Haematopoietic Effects of *Ipomoea batatas* (L) Lam. Leaf Extract on Male Wistar Rats

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Abstract

There are claims by traditional medicine practitioners that the leaves of Ipomoea batatas have haematinic effects and other folkloric usages in the treatment of ailments. Therefore the ethanol extract of the leaves of this plant was investigated to ascertain its effects on red blood cell, white blood cell, platelet counts and their differentials. Proximate, phytochemical and metal content analyses were also carried out on the powdered sample. Extraction was done by maceration, phytochemical, proximate and metal content analyses were carried out using standard procedures. Automated method was used to obtain the haematological indices of control and test rats. Increases in red blood cell count, haematocrit and haemoglobin were observed only at dose levels 1000 and 2500 mg/kg body weight of rats. Dose dependent reduction in lymphocyte counts and a significant reduction in mean platelet counts were observed. Proximate analysis revealed high moisture and crude protein content; phytochemical analysis revealed the presence of phytate, flavonoids, saponins, oxalate, tannins, total phenols, alkaloids and cardiac glycosides. The leaves of I. batatas contain high levels of magnesium, iron and potassium. Increases in red blood count, haematocrit and haemoglobin count infer haematopoietic effects; but reductions in lymphocytes could infer a negative effect on cellular immune response.

Key words: *Ipomoea batatas*; red blood cells, white blood cells, platelets, differentials, phytochemicals

Introduction

Globally, plants are used for the treatment and control of diseases; the World Health Organization (WHO) estimates that 80% of the world's population presently uses herbal medicine for some aspect of primary Health Care. *Ipomoea batatas* (f. convolvulaceae) has a lot of folkloric uses, its leaf decoction is a folk remedy for asthma, bug bites, burns, catarrh, diarrhoea, fever and tumours (Duke,1985). It has also been used as a haematinic in the treatment of sickle cell disease (Ilondu and Enwa, 2013; Woolfe, 1992). *Ipomoea batatas* (sweet potato) is a creeping plant with perennial vines and adventitious roots, some of which produce swollen tubers. The sweet potato plant originated in Central America and it is widely cultivated and consumed all over the world. Some work has been done on the medicinal properties of *I. batatas*, Mahmood *et al*. (1993) reported the presence of polyphenolics such as anthocyanins and phenolic acids such as caffeic, monocaffeoylquinic (chlorogenic), dicaffeoylqinic and tricaffeoylquinic acids in sweet potato leaves; he reported that these compounds are inhibitors of HIV replication. Okudaira *et al*. (2005) reported the hypoglycaemic effects, Islam *et al*. (2003); the radical scavenging effects and Yoshimoto *et al.* (2002), the antimutagenic properties of these compounds.

Haematology refers to the study of the numbers and morphology of the cellular elements of the blood, these are the red cells (erythrocytes), white cells (leucocytes) and the platelets (thrombocytes) and the use of these results in diagnosis and monitoring of disease. Changes in haematological parameters are often used to determine various status of the body and to determine stresses due to environmental, nutritional and/or pathological factors. This work was done to alidate the claims of herbalists that *I. batatas* has haematopoietic effects.

Materials and Methods

Plant collection and preparation: *Ipomoea batatas* leaves were collected from the University of Benin, Benin City, Edo State, Nigeria and identified by Professor, J. F. Bamidele of the plant Biology and Biotechnology Department, University of Benin, Nigeria. The leaves were air- dried, pulverized and sieved using muslin cloth (pore size, 0.5 mm).

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

Phytochemical analysis: Phytochemical screening was carried out on the powdered sample to detect the presence of secondary metabolites using standard procedures (Sofowora, 1993, Harborne, 1973, Swain, 1979, Brunner, 1984, AOAC, 1990, Adeniyi *et al*., 2009, Okeke and Elekwa, 2005).

Proximate and metal content analyses: The chemical analysis of the proximate composition of powdered *I. batatas* leaves was determined according to the methods of Association of Official Analytical Chemist (AOAC, 1990). 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 concentrations were determined using complexometric method as outlined in AOAC (1990). The Corning 421 Flame Emission photometer was used for the estimation of sodium

and potassium content (Tietz, 1995). Zinc, iron, cadmium, manganese, lead and copper concentrations were determined using the Perkin-Elmer model 403 Atomic Absorption Spectrophotometer (Skoog , 2007).

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 animals were acclimatized for two weeks before the commencement of the study. All animals were fed with commercially formulated rat feed and water ad libitum. The principles of Laboratory animal care (NIH Publication, 1985) were followed.

Chemicals: All chemicals used were of the analytical grade.

Experimental Design: Forty-eight albino rats were divided into six groups of eight rats each;
Group 1(normal control): this group of animals was given distilled water only
Group 2: animals in this group were administered 10 mg/kg of the ethanol extract of *I. batatas*.
Group 3: animals in this group were given 100 mg/kg body weight
Group 4: animals in this group were given 1000 mg/kg body weight
Group 5: animals in this group were given 2500 mg/kg body weight
Group 6: animals in this group were given 5000 mg/kg body weight
All the animals were allowed free accesses to food and water.

Blood Sample Collection: At the end of the treatment, blood samples were collected by direct cardiac puncture into sterile containers with anticoagulants (EDTA)

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

Statistical Analyses: All data were expressed as mean ± SEM. One way analysis of variance was used to test for differences among all the groups. Dunnett's multiple range tests was used to test for significant differences among the means. A p – value of < 0.05 was considered statistically significant.

Results and Discussion

Table 1 shows high moisture content; this was followed by high crude protein and crude lipid content. Crude fibre content has the lowest value.

Table 1: Proximate composition of *I. batatas*

Parameters	Proximate 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

Results are expressed as mean±SEM

Phytochemical screening of *I. batatas* as observed in Table 2 shows the presence of phytate, flavonoids, saponin, oxalate, tannins, total phenols, alkaloids and cardiac glycosides. High content of cardiac glycosides but relatively low levels of flavonoids and saponin were observed. Steroids were not present.

Table 2: Phytochemical Constituents

Phytochemicals	Qualitative	Quantitative
Phytate	Present	4.19±0.55
Flavonoids	Present	0.34±0.02
Saponin	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

Results are expressed as mean±SEM

Metal analysis revealed relatively high levels of magnesium, iron, calcium and potassium and low levels of sodium, manganese and copper. The presence of lead and cadmium were not detected.

Table 3: Metal content of *I. batatas*

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

Results are expressed as mean±SEM

Table 4: Results of red blood cell and its differentials

	0 (control)	10mg/kg	100mg/kg	1000mg/kg	2500mg/kg	5000mg/kg
RBC(x10^6/ul)	6.50±0.98^a	6.50±0.50^a	6.53±0.22^a	8.32±0.47^b	8.51±0.72^b	6.50±0.12^a
HCT (%)	44.55±0.58^a	48.03±0.68^a	39.60±9.76^a	50.87±2.36^{a,b}	54.80±1.80^b	35.85±5.85^a
HGB (g/dl)	14.10±0.32^a	15.90±0.49^{a,b}	14.08±2.28^a	17.85±0.98^b	17.70±1.00^b	13.15±0.96^a
MCV(fl)	21.69±3.22^a	24.46±0.91^a	21.56±4.11^a	21.45±3.10^a	20.79±1.72^a	20.23±0.98^a
MCHC (g/dl)	31.60±0.59^a	32.73±0.93^a	35.73±9.03^b	35.07±1.52^b	32.15±0.91^a	36.68±5.14^b
MCH (pg)	19.93±0.18^a	19.60±0.23^a	25.05±5.45^{a,b}	21.50±1.29^a	20.93±0.74^a	21.28±2.28^a
RDW (%)	16.10±1.90^a	16.92±3.11^a	16.80±2.10^a	18.63±2.20^a	21.27±0.52^b	21.73±0.88^b

Results are expressed as mean ± SEM (n=8). Values with different letters are significant ($p<0.05$).

Red blood cell, haematocrit and haemoglobin count were significantly increased at dose levels 1000 mg/kg and 2500 mg/kg, but this effect was reduced at dose level 5000 mg/kg. RDW was significantly increased at dose levels 2500 and 5000 mg/kg. The effect on MCHC was non dose dependent; other parameters were not significantly altered.

Table 5: Results of white Blood Cell and its differentials (x10^3/ul)

Treatment	WBC	Monocyte	Lymphocytes	Granulocyte
Normal control	15.68±2.73^a	1.85±0.74^a	11.05±1.85^a	1.73±0.71^a
10 mg/kg	12.13±0.74^b	1.88±0.42^a	7.70±1.45^b	2.58±0.12^b
100 mg/kg	8.70±0.68^c	0.50±0.03^b	7.32±0.80^b	0.83±0.05^c
1000 mg/kg	10.78±1.64^c	1.34±0.05^c	7.43±0.78^b	2.00±0.68^b
2500 mg/kg	13.88±1.98^b	2.50±0.55^d	7.45±0.93^b	3.95±0.78^d
5000 mg/kg	9.63±1.013^b	1.60±0.21^b	4.97±0.52^c	2.23±0.16^b

Results are expressed as mean ± SEM (n=8). Values with different letters are significant ($p<0.05$).
There were non-dose dependent reduction in white blood cell count and a dose dependent reduction in lymphocytes compared with control. The effects of *I. batatas* on monocyte and granulocyte were non-dose dependent.

Table 6: Results of platelet and its differentials

Treatment	Platelets (x10^5/ul)	PCT (%)	MPV (fl)	PDW (%)
Normal control	3.30±0.69^a	0.21±0.04^a	8.50±0.17^a	45.92±4.22^a
10 mg/kg	4.56±0.45^b	0.32±0.03^b	6.80±0.52^b	42.40±1.95^a
100 mg/kg	4.95±0.82^b	0.42±0.13^c	6.60±1.22^b	42.50±1.79^a
1000mg/kg	3.84±0.68^a	0.26±0.03^a	6.47±1.31^b	43.30±3.02^a
2500 mg/kg	3.79±0.74^a	0.27±0.06^a	6.48±0.97^b	51.87±2.75^b
5000 mg/kg	5.83±0.74^b	0.41±0.05^c	6.77±0.88^b	41.00±2.56^a

Results are expressed as mean ± SEM (n=8). Values with different letters are significant ($p<0.05$).

The effects of ethanol extracts of *I. batatas* on platelets count, plateletcrits and platelet distribution width were non-dose dependent however the mean platelet volume were significantly reduced compared with the control.

DISCUSSION

The results on proximate analysis are displayed on Table 1; these results show high moisture content of leaves of *I. batatas;* this is xteristics of green leafy vegetables (Osagie and Eka, 1999). The results also reveal relatively high ash, crude protein and crude lipid content. This is in agreement with the report of Antia *et al*., (2006). 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. Phytochemical screening of *I. batatas* shows the presence of phytate, flavonoids, saponin, oxalate, tannins, total phenols, alkaloids and cardiac glycosides (table 2). Steroids were not present. This result is in agreement with the report of Ilondu and Enwa (2013). The plant leaves have very high levels of cardiac glycosides and oxalates and low levels of flavonoids and saponins. The low content of saponin may be an advantage because saponins are often haemolytic in function. High content of cardiac glycosides and potassium is suggestive of a cardio protective nature.

Metal analysis showed the presence of sodium, potassium, calcium, magnesium, irons, zinc, manganese and copper (table 3). It contains relatively high levels of magnesium,

iron, calcium and potassium and low levels of sodium, manganese and copper. The presence of lead and cadmium were not detected. Iron is one of the principal agents used in the treatment of anaemia. Table 4 shows the effect of *I. batatas* on red blood cells and its differentials. Red blood cells, haematocrit and haemoglobin levels were significantly increased at dose levels 1000 mg/kg and 2500 mg/kg demonstrating a stimulatory effect on haematopoietic organs. This haematopoietic tendency may be due to the presence of high levels of crude protein and iron in the leaves of this plant. The protein is required in the synthesis of the non-heme component of haemoglobin; while the iron, the heme component.

This haematopoietic tendency may also be attributable to the presence of bioactive chemicals like chlorogenic acids and 3, 4, 5. Tri-o-caffeoylquinic acids isolated from the leaves of this plant (Mahmood et al., 1993). Phytochemicals can be considered as possible drugs; they have stimulating effects on enzymes. Table 5 displays the results on white blood cell and its differentials. There were non-dose dependent reduction in white blood cell count and a dose dependent reduction in lymphocytes compared with control. The effects of *I. batatas* on monocyte and granulocyte were non-dose dependent. Reductions in lymphocytes may eventually reduce the white blood cell count. This implies a negative effect on cellular immune response. Table 6 shows the results on platelets and its differentials. The effects of ethanol extracts of *I. batatas* on platelets count, plateletcrits and platelet distribution width were non-dose dependent however the mean platelet volume were significantly reduced compared with the control. Mean platelet volume (MPV) is a marker of platelet function; large platelets contain more dense granules and produce more thromboxane A_2. Reduction in mean platelet volume implies a reduction in platelet function.

CONCLUSION

The haematopoietic nature of ethanol extracts of I. batatas has been demonstrated at the 1000 and 2500 mg/kg dose levels. More work is needed in this area to determine the safe doses of administration. The haematopoietic tendency of I. batatas is attributable to a high protein and iron content and to the presence of phytochemicals.

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