HYPOGLYCAEMIC ACTIVITY OF *VERNONIA AMYGDALINA* (DEL.) EXTRACTS IN NORMAL AND ALLOXAN-INDUCED RATS

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Vernonia amygdalina (Del.) is widely cultivated in tropical Africa and known for its bitter principles. The extracts of this plant are used in folklore medicine to treat fevers, measles, tuberculosis, toothaches, parasitic infections, asthma, diarrhoea and in managing diabetes mellitus. The growing concerns arising from the treatment and management of the diabetes prompted this study. Hence, the hypoglycaemic potentials of leaf, stem and root (squeezed and methanolic) extracts of the plant were investigated in normo-glycaemic and alloxan-induced rats. The blood glucose levels in normal and diabetic rats were determined after the administration of 300mg/kg of extract and 150mg/kg of alloxan monohydrate at time (t) = 0, 1, 2 and 4 h. The hypoglycaemic activities of the squeezed extracts of leaves, stem and roots were not significant in both normal and diabetic rats. However, the methanolic extracts of the leaves and roots demonstrated significantly remarkable hypoglycaemic activities compared with the activity given by the stem extract. The methanolic extracts of leaves and root have shown to be effective in lowering blood glucose level (80% reduction after 4h) while the stem afforded a poorly 20% reduction after 4 h. The results from this study have lent scientific credence to the ethnobotanical use of the plant in the treatment and management of diabetes mellitus. However, the claims that the stem is as effective in the herbal therapy of this metabolic disease can not be supported by the results obtained from this study.

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INTRODUCTION

Medicinal plants are undoubtedly relevant in both developing and developed nations of the world as sources of herbal drugs for treating and managing various ailments and disease conditions. Herbal medicines are finished, labelled medicinal products which contain plant parts whether in the crude state or slightly processed state.[1] Hence, some plants are used for treating such high priority diseases such as cardiovascular conditions, sickle cell anaemia, HIV/AIDS, renal ailments, tumours and most especially diabetes mellitus.[2] Many patients who suffer from diabetes mellitus are experiencing difficulties in managing the disease condition due to several factors including increasing cost and uncomfortable side effects of orthodox therapy. In addition, the increasing prevalence of this condition in the third world means there are more diabetics per orthodox health personnel and also increase in visits to diabetic clinics.[3-6]

In the light of these realities, there is an urgent need for a search for herbal recipes that could be used in the treatment and management of this metabolic disorder of the body. *Vernonia amygdalina* (Del.) which is widely grown in tropical Africa and known for its 'bitter principles'[7] is used in traditional medicine to treat fevers, measles, tuberculosis, toothaches, parasitic infections, asthma, diarrhoea and in managing diabetes mellitus.[7-10] Also, the extracts of the plant have been evaluated for laxative, arbortificient, anti-helmintic, antithrombotic and anticoagulant properties.[7-14]

The plant possesses nutritional values especially in the South-Eastern parts of Nigeria where its consumption is widespread.[8,10-11] Consequently, the present study was undertaken to investigate into the hypoglycaemic potentials of the leaf, stem and root extracts of *V. amygdalina* in rats.

MATERIALS AND METHODS

Plant Collection and Identification:

Fresh leaves, stem and roots of *V. amygdalina* were collected within the precinct of the botanical gardens of the Faculty of Pharmacy, University of Uyo, Nigeria around July, 2010. Voucher specimens of the plant (Nos H75-H77) were deposited in the herbarium of the Faculty of Pharmacy, University of Uyo, Nigeria.

Extraction

The fresh leaves of *V. amygdalina* were macerated with a wooden mortal and pestle after washing with water. 200g of the macerated leaves was squeezed and the resultant mixture filtered with a filter paper (Whatman International, England). The filtrate obtained was subsequently concentrated *in vacuo* on a rotary evaporator (Buchi CH-920, Laboratorium Technic, Flawk/SG, Switzerland) and the obtained dried powder stored in a silica-gel desiccator prior to further tests. Another 200g of the leaf extract was extracted with cold 96 % aqueous methanol at room temperature (27± 2 ^{0}C) for 72h, likewise concentrated and stored. The same procedures were repeated for the stem and roots.

Preparation of rats

Permission was sought from the College of Health Sciences' Animals Ethics Committee, University of Uyo, Uyo, Nigeria and approval was granted on the 24th, July, 2010 as contained in the reference document (UU/CHS/DP/12). The animals were then subsequently used in the

hypoglycaemic studies. Albino rats of both sexes obtained from the University of Uyo, Animal House weighing on the average 110.12 ± 10.67g were made diabetic by intraperitoneal injection of alloxan monohydrate (150mg/kg). The animals were quarantined for 7days to stabilize the blood glucose level. The rats were maintained under standard laboratory conditions and had free access to feed (Pfizer Feeds, Nigeria) and water *ad libtum*.

Administration of extracts

Normoglycaemic rats

The animals were arranged into seven groups of five rats each. The rats were put through a 12 h overnight fast and the groups were subsequently treated as follows:

Group I (control) - received 1ml of saline water orally.

Group II - received 300mg/kg of squeezed leaf extract orally.

Group III - received 300mg/kg of squeezed stem extract orally.

Group IV - received 300mg/kg of squeezed root extract orally.

Group V - received 300mg/kg of methanolic leaf extract orally.

Group VI - received 300mg/kg of methanolic stem extract orally.

Group VII - received 300mg/kg of methanolic root extract orally.

Diabetic rats

The animals were rested for 12 days and made diabetic by an inter-peritoneal administration of 150mg/kg alloxan monohydrate. After 5 days, the diabetic rats (glucose level >350mg/dL or 5.0 Mmol/L) were regrouped into four groups of 5 rats each. 15 of the animals had died on the 1st and 2nd days after the alloxan injection administration. The four groups were subsequently treated as highlighted below:

Group A (control) - received 1 ml of saline water orally.

Group B - received 300 mg kg^{-1} of methanolic leaf extract orally.

Group C - received 300 mg kg^{-1} of methanolic stem extract orally.

Group D - received 300mg kg^{-1} of methanolic root extract orally.

Estimation of blood glucose level

Blood was collected from the tail vein of the rats and analysed for glucose using the One Touch Glucometer (Ames Gx Model, Germany). In both the normal and diabetic rats (i.e., *a* and *b* above), blood glucose was determined at 0, 1, 2 and 4 hours.

Statistical analysis

The data were expressed as mean ± S.D. The significance of the data was determined using student's *t* – test and were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

The percentage changes (%) in blood glucose level are as displayed in Tables 2, 4 and 6. These values were calculated as follows:

$$\% \text{ Change} = 100\, G_T / G_O$$

where

G_T = Blood glucose level at time (t) = 1, 2 and 4 h;

G_O = Blood glucose level at time (t) = 0.

Normoglycaemic rats

The squeezed extracts of leaves, stem and roots of *V. amygdalina* were tested in normoglycaemic rats at 300mg/kg. The data obtained using the student's t test showed no statistically significant difference in the normoglycaemic rats when compared to the control at t = 1, 2 and 4 h as can be seen in Table 2. This might be due to the preparation technique adopted which could have hindered the amount of plant materials filtered into these squeezed extract mixtures. However, the methanolic extracts of leaves and roots exhibited significantly ($p<0.05$) and approximately similar hypoglycaemic activities in normoglycaemic rats especially at t = 2 and 4 h which are remarkable. These observations are as displayed in Table 4,

Diabetic rats

The methanolic extracts of leaves, stem and roots were equally tested in alloxan-induced rats at 300mg/kg. It could be seen that the hypoglycaemic activities of the leaf and root extracts were highly pronounced (80% reduction in blood glucose level) but was evidently poor in the stem extract (20% reduction in blood glucose level). These observations are as presented in Table 6. The methanolic leaf and root extracts demonstrated significantly ($p<0.05$) and approximately similar hypoglycaemic activities. These observations are not surprising because the extracts of *V. amygdalina* have been found to contain saponins, cardiac glycosides, tannins, flavonoids, terpenes, sugars, proteins, fats and vitamins C which have been implicated in previous studies to be hypoglycaemic.[15,16]

It is very probable that any of these chemical constituents or a combination of them could be responsible for the hypoglycaemic activity demonstrated by the plant. Further studies might have to be done to isolate and identify these hypoglycaemic principles and mechanism of action investigated

Table 1. Blood glucose level (mmol L^{-1}) in normoglycaemic rats using squeezed extracts.

Group	Extract	Dose	0 h	1 h	2 h	4 h
I	Control	1 ml saline water	2.5	2.4	2.4	2.5
II	Leaf	300 mg kg^{-1}	2.8	2.8	2.6	2.5
III	Stem	300 mg kg^{-1}	3.1	3.1	2.9	2.8
IV	Root	300 mg kg^{-1}	2.6	2.5	2.4	2.2

Mean ± S. D. $n = 5$

Table 2. Percentage change in blood glucose level ((mmol L^{-1}) in normoglycaemic rats using squeezed extracts.

Group	Extract	Dose	%	1 h	2 h	4 h
I	Control	1 ml saline water	100	96.3	98.2	99.3
II	Leaf	300 mg kg^{-1}	100	100	92.9	89.3
III	Stem	300 mg kg^{-1}	100	100	93.6	90.3
IV	Root	300 mg kg^{-1}	100	96.1	92.3	84.6

Mean ± S. D. $n = 5$

Table 3. Blood glucose level (mmol L^{-1}) in normoglycaemic rats using methanolic extracts.

Group	Extract	Dose	0 h	1 h	2 h	4 h
I	Control	1 ml saline water	2.5	2.4	2.4	2.5
V	Leaf	300mg/kg	2.8	2.7	2.5	2.1
VI	Stem	300mg/kg	2.8	2.7	2.6	2.7
VII	Root	300mg/kg	3.3	2.9	2.9	2.6

Mean ± S. D. $n = 5$

Table 4. Percentage Change in Blood Glucose Level ((Mmol/L) in Normoglycaemic Rats using Methanolic Extracts.

Group	Extract	Dose	%	1 h	2 h	4 h
I	Control	1 ml saline water	100	96. 2	98.2	99.3
V	Leaf	300mg/kg	100	96.4	89.3	75.1
VI	Stem	300mg/kg	100	96.4	92.9	96.4
VII	Root	300mg/kg	100	87.9	87.9	78.8

Mean ± S. D. $n = 5$, $p<0.05$

Table 5. Blood glucose level (mmol L^{-1}) in alloxan-induced rats using methanolic extracts.

Group	Extract	Dose	0 h	1 h	2 h	4 h
A	Control	1 ml saline water	11.6	10.5	10.8	10.5
B	Leaf	300mg/kg	11.6	8.8	7.4	3.5
C	Stem	300mg/kg	12.5	11.1	10.8	8.9
D	Root	300mg/kg	11.8	9.8	6.5	3.8

Mean ± S. D. $n = 5$

Table 6. Percentage change in blood glucose level ((mmol L^{-1}) in alloxan-induced rats using methanolic extracts.

Group	Extract	Dose	%	1 h	2 h	4 h
A	Control	1 ml saline water	100	90.8	93.1	90.3
B	Leaf	300mg/kg	100	76.3	63.7	30.1
C	Stem	300mg/kg	100	88.3	86.0	71.2
D	Root	300mg/kg	100	83.1	55.3	32.0

Mean ± S. D. $n = 5$, $p<0.05$

Furthermore, the results from this study as displayed in Tables 4 and 6 have shown that only the leaf and root extracts have demonstrated remarkable hypoglycaemic activities. This observation clearly negates the claims in [10] that the stem in addition to the leaves and roots *V. amygdalina* are employed in the treatment and management of diabetes mellitus especially in South-Eastern parts of Nigeria. Alloxan destroys the beta cells of the pancreas leading to insulin defiency.[4-5] Therefore, it is conceivable that the hypoglycaemic activities shown by the leaf and root extracts were not related to insulin secretion by the pancreatic cells but rather by other mechanisms of action. However, close monitoring of blood glucose concentration in humans is required in the use of the leaf and root extracts of *V. amygdalina* in the herbal therapy of diabetes mellitus to avoid hypoglycaemic shock.

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