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Proximate Analysis and the Curative Properties of *Ipomoea batatas* Lam in Phenylhydrazine-induced Anaemic Rats

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Abstract
This work was done to validate the claim made by some herbalist that this plant is hematopoietic. Phytochemical, proximate and metal content analyses were carried out on the powdered leaves; forty-eight albino rats were divided into six groups of eight rats each. Group 1 (normal control) was administered distilled water only by oral gavage; groups 2 to 6 were induced with 80 mg/kg body weight, bwt, of phenylhydrazine, while group 2 was neither treated with the extract nor standard drug; groups 3 to 5 were administered 250, 500 and 750 mg/kg bwt of extract. Group 6 was administered 12.5 mg/kg bwt of Chemiron. Proximate analysis revealed high moisture and crude protein content, while phytochemical screening revealed the presence of phytate, flavonoids, saponins, oxalate, tannins, total phenols, alkaloids and cardiac glycosides. Metal analysis revealed the presence of high levels of magnesium, iron and potassium. The ethanol extract of I. batatas compared favourably with chemiron in producing ameliorative effects on haematocrit, haemoglobin and red blood cell count at dose level of 750 mg/kg bwt. The increase in white blood cells and its differentials observed in the negative control rats were normalized on administration of the extract and Chemiron.

Keywords: Chemiron, *Ipomoea batatas*, Phenylhydrazine, Red blood cells, White blood cells

Introduction
Anaemia is a disease characterized by reduction in the concentration of haemoglobin, circulating red blood cells, and its indices, and haematocrit below normal level. It may be due to reduced production of red blood cells or haemorrhage [1]. Phenylhydrazine induces anaemia by causing lipid peroxidation of red blood cell membrane. Phenylhydrazine toxicity has been linked to drug-induced oxidative stress occurring in the erythrocytes [2]. *I. batatas* (L) (sweet potato) is a tuberous-rooted perennial. Some of the roots produce elongated starchy tubers which have nutritive and pharmaceutical value, and its leafy tops are eaten as vegetable. *I.batatas* has been used traditionally in the treatment of several ailments such as diabetes, anaemia and other diseases [3 - 5]. Some works on the medicinal effect of *I. batatas* have been documented such as the antisickling activity. Studies have shown that anthocyanin extract of *I. batatas* reversed majority of sickle-shaped erythrocytes in SS blood into normal biconcave form [6]. In addition, some authors have reported that the leaves of *I. batatas* modulate various immune functions [7]. Safety assessment has been carried out on fresh leaves of this plant and no hepatic or renal damage was observed [8]. *I. batatas* leaves are efficient sources of minerals, protein and vitamins [9]. So far, little or no work has been done to determine the therapeutic effect of the leaves of *I. batatas* on anaemea; the present study was carried out to bridge this knowledge gap.

Materials and Methods
Materials
Plant collection and preparation
Ipomoea batatas leaves were obtained from a cultivated place in the Department of Biochemistry, University of Benin, Benin City, Edo State, Nigeria. The leaves were air- dried, pulverized and sieved.
Animals
Forty-eight male albino rats (180 - 200 g) used for this study were purchased from the Animal House of Ambrose Alli University, Ekpoma, Edo State, Nigeria. They were divided into six groups of eight rats each and kept in separate cages. The rats were acclimatized for two weeks before the commencement of the study, and were fed with commercially formulated rat feed and water *ad libitum*. The principles of laboratory animal care were followed [19].
Methods
Experimental design
Forty-eight albino rats were divided into six groups of eight rats each, and administration of extract was done intragastrically.

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Group 1(normal control): this group of rats was given distilled water only
Group 2 (negative control): rats in this group were induced with 80 mg phenyl hydrazine per kg bwt; they were neither treated with the crude leaf extract nor the standard drug.
Group 3 (T250): rats in this group were induced with 80 mg phenyl hydrazine per kg bwt for three days, after which they were treated with 250 mg of *I. batatas* leaf extract per kg bwt for another 3 days.
Group 4 (T500): rats in this group were induced with 80 mg phenyl hydrazine per kg bwt for 3 days after which they were treated with 500 mg/kg bwt of *I.batatas* leaf extract for another 3 days.
Group 5 (T750) : rats in this group were induced with 80 mg phenyl hydrazine per kg bwt for 3 days after which they were treated with 750 mg/kg bwt of *I. batatas* leaf extract for another 3 days.
Group 6: rats in this group were induced with 80 mg phenyl hydrazine per kg bwt for 3 days after which they were treated with Chemiron (12.5 mg/kg bwt) for another 3 days.
The rats were allowed free accesses to food and water.

Extraction and concentration

The powdered leaves (100 g) were extracted in 1000 ml absolute ethanol. Extraction was by maceration over a 72 h period; the extract was filtered with a fine muslin cloth and concentrated using a rotary evaporator. The concentrated extract was then freeze – dried by lyophilization.

Phytochemical analysis

Phytochemical screening was carried out on the powdered sample to detect the presence of secondary metabolites. The alkaloid content was estimated by the procedure described by [15]; flavonoid by the procedure described by [11]; phytate by the procedure described by [14]; oxalate by the procedure described by [15]; saponins by the procedure described by [13]; steroids and cardiac glycosides by the procedure described by [10]; tannins by the procedure described by [12], and total phenol by the procedure described by [16]

Proximate and metal content analyses

The chemical analyses of the proximate composition of powdered *I.batatas* leaves were determined according to the methods of Association of Official Analytical Chemist [14]. The parameters determined were moisture content, ash content, crude protein content, crude lipid content, crude fibre and carbohydrate content. Each parameter was determined for three replicates. Calcium and magnesium were determined using complexometric method [14]. The Corning 421 Flame Emission Photometer was used for the estimation of sodium and potassium content [17]. Zinc, iron, cadmium, manganese, lead and copper were determined using the Perkin- Elmer model 403 Atomic Absorption Spectrophotometer [18]

Blood sample collection

At the end of the treatment period, blood samples were collected by direct cardiac puncture into sterile containers with or without anticoagulants.

Biochemical analysis

Haematological indices were estimated using Sysmex-kx-21N automated haematological analyser.

Statistical analysis

Data are expressed as Mean ± SEM. The statistical analysis was performed using SPSS (16.0) and the groups compared using Dunnett's Multiple Range test. Values of $p < 0.05$ were considered statistically significant.

Results

Phytochemical analysis

The results of phytochemical analyses showed that *I. batatas* leaves contain phytate, flavonoids, saponin, oxalate, tannins, total phenols, alkaloids and cardiac glycosides. It has a high content of cardiac glycosides, total phenols and oxalate and a low content of flavonoids and saponins (Table 1).

Table1: Phytochemical analysis of *I. batatas* leaves

Phytochemicals	Qualitative	Quantitative (g %)
Phytate	Present	4.19 ± 0.55
Flavonoids	Present	0.34 ± 0.02
Saponins	Present	0.50 ± 0.02
Oxalate	Present	14.47 ± 3.21
Tannins	Present	6.67 ± 1.50
Total phenols	Present	9.74 ± 2.15
Alkaloids	Present	1.78 ± 0.04
Steroids	Absent	-
Cardiac glycosides	Present	36.25 ± 4.41

Data are expressed as Mean ± SEM.

Proximate analysis

The proximate analysis of powdered sample of *I. batatas* leaves showed high moisture content. The leaves are relatively higher in crude protein than crude lipid or ash content (Table 2).

Table 2: Proximate composition of *I. batatas* leaves

Parameters	Composition (%)
Moisture content	73.03 ± 5.22
Ash content	6.10 ± 1.13
Crude protein	10.04 ± 1.15
Crude lipid	8.09 ± 1.20
Crude fibre	4.11 ± 0.94
Carbohydrate	71.66 ± 5.48

Data are expressed as Mean ± SEM.

Metal content analysis

The metal analysis of powdered sample of *I. batatas* showed the presence of sodium, potassium, calcium, magnesium, iron, zinc, manganese and copper. Its highest constituent is magnesium followed by iron and then calcium (Table 3).

Table 3: Metal content analysis of *I. batatas* leaves

Metals	Composition (mg/kg)
Sodium (Na^{+})	5.52 ± 0.70
Potassium (K^{+})	20.90 ± 0.20
Calcium (Ca^{2+})	22.70 ± 0.07
Magnesium (Mg^{2+})	63.90 ± 5.20
Iron (Fe^{2+})	29.40 ± 3.22
Zinc (Zn^{2+})	15.90 ± 0.06
Manganese (Mn^{2+})	3.90 ± 0.01
Copper (Cu^{2+})	2.24 ± 0.01
Lead (Pb^{2+})	Not detected
Cadmium (Cd)	Not detected

Results are expressed as Mean ± SEM.

Red blood cell and its differientials

The negative control rats had significantly lower red blood cell count, haematocrit (HCT), haemoglobin (HGB) and higher mean corpuscular haemoglobin concentration (MCHC) compared with the normal control. Red blood cells (RBC) of test rats were significantly lower than the normal control. However, at dose levels 250 mg/kg b.wt and 500 mg/kg b.wt, the red blood cell count were not significantly altered compared with the negative control. Rats administered 750 mg/kg b.wt and Chemiron had significantly higher red blood cell count compared with the negative control. Rats administered graded doses of the crude drug extract and Chemiron-treated rats did not differ significantly in haematocrit when compared with the normal control but had significantly higher haematocrit than the negative control rats. Haemoglobin concentration of rats administered the graded doses of *I. batatas* leaf extract were significantly lower compared with the normal control but were not significantly altered compared with the negative control, the Chemiron treated rats did not differ significantly in haemoglobin when compared with both the normal and the negative control (Table 4a).

Table 4a: The effect of *I. batatas* leaf extract and the standard drug, Chemiron on red blood cell and its differentials in phenyl hydrazine- induced anaemic rats.

Groups	RBC (x 10^6/ µl)	HCT (%)	HGB (g/dl)
Normal control	8.23 ± 0.37^{a}	50.07 ± 5.04^{a}	16.40 ± 0.15^{a}
Negative control	3.52 ± 0.16^{b}	20.35 ± 0.55^{b}	10.35 ± 0.05^{b}
T250	3.48 ± 0.04^{b}	40.55 ± 0.05^{a}	9.75 ± 0.15^{b}
T500	3.88 ± 0.16^{b}	46.30 ± 3.60^{a}	11.50 ± 0.60^{b}
T750	5.50 ± 1.67^{c}	46.60 ± 1.98^{a}	11.95 ± 0.42^{b}
TChem	6.50 ± 1.26^{c}	49.30 ± 5.80^{a}	12.28 ± 2.35^{ab}

Results are expressed as Mean ± SEM (n = 8). Values with different superscipts are significant ($p < 0.05$). T250 = treatment with 250 mg/kg b.wt of extract; T500 = treatment with 500 mg/kg b.wt of extract; T750 = treatment with 750 mg/kg b.wt of extract; TChem =treatment with Chemiron.

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of rats

The mean corpuscular volume (MCV) of the treated rats (those administered graded doses of *I. batatas* and Chemiron) were significantly higher when compared with the MCV of both the normal and the negative control. Mean corpuscular haemoglobin (MCH) of control and test rats was not significantly altered. Mean corpuscular haemoglobin concentration (MCHC) of I.*batatas* treated rats were significantly lower compared with the negative control but insignificantly different when compared with the normal control (Table 4b).

Table 4b: Levels of MCV, MCHC, MCH and RDW in the rats

Groups	MCV (fi)	MCHC (g/dl)	MCH (pg)	RDW (%)
Normal control	59.94 ± 3.01^{a}	32.75 ± 0.38^{a}	19.93 ± 0.79^{a}	18.73 ± 1.36^{a}
Negative control	51.91 ± 0.65^{a}	50.86 ± 1.15^{b}	26.40 ± 0.85^{a}	17.35 ± 1.05^{a}
T250	116.51 ± 1.50^{b}	24.04 ± 0.40^{a}	28.01 ± 0.10^{a}	18.85 ± 0.35^{a}
T500	119.32 ± 4.55^{b}	24.84 ± 0.65^{a}	29.64 ± 0.35^{a}	19.00 ± 0.20^{a}
T750	93.19 ± 6.36^{c}	23.50 ± 0.23^{a}	21.90 ± 1.70^{a}	21.83 ± 2.37^{a}
TChem	75.83 ± 3.15^{c}	24.91 ± 2.30^{a}	18.89 ± 1.28^{a}	25.05 ± 1.95^{a}

Results are expressed as mean ± SEM (n = 8). Values with different superscripts are significant ($p < 0.05$). T250 = treatment with 250 mg/kg b.wt of extract; T500 = treatment with 500 mg/kg b.wt of extract; T750 = treatment with 750 mg/kg b.wt of extract; TChem = treatment with Chemiron.

White blood cell and its differentials

The negative control rats had significantly higher white blood cell count, monocytes, lymphocytes and granulocytes when compared with the normal control. These parameters were not significantly affected in the test rats when compared with the normal control but were significantly reduced when compared with the negative control (Table 5).

Table 5: The effect of *I. batatas* leaf extract and Chemiron on white blood cell and its differentials in phenyl hydrazine- induced anaemic rats (x 10^{3}/ µl)

Groups	WBC	Monocyte	Lymphocytes	Granulocyte
Normal control	8.65 ± 1.64^{a}	0.63 ± 0.13^{a}	6.23 ± 1.33^{a}	1.80 ± 0.35^{a}
Negative control	52.65 ± 7.15^{b}	4.10 ± 1.10^{b}	42.55 ± 1.35^{b}	6.05 ± 1.75^{b}
T250	11.40 ± 0.80^{a}	1.55 ± 0.25^{a}	8.15 ± 0.05^{a}	1.70 ± 0.50^{a}
T500	10.20 ± 1.41^{a}	0.90 ± 0.05^{a}	8.20 ± 0.08^{a}	1.10 ± 0.10^{a}
T750	11.60 ± 0.32^{a}	1.05 ± 0.03^{a}	8.28 ± 0.49^{a}	2.20 ± 0.23^{a}
TChem	10.50 ± 0.30^{a}	0.90 ± 0.01^{a}	7.50 ± 0.05^{a}	2.20 ± 0.10^{a}

Results are expressed as mean ± SEM (n=8). Values with different superscripts are significant ($p < 0.05$). T250 = treatment with 250 mg/kg b.wt of extract; T500 = treatment with 500 mg/kg b.wt of extract; T750 = treatment with 750 mg/kg b.wt of extract; TChem = treatment with Chemiron.

Platelets and its differentials

Platelet count was not significantly affected in both the control and test rats. Plateletcrits (PCT) were significantly reduced in the negative control rats and in the group of rats administered 250 mg/kg; they were not significantly altered in the other test rats. Mean platelet volume (MPV) was significantly increased only in the Chemiron -treated rats. Platelet distribution width was not significantly affected in both the control and test rats (Table 6).

Table 6: The effect of *I. batatas* leaf extract and Chemiron on platelet and its differentials in phenyl hydrazine- induced anaemic rats.

Groups	Platelets	PCT (%)	MPV (fl)	PDW (%)
Normal control	6.02 ± 0.41^{a}	0.37 ± 0.01^{a}	6.13 ± 0.13^{a}	40.63 ± 3.42^{a}
Negative control	4.42 ± 0.35^{a}	0.15 ± 0.01^{b}	6.35 ± 0.45^{a}	43.90 ± 2.22^{a}
T250	4.34 ± 0.22^{a}	0.25 ± 0.02^{b}	5.75 ± 0.05^{a}	31.60 ± 5.21^{a}
T500	4.67 ± 0.95^{a}	0.30 ± 0.05^{a}	6.60 ± 0.50^{a}	35.30 ± 1.51^{a}
T750	4.39 ± 0.68^{a}	0.28 ± 0.04^{a}	6.57 ± 0.29^{a}	33.23 ± 2.44^{a}
TChem	3.90 ± 0.01^{a}	0.33 ± 0.02^{a}	8.15 ± 0.15^{b}	43.95 ± 3.01^{a}

Results are expressed as mean ± SEM (n = 8). Values with different letters are significant ($p < 0.05$). T250 = treatment with 250 mg/kg b.wt of extract; T500 = treatment with 500 mg/kg b.wt of extract; T750 = treatment with 750 mg/kg b.wt of extract; TChem = treatment with Chemiron.

Discussion

Phytochemical screening of *I. batatas* leaves showed the presence of phytate, flavonoids, saponins, oxalate, tannins, total phenols, alkaloids and cardiac glycosides. Steroids were not present. This result is in agreement with the report of some authors [20, 21]. The plant leaves had high levels of cardiac glycosides and oxalates and low levels of flavonoids and saponins. The low content of saponins may be an advantage because saponins are often haemolytic in function. High content of cardiac glycosides and potassium is suggestive of a cardioprotective nature.

Results of proximate analysis revealed high moisture content, and this is characteristics of green leafy vegetables [22]. The results also revealed relatively high ash, crude protein and crude lipid content. This is in agreement with the report of previous studies [23]. The ash value indicates the quantity of inorganic component, the high ash content of the leaves of this plant shows that the leaves are rich in minerals. Elemental analysis revealed the presence of high levels of magnesium, iron, calcium and potassium in the leaves of this plant. Iron is one of the principal agents used in the treatment of anaemia. There were no traces of cadmium and lead in the leaves of this plant; the presence of metals such as cadmium, mercury, zinc and lead in food and drugs is regulated by law. These metals are known as heavy metals and are only allowed in trace amounts in foods and drugs [25].

Results of the effect of ethanol extracts of *I. batatas* and Chemiron on red blood cell and its differentials in phenyl hydrazine-induced rats showed significantly reduced levels of red blood cells, haematocrit and haemoglobin in those rats that were induced but not treated (negative control) compared with the normal control. This suggests that anaemia was successfully induced by phenyl hydrazine, and is in agreement with the reports of previous studies [25, 26]. Phenyl hydrazine has been reported to produce increased MCV, MCH and MCHC levels [27]. The results of this study also show increased MCHC although MCV and MCH were not significantly altered compared with the normal control. The ethanol extracts of *I. batatas* compared favourably with the standard drug, Chemiron in ameliorating the effect of phenyl hydrazine on the blood. They produced increases in red blood cells and haematocrit, although the increase observed in haemoglobin levels were not significant. The ethanol extract of *I. batatas* produced ameliorative effects on haematocrit at all dose levels, but only produced ameliorative effect over the red blood cells at dose level 750 mg/kg bwt. Increases in haematocrit without concomitant increase in red blood cells and haemoglobin observed at dose levels 250 and 500 mg/kg bwt may indicate an abnormal swelling of the red blood cell. Mean corpuscular volume (MCV) categorizes red blood cells by size. In this study, MCV was not significantly altered in the test rats. The curative effects of *I. batatas* over phenyl hydrazine-induced anaemia were observed at dose of 750 mg/kg bwt. This effect may be attributable to the presence of antioxidants such as flavonoids, saponins, tannins and other phenolic compounds. These antioxidants scavenge free radicals and hence may restore the integrity of red blood cell membrane [20].

Induction with phenyl hydrazine produced significant increases in white blood cells, lymphocytes, monocytes and granulocytes as evident in the negative control rats. The increases observed in these parameters in the negative control rats could be an immune response in the presence of a toxic chemical. Treatment with *I. batatas* and Chemiron normalized these indices. The reduction observed in platelet count in the negative control rat compared with the normal control was not significant; this is not in agreement with the report of some workers [28], who recorded a lowered blood platelet count in albino rats administered toxic chemicals. However, this result may have been significant if the treatment period was extended. Mean platelet volume and platelet distribution width were not affected in the negative control rats compared with normal control but plateletcrit were significantly reduced compared with the normal control. This effect on plateletcrit was only ameliorated at the 500 and 750 mg/kg bwt and also in the Chemiron treated rats.

Conclusion

The ethanol leaf extract of I. batatas compared favourably with Chemiron at the 750 mg/kg bwt dose level. They were able to reverse haematocrit and red blood cell count and normalize white blood cell indices and plateletcrit in phenyl hydrazine-induced anaemic rats. These effects have been attributed to the presence of phytochemicals in the leaves of *I. batatas*.

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