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Original Research

Thiamine and pyridoxine supplementations ameliorate subchronic lead-induced hepatotoxicity and nephrotoxicity: single action and cocktail effect analysis

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ABSTRACT

The study investigated the role of thiamine and pyridoxine individually and in cocktail in mitigating the biochemical alterations induced by subchronic administration of 500mg/kg of lead acetate for 6 weeks. Twenty – five Wistar albino rats divided into five groups of five animals in each group were used for this study. The first group, the negative control, received no treatment. The second group, the positive control, had 500mg of lead acetate added per kg of feed. The three other groups were given the same regimen as the second group but in addition, 300mg of thiamine per kg diet was added to the third group, 300mg of pyridoxine per kg diet added to the fourth group and 300mg of vitamin mixture containing 150mg of thiamine as well as 150mg of pyridoxine was added per kg diet to the fifth group. The rats were sacrificed after 6 weeks and sera were analysed for alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) activities. Urea and creatinine concentrations were also evaluated. The activities of ALT, AST and ALP as well as the levels of urea and creatinine were significantly lower in all groups that received vitamin supplementation when compared with the positive control. This indicates that thiamine, and pyridoxine has ameliorative effect on lead induced toxicity on liver and kidney of Wistar albino rats. The significant difference between the cocktail group and other individual vitamin groups also showed an additive effect when thiamine and pyridoxine are administered in combination.

KEY WORDS: Thiamine, Pyridoxine, Oxidative stress, Alanine transaminase, Aspartate transaminase, Alkaline phosphatase

List of abbreviations: **ALT**- Alanine transaminase; **AST**- Aspartate transaminase; **ALP**- Alkaline phosphatase; **ALAD**- δ- aminolevulinic acid dehydratase; **GSH**- Glutathione (reduced); **ROS**- Reactive oxygenated species; **SEM**- Standard Error Mean.

INTRODUCTION

Lead is a persistent ubiquitous environmental and industrial pollutant that has been detected in various phases of environmental and biological systems. Occupational and environmental exposure to lead has been a serious problem especially in industrialising and developing parts of the world.

Lead is known to induce a broad range of physiological, biochemical, and behavioural dysfunctions in laboratory animals and humans, including central and peripheral nervous systems, haemopoietic system, cardiovascular system, kidneys, liver and male and female reproductive systems[1]. The effects of lead on the peripheral nervous system are more pronounced in adults while the central nervous system is more prominently affected in children[2]. Encephalopathy can manifest in the nervous system due to lead exposure, but at high exposures, more severe manifestations which may include delirium, lack of coordination, convulsions, paralysis, coma and ataxia occur[3]. Lead directly affects the hematopoietic system through restraining the synthesis of haemoglobin by inhibiting various key enzymes involved in the heme synthesis pathway. It also reduces the life span of circulating erythrocytes by increasing the fragility of cell

membranes. The combined aftermath of these two processes leads to anemia [4,5]. Acute and chronic nephropathy is renal abnormalities that have been reported to occur due to lead exposure [6]. Lead also causes a number of adverse effects on the reproductive system in both men and women. Common effects seen in men include: reduced libido, abnormal spermatogenesis (reduced motility and number), chromosomal damage, infertility, abnormal prostatic function and changes in serum testosterone. Women on the other hand, are more susceptible to infertility, miscarriage, premature membrane rupture, pre-eclampsia, pregnancy hypertension and premature delivery [7]. Both chronic and acute lead poisoning causes cardiac and vascular damage with potentially lethal consequences including hypertension and cardiovascular disease[8].

The pathogenesis of lead toxicity is multifactorial, as lead directly interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl proteins (interrupting structural protein synthesis), alters calcium homeostasis, and lowers the level of available sulfhydryl antioxidant reserves in the body[9]. Lead-induced oxidative stress has been identified as the primary contributory agent in the pathogenesis of lead poisoning[1]. Oxidative stress has also been implicated in specific organs with lead-associated injury, including liver, kidneys and brain tissue. Reactive oxidative species (ROS) generated as a result of lead exposure have been identified in lungs, endothelial tissue, testes, sperm, liver, and brain [1].

Based on the observation that free radicals were generated during the pathogenetic processes induced by lead exposure, it was presumed that supplementation of antioxidants could be an alternative method for chelation therapy[10]. The role of vitamins particularly B, C and E has been found to be extremely significant in fighting toxicological manifestations of lead poisoning. These vitamins may chelate lead from the tissues along with restoring the pro/antioxidant balance [6]. Vitamin C, a well-known free-radical scavenger has been shown to inhibit lipid peroxidation in liver and brain tissue of lead-exposed animals[11]. In lead-exposed rats, a minimal 500 mg/L concentration in drinking water was able to reduce ROS levels by 40 percent[12]. Vitamin B6 (pyridoxine) is an important co-factor which participates in the metabolic trans-sulfuration pathway that is responsible for the synthesis of cysteine from dietary methionine. Vitamin B6 acts also as an antioxidant by stimulating the production of GSH and as a moderate chelator[13]. Supplementation with pyridoxine in lead exposed rats has been shown to improve ALAD activity and also reduce the lead levels in the blood, liver and kidney of rats[14].Vitamin B1 (thiamine) has been reported to exert protective efficacy against short term implications of lead poisoning. The protective roles of thiamine hydrochloride on lead-induced endogenous lipid peroxidation in rat hepatic and renal tissues have been documented[15,16]. Vitamin E is a fat soluble vitamin with numerous biological functions[17]. It also possesses powerful anti-oxidative properties, operative in the membrane to prevent lipid peroxidation by obstructing the free radical chain reaction. Sajithaet al.[18] demonstrated that vitamin E administered to rats counteracted the deleterious effect of lead by scavenging free radicals and thus preventing oxidative stress.

Removing lead from industry is a difficult challenge in most of the developing countries and due emphasis is given to develop ways of managing cases of lead intoxication. While few studies have explored the individual actions of the water-soluble vitamins such as thiamine and pyridoxine on subchronic lead-induced toxicity, mixture effect analysis of the two vitamins are however, lacking to underpin informed management decision of the toxicant. This study which aimed to investigate the ameliorative effects of using vitamin B1 and B6 supplements individually and in combination on lead toxicity in albino rats using liver and kidney function as indicators is one of such attempts.

METHODS

Animals and management

Five-week old Wistar rats weighing 124-130g were obtained from the Veterinary and Animal Husbandry Unit, University of Nigeria, Nssukka. The rats were fed on the compounded feeds (see Table 1)and water provided ad libitum. The experiment and animal handling were performed in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals[19].

Test chemical

Analytical grade lead (II) acetate-3- hydrate procured from BHD chemicals Poole, England was used for the study as the toxicant. High grade thiamine and pyridoxine vitamins were sourced and purchased from Impact Pharmaceuticals Limited, Trans- Ekulu, Enugu, Enugu state, Nigeria.

Experimental animal treatment

After range finding preliminary trials, treatment of animals was conducted using 25 male Wistar albino rats randomly divided into five groups of 5 rats per group. Animals in group I which received normal diet served as the negative control, group II which received 500mg lead acetate per kg feed served as the positive control, group III received 500mg lead acetate and 300mg thiamine per kg feed, group IV received 500mg lead acetate and 300 mg pyridoxine per kg feed and group V received 500mg lead acetate, 150mg thiamine and 150mg pyridoxine per kg feed for period of 42 days. The composition of the feed diets is shown in Table 1.

Table 1: Composition of the compounded diets for the experiment

	Component (g/100 - 100.80g)							
	salt	Vitamin/mineral mix	Palm oil	Calcium caseinate	Maize flour	Lead acetate	Thiamine	Pyridoxine
I (Negative control)	2.00	1.00	10.00	22.00	65.00	-	-	-
II (Positive control)	2.00	1.00	10.00	22.00	65.00	0.50		
III (Thiamine treat)	2.00	0.20	10.00	22.00	65.00	0.50	0.30	-
IV (Pyridoxine treated)	2.00	0.20	10.00	22.00	65.00	0.50	-	0.30
V (Pyridoxine + thiamine treated)	2.00	0.20	10.00	22.00	65.00	0.50	0.15	0.15

Group I and II measured in g/100g and g/100.50g, respectively, while Group III-V measured in g/100.80g

Serumbiochemical analysis

After 42 days of treatment, the animals were euthanized using chloroform and blood samples were immediately collected in to labelled test tubes by the cardiac puncture method. The blood samples were centrifuged at 300rpm for 10 minutes.The sera samples were collected and utilized for alanine transaminase, aspartate transaminase and alkaline phosphatase activity assays and urea and creatinine concentration assays using Mindray BS- 120 Chemistry autoanalyser (Bayer Express Plus, Germany)which has the measuring principles of absorbance photometry, turbidimetry and ion selective electrode technology.

Statistics

All the data collected were subjected to statistical analysis. Results were expressed as mean ±SEM and analyzed by SPSS for windows (15.0 version, SPSS Inc, Chicago, USA). The Tukey test and one-way ANOVA were used for comparison of means. The values were considered significant when $p<0.05$.

RESULTS

Table 2 shows the average feed intake for the period of exposure while Table 3 presents the lead acetate and vitamins ingested for 42 days. The rats ingested variable amounts of exposed lead and supplemented vitamins.

Biochemistry

The enzyme activities, and urea and creatinine analysed as biomarkers of hepatotoxicity and nephrotoxicity, respectively are shown in Figures 1-5 encompassing comparisons among the treatment groups.

Table 2. Average feed intakes of the ratsfor 42 days

Week	Treatment group (g/rat/day)				
	I	II	III	IV	V
1	24.00	23.90	23.80	23.70	23.80
2	24.00	23.40	23.80	23.70	23.60
3	24.20	23.40	23.70	23.60	23.60
4	24.10	23.20	23.70	23.60	23.60
5	23.80	22.80	23.60	23.50	23.50
6	23.60	22.60	23.60	23.50	23.50

Table 3: Average lead and supplemented vitamins ingested per rat for 42 days

Group	Ingestion (mg)	
	Lead	Vitamin
I (Negative control)	0.00	0.00
II (Positive control)	97.62	0.00
III (Thiamine)	99.64	59.72
IV (Pyridoxine)	99.20	59.47
V (Thiamine + pyridoxine)	99.20	59.47

Alanine transaminase activity

The bar chart presented in Figure 1 shows the ALT activity following chronic lead ingestion. There was significant increase ($p<0.05$) in alanine transaminase (ALT) activity in lead (Pb) administered group without a palliative (positive control group) when compared to the negative control group. ALT activity was also significantly higher ($p<0.05$) in the positive control group relative to thetreated groups that had their feed supplementedwith thiamine, pyridoxine and cocktail.However, ALT experimentally revealed significantly higher activity in animal groups treated with thiamine and pyridoxine when compared to the cocktail group (Figure 1).

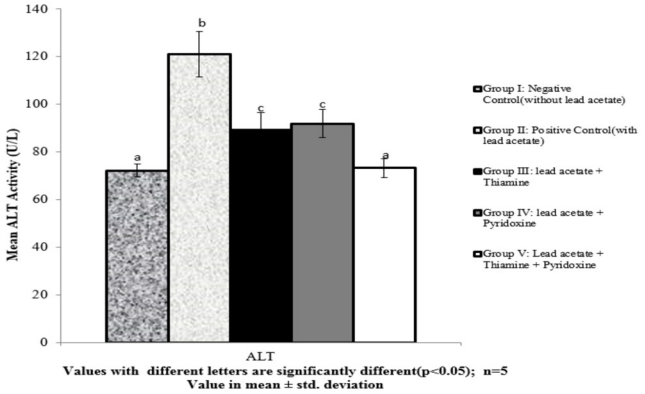

Fig 1. Effect of thiamine and pyridoxine singly and in combination on alanine transaminase (ALT) activity.

Aspartate transaminase activity

Lead ingestion in Wistar rat group without a palliative resulted in significant increase ($p<0.05$) in aspartate transaminase (AST) activity on comparison with the negative control group (Figure 2). Thiamine, pyridoxine and cocktail treated groups also demonstrated higher ($p<0.05$) AST activity taking into account the outcome of positive control group.Significantactivity of AST exists in the thiamine and pyridoxine treated groups when compared to the animals exposed to vitamin mixture (Figure 2).

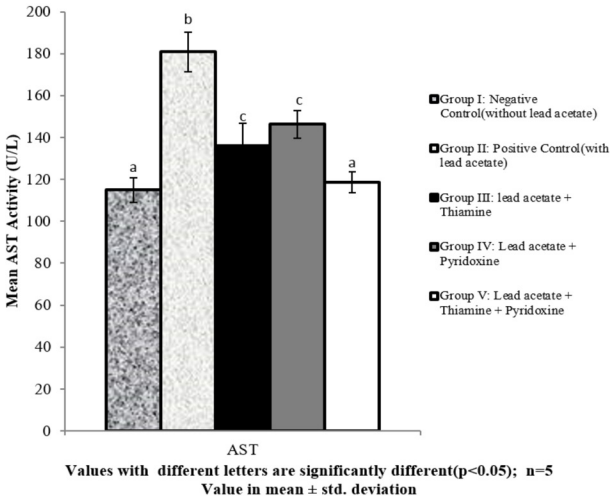

Fig 2. Effect of thiamine and pyridoxine singly and in combination on aspartate transaminase concentration.

Alkaline phosphatase activity

Figure 3 shows the effects of lead and vitamins on alkaline phosphatase activity. ALP activity significantly increased ($p<0.05$) following lead exposure in Wistar albino rat group without vitamin supplementation (positive control), effect not practically observed in the negative control. Similar increase ($p<0.05$) was recorded in ALP of positive control animal group on comparison with the animals exposed to thiamine, pyridoxine and cocktail. Conversely, lead-dosed rats treated with combined vitamins showed lower ($p<0.05$) ALP activity than the groups with single supplementation of thiamine and pyridoxine.

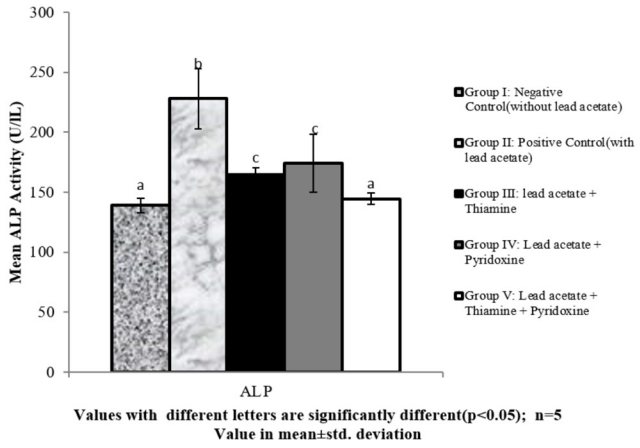

Fig 3. Effect of thiamine and pyridoxine singly and in combination on alkaline phosphatase (ALP) activity.

Serum urea concentration

There was high ($p<0.05$) urea concentration in positive control group with ordinary lead exposure compared to the groups treated with thiamine, pyridoxine and vitamin binary mixture (Figure 4).Lead-exposed groups with single vitamin supplementations (thiamine and

pyridoxine) correspondingly recorded higher ($p<0.05$) urea concentrations than the cocktail group.

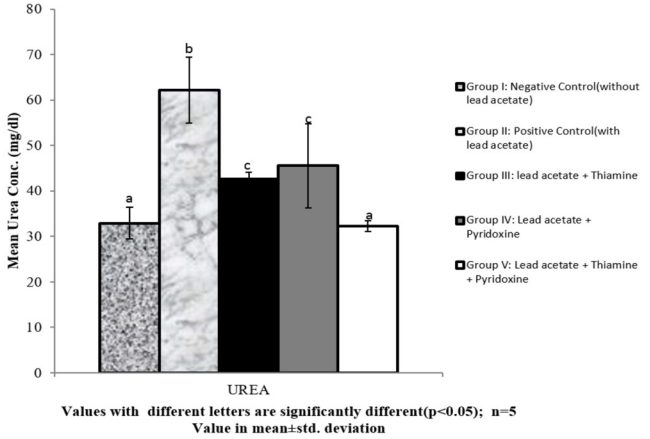

Fig 4. Effect of thiamine and pyridoxine singly and in combination on serum urea concentration

Serum creatinine concentration

Effects of subchronic lead ingestion in serum creatinine concentrations of exposed albino rats are presented in Figure 5. Positive control group had higher ($p<0.05$) creatinine concentrations than the negative control, thiamine, pyridoxine, and their mixture treated groups, respectively. Moreover, there was also a significantly higher ($p<0.05$) concentration of creatinine in the thiamine and pyridoxine treated groups when compared with the cocktail group.

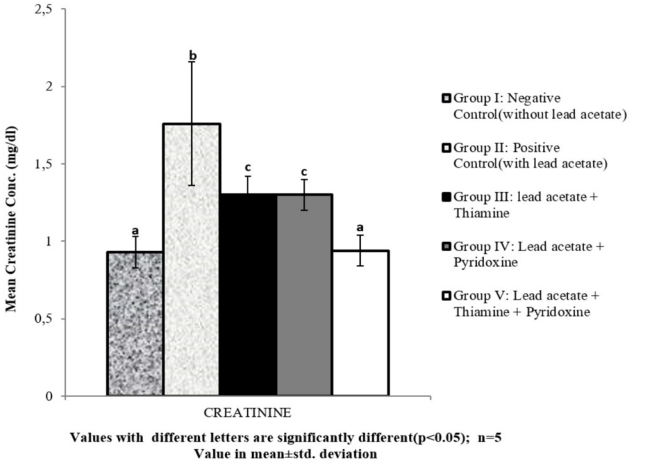

Fig 5. Effect of thiamine and pyridoxine singly and in combination on serum creatinine concentration

DISCUSSION

Lead toxicity has received high attention due to its economic applications and corresponding environmental ubiquity and is one of the most studied heavy metals in public health epidemiology. This study obviously shows the biochemical effects of subchronic lead ingestions in Wistar albino rats and similarly the toxicity of the metal to biological systems. However, vitamins in single and mixture supplementations clearly portray their alleviative roles in lead-induced toxicity in vivo.

The significantly lower activities of ALT, AST, ALP and lower levels of urea and creatinine in the thiamine treated group when compared to the positive control group is indicative of ameliorative effect of thiamine in liver and kidney lead-toxicity as they are biochemical indices of the identified organ functionalities. Parallel accounts have demonstrated the lead effects on the functional biochemistry of rat's liver and kidney[15,16] and thus confirm the current findings. Furthermore, reports on other higher vertebrates such as goats and sheep[20,21] are consistent with the present studies.

Thiamine is water soluble, sulfhydryl group containing vitamin that permits several configurations of thiamine-lead complexes and has been validated to enhance the elimination of lead from the body in diverse research documents [22,23,24]. However, it is also pointed to reduce lead absorption from gastrointestinal tract and prevents its deposition in some tissues [25,26]. Senapati et al. [16] reported that thiamine has been found to protect against lead- induced lipid peroxidation in rat liver and kidney. According to Jung and Kim[27] and as affirmed by Wang et al. [28], thiamine may scavenge for O2– and OH- and thus affect the cellular response to oxidative stress.

Pyridoxine is another potent vitamin with potential of reducing the toxicity of lead in liver and kidney. Significantly lower activities of ALT, AST and ALP, and lower levels of urea and creatinine in the serum were observed in our studies on experimental group that received pyridoxine administration. Such biochemical activity effects are however consistent with Wistar albino rats in previous report[29]. Supplementation with pyridoxine in lead exposed rats has been made known to improve ALAD activity and also reduces the lead levels in the blood, liver and kidney of rats[14]. The suggested mechanistic action of pyridoxine in lead toxicity relays to its role in the metabolic trans-sulfuration pathway, which allows for the metabolism of cysteine from methionine, the main dietary source of cysteine and the rate- limiting amino acid in glutathione production[30]. Vitamin B6 acts also as an antioxidant by stimulating the production of GSH and as a moderate chelator[13]. Chelation of lead by vitamin B6 could be ascribed to the presence of the ring in the nitrogen atom or to the interference of vitamin B6 with the absorption of lead. McGowan[29] showed that lead- exposed rats which had diet-induced vitamin B6 deficiencies had significantly lower gluthathione levels than lead-exposed rats with normal vitamin B6 levels.

Beneficial biocatalytic synergism and additivity is the next logical step in vitamin mixture suppressive potentiality on lead-facilitated toxicity on animal biochemistry.The

lower activities of ALT, AST and ALP and levels of urea and creatinine in the serum of the cocktail treated group designate the effect of combined administration of thiamine and pyridoxine in ameliorating lead-induced toxicity. The difference in biochemical activities between the experimental animal group treated with cocktail and other groups that got individual vitamin supplementation showed an additive effect of thiamine and pyridoxine combinative administration.

Reversibility of the lead-induced oxidative stress in Wistar albino rats due to vitamin supplementations is a novel marker in therapeutic efficacy for lead intoxication. Single exposure of thiamine and pyridoxine ameliorates the effects of lead toxicity in the liver and kidney and an additive effect exists when thiamine and pyridoxine are administered in combination. Studies targeting synergistic effect of thiamine and pyridoxine in vivo on lead biotoxicity through use of chemical mixture effect models are very crucial in underpinning explanatory data on lead toxicity and reduction in human poisoning.

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