

Nutritional, haematinic and biosafety evaluation of *Ipomoea batatas* (L.) Lam. (Solanales: Convolvulaceae) leaf extract on albino rats

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Abstract. This study evaluates the nutritional, Haematinic and biosafety of *Ipomoea batatas* (L.) Lam. (Solanales: Convolvulaceae) aqueous leaf extract on albino rats. Proximate and mineral compositions were determined using standard methods. Haematinic activity of the plant was done using graded doses; 125, 250, and 500 mg/kg and 40 mg/kg phenyl hydrazine hydrochloride to induce anaemia. A modified method was used for acute and sub-acute toxicological evaluations. Results from the study showed that aqueous extracts of *I. batatas* had significant increase in RBC, HCT, Hgb, MCV, MCH and MCHC at 7.19 ± 0.45 , 46.13 ± 0.08 , 13.43 ± 0.27 , 82.40 ± 0.92 , 24.15 ± 1.24 and 37.78 ± 0.20 , respectively, when compared with the control group. Acute study showed no pathological behaviour with absent mortality. Sub-acute study of the spleen, heart, liver and kidney showed a mild activation of local immune system. The extracts recorded 89.14% scavenging property compared with 94.3% ascorbate in antioxidant study. Proximate analysis had 31.56% of moisture content, 16.25% of protein, 7.64% of ash, 0.37% of crude fibre, 0.19% of fat and 43.99% of carbohydrate. Investigation of calcium, magnesium, Iron, potassium, phosphorus, sodium and zinc were 28.03, 339.61, 15.87, 4.61, 35.90, 4.21 and 0.08 mg/kg others were not detected. This may be due to stimulating mechanism of Myelo-Erythroid cell ratio in bone marrow or antioxidant effect. Result thus validates ethnobotanical uses of *I. batatas* for the treatment of anaemia.

Keywords: Sweetpotato; Anaemia; Bone marrow; Peripheral blood smear; Phenylhydrazine hydrochloride.

Introduction

Currently, complimentary/alternative use of medicines, especially the consumption of phytochemicals has rapidly increased universally. Herbal medicines have less or no damaging effect than conventional drugs. They

improve patient tolerance on prolong use (Kaliora et al., 2006). *Ipomea batatas* (L.) Lam. (Solanales: Convolvulaceae) is a native to Central and Southern America, the un-recent known plants to human. Sweet potato is herbaceous perennial vegetable with white and purple flowers and long nutritious storage roots (Antia

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et al., 2006; Ozaki et al., 2010). Recently, numbers of reports showed that, *I. batatas* phytochemicals exhibit antioxidative or radical-scavenging effect with health-promoting property in humans (Konczak-Islam et al., 2003; Suda et al., 2003; Rabah et al., 2004; Dini et al., 2009). The plant supports correct heart functions (Noda and Horiuchi, 2008). *I. batatas* has a broad Ethnomedicinal function which includes; anti-anemic, anti-inflammatory, anti-fertility, anti-carcinogenesis, anti-mutagenity, anti-diabetes, anti-hemorrhagic, and promote stable blood sugar level (Bratosin et al., 1998; Lippi et al., 2012).

Extrinsic anemia arises as hemolytic anemia in blood vessels known as intravascular haemolysis or other body parts called extra-vascular. The potential cause varies from safe anemia to life-threatening anemia. Usually, hemolytic anemia can either be classified as merited or acquired (Telford et al., 2003). Peripheral blood films stained test using Wright's stain present vital diagnosis understanding of anemia with various diseases associated to leukocytes and platelets (Lessin et al., 2006; Komolafe and Awoniyi, 2013).

The objective of this study is to evaluate the nutritional, haematinic and toxicological profile of *I. batatas* extracts.

Materials and methods

Collection of plant material

Sweet potatoes leaves (*I. batatas*) were gotten from Akure, Ondo State, Nigeria. It was identified and authenticated by Dr. O. Timothy in the Department of Plant Biology and Biotechnology, University of Benin, Benin City Edo State, Nigeria.

Preparation of plant material

The leaves were rinsed and air dried for 3 days, afterwards oven dried at 40 °C for few hours. The crunchy leaves were pulverized into powder with electrical grinder and extracted using

aqueous solvent. It was stored in an air-tight container for further application.

Determination of antioxidant activities

Free radical scavenging activity of *I. batatas* leaves extract against 1, 1-Diphenyl-2-picrylhydrazyl radical (Sigma Aldrich) was evaluated by 517 nm UV spectrophotometer. The radical scavenging property was measured using slight modified method described by Leong and Shui (2002):

$$\% \text{ inhibition} = (A_a - A_b / A_b) \times 100$$

Where A_b is absorption for the blank sample and A_a the absorption for the extract

Nutritional evaluation

Proximate analysis/composition. The proximate analysis (gross chemical composition) was determined by the recommended method of AOAC (2005). This includes:

Moisture content determination. 2 g of the dry sample was placed in a crucible of 31.990 g, the crucible then was put into the oven for 3 h at 105 °C until a constant weight was attained. The crucible was transferred to a dessicator to cool and the weight was noted to 33.786 g. The percentage moisture constituent was calculated as:

$$\% \text{ MC} = \frac{\text{WLS}}{\text{WOS}} \times 1,000$$

Where:

MC = Moisture content

WLS = weight loss by sample

WOS = weight of original sample

$$\% \text{ DM} = 100 - \% \text{ MC}$$

Where:

DM = Dry matter

MC = Moisture content

Ash content determination. 2 g weight of the dry sample was introduced in a crucible of 31.981 g. The crucible was then placed in the muffle furnace and was set at 550°C for three hours for complete ashing. The crucible was brought out from the furnace after the designated time and was taken to the dessicator to cool, after which the weight was noted to be 32.355 g. The ash constituent was calculated as:

$$\% \text{ AC} = \frac{\text{WA}}{\text{WS}} \times 100$$

Where:

AC = Ash content

WA = Weight of ash

WS = Weight of sample

$$\% \text{ OM} = 100 - \% \text{ AC}$$

Where:

OM = Organic Matter

AC = Ash content

Crude oil determination. 1 g of the dry sample was conveyed into filter paper of 1.550 g. The filter paper was then wrapped and placed in the extraction chamber of the soxhlet extractor. The extraction flask was two quarter filled with petroleum ether (organic solvent) for the extraction of oil from the sample. The extraction continued with the organic solvent extracting the oil from the sample by boiling gently and leaving it to siphon over several hours until the solvent in the chamber becomes clear. The filter paper was collected and placed in the oven at 105°C for one hour. The filter paper was then cooled in the dessicator and weighed to be 2.468g. The percentage fat was then calculated as:

$$\frac{\text{Difference in weight}}{\text{Original weight}} \times 100$$

Crude fibre determination. 1 g of the defatted sample was transferred into 260 mL conical flask. For acid digestion, 100 mL of boiling 1.25% H₂SO₄ was poured and made to boil within an minute, and then it was boiled gently for 30 min maintaining a constant volume with the help of the wash bottle. The mixture was then filtered through a poplin cloth by suction using Buchner funnel, the poplin cloth was then rinsed thoroughly with hot distilled water and the residue was transferred into another flask with spatula for alkaline. 100 mL of boiling 1.25% NaOH was added, and brought to boiling in 1 min, it was left to boil gently for 30 min. The process of filtering through a poplin cloth was repeated. The residue was thoroughly savaged from the poplin cloth with the help of spatula and the remaining residue was rinsed off from the poplin cloth into the crucible with ethanol. The crucible was then dried with sample in an oven at 105 °C for one hour and was transferred into a dessicator to cool before the weight was taken to be 30.892 g. The crucible was then transferred with the sample into muffle furnace at 300 °C for two hours and then into the dessicator to cool. The percentage crude fibre was calculated as:

$$\% \text{ CF} = \frac{\text{WD} - \text{WAS}}{\text{WS}} \times 100$$

Where:

CF = crude fibre

WD = weight of digest

WAS = weight of ash sample

WS = weight of sample

Crude protein determination

Crude protein determination of the sample was done the kjeldahl method with a little modification. 1 g of the dry sample was taken into the digestion flask with 5 g of the digestion mixture added to it. 25 mol of concentrated H₂SO₄ was added to the flask swirled in other to mix the content thoroughly before it was placed on the heating mantle for the

digestion process to commence, so that a clear mixture was achieved. The digest was allowed to chilled, poured into a 100 mol volumetric flask to the required mark before it was then filtered. Distillation of the digest was perform with 100 mL of digest being introduced into a distillation flask, 30 mol of distilled water and 10 mol of 40% NaOH was gradually added through the process. The distillation process was completed after 20 min with ammonia (NH₃) produce as ammonium hydroxide (NH₄OH), in a conical flask with 5 mol of boric acid mixed indicator. The distillate was titrated against 0.1 M HCL standard solution till pink colour was observed. The percentage crude protein of the sample was calculated as:

$$\% \text{ CP} = \% \text{ N} \times \text{Crude factor}$$

Where:

$$\% \text{ N} = \frac{S \times 0.014 \times D}{\text{Wt} \times V} \times 100$$

S = Sample titration reading
 D = Dilution of sample after digest (100 mL)
 V = Volume taken for distillation (10 mL)
 0.014 = Milli-equivalent weight of nitrogen
 Wt = Weight of sample used
 Crude factor = 6.25

Nitrogen Free Extract. The NFE was obtained by subtracting the amounts of all five fractions above from 100%. Thus NFE is determined as follows:

$$\text{NFE} = 100 - (\% \text{ water} + \% \text{ ash} + \% \text{ crude protein} + \% \text{ crude fibre} + \% \text{ crude oil})$$

Determination of mineral elements composition

Weighed 5 g of the leave powdered was ashed at 550°C in a muffle furnace for 5 h and the residue liquefied in 100 ml deionised water. Standard solutions of the minerals were prepared and used to calibrate the atomic absorption spectrophotometer (AAS) (model 969 AA, Unicamseries) using acetylene-air flame at certain wavelengths. Aliquots of the ash solutions were inserted into AAS and from the standard curve the various concentrations were obtained.

Experimental animals

Fifty four healthy Wistar albino rat weighing 180-250 g. The animals were obtained in the Animal and Environmental Biology, University of Benin animal house. They were housed in a well-ventilated woody cages in normal laboratory state (12 h light/dark cycle: 23 °C ± 2 °C) and fed with standard diet. Food and water were given *ad libitum* to the animals. They were

handled in accordance to standard protocols of National Institute of Health USA: Public Health Service policy on humane care and use of Laboratory Animals (2002).

Experimental protocol

Phenylhydrazine hydrochloride induced anaemia was grouped as follows:

Six groups, of 9 animals per group received the following treatment schedule.

Group A: Received distilled water (0.5 ml/kg p.o.) alone.

Group B: Received ferrous (iii) - hydroxide poly-maltose (5 mg/kg p.o.).

Group C: Received phenyl hydrazine hydrochloride (40 mg/kg p.o.) alone.

Group D: Received aqueous extract of *I. batatas* (125 mg/kg p.o.).

Group E: Received aqueous extract of *I. batatas* (250 mg/kg p.o.).

Group F: Received aqueous extract of *I. batatas* (500 mg/kg p.o.).

Animals were fasted overnight before administration of phenyl hydrazine hydrochloride according to body weight; they were post-treated for 14 consecutive days. Three rats were sacrificed across the groups for 0, 7 and 14 days. The necessary sample were then analyzed.

Bone marrow preparation

Bone marrows were decalcified into slides and interpreted with pictures.

Acute toxicity study

Across the groups, the animals were observed for 4 and 24 h for every behavioral changes such as hypersensitivity, sedation, hyper-respiration, hyperactivity, itching, salivation, diarrhea, convulsion, coma and dead after the administration of the extract at respective doses (125 mg/kg, 250 mg/kg and 500 mg/kg), and distilled water. Lethality or Mortality was calculated after 24 h and the lethal dose (LD₅₀) was determined. All animals were observed for 14 days for delayed mortality.

Hematology assays

Automated sysmex KX-21 hematology analyzer (Sysmex Corporation, Kobe, Japan) was used for the determination of red blood cells (RBC), hemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RCDW), white blood cell (WBC), monocytes (MO), lymphocytes (LY), platelets (PLT). Platelets crit (PCT), platelet density

width (PDW), mean platelet volume (MPV) and granulocytes (GR).

Histopathological study

Organs such as; Liver, Kidney, Heart, Spleen which were fixed in 10% (vol/vol) formaldehyde, cleaned up in xylene and, embedded in paraffin wax (melting point at 56%) for organ preservation. Tissue sections were prepared according to the method of Drury and Wallinton (2003) and stained with eosin/hematoxylin. Photomicrographs were taken at ×400 magnifications using digital camera.

Statistical analysis

Data were presented as (SEM) of the respective replicates. (ANOVA) was used to compare means of different sample significance; Duncan Multiply Range test shows the source of significance (Post hoc test). SPSS 21.0 computer software package. Microsoft Excel 2013 version was used for analysis. $P \leq 0.05$ (95% confidence interval) was considered significant, while $P \leq 0.01$ (99% confidence interval) was considered as highly significant.

Results

Antioxidant activity

DPPH scavenging activity of *I. batatas* leaf extracts. The results of the total Antioxidant activity of aqueous extract of *I. batatas* indicated in Figure 1. Aqueous extract was observed to possess the highest radical scavenging potential with inhibition of 77.97% at 80 µg/mL and 89.14% at 100 µg/mL, when compared with standard (ascorbic acid) 92.91% at 80 µg/mL and 94.3% at 100 µg/mL.

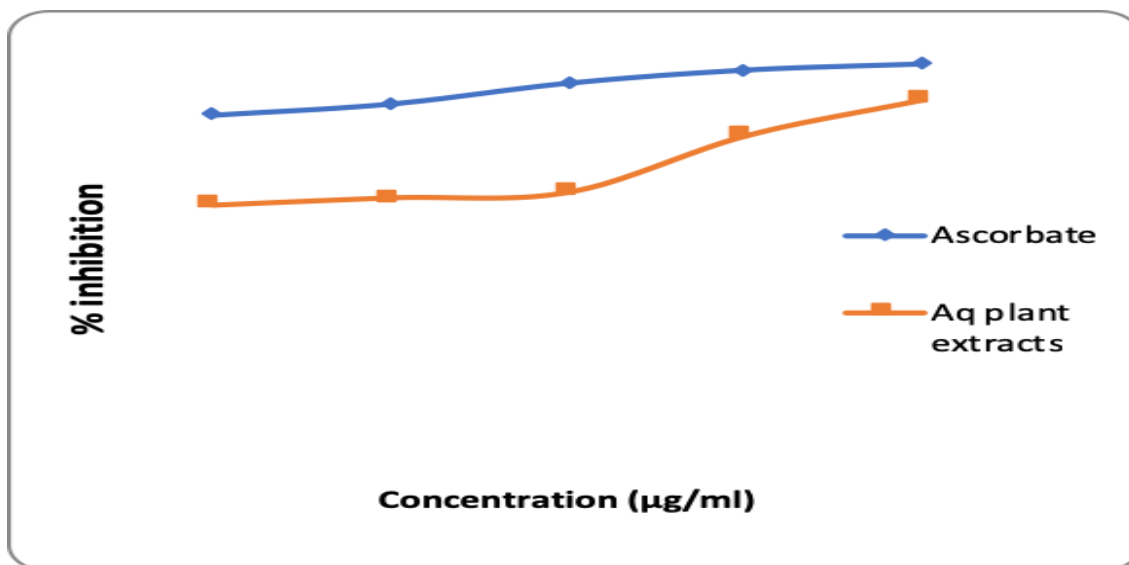


Figure 1. DPPH scavenging activities of crude aqueous extracts of *I. batatas* leaves and ascorbic acid.

Nutritional evaluation value for *I. batatas* aqueous extracts

The percentage composition on dry weight level as shown in the Figure 2,

below indicated that *I. batatas* contain moisture content, protein, ash content, crude fibre, fat and carbohydrate at a moderate amount.

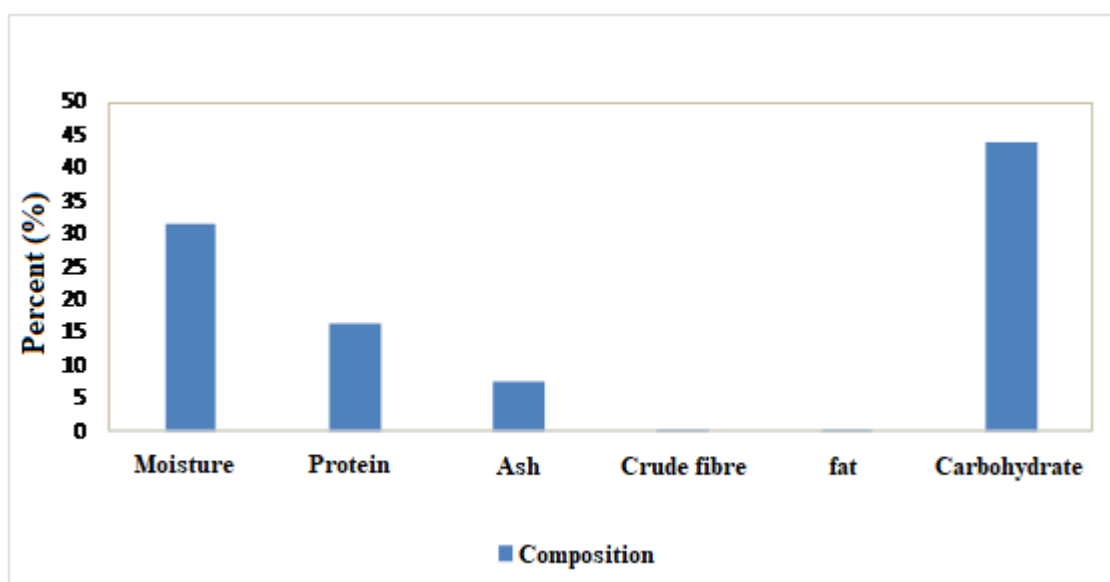


Figure 2. Proximate composition of *I. batatas* aqueous leaf extract.

Mineral element composition on aqueous extract of *I. batatas*

Figure 3 revealed that the leaf extract of *I. batatas* has a reasonable

percentage of magnesium, phosphorus, calcium and iron with a fair percentage of potassium, and sodium, and a very low percentage of zinc.

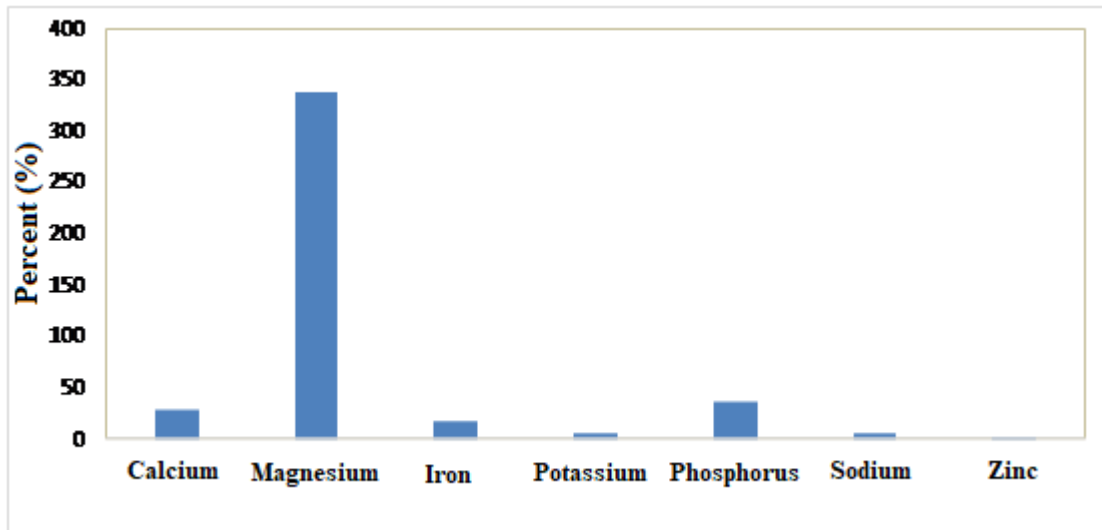


Figure 3. Elemental compositions of *I. batatas* leaf extract.

Hematology analysis

Table 1 shows significant decrease in Red blood cell count across 40 mg/kg Phenylhydrazine, 10 mg/kg ferrous (iii) - hydroxide and treated

groups in day 1 of the experiment with subsequent onset of action by an increased in day 7 and 14 of the treated group with aqueous leaves extract of *I. batatas*.

Table 1. Effect of aqueous leaves extract of *I. batatas* on red blood cell count (RBC) $\times 10^6/\mu\text{l}$ in phenyl hydrazine-induced anemia.

Treatment	Doses (mg/kg)	Day 1	Day 7	Day 14
Phenylhydrazine	40	3.77 \pm 0.39	3.68 \pm 0.33	4.33 \pm 0.19
Ferrous (iii) - Hydroxide	10	4.96 \pm 0.50 ^a	5.01 \pm 0.48 ^a	6.19 \pm 0.47
Control	DW	5.01 \pm 0.28 ^a	5.41 \pm 0.17 ^b	7.96 \pm 1.16 ^b
<i>Ipomoea batatas</i>	125	5.05 \pm 0.06 ^a	5.02 \pm 0.03 ^a	6.20 \pm 0.69
<i>Ipomoea batatas</i>	250	5.09 \pm 0.05 ^a	5.12 \pm 0.04 ^b	6.42 \pm 0.67
<i>Ipomoea batatas</i>	500	5.13 \pm 0.06 ^a	5.17 \pm 0.06 ^b	7.19 \pm 0.45 ^a

Values are mean \pm SEM with p-value < 0.05. DW = Distilled water.

Table 2 shows significant decrease in Packed Cell Volume across 40 mg/kg Phenylhydrazine, 10 mg/kg ferrous (iii) - hydroxide and treated

groups by day 1 of the experiment with subsequent increase in day 7 and 14 of the experiment after treatment with aqueous leaves extract of *I. batatas*.

Table 2. Effect of *I. batatas* aqueous leaves extract on Haematocrit (HCT)% in phenyl hydrazine-induced anemia.

Treatment	Doses (mg/kg)	Day 1	Day 7	Day 14
Phenylhydrazine	40	36.39±1.83	35.42±1.80	35.73±2.37
Ferrous (iii) - Hydroxide	10	39.04±0.58	41.01±0.61 ^a	45.35±2.69 ^a
Control	DW	41.15±0.46 ^b	42.36±0.33 ^b	53.83±1.16 ^c
<i>Ipomoea batatas</i>	125	42.29±0.37 ^b	42.71±0.89 ^b	46.13±0.08 ^b
<i>Ipomoea batatas</i>	250	41.75±0.38 ^b	42.60±0.94 ^b	45.58±2.55 ^a
<i>Ipomoea batatas</i>	500	40.74±0.37 ^a	41.74±1.17 ^b	44.10±1.10 ^a

Values are mean ± SEM with p-value < 0.05. DW = Distilled water.

Table 3 shows significant decrease in hemoglobin across across 40 mg/kg Phenylhydrazine, 10 mg/kg ferrous (iii) - hydroxide and treated

groups by day 1 of the experiment with subsequent increase in day 14 of the experiment after treatment with aqueous leaves extract of *I. batatas*.

Table 3. Effect of *I. batatas* aqueous leaves extract on Hemoglobin (Hg) g/dL in phenyl hydrazine-induced anemia.

Treatment	Doses (mg/kg)	Day 1	Day 7	Day 14
Phenylhydrazine	40	11.49±0.35	12.19±0.25	12.18±0.60
Ferrous (iii) - Hydroxide	10	12.15±0.14	13.23±0.35 ^a	14.35±1.36
Control	DW	13.30±0.23 ^c	13.83±0.13 ^b	14.76±1.15
<i>Ipomoea batatas</i>	125	12.72±0.20 ^b	13.43±0.27 ^a	14.40±0.92
<i>Ipomoea batatas</i>	250	12.87±0.15 ^b	13.21±0.23 ^a	14.35±0.20
<i>Ipomoea batatas</i>	500	12.14±0.07	12.77±0.14	13.95±0.55

Values are mean ± SEM with p-value < 0.05. DW = Distilled water.

Table 4 shows significant decrease in Mean Corpuscular Volume across 40 mg/kg Phenylhydrazine, 10 mg/kg ferrous (iii) - hydroxide and

treated groups in day 1 of the experiment with subsequent increase in 14 of the experiment after treatment with aqueous leaves extract of *I. batatas*.

Table 4. Effect of *I. batatas* aqueous leaves extract on Mean Corpuscular Volume (MCV) fl in phenyl hydrazine-induced anemia.

Treatment	Doses (mg/kg)	Day 1	Day 7	Day 14
Phenylhydrazine	40	56.76±6.21	57.96±5.90	67.16±2.40
Ferrous (iii) - Hydroxide	10	67.18±3.19	70.22±0.41 ^a	74.10±1.39
Control	DW	69.92±1.34 ^a	71.20±0.71 ^b	84.75±2.31 ^c
<i>Ipomoea batatas</i>	125	70.36±0.38 ^a	71.17±0.35 ^b	75.13±2.96
<i>Ipomoea batatas</i>	250	69.48±0.62 ^a	70.40±0.45 ^a	82.40±0.92 ^c
<i>Ipomoea batatas</i>	500	71.00±0.26 ^a	71.14±0.27 ^a	76.10±0.64 ^a

Values are mean ± SEM with p-value > 0.05. DW = Distilled water.

Table 5 shows significant decrease in Mean Corpuscular Haemoglobin Concentration across 40 mg/kg Phenylhydrazine, 10 mg/kg ferrous (iii) - hydroxide and treated groups in day 1 of

the experiment with subsequent increase in day 14 of the experiment after treatment with aqueous leaves extract of *I. batatas*.

Table 5. Effect of *I. batatas* aqueous leaves extract on Mean Corpuscular Hemoglobin (MCH) pg in phenyl hydrazine- induced anemia.

Treatment	Doses (mg/kg)	Day 1	Day 7	Day 14
Phenylhydrazine	40	16.58±0.70	19.14±0.63	19.20±0.45
Ferrous (iii) - Hydroxide	10	18.81±2.91	21.94±0.33 ^b	23.35±1.53
Control	DW	22.33±0.45 ^a	22.85±0.52 ^c	25.83±2.89 ^a
<i>Ipomoea batatas</i>	125	21.76±0.42	22.39±0.40 ^b	23.55±0.26
<i>Ipomoea batatas</i>	250	22.04±0.58 ^a	22.43±0.46 ^b	24.15±1.24 ^a
<i>Ipomoea batatas</i>	500	21.30±0.45	21.56±0.41 ^a	22.45±0.90

Values are mean ± SEM with p-value >0.05. DW = Distilled water.

Table 6 shows significant decrease in Mean Corpuscular Haemoglobin Concentration across 40 mg/kg Phenylhydrazine, 10 mg/kg ferrous (iii) - hydroxide and treated

groups in day 1 of the experiment with subsequent increase in day 7 and 14 of the experiment after treatment with aqueous leaves extract of *I. batatas*.

Table 6. Effect of *I. batatas* aqueous leaves extract on Mean Corpuscular Hemoglobin (MCHC) pg in phenyl hydrazine- induced anemia.

Treatment	Doses (mg/kg)	Day 1	Day 7	Day 14
Phenylhydrazine	40	27.35±1.50	31.92±0.89	32.25±0.56
Ferrous (iii) - Hydroxide	10	31.47±0.48 ^b	34.47±0.71 ^a	34.81±0.99 ^a
Control	DW	32.84±0.65 ^b	36.74±0.60 ^c	37.09±0.31 ^c
<i>Ipomoea batatas</i>	125	31.99±0.56 ^b	36.09±0.50 ^b	36.43±0.81 ^b
<i>Ipomoea batatas</i>	250	32.34±0.34 ^b	37.44±0.29 ^c	37.78±0.20 ^c
<i>Ipomoea batatas</i>	500	32.05±0.21 ^b	37.45±0.33 ^c	37.48±0.29 ^c

Values are mean ± SEM with p-value < 0.05. DW = Distilled water.

Peripheral blood smear

From Figure 4, administration of graded dose of aqueous extracts induced normochromic and normocytic cells on damage red blood cells on day 14 showing effectiveness of the extract on red blood cells when compared across

40 mg/kg phenylhydrazine hydrochloride and 5 mg/kg of ferrous (iii) - hydroxide induced hypochromic macrocytes and normochromic and normocytic cells to 5 mg/kg of ferrous (iii) - hydroxide group.

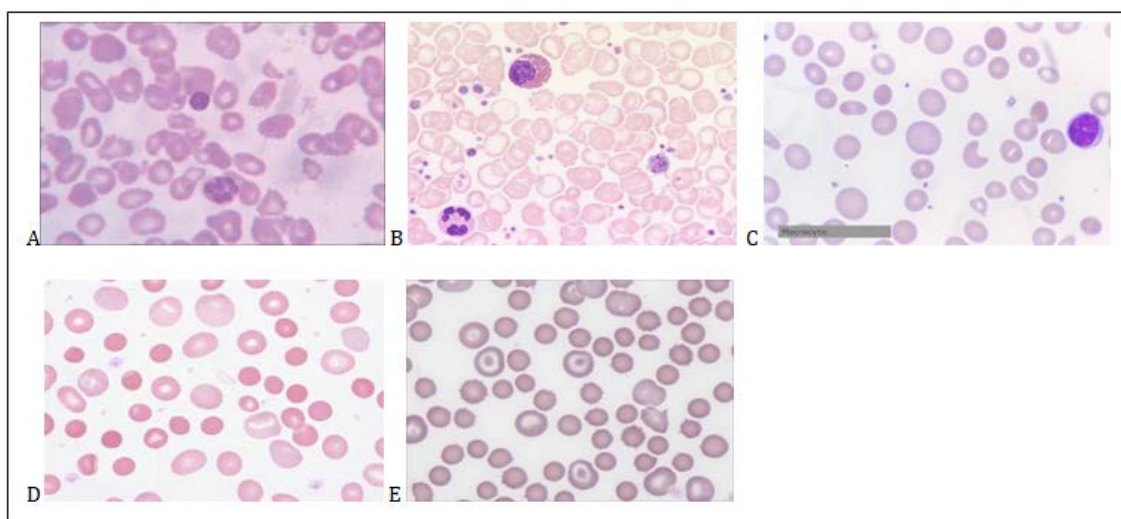


Figure 4. (A) Phenylhydrazine alone A, showed macrocytes. B, hypochromic macrocytes and C Platelet, (B) 5 mg/kg of ferrous (iii) - hydroxide A, showed normochromic and normocytic cell. B, multi-segmented neutrophil, (C) 125 mg/kg aqueous A, showed Normochromic macrocytic. B, helmet fragmented red cells C, normocytic red cell, (D) 250 mg/kg aqueous A, showed hypochromic macrocytic cells and partly normocytic cells and (E) 500 mg/kg aqueous plant extract A, showed normochromic macrocyte. B, burr cell and largely normocytic cells.

Bone marrow

From Figure 5, administration of graded doses of aqueous extract of *I. batatas* induced myelo-erythroid cells with mildly increased of about 60% for

125 mg/kg, 65% for 250 mg/kg and 70% for 500 mg/kg, when compared with Distilled water induced myelo-erythroid cells up to 75% which is normal, and less than 40% for 40 mg/kg phenyl hydrazine

HCl group showing severe anemic condition in rats and 5 mg/kg of ferrous (iii) - hydroxide induced myelo-

erythroid cells up to 70% at day 14 in anaemic treated rats.

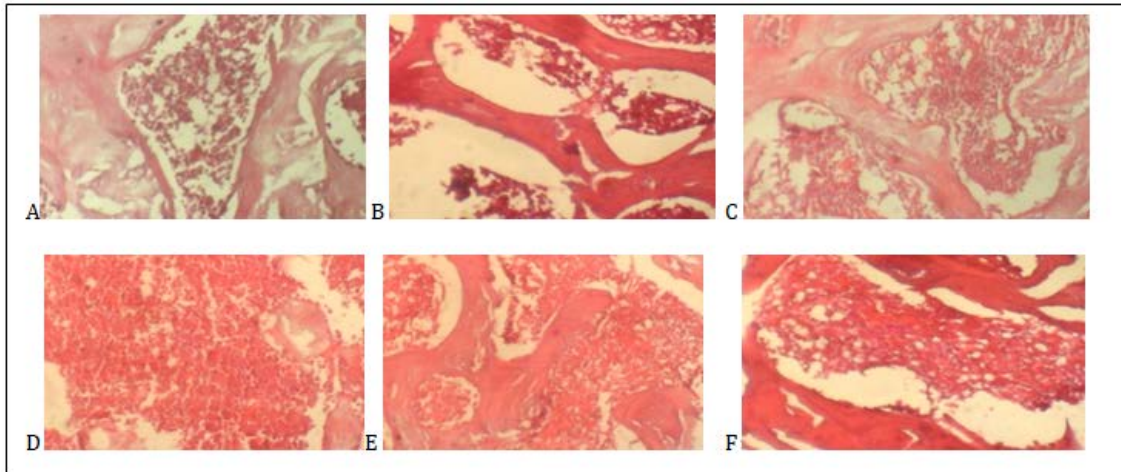


Figure 5. (A) Control Rat bone marrow composed of megakaryocyte, Bone and Myelo-Erythroid cells, fat vacuoles (B) Phenylhydrazine alone showed moderately decreased marrow, fat ratio, bone trabecula (C) 5 mg/kg of ferrous (iii) - hydroxide showed normal marrow, fat ratio (D) 125mg/kg aqueous extract showed moderately increased marrow, fat ratio (E) 250mg/kg aqueous extract showed moderately increased marrow, fat ratio and (F) 500mg/kg aqueous extract showed moderately increased marrow, fat ratio at day 14 (H&E x 100).

Acute toxicological study

Results from the observed acute toxicity studies revealed that aqueous extract was safe up to the highest dose of 500mg/kg. No harmful signs and symptoms or behavioural adverse effect was observed also, no mortality was recorded during the period of the study.

Histopathological profile

From Figure 6, administration of graded doses of aqueous extract of *I. batatas* induced moderate vascular dilation of the heart compared to graded doses of Control (distilled water), 40 mg/kg phenyl hydrazine hydrochloride and 5 mg/kg of ferrous (iii) - hydroxide poly-maltose induced dilated coronary vessel and mild vascular congestion of the heart.

Administration of graded doses of aqueous extract of *I. batatas* induced normal follicular architecture on spleen when contrasted with graded doses of Control (distilled water), 40 mg/kg phenyl hydrazine hydrochloride and 5mg/kg of ferrous (iii) - hydroxide poly-maltose induced severe sinus dilation and mild follicular atrophy on spleen.

From Figure 7, administration of graded doses of aqueous extract of *I. batatas* induced normal follicular architecture on spleen when contrasted with graded doses of Control (distilled water), 40 mg/kg phenyl hydrazine hydrochloride and 5 mg/kg of ferrous (iii) - hydroxide poly-maltose induced severe sinus dilation and mild follicular atrophy on spleen.

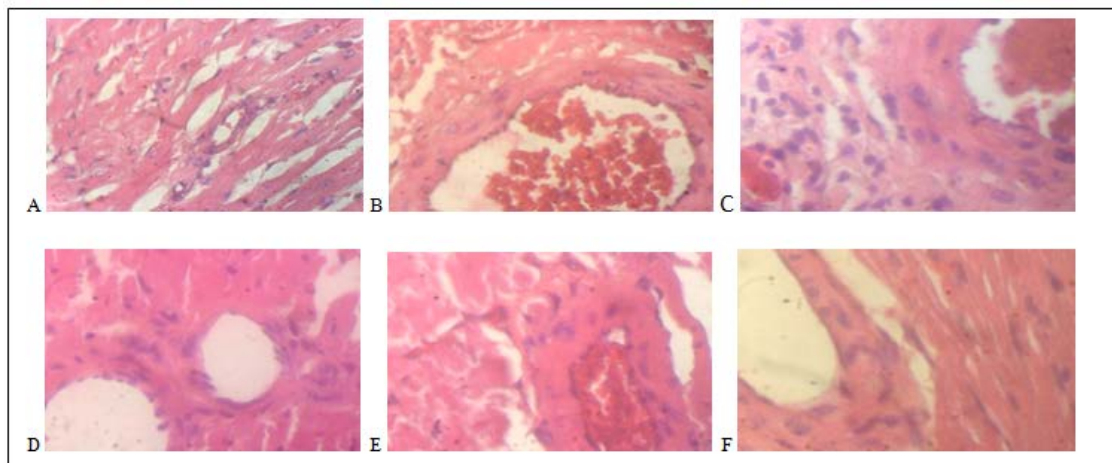


Figure 6. (A) Control Rat heart composed of; A, bundles of myocardial fibres, B, coronary vessel and C, interstitial space, (B) Phenylhydrazine alone showed; A, dilated coronary vessel with B, mild vascular congestion, (C) 5 mg/kg of ferrous (iii) - hydroxide showing; A, dilated coronary vessel B, mild vascular congestion and C, heavy infiltrates of inflammatory cells, (D) 125mg/kg aqueous extract showed A, moderate vascular dilation, (E) 250mg/kg aqueous extract showed A, moderate vascular congestion, and (F) 500mg/kg aqueous extract showed A, moderate vascular dilatation (H&E) at day 14.

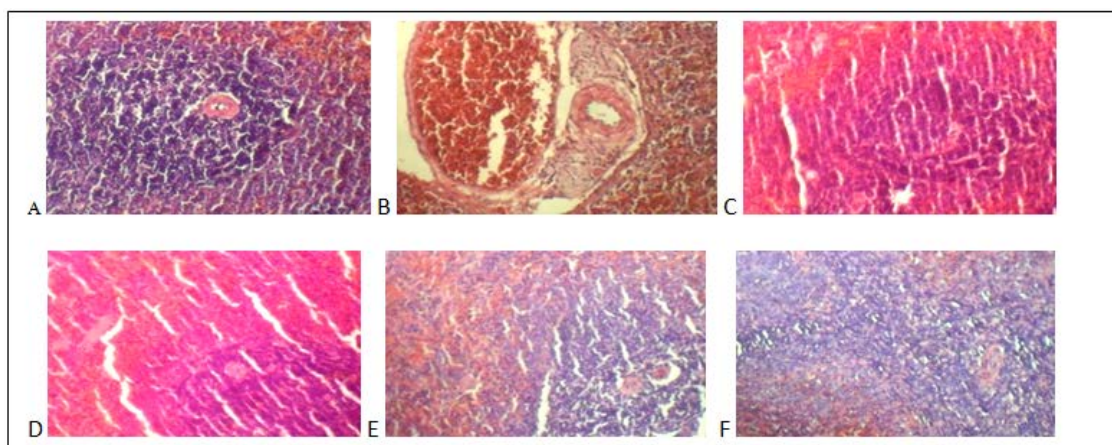


Figure 7. (A) Control Rat spleen composed of A, white pulp, B, red pulp and C, arteriole, (B) Phenylhydrazine alone showed A, severe sinus dilation and B, moderate follicular atrophy, (C) 5mg/kg of ferrous (iii) - hydroxide poly-maltose showed A, mild follicular atrophy, (D) 125mg/kg aqueous extract showed A, mild follicular atrophy, (E) 250mg/kg aqueous extract showed A, normal follicular architecture and (F) 500mg/kg aqueous extract showed A, normal follicular architecture (H&E x 100) at day 14.

Administration of graded doses of aqueous extract of *I. batatas* induced mild vascular congestion and moderate Kupffer cell activation to the liver when compared with Control (distilled water), 40 mg/kg phenyl hydrazine hydrochloride and 5mg/kg of ferrous (iii) - hydroxide poly-maltose induced patchy

vascular intimal ulceration and moderate vascular congestion in liver cells.

From Figure 8, administration of graded doses of aqueous extract of *I. batatas* induced mild vascular congestion and moderate Kupffer cell activation to the liver when compared with Control (distilled water), 40mg/kg

phenyl hydrazine hydrochloride and 5 mg/kg of ferrous (iii) - hydroxide poly-maltose induced patchy vascular intimal ulceration and moderate vascular congestion in liver cells.

From Figure 9, administration of graded doses of aqueous extract of

I. batatas induced mild interstitial congestion in the kidney when compared with Control (distilled water), 40 mg/kg phenyl hydrazine hydrochloride and 5 mg/kg of ferrous (iii) - hydroxide poly-maltose induced interstitial space and focal vascular intimal to the kidney.

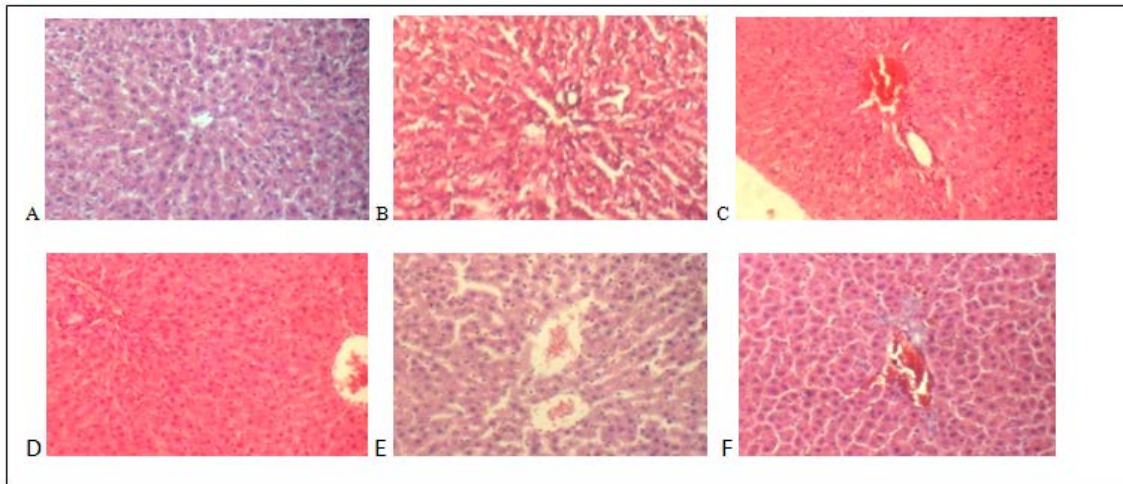


Figure 8. (A) Control Rat liver composed of central vein, hepatocytes and sinusoids (B) Rat liver treated with Phenylhydrazine alone showed patchy vascular intimal ulceration (C) Rat liver treated with 5 mg/kg of ferrous (iii) - hydroxide poly-maltose showed moderate vascular congestion (D) 125 mg/kg aqueous extract showed mild vascular congestion (E) 250 mg/kg aqueous extract showed mild vascular congestion and (F) 500 mg/kg aqueous extract showed mild vascular congestion and moderate Kupffer cell activation (H&E x 100) of hepatocytes.

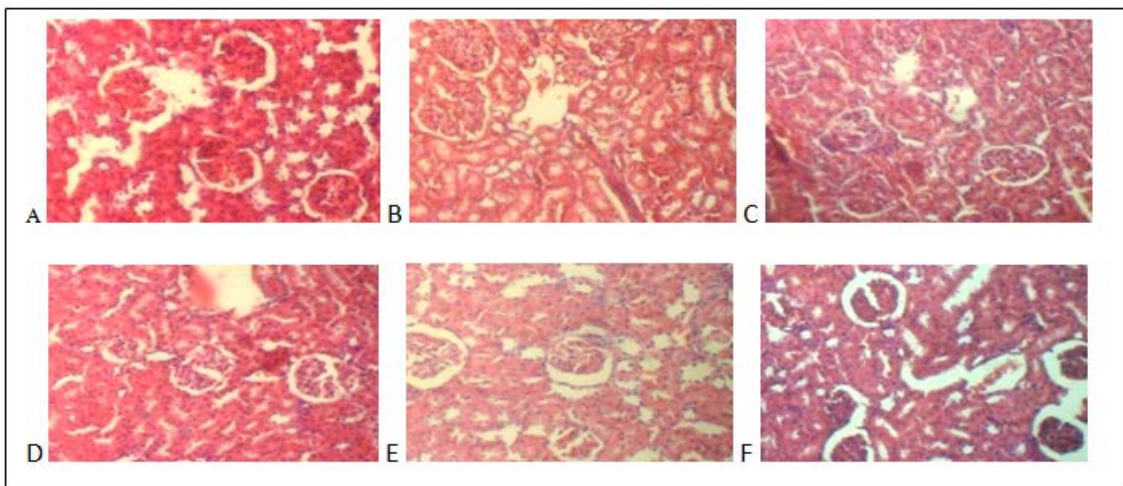


Figure 9. (A) Control Rat kidney composed of glomerulus, tubules and interstitial space (B) Phenylhydrazine alone showed focal vascular intimal erosion (C) 5mg/kg of ferrous (iii) - hydroxide poly-maltose showed mild interstitial congestion (D) 125mg/kg aqueous extract showed mild interstitial congestion (E) 250mg/kg aqueous extract showed mild interstitial congestion and (F) 500 mg/kg aqueous extract showed mild interstitial congestion (H&E x 100) in kidney.

Discussion

I. batatas showed its efficacy in radical-scavenging property useful in health-promoting activity. The plant extracts triggered radical scavenging activity against the produced oxygen derivatives in DPPH assay. Aqueous extract of *I. batatas* showed an increased in antioxidant activity when compared with the standard (ascorbic acids). The capability of the extract to inhibit oxidation from DPPH, signifies protons donor and scavenging effect for other stable radicals which is in line with the findings of Falodun and Irabor (2008), worked with methanolic extract of *Calliandria surinamensis*.

Proximate constituent of a particular plant is an absolute source of nutrients in that plant. The present of protein in the extract of *I. batatas* may be needed to stimulate necessary hormone required in facilitating bone marrow for blood cells production (AOAC, 2005).

Moisture and ash content are valuable in their stability, quality and purity of powered crude drug. The moisture content of *I. batatas* leaf was high when compared to *Microdesmis keyana* root as reported by Odesanmi et al. (2012).

Macronutrients display important activity in metabolism and enzymatic reaction when their functions are related to co-enzymes and co-factors. Magnesium, calcium, iron and phosphorus concentration in the leaf samples of *I. batatas* when compared with *Microdesmis keyana* root (Moswa et al., 2005). Magnesium, calcium, Iron and phosphorus is helpful for further development of bone marrow and to activate certain hormones (erythropoietin) for blood cells synthesis (Odesanmi et al., 2012; Moswa et al., 2005). High level of calcium and iron in the leaf of *I. batatas* was vital as it may aid protection of structural rigidity in the skeleton, thereby enhances red blood cells production (Moswa et al., 2005).

The present of zinc can be responsible for the stimulating effect of the plant in boosting the immune system (Sen et al., 2005).

Hemolytic anemia was induced with phenylhydrazine hydrochloride in *Wistar* rats and treated at 0, 7 and 14 days using 10 mg/kg ferrous (iii) - hydroxide and graded dose of *I. batatas* extract (125, 250 and 500 mg/kg). Aqueous extract of *I. batatas* leaf enhanced blood production in treated groups (Yamoto and Maede 2002; Yakubu et al., 2007). Results from the hematological index (Hgb, HCT, RBC, MCHC, MCV and MCH) in treated groups significantly increased hemoglobin, HCT and red blood cells (14.40 ± 0.92 , 46.13 ± 0.08 and 7.19 ± 0.45) when compared with untreated group (12.18 ± 0.60 , 35.73 ± 2.37 and 4.33 ± 0.19) (Tables 1, 2 and 3). The Mean Corpuscular Volume (MCV) and Mean corpuscular hemoglobin concentration (MCHC) are constants for typical anemia. MCV decreased significantly in untreated group following the administration of phenylhydrazine that showed microcytosis. The decreased counteracted 14 days treated and untreated group. In addition, recompense was quicker with ferrous (iii) - hydroxide and *I. batatas* extract implying that diverse mechanisms are implicated in erythropoiesis. Recovery effect of blood cells probably could be as a result of erythropoiesis secreted from the kidney to regenerating blood cells in bone marrow. Hemoglobin (oxygen carrying capacity of the blood), MCV, MCH AND MCHC exhibited a significant increase in treated group, this finding agrees with the work of Cole (2006) and Adebayo et al. (2005) on ethanolic extract of *Bougainvillea spectabilis* (Yakubu et al., 2007; Yakubu and Afolayan, 2009) on aqueous extracts of *Fadogia arggregristis* stem and *Bulbine natalensis* stem. Extracts from *I. batatas* regenerates blood production with normal morphology when compared with

untreated and reference drug groups in peripheral blood smear (Figure 4). Aqueous extract of *I. batatas* triggers myelo-erythroid cells at 60%, 65% and 70% across treated groups when compared with reference drug (75%) having normal regenerated blood cells, and 40% in untreated group which is an indication of severe anaemia. These findings consent with earlier study of Sen et al. (2005) and Claro et al. (2006) on significant changes and increase in red blood cells percentage and morphology in myelo-erythroid cells (Figure 5).

No adverse toxicological effect of aqueous extract of *I. batatas* was observed with absent mortality (no mortality). It can be suggested that lethal dose (LD₅₀) of the extract is above 500 mg/kg since no death was recorded. This finding is in line with earlier study of Gasting et al. (2010) with no mortality from aqueous leaf extract of *Alchornea cordifolia* at 3,200 mg/kg, methanolic and aqueous leaf extracts of *Emilia coccinea* at 8,000 mg/kg were administered (Idu et al., 2010).

Histological study of selected organs on day 14 following the administration of graded doses of the plant extracts showed vasoactive effects of the vessels (blood flow increase) when compared with untreated group (vascular hypertrophy in the kidney, heart, spleen and liver) which agrees with the findings of (Smith et al., 2002). The treated groups induced dilatation of coronary vessel with a mild vascular congestion as shown in Figure 6, compared with untreated group. The spleen showed normal follicular architecture in Figure 7 across graded doses. Figure 8 and Figure 9 showed a mild follicular activation and lymphocytosis of local immune system of normal liver and renal architecture. This finding is in line with previous study of (Smith et al., 2002) exposed to *Cissus populnea*.

Conclusion

It is relevant to consider that this research study validate the folklore use and showed essential preclinical information progressing to the development of anti-anemic herbal remedy in the country. Thereby the possibility of the isolation and feature of the bioactive component (s) accountable for the diverse pharmacological activities of the leaf extract of *I. batatas* should be determined in a bioactive guide to assay.

Conflicts of interest

Authors declare that they have no conflict of interests.

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