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Original research

Clinical haematobiochemical parameters during experimental Lead poisoning in Goats: effects of methanol extract of Tiger nuts (*Cyperus esculentus)*

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ABSTRACT

The effects of methanol extract of tiger nut on haematobiochemical parameters during experimental lead poisoning in Red Sokoto goats were investigated. The seeds of tiger nuts were extracted with 90% methanol. Phytochemical analyses were carried out using standard procedures. A total of twenty-four adult Red Sokoto goats of both sexes were separated randomly into four groups for acute and chronic studies. Three goats in both acute and chronic studies were housed per group. In the acute and chronic studies DW groups was administered an equivalent amount of distilled water (400 and 200 ml/kg). Group II was only administered lead acetate (400 and 200 mg/kg). Group III was administered with methanol extract of tiger nuts (400 and 200 mg/kg) combined with lead acetate (400 and 200 mg/kg) respectively. Group IV was administered with tiger nuts (400 and 200 mg/kg) only for a period of 2 and then 20 weeks for acute and chronic phases respectively. Haematobiochemical parameters were determined. The lead acetate and lead acetate combined with methanol extract of tiger nut in acute and chronic groups showed microcytic normocytic and microcytic hypochromic anaemia respectively with the latter suggestive that with chronicity, an enzymatic inhibition of haemoglobin synthesis led to iron deficiency. Alterations in serum biochemical enzymes and other parameters indicative of hepato-renal injury were observed and ameliorated by methanol extract from tiger nuts.

KeyWords: Anaemia, haematobiochemical, hepato-renal, lead, tiger nuts.

INTRODUCTION

Lead is one of the most abundant toxic heavy metals detected in all parts of the environment and biological system (Xia *et al.,* 2010). It is an abundant, ubiquitous, dangerous, and toxic environmental contaminant of global concern due to its significant role in the modern industry (Sainath *et al.,* 2011; Valverde *et al.,* 2001). Haematological parameters in some instances gave an indication of lead intoxication with anaemia being the major effect of lead poisoning, due to inhibition of heme synthesizing enzymes with an increased level of protoporphyrin (Gani *et al.,* 2017). 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 (Ercal *et al.,* 2001**)**. The mechanisms of lead toxicity include its ability to interact with proteins and change their functions or inhibit or mimic the action of calcium, replace zinc as a co-factor in enzymes, and cause oxidative stress (Hsu and Guo, 2002). Lead also inhibits the activity of δ-aminolevulinic acid dehydrogenase (ALAD) and ferrochelatase and consequently, affects the haeme biosynthesis. Low haeme synthesis is a sign associated with an inhibitor of δ-aminolevulinic acid synthetase activity, so lead toxicity causes an increase in plasma and urinary δ-aminolevulinic acid (Carocci *et al.,* 2015). Lead prevention of ferrochelatase results in the accumulation of zinc, rather than iron in the porphyrin moiety (Lubran, 1980). Sodium ethylenediamine tetraacetic acid is a long-established artificial lead chelating agent but found toxic to the kidneys and liver (Enagbonma *et al.,* 2016). Numerous plants and seeds have antioxidant properties as they scavenge free radicals and inhibit lipid peroxidation (Scartezzini and Speroni 2000; Tapiero *et al.,* 2002), therefore, supplementation with an exogenous source

of antioxidants is likely to alleviate or protect oxidative pressure and tissue damage caused by lead poisoning. The present study was designed to investigate the ameliorative effect of methanol extract of Tiger nuts on the hematobiochemical parameters during lead poisoning of Red Sokoto goats.

MATERIALS AND METHOD

EXPERIMENTAL ANIMAL

Twenty-four Red Sokoto goats of both sexes weighing between 20.92 ± 0.97 kg of about one (1) year were used. They were procured from the Maiadua goat market in Katsina State, Nigeria. The animals were acclimatized for two weeks and vaccinated against *Pestis de petit ruminant* (PPR) prior to the commencement of the experiment. The animals were kept in the animal house of the Department of Veterinary Physiology, Ahmadu Bello University, Zaria, Kaduna State. They were fed with grass, hay, wheat bran, and groundnut leaves at intervals during the early hours of the day and supplemented with 2 kg of concentrate feeds later in the day, and were given free access to water. The animals were weighed on arrival using a weighing balance. The blood and fecal samples were taken to ascertain the health status of the animals prior to the commencement of the experiment.

EXPERIMENTAL DESIGN

The median lethal dose (LD_{50}) of lead acetate and the methanol extract of tiger nut was determined using Wistar rat as described by Lorke (1983). The dosage of lead acetate and tiger nut was determined using $1/10^{th}$ of 4000 mg/kg lead acetate and 2000 mg/kg methanol extract of tiger nut. The actual dose was calculated as the ratio of dosage per body weight and concentration. Twenty-four Red Sokoto goats were separated randomly into four groups. The acute study group (DW, LA, LA+TN and TN) had three goats per group. The chronic study groups (DW, LA, LA+TN and TN) had three goats per group. Group, I was administered distilled water (200 ml/kg). Group II was administered lead acetate (200 mg/kg) only. Group III was administered tiger nut (200mg/kg) and lead acetate (200 mg/kg). Group IV was administered methanol extract of *C. esculentus* (200 mg/kg) only. The different regimens were administered per *os* once daily using a 20 ml syringe for a period of 2 and then 20 weeks for acute and chronic phases respectively.

Ethical approval for the use of Red Sokoto goats for this study was obtained from the Ahmadu Bello University Committee on Animal Use and Care, Ahmadu Bello University, Zaria Nigeria with Registration Number ABUCAUC/2017/033.

BLOOD COLLECTION AND HAEMATOLOGICAL ANALYSIS

In this study, 5 mL of a blood sample was collected through jugular venepuncture from each animal as described by Adam *et al.,* 2019 prior to the beginning of the experiment to obtain the baseline values. Two milliliters (2 mL) of a blood sample obtained were transferred to a sterile capped tube, containing ethylenediaminetetraacetic acid anticoagulant (Greiner Bio-One, Frickenhausen, Germany) and used for haematological evaluation. In the acute toxicity study, blood was collected every day for a period of 2 weeks while for the chronic lead toxicity study, blood was collected 2 times weekly for 150 days. All the blood samples were collected in plastic test tubes, containing anticoagulant (EDTA). The automated hematological analyzer (Dhanwantari medical systems, DMS, India) was used to determine the haematological parameters, including total erythrocyte count (RBC), total leucocyte count (WBC), packed cell volume (PCV), haemoglobin concentration (Hb), and platelets; then the erythrocytic indices-mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and the number of platelets was calculated as described by Coles (1980).

ANALYSIS OF SERUM BIOCHEMICAL PARAMETERS

Five milliliters of blood were collected through the jugular veins and transferred into non-anticoagulant sample bottles as described by Adam *et al.,* 2019. These blood samples were processed for the evaluation of biochemical parameters as described by Saeed, 2015. The obtained serum samples will be used for the evaluation of liver enzymes such as alanine aminotransferase (ALT), aspartate transferase (AST), total protein (TP), urea, and creatinine. The estimation of the biochemical parameters will be measured using Clinical Chemistry Analyzer (Merck, Ltd., Germany).

STATISTICAL ANALYSIS

Values obtained as mean ± SEM were subjected to one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* multiple comparisons using Graph Pad Prism version 4.0 for windows from GraphPad Software, San Diego, California, USA). Values of $P < 0.05$ were considered significant.

RESULTS

Effect of methanol extract of Tiger nuts on the haematological parameters; from the Table1, there was a significant ($P < 0.01$) lower PCV in the LA group when compared to LA (10.633 ± 0.203) with LA + TN (17.30 ± 0.379). However, there was no significant ($P > 0.05$) difference in PCV values when comparing TN (21.97 ± 1.037) with LA + TN (17.30 ± 0.379). Again, the

comparison between haemoglobin concentration in LA (4.967 ± 0.145) with LA + TN (5.833 ± 0.167) group showed no significant (P > 0.05) difference. There was a significant (P < 0.01) decrease in haemoglobin concentration in LA + TN when comparing TN (7.500 ± 0.289) with LA + TN (5.833 ± 0.169) group. As shown in Table I again, there was no significant (P > 0.05) difference in RBC counts when comparing LA (6.46 ± 0.260) with LA + TN (7.200 ± 0.153) group. However, the comparison between TN (10.40 ± 0.208) with LA + TN (7.200 ± 0.153) showed significant (P < 0.01) lower RBC counts in LA + TN group. Table I also showed no significant (P > 0.05) difference in MCV values when comparing LA (17.83 ± 0.601) with LA + TN (18.50 ± 0.289). However, there was a significant (P < 0.01) decrease in MCV value in LA + TN group when compared to TN (20.43 ± 0.433) with LA + TN (18.50 ± 0.289). As shown in Table I again, there was a significant (P < 0.01) decrease in MCH in the LA group when compared LA (5.500 ± 0.289) with LA + TN (7.000 ± 0.289) group. However, there was no significant (P > 0.05) difference when comparing TN (8.167 ± 0.167) with LA + TN (7.000 ± 0.289) group (Table I).

As shown in Table II, there was highly significantly (P < 0.0001) lower PCV in the LA group when compared lead acetate (11.23 ± 0.120) with distilled water (22.30 ± 0.058), tiger nut (21.43 ± 0.678) or lead acetate supplemented with tiger nut (12.07 ± 0.120) group. There was a highly significant (P < 0.001) lower haemoglobin concentration in the LA group when compared DW (9.033 ± 0.573) with LA (5.667 ± 0.203) and significantly (P < 0.01) lower haemoglobin concentration in the LA group when compared LA (5.667 ± 0.203) with TN (8.000 ± 0.577) groups as shown in table I. However, there was no significant (P > 0.05) difference in haemoglobin concentration when compared DW (9.033 ± 0.573) with LA + TN (7.100 ± 0.322) or TN (8.00 ± 0.577). Also, the comparison in haemoglobin concentration between LA + TN (7.100 ± 0.322) and TN (8.000 ± 0.577) groups showed no significant (P > 0.05) difference (Table II). In Table II, there were highly significantly (P < 0.001) lower red blood cell counts in LA or LA + TN groups when compared DW (10.17 ± 0.601) with either LA (7.133 ± 0.187) or LA + TN (7.667 ± 0.167) group (Table II).

There was no significant (P > 0.05) difference in MCV when compared to DW (23.67 ± 0.440) with either LA + TN (23.50 ± 0.289) or TN (23.50 ± 0.289) groups. In table I, there was no significant (P > 0.05) difference in MCHC when compared DW (33.17 ± 0.272) with either LA (30.00 ± 0.577) or LA + TN (29.00 ± 0.577) groups (Table II).

As shown in Figure I, there was a significant (P < 0.01) increase in absolute WBC counts in the LA group when comparing LA

Table I: Haematological values of Red Sokoto goats acutely intoxicated with lead acetate and treated with methanol extract of *Tiger nuts*

a, b, c = Values of mean ± SEM in the same row with different superscripts are significantly (P < 0.001) different. Packed cell volume (PCV), haemoglobin (Hb), Red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), distilled water (DW), lead acetate (LA), lead acetate combined with a tiger nut extract (LA + TN), tiger nut extract (TN).

Parameters	DW	LA	LA + TN	TN
PCV (%)	21.67 ± 0.882^{a}	10.63 ± 0.203^{b}	17.30 ± 0.3786^{ab}	21.97 ± 1.037^{a}
Hb (g/dl)	8.167 ± 0.441^{a}	4.967 ± 0.145^{b}	5.833 ± 0.167^{b}	7.500 ± 0.289^{a}
RBC ($\times 10^{12}$/L)	10.63 ± 0.441^{a}	6.467 ± 0.260^{b}	7.200 ± 0.153^{b}	10.40 ± 0.208^{a}
MCV (f/cell)	20.50 ± 0.289^{a}	17.83 ± 0.601^{b}	18.50 ± 0.289^{b}	20.43 ± 0.433^{a}
MCH (pg/cell)	8.000 ± 0.289^{a}	5.500 ± 0.289^{b}	7.000 ± 0.289^{a}	8.167 ± 0.167^{a}
MCHC (g/dl)	32.60 ± 0.306^{a}	30.50 ± 1.041^{a}	30.70 ± 0.651^{a}	31.83 ± 0.440^{a}

Table II: Haematological values of Red Sokoto goats chronically intoxicated with lead acetate and treated with methanol extract of *Tiger nuts*

a, b, c = Values of mean ± SEM in the same row with different superscripts are significantly (P < 0.001) different. Packed cell volume (PCV), haemoglobin (Hb), Red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), distilled water (DW), lead acetate (LA), lead acetate combined with a tiger nut extract (LA + TN), tiger nut extract (TN).

Parameters	DW	LA	LA + TN	TN
PCV (%)	22.30 ± 0.058^{a}	11.23 ± 0.102^{b}	12.07 ± 0.102^{b}	21.43 ± 0.677^{a}
Hb (g/dl)	9.033 ± 0.573^{a}	5.667 ± 0.203^{b}	7.100 ± 0.322^{ab}	8.000 ± 0.577^{a}
RBC ($\times 10^{12}$/L)	10.17 ± 0.601^{a}	7.133 ± 0.186^{b}	7.667 ± 0.167^{b}	10.50 ± 9.289^{a}
MCV (f/cell)	23.67 ± 0.440^{a}	21.00 ± 0.058^{b}	23.50 ± 0.289^{a}	23.50 ± 0.289^{a}
MCH (pg/cell)	8.167 ± 0.167^{a}	6.167 ± 0.167^{b}	7.167 ± 0.333^{ba}	8.100 ± 0.100^{a}
MCHC (g/dl)	33.17 ± 0.272^{a}	30.00 ± 0.577^{b}	29.00 ± 0.577^{b}	32.83 ± 0.441^{a}

(23 x 10^3µ/L) with LA + TN (18 x 10^3µ/L) group. However, there was no significant (P > 0.05) difference when comparing LA + TN (18 x 10^3µ/L) and TN (13 x 10^3µ/L). There was also no significant (P > 0.05) difference in absolute neutrophil counts when comparing LA (12 x 10^3µ/L) with LA + TN (9 x 10^3µ/L). However, there was a significant (P < 0.01) increase in absolute neutrophil counts in LA + TN group when compared to LA + TN (9 x 10^3µ/L) with TN (7 x 10^3µ/L) group (Figure I). Again in Figure I, there was a significant (P < 0.01) increase in absolute lymphocyte counts in the LA group when comparing LA (17 x 10^3µ/L) with LA + TN (12 x 10^3µ/L) group. There was also a significant (P < 0.01) increase in absolute lymphocyte counts in LA + TN group when compared LA + TN (13 x 10^3µ/L) with TN (8 x 10^3µ/L) group (Figure I).

`There was a significant (P < 0.01) increase in absolute WBC counts in the LA group when comparing LA (23 x 10^3µ/L) with LA + TN (18 x 10^3µ/L) group (Figure I). There was also a significant (P < 0.01) increase in absolute WBC counts in LA + TN group when comparing LA + TN (19 x 10^3µ/L) with TN (13 x 10^3µ/L) group (Figure I). However, there was no significant (P > 0.05) difference in absolute neutrophil counts when comparing LA (12 x 10^3µ/L) with either LA + TN (10 x 10^3µ/L) or TN (8 x 10^3µ/L) groups (Figure I). As shown in Figure I, there was no significant (P > 0.05) difference in absolute lymphocyte counts when comparing LA (16 x 10^3µ/L) with LA + TN (14 x 10^3µ/L) group. However, there was a significant (P < 0.01) increase in lymphocyte counts in LA + TN when compared LA + TN (14 x 10^3µ/L) with TN (8 x 10^3µ/L) group (Figure I).

Effects of methanol extract of *Tiger nuts* on liver enzymes; as shown in Table III, there was no significant (P > 0.05) difference when comparing LA (25.00 ± 3.000) with LA + TN (22.63 ± 6.438). However, there was a highly significant (P < 0.001) higher ALT in LA + TN when compared LA + TN (22.63 ± 6.438) with TN (8.57 ± 1.212) group. Again, there was a significant (P < 0.01) higher TP in LA + TN when compared LA (4.53 ± 0.291) with LA + TN (5.400 ± 0.306) group. However, there was a significant (P < 0.01) decrease in TP in LA + TN when compared to LA + TN (5.410 ± 0.306) with the (7.00 ± 0.289) group (Table III).

Table IV showed a significant (P < 0.01) higher ALT in the LA group when compared LA (30.0 ± 5.34) with LA + TN (25.67 ± 15.39) group. Again, there was a significant

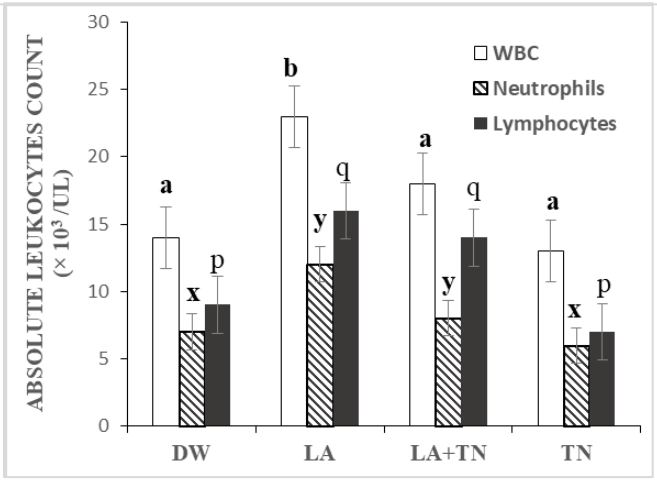

Figure I: Mean absolute leukocyte count of Red Sokoto goats in different experimental groups during acute toxicity study.

Values of mean ± SEM on the bar chart with no similar superscripts are significantly (P < 0.001) different. Distilled water (DW), lead acetate (LA), lead acetate combined with tiger nut extract (LA + TN), tiger nut extract (TN), white blood cell (WBC), neutrophil (NEUT), lymphocyte (LYMP)

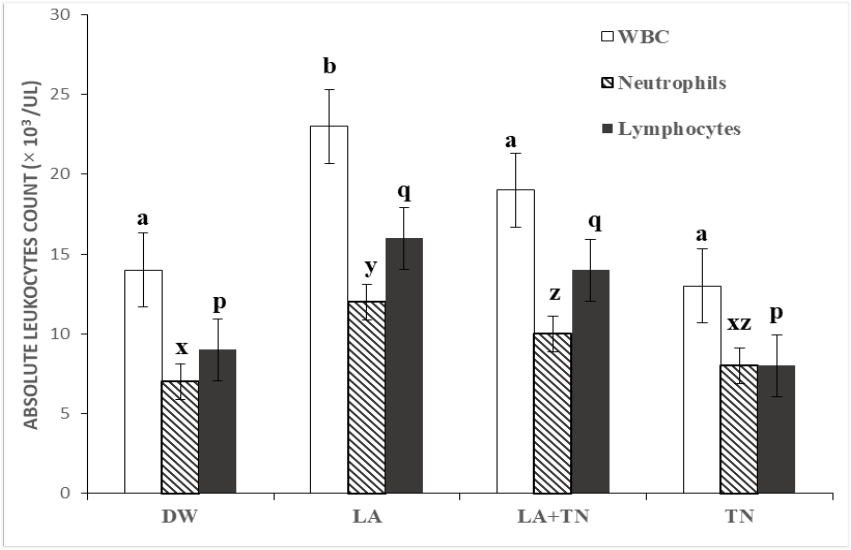

Figure II: Mean absolute leukocyte count of Red Sokoto goats in different experimental groups during the chronic toxicity study.

Values of mean ± SEM on the bar chart with no similar superscripts are significantly (P < 0.001) different. Distilled water (DW), lead acetate (LA), lead acetate combined with a tiger nut extract (LA + TN), tiger nut extract (TN), white blood cell (WBC), neutrophil (NEUT), lymphocyte (LYMP)

Table III: Effect of methanol extract of tiger nut on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, and in Red Sokoto goats treated with lead acetate at 400 mg/kg for 2 weeks (acute studies) Distilled water (DW), lead acetate (LA), lead acetate combined with a tiger nut extract (LA + TN), tiger nut extract (TN)

a, b, c = Values of mean ± SEM in the same row with different superscripts are significantly ($P < 0.001$) different.

Parameters	DW	LA	LA + TN	TN
ALT(U/L)	10.10 ± 1.212^{a}	25.00 ± 3.000^{b}	22.63 ± 6.438^{b}	8.57 ± 1.212^{a}
AST (U/L)	20.11 ± 2.249^{a}	36.04 ± 2.500^{b}	30.57 ± 1.167^{b}	18.36 ± 2.891^{a}
Urea (mg/dl)	24.83 ± 4.107^{a}	48.93 ± 3.906^{b}	35.32 ± 3.477^{abc}	20.37 ± 1.157^{a}
Creatinine (mg/dl)	2.333 ± 0.521^{a}	4.500 ± 1.735^{a}	3.083 ± 0.060^{a}	1.533 ± 0.267^{a}
TP (g/dl)	6.833 ± 0.441^{a}	4.533 ± 0.291^{b}	5.400 ± 0.306^{a}	7.000 ± 0.289^{b}

Table IV: Effect of methanol extract of tiger nut on serum alanine amino transferase (ALT), aspartate amino transferase (AST), urea, and creatinine in Red Sokoto goats treated with lead acetate at 200 mg/kg for 20 weeks (chronic studies). Distilled water (DW), lead acetate (LA), lead acetate combined with a tiger nut extract (LA + TN), and tiger nut extract (TN).

Parameters	DW	LA	LA + TN	TN
ALT(U/L)	9.67 ± 0.219^{a}	30.00 ± 5.340^{b}	25.67 ± 15.390^{a}	8.00 ± 0.145^{b}
AST (U/L)	19.17 ± 6.660^{a}	45.00 ± 3.124^{b}	30.01 ± 2.988^{ab}	17.65 ± 5.788^{ab}
Urea (mg/dl)	25.04 ± 1.031^{a}	50.67 ± 4.410^{b}	35.77 ± 7.098^{ab}	20.83 ± 4.868^{ab}
Creatinine (mg/dl)	1.700 ± 0.473^{a}	6.421 ± 1.830^{b}	5.867 ± 0.467^{b}	1.633 ± 0.584^{a}

($P < 0.01$) increase in ALT in LA **+ TN** group when comparing LA + TN (25.67 ± 15.39) with TN (8.00 ± 0.145) group. Table IV also showed no significant ($P > 0.05$) difference in TP when compared LA (5.033 ± 0.203) with either LA + TN (5.633 ± 0.088) or TN (6.533 ± 0.260) or LA + TN (5.633 ± 0.09) group (Table IV). As shown in Table IV, the comparison between LA (5.033 ± 0.203) with DW (6.500 ± 0.289) or TN (6.533 ± 0.260) showed no significant ($P > 0.05$) difference in total protein. Also, there was no significant ($P > 0.05$) difference in total protein when compared LA + TN (5.633 ± 0.088) with either DW or TN or LA group (Table IV).

Effects of methanol extract of *Tiger nuts* on kidney function parameters; from Table III, there was a significant ($P < 0.01$) increase in urea level in the LA group when comparing LA (48.93 ± 3.906 mg/dl) with LA + TN (35 ± 3.477 mg/dl) group. However, there was no significant ($P > 0.05$) difference when comparing DW (24.83 ± 4.107 mg/dl) and TN (20.37 ± 1.157 mg/dl). Again, there was no significant ($P > 0.05$) difference in the level of creatinine in lead acetate group when comparing LA (4.500 ± 1.735 mg/dl) with LA + TN (3.083 ± 0.060 mg/dl) group. Also, from Table IV, there was a highly significant ($P < 0.001$) decrease in urea level in lead acetate when compared LA + TN (35.77 ± 7.098 mg/dl) group with LA (50.67 ± 4.410 mg/dl) group. However, there was no significant ($P > 0.05$) difference in the level of creatinine in lead acetate group when comparing LA (6.421 ± 1.830 mg/dl) with LA + TN (5.867 ± 0.467 mg/dl) group.

DISCUSSION

The low values of packed cell volume, haemoglobin, total protein, and red blood cells obtained for the acute and chronic studies suggested that the administration of lead acetate causes anaemia. The result of this study agrees with the report of Beckman and Ames, (1997) who attributed the cause of anaemia due to lead toxicity to shortened erythrocyte lifespan and impairment of heme biosynthesis. Gani *et al.,* (2017) also associated anaemia as a result of lead toxicosis to inhibition of activities of aminolevulinic acid dehydratase in haeme synthesis. In animals, the packed cell volume was directly related to the red blood cell and haemoglobin contents (Beckman and Ames, 1997).

A significant decrease ($P < 0.01$) in haemoglobin concentration was also observed in lead-acetate-treated acute and chronic groups respectively, which implies oxygen deficit in the body leading to tissue necrosis and apoptosis in lead acetate groups. Also, the conversion of haemoglobin into met-haemoglobin as a result of lipid peroxidation of the erythrocyte cell membrane could reduce the Hb content, as observed in the report of Yartireh and Hasheiman (2013). Also, a decrease in the total erythrocyte counts in an experimental lead acetate toxicity study rat (Ekanem *et al.,* 2015) which was attributed to the adverse effects of lead acetate on the haematopoietic system, impaired absorption of

essential vitamins and minerals from the intestines and destruction of the red blood cells in the body. These damages could result in reduced oxygen supply to tissues, thereby multiplying the oxidative stress of lead (Ekanem *et al.*, 2015). Haemoglobin is the iron-containing oxygen transport metallo-protein in the red blood cells and its reduction would lead to symptoms of anaemia, as well as decreased heme synthesis resulting in hypochromic red blood cells (reduced haemoglobin pigment) and microcytic red blood cells (smaller than normal) (Kunkomawa *et al.*, 2015).

An obvious decrease in red blood cell counts occurred in the lead acetate and lead acetate combined with methanol extract of tiger nut groups of acute and chronic studies respectively. The observed decrease in red blood cell counts under the influence of lead acetate might be attributed to the binding of lead to red blood cells, which increases membrane fragility and destruction of red blood cells (Saeed, 2015) leading to hemolysis. There are mainly three mechanisms underlying external substance-induced haemolysis; oxidative, immune, and non-immune haemolysis. The main cause of oxidative haemolysis is that red blood cells are extremely sensitive to oxygen-derived free radicals, easily leading to functional damage and shortening their lifespan (Xiaoyang *et al.,* 2017). This implies that the effect of lead acetate could result in red blood cell morphological changes, shrinkage, and even fragmentation suggesting that there may be a direct haemolytic risk of the lead acetate on red blood cells.

The significant decrease in the values of MCV, MCH, and MCHC in the lead acetate and also in lead acetate combined with methanol extract of tiger nut showed a microcytic normochromic and microcytic hypochromic anaemia respectively in the acute and chronic groups in the Red Sokoto goat. This means that in the acute study the size of red blood cells was small with some level of efficient haemoglobin but in the chronic study, the haemoglobin became reduced and inefficient with small red blood cell size. But it was established that lead has the tendency to inhibit the enzyme ferrochelatase that is responsible for iron synthesis, with chronicity an enzymatic inhibition of haemoglobin synthesis led to iron deficiency. This result agrees with the observed haematological effects of lead poisoning in albino rats (Saeed, 2015; Mohammadhosein *et al.,* 2003). Through this study basophilic stippling was not observed with all types of stains applied; this is consistent with the observation that basophilic stippling was not a common finding in ruminants (Cowell, 2004).

The low serum total proteins (TP) observed in acute and chronic studies establishes hypo-proteinemia in lead toxicity in Red Sokoto goats. This implies that low serum total protein levels were probably caused by a combination of reduced hepatic protein synthesis and high urinary protein loss as a result of hepatic and renal injury (Soliman *et al.*, 2013) to lead poisoning. This agrees with the result of Sujatha *et al.,* (2011).who reported low serum total proteins in lead poisoning in Wistar rats as also reported by Saeed *et al.,* (2017) in humans. It was established that almost all serum proteins are produced and secreted by hepatocytes. The major exceptions are the immunoglobulins that are produced by the immune system consisting of the reticuloendothelial tissues, lymphoid, and plasma cells (Eckersall, 2008). Non-hepatic tissues, including the intestine, lung, adipose tissue, and mammary gland, also have the capability of synthesizing some serum proteins for specific functions (Tothova *et al.,* 2016). Proteins are involved in almost all of the reactions occurring in the organism, including the maintenance of the colloid osmotic structure, catalysis of biochemical reactions, and buffering acid-base balance. Some of the proteins act as carriers of lipids, hormones, vitamins, and minerals in the circulatory system, and are involved in the regulation of cellular activity and the immune system (Tothova *et al.,* 2016), while others play important roles as enzymes, complement components or protease inhibitors, essentials for haemostasis, platelet adhesion and aggregation, as well as coagulation (Tothova *et al.,* 2016).

The increased enzyme activity of alanine amino-transferase (ALT) and aspartate amino-transferase (AST) in the lead acetate-treated group in this study showed the toxic effect of lead poisoning in the liver. This means the liver was unable to metabolize and detoxifies lead acetate that eventually accumulated and caused hepatic tissue damage. The result of this study agrees with the report of El- Hameed *et al.,* (2008); Dalia, 2010 and Zaki *et al.,* (2010). The patho-mechanism of lead is to damage the hepatocytes thereby allowing the leakage of the liver enzymes such as ALT and AST from degenerated hepatocytes into the blood circulation (Haousa *et al.,* 2015). The increased activity of these liver enzymes might be due to the continual destruction of liver cells as a result of prolonged administration of lead acetate which did not allow hepatic cell regeneration. However, the lead acetate combined with the methanol extract of tiger nut showed a significant decrease in the level of liver enzymes. This means that the methanol extract of tiger nut was able to ameliorate the damaging effects of lead on hepatocytes. In other words, these findings might be attributed to the antioxidant effect of tiger nuts. This postulation is in accordance with the findings of Bamishaiye & Bamishaiye (2011).

In this study, creatinine and urea level were elevated ($P < 0.001$) in the lead acetate-treated groups with a significant decrease in the creatinine and urea level in lead acetate combined with methanol extract of tiger nut in the Red Sokoto goats. This increment in the level of creatinine and urea showed damage to renal tissue as a result of lead

toxicity. This result is in line with the report of Sajid *et al.*, (2017), who reported an increase in urea and creatinine in lead poisoning in rats. However, lead has the affinity to damage the brush border of the proximal convoluted tubule which made creatinine and urea impermeable during glomerular filtration thereby causing the accumulation of urea and creatinine in blood circulation (El-Nekeety *et al.*, 2009). Although, serum creatinine is a muscle metabolite that is excreted by kidneys, an elevated level of this protein is a sign of renal dysfunction (Jayasunder and Macnab, 2012). The previous report associated renal dysfunction with damage to glomerular function and renal tubular destruction.In this study, white blood cells (WBCs) and lymphocytes were significantly higher ($P < 0.001$) in all the groups when compared to the level of neutrophils in all the groups. But the high values of WBC and lymphocytes in the lead acetate-treated groups might be suggestive of activation of immune response which agrees with Boskabady *et al.*, (2012) who observed that inhaled lead acetate increased WBC counts. However, there was a significant increase ($P < 0.01$) in neutrophil counts in the lead acetate and lead acetate combined with the methanol extract of the tiger nut group. This increase might be due to the inflammatory response of the body system against damages inflicted as a result of lead injury. This result is contrary to the report of Khan *et al.*, (2008) who observed a decrease in total leucocyte count in lead acetate-exposed rats.

CONCLUSION

In conclusion, the hematological changes of acute and chronic lead acetate as well as lead acetate combined with methanol extract of tiger nut caused microcytic normochromic and microcytic hypochromic anaemia in the acute and chronic studies respectively. This may be due to the effect of lead on the enzyme that involves in heme synthesis. But the exposure to lead as well caused an increase in the serum level of liver and kidney enzymes as an indication of hepato-renal damage of lead on those organs. However, Lead acetate combined with methanol extract from tiger nut showed decreased serum levels of liver and kidney enzymes. It is therefore suggestive of the ameliorative effect of the methanol extract of tiger nut on lead poisoning.

CONFLICT OF INTEREST

There was no conflict of interest among the authors

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