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Assessment of Hematological Parameters to Study the Effect of Thiamine Hydrochloride on Lead Acetate Induced Toxicity in Wistar Rats

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Abstract

The effect of thiamine hydrochloride against lead induced acute toxicity was experimentally studied in rats. Hematological parameters such as WBC count and differential count were determined and a decrease in their levels were observed throughout study period in the lead acetate induced animals. Cholesterol level was also found to be decreased throughout the period of study, in lead acetate induced animals. Biochemical alterations were reversed on treatment with thiamine hydrochloride due to its ameliorative role in lead induced rats.

Keywords: Lead acetate; Thiamine hydrochloride and WBC Count.

Introduction

Lead is a ubiquitous environmental pollutant that has been detected in various phases of environmental and biological systems. Lead induces a broad range of physiological, biochemical, and behavioural dysfunction in research animals and humans, including central and peripheral nervous systems, haemopoietic system, cardiac system, liver, kidneys and human reproductive systems [1]. Lead has its effects on the peripheral nervous system in adults while in children the central nervous system is much more affected [2]. Encephalopathy, lack of coordination, convulsions, paralysis and coma are the effects of lead on central nervous system [3]. It inhibits various key enzymes involved in the heme synthesis and affects the hematopoietic system. It increases the fragility of cell reducing the lifespan of erythrocytes. These two processes results in anemia [4,5]. Acute and chronic nephropathy is the renal abnormality that occurs due to lead exposure [6].

Common effects of lead seen in men include: abnormal spermatogenesis, chromosomal damage, infertility, altered prostatic function and changes in serum testosterone. Infertility, premature membrane rupture, pre-eclampsia, pregnancy hypertension and premature delivery [7] are the effects of lead on women.

Lead exposure induces free radicals generation that results in the pathogenesis, which could be overcome by antioxidant supplementation, an alternative for chelation therapy [8]. This vitamin may chelate lead from the tissues. The pathogenesis of lead toxicity might be due to its direct interruption in enzyme activation, competitive inhibition of trace mineral absorption, interrupts structural

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protein synthesis by binding to sulfhydryl proteins, alteration in calcium homeostasis, and lowers the level of available sulfhydryl antioxidant reserves in the body [9,10], reported that thiamine scavenges superoxide and hydroxyl radicals thereby reduces the oxidative stress. Thiamine is a water soluble sulphydryl group containing vitamin, the most recommended therapeutic agent for the lead toxicity studies. Researchers have postulated that thiamine plays role in the decrease in lead absorption and stimulates its excretion [11]. The present study aimed to investigate the ameliorative effects of using thiamine hydrochloride on lead toxicity in albino rats using blood parameters as indicators of oxidative stress.

Materials and Methods

Male Albino rats with the weights ranging from 100-160g, were purchased from Agricultural University Extension Centre, Kattupakkam, Chennai, were kept at room temperature (32 ± 2^0C) at L:D (12:12) cycles. Experiments were done in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" [13]. Animals were categorized into four groups of six animals in each group (Group I – control, Group II – lead acetate-treated, Group III – lead acetate and thiamine hydrochloride treated, Group IV – thiamine hydrochloride treated). All animals were acclimatized to laboratory conditions before the experiment. Animals were maintained in polypropylene cages and provided with standard food pellets and *ad libitum*. [CPCSEA No – IAEC 1/2008/02]. Thiamine hydrochloride was purchased from Sisco Research Laboratories Private Limited, Mumbai India. All chemicals inclusive of lead acetate used were of analytical grade. Group I animals served as control. Group II animals were administered with lead acetate intraperitoneally (100 mg/kg) every day for 14 days [14]. Group III animals were administered with Thiamine hydrochloride (150 mg/kg) (ip) [15] and lead acetate Intraperitoneally (ip) (100 mg/kg) every day for 14 days. Group IV animals received Thiamine hydrochloride (150mg/kg) (*ip*) for 14 days. Biochemical determinations were performed after 14 days of lead acetate and/or thiamine hydrochloride administration. At the end of experimental period (14 days) animals from all groups were sacrificed by cervical dislocation. Blood samples were collected from each group of rats. In one tube blood was collected and WBC Count [16], Differential count [17] was performed. In another tube blood was collected and left aside to clot. Cholesterol estimation was done by [18].

Analysis of variance followed by Least Significant Difference test was carried out to detect the significant differences between control and the other groups.

Results

Table 1: Total WBC count of control and experimental rat (*Rattus norvegicus*) exposed to lead acetate and thiamine hydrochloride.

Parameter	Exposure period (No. of days)	Control (cells/cu.mm)	Lead Acetate (cells/cu.mm)	Lead Acetate+ Thiamine Hydrochloride (cells/cu.mm)	Thiamine Hydrochloride (cells/cu.mm)
WBC Count (cells/cu.mm)	7th Day	2.2 ± 0.04	2.6 ± 0.02	1.2 ± 0.05	1.9 ± 0.07
	14th Day	1.6 ± 0.01	2.8 ± 0.01	1.15 ± 0.02	1.62 ± 0.05

Values are expressed as Mean ± SD

Students 't' test

p<0.05, 0.01-significant in all experimental groups.

Table 2: Differential leucocyte Count of control and experimental rat (*Rattus norvegicus*) exposed to Lead Acetate and Thiamine Hydrochloride

Parameter	Exposure period (Days)	Control (%)	Lead acetate (%)	Lead acetate + Thiamine Hydrochloride (%)	Thiamine Hydrochloride (%)
Lymphocyte	7th Day	65.25 ± 3.18	43.75 ± 3.03	51.25 ± 3.04	61.25 ± 1.09
	14th Day	70.75 ± 1.02	51.75 ± 2.38	35 ± 1.2	32 ± 1.92

Values are expressed as Mean ± SD

Students 't' test

p<0.05, 0.01-significant in all experimental group

Table 3: Differential leukocyte count of control and experimental rat (*Rattus norvegicus*) exposed to lead acetate and thiamine hydrochloride.

Leukocytes	Exposure period (Days)	Control (%)	Lead Acetate (%)	Lead acetate + Thiamine Hydrochloride (%)	Thiamine Hydrochloride (%)
Neutrophil	7th Day	41.5 ± 1.25	40.75± 1.16	37 ± 1.22	32 ± 0.70
	14th Day	30.5 ± 1.11	47 ± 1.22	37 ± 1.41	28.5 ± 1.11

Values are expressed as Mean ± SD

Students 't' test

p<0.05, 0.01-significant in all experimental groups

Table 4: Differential leukocyte count of control and experimental rat *(Rattus norvegicus*) exposed to lead acetate and thiamine hydrochloride.

Leukocytes	Exposure period (Days)	Control (%)	Lead Acetate (%)	Lead acetate + Thiamine Hydrochloride (%)	Thiamine Hydrochloride (%)
Basophil	7th Day	5.5 ± 1.11	3 ± 1.11	3.25 ± 0.90	4.75 ± 0.70
	14th Day	6.25 ± 0.90	3.75 ± 0,24	5.5 ± 0.5	5 ± 0.70

Values are expressed as Mean ± SD.

Students 't' test

p<0.05, 0.01-significant in all experimental groups.

Table 5: Differential leukocyte count of control and experimental rat *(Rattus norvegicus*) exposed to lead acetate and thiamine hydrochloride.

Leukocytes	Exposure Period (Days)	Control (%)	Lead Acetate (%)	Lead acetate +Thiamine Hydrochloride (%)	Thiamine Hydrochloride (%)
Eosinophil	7th Day	5.5 ± 0.65	2.75±0.43	5.5 ± 1.65	3.25 ± 0.83
	14th Day	6.75±0.43	4.25 ± 0.78	5.75 ± 0.43	5.5 ± 1.65

Values are expressed as Mean ± SD.

Students 't' test

p<0.05, 0.01-significant in all experimental groups.

Table 6: Differential leukocyte count of control and experimental rat *(Rattus norvegicus*) exposed to lead acetate and thiamine hydrochloride.

Leukocytes	Exposure Period (Days)	Control (%)	Lead Acetate (%)	Lead acetate +Thiamine Hydrochloride (%)	Thiamine Hydrochloride (%)
Monocyte	7th Day	6.75 ± 1.11	4 ± 0,70	4.25 ± 0.83	4.25± 0.83
	14th Day	5.5 ± 1.11	4.25 ± 0.82	5.5 ± 0.5	5.5 ± 0.5

Values are expressed as Mean ± SD.

Students 't' test.

p<0.05, 0.01 –significant in all experimental groups.

Table 9: Cholesterol Level in control and experimental rat exposed to lead acetate and thiamine hydrochloride

Exposure Period (Days)	Control (mg/dl)	Lead Acetate (mg/dl)	Lead acetate + Thiamine Hydrochloride (mg/dl)	Thiamine Hydrochloride (mg/dl)
7th Day	154.1 ± 0.05	137.6 ± 0.04	85.55 ± 0.02	120.1 ± 0.01
14th Day	102.6 ± 0.04	80.3 ± 0.02	111.2 ± 0.04	128.5 ± 0.03

Values are expressed as Mean ± SD

Students 't' test

p<0.05, 0.01-significant in all experimental groups.

Discussion

Lead is one of the toxic heavy metals of much significance. Exposure to heavy metals such as lead may cause chronic diseases (diabetes, renal disease, cancer, male infertility etc.) [19]. Oxidative stress represents an imbalance between the production of free radicals and the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage [7]. It has been reported as a major mechanism of lead induced toxicity [6]. Under the influence of lead, onset of oxidative stress occurs on account of two different pathways operative simultaneously. First, the generation of reactive oxygen species, ROS and second, the antioxidant reserves become depleted [20]. Apart from targeting the sulfhydryl groups, lead can also replace the zinc ions that serve as important cofactors for these antioxidant enzymes and inactivate them [21]. Lipid peroxidation, another indicator of oxidative stress occurs as a result of the action of ROS on lipid membranes [22]. Products of lipid peroxidation disrupts the physicochemical properties, fluidity, and integrity of cell membranes, increasing susceptibility to lipid peroxidation and cell necrosis. Many reports have showed that thiamine detoxifies lead by the formation of complexes with lead [22,23]. lead toxicity has not been clearly elucidated until now. It might be attributed to the formation of complexes between thiamine and lead followed by its excretion. Thiamine also has been found to protect against lead-induced lipid peroxidation in rat liverand kidney [11].

In our study with lead acetate, WBC Count decreased during the entire period of study. The term leucopenia describes a condition characterized by a low white blood cell count. Researchers reported low count of WBC in the disorders of liver and spleen. Hence the decreased WBC Count in the present investigation may be due to damage of the liver caused by lead toxicity. Similar results have been reported when mice were treated with lead chromate [24]. Results show that the lead acetate increased significantly the levels of cholesterol in Group II rats which might be due to oxidative stress caused by lead acetate.

Conclusion

Thus the present study showed that lead acetate induces free radical formation in rats and this condition reverted to the normal as that of control by the treatment with thiamine hydrochloride, which proved the anti protective role of thiamine against the lead toxicity.

References

1. Aly MH, Kim HC, Renner SW, Boyarsky A, Kosmin M, Paglia DE.. Hemolytic anemia associated with lead poisoning from shotgun pellets and the response to succimer treatment. American Journal of Hematolology. 1993;44:280–83.
2. Flora SJS, Pande M, Mehta A. Beneficial effect of combined administration of some naturally occurring antioxidants (vitamins) and thiol chelators in the treatment of chronic lead intoxication. Chemico-Biological Interactions. 2003;145:267-80.
3. Flora G, Gupta D, Tiwari A. Toxicity of lead: A review with recent updates. Inter discip Toxicol. 2012;5:47–58.
4. Cornelis R, Caruso J, Crews H, Heumann K. Handbook of elemental speciation II: species in the environment, food, medicine & occupational health: John Wiley & Sons, Ltd 2005.
5. Guidotti TL, McNamara J, Moses MS. The interpretation of trace element analysis in body fluids. Indian J Med Res. 2008;128:524-32.
6. Flora G, Gupta D, Tiwari A. Toxicity of lead: A review with recent updates. Inter discip Toxicol 2012;5:47–58.
7. Flora SJS, Pachauri V, Saxena G. Arsenic, cadmium and lead. Reproductive and Developmental Toxicology 2011: Academic Press.
8. Flora SJS, Pande M, Mehta A. Beneficial effect of combined administration of some naturally occurring antioxidants (vitamins) and thiol chelators in the treatment of chronic lead intoxication. Chemico-Biological Interactions. 2003;145:267-80.
9. Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Current Topics in Medicinal Chemistry. 2001;1:529-539.
10. Wang C, Liang J, Zhang C, Bi Y, Shi X, Shi Q. Effect of ascorbic acid and thiamine supplementation at different concentrations on lead toxicity in liver. Annals of Occupational Hygiene. 2007;51:563-69.
11. Senapati SK, Dey S, Dwivedi SK, Patra RC, Swarup D. Effect of thiamine hydrochloride on lead induced lipid peroxidation in rat liver and kidney. Vet Hum Toxicol. 2000;42:236-37.
12. KIM JS, Blakeley HR, Rousseaux CG. The effects of thiamine on tissue distribution of lead. J.Appl Toxicol. 1990;10:93-97.
13. National Institutes of Health Guide for Care and Use of Laboratory Animals. Guide for the care and use of laboratory animals, DHEW Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892.

14. Lubbad MY, Al-Quraishy S, Dkhil MA. Antimalarial and antioxidant activities of *Indigofera oblongifolia* on Plasmodium chabaudi-induced spleen tissue injury in mice. Parasitol Res. 2015.p.10–14.
15. Morrison AB, Campbell JA. Vitamin absorption studies. 1. Factors influencing the excretion of oral test doses of thiamine and riboflavin by human subjects. J Nutr. 1960;72:435–40.
16. Crosland-Taylor Pl. The micro PCV. In: van Assendelft OW, England 1M, eds. Advances in Haematological Methods: the Blood Count. Boca Raton, Florida: CRC Press, 1982.pp.85-92.
17. MacGregor RG. Scott RW and Loh GL., The differential leucocyte count. J Pathol Bacteriol. 1940; 51:337–68.
18. Zlatkis A., Zak B., and Boyle, A.J. A new method for the direct determination of serum cholesterol. J. Lab. Clin. Med. 1953;41:486-91.
19. Olkowski AA, Gooneratne SR, Christensen DA. The effects of thiamine and EDTA on biliary and urinary lead excretion in sheep. Toxicology Letters 1991;59:153-59.
20. Flora S.J.S. Nutritional components modify metal absorption, toxic response and chelation therapy. J. Nutr. Environ. Med. 2002;12 53–67.
21. Flora S.J., Flora G., Saxena G., Mishra M. Arsenic and lead induced free radical generation and their reversibility following chelation. Cell Mol. Biol. 2007 Apr 15;53(1):26-47.
22. Kim JS, Blakeley HR, Rousseaux CG. The effects of thiamine on tissue distribution of lead. J. Appl Toxicol. 1990;10:93-97.
23. Orisakwe O.E. Lead and Cadmium in Public Health physicians neglect and pitfall in patient management. N. Am. J. Med. Sci. 2014;6:70–61.
24. Chakarvarthy G, Goyal R.P, Sharma A. Haematological and Biochemical changes induced by lead chromate in swiss albino mice. Indian J. Environ. Sci. 2005.