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The Hypoglycemic and Hypolipidemic Effects of the Aqueous Extract of Vernonia Amygdalina Leaves on Alloxan Induced Diabetic Albino Rats

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Abstracts: The study was conducted to determine the hypoglycemic and hypolipidemic effects of the aqueous extract of *vernonia amygdalina* leaves extract. Healthy albino rats weighing between 200g and 230g were used. The rats were divided in to four different groups each containing five albino rats respectively. Three of the groups (b, c and d) were induced with diabetes by the administration of alloxan monohydrate at a concentration of 150mg/kg through intraperitoneal injection. The fasting blood glucose of the rats and their weights were measured before and after the induction of diabetes using glucose metre. After the induction of diabetes the rats were treated using the aqueous extract of *vernonia amygdalina* leaves at different concentrations (100, 150 and 200mg/kg) respectively according to group daily, where as, the other group (a) was not given any treatment and this served as the normal control, providing a baseline data. The experiment lasted for two weeks after which the animals were sacrificed by surgical dislocation of the neck following 16 hours fasting. The serum was obtained and used for the analysis of lipid profile and fasting blood glucose. All the treatment groups showed a significant decrease in fasting blood glucose level ($p<0.05$) when compared with their respective alloxan induced diabetic values. Also, all the treatment groups did not have significantly different values from the normal control ($p>0.05$) in the case of the parameters of lipid profile. Therefore, it was concluded that the aqueous extract of *vernonia amygdalina* leaves had both hypoglycemic and hypolipidemic effects on alloxan induced diabetes.

Keywords: *Vernonia amygdalina*, alloxan induced diabetes, hypoglycemic, hypolipidemic

INTRODUCTION

The practice of traditional herbal medicine is as old as man, and has been employed in the treatment and prevention of various diseases and illnesses (Bullough and Leary, 1982).The technique usually requires using plant parts such as roots, leaves, barks or flowers to prepare decoctions, syrups, extracts, or tincture for medicinal purposes. Many of these medicinal plants are part of our diet as spices, vegetables and fruits. The scope of herbal medicine is sometimes extended to fungal and bee products as well as mineral, shell and certain animal parts (Acharya and Anshu, 2008). This technique works by exploring various reactive ingredients possessed by plants, which work together to bring about a beneficial effect of medicinal value (Ahmad and Owais, 2006).

In the 19th century when chemical analysis first became available scientists began to extract and modify the active ingredients from plants. Later chemists began making their own versions of plant compounds and overtime the use of herbal remedies declined in favor of formulated drugs (Aguwa, 1996). But, recently, the WHO estimated that 80% of people worldwide rely on herbal medicine for some parts of their primary health care (Iwu, 2002).

Vernonia amygdalina, a member of *Asteracea* family is a leafy vegetable that grows up to 5m high, with abrogate to oblanceolate leaves. This species is usually found in gardens, and commonly found in Nigeria, Cameroon, Gabon and Congo (Dalzrel, 1936). The leaves are green with a characteristic bitter taste. No seeds are produced and the tree has, therefore, to be distributed through cutting. The plant is widely distributed in west coast of Africa where it grows wild and as a domestic browse plant (Farombi, 2003). This plant has, however, been named differently by different ethnics around the world: Ewuro in Yoruba, Etidot in Ibibio, Onugbu in Igbo, Ityuna in Tiv, Deri in Waja, La`yin Tulin in Dadiya and Chuwaka in Hausa.

V. amygdalina is generally raised by stem cutting and are planted at an angle of 45 to obtain faster

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sprouting (Akinpelu, 1999). It is medically of value in curing fever, lascative, pile (haemorrhoids) gastrointestinal trouble and diabetes (Akah and Okafor, 1992). In fact, all parts of the plant have been known to be pharmacologically useful. Oral administration of the aqueous leaf extract of the plant was found to relieve pain (Bullough and Leary, 1982).

Nutritionally, *V. amygdalina* is used mainly in soup making in the tropics and also as an appetizer and febrifuge (Eleyinmi *et al*., 2008), and has proven to be a successful supplement in many food (Ojiako and Nwanjo, 2006).

V. amygdalina can be classified as a healthy food because it promotes the healthy development of the body. It contains not only the active drug molecules but also other substances that are necessary for maintaining health and physiological functions of the body without manifestation of toxicity (Iwu, 2002). As a result, *V. Amygdalina* serves as a low cost and a readily available source of important nutrients to humans. In Nigeria it is a major vegetable of the celebrated "bitter leaf soup". The leaves may be consumed either as a vegetable (after removing its bitter taste through soaking in several changes of water or by boiling) or an aqueous extract as tonic for treatment of various illnesses (Iwu, 2002). *V. Amygdalina* has been shown to contain significant quantities of lipid (Ejoh *et al*., 2007) and protein with high essential amino acids (Eleyinmi et al., 2008). The plant has also been shown to contain appreciable quantity of ascorbic acid and caroteinoids (Udensi *et al*., 2002; Ejoh *et al*., 2007). *V. Amygdalina* produces a variety of flavonoids and bitter sesquiterpene lactones which contributes to its bioactivity (Nangedo *et al*., 2002).

Igile *et al*., (1994), reported that traditional medicine practitioners use the plant as anti-helminthes, anti-malaria and as a laxative. Others use it as a digestive tonic, appetizer, and for the tropical treatment of wounds (Iwu, 1986). Dalzrel (1936), was the first to report that the root and twig of the plant are used for the treatment of stomach and gastro intestinal problems by the Hausa`s of northern Nigeria, while the decoction from the leaves is used in treating malaria fever in Guinea and cough in Ghana. In some parts of Nigeria, the stems are used as Chewing sticks for oral hygiene and for management of some dental problems. In Malawi and Uganda, *V. Amagadalina* is used by traditional birth attendants to aid the expansion of the placenta after birth, aid post-partum uric contraction, induced lactation and control harmorrhage (Bullough and Leary, 1982). Among the people of southern Nigeria, *V.amygdalina* has a high reputation for use in the traditional management of diabetes mellitus; scientific studies have also confirmed it as an anti-hyperglycemic agent (Akah *et al*., 2004). The aqueous leaf extract has been shown to posses anti-hyperlipidemic and hypolipidemic effects on diabetic rats (Atangwho *et al*., 2007). Recent studies indicate that extract of Bitter leaf exerts antibiotic action against drug resistant micro-organisms and can prevent or delay the onset of breast cancer. Bitter leaf extracts at physiologically relevant concentration inhibited DNA synthesis in a breast cell line (Jisaka *et al*., 1993).

Diabetes mellitus is a global epidemic affecting essentially biochemical activities in almost every age group and also the most severe metabolic pandemic of 21st century, affecting biochemical activities in almost every cell in the body (Aguwa, 1996). It was estimated by WHO that about 30 million people suffered from diabetes in 1985, and in the year 2000, 171 million people had diabetes (Tiwari and Rao, 2002). This is expected to double by the year 2030 and this large increase is expected to occur in developing countries, especially in people of ages ranging from 45 and 65 years (Boon et al., 2006). Diabetes mellitus is a multifunctional disease which is characterized by hyperglycemia (Ugochukwu and Babady, 2003), lipoprotein abnormalities and raised basal metabolic rate (Owu *et al*., 2006). It is a heterogeneous primary disorder of carbohydrate metabolism with varied etiology culminating in absolute relative insulin deficiency or insulin resistance or both. There is a reservoir of basic information that suggests the involvement of oxidative stress in pathogenesis of diabetes mellitus. This can increase lipid peroxidation and development of insulin resistance, which promote the development of complications in diabetes mellitus (Demozay *et al*., 2008).

Alloxan is a toxic glucose analogue, which selectively destroys the insulin producing cells (beta cells) in the pancreas (Prince and Menon, 2000). Alloxan is used to induce experimental diabetes by selectively destroying the beta cells of the pancreas. It is taken up by the pancreatic beta cells and subsequently generates reactive oxygen species (ROS), which contribute to the DNA fragmentation and evoke other deleterious changes in the cells (Lenzen and Panten, 1998). This research is aimed at determining the hypoglycemic and hypolipidemic effects of the aqueous extract of *Vernonia amygdalina* leaves on alloxan induced diabetic albino rats.

MATERIALS AND METHODS

Plant

The plant used in this experiment is leaves of *Vernonia amygdalina* (bitter leaf) obtained from a local market in kwadon, Gombe state, Nigeria. It was then identified by Prof. A.G Ezra, Department of

Biological Sciences Gombe State University. Healthy fresh leaves were sorted and washed to remove debris and dust particles without squeezing and was air dried. The dry leaves were then pounded into fine powdery form using Pestle and Mortar.

Extraction of the aqueous extract of the plant
Fourty (40) gram (40 g) of the dried leaves powder was soaked in 800ml of distilled water and it was left to stand for 24hours before filtering through a muslin cloth. The filtrate was concentrated using a rotary evaporator maintained at 45^0C and further air drying. Four (4) gram of the aqueous extract obtained from above was weighed and dissolved in 50ml distilled water to get 8% of the solution.

Experimental animals
Twenty (20) healthy albino rats of both sexes weighing between 200g and 230g were used for this experiment. They were obtained from the National Research Institute Vom. They were kept in the biological farm of the Biological Sciences Department of Gombe State University under standard conditions. The rats were maintained on a diet of poultry grower's marsh (Vital feed Jos, Plateau State) containing 14.50% crude protein, 7% fats, 7.20% crude fibre, 0.8% calcium, 0.4% available phosphorus 40% minerals, 1% vitamin and 54% carbohydrates. They have access to clean water. The animals were kept for two (2) weeks to acclimatize prior to the commencement of the experiment. The rats were divided in to four groups, each consisting of five rats, on the basis of their weights. The rats were grouped as follows:

Group A (Normal control) - The five rats in this group were given growers' mash and normal saline throughout the experimental period. Parameters from this group served as baseline data.

Group B (Diabetic + 100mg/kg of extract)- The rats in this group were maintained on the same feed as the normal control but in addition they were diabetic and given the plant extract at a concentration of (100mg/kg) throughout the period of the research.

Group C (Diabetic + 150mg/kg of the extract)-The rats in this group were maintained on the same diet as group B, they were also diabetic but were treated using 150mg/kg of the extract throughout the period of the research.
Group D (Diabetic + 200mg/kg of the extract) - The rats in this group were also diabetic and were maintained on the same diet as other groups but were treated using 200mg/kg of the extract.

Induction of Alloxan
Diabetes was induced by a single injection of freshly prepared Alloxan monohydrate (150mg alloxan per kg). It was dissolved in distilled water and administered intraperitoneally (IP) to rats after fasting for at least 16 hours. Blood glucose level was measured 72 hours after alloxan administration using Glucose metre. Rat with fasting blood glucose of more than 200mg/dL is considered to be diabetic and hence use in this research as suggested by other researchers (Stanley and Venogopal, 2001).

Blood sample collection
After 14 days of the treatment, the rats were fasted for 16 hours and made unconscious with 10% chloroform before sacrificing them by surgical dislocation of the neck and allowing free flow of blood into sterile universal bottles. The blood sample was allowed to clot and serum separated and was used to determine the lipid profile parameters.

Methodologies for determination of biochemical parameters
Fasting Blood Glucose test
This was done using glucometer by fixing the glucometer test strips on the glucometer while a drop of blood was applied on the tip of the strips for the determination of the glucose level. This test was done before the induction of diabetes; 3days after the induction of diabetes and on the 14^{th} day of the treatment of diabetes, by pricking the tail to get the blood sample after the animal must have fasted for at least 16 hours.

Serum Lipid Estimation
Triglycerides by the method of (Cole *et al*., 1997).
Principle:
The triglycerides were determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen-peroxide 4-amino phenazone and 4- chlorophenol under the catalytic influence of Peroxidase.

Triglycerides + H_20 $\xrightarrow{\text{lipase}}$ glycerol +fatty acids

Glycerol +ATP $\xrightarrow{\text{cholesterol esterase}}$ glycerol-3-phophate + ADP

Glycerol-3-phosphate+0_2 $\longrightarrow$ dihydroxyacetone + phosphate +H_20_2

$2H_20_2$ + 4-aminophenozone + 4-chlorophenol $\longrightarrow$ quinoneimine + HCl + $2H_2O$

Procedure
Reagents and samples were brought to room temperature in to test tube arranged and labeled as test, standard and blank, ten microliter (10ul) of the serum and standard were added to the appropriate test tubes and 1000ul of the reagent was added to all test tubes. The content of the tubes were mixed and incubated at 25^0C for 20 minutes. Absorbance of the sample and standard were measured within 60 minutes against reagent blank at a wave length of 500nm using a spectrophotometer.

Total cholesterol by the enzymatic end point manual method of Trinder, (1969).

A prepared kit for cholesterol estimation was used.

Principle: Cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator is quinoneimine formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxide.

Cholesterol ester $+H_20$ Cholesterol esterase cholesterol + fatty acid

Cholesterol + 0_2 cholesterol 3-0ne + H_20_2

$2H_20_2$ + phenol + 4 –aminoantipyrine quinoneimine + $4H_20$

Procedure:

To the test tubes arranged and labeled as test, standard and blank, 10ul of test and standard were added to the appropriate tubes; then 100ul of reagent was added to all test tubes. The content of the tubes were mixed and incubated for 5 minutes at 37^0C. The absorbance of the sample was measured at a wavelength of 500nm against blank within 60 minutes using a spectrophotometer.

Determination of HDL – cholesterol using the enzymatic end point manual method of Trinder, (1969).

After precipitation of other fractions with magnesium chloride, the HDL- cholesterol content in the supernatant fluid is estimated as for total cholesterol using the method of Trinder (1969)

LDL-cholesterol estimation using the Friedewald equation (Friedewald *et al*., 1972).

Concentration of LDL Cholesterol= Total cholesterol-(HDL Cholesterol + Triglyceride/5)

RESULTS

The results of the fasting blood glucose are summarized in tables 1, 2 and 3. In Table 1, the fasting blood glucose was compared before the induction of alloxan and 3days after the induction of alloxan. When the normal control was compared before and after the induction, there was no significant difference ($p> 0.05$) but when the test group were compared before and after induction, there was significant difference ($p<0.05$).

From Table 2, the fasting blood glucose of rats was compared 3days after the induction of alloxan and after the experiment; the summarized result showed a significant difference when compared after the induction of alloxan and after the treatment with extract ($p<0.05$). From Table 3 which showed the fasting blood glucose of rats before and after the experiment, it can be deduced also that there is equally a significant difference in the fasting blood glucose before and after the experiment ($p<0.05$).

The results of the body weight of rats were summarized in Tables 4 and 5. In Table 4; the rats' body weight before the induction of alloxan and 3days after the induction of alloxan were compared, there was no significant difference ($p>0.05$) in the body weights of the rats in all the groups.

From Table 5, the rats' body weights were compared before the experiment and after the experiment. In the normal control group (group A) there was significant difference ($p<0.05$) before and after the experiment on the other hand there was also a significant difference between the members of the test groups.

The result of the lipid profile was summarized in Table 6. The total cholesterol; when the normal control was compared with the test groups there was significant difference with group B, when it was compared with other test groups but as the dosage was increased (150mg/kg and 200mg/kg) that is in groups C and D, there was no any significant difference with the normal control and between them ($p>0.05$).

Triglyceride revealed significant difference when the normal control was compared with groups B and C but when compared with group D there was no any significant difference but when comparison was made within the groups, there was a significant difference ($p <0.05$).

High density lipoprotein showed no any significant difference ($p>0.05$) when test groups were compared with the normal control but group B showed significant difference ($p<0.05$) when compared with the normal control and within the group.

Low density lipoprotein showed no any significant difference ($p>0.05$) when the normal control was compared with the test groups and also when comparison was made within the test groups.

Table 1. The fasting blood glucose of the rats in mMol/L before the induction of alloxan and 3 days after the induction of alloxan.

Period	A (normalcontrol)	B(test group+100mg/kg)	C(testgroup+150mg/kg)	D(test group+100mg/kg)
Before	4.89 ± 0.54^a	4.12 ± 0.72^a	4.20 ± 0.91^a	4.72 ± 0.36^a
After	4.89 ± 0.54^a	20.07 ± 4.00^b	17.65 ± 2.10^b	19.98 ± 3.18^b

Values are mean ± S.D of 4 different replicates. Values with different superscripts down the group are statistically different at ($p < 0.05$).

Table 2. The fasting blood glucose of the rats in mMol/L 3days after the induction of alloxan and after the experiment

Period	A (Normal control)	B(Testgroup+100mg/kg)	C(Testgroup+150mg/kg)	D (Test group+ 200mg/ kg)
Before	4.89±0.54[a]	20.07±4.00[a]	17.65±2.16[a]	19.98±3.18[a]
After	4.71±0.86[a]	13.90±0.10[a]	15.12±2.23[a]	12.16±0.5[b]

Values are mean ± S.D of 4 different replicates. Values with different superscripts down the group are statistically different at ($p < 0.05$).

Table 3. The fasting blood glucose of the rats in mMol/L before the experiment and after the experiment.

Period	A (normal control)	B (test group+100mg/kg)	C(testgroup+150mg/kg)	D (test group+ 200mg/kg)
Before	4.89±0.59[a]	4.12±0.09[a]	4.20±0.91[a]	4.72±0.36[a]
After	4.51±0.86[a]	13.90±1.00[b]	15.12±2.23[b]	12.16±0.52[b]

Values are mean ± S.D of 4 different replicates. Values with different superscripts down the group are statistically different at ($p < 0.05$).

Table 4. The body weight in gram of the rats before the induction of alloxan and 3 days after the induction of alloxan.

Periods	A (Normal control)	B (test group +100mg/kg)	C (test group+150mg/kg)	D (testgroup+200mg/kg)
Before	106.75±9.29[a]	176.37±23.46[a]	195.25±3.18[a]	154.67± 11.22[a]
After	109.00±8.64[a]	171.67±22.91[a]	191.00±30.50[a]	149.33±11.37[a]

Values are mean ± S.D of 4 different replicates. Values with different superscripts down the group are statistically different at ($p < 0.05$).

Table 5. The body weight in gram (g) of rats before the experiment and after the experiment

Period	A (normal control)	B (test group+ 100mg/kg)	C (test group+ 150mg/kg)	D (test group+200mg/kg)
Before	106.75±9.29[a]	176.37±23.46[a]	195.25±3.18[a]	154.67±11.22[a]
After	150.50±15.15[b]	155.67±19.55[b]	172.00±27.75[b]	122.33±9.87[b]

Values are mean ± S.D of 4 different replicates. Values with different superscripts down the group are statistically different at ($p < 0.05$).

Table 6. Lipid profile of the rats

Group of rats	Cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
A (normal control)	71.94±14.05[a]	106.18±20.84[ab]	35.65±9.53[ab]	15.11±0.59[a]
B(test group+ 100mg/kg)	45.44±5.74[b]	53.13±22.52[abc]	23.24±4.26[ab]	11.53±3.11[a]
C (test group+ 150mg/kg)	67.76±8.74[a]	74.87±11.26[abc]	38.69±3.04[abc]	14.29±8,69[a]
D(test group+ 200mg/kg)	69.12±5.79[a]	105.92±3.09[ab]	34.58±10.34[ab]	13.35±12.87[a]

Values are mean ± S.D of 4 different replicates. Values with different superscripts down the group are statistically different at ($p<0.05$).

DISCUSSION

The hypoglycemic effect of *Vernonia amygdalina* on alloxan induced diabetic rats was examined in this experiment. The most routine and biochemical marker used in the diagnosis and progress monitoring during management of diabetes mellitus in both clinical and experimental settings is serum glucose concentration (Mayfield, 1998). This was measured in this study and was seen to increase significantly ($p<0.05$) in all the groups administered with alloxan, where as, there was no any increase in the normal control group (Table 1).This indicated that all the treatment groups have been induced with diabetes mellitus evident by increased Fasting blood glucose concentration observed in the groups (Table 1). However, after treatment with the leaves extract of *V. amygdalina* there was decrease in fasting blood glucose level in the groups given 100mg/kg and

150mg/kg though not statistically significant ($p > 0.05$) as is also the same with the normal control group, but there was significant decrease in fasting blood glucose in the group given 200mg/kg of the extract ($p < 0.05$) (Table 2) .This suggests that the effect of the extract in lowering the blood glucose level is dose dependent. The result clearly showed that after the administration of *V. amygdalina* at varying levels of 100mg/ kg, 150mg/ kg and 200mg/kg there was observed hypoglycemic effects. This study, therefore, confirmed the effectiveness of this plant in the ethno therapy of diabetes mellitus as shown by past research (Akah and Okafor 1992). According to the report of Igile *et al.*, (1994), the leaves of *V. amygdalina* contains biflavonoids such as luteolin 7-o-B, glucoside and luteolin 7-oB-glucoronide, besides several stimastine type saponins such as vernonioside A1,A2,A3,D3 and C which have also been isolated from the leaves of *V. amydalina* (Jisaka *et al*., 1992). In addition *V. amygdalina* leaves have been reported to contain bioactive sequistarpenes lactones such as vernolide and vernodalol (Erasto *et al.*, 2006). Therefore, it might be suggested that the hypoglycemic activity of *V. amygdalina* as reported in this study may be a function of its rich flavonoids content.
More also, study conducted by Ojewale, (2006), showed that flavonoids such as quercetin improves hyperglycemia and islet morphology in streptomycin induced diabetic rats. Besides Adewole *et al*., (2009), reported the beneficial effects of the aqueous extract of *Annona muricatalin* leaves on blood glucose of streptomycin induced diabetic rats. They concluded that plants bioflavonoids and coumarins may play important roles in the establishment of normal glyceamia in diabetic rats. Similar reference had been drawn by (Ojewale 2006; Akinola *et al.*, 2009).

Although not fully understood, several reports have attempted insights into the hypoglycemic mechanisms of plant extract; Sonia and Scrinivason, (1999), in their report presupposed increased peripheral glucose uptake by inhibition of the action of insulin by inhibitory glycogenesis. Also, Atangwho *et al*., (2007b), had suggested in their earlier report on *V. amygdalina* that two possible mechanisms exist: one targeting insulin production from the islet cells and the second on peripheral carbohydrates; mechanisms involving insulin productions are usually more potent.

In diabetes the obligatory renal water loss combined with hyper osmolarity tends to deplete intracellular water, triggering the osmo receptor of the thirst centre of the brain and polydipsia which will lead to water intake (Boon *et al*., 2006). The catabolic effect then prevails resulting in weight loss. This was also observed in this study three days after the induction of diabetes with alloxan there was decrease in weight in all the groups administered with alloxan though not significant ($p>0.05$), but this is not the case with the normal control group which does not show any decrease in weight (Table 4).But following the administration of the leaves extract of *V. amygdalina*, the frequent urination decreased, and water consumption also decreased. But there was significant decrease in weight in all the treatment groups and significant increase in weight in the normal control group (Table 5). The fact that the fasting blood glucose level and weight of all the treatment groups did not return to the pre treatment level as can be seen in Tables 3 and 5 respectively, could be linked to the fact that *V. amygdalina* alone could not return the serum glucose level to normal glycaemia and also the weight of diabetic rats to pre treatment level after just 14days of treatment as established by past research (Atangwho *et al.*, 2007b).

High level of triglycerides, LDL- cholesterol, VDL-cholesterol and low level of HDL have been associated with heart disease, insulin resistance and diabetes mellitus (Atangwho *et al*., 2007a). The abnormally high concentration of serum lipids in diabetes is mainly due to increase in the mobilization of fatty acid from the peripheral fat deports (Akah *et al*., 2004). In this study, the values obtained for all the parameters of lipid profile; Cholesterol, LDL, TG, and HDL (Table 6), appeared to show no significant difference when comparison was made within each treatment group and between each treatment group and the normal control especially at 200mg/kg of the extract. This indicates that the leaves extract of *V. amygdalina* can serve as a cure to heart diseases induced as a result of diabetes. In this study the aqueous extract of *V. amygdalina* has been shown to have hypolipidemic effect in the diabetic rats since the values of the test groups especially at a concentration of 200mg/kg and the normal control showed no significant difference, this is in line with the report of Akah *et al*., (2004).

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