

Full Length Research Paper

The effect of *vernonia amygdalina* on alloxan induced diabetic albino rats

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The hypoglycemic effect of the plant extract of *Vernonia amygdalina* was determined using alloxan induced diabetic rats. The rats were divided into six groups of five rats each. Four of the groups were induced with diabetes and treated with different doses of the plant extract i.e. 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg which was administered for a period of two weeks. The plant extract was observed to have hypoglycemic effect on each group of diabetic rats as it reduced their fasting blood sugar (FBS) levels from 277.6mg/dl to 142.2mg/dl (Group1), 284.8mg/dl to 102.0mg/dl (Group2), 256.4mg/dl to 86.3mg/dl (Group3) and 265.6mg/dl to 82.1mg/dl (Group 4) over a period of two weeks. The fifth group consisting of non-induced rats was administered with 400mg/kg of *Vernonia amygdalina* for a period of two weeks and the FBS level was compared with that of the control group. There was no significant difference ($P>0.05$) between the mean FBS of the fifth group 81.6mg/dl and that of the control (86.9mg/dl). However, the hypoglycemic effect of the plant extract of *Vernonia amygdalina* was felt as this brought the mean FBS level from 91.4mg/dl to 81.6mg/dl as compared with the control. Table 4. Phytochemical screening on the leaves of *Vernonia amygdalina* revealed the presence of alkaloids, tannins, saponins and cardiac glycosides. The liver, kidney and pancreas of the experimental animals were examined histologically to ascertain if 400mg/kg of the plant extract is toxic to the organs or not. The plant extract had no adverse effect when administered on normal rats except for a marked congestion of the mesenteric blood vessel; the extract in fact reduced the level of damage to the kidney, liver and pancreas when administered on diabetic rats.

Keywords: *Vernonia amygdalina* leaf extract, Alloxan-induced diabetic rats.

INTRODUCTION

“Diabetes” is a very popular disease that cuts across the web and is not a respecter of an individual’s status. Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia Ugochukwu *et al.* (2003), hypercholesterolemia and hypertriglyceridemia Njai Kumar *et al.* (2010), lipoprotein abnormalities Scoppola *et al.* (2001), raised basal metabolic rate Owu *et al.* (2006), defect in reactive oxygen species scavenging enzymes Kesavulu *et al.* (2000), and high oxidative stress induced damage to pancreatic beta cells (Nayeemunnisa, 2009). Diabetes mellitus is ranked seventh among the leading causes of death and is considered third when its fatal complications are taken into account Trivedi *et al.* (2004). A person having

this disease can still live very long, it all depends on careful handling, discipline and adherence to prescription and advice of the physician. On the other hand this disease can be lethal and can kill or maim a diabetic person, within a short time and that is why several measures have been taken by doctors, scientist, and researchers alike to curb this disease. Although some very potent drugs have been developed to manage this illness, research is still on, as to how this illness can be totally cured, and several plants have been identified as having anti-diabetic properties and available literature indicate that there are more than 800 plant species showing hypoglycaemic activity (Rajagopal *et al.*, 2008). In Nigeria there has been active development of phytopharmaceuticals with proven efficacy in a variety of medicinal problems. There has been increasing demand for the use of plant products with antidiabetic activity due to low cost, easy

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Table 1. Proximate Composition of *V. amygdalina*

Content	% Dry matter basis
Crude protein	21.54
Crude fibre	11.47
Ether extract	5.79
Ash	15.54
Nitrogen free extract	35.62
Dry matter	89.96

Source: Ekeocha (2010)

availability and lesser side effects. Therefore, plant materials are continuously scrutinized and explored for their effect as hypoglycemic agents. One of such plant is *Vernonia amygdalina* just like *Ficus glomerata* which has been used in traditional system of medicine for treating liver diseases, diarrhoea, piles, asthma, leprosy and other ailments (Warrier *et al.*, 1996).

In the present study, the hypoglycemic action of *V. amygdalina* against alloxan induced diabetic rat was reported.

MATERIALS AND METHODS

Thirty (30) healthy albino rats weighing between 150g and 200g were procured from the Veterinary Physiology animal house, University of Ibadan. These rats were allotted into six groups, each consisting of five albino rats. Group 1-4 were injected with the drug Alloxan, leading to their development of the disease diabetes mellitus. These albino rats were then treated with 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg dose of *Vernonia amygdalina* for two weeks. The rats in group 5 were not induced with the disease, diabetes mellitus, but were administered with 400mg/kg of the plant extract of *Vernonia amygdalina*. The last group (group 6) was left as the positive control, because they were neither induced with the drug alloxan to cause the disease diabetes mellitus nor were they administered with the plant extract of *Vernonia amygdalina*. Before initiation of experiment, the rats were acclimatized for a period of 7days. Standard environmental conditions such as temperature ($27 \pm 2^{\circ}\text{C}$), relative humidity (47-57%) and 12hrs dark/light cycle were maintained in the quarantine. All the rats were fed with rabbit pelletized diet (Ladokun Feed Mill, Nigeria Ltd.), water was allowed *ad-libitum* under strict hygienic conditions and routine vaccination and medication administered.

Alloxan

Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative (Lenzen, 2008) and was originally isolated in 1818 by Brugnatelli and got its name in 1838 by

Friedrich Wöhler and Justus von Liebig Lenzen *et al.* (1998). Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type 1 diabetes in humans (Lenzen, 2008). Alloxan monohydrate was obtained from Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan and all the other chemicals used were of analytical grade and were acquired from commercial sources. 10% Alloxan dissolved in physiological saline was used to induce the diabetic condition in the diabetic rats. This was by weighing 10g of fresh samples of Alloxan on a mettler's balance and dissolving in 100mls of 0.15m physiological saline solution. The dose administered was a standard dose of 100mg/kg weight of the rats.

$$\text{Volume to be Administered} = \frac{\text{Weight of animal (g)} \times \text{Dose of drug (mg/kg)}}{1000 \times \text{Concentration of drug (mg/ml)}}$$

The plant material

Fresh plants of *V. amygdalina* were obtained from the International Institute of Tropical Agriculture (IITA) Ibadan. The leaves were air dried in a shade and later ground to a powdery form using a blender. A concentration of 10mg/ml of the aqueous extract was used for the experiment. This was produced in a large quantity by weighing 1g of the powdered herb and soaking in 100mls of distilled water. The soaking was done overnight i.e. Twelve (12) hours in order to get the aqueous extract. The remaining extract was stored in a refrigerator, at a temperature of 4°C . Different doses of 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg of the plant extract was used for the experiment. The amount of extract to administer to the rats was same as for Alloxan.

Blood sugar level

The basal blood sugar (BBS) levels of all the albino rats

Table 2. Mineral Profile / Content of *V. amygdalina*

Mineral content	Quantity (mg/kg)
Calcium (Ca)	3.324
Phosphorus (P)	1.288
Potassium (K)	2.009
Sodium (Na)	3.746
Magnesium (Mg)	1.100
Iron (Fe)	468.16 (mg/g)
Manganese (Mn)	449.44
Copper (Cu)	26.22
Zinc (Zn)	580.52

Source: Ekeocha (2010)

Table 3. Mean Basal Blood Sugar mg/dl and Mean Fasting Blood Sugar mg/dl of Diabetic Rats.

Parameter (mg/dl)	Group1	Group2	Group3	Group4
Mean BBS level	88.0±0.16	89.2±0.23	85.2±0.27	85.8±0.25
Mean FBS after injection Of Alloxan	277.6±6.55	284.8±3.80	256.4±1.39	265.6±4.12

were obtained after fasting them overnight. This was done before they were injected with alloxan to make them diabetic. Table 3. The purpose of this is to know their initial blood sugar level before the commencement of the experiment so as to compare the results of the basal blood sugar (BBS) level when they are not diabetic with the fasting blood sugar (FBS) level when they are made diabetic and this was done by using a glucometer (which is a device used for measuring the blood sugar level). The procedure involved cutting a bit of their tails for a drop of blood and dropping this blood on a specified space on the glucometer.

Groups 1-4 of the six groups of albino rats were injected with 100mg/kg of alloxan, intramuscularly and their FBS were checked forty eight (48) hours after and those with FBS varying above 100-500mg/dl were considered diabetic. These were subsequently treated with varying doses of the aqueous extract of *Vernonia amygdalina* twice daily for fourteen days (i.e two weeks) on every other day and on seven (7) different occasions. The administration was done orally with the aid of an oral canola and their FBS was checked every two (2) days and recorded. The fifth group, the normal rats which were not injected with Alloxan, was administered with 400mg/kg of *Vernonia amygdalina* and their FBS were also recorded every two days for two weeks. The last group i.e. the 6th group of rats used as control were only fed with commercial feeds and given water daily. Their FBS were also checked every two days and compared with the FBS of the other groups.

Phytochemical analysis

The leaves of *V. amygdalina* were air dried and ground to powdery form to make a sample and a small quantity was taken to the laboratory of the Department of Phamacognosy, Faculty of Pharmacy, University of Ibadan for phytochemical analysis. This was to determine the presence of Alkaloids, Antraquinones, Saponins, Tannins and Cardiac glycosides.

Histopathological test

Two rats were selected from each of the various groups and were slaughtered and the following visceral organs were harvested i.e. Pancreas, liver and the kidney and these were stored separately in sample bottles containing formaldehyde for their preservation. These were taken to the histo-pathology laboratory at the Department of Veterinary Medicine, University of Ibadan. The tissues were processed into slides using the automatic tissue processing (Histo-kinette) machine. The slides of these organs were prepared and taken to the Department of Anatomy, University of Ibadan for Photomicrograph.

Statistical analysis

The data collected were subjected to the analysis of variance procedure of SAS (2000) and significant

Table 4. Fasting Blood Sugar (mg/dl) of normal and induced-diabetic albino rats administered with varying doses of *Vernonia amygdalina* leaf extracts

Number Of days	Group1 50mg/kg	Group2 100mg/kg	Group3 200mg/kg	Group4 400mg/kg	Group5 400mg/kg	Group6 Control
2	277.6±6.55	284.8±3.80	256.4±1.39	265.6±4.01	91.4±0.38	87.1±0.21
4	237.4±5.22	243.5±2.76	203.2±0.62	201.8±3.20	85.2±0.11	87.5±0.16
6	231.2±5.27	226.6±1.76	151.6±0.95	143.2±0.56	84.6±0.25	88.3±0.88
8	217.1±1.93	200.0±1.94	106.0±0.73	94.0±0.83	83.5±0.13	87.9±1.33
10	196.0±4.57	176.0±1.97	89.0±0.58	87.3±0.23	82.2±0.36	89.4±1.25
12	163.4±4.01	132.0±1.21	87.3±0.55	84.8±0.15	81.8±0.43	86.2±0.21
14	142.2±3.34	102.0±0.88	86.3±0.55	82.1±0.31	81.6±0.19	86.9±0.23

Table 5. Mean values of body weights of the different groups of rats (g)

Body Weight	Group1 50mg/kg	Group2 100mg/kg	Group3 200mg/kg	Group4 400mg/kg	Group5 400mg/kg	Group6 Control
Initial	186±3.53	194±4.60	188±4.31	202±5.07	192±1.96	190±2.03
Final	188±3.42	203±5.14*	198±3.47*	226±6.32*	198±3.11*	210±4.55*

Initial: Group Initial Body weight (g)

Final: Body weight (g) after 14 d of experiment

*Significant differences appeared between the groups ($p < 0.05$).

means were separated using Duncan's Multiple Range Test of the same package.

RESULTS

Two days after the injection of alloxan, the mean fasting blood sugar (FBS) levels of the groups of diabetic rats increased significantly ($P < 0.05$) from 88.0, 89.2, 85.2, 85.8mg/dl (basal reading) to 277.6, 284.8, 256.4 and 265.6mg/dl respectively (Table 3).

The results obtained from Table 4 showed that there is significant difference ($P < 0.05$) in the FBS among diabetic groups (i.e. 1-4) 142.2mg/dl – 82.1 mg/dl. There was a marked reduction in FBS level from day 2 to day 14 among diabetic groups and the reduction was more glaring in group 4, 265.6mg/dl to 82.1mg/dl (Table 4) as compared with 277.6mg/dl to 142.2mg/dl in group 1. However, there was no significant difference ($P > 0.05$) between group 5 and control group although there was a reduction in the FBS level in group 5 from 91.4mg/dl to 81.6mg/dl (Table 4).

The effect of this is seen as it took a longer period of time for the fasting blood sugar level of the rats administered with 50mg/kg (Group 1) of the plant extract of *Vernonia amygdalina* to be reduced than it did for the rest of the other groups and the no significant difference ($P > 0.05$) in the weights (186±3.53 - 188±3.42) of rats in group 1 (Table 5) after two weeks of administration of VAL is an indication that the healing process was slow.

DISCUSSION

The result confirms that *V. amygdalina* has anti-diabetic properties and this was seen in the experiment conducted on the various groups of rats. All the rats injected with Alloxan became diabetic, as their fasting blood sugar (FBS) levels exceeded the normal range of between 80 - 100mg/dl. (Table 3) but when subsequently, they were given the plant extracts, their FBS levels were significantly ($P < 0.05$) lowered. (Table 4). The rats were considered treated when their FBS returned to almost their basal blood sugar levels especially those in group 4 and this took a period of two weeks. In the present study, oral administration of *V. amygdalina* decreased serum glucose and enhanced insulin level in diabetic rats. This hypoglycaemic effect may be due to depression of key gluconeogenic or the increase in the levels of glucose transports and stimulation of uptake in peripheral tissues (Ji su kim *et al.*, 2006). Another effect of this plant extract may be that it preserves the cells of islets of langerhans of β - cells functions, which result in a significant increase in insulin activity (Hossain M Z *et al.*, 1992; Yoshikawa *et al.*, 1995; Soon *et al.*, 2002; Kamiya *et al.*, 2008). It was observed that the rats in group 4 got treated quickly as compared to those in the other groups (Table 4) probably because the rats in group 4 were administered with a higher dose of the plant extract (400mg/kg) as compared with the rats in the other groups. It was also observed that the blood sugar levels of the rats in

group1 reduced gradually and slowly as compared with the rats in the other groups in the experiment. The histopathological studies conducted on the tissues of the diabetic rats administered with 50mg/kg of the plant extract revealed a severe diffuse degeneration of hepatocyte cells with the presence of large vacuoles within the hepatocytes throughout the parenchyma. There was a marked congestion of portal arteries, a degeneration of islet cells and deposition of eosinophilic hyaline materials in the surrounding tissues of the pancreas and kidney. There was also deposition of eosinophilic hyaline materials in the glomerular tufts of a few glomeruli and this was due to the diabetic condition of the rats and the slow healing process of the pancreas, kidney and the liver. Sections of the liver of the diabetized rats treated with 400mg/kg of the plant extract revealed a moderate diffuse fatty degeneration of hepatocyte cells throughout the liver parenchyma and a mild congestion of portal arteries while sections of the kidney revealed the presence of eosinophilic hyaline deposits in some glomerular mesangial cells within the renal cortex though no significant lesion was seen in the pancreas islets. When this observation was compared with sections of the liver, kidney and pancreas of the diabetic rats which were treated with 50mg/kg of the plant extract, a significant difference ($P < 0.05$) was noticed and this implies that *V. amygdalina* combats the effect of alloxan and thus reversed the damaged organs. The inference obtained from sections of the pancreas, kidney and the liver of the normal rats which were administered with 400mg/kg of the plant extract revealed no adverse effect on the tissues and organs. The administration of the plant extract on the normal rats (Group 5) was aimed at observing its hypoglycemic effect and also to know if the plant extract is lethal to animal organs and tissues. At the end of the two weeks of administration of the plant extract, the mean FBS level of the normal rats reduced significantly, from 91.4 mg/dl to 81.6mg/dl. Hence it can be concluded that the plant extract had a hypoglycemic effect on the normal rats and rats should be monitored to know when they have fallen below normal glycemia. Phytochemical screening of *Vernonia amygdalina* revealed the presence of alkaloids, tannins, saponins and cardiac glycosides though in infinitesimal amount while anthraquinones were absent.

CONCLUSION AND RECOMMENDATION

The plant *Vernonia amygdalina* has hypoglycemic effect as demonstrated in this research. It has been observed in this research that the dose of the plant administered affects the duration of the time it takes to treat the diabetic condition. Hence appropriate dosage for the human body and duration of administration depends on the degree of diabetes. *Vernonia amygdalina* has been observed to treat diabetic mellitus; there are possibilities that it still treats various other diseases of

which people are still afflicted with, like high blood pressure (hypertension). This was observed in the histological section of the liver of a diabetic rat but treated with 400mg/kg of *Vernonia amygdalina* as there was a moderate diffuse fatty degeneration of hepatocytes throughout the liver parenchyma. Research should however go on to discover the other medicinal uses of this plant so that people afflicted with different ailments and diseases can be cured.

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