Original Research Article

INVESTIGATION OF HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECTS OF METHANOLIC EXTRACTS OF BITTER LEAF [*Vernonia amygdalina*] IN MALE RATS

ABSTRACT

Different agents used for treatment of diabetes mellitus are associated with serious adverse effects. This necessitates the scientific quest for substitutes that are comparatively less noxious- usually medicinal plants (e.g. *Vernonia amygdalina*). Diabetes was induced in rats in groups A-D using alloxan. Group E served as control. Once daily for 28 days, diabetic rats in groups B and C were treated with 50 & 100 mg/kg BW of methanolic extracts of *Vernonia amygdalina* respectively while group D rats were administered with metformin hydrochloride (250 mg/Kg BW). Rats in group A served as untreated diabetic group. Fasted blood samples were collected for estimation of fasting blood glucose (FBG); total cholesterol (TC), triglyceride (TG), and high density lipoprotein-cholesterol (HDL-C) using standard biochemical methods. Statistical analysis was carried out using Student's t test, $P<0.05$ was considered significant. After treatment, there were significant decreases in levels of FBG, TC, TG, LDL-C but increase level of HDL-C of B, C, & D when compared with group A. results of the study suggest that methanolic extract of *V. amygdalina* possesses antidiabetic properties and anti-hypolipidemic effects; this may explain why this plant is traditionally used for the management of diabetes mellitus and its complications.

Keywords: *Vernonia amygdalina;* diabetes mellitus; lipid profile; blood glucose

1.0 INTRODUCTION

Plants serve various purposes and their usefulness to man is not limited to their role as sources of raw materials for industries; they are also consumed as food and sometimes used as medication. For ages, plants have provided man with diverse means of healing. In fact, many parts of plants such as fruits, seeds, barks, roots, and flowers have been used as medication to provide alternative therapies for various diseases that affect man and animals [1]. In Asian countries like China and Indian, plants and plants products are used to support the primary healthcare system especially in the treatment of many diseases [2].

Medicinal plants contain potentially useful chemicals that are currently used for the manufacturing of modern therapeutic agents [3]. The evaluation of medicinal plants, used traditionally in treating diabetes is of growing interest. The World Health Organization [4] recommended and even encouraged this practice especially in countries where access to conventional treatment of diabetes is inadequate.

Diabetes mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycemia that occurs from defective insulin secretion, insulin action or both, and consequently result in impaired carbohydrate, lipid and protein metabolism. Currently, diabetes mellitus is one of the main causes of serious maladies in the 21st century. The world population of diabetes mellitus in year 2008 was approximately 150 million and the population of this pandemic was expected to more than double by the year 2025 [4-7].

Diabetes has been implicated to be the leading cause of secondary hyperlipidemia.

Hyperlipidemia involves abnormally elevated levels of any or all lipids and/or lipoproteins in the blood [8]. Other complications of diabetes mellitus include; retinopathy, neuropathy, micro-vascular and macro-vascular diseases, nephropathy etc. The treatment of diabetes without adverse effects still poses a challenge. Hence, medicinal plants continue to play a crucial role in the discovery of new compounds for the management of this disease.

Vernonia amygdalina contains a wide range of phytochemicals with significant antioxidant potential. No doubt, this accounts for its use in folkloric medicine for treatment of diseases such as eczema, measles and anemia [6]. This study was designed to provide information on *Vernonia amygdalina* as a source of treatment for diabetes and one of its metabolic complications- hyperlipidemia.

2.0 METHODOLOGY

2.1 Experimental animals:

Thirty (30) male adult albino wistar rats weighing averagely 160 g were used for the study. The study was conducted in the Animal House of Ladoke Akintola University of Technology, Mercyland campus, Osogbo. The rats were allowed to acclimatize for two weeks after which they were divided into five groups of six rats each. Their access to water and standard pellet feed was without any restriction. The animal study was carried out in conformity with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research; as promulgated and adopted by United States Institutes of Health (1985).

2.2 Preparation of extract:

Fresh leaves of *Vernonia amygdalina* was plucked from Ifon village, Osun State (Nigeria). It was washed, air-dried and crushed into coarse powder using mortar and pestle. Approximately 1650 g of the powder was loaded into a thimble and continuously extracted with 95% methanol in a Soxhlet extractor for 24 hours. The solvent was distilled off in the rotary evaporator to obtain a solid residue. The concentrates were left opened in a water bath (40°C) for complete dryness before extract was transferred in airtight container and then stored in a refrigerator at 4°C. The percentage yield from 1650 g of powder was 16.36% w/w (27 g).

2.3 Qualitative Determination of Phytochemicals:

Qualitative estimation of phytochemical contents in methanolic extracts of *V. amygdalina* was carried out using the method of Ghambal et al. [9]. *V. amygdalina was* screened for flavonoids, tannins, saponins, deoxy-sugar, alkaloids, anthraquinones, terpenoid,

phlobatanins, and cardiac glycosides. Results were described qualitatively as present, highly present, more highly present or not detected.

2.4 Induction of diabetes:

Experimental diabetes was induced in male wistar strain albino rats by intraperitoneal injection of alloxan monohydrate dissolved in saline at a concentration of 100 mg/mL as described by Rohilla and Ali [10]; the dosage level chosen was 100 mg/kg of body weight. In the first 24 h after alloxan injection, the animals were given unrestricted access to 5% glucose solution to prevent alloxan-induced hypoglycemia that is capable of causing mortality. Fasting blood was collected from the tail snips of the rats and used to confirm diabetes after 72 hours of induction using accu-check active glucose meter (Roche Diagnostic GmbH Sandhofer Strasse, Ref:05234441049, Germany). Animals with blood glucose >200 mg/dL were considered diabetic.

2.5 Experimental design/treatment/blood sample collection:

The rats with blood glucose >200 mg/dL were divided into groups A – D. While group A served as the diabetic control (untreated), methanolic extract of *Vernonia amygdalina* was administered to Groups B and C at dosage levels of 50 & 100 mg/kg body weight (BW) respectively. At dosage level of 250 mg/Kg BW, Glucophage (metformin hydrochloride) was administered to Group D. In all cases, route of administration was by gastric gavage. Group E served as the control. The treatment was given once daily for 28 days and the body weight of the rats were monitored weekly.

On day 29 after an over-night fast, the rats were sacrificed by cervical dislocation. Blood samples were collected through cardiac puncture into anti-coagulant free tubes and fluoride oxalate bottles (for glucose estimation). The samples collected into anti-

coagulant free tubes were centrifuged at 3,500 rpm for 10 minutes to obtain sera which were used for the estimation of lipid profile.

2.6 Biochemical analyses:

All chemicals used were of high purity grade and was supplied by Sigma-Aldrich Inc. (St. Louis, MO, USA). Fasting blood glucose was estimated using commercial kit from AGAPPE Diagnostics Switzerland GmbH Knonauerstrasse 54 - 6330 Cham – Switzerland. Total cholesterol [TC] determination was by the enzymatic method described by Allain *et al.*, [11]. Triglyceride [TG] was determined by the method of Buccolo and David [12]. Concentration of low density lipoprotein [LDL-C] was obtained using the Friedewald's formula [13] expressed as:

$$\text{LDL-C} = \text{Total cholesterol} - \text{HDL Cholesterol} - \frac{TG}{2.2}$$

2.7 Statistical analysis: Results were reported as mean ± standard error of mean. Mean values were analyzed using Student's t test, with statistical package for social sciences (SPSS) version 18.0. Results were regarded as significant at $p<0.05$.

3.0 RESULTS

The results of the study are shown in Figures 1-2 and Tables 1-4 below. The results of fasting blood glucose and lipid profile are summarized in Figures 1 and 2. Table 1 shows the mean ± standard error of mean values of fasting blood glucose (FPG) levels of the wistar rats in groups A-E. There was significant increase in blood glucose level of wistar rats following the administration of alloxan compared with group E (control). There was a significant decrease ($p < 0.05$) in fasting blood glucose level in the diabetic rats treated with 50 mg/kg BW and 100 mg/kg BW extract of *V. Amygdalina* and rats in group D (treated with glucophage) when compared with group A (untreated diabetic group).

Significant decrease in the mean value of fasting blood glucose level ($p < 0.05$) was observed in group C treated with 100 mg/kgBW extract of *V. amygdalina* as compared with group D treated with standard ant-diabetic drug (glucophage). However, no significant difference was seen in group B treated with 50 mg/kg BW of *V.amygdalina* extract when compared with group D treated with glucophage.

Table 2 shows the mean ± standard error obtained for the lipid profiles of the five groups of the wistar rats. The results showed significant ($p < 0.05$) increases in the mean values of TC, TG, LDL-C and decrease in HDL-C of the test groups (A, B, C, D) when compared with group E (control). On the other hand, there were significant ($p < 0.05$) decreases in the mean values of TC, TG, LDL-C and increase in HDL-C of the test groups (B, C, D) when compared with group A (untreated diabetic group).

Table **3** shows the mean ± standard error of mean of the initial and final body weight of

the experimental rats. The results showed significant ($p < 0.05$) increase in the body weight of rats in Groups B-E when initial body weight of each group was compared with final weight whereas the body weight of rats in Group A was significantly reduced at the end of the experimental i.e. when final weight was compared with initial weight ($P<0.05$).

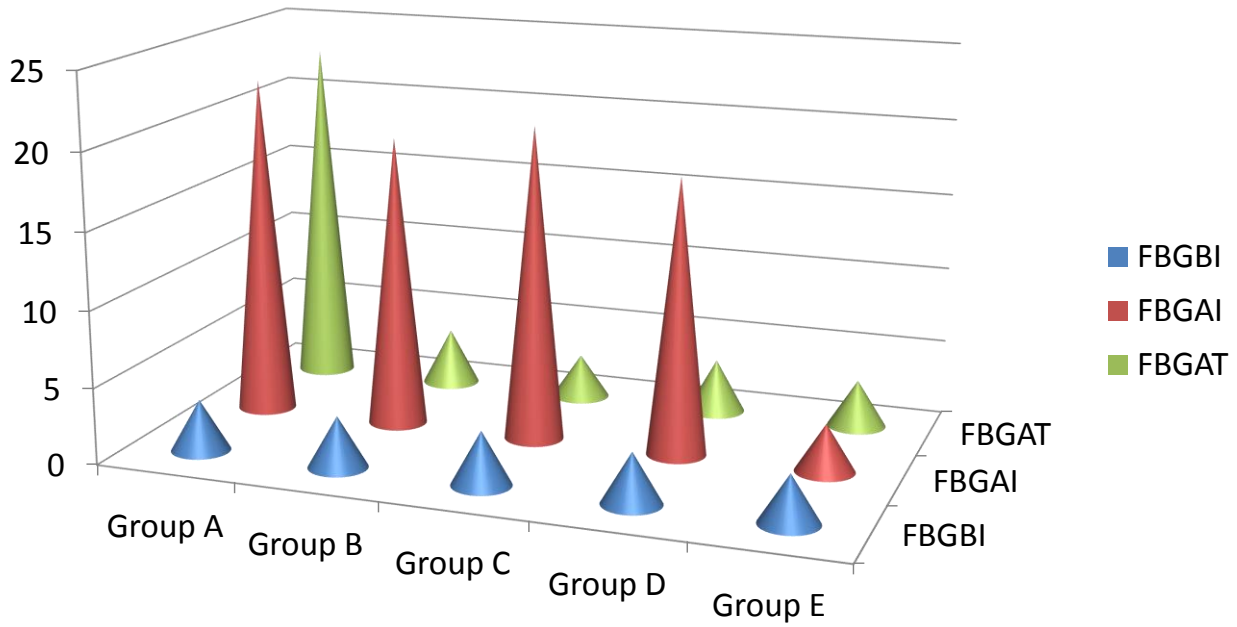

Figure 1: Graphic summarization of fasting blood glucose of experimental rats. Results are expressed in mmol/L. Abbreviations: FBGBI- fasting blood glucose before induction; FBGAI- fasting blood glucose after induction; FBGAT- fasting blood glucose after treatment.

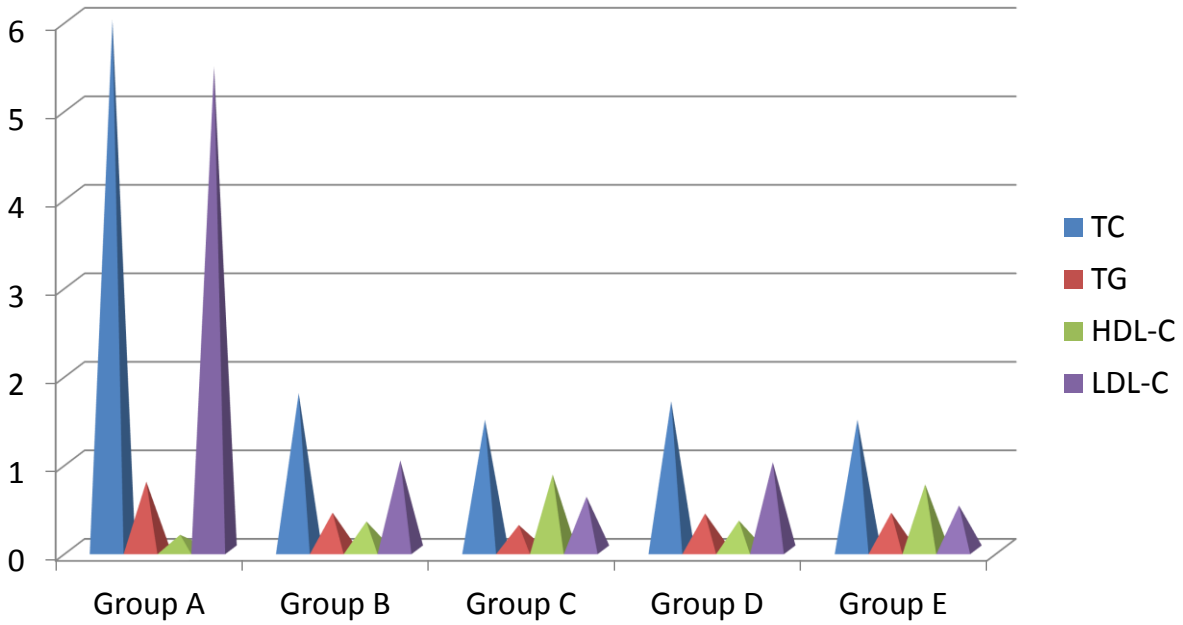

Figure 2: Graphic summarization of lipid profile of experimental rats. Results are expressed in mmol/L. Abbreviations: TC- total cholesterol; TG- triglycerides; HDL-C- high density lipoprotein- cholesterol; LDL-C- low density lipoprotein- cholesterol.

Table 1: **Blood glucose levels of alloxan-induced diabetic male wistar rats dosed with methanolic extract of *V. amygdalina.***

GROUP	FASTING BLOOD GLUCOSE BEFORE INDUCTION	FASTING BLOOD GLUCOSE AFTER INDUCTION	FASTING BLOOD GLUCOSE AFTER TREATMENT
A	3.36 ± 0.25	22.39 ± 2.07*	22.89± 5.07
B	3.36 ± 0.45	19.19 ± 2.44*	3.70 ± 0.36†
C	3.52 ± 0.22	20.64 ± 0.69*	2.86 ± 0.10†§
D	3.32 ± 0.38	18.10 ± 1.25*	3.50 ± 0.19†
E	3.14 ± 0.42	3.14 ± 0.02	3.10 ± 0.40

Results are presented as mean ± standard error of mean. *P < 0.05 considered significant when groups A, B, C, D were compared with group E (control). †P < 0.05 considered significant when B, C, D were compared with group A (diabetic group). §P < 0.05 considered significant when Groups B and C were compared with group D.

Table 2: Effect of *V. amygdalina* extract on lipid profiles of the alloxan-induced diabetic male wistar rats

GROUP	LIPID PROFILE			
	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
A	5.99 ± 0.54*	0.77 ± 0.11*	0.17 ± 0.06*	5.46 ± 0.48*
B	1.77 ± 0.09*†	0.42 ± 0.09*†	0.32 ± 0.06*†	1.01 ± 0.10*†
C	1.47 ± 0.06*†§	0.28 ± 0.13*†§	0.85 ± 0.06*†§	0.60 ± 0.08*†§
D	1.68 ± 0.34*†	0.41 ± 0.06*†	0.33 ± 0.05*†	0.99 ±0.08*†
E	1.47 ± 0.10	0.42 ± 0.27†	0.74 ± 0.09	0.50 ±0.13

Results are presented as mean ± standard error of mean. *P < 0.05 considered significant when groups A, B, C, D were compared with group E (control). †P < 0.05 considered significant when B, C, D were compared with group A (diabetic group). §P < 0.05 considered significant when Groups B and C were compared with group D.

Table 3: Initial and final body weight of the experimental wistar rats

GROUP	INITIAL WEIGHT (g)	FINAL WEIGHT (g)
A	172.67 ± 4.89	143.00 ± 8.05*
B	150.67 ± 2.94	153.17 ± 5.42*
C	159.50 ± 3.01	163.50 ± 4.80*
D	166.00 ± 4.04	168.83 ± 5.46*
E	153.33 ± 2.16	161.83 ± 5.53*

Results are presented as mean ± standard error of mean. *P < 0.05 considered significant

Table 4: Phytochemical results

Phytochemicals	Inference (Remarks)
Flavonoids	++ (highly present)
Tannins	++ (highly present)
Saponins	++ (highly present)
Deoxy-sugar	++ (highly present)
Alkaloids	+++ (more highly present)
Anthraquinones	+ (present)
Terpenoid	++ (highly present)
Cardiac glycosides	+++ (more highly present)

4.0 DISCUSSION

Diabetes is a debilitating disease that is usually associated with very many severe metabolic alterations that may cumulate into myriad of complications. While different standard treatments are available, many of them are associated with various side effects. This is the basis of many ethno-pharmaceutical approaches to the management of this disease. *V. amygdalina* has been used for the treatment of various diseases. Results of the study revealed that its phytochemical constituents include alkaloid, tannins, saponins, cardiac glycosides, terpenoids, and reducing sugars. The phytochemical contents (Table 4) of the present study extract show considerable close similarities with those of past studies [14, 15].

The significant decreases in the levels of glucose may not be unassociated with phytochemical contents of the extract. Through its insulin-like activity and inhibition of gluconeogenesis and glycogenolysis, terpenoids has been reported to reduce blood glucose [16]. On the other hand, saponin is known for its ability to improve hyperglycemia associated oxidative stress in type 2 diabetes [17]. Saponins do this because they have insulin-like characteristics, which stimulate glucose uptake enhancing Glut4 expression and thereby participating in storage of glucose as glycogen in adipocytes [18].

There is sufficient evidence that tannins extracted not only from *V. amygdalina* but also from different plant materials exhibited α-amylase and α-glucosidase inhibitory activities [19], this invariably results in decrease glucose transport through the intestinal

epithelium. Meanwhile, flavonoids [20] and alkaloids [21] in like manner are recognized for their anti-diabetic property via inhibitory property on α-glucosidase. Cumulatively, these various effects added up to remarkable reduction in glucose level in the diabetic wistar rats treated with 50 & 100 mg/kg of *V. amygdalina.*

The results of the study are in agreement with those of Akah and Okafor [22] and Akah *et al.* [23] especially with regards to the hypoglycemic effect of *V. amygdalina* extract on diabetic rats as shown in Table 1. Alloxan, a diabetogenic agent induces diabetes by damaging the pancreatic β-cells of the islets leading to hyperglycemia. The present findings are in accord with previous observations [24; 25], where antidiabetic medicinal plants profoundly lower the high blood glucose level occasioned from alloxan related diabetes.

The reduced HDL-C levels in the diabetic control may be as a result of insufficiency in fatty acid metabolism, increased gluconeogenesis, and high production of ketone bodies in the diabetic state, which ultimately resulted in hypercholesterolemia and hypertriglyceridemia- the most frequently encountered lipid abnormalities in diabetes [26]. Dyslipidaemia is a recognized complication commonly encountered in poorly managed diabetes [27]. The elevated levels of serum TG, TC, and LDL-C but low levels of HDL-C observed in diabetic rats compared to control rats is consistent with earlier observations from various researchers [27-30]. This no doubt suggests that elevation in glucose level on induction of diabetes consequently leads to a corresponding upsurge in blood lipids. According to Ayinla *et al.* [27] the characteristic dyslipidaemia of diabetes occurs as raised levels of cholesterol, phospholipids, triglycerides and other lipoproteins.

The metabolic changes that eventually result in these have been linked to increased mobilisation of free fatty acids from peripheral fat depots due to loss of inhibition of the hormone-sensitive lipase from insulin absence [31]. Increased mobilization of fatty acid from the adipocytes might have been the basis of the significant weight loss in diabetic group when initial and final weights were compared. Deficiency of insulin (either from defect in secretion or action) usually enhances the activities of catecholamines, glucagon and other hormones which then promote lipolysis. The metabolic aberration that results in excess fatty acids production proceeds to their conversion to cholesterol and phospholipids which, together with excess triacylglycerols produced at the same time in the liver are released into the blood stream in the form of lipoproteins.

Tavasoli *et al.* [32] also concurred especially with the role hormones play in abnormal lipid profile of diabetes mellitus. According to them, hyperlipidaemic condition in diabetic state as observed in untreated diabetic rats is a possible consequence of uninhibited actions of lipolytic hormones in fat depots. Dyslipidaemia that is featured as elevated levels of TC and LDL-C, is one of the most important coronary risk factors, and has been identified as a significant cause of morbidity and mortality in diabetic patients. That rats administered with methanolic extract of *V. amygdalina* showed alleviations of all the lipid profile parameters is an indication of its hypolipidaemic effects (Table 2). This may be attributed to the phytochemical constituents of bitter leaf which probably inhibited cholesterol and/or bile acid absorption. Moreover, the high free radical scavenging property of *V. amygdalina* earlier reported is a possible suggestion for its

potent antilipidaemic effect.

It has been affirmed by the World Health Organization that the prevalence rate of diabetes is 8.5%, and that the number of adults living with diabetes has increased by almost four times to approximately 422 million adults from 1980 to 2015 [4-6]. According to International Diabetes Federation, globally on an average, a person dies from diabetes every 6 s [4; 5]. Meanwhile statistics of diabetes is equally discouraging in Nigeria since as many as 5 million individuals are currently living with the disease [5; 6]. With such glum statistics, the results of this study become crucial because even though considerable advancement has been made so far on basic and clinical investigations into diabetes, there exists no ultimate therapy. Moreover, the fact that the existing therapeutic options are limited will make the present study relevant in the discourse of diabetes management. Especially, as the current options are bedeviled with several grievous side effects such as liver problems, diarrhoea and lactic acidosis [33; 34].

Conclusion

Methanolic extract of *V. amygdalina* possesses antidiabetic properties and anti-hypolipidemic effects, this may explain why this plant is traditionally used for the management of diabetes mellitus and its complications.

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