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Ameliorative effects of aqueous and methanol extracts of *Hibiscus sabdariffa* calyx (Zobo) on haematology and lipid profile of Wistar rats exposed to lead-induced toxicity

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Running title: *Hibiscus sabdariffa* calyx extract and lead toxicity

Abstract

Exposure to lead toxicity has been reported to cause harmful effects to humans and animals, with the most deleterious effects on the haematopoietic cells. In this study, therefore, the potential ameliorative effects of a local plant extract, *Hibiscus sabdariffa* (Zobo), on lead toxicity were evaluated. Forty-two 6-week-old male Wistar rats with an average weight of 140g were used for the study. The rats were divided into 7 groups of 6 rats per group. Group A, served as the control, received only distilled water. Group B received 100 mg/kg of lead acetate only, while Group C received 100 mg/kg of lead acetate and 200 mg/kg of aqueous extract of *Hibiscus sabdariffa*. Group D received 100 mg/kg of lead acetate and 400 mg/kg of aqueous extract of *Hibiscus sabdariffa*, Group E received 100 mg/kg of lead acetate and 50 mg/kg of *Hibiscus sabdariffa* methanol extract while Group F received 100 mg/kg of lead acetate and 100 mg/kg of *Hibiscus sabdariffa* methanol extract, Group G was given 100 mg/kg of lead acetate and 100 mg/dL of ascorbic acid, concurrently, per os per day for 28 days by gastric gavage. Blood samples were collected at days 14 and 28 for haematology, erythrocyte osmotic fragility and plasma biochemistry. The results showed that 200 mg/kg of aqueous extracts and 50 and 100 mg/kg of methanol *Hibiscus sabdariffa* ameliorated anaemia (haematinic activity), lymphocytosis and increased erythrocytes' osmotic fragility caused by both acute and subacute lead toxicity. The results from plasma biochemistry confirmed the antilipidaemic and anticholesterolaemic effects of the extracts. In conclusion, the study demonstrated that lead toxicity induced anaemia, dyslipidaemia and hypercholesterolaemia, which were ameliorated by low doses of *Hibiscus sabdariffa* calyx (Zobo) extracts.

KEYWORDS: Lead, Anaemia, Antilipidaemic, Anticholestrolaemic, *Hibiscus sabdariffa*

Introduction

Global awareness on environmental toxicological studies, especially on the effects of heavy metals, organophosphates and Bisphenol A, has been on the rise for some time now (James and Oshaughnessy, 2023; Wang, 2023). This is not unconnected with environmental changes associated with global warming and unexpected climate changes that have spurred people to determine or evaluate primary and remote causes of all the environmental changes.

Heavy metals are metals and metalloids with a specific density above 5 g/cm^3 and which are distributed widely in the earth's crust with the low concentration in the body (Alisa and Ferns, 2011; Briffa *et al.,* 2020). They can also be described as naturally occurring elements with a high atomic weight and a density 5 times greater than that of water (Tchounwou *et al.*, 2014). Examples of heavy metals commonly found on the environments and diets are lead, antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium and zinc (Mitra *et al*., 2022; Munir *et al*., 2022). Heavy metals like lead, arsenic, cadmium, chromium, and mercury are regarded as one of the main threats to health. They have been known to induce multiple organ damage at low levels of exposure (Tchounwou *et al*., 2014).

Lead (Pb) is a heavy metal with the relative atomic mass of 207.2 and a non-physiological metal that has been in use by humans for many years. The use ranges from wine sweetening in ancient Greece to more recently as an additive in paint and gasoline (Olufemi *et al*., 2022). Lead (Pb) was reported to be one of the most abundant natural substances on earth and it is being put to widespread industrial uses because of its physical properties; including high malleability and low melting point (327.5℃) (Tchounwou *et al*., 2014). Its use has been associated with more than 900 industries, including mining, smelting, refining, battery manufacturing, and many more (Olufemi *et al*., 2022). Lead is also known as an important environmental toxicant and nerve poison that can cause damage to many functions of the nervous system (Hou *et al*., 2013; Collin *et al*., 2022).

Due to the large-scale production and lead mining with the lack of regulations, lead is emitted largely into the environment through wastewater irrigation, solid waste disposal, sludge application, vehicular exhaust and atmospheric deposition (Alankarage and Juhasz, 2023).

Exposure to lead toxicity has been reported to cause harmful effects to humans, with the most deleterious effects on the haematopoietic, hepatic, nervous, and renal systems through generation of free radicals resulting in oxidative damage to lipids, proteins, critical biomolecules and DNA (Boskabady *et al*., 2018). Lead toxicity disrupts almost all the functions e.g., nervous system, respiratory system, reproductive system, digestive system, kidney, Red blood cells, etc, but the CNS is mostly affected (Pal *et al*., 2015; Collin *et al*., 2022).

Hibiscus sabdariffa belongs to the family of *Malvaceae* and grown in almost all part of the world for its fibre, seed, leaf or fleshy calyx (Mohamed, 2021). The most important economic parts of *Hibiscus* *sabdariffa* are the sepals (calyx and epicalyx) which are useful as food (jam and jelly) and in cosmetic industries as a raw material for natural colouring agent (Salami and Afolayan, 2020; Apaliya *et al*., 2021; Mohamed, 2021).

Hibiscus sabdariffa seeds are roasted or grounded into the powder and used in meals as oily soups and sauces in some parts of Africa (Da-costa-rocha *et al*., 2014). The leaves are used for animal fodder and fibre (Plotto, 2004). It seeds are useful for feeding poultry and sheep and the residues from the seed oil extraction can also be used to feed chicks and cattle (Da-costa-rocha *et al*. 2014). It has also been used widely as local medicines. For example, the infusions of the leaves or calyces are used traditionally in Africa, India and Mexico for their hypotensive, diuretic, febrifugal and cholerectic effects because of its ability to decrease the viscosity of the blood and to stimulate intestinal peristalsis (Da-costa-Rocha *et al*., 2014; Almajid *et al*., 2023). In Egypt, the calyces' preparations have been used to treat Cardiac and nerve diseases as well as diuretic (Da-costa-Rocha *et al*., 2014). Other studies have reported that *H. sabdariffa* reduced serum cholesterol and body weight significantly (Nnamonu *et al*., 2013).

Materials and Methods

Experimental animals

Forty-two 6 weeks old male Wistar rats with average weight of 140g were used for the study. The rats were divided into 7 groups consisting of 6 rats per group.

Aliyu *et al*., 2014 reported that blood pressure and heart rate were reduced by the presence of aqueous *Hibiscus sabdariffa* through the inhibition of systemic vascular resistance mediated by the sympathetic nervous system. In the context of the pharmacological effects of *Hibiscus sabdariffa*, the main constituents are organic acids, anthocyanins, polysaccharides and flavonoids (Choong *et al*., 2019). Anthocyanin pigments which responsible primarily for red colour in *Hibiscus sabdariffa* were reported to contain delphinidin-3-glucoside and cyanidin-3-glucoside (Choong *et al*., 2019), it also possesses antioxidant activity comparable to ascorbic acid (Tounkara *et al*., 2014; Collin *et al*., 2022).

Despite the fact that the current lead usage has been drastically minimized because of the recognition of the damaging effects of lead toxicity, human and animal lead exposure remains a clear and present danger especially in the developing economies with significant population of artisanal miners. This study therefore evaluated the ameliorative effects of *Hibiscus sabdariffa* calyx extracts on both acute and sub-acute lead exposure on blood parameters in Wistar rats.

Group A, which was the control received only distilled water. Group B received 100mg/kg body weight of Lead acetate only while group C received 100 mg/kg body weight of Lead acetate and 200 mg/kg body weight of aqueous extract of *Hibiscus sabdariffa* concurrently. Group

D received Lead acetate and 400 mg/kg of *Hibiscus sabdariffa* aqueous extract. Group E received Lead acetate and 50 mg/kg of *Hibiscus sabdariffa* methanol extract. Group F received Lead acetate and 100 mg/km of *Hibiscus sabdariffa* methanol extract while Group G was given Lead acetate and 100 mg/kg of Ascorbic acid. The Lead acetate, the extracts, ascorbic acid and the distilled water were given per os by gastric gavage. The experiment covered a period of 28 days; however, blood samples were collected at day 14 for haematology and erythrocyte osmotic fragility. The final sample collection took place when the study was terminated at day 28. Ethical guidelines on laboratory animals handling were adhered to during the experiment.

Plant material and extract preparation

Methanol extraction procedure

Dried calyces of *H. sabdariffa* was procured from the local market (Bodija market), Ibadan, Oyo State, Nigeria (7.4351º N, 3.9143º E). The calyces were identified at the herbarium, Department of Botany, University of Ibadan with the voucher number (UIH-22792). The dried *H. sabdariffa* then pulverized into powder using a laboratory milling machine (Thomas Willey model 4, USA). About 1.5kg of the powdered plant material was introduced into a flask and 500ml (96%) methanol was added. The content was left for 8 days with occasional shaking to increase the extraction capacity (Abdallah, 2016). The macerated calyxes were filtered and concentrated in a rotary evaporator at 38ºC

which was weighed and re-dissolved in normal saline according to the body weights of the animals for oral administration.

Aqueous extraction procedure

About 1.5 kg of the powdered plant material was introduced into 2000 ml flat bottom flask and 500ml of distilled water was added. The content was mixed thoroughly and left for 24 hours with an occasional shaking to increase the extraction capacity. Thereafter, the soaked substance was filtered with Whatman filter paper (grade 1: 11 µm) and the resulting filtrate was dried into powder using a rotary evaporator (Stuart, model RE-300, UK). The solid extract was weighed and re-dissolved in normal saline according to the body weights of the animals for oral administration.

Blood sample collection

Blood sample was collected from each animal from the retro-orbital venous plexus into lithium heparinized tubes. From the blood samples collected, packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), total and differential white blood cell count and platelets count were determined. The plasma was obtained after centrifugation of the blood in a macro centrifuge (MSE England) at 3000 revolutions per min for 10 minutes.

Erythrocyte osmotic fragility

Erythrocyte osmotic fragility was determined according to the method described by Oyewale (1992). Briefly, 0.02 ml of blood was added to tubes containing increasing concentration of phosphate-buffered sodium chloride (NaCl) solution at pH 7.4 (0, 0.1, 0.2, 0.3, 0.5, 0.7, 0.8, and 0.9%). The tubes were gently mixed and incubated at room temperature (29 ºC) for 30 minutes. The content of each tube was then centrifuged at 1500 rev/ min for 10 minutes and the supernatant decanted. Optical density of the supernatant was determined spectrophotometrically at 540nm using SM22PC Spectrophotometer (Surgienfield Instruments, England). Haemolysis in each tube was expressed as a percentage, taking haemolysis in distilled water (0% NaCl) as 100%.

Determination of serum biochemical parameters

Sodium and potassium serum levels were determined using the Corning 410 Clinical Flame photometer method. Chloride and bicarbonate were determined by titrimetric methods of Schales and Schales (1941). Urea and creatinine were determined by spectrophotometer with the methods of Coloumbe and Farreau (1963) and Taussky (1956) respectively. Alkaline phosphatase activity was determined by the method of Bessey *et al*., (1946). Samples, standard and controls were incubated with p-nitrophenyl phosphate as substrate for 15 minutes at 37℃. The reaction was stopped by the addition of 5.0 mL of 0.5 M NaOH and the absorbance read at 405 nm using a RA-50 spectrophotometer (Ames/Technicon, France).

The activities of ALT and AST were determined by the method of Reitman and Frankel (1957). ALT and AST substrates (500 μL) were preincubated at 37℃ for 5 minutes. 100 µl of samples and control were added and the mixture further incubated for 30 minutes (ALT) and 60 minutes (AST) respectively. The reaction was terminated by adding 500 μL of 1 mmol 2, 4-dinitrophenylhydrazine and allowed to stand at room temperature for 20 minutes. The colour was developed by addition of 5 mL of 0.4 M NaOH and absorbance read at 505 nm using a RA-50 spectrophotometer (Ames/Technicon, France).

The serum concentration of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) were determined by enzymatic colorimetric method using the following Roche kits for Cobas Integra 400 plus autoanalyzer: TG, TC second generation, HDL-C and LDL-C plus second generation.

Statistical analyses

All values are expressed as mean ± S.D. One-way ANOVA followed by Tukey's post-hoc test was used to performed data analyses and compare the data between groups using GraphPad Prism version 7.00 (https://graphpad.com). The probability value of $P < 0.05$ was considered

Results

Haematology parameters in acute lead toxicity

Acute exposure of rats to lead for 14 days as shown in Table 1 revealed that the packed cell volume (PCV) of groups D, E and G that received lead + 400 mg/kg aqueous extract of HSA, lead + 50 mg/Kg methanol extract of HSM and lead + 100 mg/kg ascorbic acid, respectively, were significantly lower (P<0.05) than the control (group A) that received only distilled water. Similarly, groups D, E, F and G had significantly lower PCV than that of group B that received lead only, while, groups D, E and G were also significantly lower than that of group C that received lead and 200 mg/Kg of aqueous extract of *Hibiscus sabdariffa*.

The haemoglobin concentration and red blood cell count of the control group A were higher significantly (P < 0.05) than group G. They were also higher significantly in group B than groups D, E, F and G. The haemoglobin concentration was also higher significantly in group C than groups D, E and G, while, red blood cell count for group C was significantly higher than group G only. There was no significant difference in mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration within the groups.

Haematology parameters in sub-acute lead toxicity

When compared with the erythrocytic parameters in acute toxicity in Table 1, sub-acute exposure (Table 2) to lead for twenty-eight days results in significant decreases (P < 0.01) in the values of PCV, RBC counts, haemoglobin concentration while MCV, MCH and MCHC remain unchanged (Table 2). As shown in Table 2, the PCV, RBC and Hb concentration in group D (lead + 400 mg/kg aqueous extract of HSA) was significantly lower than of the control (group A) but only marginal decreases that were not significant were observed in the other parameters when compared with the positive control and group B that received lead only.

Erythrocyte osmotic fragility in acute lead toxicity

Acute exposure to lead also affected the erythrocyte osmotic resistance in hypotonic solutions (Fig.1). For example, at 0.1% NaCl concentration, the erythrocyte osmotic fragility of the rats in groups C, D, E and F were higher significantly (P<0.05) than the control group (Group A). At 0.5% NaCl, the rats in groups A, C, E and F had significantly lower erythrocyte osmotic fragility compared to the rats in group B (Fig. 1). At 0.7% NaCl concentration, the erythrocyte osmotic fragility of groups A, B, D, E and G were significantly lower that of group F, while, that of group B was significantly lower than the group C.

In like manner, at 0.9% NaCl concentration, erythrocyte osmotic fragility of the rats in groups C, F and G were significantly lower than the group A, while of groups C and F were significantly higher than the group B, but groups D, E, F and G had erythrocyte osmotic fragility that were significantly lower than group C.

Erythrocyte osmotic fragility in sub-acute lead toxicity

Fig. 2 shows the effects of sub-acute exposure of rats to lead toxicity for 28 days on the erythrocyte osmotic fragility. At 0% NaCl concentration, the erythrocyte osmotic fragility of the rats in groups A, B, C, E, F and G were significantly higher ($P<0.05$) than the group D. Similarly, at 0.1% NaCl concentration, groups B and F had the erythrocyte osmotic fragility that were significantly higher ($P<0.05$) than group D. Furthermore, at 0.7% NaCl concentration, groups C, F and G had significantly higher erythrocytes osmotic fragility than either of the group A, or group B.

At 0.9% NaCl, erythrocytes osmotic fragility values in groups C and G were significantly higher ($P<0.01$) than the values in the unexposed control (group A) while that of group B was significantly lower ($P<0.05$) than group G, whereas erythrocyte osmotic fragility in group D was significantly lower than that of group C.

Leucocyte and platelets parameters values in acute lead toxicity

Table 3 shows the leucocyte and platelet parameters values in acute lead toxicity as modulated by *Hibiscus sabdarifa* extracts and vitamin C. The total WBC count in group B that were treated with lead only was significantly higher ($P<0.05$) than those of groups C and F. Groups C and F also had significantly higher ($P<0.05$) WBC than were those rats in groups D, E and G. But all the lead exposed groups had higher ($P<0.05$) WBC counts than that of the untreated control (group A)

In a manner similar to that of the WBC, the total lymphocyte counts in group B (lead only) was significantly higher than that of the control (group A). It was also higher than those other those in groups C – G. Lymphocytes counts in groups C and D were also lower than those values obtained in group E, F and G.

The absolute lymphocyte counts of group A was higher significantly than group C, the groups C and F were significantly lower than group B, while, the groups D and E were significantly were significantly higher than group C and groups F and G were lower significantly than group E. There were no significant variations in the neutrophil and eosinophil counts. However, the absolute monocyte counts of group B was significantly higher than group D

While looking at the platelet counts, the platelets count of rats in the control group was significantly lower than those of the lead exposed groups except that of group F, which received lead and vitamin C, which had lowest platelets counts.

Leucocyte and platelet parameters in rats exposed to sub-acute lead toxicity

Table 4 shows the leucocyte and platelets parameters values in sub -acute lead toxicity as modulated by *Hibiscus sabdariffa* extracts and vitamin C. Total WBC in a manner similar to the results observed in acute toxicity, there was a significant increase ($P<0.05$) in the WBC in the lead treated groups. It was significantly higher than the values in the untreated control, and group C exposed to lead and 100mg/kg aqueous extract of *H. sabdariffa*.

Biochemical parameters in acute lead toxicity

As shown in Table 5, sodium ion concentrations in groups A and E were significantly lower ($P<0.05$) than the group G. The bicarbonate ion concentration of the groups A and B were significantly lower than group F, group C was significantly lower than the values in groups E and F, groups D and E were significantly lower than group F while, groups E and F were significantly higher than group G.

Biochemical parameters in sub-acute lead toxicity

As shown from Table 6, Sodium ion concentration in groups A, D and E were significantly higher (P<0.05) than the value in group G.

Lipid profile in acute lead toxicity

The lipid profile parameters, total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and low-density lipoprotein (LDL) could be seen following the previous trend observed in the other parameters mentioned above (Table 7). The rats in group B and those exposed to lead and extract had higher TC, TG, HDL and LDL than those of the control (group A), but were corrected in group F (TG), and group E (TG and HDL). The concentration of HDL of groups D and F were significantly higher (P<0.05) than group A.

Lipid profile in sub-acute lead toxicity

The lipid profile parameters, total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in sub- acute lead exposure and modulatory effects of *H. sabdariffa* extracts are shown in Table 8. The concentrations of HDL in groups B, D and E were significantly higher than group A. However, the HDL concentrations were brought down significantly (P<0.05), even below that of the control in those rats in groups C, F and G, while, group G was significantly lower than the groups B, C, D and E. The LDL concentration of group G was significantly lower than the groups A, B and D.

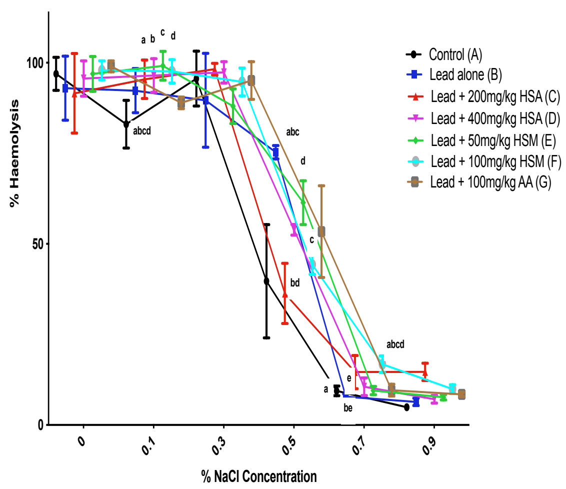

Fig.1. Erythrocytes osmotic fragility of rats exposed to acute lead toxicity and concurrent treatment with *Hibiscus sabdarifa* methanol extracts and ascorbic acid. Values are expressed as mean ± SD, n = 5.

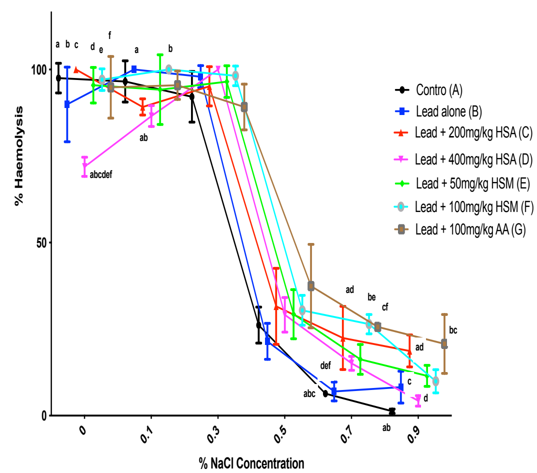

Fig. 2. Erythrocytes osmotic fragility of rats exposed to sub -acute lead toxicity for 28 days and concurrent treatment with *Hibiscus sabdarifa* aqueous and methanol extracts and ascorbic acid. Values are expressed as mean ± SD, n = 5.

Table 1. Effects of acute lead toxicity on erythrocyte parameters in Wistar rats as modulated by aqueous and methanol extracts of *Hibiscus sabdariffa*

Parameters	A – Control	B - Lead alone	C - Lead + 200mg/kg HSA	D - Lead + 400mg/kg HSA	E - Lead + 50mg/kg HSM	F - Lead + 100mg/kg HSM	G - Lead + 100mg/kg AA
PCV (%)	48.67 ± 5.51^{abc}	51.75 ± 1.71^{defg}	49.00 ± 4.55^{hij}	38.25±2.63^{adh}	37.25 ± 0.96^{bei}	42.50±3.11^{f}	35.60 ± 2.61^{cgj}
Hb (g/dL)	14.83 ± 2.11^{a}	17.52 ± 1.24^{bcde}	16.02±1.27^{fgh}	12.45 ± 0.95^{bf}	12.72 ± 1.14^{cg}	13.68±1.65^{d}	11.74 ± 0.96^{aeh}
RBC (10^{-6}µL)	7.47 ± 0.87 ^{a}	8.58 ± 0.38 ^{bcde}	7.79 ± 0.91^{f}	6.58 ± 0.51^{b}	6.54 ± 0.52^{c}	7.18 ± 0.50^{d}	5.93 ± 0.52 ^{aef}
MCV (fl)	61.13 ± 2.42	62.67 ± 3.74	60.90 ± 1.84	60.46 ± 2.00	59.29 ± 1.23	59.91 ± 1.68	60.11 ± 1.67
MCH (pq)	19.74 ± 0.45	20.42 ± 0.97	20.08 ± 0.74	19.71 ± 0.78	19.44 ± 0.53	20.08 ± 0.56	19.82 ± 0.70
MCHC (g/dl)	32.4 5± 0.67	19.74 ± 0.45	20.42 ± 0.97	32.59 ± 0.81	32.79 ± 0.67	33.51 ± 0.32	32.96 ± 0.43

Values are presented as mean ± SD while superscript alphabets along the same row are significantly different at P<0.05. Note: HSA = aqueous extract of *Hibiscus sabdariffa,* HSM = methanol extract of *Hibiscus sabdariffa,* AA = Ascorbic acid, PCV = Packed cell volume, Hb = Haemoglobin concentration, RBC = Red blood cell count, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration

Table 2. Effects of sub-acute lead toxicity on erythrocyte parameters in Wistar rats as modulated by aqueous and methanol extracts of *Hibiscus sabdariffa*

Parameters	A – Control	B - Lead alone	C - Lead + 200mg/kg HSA	D - Lead + 400mg/kg HSA	E - Lead + 50mg/kg HSM	F - Lead + 100mg/kg HSM	G - Lead + 100mg/kg AA
PCV (%)	38.20±2.68	35.40±3.29	35.80 ± 2.68	32.60 ± 2.79	36.00±3.81	35.25 ± 2.22	36.00±2.00
Hb (g/dL)	12.90 ± 0.78	11.88 ± 1.14	11.80 ± 0.93	10.88 ± 0.79	11.82 ± 1.43	12.03 ± 0.61	11.88±0.65
Rbc ($10^{-6/}$µL)	6.24 ± 0.20	6.05 ± 0.39	5.99 ± 0.48	5.31 ± 0.65	5.93 ± 0.64	5.82 ± 0.59	5.86 ± 0.60
MCV (fl)	59.36 ± 1.24	62.09 ± 4.41	59.83 ± 2.05	61.69 ± 2.88	60.56 ± 1.50	59.94 ± 2.87	61.64±3.34
MCH (pq)	20.07 ± 0.32	20.83 ± 1.00	19.73 ± 0.98	20.61 ± 1.24	19.87 ± 0.91	20.39 ± 1.22	20.35±1.27
MCHC (g/dl)	34.20 ± 1.13	35.56 ± 0.83	32.96 ± 0.67	33.41 ± 0.75	32.79 ± 0.87	34.01 ± 0.90	33.00±0.55

Values are presented as mean ± SD, Number of animals = 5 per group. Note: HSA = aqueous extract of *Hibiscus sabdariffa,* HSM = methanol extract of *Hibiscus sabdariffa,* AA = Ascorbic acid, PCV = Packed cell volume, Hb = Haemoglobin concentration, RBC = Red blood cell count, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration

Table 3. Effects of acute lead toxicity on leucocyte and platelet parameters in Wistar rats as modulated by aqueous and methanol extracts of *Hibiscus sabdariffa*

Parameters	A – Control	B - Lead alone	C - Lead + 200mg/kg HSA	D - Lead + 400mg/kg HSA	E - Lead + 50mg/kg HSM	F - Lead + 100mg/kg HSM	G - Lead + 100mg/kg AA
TWBC (x 10^3/µL)	3.91 ± 5.4 ^{abcde}	8.08 ± 2.3^{af}	5.01 ± 0.4^{fg}	7.00 ± 0.6^{b}	7.03 ± 0.3^{c}	6.45 ± 0.4^{d}	7.82 ± 0.3^{eg}
LYM (x 10^3/µL)	2.60 ± 0.2	6.00 ± 1.6^{ab}	3.68 ± 0.3^{cde}	3.37 ± 0.3^{c}	4.90 ± 0.3^{ad}	3.86 ± 0.1	4.95 ± 0.3^{be}
NEU (x 10^3/µL)	1.00 ± 0.0	1.80±0.6^{abc}	1.21 ± 0.2^{def}	2.16 ± 0.4^{ad}	2.41 ± 0.2^{be}	2.13 ± 0.1	2.63 ± 0.1^{cf}
MONO (x 10^3/µL)	0.06 ± 0.02	0.10 ± 0.02	0.11 ± 0.02	0.18 ± 0.06	0.10 ± 0.03	0.07 ± 0.01	0.09 ± 0.01
EOS (x 10^3/µL)	0.11 ± 0.01	0.24 ± 0.07	0.12 ± 0.02	0.07 ± 0.01	0.10 ± 0.05	0.14 ± 0.02	0.11 ± 0.04
Platelet (x 10^3/µL)	126.7±10.8^{abc}	172.3 ± 8.6^{d}	180.0±5.5^{ae}	210.0±13.5^{bf}	178.0 ± 5.6^{g}	197.3±18.5^{ch}	86.3±40.2^{defgh}

Values are presented as mean ± SD, n = 5. Values with superscript alphabets along the same row are significantly different at P<0.05 Note: TWBC = Total white blood cell count, NEU = Neutrophil count, LYM = Lymphocyte count, MONO = Monocyte count, EOS = Eosinophil count.

Table 4. Effects of sub-acute lead toxicity on leucocyte and platelet parameters in Wistar rats as modulated by aqueous and methanol extracts of *Hibiscus sabdariffa*

Parameters	A – Control	B - Lead alone	C - Lead + 200mg/kg HSA	D - Lead + 400mg/kg HSA	E - Lead + 50mg/kg HSM	F - Lead + 100mg/kg HSM	G - Lead + 100mg/kg AA
TWBC (x 10^3/µL)	6.23 ± 0.3	7.75 ± 1.3^{ab}	5.01±0.7^{acde}	7.10 ± 0.2^{cf}	7.12 ± 0.2^{dg}	5.25 ± 0.3^{bgfh}	7.04 ± 0.7^{eh}
Lym (x10^3/µL)	3.99 ± 0.1	4.65 ± 0.8	2.84 ± 0.4	4.00 ± 0.2	4.88 ± 0.1	3.28 ± 0.3	3.81 ± 0.2
Neu (x 10^3/µL)	1.71 ± 0.1^{a}	2.77 ± 0.5	1.69 ± 0.3^{b}	2.53±0.3^{abcde}	1.77 ± 0.1^{c}	2.41 ± 0.2^{d}	2.50 ± 0.1^{e}
Mono (x 10^3/µL)	0.10 ± 0.03	0.17 ± 0.07	0.07 ± 0.02	0.11 ± 0.06	0.11 ± 0.03	0.14 ± 0.03	0.15 ± 0.01
Eos (x 10^3/µL)	0.14 ± 0.01	0.16 ± 0.10	0.10 ± 0.03	0.10 ± 0.04	0.01 ± 0.04	0.21 ± 0.04	0.12 ± 0.04
Platelet (x 10^3/µL)	126.33±3.33^{ab}	171.33±17.01^{acdefg}	139.0±1.00^{chij}	108.7±8.1^{dhk}	98.33±11.6^{beijl}	137.00±4.6^{fklm}	112.60±5.7^{gm}

Values are presented as mean ± SD, n = 5. Values with superscript alphabets along the same row are significantly different at P<0.05 Note: TWBC = Total white blood cell count, Neu = Neutrophil count, Lym = Lymphocyte count, Mono = Monocyte count, Eos = Eosinophil count.

Table 5. Effects of acute lead toxicity on plasma electrolyte and biochemical of Wistar rats as modulated by aqueous and methanol extracts of *Hibiscus sabdariffa*

Parameters	A – Control	B - Lead alone	C - Lead + 200mg/kg HSA	D - Lead + 400mg/kg HSA	E - Lead + 50mg/kg HSM	F - Lead + 100mg/kg HSM	G - Lead + 100mg/kg AA
Na^+mmol/L	134.00±2.00^{a}	139.70±0.58	140.0 ± 1.0	147.0 ± 2.0	134.00±3.00^{b}	137.70±3.22	141.00±1.00^{ab}
K^+ mmol/L	3.43 ± 0.49	3.90 ± 0.20	4.03 ± 0.21	3.83 ± 0.15	3.40 ± 0.26	3.50 ± 0.25	4.13 ± 0.15
Cl^-mmol/L	103.30 ± 2.89	106.70±2.89	106.7 ± 2.89	105.0 ± 5.0	98.37 ± 2.89	102.70 ± 2.52	106.70 ± 2.89
HCO_3^- mmol/L	24.00 ± 2.00^{a}	22.67±1.53^{b}	21.33 ± 1.53^{cd}	21.67±2.03^{e}	25.67±0.58^{cfg}	33.67±0.58^{abdefh}	21.00±1.00^{gh}
TP (g/L)	6.53 ± 0.35	6.97±0.32	7.20±0.20	7.10±0.36	6.53 ± 0.35	6.60 ± 0.20	7.20 ± 0.20
Alb (g/L)	3.57 ± 0.21	3.93 ± 0.31	4.13±0.06	3.97±0.25	3.70 ± 0.17	3.77 ± 0.25	4.07 ± 0.12
Glob (g/L)	2.97 ± 0.15	3.03 ± 0.06	3.07±0.21	3.13±0.12	2.83 ± 0.21	3.17 ± 0.21	3.13 ± 0.23
Urea (mmol/L)	17.00±3.61^{abcd}	30.00±2.00^{a}	30.33 ± 1.53^{b}	30.33 ± 8.15^{c}	21.00 ±2.65^{e}	23.00 ± 2.00	34.33 ± 4.51^{de}
Creatinine (µmol/L)	0.47 ± 0.12^{abc}	0.67 ± 0.06	0.73±0.06^{a}	0.73±0.12^{c}	0.53 ± 0.06	0.60 ± 0.00	0.70 ± 0.06^{c}

Values are presented as mean ± SD, n = 5. Values with superscript alphabets along the same row are significantly different at P<0.05

Table 6. Effects of sub-acute lead toxicity on electrolyte and biochemical parameters in Wistar rats as modulated by aqueous and methanol extracts of *Hibiscus sabdariffa*

Parameters	A – Control	B - Lead alone	C - Lead + 200mg/kg HSA	D - Lead + 400mg/kg HSA	E - Lead + 50mg/kg HSM	F - Lead + 100mg/kg HSM	G - Lead + 100mg/kg AA
Na^+ mmol/L	140.30±0.58^{a}	137.30 ± 2.08	135.3 ± 3.51	140.0±1.00^{c}	140.00 ± 1.00^{c}	139.00±1.73	133.00±3.00^{abc}
K^+ mmol/L	4.00 ± 0.17	3.53 ± 0.25	3.50 ± 0.25	3.93 ± 0.06	3.93 ± 0.21	3.87 ± 0.25	3.40 ± 0.44
Cl^- mmol/L	108.30±2.89	101.70 ± 2.89	101.7 ± 2.89	108.3 ± 2.80	108.30 ± 2.89	108.30±2.89	103.30 ± 2.89
HCO_3^- mmol/L	21.67 ± 1.16	25.00 ± 1.00	24.33 ± 1.53	21.67 ± 1.16	22.67 ± 1.16	23.00 ± 2.65	24.67 ± 1.53
TP(g/L)	7.20 ± 0.20^{a}	6.53 ± 0.15	6.50 ± 0.3	6.90 ± 0.4	7.03 ± 0.15	6.70 ± 0.50	6.23 ± 0.12^{a}
Alb (g/L)	4.20 ± 0.20^{a}	3.50 ± 0.17^{a}	3.73 ± 0.31	4.07 ± 0.21	3.97 ± 0.21	3.62 ± 0.23	3.57 ± 0.23
Glob (g/L)	3.00 ± 0.00	3.03 ± 0.12	2.77 ± 0.49	2.83 ± 0.21	3.07 ± 0.12	3.03 ± 0.38	2.90 ± 0.23
Urea (mmol/L)	33.33 ± 4.51^{a}	22.33 ± 2.08	21.33 ± 3.51	27.67 ± 4.16	28.67 ± 2.08	22.00 ± 9.00	17.67 ± 3.79^{a}
Creatinine (µmol/L)	0.73 ± 0.06^{a}	0.57 ± 0.06	0.53 ± 0.12	0.70 ± 0.10	0.73 ± 0.06^{b}	0.60 ± 0.20	0.43 ± 0.06^{ab}

Values are presented as mean ± SD, n = 5. Values with superscript alphabets along the same row are significantly different at P<0.05

Table 7. Effects of acute lead toxicity on lipid profile in Wistar rats as modulated by aqueous and methanol extracts of *Hibiscus sabdariffa*

Parameter	Control	Lead alone	C - Lead + 200mg/kg HSA	D - Lead + 400mg/kg HSA	Lead + HSM (50mg)	Lead + HSM (100mg)	Lead + AA (100mg)
TC	117.70±9.29	138.00 ± 2.52	151.30±12.90	162.70±23.01	145.30±6.51	158.30±45.37	146.70±13.32
TG	51.67 ± 5.03	68.33 ± 4.04	73.33 ± 11.50	69.00 ± 14.00	60.67 ± 3.06	57.67 ± 2.08	74.00 ± 10.15
HDL	30.67±2.08^{ab}	45.67 ± 4.93	45.67 ± 4.51	48.33 ± 5.51^{a}	39.00 ± 2.00	47.33 ± 10.21^{b}	43.33 ± 5.51
LDL	61.00 ± 3.00	89.00 ± 1.00	93.67 ± 6.03	103.70 ± 7.10	84.67 ± 8.37	101.00±35.50	85.00 ± 9.54

Values are presented as mean ± SD, n = 5. Values with superscript alphabets along the same row are significantly different at P<0.05. Note: TC = Total cholesterol, TG = Triglycerides, HDL = High density lipoprotein, LDL = Low density lipoprotein.

Discussion

Acute and sub-acute exposure to lead resulted in normocytic normochromic anaemia as evidenced by significant reduction in PCV, RBC and haemoglobin parameters, while the MCV, MCH and MCHC were similar in the acute phase, but sub-acute exposure for 28 days resulted in microcytic hypochromic anaemia. This is in agreement with previous reports of Nikolic *et al*. (2015) and Mazumdar and Goswami (2016) who reported similar normocytic normochromic anaemia in occupationally exposed individuals in commercial enamel paint industry and lead battery manufacturing plants (Bagepally *et al*., 2016). This activity of lead is associated with its ability to bind with haemoglobin in replacement of iron, thus leading to iron deficiency anaemia and elevation of plasma iron content (Bagepally *et al*., 2016). Lead also causes anaemia through oxidative stress mediated destruction of erythrocytes as a result of its bioaccumulation in the body tissues (Lee *et al*., 2019) especially in bones. Exposure to lead and its toxicity has been a problem since antiquity, such that anthropological and archaeological findings from human bones and remains still shows considerable signs of lead toxicity (Sguazza *et al*., 2016) and it has been a source of death to many, especially in developing countries. Concurrent administration of *Hibiscus sabdariffa* calyx (zobo) extract especially at 50 and 100mg/kg of methanol extract and 200mg/kg aqueous extract considerably ameliorated the toxic effects of lead on the PCV, RBC and Hb values, thereby reducing the lead induced anaemia. *Hibiscus sabdariffa* extract has been previously reported to show haematinic properties by stimulating haematopoiesis (Vasavi *et al*., 2019), and it is in general use in Nigeria as a replacement drink for most of the fizzy drinks that have been associated with excess calories (Vasavi *et al*., 2019).

Lead exposure, both acute and sub-acute also caused significant leucocytosis that was actually powered by lymphocytosis due to elevated lymphocyte values in all the lead treated rats. The effect was also ameliorated by low doses of the extract in a manner that is similar to that of vitamin C. Lymphocytosis is a common feature during acute stress (Mills *et al*., 1995) and it is a common marker of oxidative stress in many animals, especially the avian species. High doses of the extract however did not show much protective effect against lead toxicity.

In a manner that is consistent with the observations in the haematological parameters, the erythrocytes osmotic fragility was observed to increase in lead treated rats only and corrected by low doses, 50mg/kg of methanol extract and 200mg/kg of aqueous extract, whereas, high dosages of the extract probably exacerbated the effects of lead on the rats. Due to the damaging effects of free radicals and ROS on erythrocyte membrane proteins and lipids, resulting in increased fluidity and susceptibility to osmotic lysis, erythrocyte osmotic fragility has been used as a marker of oxidative stress induced by several factors such as aging (Brzezińska-Ślebodzińska, 2001, Azeez *et al*., 2011), exercise (Şentürk *et al*., 2001), transportation (Adenkola and Ayo, 2009) and haemodialysis (Candan *et al*., 2002). Increased erythrocyte osmotic fragility that were observed in those rats treated with higher dosages of *H. sabdariffa* extracts may have been due to high concentration of saponin, tannin and cardiac glycosides .

Changes in plasma electrolytes concentrations, urea and creatinine levels serve as clinical indicators of the health status of the kidneys while liver enzymes AST, ALT, ALP and more specifically GGT serve as indicators of liver damage (Kuatsienu *et al*., 2017). Many of these toxic effects are seen in plants that are commonly used as medicinal plants when evaluated, and contrary to the general believe of the users. In the present study, plasma sodium ions, creatinine and urea were elevated in acute lead toxicity in the Wistar rats. While liver enzymes, AST, ALT, ALP and GGT were also found to be higher in lead treated rats. Significant reductions in these enzymes were only observed in rats treated with 200mg/kg of the aqueous extract of *H. sabdariffa* and 50mg/kg of the methanol extract, even in a manner that is better than the modulatory or ameliorative effects of vitamin C, a natural antioxidant that was used as the control. This indicates that lead toxicity resulted in liver and renal damages, which were ameliorated by low doses of *H. sabdariffa*. This finding is in agreement with the reports of Okoko and Oruambo (2008) and Lee *et al*. (2012) who observed significant protective effects of *H. sabdariffa* calyx extract on cisplastin and acetaminophen induced liver and renal damages in rats. *Hibiscus sabdariffa* has also been reported to show anticholesterolaemic activities, and protect against high fat diet induced obesity and liver damage in hamster (Huang *et al*., 2015) by inhibiting hepatic lipogenesis and preadipocytes adipogenesis (Kao *et al*., 2016) and modulation of oxidative stress, which has been demonstrated even in human subjects (Soto *et al*., 2016). The observed effects in the present study may not be unconnected with the antioxidant potential of the plants that has been widely reported by several authors (Pérez-Torres *et al*., 2019).

Lead toxicity also had significant effects on the lipid profiles in the Wistar rats in the present study, for example, acute and sub-acute lead toxicity increased total cholesterol, triglyceride, HDL and LDL values significantly. But it was only 200 mg/kg aqueous extract and the methanol extract treated rats that showed lowered cholesterol, HDL and LDL values. This further reinforces the previous anticholesterolaemic and anti lipidaemic activities of *H. sabdariffa* that has been reported by other authors including

Huang *et al*. (2015) and Kao *et al*. (2016) who showed that *H. sabdariffa* calyx extracts have anti-lipidaemic and anti-lipogenesis effects. Furthermore, the methanol extract at 50mg/kg body weight also significantly reduced the stress hormone, cortisol level in the acute phase whereas, there was no sign of elevated cortisol in the sub-acute phase of the study, although, the plasma cortisol value in the sub-acute phase was higher than the values in the acute phase in all the group of the rats.

CONCLUSION

It was demonstrated in this study that both methanol and aqueous extracts of *Hibiscus sabdariffa* calyx extracts at low doses (50 and 100 mg/kg of methanol and 200 mg/kg of aqueous) ameliorated the anaemia, lymphocytosis and increased erythrocytes osmotic fragility caused by both acute and sub-acute lead toxicity.

Authors contributions

The authors have materially participated in the research and article preparation. The authors have approved the final article. The idea for the paper was conceived, designed and supervised by Azeez, O. I. The experiment was performed by Oyedeji, O. T. The data were analysed by Oyedeji O.T and Azeez O. I. The paper was jointly written, interpreted and edited by Azeez, O. I. Oyedeji, O. T. and Ake, A.S.

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Conflict of interest statement

The authors wish to declare that there are no competing interests existing regarding the manuscript publication.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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