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Mitigation of Lead Acetate Induced Toxicity by Ginger (*Zingiber officinale*)

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Abstract

Lead is one of the main environmental contaminants which can threaten the living organisms in many ways. Lead toxicity is associated with a number of physiological, hematological and biochemical alterations. The study aimed to investigate mitigation of lead acetate induced toxicity by ginger (*Zingiber officinale*) on the risk which may result from intraperitoneal exposure to dose of lead acetate on hematological indices, function of liver and kidney. The experiment was performed on twenty male rats. They were maintained on standard healthy laboratory conditions and had free access to food and drinking water ad libitum. The rats were divided into four equal groups A-D. The group A represented the healthy control rats, while groups B, C, and D were administered doses of lead acetate alone (intraperitoneally), ginger alone (orally), and combination of lead acetate with ginger (intraperitoneally and orally) respectively for 2 weeks. At the end of the experimental period, blood was collected for hematological studies while serum was used for biochemical analysis. The results indicated that rats treated with lead acetate alone showed significant reduction in TEC, PCV, MCV, MCH and MCHC compared to the healthy control ones while there was significant elevation in TLC. These was also showed significant increase in the activities of ALT, AST as well as marked hypoproteinemia, hypoalbuminemia and azotaemia indicating liver and kidney dysfunction. However upon mitigation with *Zingiber officinale* (group D) the results showed that it was able to reverse the decrease PCV, MCV, MCH, MCHC, RBC counts as well as reduce the white cell count compared to group B that was exposed to lead acetate alone. The azotaemia, hypoproteinemia, hypoaluminemia and activities of AST and ALT were also reversed. The study revealed that *Zingiber officinale* is capable of mitigating lead acetate induced toxicity which is due to it antioxidant potential.

Keywords: Blood; kidney; lead; liver; *Zingiber officinale*

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Introduction

Lead is one of the heavy metals that are ubiquitous in nature. It is found in food, water, air and soil. Environmental sources of lead include inhalation of automobile exhaust from gasoline containing alkyl lead additives from ingestion of dust contaminated with lead and from drinking water that had passed through lead piping [1]. It is a dangerous heavy metal and harmful even in small amounts. Humans get exposed to lead through their environment and diet [2]. Lead exposure occurs mainly through the respiratory and gastrointestinal tracts, once absorbed majority of the lead is bound to erythrocytes and is dispersed into soft tissues such as liver, renal cortex, aorta, brain, lungs, spleen, teeth, and bones [3]. The manifestations of lead poisoning are nonspecific. They may include weight loss, anemia, nephropathy, infertility, liver, testis and heart damages [3-5].

Ginger is an underground rhizome plant belonging to the family *Zingiberaceae*. Chemically, ginger contains several classes of compounds. The chemical composition of dried ginger is as follows: starch 40% to 60%, proteins 10%, fat 10%, fiber 5%, inorganic material 6%, residual moisture 10%, and essential oil 1% to 4% [6]. In all, more than 200 different volatile substances have been characterized in the essential oil fraction wherein the pharmacologically active compounds are to be found. The volatile oil contains oleoresin, which is responsible for the pungency in ginger. The aromatic and pungent characteristics of ginger make it desirable in the culinary art. The economic importance of ginger centers on its use in the preparation of medicines and its constituents are reported to have antiemetic, antithrombotic, anti hepatotoxic, anti-inflammatory, stimulant, cholagogue, androgenic and antioxidant properties [7,8].

Ginger has been reported to improve fertility and has antioxidant properties [8]. Hematological and biochemical variables are among the most significant physiological indicators of health, stress and welfare. Hence this study focuses on whether oral administration of ginger can mitigate

lead acetate induced perturbations in some hemato-biochemical parameters in adult male rats.

Materials and Methods

Tested plant

Powdered rhizomes of ginger (*Zingiber officinale*) which is the underground stem were sourced from Sango Market in Ibadan, Oyo State.

Animal ethics

All experimental protocols carried out on the animals were in accordance with the international accepted principles for laboratory animal use and were approved by the Ethics Committee (UIL/FVERC/001/2018) on Laboratory animal use of the Faculty of Veterinary Medicine, University of Ilorin.

Experimental animals

A total of twenty male Wistar rats were used for this investigation. The average weight of the rats was 152 ± 3.50 g. They were provided with laboratory animal feed (Fat/oil 6%, crude fibre 5%, calcium 1%, Available phosphorus 0.4%, Lysine 0.85%, Methionine 0.35%, Salt 0.3%, Crude protein 18%, Metabolisable Energy 2900 $Kcal.kg^{-1}$, Manufactured by TOPFEEDS®, Lagos, Nigeria) and water provided. Experimental animals were acclimatized to their environment before the start of the experiment.

The rats were randomly divided into four groups (A-D) of five animals per group: Group A) was the control non-exposed group and received water for two weeks. Group B) received 5mg/kg body weight of lead as lead acetate intraperitoneally for two weeks. Group C) received 50 mg/kg body weight of ginger (*Zingiber officinale*) orally daily for two weeks. Group D) received 5 mg/kg body weight of lead as lead acetate intraperitoneally and 50 mg/kg ginger (Zingiber officinale) orally for two weeks.

Sample collection

At the end of two weeks of exposure to both the lead acetate and *Zingiber officinale*, blood samples were collected directly from the heart and divided into two tubes *viz.* EDTA tube for hematological studies and plane tube to obtain the serum for biochemical studies.

Hematological studies

Packed cell volume, red and white blood cells counts are determined as described by Jain [9].

Serum preparation

The blood samples were centrifuged at 4000 rpm for 10 min to separate the serum from the cellular components. The serum was then removed and stored in Eppendorf tubes for further analysis.

Biochemical studies

Total plasma protein and albumin concentrations were estimated according to the method of Tietz [10] and Grant, [11] respectively, while blood urea nitrogen and creatinine concentrations were estimated as described by Henry, [12] using commercial kits (Randox®, Spain). The enzymes aspartate and alanine aminotransferases activities were assayed as described by Reitman and Frankel [13].

Statistical analysis

Results were expressed as mean ± SEM. Analysis of the data was done using one-way analysis of variance followed by the Duncan multiple range test. A P value <0.05 was considered significant in all cases.

Result

Table 1 shows the mean packed cell volume, red blood cells count and white blood cells count of male Albino rats treated with lead acetate and mitigated by ginger (*Zingiber officinale*). There was significant ($P<0.05$) decrease in Packed Cell Volume (PCV), Red Blood Cells counts (RBC counts) and White Blood Cells counts (WBC counts) of rats exposed to lead acetate compared to the control group, however there was no significant change in those exposed to the *Zingiber officinale* alone and those exposed to both *Zingiber officinale* and lead acetate compared to the control group.

Table 2 shows the mean erythrocyte indices of male Albino rats treated with lead acetate and mitigated by ginger (*Zingiber officinale*). There was a significant decrease ($P<0.05$) in Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and mean corpuscular hemoglobin concentration of rat exposed to lead acetate compared to the control group, however there was no significant change in the MCH in the groups exposed to *Zingiber officinale* alone and *Zingiber officinale* with lead acetate.

Table 3 shows some biochemical parameters of male Albino rats exposed to lead acetate and mitigated by *Zingiber officinale*. There was significant increase($P<0.05$) in the activities of serum Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) of lead acetate exposed group compared to the control group. The serum creatinine and blood urea nitrogen concentrations were also significantly elevated. However, the serum protein and albumin concentration was significantly decreased.

There was a significant decrease in the activities of both the ALT and AST of group exposed to lead acetate and *Zingiber officinale* compared to the group exposed to *Zingiber officinale* alone.

Discussion

Lead is a widespread natural element in the environment and considered as one of the main persistent and common environmental pollutants. Owing to its toxic cumulative action in the environment, it can affect all biological system via exposure from different sources thus having deleterious impact on most organs of animal body. It has been reported that lead enters into the body through digestive, respiratory and dermal. When it is absorbed into the blood, some of it bound to erythrocytes and the remaining stays in the plasma to be distributed to other tissues [14,15].

Zingiber officinale is one of the most widely used culinary medicinal plants because of it numerous phytochemical constituents which is of valuable therapeutic agents in preventing and combating ailments [16,17]. It possesses numerous health benefits like antimicrobial, antiviral, gastroprotective, antidiabetic, anti-hypertensive, cardioprotective, antioxidant, anticancer and immunomodulatory properties. Additionally, it possesses hepatoprotective constituents, and thus protects the liver against the toxic effects of diverse class of xenobiotic agents like alcohol, acetaminophen, heavy metals, bromobenzene and lead [18-20].

The reduction observed in PCV, MCV, MCH, MCHC and RBC counts, observed in the group administered lead acetate result in microcytic hypochromic anemia which is consistent with Mugahi et al. [21] and Suradkar et al. [22]. The reduction of hematological values might be attributed to binding of lead to RBCs, which increase

Table 1: The mean packed cell volume, red blood cell counts and white blood cell count of male Albino rats treated with lead acetate and ameliorated by ginger (*Zingiber officinale*).

GROUPS	PCV (%)	RBC COUNT (10^6/mm^3)	WBC COUNT (10^3/mm^3)
Group A	42.14 ± 1.41[a]	6.52 ± 0.11[a]	13.40 ± 1.71[a]
Group B	33.83 ± 1.47[b]	6.31 ± 0.06[b]	19.52 ± 1.34[b]
Group C	39.57 ± 1.08[a]	6.49 ± 0.12[a]	13.45 ± 1.22[a]
Group D	36.17 ± 1.60[a]	6.40 ± 0.10[a]	14.30 ± 1.19[a]

Values are mean ± SEM. Values within the same column with different superscripts are significantly different at p<0.05

Table 2: Erythrocytes indices of male Albino rats exposed to lead acetate and mitigated by *Zingiber officinale*.

GROUPS	MCV (fl)	MCH (Pg)	MCHC (g/dl)
Group A	57.08 ± 1.01[a]	21.25 ± 0.31[a]	16.44 ± 1.71[a]
Group B	45.83 ± 0.11[b]	16.01 ± 0.16[b]	11.12 ± 1.34[b]
Group C	56.07 ± 0.08[a]	19.59 ± 0.72[a]	14.55 ± 1.22[a]
Group D	55.17 ± 0.60[a]	17.40 ± 0.10[b]	15.30 ± 1.19[a]

Values are mean ± SEM. Values within the same column with different superscripts are significantly different at p<0.05

Table 3: Mean biochemical parameters of male Albino rats exposed to lead acetate and mitigated by *Zingiber officinale*.

GROUPS	ALT (U/L)	AST (U/L)	Creatinine (mg/dl)	BUN (mg/dl)	Protein (g/dl)	Albumin (g/dl)
Group A	22.80 ± 1.20[a]	26.70 ± 0.13[a]	0.93 ± 0.03[a]	16.87 ± 1.06[a]	6.94 ± 0.34[a]	4.02 ± 0.21[a]
Group B	36.41 ± 1.70[b]	38.50 ± 0.24[b]	1.27 ± 0.29[b]	19.11 ± 1.68[b]	4.01 ± 0.22[b]	2.31 ± 0.11[b]
Group C	24.50 ± 1.50[a]	27.40 ± 0.07[a]	1.10 ± 0.11[a]	15.04 ± 1.21[a]	5.69 ± 0.21[b]	3.45 ± 0.13[c]
Group D	20.15 ± 1.20[c]	25.80 ± 0.14[a]	0.89 ± 0.20[a]	15.62 ± 1.03[a]	5.84 ± 0.11[b]	3.51 ± 0.10[c]

Values are mean ± SEM. Values within the same column with different superscripts are significantly different at p<0.05

membrane fragility and destruction. Lead has a destabilizing effect on cellular membranes and thus decreases red cell membrane fluidity and increases the rate of erythrocytes hemolysis. Hemolysis appears to be the end result of reactive oxygen species generated and lipid peroxidation in the red blood cells membrane [23]. However upon mitigation with *Zingiber officinale* the result shows that it was able to reverse the decrease PCV, MCV, MCH, MCHC and RBC counts due to lead acetate to that of the control which was not statistically different. It is an indication that *Zingiber officinale* antioxidant property was able to quench the free radicals generated by lead and thus stop the lipid peroxidation that might be generated by lead. It is not unlikely that *Zingiber officinale* was able to bind with the lead and excreted along with the urine or faeces of the rats. The antioxidant properties were able to protect the erythrocytes membrane and thus prolong the life span of the red cell. Miller et al. [24] and Ahmed et al. [25] reported that ginger have a broad range of biological activities, especially antioxidant activities that significantly lowered lipid peroxidation.

Analysis of total leucocytes counts revealed leukocytosis in lead acetate treated rats compared to the control group. The increase is attributed to the toxic action of lead on leucopoiesis in lymphoid organs. This suggests that the increase in total leucocytes count is directly related with their increased production from the germinal center of lymphoid organs under the influence of lead toxicity. Treatment with lead, induced inflammation which leads to increase in white blood cells count. Mugahi et al. [21], Das and Mukherjee [26] also reported leukocytosis in lead treated rats which was due to enhance release of lymphocytes from the lymphoid tissue. *Zingiber officinale* has the potential to ameliorate lead induced toxicity by reducing the level of white blood cell count which was not significantly different from that of the control group. The reduction might be due to presence of bioactive phytochemicals like gingerols, shogaols, paradols, gingerdiols and zingerone [27]. Zingerone scavenges superoxide anion; 6-gingerol and zingerone are reported to be good scavengers of peroxyl radicals. 6-shogoal also inhibited the production of nitric oxide. 6-gingerol is the major bioactive constituent responsible for the anti-inflammatory, anti-tumour and antioxidant activities of ginger [28]. There are many evidences that lead is a poisonous element, which targets numerous organs such as nervous system, immune system, hematopoietic system, kidneys and liver. It toxicity is associated with a number of physiological, morphological, and biochemical alterations such as nervous system disturbances, and impairment of renal system functions [22,29-31].

Effect of lead on liver function was assessed by the activities of serum AST and ALT. AST is widely used to evaluate the liver function. ALT is a cytoplasmic enzyme, while AST is found in both mitochondria and cytoplasm. Treatment with lead acetate in this study was found to induce ALT and AST activities. The elevation in the enzymatic activity of ALT and AST might be owing to the increase in cell membrane permeability or cell membrane damage of hepatocytes under the influence of lead. These results concur with previous studies that reported an elevation in AST and ALT levels after treatment with lead caused by acute hepatitis, jaundice, and liver cirrhosis [32-33]. Lead has hepatotoxic effect resulting in liver cell damage, which causes increase in serum levels of AST and ALT [35]. It has been observed that lead has toxic effects on rat liver, leading to liberation of AST and ALT [2]. The high activities of plasma AST and ALT are attached by high liver microsomal membrane fluidity, production of free radicals, and alteration in the liver cells when animals were treated with lead acetate [36]. Increase in ALT and AST enzymatic activities might be resulting from lead acetate toxicity, which causes increased cellular basal metabolic rate, irritability, and destructive alteration of liver [31,37]. These deleterious effects of lead on the activities of AST and ALT were reversed by ginger showing the ability of ginger to mitigate the toxic effects of lead. Ethanolic extract of ginger was reported to be effective in reducing the serum levels of

AST, ALT, ALP, GGT and the levels of tissue lipoygenase in alcohol induced hepatotoxicity, carbon tetrachloride hepatoxicity [38]. It might be due to the antioxidant property of the ginger which was able to mitigate the membrane lipid peroxidation of the hepatocyte membrane as well as serve as a scavenger of the free radicals generated by lead [39,40].

The site of protein synthesis is also the liver; since the liver was adversely affected due to lead toxicity it markedly produces hypo proteinaemia and hypoalbuminaemia. These were reversed by the *Zingiber officinale* because of its ability to enhance protein synthesis as a result of it mitigating ability against the lead.

Exposure to lead was able to produce azotaemia which is an indication of kidney dysfunction and a functional evidence of lead-induced nephrotoxicity [36,41,42]. The azotaemia was equally reversed upon treatment with ginger because of it detoxifying ability.

In conclusion, exposure to lead produces deleterious effects on the hematological and some biochemical parameters which were mitigated by the ginger in its ability to reverse the hematological and some of these biochemical parameters to that similar to the control group. This might be due to the antioxidant and scavenging ability of the ginger.

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