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Research Article

Anti-hyperglycaemic Effect of Aqueous and Ethanolic Extract of *Vernonia amygdalina* on Alloxan Induced Albino Wistar Rat

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ABSTRACT

Diabetes continue to threaten global health especially type II which is responsible for majority of the disease mortality. The continual search for solution has prompted the investigation of plant medicinal properties. *Vernonia amygdalina* among others has been shown to possess anti- diabetic properties in Nigeria but little or nothing has been reported on that cultivated in Enugu, hence the aim of this study. Twenty four wistar albino rats were divided into six groups. Group 1 to 5 animals were induced diabetes with alloxan and administered varying doses of aqueous and ethanol extract respectively for nine days expect for group 5 which served as negative control. Group 6; the positive control was non diabetic and did not receive plant extract. Serum was collected from whole blood, assayed for blood sugar concentration and the result was statistically analyzed. A significant difference was considered at $p \leq 0.05$. After alloxan induction, the mean blood sugar level increased significantly ($p < 0.05$) in groups 1 to 5 within a range of 269. 25 to 503.50 mg/dl compared to the positive control. And after, extract was administered, there was a significant reduction ($p < 0.05$) in the mean sugar level in groups 1 to 4 (the extract administered groups) compared to the negative control. These results suggest that *Vernonia amygdalina has* an anti-hyperglycaemic effect and the ethanol extract was more effective than the aqueous extract in lowering blood sugar concentration in rats. Thus, this study confirms the anti-hyperglycaemic effect of *Vernonia amygdalina* cultivated in Enugu and recommends its consumption for the control and management of diabetes.

Keyword: *Vernonia amygdalina,* anti hyperglycaemic effect, diabetes, Enugu, extract, blood sugar.

INTRODUCTION

Diabetes mellitus, a metabolic disorder characterized by high blood glucose level than normal in human is a major health problem[1]. It is now one of the most common non-communicable diseases in the world rated as the fourth leading cause of death in most high-income countries[2]. The International Diabetes Federation (IDF) estimated in 2011 that 366 million adults, aged 20–79 years of the world's 7 billion population have diabetes giving a comparative prevalence of 8.5%[3,4]. A study conducted in 2000 project this infected number of diabetes by 2030[5] but this has been attained much earlier suggesting that diabetes will continue to increase if not sufficiently managed. There is substantial evidence that diabetes is also epidemic in many low- and middle-income countries[2]. From the African continent counts, approximately 13.6 million people are diabetic with Nigeria having the highest number of people with

diabetes (about 1,218,000 people affected). Nigeria also has the highest number of people with impaired glucose tolerance of an estimated 3,85 million people[3].

Treatment of type I diabetes is possible and effective with the use of anti-diabetic drugs like sulfonylureas, biguanides and intravenous insulin[6]. However, this is not usually the case for type II diabetes. In type II diabetes where insulin is no longer recognize for sugar metabolism, treatment of diabetes is complicated[7]. Also, with the challenges of the existing conventional medicine, it becomes necessary to seek for possible alternative treatments which can lower the blood sugar level and facilitate sugar excretion. Better still, methods for control and management are paramount to curb the challenges.

Plants serve as food to man and are consumed on daily basis. In addition to their nutritional values, plants have shown to have health and therapeutic benefits to the body[8]. Numerous plants worldwide have shown to have medicinal properties in lowering blood sugar in body and have locally been used in the management of diabetes[8, 9]. Lowering the blood sugar level is of a major public health concern since it will not only serve for treatment but also for the control and management of the disease.

Vernonia amygdalina is a plant that grows throughout tropical Africa and has been domesticated in some parts of Nigeria. It is one of the most common plants consumed as food. The leaf of this plant commonly known as "bitter leaf" is highly used for the preparation of local dishes and consumed on daily basis. *Vernonia amygdalina* is rich in phytochemicals such as alkaloids, saponins, tannins, and glycosides responsible for its bitter taste and has been shown to possess medicinal properties in several studies[10]. However, the effectiveness or healing power of a plant may depend on the region of cultivation as regional variations such as altitude, temperature have shown to affect the functioning and medicinal properties of plants[11]. Previous studies have shown this plant to have anti-hyperglycaemic properties in various locations in Nigeria[12-15] but little or nothing has been reported on the Enugu grown plant. Thus, this study investigated the anti-hyperglycaemic property of *Vernonia amygdalina* grown in Enugu using two solvents for extraction with varying doses in wistar albino rats.

MATERIALS AND METHODS
Plant material: Fresh leaves of *Vernonia amygdalina* were collected from their natural habitat in Coal Camp of Enugu North Local Government area and transported to the Chemistry laboratory of Godfrey Okoye University, Enugu State of Nigeria.

Preparation of aqueous and methanol extract: The leaves were dried at room temperature in the laboratory for 14 days after which the dried plant materials were pounded into fine powder using pistle and mortar, and packed into an air tight container until required. Two portions of 200g each of the plant powder were suspended in 700 ml of water and 1000ml of ethanol and macerated overnight for 48 hours at room temperature. The supernatant was filtered using Whatman grade no. 3 filter paper and the filtrate was concentrated by heating on water bath at 37°C for 48 hours. The solid residue was collected and preserved in a refrigerator at 4°C for experimentation.

Selection of animals and care: Matured wistar strain albino rats, 3 months of age were used for this experiment. All the animals were acclimatized for a period of 15 days under laboratory conditions prior to the experiment. Rats were kept at an ambient temperature of $25 \pm 2°C$ with a 12 hour light to 12 hour dark cycle. Rats were provided rich standard feed (Pfizer feeds, plc, Nigeria) and water *ad libitum*. The care provided was in conformity to the principles and guidelines of laboratory animal care and ethics.

Induction of diabetes mellitus: Diabetes mellitus was induced by a single intraperitoneal injection of alloxan monohydrate at a dose of 120 mg/kg body weight suspended in normal saline. Three days later, diabetes was confirmed using glucometer (one touch, life scan limited), Animals were considered diabetic if the blood glucose level was 200 mg/dl.

Experimental Design: Before the induction of alloxan, the body weight and the normal fasting blood glucose (FBS) level of the rats was taken. Only animals with normoglycaemic level were selected for the study.

At day zero, twenty four rats were divided into six groups of four animals each and induced alloxan. Three days after animals were confirmed to be diabetic (day 2), they were administered plant extract of varying doses as follows;

Group 1: Diabetic rats administered 200mg/kg of aqueous extract
Group 2: Diabetic rats administered 400mg/kg of aqueous extract
Group 3: Diabetic rats administered 200mg/kg of ethanol extract
Group 4: Diabetic rats administered 400mg/kg of ethanol extract
Group 5: Diabetic rats administered distilled water (negative control)

Group 6: Normal rats administered distilled water (positive control)
The plant extract was administered twice daily for nine days by oral compulsion. At day 12, the animals were anaesthetized with chloroform and blood was collected by cardiac puncture.

Blood sugar quantification: The blood samples were centrifuged to obtain serum and the sugar level was assayed by the enzymatic glucose oxidase method according to the instruction manual of Teco Diagnostic Glucose Oxidase Kit using the Spectrum lab 23A spectrophotometer model no: 23AO9254. The experimental design is summarized in Figure 1.

Statistical analysis: All data were expressed as Mean ± SEM (Standard Error of the Mean). The differences between the groups was compared using Analysis of variance (ANOVA) followed by the Post-Hoc multiple within group comparison test and differences were considered significant at $P \leq 0.05$.

RESULT AND DISCUSSION
Weight differences of animals in experimental research studies may affect the outcome by creating bias. Hence, it is important to ensure that the weights of the animals used are similar before experimentation. In this study, the initial weight of the animals ranged from 180 to 230kg and there was no significant differences ($p = 0.233$) between the group mean (see table 1). This implies that there was no group difference prior to experimentation.
Alloxan is widely used to induce diabetes in experimental animals by generation of reactive oxygen species that cause damage to - cells [16]. The actions of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration cause rapid destruction of -cells and thus, increase the blood sugar level[17]. Before alloxan administration (BAI), the mean glucose level for all the groups ranged between 104.00 and 142.25 mg/dl and was not significantly different among the groups ($p =.620$). After alloxan induction (AAI), the blood sugar levels increased significantly ($p < 0.05$) in all the alloxan administered groups (1 to 5) with the mean ranging from 269. 25 to 503.50 mg/dl compared to the positive control (group 6). This data confirms the action of alloxan in inducing diabetes as earlier shown in previous studies [18-20](see table 2).
Diabetes continue to threaten global health especially type II which is responsible for the majority of mortality[21, 22]. The world has continued to seek for solutions of which in recent time, herbal remedies have been proclaimed to be useful in the treatment and management of the disease[23-25]. Plants by nature are rich in phytochemicals which have shown to possess medicinal properties. Certain plants have been shown to possess antidiabetic properties when tested on animals and some have been made available for consumption as remedy for the treatment of diabetes[26]. One of such plants is *Vernonia amygdalina* which has been shown in previous studies have antidiabetic properties[27-29]. In this study, nine days after extract administration (AEA) showed a reduction in the mean sugar level in groups 1 to 4 (the extract administered groups) with the lowest concentrations recorded in group 3 and 4 (ethanol administered) groups and the concentrations were similar and not significantly different from the positive control ($p > 0.05$). But, the sugar level in group 5 (negative control) animals which were not administered extract remained high and was significantly different ($p < 0.05$) from the positive control (Table 2 and Figure 1).
These results suggest that *Vernonia amygdalina* grown in Enugu possess anti-hyperglycaemic properties as long term administration of extract lowered the blood sugar level in diabetic rats and is similar to those obtained in different locations in Nigeria [27- 31]. The ethanol extract was more effective in lowering blood sugar levels in rats than the aqueous extract of *Vernonia amygdalina*. Ethanol has been show in various studies to act as a better solvent than water in extracting phytochemicals from plant thus have mostly been used for animal studies to evaluate the effect of plant extracts[32]. The effect was also dose dependent as the 400mg/kg was more effective than the 200mg/kg for both the aqueous and ethanol extract in lowering blood sugar. The antihyperglycaemic property is attributed to the presence of phytochemicals present in the plant particularly alkaloid which has been shown to be responsible for the medicinal properties[33].
Regional variation such as altitude, temperature stress can affect the medicinal properties of plants. Some studies have demonstrated that temperature stress can affect the secondary metabolites and other compounds that plants produce which are usually the basis for their medicinal activity[34,35]. *Vernonia amygdalina* cultivated in Enugu showed similar anti-hyperglycaemic activity in animals as reported in other locations in Nigeria suggesting that the activity of this plant is not altered by the regional variation.

CONCLUSION
Vernonia amygdalina grown in Enugu equally possess anti-hyperglycaemic effect as shown in other locations in Nigeria. Since this plant commonly serves as food, we encourage its consumption in this part of the country for the control of blood sugar level and management of diabetes.

Table 1
Mean group weight of animals in grams

Group	1	2	3	4	5	6
Mean±S.E.M	219.84±19.67	194.81±26.50	206.89±20.03	196.89±10.86	212.08±17.76	190.87±19.1

Table 2
Mean glucose level in mg/dl in various animal groups

Group	1	2	3	4	5	6
BAI	122.25±41.54	104.00±7.75	120.50±10.65	118.75±3.96	111.75±8.06	102.25±8.94
AAI	450.25±34.06[b]	269.25± 49.14[b]	377.75±76.45[b]	447.50±65.12[b]	503.50±38.75[b]	106.50±6.88
AEA	202.67±42.21[a]	134.50±2.50[a]	116.50±31.66[a]	128.50±16.50[a]	475.00±28.02	113.25±7.68[a]

Legend: **BAI**: Before Alloxan Induction; **AAI**: After Alloxan Induction; **AEA**: After Extract Administration; **a:** indicates significant difference with group 5, **b**: significant difference with group 6; S.E.M: Standard Error of the Mean
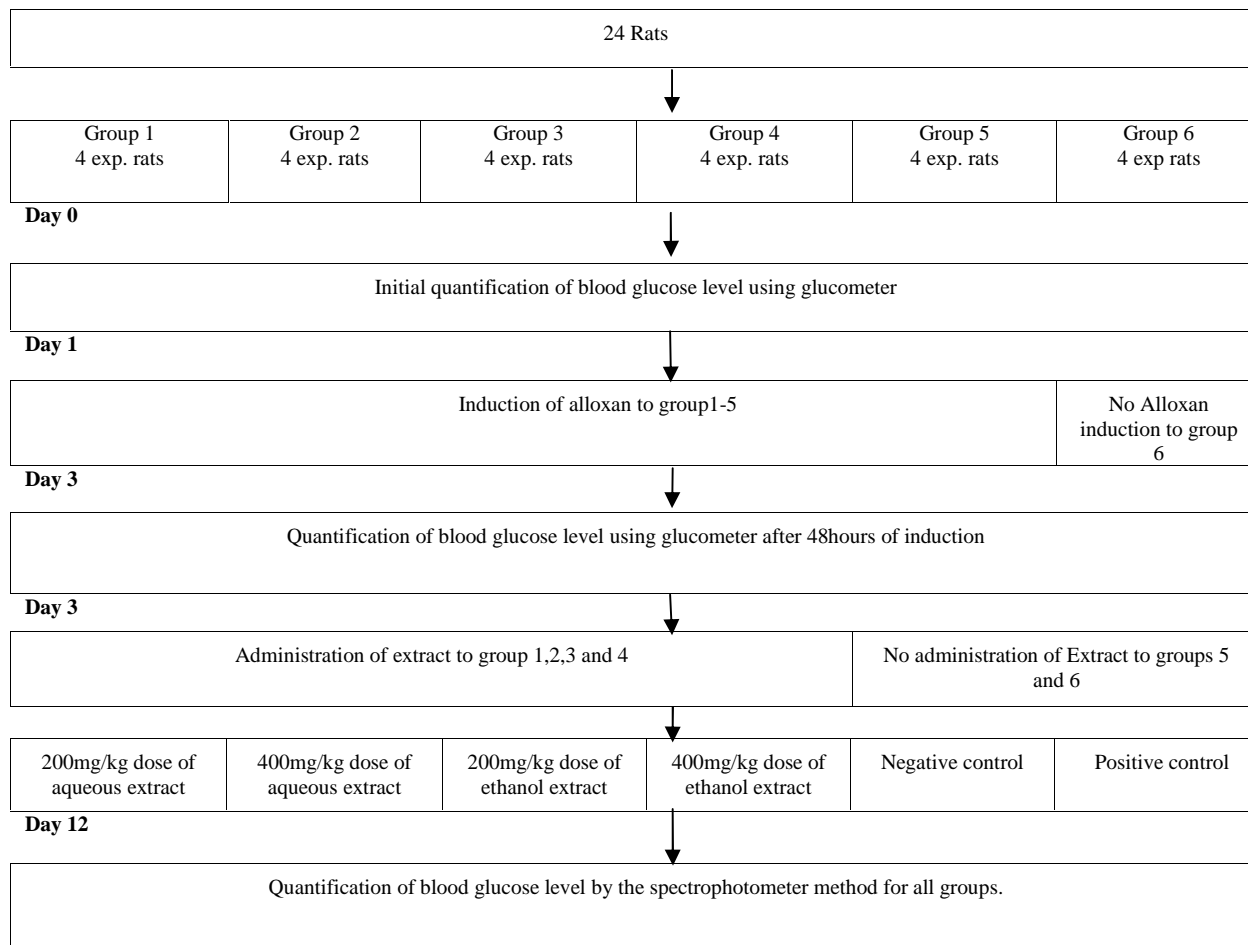
24 Rats

Group 1 4 exp. rats	Group 2 4 exp. rats	Group 3 4 exp. rats	Group 4 4 exp. rats	Group 5 4 exp. rats	Group 6 4 exp rats

Day 0

Initial quantification of blood glucose level using glucometer

Day 1

Induction of alloxan to group1-5	No Alloxan induction to group 6

Day 3

Quantification of blood glucose level using glucometer after 48hours of induction

Day 3

Administration of extract to group 1,2,3 and 4	No administration of Extract to groups 5 and 6

200mg/kg dose of aqueous extract	400mg/kg dose of aqueous extract	200mg/kg dose of ethanol extract	400mg/kg dose of ethanol extract	Negative control	Positive control

Day 12

Quantification of blood glucose level by the spectrophotometer method for all groups.

Figure 1
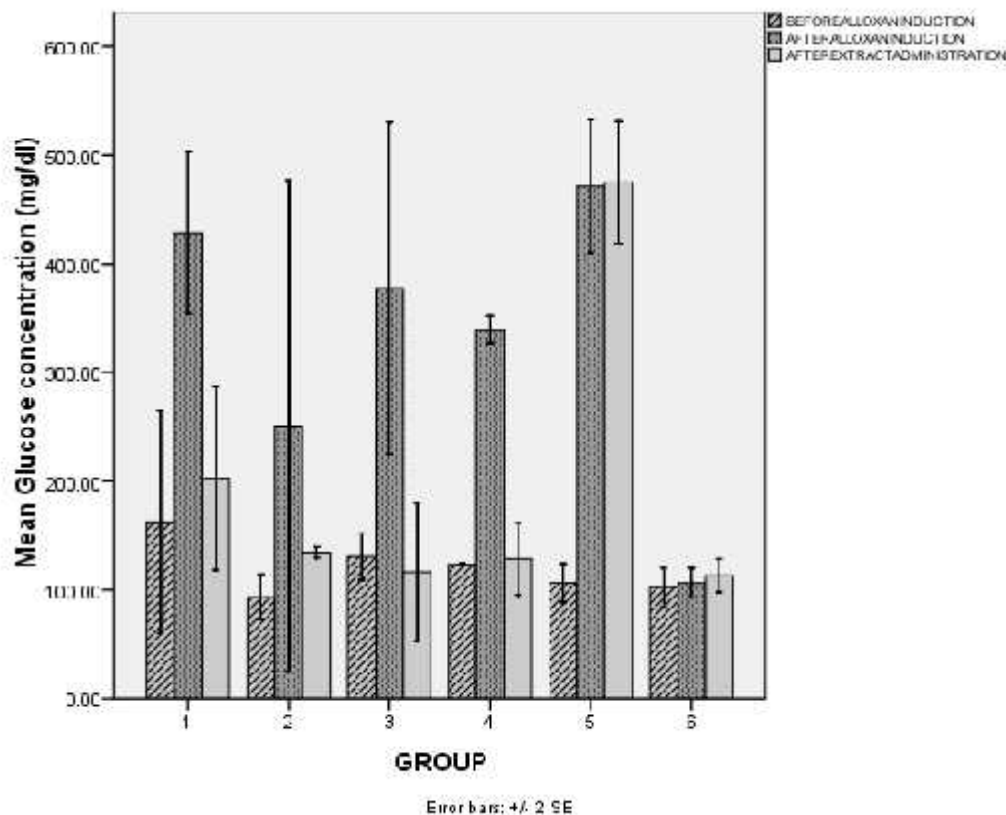**Experimental design chart**

Figure 2
Blood sugar level before and after alloxan induction and after extract administration
in various animal groups

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