SAFETY ASSESSMENT OF *IPOMOEA BATATAS* (L). LAM LEAF EXTRACT, A TRADITIONAL HAEMATINIC; IN MALE WISTAR RATS

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ABSTRACT

Plants have been the basis for medicinal treatments through much of human history. Ipomoea batatas (family: convolvulaceae) is claimed to have a number of therapeutic uses especially in the treatment of sickle cell anaemia. The ethanol extract of the leaves were investigated to ascertain its effects on body and organ weight, acute toxicity and sub-acute toxicity. For the sub-acute toxicity studies, forty-eight rats were divided into six groups of eight rats each, the first group serves as control while the remaining five groups were given 10, 100, 1000, 2500 and 5000 mg drug extract per kg body weight of rats. Losses in weights of experimental rats were observed with corresponding reduction in feed intake. Reductions were observed in the absolute weights of the liver, heart, kidneys and pancreas. Acute toxicity tests recorded no mortality and no visible sign of toxicity. Increases were observed in ALT activities compared with the control at the 1000, 2500 and 5000 mg/kg dose levels, ALP activities were significantly increased at the 2500 and 5000 mg/kg. Creatinine levels were significantly increased at the 2500 and 5000mg/kg. Although acute toxicity tests indicate that it is safe, long term treatment with I. batatas above 1,000 mg/kg dose levels may produce deleterious effects on vital organs like the liver and the kidney.

INTRODUCTION

Ipomoea batatas (f. convolvulaceae) is a creeping plant with perennial vines and adventitious roots, some of which produce swollen tubers. It is also referred to as sweet potato. *I. batatas* has a lot of nutritional and therapeutic uses; its leaf decoction is a folk remedy for asthma, bug bites, burns, catarrh, diarrhea, fever and tumours (Antia *et al*., 2006, Duke, 1985). Also, it has been reported that this plant has an anti-sickling effects and has been used in the treatment of sickle cell anaemia and other related ailment (Ilondu and Enwa, 2013).

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), dicaffeoylquinic and tricaffeoylquinic acids in *I.batatas* leaves. He reported that these phytochemicals 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 phytochemicals. There is a growing interest in the use of plant and plant parts for the treatment of ailments, much claims made by traditional medicine practitioners on the

therapeutic uses of different plants have been validated and results documented. However, there is a need for safety assessment on the use of these plants.

Scientific investigation is necessary not only because of the need to discover new drugs but also to assess the toxicity risks faced by the users. Plants contain some chemicals that are known to be toxic to both animals and humans. Some of these chemicals evolved in plants to protect them from insects, plant pathogens and other organisms. The adverse effects of toxic chemicals in plants are related to interference with nutrient availability, metabolic processes, detoxification mechanisms and allergic reactions in animals and humans. Little work has been done on toxicological assessment of *I. batatas* leaves. Ali and Tageldin., (1999) reported a reduction in body weight of rats on administration of *I. batatas*. To the best of our knowledge; no work has been done on the acute or sub-acute toxicity of the leaf extract of *I. batatas*.

Materials and Methods

Plant Collection and Preparation:

Ipomoea batatas leaves were obtained 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. A copy of the plant has been deposited in the herbarium at the University of Benin, Plant Biology and Biotechnology Department (herbarium number; UBHc0292). The leaves were air – dried, pulverized and sieved.

Extraction and Concentration

100g of the powdered leaves were extracted in 1000 ml concentrated 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.

Animals

Sixty male wistar albino rats (200 – 250 g) used for this study were purchased from the Animal House of Ambrose Alli University, Ekpoma, Edo State, Nigeria. 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. Kits used for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, total and direct bilirubin, blood urea nitrogen (BUN), and creatinine were obtained from Randox Laboratories (Crumlin, Co- Anthrim, Spain).

Acute Toxicity Determination

The Lorke's method (1983) was used. The procedure was conducted in two phases. In the first phase, 9 rats were divided into 3 groups of 3 rats each. The groups were administered 10, 100, and 1000 mg ethanol extracts per kg body weight of rats respectively. All the rats were kept under the same conditions and observed for toxicity signs or mortality for 24 hours. In the second phase, a total of 3 rats were used, the rats were divided into 3 groups of 1 rat each and were administered 1500, 3000 and 5000 mg/kg b.wt of rats and observed for toxicity and mortality signs after 24 hours. The acute toxicity LD_{50} is calculated as the arithmetic mean of the smallest dose that resulted in mortality and the highest dose which caused no mortality at all.

Sub-acute Toxicity Studies

Wistar rats (48) used for this study were divided into 6 groups of eight rats each. The first group served as the control while the remaining five groups were administered 10, 100, 1000, 2500 and 5,000 mg extract per kg body weight of animals. All animals were allowed free accesses to food and water. Weekly measurements of weights were recorded.

Blood Sample Collection

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

Biochemical Analysis

Plasma aspartate transaminase ({EC2.6.1.1}, AST), alanine transaminase ({EC2.6.1.2}, ALT), alkaline phosphatase ({EC 3.1.3.1}, ALP), total proteins, albumin, total and direct bilirubin, urea and creatinine were determined using the standard ready- to- use kits and methods of Randox Laboratories. The manufacturer's instructions for each biochemical parameter were strictly followed in the course of the investigations. Serum Na^{+}, K^{+}, HCO_3^{2-} and Cl^{-} were estimated using ion selective electrode(an automated method). Catalase (EC 1.11.1.6) activity was determined by the method of Sinha (1972), superoxide dismutase ({EC.1.15.1.1}, SOD) by the method of Misra and Fridovich (1972) while malondialdehyde levels were measured by the method of Gutheridge and Wilkin (1982).

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

Table 1: Effect of *I. batatas* leaf extract on body weight changes and feed intake

Treatment (mg/kg)	Weight Change (g)	Feed Intake (g)
0 (Control)	10.23±2.02[a]	97.83±14.05[a]
10	-6.10± 1.65[b]	65.71±16.27[b]
100	-10.83±2.74[b]	65.08±17.54[b]
1000	-18.87±4.14[c]	61.79±11.89[b]
2500	-15.07±3.88[c]	59.42±3.92[d]
5000	-15.00±3.72[c]	45.54±8.94[d]

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

Table 2: Effects of ethanol leaf extract of *I. batatas* on absolute organ weight

Treatment (mg/kg)	Heart	Kidneys	Liver	Pancreas	Spleen
0 (Control)	0.80±0.06[a]	1.28±0.05[a]	7.48±0.19[a]	0.58±0.07[a]	0.90±0.02[a]
10	0.65±0.03[b]	1.13±0.06[a]	6.28±0.18[b]	0.39±0.02[b]	0.93±0.10[a]
100	0.60±0.06[b]	0.92±0.04[b]	6.24±0.54[b]	0.33±0.02[b]	0.64±0.06[b]
1000	0.63±0.05[b]	1.08±0.10[b]	6.18±0.59[b]	0.33±0.11[c]	0.90±0.11[a]
2500	0.48±0.06[c]	0.82±0.08[b]	5.68±0.20[c]	0.32±0.06[b]	1.06±0.22[a]
5000	0.42±0.02[c]	0.68±0.04[c]	4.34±0.57[c]	0.23±0.01[b]	0.62±0.07[b]

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

Table 3: Results of acute toxicity tests on the ethanol leaf extract of *I. batatas*

First Phase:

Treatment (mg/kg)	No of rats	Mortality recorded	Mortality rate	Observation
10	3	0	-	No visible sign of toxicity
100	3	0	-	"
1000	3	0	-	"

Second Phase:

Treatment (mg/kg)	No of rats	Mortality recorded	Mortality rate	Observation
1500	1	0	-	No visible sign of toxicity
3000	1	0	-	"
5000	1	0	-	"

Table 4: Effects of ethanol extract of the leaves of *I. batatas* some liver function indices

Treatment (mg/kg)	Albumin (g/l)	Direct Bil. (µmol/l)	T. bil. (µmol/l)	ALT (µ/l)	AST (µ/l)	ALP (µ/l)
0	10.31 ± 1.76^{a}	9.68 ± 1.23^{a}	18.69 ± 4.11^{a}	30.03 ± 1.28^{a}	58.00 ± 8.00^{a}	78.10 ± 5.11^{a}
10	19.61 ± 2.94^{b}	9.27 ± 0.22^{a}	20.17 ± 5.29^{a}	35.07 ± 3.80^{a}	57.00 ± 7.50^{a}	64.35 ± 7.10^{a}
100	5.26 ± 0.50^{c}	8.04 ± 0.87^{a}	15.42 ± 2.67^{a}	31.59 ± 1.48^{a}	54.33 ± 7.88^{a}	46.95 ± 4.33^{b}
1000	18.99 ± 1.81^{b}	11.48 ± 1.78^{b}	19.81 ± 1.57^{a}	45.83 ± 2.73^{b}	47.67 ± 7.15^{b}	56.50 ± 2.51^{b}
2500	1.36 ± 0.22^{c}	8.88 ± 1.67^{a}	19.47 ± 2.12^{a}	48.91 ± 2.20^{b}	56.67 ± 2.18^{a}	127.88 ± 9.30^{c}
5000	3.47 ± 0.59^{c}	14.06 ± 1.29^{b}	15.13 ± 3.08^{a}	52.51 ± 2.17^{b}	42.00 ± 6.00^{b}	299.75 ± 32.00^{d}

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

Table 5: Effects of ethanol leaf extract of *I. batatas* on some renal function indices

	0 (Control)	10 mg/kg	100 mg/kg	1000 mg/kg	2500 mg/kg	5000 mg/kg
Urea	5.70 ± 0.22^{a}	4.80 ± 0.20^{a}	5.90 ± 0.17^{a}	6.73 ± 0.49^{a}	5.60 ± 0.56^{a}	4.70 ± 6.00^{a}
Na^{+}	137.67 ± 2.19^{a}	139.33 ± 1.67^{a}	136.33 ± 0.88^{a}	134.33 ± 0.33^{a}	136.67 ± 1.20^{a}	136.33 ± 0.88^{a}
K^{+}	8.20 ± 0.30^{a}	5.47 ± 0.57^{a}	6.60 ± 0.42^{a}	7.83 ± 0.75^{a}	9.07 ± 0.69^{a}	7.10 ± 0.53^{a}
HCO_3^{2-}	22.33 ± 2.19^{a}	18.33 ± 1.33^{a}	18.67 ± 0.67^{a}	15.33 ± 0.67^{a}	17.67 ± 1.45^{a}	15.77 ± 4.73^{a}
Cl^{-}	109.33 ± 3.33^{a}	110.00 ± 1.73^{a}	109.00 ± 0.58^{a}	107.00 ± 0.38^{a}	109.67 ± 1.76^{a}	107.67 ± 0.88^{a}
Creat.	0.47 ± 0.07^{a}	0.40 ± 0.06^{a}	0.47 ± 0.03^{a}	0.47 ± 0.12^{a}	0.60 ± 0.12^{b}	0.63 ± 0.03^{b}
Tot. prot.	86.09 ± 4.27^{a}	78.26 ± 6.88^{a}	80.15 ± 4.59^{a}	89.31 ± 8.58^{a}	80.51 ± 8.54^{a}	18.92 ± 1.72^{b}

Results are expressed as mean ± SEM (n=8). Values with different superscripts are significant (p<0.05).
{urea, creatinine and total prot. in g/l;Na^{+}, K^{+}, HCO_3^{2-},and Cl^{-} in mmo/l}

Table 6: Effects of ethanol leaf extract of *I. batatas* on the oxidative status of rats

Treatment (mg/kg)	SOD (unit/ml)x 10^3	Catalase (unit/ml)x 10^3	MDA (unit/ml) x 10^{-3}
0 (control)	1.11 ± 0.05^{a}	0.24 ± 0.01^{a}	42.00 ± 4.16^{a}
10	1.12 ± 0.25^{a}	0.23 ± 0.01^{a}	20.15 ± 0.05^{b}
100	0.78 ± 0.15^{b}	0.14 ± 0.01^{b}	20.00 ± 0.80^{b}
1000	1.14 ± 0.56^{a}	0.15 ± 0.02^{b}	5.10 ± 0.05^{c}
2500	1.00 ± 0.34^{a}	0.14 ± 0.05^{b}	40.18 ± 0.10^{a}
5000	1.95 ± 0.47^{c}	0.15 ± 0.05^{b}	56.70 ± 8.82^{d}

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

Table 1 shows the effect of ethanol leaf extract of *I. batatas* on body weight changes. There were significant reductions in body weight of rats compared with control. There were also corresponding reductions in feed intake of experimental rats compared with control. Table 2 shows the effect of ethanol extract of the leaves of *I. batatas* on absolute organ weights. There were significant reductions in the absolute weights of the heart, liver, kidneys and pancreas compared with control. The effects observed on the weights of the spleen were not dose dependent. Results of acute toxicity studies (first and second phase) are presented in table 3. No mortality was observed, no visible clinical sign of toxicity was also observed. Results of sub-acute toxicity tests are presented in tables 4, 5 and 6.

Table 4 shows the effect of *I. batatas* leaves on biochemical indices of liver function. Non dose dependent response was observed in albumin, direct bilirubin and AST activities. ALT activities were significantly increased at the higher doses of 1000, 2500 and 5000 mg/kg while ALP activities were significantly increased at dose levels 2500 and 5000 mg/kg. Table 5 shows the effect of ethanol extract of sweet potato leaves on renal function indices. Parameters like the urea, Na^{+}, K^{+}, HCO_3^{2-}, Cl^{-} were not significantly altered. Creatinine levels were significantly increased at dose levels, 2500 and 5000 mg/kg while total protein levels were only significantly reduced at dose levels 5000 mg/kg. Effects of ethanol extract of *I.batatas* leaves on lipid peroxidation status are shown in table 6. There were dose dependent reductions in catalase activities compared with the control. Significant reductions in MDA concentrations were observed at dose levels 10, 100 and 1000 mg/kg but significant increase at dose levels 5000 mg/kg compared with the control. A non- dose dependent effect on SOD activity was observed.

DISCUSSION

Toxicological assessment of rats administered graded doses of ethanol extract of sweet potato revealed a significant reduction in weight of experimental rats at all dose levels of administration (Table 1). This result is in agreement with the report of Ali and Tageldin (1999) and Taiwo *et al*., (2005). Corresponding reductions were also observed in feed intake of experimental rats. This is consistent with the report of Hill et al., (1990) who reported that consumption of the *I.batatas* leaf extract before a meal reduces energy intake in healthy animals. Oral administration of sweet potato leaf extract may stimulate cholecystokinin (CCK) release and reduce caloric intake (Little *et al*., 2005).

Table 2 shows the effect of ethanol extract of the leaves of *I. batatas* on absolute organ weights. There were significant reductions in the absolute weights of the heart, pancreas, liver and the kidneys. Greater reductions in weights were observed at the 2500 and 5000 mg/kg dose levels. The differences observed in the absolute weights of the spleen were non-dose dependent. Increase or decrease in either absolute or relative weight of an organ after administering a chemical or substance is an indication of the toxic effect of that substance (Orisakwe et al., 2003).

Results of acute toxicity studies (first and second phases) are presented in tables 3. No mortality was observed in the different groups of rats, no visible clinical sign of toxicity was observed. Acute toxicity studies are designed to determine the dose that will produce mortality or serious toxicological effects when given once or over a few administrations. They also provide information on the range of doses that could be used in subsequent toxicity testing. Results of acute toxicity tests are shown in table 3. There was no mortality or visible sign of toxicity even at the highest dose level of 5000 mg/kg.

However, acute toxicity data are of limited clinical applications since cumulative toxic effects do occur even at low doses. Sub-acute and chronic toxicity are useful in evaluating the safety profile of drug extracts; results of sub-acute toxicity tests are presented in tables 4, 5 and 6. Table 4 shows the effect of the leaves of I. *batatas* on biochemical indices of liver function. Non- dose dependent response was observed in albumin, direct bilirubin levels and AST activities. The

enzymatic activities of ALT, AST and ALP were studied to evaluate liver malfunctions.

ALT activities were significantly increased at higher doses of 1000, 2500 and 5000 mg/kg dose levels, while AST activities were not significantly affected. ALP activities were reduced at lower concentrations of 10, 100 and 1000 mg/kg, indicating reduction in growth and increased at 2500 and 5000 mg/kg indicating biliary tract obstruction. Body cells contain more AST than ALT (Mayne, 1996), AST appears in higher concentrations in a number of tissues e.g. Liver, kidney, heart and pancreas and is released slowly in comparison to ALT. ALT is localized primarily in the cytosol of hepatocytes; hence this enzyme is considered a more sensitive marker of hepatocellular damage than AST (Al-Mamary *et al*., 2002). Therefore significant elevation in ALT activities observed is indicative of hepatocellular damage.

Total protein levels were significantly reduced at the highest dose of 5000 mg/kg (table 5). Any change in the concentration of serum protein and albumin indicates a change in the normal liver functions (Ahmed et al., 1992). Urea, sodium, potassium, chloride and bicarbonate ion levels analyzed were not significantly altered, but creatinine levels were significantly increased at dose levels 2500 and 5000 compared with control (table 5). Imafidon (2006) reported an increase in creatinine levels on feeding rats with fresh leaves of *I. batatas*. High creatinine levels suggest possible kidney malfunction, this may occur as a result of reduction in glomerular filtration rate (Arora et al., 2006).

Effects of ethanol extract of the leaves of sweet potato on the oxidative status of rats are shown in table 6. There were dose dependent reductions in catalase activities compared with the control. In spite of the reductions in catalase activities, significant reductions in MDA concentrations were observed at dose levels 10, 100 and 1000 mg/kg, this shows that other antioxidants were present. The results on SOD activity were non-dose dependent, MDA levels were only significantly increased at dose level 5000 mg/kg suggesting a high lipid peroxidation status of cells at this dose level.

CONCLUSION

At low doses of 10 and 100 mg/kg, the plant is relatively safe and might be used as component of weight reducing formula since at this dose levels, there were significant reductions in body weight. However at higher doses above 1000mg/kg, ethanol extract of I. *batatas* may elicit toxic effects on vital organs especially the liver and kidney.

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