International Journal of Biochemistry, Bioinformatics and Biotechnology Studies
Vol.6, No.1, pp.1-16, 2021
Print ISSN: 2397-7728,
Online ISSN: 2397-7736

PHYTOCONSTITUENTS AND ANTIDIABETIC ACTIVITY OF *VERNONIA AMYGDALINA* (ASTERACEAE) IN STEPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT: *Vernonia amygdalina (VAM) is a medicinal plant that has been use traditionally in the management of diseases especially diabetes. Phytochemical screening and GC-MS analysis were carried out while 25 male albino wistar rats (137-223 g) were used to evaluate the andiabetic activity. The animals were randomly divided into five groups (n=5). Group I (control) received normal feed and water, while Groups II, III, IV and V were diabetes induced with single dose of 45 mg/kg b.wt streptozotocin (STZ) intraperitoneally. After three days, group III was treated with metformin (MET) whereas, groups IV and V were treated with 150 and 300 mg/kg b.wt VAM respectively for another seven days. Phytochemical screening showed the presence of most common phytochemicals except anthraquinone and GC-MS analysis revealed the presence of 10 phytoconstituents majorly fatty acids and esters, and phytol. The FBG levels of diabetic-induced rats treated with doses of VAM and MET were significantly reduced ($p<0.05$). There was observed significant ($p<0.05$) decrease in the levels of plasma aspartate aminotransaminase (AST, γ-glutamyl transferase (GGT) and lactate dehydrogenase (LDH), and non-significant ($p>0.05$) decrease in alanine aminotransaminase (ALT) in diabetic induced rats compared to control. MET treatment reversed the order in GGT and LDH while VAM doses could only reverse the order in LDH. At high dose, VAM significantly ($p<0.05$) increased the concentration of plasma total protein (TTP), creatinine (CRE), bilirubin (BIL) whereas, at low dose, VAM significantly ($p<0.05$) increased the concentration of plasma triglyceride (TRIG) and cholesterol (CHOL) compared to the STZ and control groups. In conclusion, this study suggests that VAM leaf extract possess some phytoconstituents which could be responsible for its antidiabetic activity.*

KEYWORDS: *Vernonia amygdalina*, antidiabetic, streptozotocin, GC-MS, rats

INTRODUCTION

Diabetes mellitus is the third leading cause of death, after heart attack and cancer. It is one of the most common endocrine dysfunction in the world resulting from a defect in insulin dynamics and

has caused significant morbidity and mortality due to microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications with no known cure [**1,2**]. Diabetes mellitus (DM) affects millions of people worldwide and its prevalence increases and is projected to reach 500 million by the year 2030 [**3**]. DM is a group of metabolic disease characterized by high glucose (sugar) level in the blood and urine due to inability to produce, metabolize and regulate the activity of the hormone insulin. It is a prevalent and lethal disease caused by dysfunction of carbohydrate metabolism and inadequate response of target cells to hormone insulin which affects citizens of under developed, developing and developed countries [**4**]. Pharmacological treatment of diabetes is composed of both insulin and oral glucose lowering drugs and in some instances complementary and alternative medicine.

Vernonia amygdalina is one of the most popular antidiabetic traditional herbal remedy in Nigeria [**5**]. *V. amygdalina*, also known as ‘‘African bitter leaf’’, is a plant vegetable used for both food and traditional treatment of diseases that is, the leaves are macerated and used in cooking, while the extracts are used as tonic for prevention of certain illness [**6**]. *V. amygdalina* is a valuable medicinal plant that is widespread in West Africa, it is known as bitter leaf due to its characteristic bitter taste and flavour, and can be used as an active anticancer, antibacterial, antimalarial, anti-diabetic and anti-parasitic agent [**7**].

V. amygdalina contains complex active components (phytochemicals) that are useful pharmacologically [**7**]. Phytochemicals are natural occurring bioactive compounds known for their health benefits. They are majorly responsible for the colour, flavour and aroma of fruits and notably vegetables [**8**]. The phytochemical studies of *V. amygdalina* reveals the presence of saponins, flavonoids, alkaloids, terpenes, steroids, coumarins, phenolic acids, lignans, xanthones, anthraquinones, edotides and sesquiterpenes [**9**]. Some other phytochemicals havee been isolated and characterized from the leaves of the plant which include vernonioside D, vernodalol, luteolin, vernodalin, vernolepin and luteolin 7-O- β-glucoside [**10**].

This study is to investigate the phytoconstituents by GC-MS analysis and antidiabetic activity of 70% methanol leaf extract of *V. amygdalina* in streptozotocin-induced diabetic albino wistar rats

MATERIALS AND METHODS

Collection of plant material and preparation of extract

V. amygdalina was collected from an individual farm at Imushin, Ijebu-Ode, Ogun state in February 2019. The leaves were separated from the stem and air-dried for two weeks. They were then pulverized by means of blender and stored in air-tight jar. 85 g of the powder was weighed and macerated in 70% methanol (300 mL) with occasional shaking at room temperature for 72 hours, this was repeated twice. The mixture was filtered using muslin cloth and filtrate concentrated at 60°C to about one-tenth the original volume using a rotary evaporator. The concentrate was kept in the oven (40°C) for complete dryness to obtain the methanol leaf extract of *V. amygdalina* (VAM) which was then stored in a refrigerator at −4°C until used.

Phytochemical screening

The methanol leaf extract of VAM was tested for the presence of bioactive compounds using standard procedures as described by Sofowora [**11**] and Trease and Evans [**12**]. Phytochemicals tested include alkaloids, carbohydrates, glycosides, saponins, terpenoids, phenol, flavonoids, tannins, proteins, phytosterols, polyphenols, fats and oils, and anthroquinones. Detailed procedures have been reported in our previous researches [**13,14**]

GC-MS analysis

Further phytochemical analysis was carried out using Gas chromatography-mass spectrometry analysis (GC-MS). Hewlett Packard Gas Chromatograph (Model 6890 series) equipped with a flame ionization detector and Hewlett Packard 7683 series injector was used to obtain the phytocompounds present in the extract. We have earlier reported the detailed procedure used in this analysis [**13–15**].

Animals

Twenty five (25) male albino wistar rats (137-223g) were obtained from the Animal House, College of Basic and Applied Sciences, Mountain Top University, Ibafo, Ogun State, Nigeria. The animals were given free access to feed and water *ad libitum* under clean environmental conditions (31 ± 1°C, with 65 ± 2% humidity and 12 h / 12 h light / dark cycles) and were allowed to acclimatize for 7 days before the commencement of the experiment. The ethical regulations in accordance with National and Institutional guidelines for the protection of animals' welfare were strictly adhered to during the experiments.

Experimental design

The twenty five rats were randomly distributed into five groups of five rats per group in well ventilated cages. The experimental animals received the following treatments with stipulated feed and water:

Group I (Normal control): No treatment was administered.

Group II (Negative control): Received a single dose of 45 mg/kg b.wt. streptozotocin (STZ).

Group III (Positive control): Received a single dose of 45 mg/kg b.wt. STZ) + treatment with 45mg/kg b. wt. metformin (MET) for seven days.

Group IV (Test group): Received a single dose of 45 mg/kg b.wt. STZ + treatment with 150 mg/kg b.wt. extract of *V. amygdalina* (VAM) for seven days.

Group V (Test group): Received a single dose of 45 mg/kg b.wt. STZ + treatment with 300 mg/kg b.wt. extract of *V. amygdalina* for seven days.

Induction of diabetes

Diabetes was induced experimentally (group II-V) by intraperitoneal administration of 45 mg/kg b.wt. STZ dissolved in citrate buffer (0.01 M, pH 4.5) to overnight fasted rat (12 hours after last feeding). Injected rats were returned to their cages and provided with 5% glucose solution for 12 hours to overcome STZ-induced hypoglycemia. After 72 hours of STZ administration, fasting blood glucose (FBG) level was measured using glucometer (Accu-Chek® Active, Blood Glucose Monitoring System, Roche Diabetes Care, Inc. Indianapolis, USA).

Drug administration

After 72 hours of administration of STZ and measurement of FBG, diabetic-induced rats, group III was given 45 mg/kg b.wt. MET while groups IV and V were treated with 150 and 300 mg/kg b.wt. VAM respectively for 7 days. The extract and metformin were suspended in distilled water and administered by oral gavage. Normal (group I) and diabetic control (group II) group were administered only with vehicle. At the end of this period, FBG was measured again, before the animals were sacrificed.

Collection and preparation of blood plasma sample

At the end of the experiment, the animals were fasted overnight and sacrificed by cervical dislocation under 10% chloroform anaesthesia. The blood sample collection was through the ocular into a set of heparin bottles, then centrifuged at 2500 rpm for 10 minutes to obtain the blood plasma.

Biochemical assays

The blood plasma was used to determine the concentrations of aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), total protein (TTP), creatinine (CRE), bilirubin (BIL), triglyceride (TRIG) and cholesterol (CHOL) levels using standard laboratory kit from Randox laboratories, UK.

Statistical analysis

The statistical analysis was done using GraphPad Prism 7.0. The results were reported as mean ± SEM (standard error of mean). The data collected were subjected to Analysis of Variance (ANOVA) to test for variations of the different parameters observed in the study. Results were considered statistically significant when the value of $p<0.05$.

RESULTS

Phytochemical constituents of VAM

The result of the phytochemical screening of 70% methanol leaf extract of VAM is shown in **Table 1**. It shows the presence of alkaloids, carbohydrates, proteins, fat and oils, terpenoids, phenol, flavonoids, tannins, glycosides, phytosterols, polyphenols and saponins, and absence of

anthraquinones. The GC-MS analysis results are shown in **Figure 1** and **Table 2**. The GC-MS chromatogram (**Figure 1**) identified the peaks while **Table 2** revealed the 10 bioactive compounds in methanol extract of VAM which are mostly fatty acids, fatty acid esters and phytol. The mass spectrum and molecular structure of five major bioactive compounds with more than 10% of total as revealed by GC-MS are hexadecanoic acid, methyl ester-16.26% (**Figure 2**), 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester-11.96% (Figure 3), cis-13-Octadecenoic acid, methyl ester-14.07% (Figure 4), phytol-10.52% (Figure 5) and 9, 12-Octadecadienoic acid-11.96% (**Figure 6**).

Table 1: Phytochemical screening

NO.	PHYTOCHEMICALS	RESULTS
1.	Alkaloid	Positive
2.	Carbohydrate	Positive
3.	Protein	Positive
4.	Fat and oil	Positive
5.	Terpenoids	Positive
6.	Phenol	Positive
7.	Flavonoid	Positive
8.	Tannin	Positive
9.	Glycoside	Positive
10.	Phytosterol	Positive
11.	Polyphenol	Positive
12.	Saponin	Positive
13.	Anthraquinone	Negative

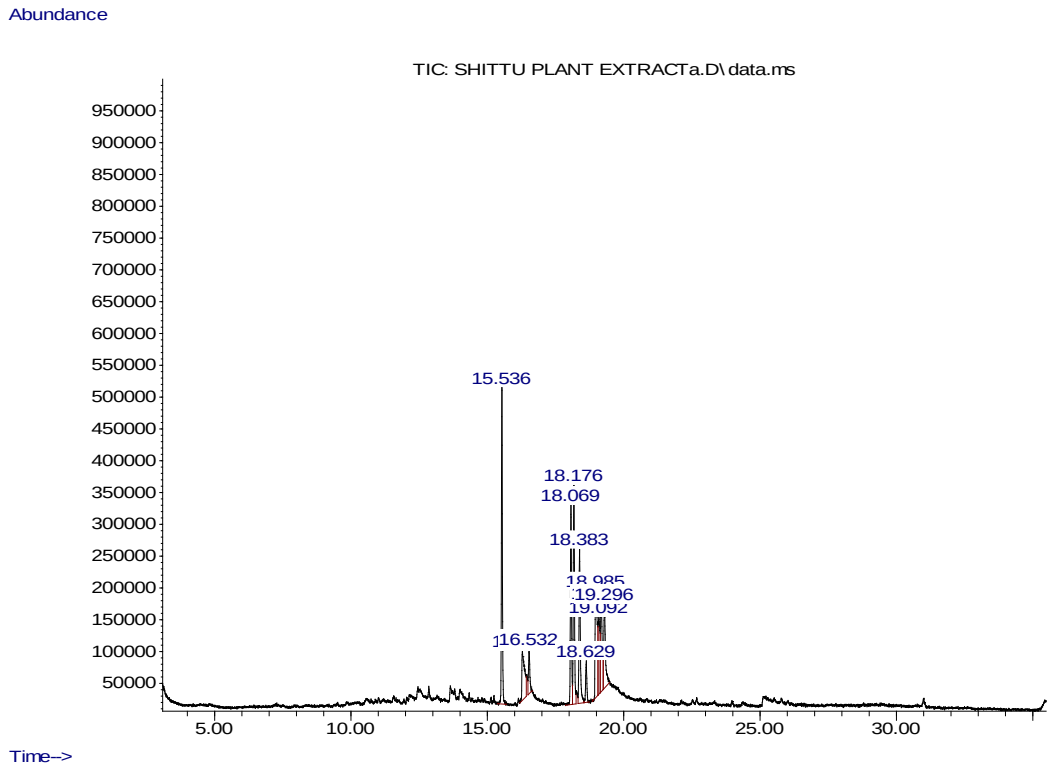

Figure 1. GC-MS chromatogram of hydro-methanolic extract of VAM

Table 2: Result of GC-MS analysis of VAM

Peak	Retention time	Library ID	% of total	Chemical formula
1	15.536	Hexadecanoic acid, methyl ester	16.262%	$C_{17}H_{34}O_2$
2	16.288	n-Hexadecanoic acid	6.841%	$C_{16}H_{32}O_2$
3	16.532	Hexadecanoic acid, ethyl ester	3.197%	$C_{18}H_{36}O_2$
4	18.069	9, 12-Octadecadienoic acid (Z, Z)-, methyl ester	11.832%	$C_{19}H_{34}O_2$
5	18.176	cis-13-Octadecenoic acid, methyl ester	14.075%	$C_{19}H_{36}O_2$
6	18.383	Phytol	10.528%	$C_{20}H_{40}O$
7	18.629	heptadecanoic acid, 16-methyl-, methyl ester	2.611%	$C_{19}H_{38}O_2$
8	18.985	9, 12-Octadecadienoic acid	11.968%	$C_{18}H_{32}O_2$
9	19.180	Linoleic acid ethyl ester	8.203%	$C_{20}H_{36}O_2$
10	19.296	9, 12, 15-Octadecatrienoic acid, ethyl ester (Z, Z, Z)	7.828%	$C_{20}H_{34}O_2$

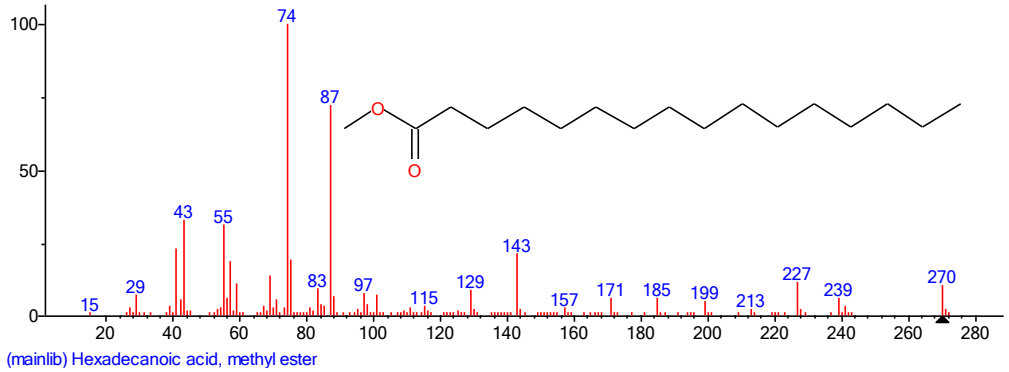

Figure 2. Mass spectrum and molecular structure of hexadecanoic acid, methyl ester

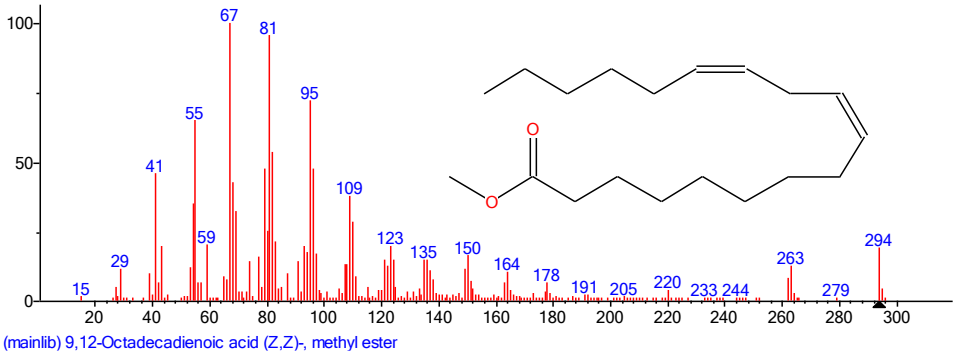

Figure 3. Mass spectrum and molecular structure of 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester

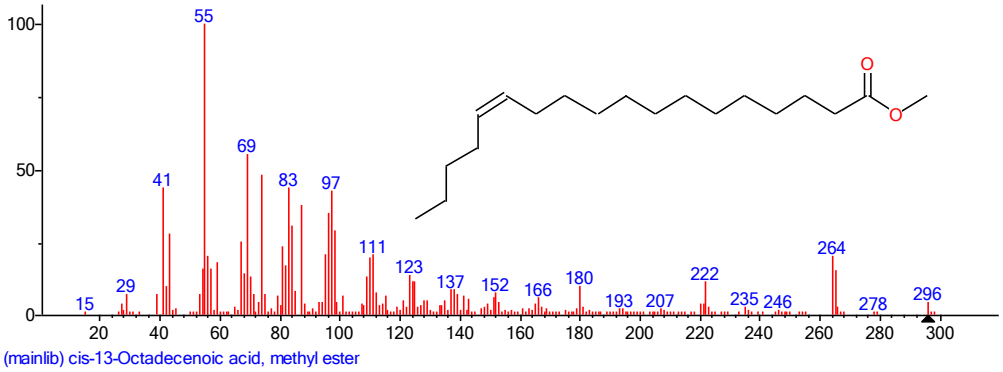

Figure 4. Mass spectrum and molecular structure of cis-13-Octadecenoic acid, methyl ester

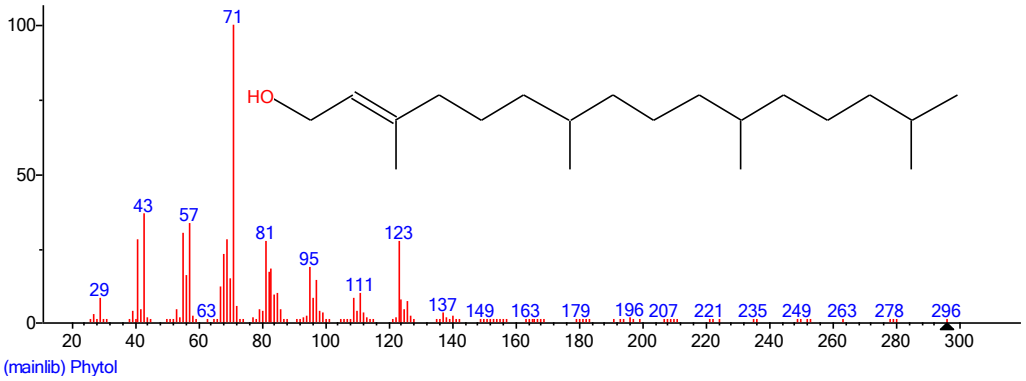

Figure 5. Mass spectrum and molecular structure of phytol

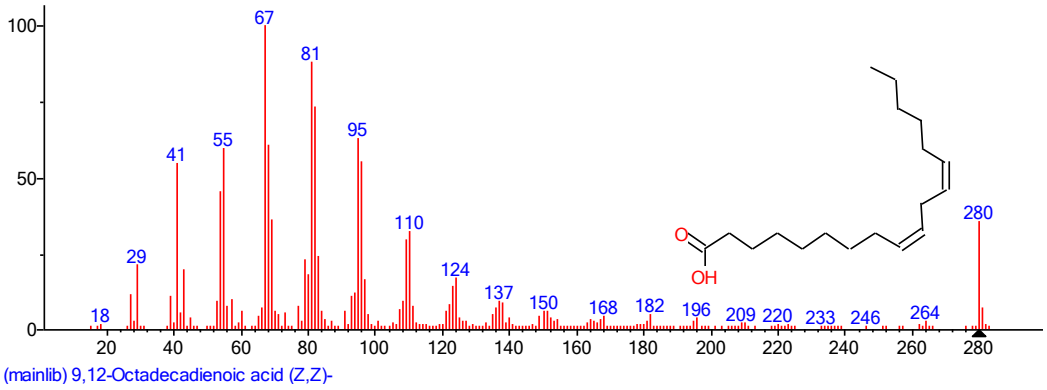

Figure 6. Mass spectrum and molecular structure of 9, 12-Octadecadienoic acid

Antidiabetic activity of VAM

The antidiabetic activity of methanol leaf extract VAM on STZ-induced diabetic rats is given in **Figure 7**. Fasting blood glucose (FBG) level of rats before induction of diabetes with STZ was compared with the FBG level of rats 72 hours after diabetes induction and after 7 days treatment of VAM and MET. The FBG level in groups II-V was observed to significantly ($p<0.05$) increased compared to the normal control rats after 72 hours of STZ administration while the FBG levels in MET and VAM treated groups were noticed to reduced significantly ($p<0.05$) after 7 days of treatment compared to the diabetic group and no significant ($p>0.05$) change when compared with the normal control.

Effect of VAM on plasma enzymes activities

Table 3 shows the effect of VAM on the activities of plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT) and lactate dehydrogenase (LDH) in STZ-induced diabetic rats. Administration of STZ significantly ($p<0.05$) increase ALT and reduce AST, GGT and LDH activities compared with the normal control. Treatment with 150 mg/kg VAM significantly ($p<0.05$) reduced the activities of AST, GGT and LDH compared with normal control and ALT when compared with STZ group. Whereas, treatment with 300 mg/kg VAM was observed to reduce significantly ($p<0.05$) the activities of AST, ALT and GGT compared with normal control but no significant ($p>0.05$) change in activities when compared with the diabetic rats. The LDH activity was however increased significantly ($p<0.05$) when compared with the diabetic rats. The metformin treated group was observed to have significant ($p<0.05$) increase in activity of LDH and no significant ($p>0.05$) change in activities of AST, ALT and GGT compared with the diabetic rats.

Effect of VAM on plasma total protein, creatinine and bilirubin

Figures 8, 9 and **10** show the effect of VAM on the concentrations of plasma total protein (TTP), creatinine (CREA) and bilirubin (BIL) respectively. It was observed that increased dose of VAM at 300 mg/kg significantly ($p<0.05$) increased the concentrations TTP, CREA and BIL compared with diabetic (STZ) control while treatment with 150 mg/kg have no significant ($p>0.05$) change in the concentrations of TTP, CREA and BIL compared with the diabetic control rats.

Effect of VAM on plasma triglycerides and cholesterol levels

Figure 11 and **12** depict the effect of VAM on the levels of plasma triglycerides and cholesterol respectively, in STZ-induced diabetic rats. Administration of STZ was observed to significantly ($p<0.05$) reduce the level triglyceride compared with the normal control rats. Treatment with 150 mg/kg and 300 mg/kg VAM doses, then significantly ($p<0.05$) elevated the triglyceride level, whereas the increase in cholesterol was not significant compared with the diabetic and normal control rats.

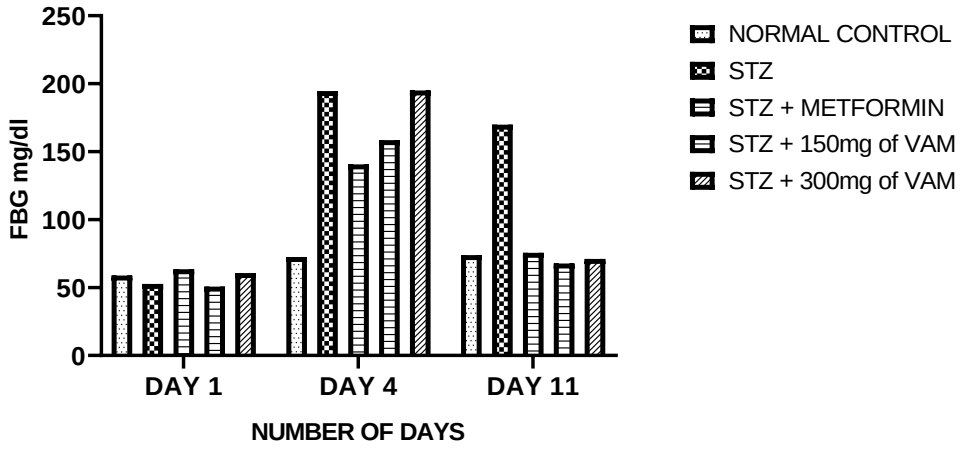

Figure 7. Effect of VAM on concentration of fasting blood glucose (FBG) in streptozotocin (STZ)-induced diabetic rats. Values represent mean ± SEM (standard errorof mean), (n=5).

Table 3: Effect of VAM on plasma enzymes in streptozotocin-induced rats

GROUPS	AST (U/L)	ALT (U/L)	GGT (U/L)	LDH (U/L)
Normal Control	28.60±0.98[a]	4.00±0.00[a]	14.36±1.35[a]	33.84±0.83[a]
STZ	21.25±3.33[b]	5.00±1.00[b]	5.21±0.75[b]	16.51±4.13[b]
STZ + Metformin	19.75±3.95[b]	5.00±1.00[b]	30.69±1.53[c]	20.64±3.77[b]
STZ + 150 mg/kg VAM	15.40±2.21[b]	4.00±0.00[a]	4.17±1.58[b]	26.41±2.10[c]
STZ + 300 mg/kg VAM	23.60±1.40[b]	5.6±0.98[b]	3.94±0.69[b]	26.41±2.10[c]

Data are expressed as mean ± SEM (standard error of mean), (n=5). Groups with different superscripts are significantly different at p≤0.05. Steptozotocin (STZ); *V. amygdalina* (VAM); Aspartate aminotransferase (AST); Alanine aminotransferase (ALT); Gamma-glutamyltransferase (GGT); Lactate dehydrogenase (LDH)

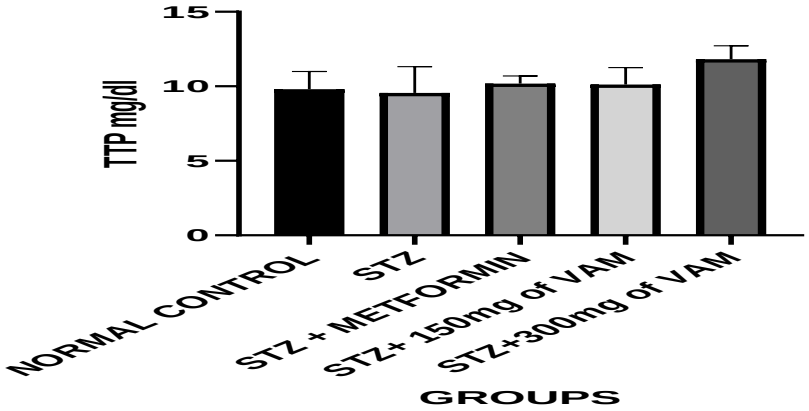

Figure 8. Effect of VAM on plasma total protein (TTP) in streptozotocin (STZ)-induced rats. Values represent mean ± SEM (standard error of mean), (n=5).

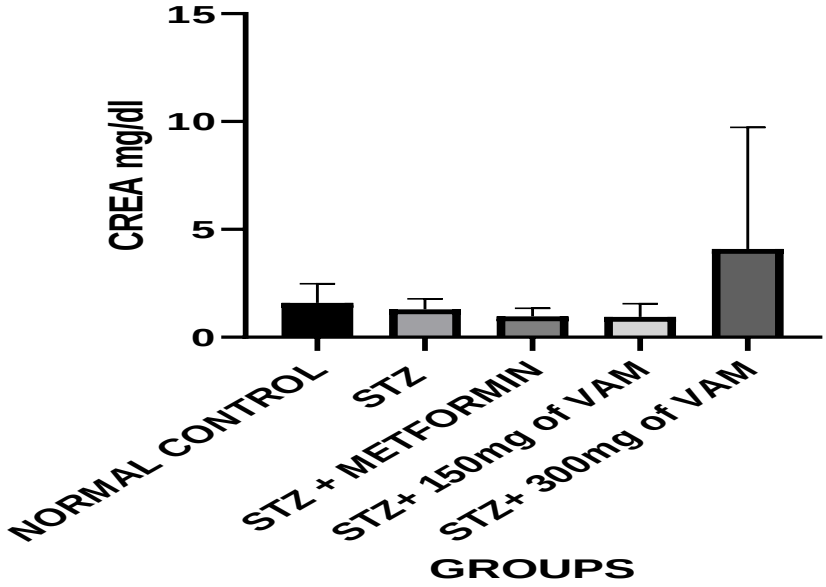

Figure 9. Effect of VAM on plasma creatinine (CREA) in streptozotocin (STZ)-induced rats. Values represent mean ± SEM (standard error of mean), (n=5).

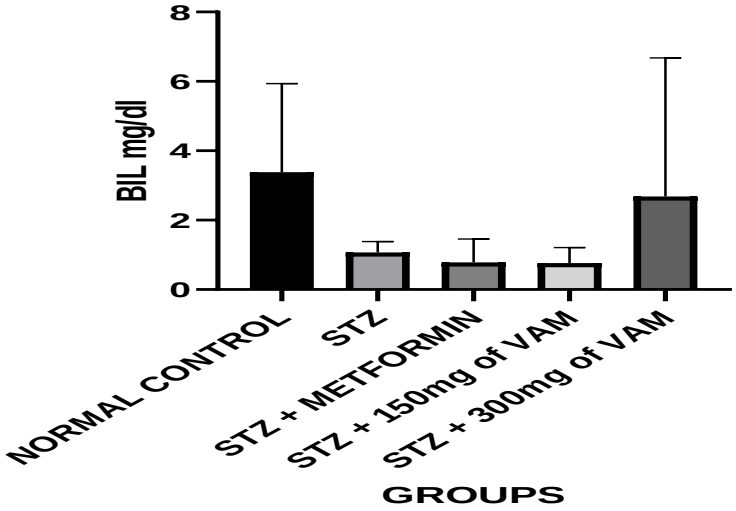

Figure 10. Effect of VAM on plasma bilirubin (BIL) in streptozotocin (STZ)-induced rats. Values represent mean ± SEM (standard error of mean), (n= 5).

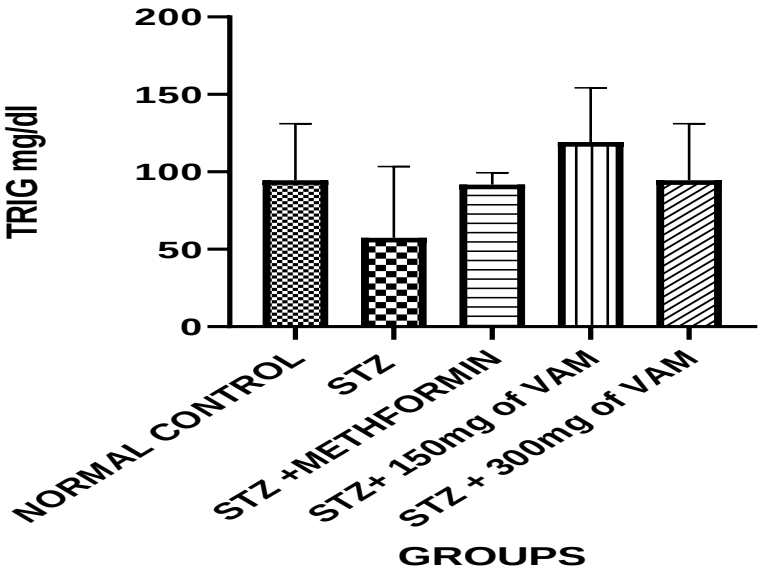

Figure 11. Effect of VAM on plasma triglyceride (TRIG) in streptozotocin (STZ)-induced rats. Values represent mean ± SEM (standard error of mean), (n=5).

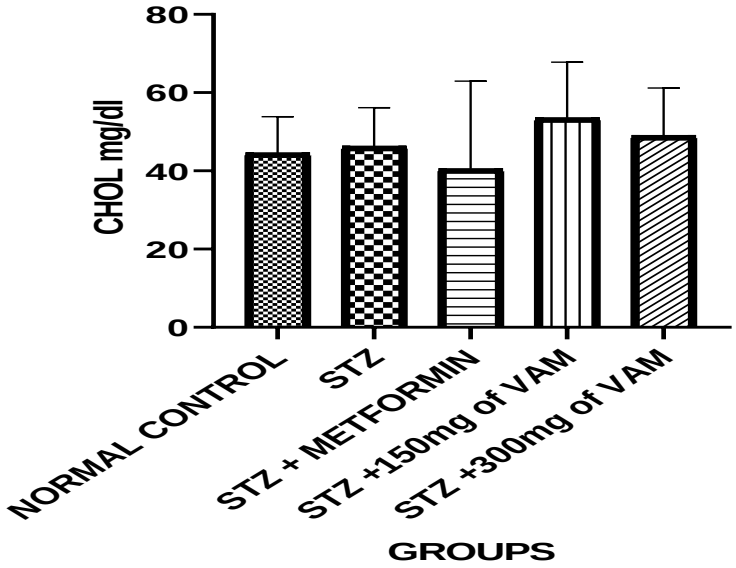

Figure 12. Effect of VAM on plasma total cholesterol (CHOL) in streptozotocin (STZ)-induced rats. Values represent mean ± SEM (standard error of mean), (n=5).

DISCUSSION

There are naturally occurring substances called phytochemicals that have been discovered in plants parts such as roots, stems and leaves. These substances are ubiquitous and serve as protective factors in plants against heat, ultraviolent light and external pathogens [**16**]. *V. amygdalina* was reported to have high content of pharmacologically active phytochemicals such as saponins, tannins, alkaloids and flavonoids, triterpenoids, steroids and cardiac glycosides that are also effective as supplements in human and animal nutrition [**7,17**]. Several compounds with different bioactivities which belong to various classes of compounds have been isolated and characterized from the plant [**18**]. This study has again confirmed the presence of alkaloids, carbohydrates, proteins, fat and oils, terpenoids, phenol, flavonoids, tannins, glycosides, phytosterols, polyphenols and saponins phytochemicals and absence of anthraquinones in VAM. The GC-MS analysis has also revealed hexadecanoic acid, methyl ester, 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester, cis-13-Octadecenoic acid, methyl ester, phytol and 9, 12-Octadecadienoic acid as some of the important bioactive phytoconstituents in the plant.

Diabetes mellitus is characterized by high glucose levels in the blood as a result chronic endocrinological disorder in pancreas by insufficient secretion of insulin or inadequate utilization of insulin by the target cells [**18**]. Monitoring of blood glucose level has remained a common denominator for diabetic status index by following a progressive plan of hyperglycemia treatment of diabetes mellitus [**18**]. In this study, blood glucose level was measured following diabetes inducement and treatment with MET and VAM for 7 days in the rats. The observed reduction in the fasting blood glucose level of diabetic rats treated with VAM was comparable with the fasting

blood glucose level of positive control rats treated with MET which agreed with previous reports [**19–23].** Phytochemicals such as flavonoids, glycosides, tannins and phytosterols have been implicated in hypoglycaemic and antihyperglycemic action [**24**]. Studies have also shown that flavonoids can act as an insulin secretagogues or insulin mimetics [**25**]. Flavonoids are likely to influence the pleiotropic mechanisms to attenuate diabetic complications thereby protecting β-cells against ROS mediated damage and minimizing hyperglycemia in STZ-induced diabetes [**25**]. Therefore, VAM may have insulin like activity.

V. amygdalina was shown to lower ALT, AST in the serum diabetic rats [**19, 26**]. It was observed in this case that while ALT was significantly reduced at the low dose of VAM, AST and GGT were reduced non significantly whereas LDH was significantly increased compared with the diabetic control rats. There was ameliorative increased levels of TTP, CREA, BIL, TRIG and CHOL compared with the diabetic rats. This shows that VAM may have ameliorated the drug-induced damage to the liver cells as observed in diabetic untreated rats.

In conclusion, this study shows that VAM possessed antidiabetic activity. The antidiabetic property could be due to the high content of flavonoids and terpenoids present in the plant leaf extract.

Ethical approval

This research study was approved by the Research and Ethics Committee of the College of Basic and Applied Sciences, Mountain Top University, Makogi, Ibafo, Ogun State, Nigeria.

Disclosure statement

There is no conflict of interest in this research work

Funding

The study was funded by the authors and supported by Mountain Top University.

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