydrochloride

Parameter	Experiment (No. of days)	Control	Lead acetate	Thiamine Hydrochloride	Lead acetate + Thiamine Hydrochloride
Protein (g/dl)	7	7.5 ± 0.070	10.25 ± 0.1118	8.5 ± 0.192	6.75 ± 0.070
	14	7.25 ± 0.111	9.7 ± 0.147	7.75 ± 0.05	7.57 ± 0.072

Values are expressed as Mean ± SD.
Students 't' test
P<0.05, 0.01-significant in all experimental groups.

Table 5: Estimation of urea or control and experimental animal exposed to lead acetate and thiamine hydrochloride

Parameter	Experiment (No. of days)	Control	Lead acetate	Thiamine Hydrochloride	Lead acetate +Thiamine Hydrochloride
Urea (mg/dl)	7	23.5 ± 2.5	34.75 ± 1.09	26.25 ± 1.39	20.5 ± 1.11
	14	24 ± 1.3	40.5 ± 1.32	26.5 ± 2.69	30.75 ± 2.86

Values are expressed as Mean ± SD.
Students 't' test
P<0.05, 0.01-significant in all experimental groups.

Table 6: Estimation of triglyceride of control and experimental rat (rattus norvegicus) exposed to lead acetate and thiamine hydrochloride

Parameter	Experiment (No. of days)	Control	Lead acetate	Thiamine Hydrochloride	Lead acetate + Thiamine Hydrochloride
Triglyceride (mg/dl)	7	44.25 ± 2.86	31 ± 2.54	40 ± 1.41	43.25 ± 2.046
	14	45.2 ± 1.786	30.75 ± 1.457	42.25 ± 2.165	31.5 ± 1.118

Table 7: Assay of alkaline phosphatase of control and experimental rat exposed to lead acetate and thiamine hydrochloride

Parameter	Experiment (No. of days)	Control	Lead acetate	Thiamine Hydrochloride	Lead acetate + Thiamine Hydrochloride
Alkaline Phosphatase (IU/L)	7	11.31 ± 0.070	16.15 ± 0.070	24.29 ± 0.251	10.07 ± 0.09
	14	10.9 ± 0.050	20.11 ± 0.007	26.2 ± 0.070	8.06 ± 0.0132

Values are expressed as Mean ± SD, Students 't' test, P<0.05,0.01-significant in all experimental groups.

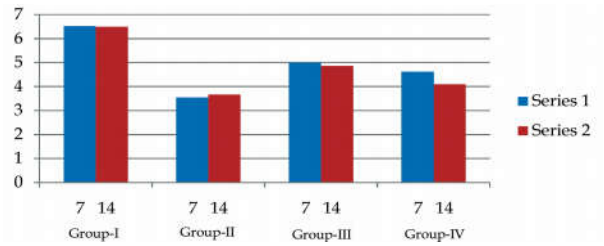


Fig. 1:

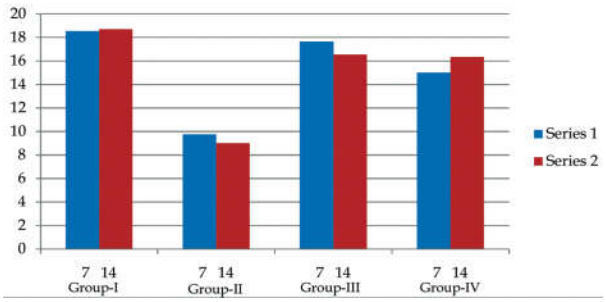
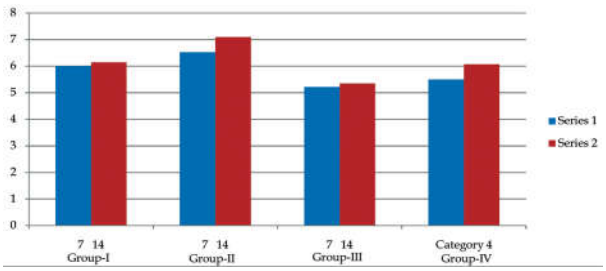


Fig. 2:



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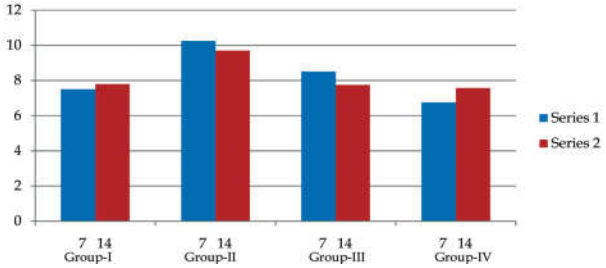

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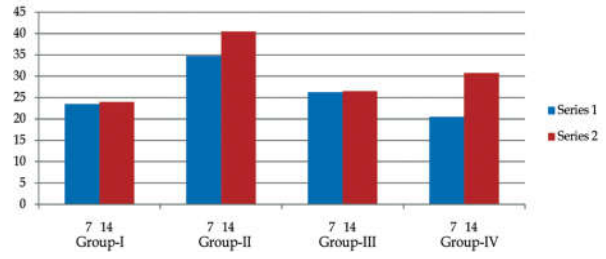

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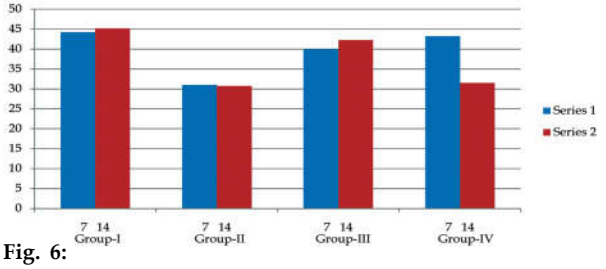

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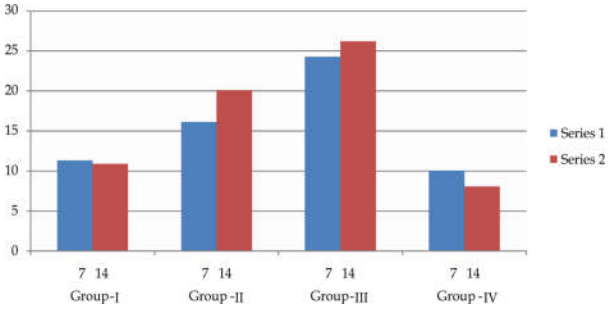

Fig. 6:

Discussion

Lead is a cumulative toxic element of increasing use in industry (Demayo et al). Several reports during past decades have revealed human population at high risk for metal toxicities through environment pollution (Dorn et al., Creason et al ., Lnadrigan et al .).

Industrial and experimental evidence showed lead to be one of the most hazardous metals and increasing use in industry (Goyer, et al).

In the present study with lead acetate, the RBC count was significantly decreased in experimental animals compared to control. Lead has been shown to produce acute haemolytic crisis which results in severe anaemia, haemoglobinuria and stippling of blood cells in mammals (Albahary). The animals which were dosed with thiamine against lead intoxication, maintain the normal RBC count. Thiamine has got erythropoietic activity, helps to maintain normal RBC count and improves circulation.

The haemoglobin content the lead induced rates showed a significant decreases. A fall in the concentration of haemoglobin might be due to depleted rate of haemoglobin synthesis. Depletion in the haemoglobin synthesis rate begins in the polychromatic normoblasts stage in the process of erythropoieses in bone marrow (Lewis). Impaired haemoglobin synthesis might be due to decrease in iron available or reduced iron uptake by developing erythrocytes. Similar results have also been obtained when mice were dosed with lead chromate (Chakravarthy et al).The thiamine antagonistic action lead intoxication, showed an elevation in haemoglobin content.

Lead intoxication also significantly augments the uric acid concentration. Uric acid is the end product of the catabolism of tissue nucleic acid, i.e, purine and pyrimidine bases metabolism (Sharma). The increments in uric acid concentration may be due to

the degradation of purines or to an increase of uric acid levels by either overproduction or inability of excretion. Similar results have been obtained in mice dosed with lead chromate (Chakravarthy et al). Thiamine administration resulted in decrease in uric acid level.

In the lead acetate induced animals, there was an elevated level of protein, followed by a decrease, which clearly depicts the disturbance in liver function depresses the serum protein production and thus results in hypoproteinemia in animals (Thompson et al). Presumably, the effects results have also been obtained in the lead loaded albino rats treatment with chelating agents and natural oils (Binger).

The elevation of urea occurs in the lead acetate induced rats. Urea is the principle product of protein catabolism. Enhanced protein catabolism together with accelerated amino acid deamination for gluconeogenesis is probably an acceptable postulate to interpet the elevated urea. Similar results have also obtained on the lead loaded albino rats and treatment with chelating agents and naturlal oils (Binger).

The triglyceride contents of serum decreased when compared to control in lead induced albino rats. Changes in triglyceride levels are reported in the diseases of liver and biliary tract (Singh et al). They are considered as valuable indicators of drug induced distribution of lipid metabolism, development of fatty liver and impairment biliary secretion (Sharma). In this study, the decreased triglyceride levels might be due to impairment of liver function .

In the present study, the alkaline phosphatase level was increased. It is an intercellular enzyme found primarly in the liver. It is also produced in small amounts by cells lining the intestine, the placenta and the kidney. The primary importance of measuring alkaline phosphatise is to check the possibility of liver damage. When liver and bile duct system are not functioning properly, this enzyme is not excreted through the bile duct as a result ,the enzyme is released into the blood stream. Similar results have also been obtained in mice dosed with lead chromate (Chakarvathy et al). Thiamine hydrochloride has got antagonistic action against lead acetate and hence enzymes level was slightly back to normal as it has got regularly role in all metabolism .

The haematological and serological findings in the present investigation suggests that lead acetate adversely affects the blood cells of the animals and causes improper functioning of the liver. On the other hand, thiamine acts an antagonistic and almost and reverses the condition .

It is necessary to generate awareness among people regarding the toxic effects of lead acetate, which is classified as a human carcinogen.

Conclusion

Lead, a heavy metal was selected though it is a non- essential element yet a carcinogen and hence it's effect was studied in the Swiss albino rats. The effect of heavy metal and vitamin B1 on the haematological and biochemical parameters was studied. Total count of RBC, haemoglobin content, triglyceride level decreased throughout the period of study, in lead induced animals. Protein, urea, uric acid levels are increased throughout the study when treated with lead acetate. On the contrary, Thiamine hydrochloride acts as an antagonist to the lead acetate and the results were reversed.

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