Ameliorative potentials of aqueous extracts of leaf and stem of *Ipomoea involucrata* on selected biochemicals in experimental diabetic rats

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**ABSTRACT**

**Background:** Diabetes mellitus is a chronic metabolic disorder characterized by derangements in carbohydrate, protein and lipid metabolisms, due to deficiency in insulin secretion and action. This research evaluates the ameliorative potentials of aqueous extracts of leaves and stem of *Ipomoea involucrata* on selected biochemicals in experimental diabetic rats.

**Methods:** Diabetes mellitus was induced by single intraperitoneal injection of 150 mg/kg body weight of alloxan and the animals were orally administered with gilanil (4 mg/kg) for positive control, 100, 200 and 300 mg/kg bw aqueous extract of leaves (groups 4-6) and stem (groups 7-9) of *Ipomoea involucrata* once daily for 21 days. Biochemical parameters were analysed using standard methods.

**Results:** The median lethal dose was established at 648 mg/kg (leaves) and 547 mg/kg (stem). The negative group (untreated) showed significant increase in glucose concentration compared to the other groups. After 2 to 3 weeks there was significant (p<0.05) decrease in glucose concentration of the extract and glibenclamide (positive group) treated groups when compared with the negative group. Diabetes control rats showed significant (p<0.05) high serum lipid profile (except for high density lipoprotein), liver enzymes/ indices and renal indices when compared with non-diabetic control rats. However, these alternations were reversed with the positive group and the groups treated with aqueous extracts of both samples. The differences observed in the electrolytes were not significant in all groups.

**Conclusions:** The results suggest that aqueous extract of leaves and stem of *I. involucrata* is considerably safe and a potential therapy for management of complications associated with diabetes mellitus.

**Keywords:** Ameliorative potentials, Biochemicals, Diabetic rats, *Ipomoea involucrata*, Leaves, Stem

**INTRODUCTION**

The utilization of any material can be achieved through scientific research which reveals its components and their properties. The knowledge revealed determines the specific function of the material. Most plant materials have been proven to have both nutritional and therapeutic properties. The use of phyto-medicine has been identified as a means of studying the potentiality of future medicines. *Ipomoea involucrata* has been in continuous use for different purposes such as nutritional, medicinal and agricultural. In Guinea, the leaves and stem are eaten as food, while the Ghanian herbalists use the leaves in the treatment of anaemia. The leaves and stem decoction of *Ipomoea batatas* is used in folk remedies for tumours of the mouth and throat. The stem and leaves extracts has been shown to support blood pressure control. Diabetes mellitus is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycaemia and glucose intolerance as a result of lack of insulin, defective insulin action or both. Such
complications arise due to derangements in the regulatory system associated with storage and mobilization of metabolic fuels.\textsuperscript{9} Diabetes mellitus and lesser forms of glucose intolerance can now be found in almost every population in the world. Epidemiological evidence suggests that without effective prevention and control programmes, diabetes will likely continue to increase globally. The World Health Organization (WHO) estimates that 80\% of the world population presently used herbal medicine for some aspects of primary health care.\textsuperscript{10} Several treatments have been made and success recorded, but the adverse effects associated with synthetic medicine are rather creating fears than remedy; for example, the use of sulphonyleurea (glibenclamide). The adverse effect of this drug at slightest dereliction or contraindication are severely consequential.\textsuperscript{2} In line with the increased interest in medicinal plants in recent times, it is imperative to investigate and explore aqueous extract of leaves and stem of \textit{I. involucrata} on selected biochemicals in experimental diabetic rats.

**METHODS**

**Collection of test plant**

The leaves and stem of \textit{I. involucrata} were obtained from Rukpokwu in Obio/Akpor Local Government Area of Rivers State, Nigeria. Plant identification, authentication and voucher referencing (UBH 1336) were done at the taxonomy section of the Departments of Plant Science and Biotechnology, University of Benin, Benin city, Edo State Nigeria by Dr. Adeyemi Akinnibosun.

**Preparation of plant extract**

The leaves and stem of \textit{I. involucrata} were sorted, air dried and ground to fine powder using laboratory Hammermill. Five hundred grams obtained from each sample were percolated in two liters of distilled water in a conical flask with constant shaking and left standing for 24 hours at room temperature. The samples were thereafter filtered using Whatman no. 1 filter paper and the filtrate concentrated on a thermostat bath at 500°C in an evaporating dish. The stem and leaves extract were separately removed from the evaporating dish using a spatula and collected into dry sterile container and stored at 40°C until required for analysis.

**Acute toxicity study**

Toxicity of the leaves and stem extract was determined by the method of Lorke and Olowu et al, with slight modifications.\textsuperscript{11,12} Briefly described, a total of thirty-nine rats of both sexes where randomly allotted into 6 groups of three rats each for the leaves and stem extracts. The normal control group, also contain 3 rats. Apart from the control which received feed and water, the treatment groups received orally, 100 mg/kg, 300 mg/kg, 400 mg/kg, 500 mg/kg, 600 mg/kg and 700 mg/kg body weight (b.w) of either the leaves or stem extract.

Observation for signs of toxicity and mortality was made for 48 hours.

**Experimental animal design**

Fifty adult albino rats weighing between 185-200 g were obtained from the animal house of Biochemistry Department, University of Port Harcourt with Institutional Animal Ethics Committee approval number: UPH/CEREMAD/REC/MM60/040. They were fed with normal rat pellet and water ad libitum. The rats were all allowed 21 days of acclimatization before experimentation. All procedures in the study were performed according to NIH guide for the care and use of laboratory animals.\textsuperscript{13} They were grouped into ten of five rats in each group in a disinfected metal cage. The animals were grouped based on different treatment viz; control, leaves and stem extracts. Group 1 served as the normal control which received feed and water only, group 2 (negative control) served with alloxan (150 mg/kg bw), groups 3 (positive control) served with alloxan (150 mg/kg bw) and standard drug (4 mg/kg Glandil). While groups 4, 5 and 6 received leaves extracts at 100, 200 and 300 mg/kg b.w and groups 7, 8 and 9 received stem extracts at 100, 200 and 300 mg/kg b.w respectively. The rats were orally treated daily for 21 days.

**Induction of diabetes mellitus**

Diabetes was induced by administering intraperitoneally injection of a freshly prepared solution of alloxan monohydrate (150 mg/kg) diluted in citrate buffer (pH 4.5) to the overnight fasted rats.\textsuperscript{14} Control rats were injected with citrate buffer only. In view of the fact that, alloxan is capable of producing fatal hypoglycaemia as a result of the massive paracreatic insulin released rats were treated with 80\% glucose solution intraperitoneally after 6 hours.\textsuperscript{15} The animals were then allowed to drink 5\% glucose solution for the next 24 hours to prevent hypoglycaemia. Those with blood glucose concentrations above 180 mg/dl were used for the experiment.

**Blood collection**

At the end of 21 days treatment period, the rats were deprived of pellet but had free access to drinking water for 24 hrs. before being sacrificed under inhaled chloroform anaesthesia. Blood samples were collected through jugular puncture into non-heparinized containers for biochemical analysis. Blood samples for glucose level determination were collected from the tail tips of the rats. The pancreases of the experimental rats were surgically removed.

**Biochemical parameters**

The blood glucose level was determined for all the sample groups by the glucose oxidase method. Lipid profile was estimated using Randox diagnostic reagent...
kit. Liver enzymes were assayed using the method of Reitman and Franklin. Biuret method was used for total protein. Serum total bilirubin was analysed at 578 nm, while creatinine and urea were measured using Prietest EasyLab Biochemistry Analyzer. Electrolytes were estimated using automated EUC machine (Mindrays BS 800 Chemistry). 16,17

Histological examination

The pancreas of the experimental animals were subjected to histological analysis using the method of Kumar et al. 18

Statistical analysis

The data were subjected to statistical analysis using Statistical Package of Social Sciences (SPSS) software version 20.0. Values are reported as mean±standard error of mean (SEM) using one-way analysis of variance (ANOVA) test. Significance level was considered at 95% confidence level (p<0.05).

RESULTS

The results of the acute toxicity test of the aqueous leaves and stem extract of *I. involucrata* leaves and stem show that oral administration of aqueous leaves and stem extract did not induce lethality or mortality up to 600 and 500 mg/kg b.w for leaves and stem extract respectively. The medium lethal dose (LD50) was calculated as the square root of the product of the highest dose that causes zero mortality (LD0) and least dose that caused hundred percent mortality (LD100).

\[ LD_{50} = \sqrt{LD_{0} \times LD_{100}} \]

Thus, for the leaves extract \( LD_{50} = \sqrt{600 \times 700} = 648 \text{ mg/kg} \)

For the aqueous stem extract \( LD_{50} = \sqrt{500 \times 600} = 547 \text{ mg/kg} \)

Table 1 shows the effect of aqueous extracts of *I. involucrata* leaves and stem on blood glucose concentration of diabetic rats. One week after treatment, the negative control (group 2) showed significant (p<0.05) increase in glucose concentration compared to the other groups. By weeks two and three, there was significant decrease in glucose concentration in group 3, treated with standard drugs (positive control) and the extract treated groups when compared with the negative control (group 2). Similar trends were observed for both leaves and stem extracts.

### Table 1: Effect of *I. involucrata* leaves and stem extract on blood glucose (mg/dl) of diabetic rats.

| Week | Groups | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|------|--------|---|---|---|---|---|---|---|---|---|
| 1    |       | 85.00±0.66 & 284.60±1.84 & 259.60±1.07 & 256.50±0.85 & 267.60±0.85 & 254.70±1.40 & 260.80±3.45 & 266.33±0.67 & 265.00±1.93 |
| 2    |       | 88.30±0.70 & 271.66±3.20 & 156.30±1.45 & 200.35±1.20 & 173.44±1.45 & 224.70±1.18 & 185.47±1.20 & 227.60±3.28 & 235.20±2.08 |
| 3    |       | 83.40±0.45 & 256.33±2.33 & 132.70±1.69 & 190.50±1.60 & 138.35±1.11 & 212.33±2.40 & 132.71±1.68 & 208.25±2.80 & 222.33±2.40 |

Values are mean±SEM triplicate determination. Values in the same row with different superscripts (a-e) are significantly different at p<0.05.

Table 2: Effect of aqueous extracts of *I. involucrata* leaves and stem on lipid profile of diabetic rats.

| Parameter         | Groups | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|-------------------|--------|---|---|---|---|---|---|---|---|---|
| Triglycerides     | 1.23±0.03a & 2.70±0.05a & 0.81±0.03c & 1.00±0.04f & 0.86±0.04b & 0.97±0.01i & 1.02±0.01e & 0.99±0.03c & 0.97±0.10c |
| Total cholesterol | 3.03±0.01c & 3.89±0.03e & 2.88±0.11c & 3.30±0.05e & 2.97±0.06 & 3.08±0.12c & 0.93±0.29 & 2.93±0.06e & 3.00±0.12c |
| HDL               | 0.60±0.00a & 0.26±0.00b & 0.72±0.1a & 0.66±0.03b & 0.69±0.01c & 0.57±0.01c & 0.60±0.01c & 0.64±0.06a & 0.62±0.14c |
| VLDL              | 0.56±0.00a & 1.23±0.03b & 0.37±0.01c & 0.45±0.03a & 0.39±0.00c & 0.44±0.04c & 0.46±0.05ac & 0.85±0.00ae & 0.44±0.00ae |
| LDL               | 1.87±0.08a & 2.40±0.01b & 1.79±0.00a & 2.09±0.03b & 1.98±0.11c & 2.07±0.02d & 1.87±0.14a & 1.84±0.0a & 1.94±0.02d |

Values are mean±SEM triplicate determination. Values in the same row with different superscripts (a-e) are significantly different at p<0.05.

The effect of the extracts on the lipid profile parameters of alloxan induced diabetes is presented in Table 2. Group 3 and the extract treated groups showed a significant (p<0.05) decrease in the concentration of serum triglycerides, total cholesterol, low density lipoprotein (LDL) and very low-density lipoprotein (VLDL) and a significant increase (p<0.05) in serum high density lipoprotein (HDL) concentrations when
The effect of leaves and stem extract on some biochemicals is shown in Table 3. There was significant (p<0.05) decrease in total protein of the treated group when compared with the negative control and group 4. No significant decrease in the bilirubin level of all the groups.

The extracts of both leaves and stem did not show any significant difference in the electrolyte levels of the diabetic rats.

**Histological studies**

The histopathological findings of the pancreases showed that the group that was administered with standard drug shared increase in islet cell mass, while the negative groups showed reduced islet cell mass. The islet cell mass shown in the treated groups varied and was not concentration dependent (Figure 1).

**DISCUSSION**

The results of the oral lethal dose of the aqueous extracts of *I. involucrata* leaves and stem showed the oral LD50 of the aqueous leaves extract of *I. involucrata* to be 648 mg/kg while the LD50 of the aqueous stem extract of *I. involucrata* was at 547 mg/kg respectively. These results appeared to be relatively similar to that earlier obtained for the ethanolic extract of *I. involucrata* leaves (760 mg/kg) in male albino rats. According to Clarke and Clarke, any compound with an estimated LD50 equal or greater than 1000 mg/kg/oral can be considered relatively safe.
Figure 1: Histology of the pancreas of the (A) Normal control H and E X 200 showing normal Islet cells (NI) and acinar cells (AC), (B) Negative control H and E X 100 showing reduced islet cell mass (IC) and acinar cells (AC), (C) Positive group (induced) rats administered with the standard drug H and E X 200 showing increase in islet cell mass (ICM), (D) Group 4 (induced) rats administered with 100 mgkg-1 of the aqueous extract of I. involucrata leaves H and E X 200 showing reduced islet cell mass, (E) Group (induced) rats administered with 100 mgkg-1 of the aqueous extract of I. involucrata stem showing increased islet cell mass (ICM), (F) Group (induced) rats administered with 200 mgkg-1 of the aqueous extract of I. involucrata leaves showing slight decrease in islet cell mass, (G) Group (induced) rats administered with 200 mgkg-1 of the aqueous extract of I. involucrata stem showing reduced islet cell mass (ICM), (H) Group 6 (induced) rats administered with 300 mgkg-1 of the aqueous extract of I. involucrata leaves showing reduced islet cell mass (ICM), (I) Group 6 (induced) rats administered with 300 mgkg-1 of the aqueous extract of I. involucrata leaves showing reduced islet cell mass (ICM).
Similarly, according to the American Society for Testing and Materials, any chemical substance or test compound with LD50 estimate greater than 2000-5000 mg/kg body weight/oral route could be considered of low toxicity and safe. Numerous studies have previously observed increases in glucose level in diabetic conditions. Consequently, the significant increase in blood glucose level in diabetic (not treated) rats could be a consequence of the intrinsic oxidative stress generated as a result of the destruction of beta cells in rats administered alloxan. However, a significant decrease (p<0.05) in blood glucose levels was observed in groups administered the standard drug (positive) and groups treated with leaves and stem extracts of *I. involucrata* respectively in 2nd and 3rd week of treatment compared with the negative control. These hypoglycaemic effects could have been mediated either via induction of hyperinsulinenia (increased pancreatic insulin secretion) or increased peripheral utilization of glucose. The latter appears more reasonable as the b-pancreatic cells were already destroyed by alloxan injection. It has been shown that streptozotocin and alloxan induced diabetes mellitus by causing pancreatic b-selective cytotoxicity, via disruption of the cell membrane integrity. The pancreatic b-cells are involved in the synthesis, storage, and release of insulin, the peptide hormone that regulates carbohydrate, protein, and lipid metabolism. Furthermore, the oral hypoglycaemic effect of the aqueous leaves and stem extract of *I. involucrata* could be a consequence of the phytochemicals present in these extracts. It has been shown that the biological activities of alkaloids and flavonoids include hypoglycaemic, hypolipidaemic, hypotension among other biological activities. Garzon de la Mora et al, reported that alkaloid extract of *Lupinexaltatus* decreased glyceremia and blood cholesterol in alloxan induced diabetic wistar rats.

Lipids play a vital role in the pathogenesis of diabetes mellitus. In diabetic condition, the increase in blood glucose level is usually accompanied by an increase in plasma cholesterol, TGs, LDL and decrease in HDL. The increased level of serum lipid in diabetes represents a risk factor for coronary heart disease. In insulin deficient diabetes, the concentration of serum free fatty acids is elevated as a result of free fatty acids out flows from fat depots where the balance of the free fatty acid esterification triacylglycerol lipolysis cycle is displaced in favour of lipolysis. Thus an excess fatty acid in the plasma produced by alloxan induced diabetes promotes the conversion of excess fatty acids into phospholipids and cholesterol in the liver. These two substances along with excess TGs formed in the liver may be discharged in the liver in the form of lipoproteins. Oral administration of the aqueous leaves and stem extracts of *I. involucrata* significantly decreased the lipid profile (except for HDL) levels in comparison with the negative control (Table 2). The high concentration of triglyceride, total cholesterol, low density lipoprotein and decrease in high density lipoprotein observed in diabetes untreated rats compared to normal rats group, agreed with reports of some researchers which suggests that an elevated glucose level will occur upon induction of diabetes, and this would give rise to corresponding increase in plasma lipids concentration. Hyperlipidaemia is a factor of cardiovascular diseases associated with diabetes mellitus. It is often characterized by elevated cholesterol, triglycerides, phospholipids and other lipoproteins. This outcome suggests that the extract could be useful in combating complications associated with diabetes. The liver is an important insulin dependent tissue which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes. The results of the current study demonstrated alloxan administration to the experimental rats resulted in elevations in the levels of serum AST, ALP and ALT which is indicative of hepatocellular damage. Even though significant elevations was only observed in AST and ALT levels in the positive control in comparison with the negative control. This might possibly be due to the release of these enzymes from the cytoplasm into the blood circulation rapidly after rupture of the plasma membrane and cellular damage. Several studies have reported similar elevation in the activities of serum AST, ALP and ALT during alloxan administration. There was no significant difference (p>0.05) in serum protein and total bilirubin levels as shown in Table 3 when compared to the normal control. This might be as a result of some proteins forming intrachains or interchains disulfide bridges between cysteine residues. The cross-links in this way help to protect the native conformation of the protein molecule from the intensity of oxidative stress. Treatment with *I. involucrata* aqueous leaves and stem extract significantly reduced the activities of ALT enzymes in alloxan induced diabetic rats. This shows that extracts may tend to prevent liver damage by maintaining the integrity of the plasma membrane thereby suppressing the leakage of the enzymes through the membrane, thereby exhibiting hepatoprotective activity. A number of scientific reports indicate that certain flavonoids, phenols and steroids have protective effect on liver due to its antioxidant properties.

Increase in urea levels in diabetes may be attributed to enhanced catabolism of both the liver and plasma proteins that go together with gluconeogenesis. The concentration of renal function markers such as urea and creatinine which significantly increased in diabetic (untreated rats) as shown in the results was significantly reduced after oral administration of the aqueous leaves and stem extracts of *I. involucrata* and the standard drug Glanilglibenclamide. This outcome suggests possible anti-diabetic activity of these extracts probably due to improvements in impaired liver function caused by the induction of diabetes.

The results of the current study demonstrated reductions in the islet cell mass (Plate 2) in the diabetic control as a result of destruction of β cells caused by alloxan injection. However, the leaves and stem extracts of *Ipomoea*...
involutrata} relatively ameliorated this damage as a result of the increase in Islet cell mass in diabetic groups administered 100 mg/kg stem and 300 mg/kg of aqueous leaves and stem extracts of *I. involucrata*.

**CONCLUSION**

The study demonstrated antidiabetic, antilipidemic and hepatoprotective potentials of the aqueous extract of *I. involucrata* stem and leaves. This provides claims that the leaves and stem of *I. involucrata* was able to ameliorate abnormalities associated with diabetes mellitus.

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