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Hypoglycaemic activity of *Vernonia amygdalina* (chloroform extract) in normoglycaemic and alloxan-induced hyperglycaemic rats

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Abstract

The study was carried out to evaluate the hypoglycaemic effect of the crude chloroform extract of *Vernonia amygdalina* (leaves) on blood glucose concentration (BGC) of normoglycaemic and alloxan-induced hyperglycaemic rats. Adult male albino rats were randomly allocated into four groups. Group I animals served as untreated controls to normoglycaemic group II animals. Alloxan-induced hyperglycaemic rats of group III served as untreated controls to hyperglycaemic rats of group IV. Groups II and IV animals were treated with a single intraperitoneal (IP) dose of 750 mg/kg of crude chloroform extract of *V. amygdalina*. Control animals of groups I and III were administered equi-volume (per kilogram of body weight) of normal saline IP. The BGCs of all animals in each group were determined at 0, 0.5, 1, 4, 8 and 24 hours post-treatment with normal saline (controls) or the crude chloroform extract (treated groups). There was significant ($P < 0.05$) lowering of BGCs between 1 - 4 hours (for normoglycaemic rats) and 1 - 8 hours (for hyperglycaemic rats). Thus the crude chloroform extract of the leaves of *V. amygdalina* has a hypoglycaemic activity in both normoglycaemic and alloxan induced hyperglycaemic rats. This study lends support to the claim by herbalists of Plateau and Nassarawa States, that *V. amygdalina* may have an antidiabetic effect in diabetes mellitus.

Keywords: *Vernonia amygdalina*, normoglycaemia, alloxan-induced hyperglycaemia, hypoglycaemic activity, diabetes mellitus

Introduction

Many patients in developing countries that suffer from diabetes mellitus (DM) are finding it increasingly difficult to manage the disease condition due to several factors including: increasing cost and side effect of orthodox therapy and apparent vitiation of therapeutic effectiveness of the drugs such that normal blood glucose concentrations (BGC) cannot be adequately maintained. In addition, the increasing prevalence of DM amongst third world communities means that there are more diabetic patients per orthodox health care personnel, as well as increase in the number of visits to diabetic clinics (Campion, 1993). Consequently more rural diabetic patients are relying more and more

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on traditional remedies (Gyang and Sokomba, 2000).

Frequent claims by herbal medicine practitioners of the effectiveness of herbal medicines have stimulated the interest of diabetologists and biomedical scientists to evaluate the commonly used phytomedicines for anti-diabetic activity.

Currently over 400 plant-based preparations world wide have been evaluated and found to possess some anti-diabetic properties (Bailey and Day, 1989; Ezugwu and Nze, 1998; Klepser 2001).

For example, the Nutrition and Metabolism Department of Trinidad Ministry of Health, in an extensive study of 622 diabetic patients, 103 plant products were reported to have been used to control the condition. Herbal preparations used include *Mormodica charantia, Aloe babadensis, Bonita daphnoides* and *Phyllanthus amarus* all of which were confirmed to be effective (Mohabir *et al.*, 1977). Also several other plants from Zimbabwe including the leaves of *Opuntia megacantha* and *Morus alba,* the bark of *Ficus thoningii*, and the bulbs of *Allium sativum* have all been shown to posses significant anti diabetic activity. These plant products were reported to lower blood glucose level by 17% to 31% and by 17% to 21% in normoglycaemic and alloxan induced hyperglycaemic rats respectively (Bwititi and Musabayane, 1977). The protective effect of the aqueous extract of the Cameroonian plant *Anacardium occidentale* against streptozotocin-induced diabetes mellitus in rats has been demonstrated (Kamtchouing *et al.*, 1998). Most recently the aerial parts of *Equisetum myriochaetum* have been reported to have hypoglycaemic effect in streptozotocin-induced diabetic rats (Cetto *et al.*, 2000)

Vernonia amygdalina (Family Asteraceae) is a shrub grown in most parts of Nigeria and bears the common name "bitter-leaf". Medicinally, the leaves are used for the herbal treatment of asthma, malaria, measles, diarrhoea, tuberculosis and abdominal pain (Adjanahoun *et al.*, 1993). The leaves and the roots are also used to treat malaria, sickle-cell anaemia, eruptive fever and gastro-duodenal ulceration (Adjanahoun *et al.* 1991).

In a recent survey (Gyang, 1994), of herbal therapy of DM, it was observed that over 60% of the 83 herbal medicine practitioners in Nigeria's central states of Plateau and Nassarawa, used *V. amygdalina* leaves for the treatment of DM. All of these herbalists confirmed the effectiveness of the leaves of *V. amygdalina* in their treatment of DM. The claim of the herbalist induced the authors to evaluate the anti-diabetic activity of the leaves of *V. amygdalina.*

Experimental

Plant Material. The fresh leaves of *V. amygdalina* were collected from Jos, Nigeria and authenticated by D.D. Nyam of the Department of Botany University of Jos, Nigeria, where voucher sample. (No. SSG / DM/Herb. Therap.1) has been preserved for future reference. Preliminary phytochemical analysis was carried out on a sample voucher and the remaining voucher used to obtain the crude chloroform extract.

Extraction of Plant Material. The fresh leaves of *V. amygdalina* were macerated using a wooden mortar and pestle. 120g was mounted in a Soxhlet apparatus and extracted with 250mL of chloroform (Analar grade) for 48 hours using the method described by Sofowora (1993). The extract was subsequently concentrated and dried by placing over a hot plate maintained at 50°C. The dried extract which weighed 13.7g (14.4% yield) was then stored in a refrigerator at -4°C until used.

Fractionation of phytochemical constituents. Preliminary screening of the phytochemical constituents for hypoglycaemic activity revealed that only the glycosidal and alkaloidal fractions possess

hypoglycaemic activity. To evaluate the antidiabetic activity of each of these fractions (glycosidal and alkaloidal) the procedure outlined below was employed.

Procedure for fractionation of alkaloids. An amount of the crude chloroform extract weighing 9.53g was made alkaline and gently shaken by hand for about one hour. Up to 25ml of chloroform was added, followed by 25ml of distilled water. To the chloroform phase (which contained the alkaloidal fraction as well as waxes) was added 30ml of dilute (0.1N) hydrochloric acid. Using a separatory funnel, the acidic phase (upper) which contained alkaloidal salts was transferred into a beaker and neutralized using sufficient ammonium hydroxide (until litmus paper test show that pH has risen to 7). The contents of the beaker were then dried to constant weight by gently heating over a water bath maintained at 45°C. Dragendorff reagent was used to confirm presence of alkaloids (Turner and Brain, 1975). This fraction was then screened for hypoglycaemic activity in both normoglycaemic and alloxan-induced hyperglycaemic rats.

Procedure for fractionation of glycosides. A portion of the crude chloroform extract (9.5g) was put in a test tube and 25ml of 70% ethanol added and hand shaken vigorously. The test tube contents were then warmed over a bath at 45°C for 5 minutes. After cooling, 5 % lead acetate was gradually added until a precipitate was formed. The precipitate was then filtered out and the filtrate evaporated to dryness over a water bath at 45°C. The solute left on evaporation was tested for presence of glycosides using the method described by Sofowora (1993). The glycosidal fraction was then screened for hypoglycaemic activity in both normoglycaemic and alloxan-induced hyperglycaemic rats.

Animals. A total of twenty four (24) male apparently healthy albino rats (Wistar strain) weighing between 87-141g were used in the study. The animals which were obtained from the animal house of Department of Pharmacology and Clinical Pharmacy were prior to use fed on standard rat animal feed (Pfizer, Jos, Nigeria) and allowed free access to food water.

Administration of the crude chloroform extract to the rats. The effect of the chloroform extract of *V. amygdalina* on the BGC of normoglycaemic rats, twelve rats were randomly allocated to two groups of 6 each and labelled as groups I and II as shown in Table 1. Rats in group I (controls) were administered equi-volume (per kg body weight) normal saline, while rats in group II were administered 750 mg/kg IP crude chloroform extract (dissolved in normal saline).

After a 12 - hour overnight food fast, the BGC of each rat in groups I and II was measured at 0, 0.5, 1, 2, 4, 8 and 24 hours by cutting the tip of the animal's tail and a drop of fresh blood squeezed out on to the sensor pad of a specified strip of the glucose measuring meter (Glucometer GX model, Ames Incorporated Germany). The procedure was repeated with another set of twelve rats randomly allocated to two groups III and IV) of 6 animals each, as shown in Table 1.

Three days prior to administration of the crude chloroform extract, animals in groups III sand IV were made hyperglycaemic by an intraperitoneal administration of 100 mg/kg alloxan (dissolved in distilled water). Animal in group IV were administered a single dose of 750 mg/kg IP of the crude chloroform extract of *V. amygdalina* while animals in group III were each give an equi-volume (per kg body weight) of normal saline to serve as the untreated hyperglycaemic control group.

The hypoglycaemic activity of the alkaloidal fraction of the crude chloroform extract of *V. amygdalina* normoglycaemic rats was screened by using a group of 6 rats. Each rat was administered (after a 12 hour

overnight food fast) intraperitoneally 500 mg/kg the alkaloidal fraction and BGCs taken at 0, 0.5, 1, 2, 4, 8, and 24 hours. In similar manner using a group of 6 rats was administered (after a 12 hour over-night food fast) 500 mg/kg IP the glycosidal fraction of *V. amygdalina* and BGCs measured at 0, 0.5, 1, 2, 4, 8, and 24 hours after treatment. The procedure was repeated with another group of 6 rats, but the animals were administered equi-volume (per kg of body weight) normal saline to serve as the untreated control group.

This procedure was repeated with rats in the two groups previously made hyperglycaemic by intraperitoneal administration of 100 mg/kg alloxan 3 days before the start of the experiment. The BGCs for both normoglycaemic and alloxan-induced hyperglycaemic rats are presented in Table 2.

The procedure of screening for hyperglycaemic activity of the alkaloidal fraction of the crude extract of *V. amygdalina* was repeated in screening the glycosidal fraction of the same crude extract, the results are also presented in Table 3.

Results and Discussion

In this study, preliminary qualitative phytochemical analysis of the leaves of *V. amygdalina* revealed the presence of alkaloids saponins, tannins, and glycosides. The chloroform extract yielded 13.7g of solid from 120g of the leaves.

The crude chloroform extract exhibited hypoglycaemic activity in both normoglycaemic and alloxan-induced hyperglycaemic rats (Table 1). Similarly both the alkaloidal and glycosidal fractions exhibited hypoglycaemic activity in both normoglycaemic and alloxan-induced hyperglycaemic rats (Table 2 and 3 respectively). The normoglycaemic controls did not show any change in BGCs while the BGCs of alloxan-induced hyperglycaemic controls remained above the normal range for rats (60-120mg/dL).

The crude chloroform extract of *V. amygdalina* leaves exhibited significant ($P<0.05$) lowering of blood glucose concentrating in both normoglycaemic and alloxan-induced hyperglycaemic rats. The onset and duration of this activity was ½ and 8 hours respectively.

These findings suggest that the crude chloroform extract of *V. amygdalina* may lower BGC in diabetic patients, this likely to cause hypoglycaemia in normoglycaemic individual. It therefore means that the use of the leaves of *V. amygdalina* in the herbal therapy of DM may require close monitoring of BGC (e.g. 60 - 120 mg/dL in humans) to avoid hypoglycaemic shock.

In alloxan-induced hyperglycaemic rats however, the hyperglycaemic activity (onset 30 minutes and duration 8 hours) was even more pronounced. The extent of reduction in the blood glucose level was more prominent in alloxan induced hyperglycaemic than in normoglycaemic rats. Furthermore, it can be inferred from the results that the efficacy of the extract as an anti-diabetic remedy is more pronounced in the hyperglycaemic state since the hyperglycaemic activity was more pronounced in alloxan-induced hyperglycaemic than normoglycaemic rats.

The hypoglycaemic activity of the leaves of *V. amygdalina* resides in the alkaloidal and glycosidal phytochemical constituents. The reduction in BGC by the glycosidal fraction in normoglycaemic rats at a dose of 500 mg/kg was not statistically significant. In alloxan-induced hyperglycaemic rats on the other hand, the glycosidal fraction showed a significant ($P<0.05$) reduction in BGC an hour after treatment. This suggests that at a single dose of 500 mg/kg, the glycosidal fraction only elicit appreciable anti-diabetic activity in alloxan-induced hyperglycaemic animals.

In summary, it can be said that the hypoglycaemic activity of the crude

chloroform extract of *V. amygdalina* in hyperglycaemic and alloxan induced hyperglycaemic rats resides mainly in the alkaloidal phytochemical constituents of the leaves of this plant. It may therefore be concluded that the claim (by herbal medicine practitioners in Plateau and Nassarawa States of Nigeria) of the effectiveness of the leaves of *V. amygdalina* in the herbal therapy of DM in humans is supported by the findings in this study.

Table 1. Blood glucose concentrations (BCG) at different time intervals following administration of a single dose (750 mg/kg IP) of the chloroform extract of *V. amygdalina* in normoglycaemic and alloxan-induced hyperglycaemic rats.

Post treatment time (h)	BGC (mean ± SEM; n = 6) mg/dL			
	Normoglycaemic rat		Hyperglycaemic rats	
	Control (Group I)	Treated (Group II)	Control (Group III)	Treated (Group IV)
0	73± 2.3	78 ± 4.4	147 ± 5.2	151 ± 4.4
0.5	61 ± 4.9	53 ± 3.9	138 ± 4.6	112 ± 3.2
1	64 ± 2.7	39 ± 2.9*	155 ± 4.0	58 ± 4.6*
2	73 ± 5.1	42 ±3.5*	146 ± 3.7	45 ± 3.0*
4	62 ± 2.7	39 ± 3.2*	163 ± 3.5	56 ± 2.8*
8	75 ± 3.2	62 ± 4.6	161 ± 5.1	68 ± 2.1*
24	82 ± 4.3	79 ± 3.7	149 ± 4.3	137 ± 4.8

* Statistically significant ($P < 0.05$)

Table 2. Blood glucose concentrations (BCG) at different time intervals following administration of a single dose (500 mg/kg IP) of the alkaloidal fraction of *V. amygdalina* in normoglycaemic and alloxan-induced hyperglycaemic rats.

Post treatment time (h)	BGC (mean ± SEM; n = 6) mg/dL			
	Normoglycaemic rat		Hyperglycaemic rats	
	Control	Treated	Control	Treated
0	73± 2.3	63 ± 5.0	147 ± 5.2	156 ± 3.4
0.5	61 ± 4.9	58 ± 4.6	138 ± 4.6	141 ± 4.4
1	64 ± 2.7	51 ± 2.1	155 ± 4.0	65 ± 2.7*
2	73 ± 5.1	48 ± 3.2*	146 ± 3.7	41± 3.2*
4	62 ± 2.7	45 ± 5.1	162 ± 3.5	43 ± 3.8*
8	75 ± 3.2	49 ± 3.5	161 ± 5.1	76 ± 4.2*
24	82 ± 4.3	66 ± 4.9	149 ± 4.3	117 ± 5.6

* Statistically significant ($P < 0.05$)

Table 3: Blood glucose concentrations (BCG) at different time intervals following administration of a single dose (500 mg/kg IP) of the glycosidal fraction of *V. amygdalina* in normoglycaemic and alloxan-induced hyperglycaemic rats.

Post treatment time (h)	BGC (mean ± SEM; n = 6) mg/dL			
	Normoglycaemic rat		Hyperglycaemic rats	
	Control	Treated	Control	Treated
0	73± 2.3	68 ± 5.2	147 ± 5.2	165 ± 7.1
0.5	61 ± 4.9	69 ± 4.3	138 ± 4.6	132 ± 4.8
1	64 ± 2.7	54 ± 2.5	155 ± 4.0	86 ± 2.4*
2	73 ± 5.1	55 ± 5.1	146 ± 3.7	75 ± 4.1*
4	62 ± 2.7	47 ± 3.4	163 ± 3.5	69 ± 3.7*
8	74 ± 3.2	57 ± 4.6	161 ± 5.1	65 ± 4.5*
24	82 ± 4.3	74 ± 3.6	149 ± 4.3	137 ± 6.1

* Statistically significant ($P < 0.05$)

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