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Anti-diabetic Effect of *Anthocleista vogelii* Ethanolic Root Extract and Fractions in Streptozotocin-induced Diabetic Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author RMS performed the experiments, performed the statistical analysis and wrote the first draft of the manuscript. Authors OAA and JAI carried out the histological examinations and contributed to the writing of the manuscript (protocol). Authors LAA, OJO and BOI contributed to the protocol. Author EMO provided technical assistance, supervised the work and contributed to the protocol. Author ORI supervised the work and contributed to the protocol. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the anti-diabetic effect of *Anthocleista vogelii* root ethanolic extract (EE) and fractions (ethyl acetate [EF], dichloromethane [DF] and *n*-hexane [HF] fractions) in streptozotocin (STZ)-induced diabetic albino rats.
Study Design: The antidiabetic effect of *A. vogelii* root extracts was investigated in Albino rats by measuring some biochemical parameters including fasting blood glucose levels and also by examining the histology of the pancreas, liver and kidney.
Place and Duration of Study: Medicinal Plants Section, Bioresources Development Centre, Ogbomoso and Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria between January and April, 2015.
Methodology: The control group was administered 10 ml/kg distilled water, the standard drug group was administered 5 mg/kg glibenclamide, the test groups were administered 100, 200 and 400 mg/kg ethanolic extract and 200 mg/kg of each of the fractions (EF, DF and HF) orally to STZ-induced (60 mg/kg; interperitoneal) diabetic rats. Fasting blood glucose levels (FBGL), biochemical parameters, changes in body weight, food intake, water intake and the histology of the pancreas, liver and kidney of diabetic rats were examined.
Results: The result of the study showed that EF exerted a more significant ($P<0.05$) reduction in FBGL more than the other fractions when compared with the control. The EE and fractions significantly ($P<0.05$) decreased food intake, water intake, serum cholesterol, triglyceride, low density lipoprotein, alanine aminotransferase, aspartate aminotransferase and creatinine concentrations and increased high density lipoprotein levels of diabetic treated rats when compared with the control. The extracts also caused the regeneration of cells of the pancreas, kidney and liver of diabetic treated rats.
Conclusion: The study concluded that *A. vogelii* ethanolic root extract and fractions exerted potent anti-diabetic and anti-hyperlipideamic activity in STZ-induced diabetic rats and thus, the plant may be used for the treatment and management of diabetes.

Keywords: Anthocleista vogelii; diabetes; Albino rats; streptozotocin; root.

1. INTRODUCTION

There is a recent increase in medicinal plants research in the last few decades and the demand to use herbal formulations in the treatment/management of diseases is also on the increase. Globally, it has been well acknowledged that traditional medicine-derived plants play an important role in the treatment/management of a number of diseases including diabetes mellitus [DM] [1]. DM is a metabolic disorder characterized by chronic hyperglycemia, with alterations in carbohydrate, fat and protein metabolism either because pancreatic beta cells do not produce insulin [Type 1 DM] or because the insulin-target cells are not sensitive to the hormone [Type 2 DM] [2]. According to World Health Organization (WHO), the global prevalence of DM was estimated to be 2.8% (171 million persons) in the year 2000; with projections of 4.8% (366 million persons) by 2030 [3]. However, the International Diabetes Federation (IDF) recently revealed that this number had already been reached in 2011 and they expect an even more than 552 million affected persons in 2030 [4].

WHO also estimated that approximately 80% of the world's population depends on herbal formulations and medicinal plants for their primary health care [5]. More so, studies have shown that the selection of scientific and systematic approach for the biological evaluation of plant products based on their use in the traditional system of medicine forms the basis for an ideal approach in the development of new drugs with little or no side effects from medicinal plants [6]. *Anthocleista vogelli* is used in ethno-medicine for the treatment and management of various diseases including diabetes. Studies carried out on the antidiabetic effect of *A. vogelii* aqueous root extract and the ethanolic extract concluded that *A. vogelli* exerted significant decrease in fasting blood glucose level (FBGL) in Albino rats [7,8].

In this study, the antidiabetic and antihyperlipidemic properties of the ethanolic

extract and fractions of *Anthocleista vogelli* root was investigated in streptozotocin-induced diabetic Albino rats.

2. METHODOLOGY

The animal experiments were performed according to the approved guidelines of Obafemi Awolowo University research ethics committee.

2.1 Plant Collection and Identification

Root of *Anthocleista vogelii* were collected from the premises of National Biotechnology Development Agency, Bioresources Development Centre, Ogbomoso, Nigeria. The plant was identified and authenticated at Ife-Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State. A voucher specimen (No. 17399) was deposited at Ife-Herbarium.

2.2 Ethanolic Extraction

A. vogelii root was washed, air dried, pulverized and macerated in distilled water for 72 hours before undergoing filtration using muslin cloth and cotton wool in funnel. The filtrate was filtered again using filter paper. The filtrate was then concentrated into a solid paste *in vacuo* at 45°C using a rotary evaporator [9]. The solid paste was freeze dried and the percentage yield for the extract was calculated. The freeze dried extract was stored in a refrigerator at 4°C prior to use. The dried extract was reconstituted in distilled water (4 mg/ml).

2.3 Fractionation of *A. vogelii* Root Ethanolic Extract

A. vogelii root ethanolic extract was partitioned using various solvents of different polarities (*n*-hexane, dichloromethane and ethylacetate) to obtain their respective fractions. Each of the filtrate was then concentrated *in vacuo* at 45°C using a rotary evaporator. The fractions were freeze dried and each dry fraction was labeled accordingly and stored in a refrigerator at 4°C prior to use.

2.4 Animals

Albino rats (both sexes) weighing between 150-200 g were obtained from Animal House, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. They were kept in well ventilated aluminium cages and fed with Vita feed. The animals were also given water *ad libitum.* The rats were allowed to acclimatize with the environment at ambient temperature under natural day light/night conditions for two weeks before the start of the experiment.

2.5 Acute Toxicity Studies (Median Lethal Dose [LD_{50}] Determination)

The (LD_{50}) of the root extract was determined in Albino rats through oral route (p.o.) [10].

2.6 Glucose Loading

The mass of 10 g/kg body weight (p.o.) of glucose was administered to Albino rats that were fasted overnight. After 30 minutes of glucose administration, the blood glucose level was checked using glucometre and glucose strip [11]. Rats with blood glucose level above 7.0 mmol/l were taken for the test. Group 1 (control) diabetic untreated rats were administered 10 ml/kg distilled water; group 2-4 diabetic treated rats were administered 100, 200 and 400 mg/kg *A. vogelii* ethanolic root extract [EE]; group 5, 6 and 7 diabetic treated rats were administered 200 mg of fractions (ethyl acetate [EF], dichloromethane [DF] and *n*-hexane [HF] fractions respectively) and group 8 diabetic treated rats were administered 5 mg/kg glibenclamide acutelly to glucose loaded rats.

2.7 Induction of Diabetes using Streptozotocin (STZ)

The Albino rats were fasted overnight and diabetes was induced by a single intraperitoneal injection of freshly prepared solution of STZ (60 mg/kg). The animals were given food and water immediately after induction to overcome the drug induced hypoglyceamia. Seventy two (72) hours later rats with fasting blood glucose levels (FBGL) above 11.1 mmol/L (200 mg/dL) were considered diabetic and selected for the experiment [12,11]. Group 1 (control) diabetic untreated rats were administered 10 ml/kg distilled water; group 2-4 diabetic treated rats were administered 100, 200 and 400 mg/kg *A. vogelii* ethanolic root extract [EE]; group 5, 6 and 7 diabetic treated rats were administered 200 mg of fractions (ethyl acetate [EF], dichloromethane [DF] and *n*-hexane [HF] fractions respectively) and group 8 diabetic treated rats were administered 5 mg/kg glibenclamide daily for 14 days.

2.8 Reagents

Assay kits for the estimation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CRT), cholesterol (CHOL), triglyceride (TRIG) and high density lipoprotein (HDL) concentration were purchased from Randox Laboratories Limited, U.K.

2.9 Determination of Biochemical Parameters

The FBGL was measured at 0, 30, 60, 120 and 240 minutes and also on day 1, 4, 7, 10 and 14 [13,9] using glucometer and glucose strips (Accu-Check Active Glucometer, model: GC0088, Mannheim Germany). The animals were fasted overnight on the 14th day before they were sacrificed on the 15th day. On the 15th day, the rats one at a time were euthanized in an air tight glass chamber saturated with diethyl ether, they were dissected and blood samples were collected by cardiac puncture into plain bottles. Blood samples in plain bottles were centrifuged at 2500 rpm for 25 minutes and the serum was used for biochemical analysis these include; total cholesterol (CHOL) concentration [13-16], triglyceride (TG) concentration [17,16,18], high density lipoprotein (HDL), low density lipoprotein (LDL) [19], creatinine [20], aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [21,22].

2.10 Histopathology

The sections of the pancreas, kidney and liver were placed in a tissue cassette and fixed in 10% buffered formalin. The tissues were then processed routinely and were embedded in paraffin wax. Histological sections were cut at 5-6 µm and stained with routine Haematoxylin and Eosin for microscopic assessment. Photomicrograph was taken at X 400 magnification.

2.11 Statistical analysis

All quantitative data were expressed as the mean ± standard error of mean (SEM). Statistical analysis was carried out using one way analysis of variance and significant difference between means was assessed using Bonferroni t-test at 95% level of significance. The software used for the statistical analysis is Primer (version 3.01).

3. RESULTS AND DISCUSSION

3.1 Acute Toxicity Studies (Median Lethal Dose [LD_{50}] Determination)

There was no mortality after administration of the ethanolic extract and fractions upto a dose of 5000 mg/kg body weight. The median lethal dose (LD_{50}) of the ethanolic root extract and each of the fractions was ≥ 5000 mg/kg (p.o.).

3.2 Effect of *A. vogelii* ethanolic Root Extract and Fractions on % FBGL in Glucose Loaded Rats and STZ-induced Diabetic Rats

The ethanolic extract (EE) at 200 mg/kg body weight exerted a significant ($P < 0.05$) decrease in FBGL (at 30, 60, 120 and 240 minutes) more than the other doses of the EE when compared to the control (Fig. 1). Also, the ethylacetate fraction (EF) exerted a significant ($P < 0.05$) decrease in FBGL (at 30, 60, 120 and 240 minutes) more than the other extract fractions when compared to the control and glibenclamide (Fig. 2).

The ethanolic extract and the extract fractions exerted a significant decrease ($P < 0.05$) in FBGL on day 4, 7, 10 and 14 when compared with the control and glibenclamide. The EE at 100 mg/kg and the EF exerted a more significant decrease ($P < 0.05$) in FBGL than the other extract fractions (Table 1).

The FBGL of *A. vogelii* treated diabetic rats was reduced significantly at 30 minutes and on the 4th day onwards all through the period of the experiment. Studies carried out on the stem bark of *A.vogelii* showed that the acetone fraction possesses more hypoglycemic activity in both normoglyceamic and alloxan-induced diabetic animals than the other extract fractions [23]. In this study, *A.vogelii* root ethylacetate fraction exerted a higher reduction in FBGL than the other fractions in glucose loaded and STZ-induced diabetic treated rats. 200 mg/kg ethanolic extract elicited a higher reduction in FBGL than the other ethanolic extract doses in glucose loaded rats while 100 mg/kg elicited a bigger FBGL decrease in STZ-induced diabetic treated rats. The healthy animals (normoglyceamic rats administered 10 mL/kg distilled water) were normoglyceamic all through the experimental period.

Glibenclamide, a sulphonylureas, which was used as the standard drug in this study has been proposed to produce anti-hyperglycaemic effects through secretion of insulin [24]) and has been widely accepted as a good model in diabetic animal experiments associated with mild or moderate hyperglycaemia [25]. It has been proposed that sulphonylureas produce anti-diabetic effects through secretion of insulin [26,24]. The result of this study confirms the use of the plant root in ethno-medicine for the management of diabetes. The beneficial effects of *A. vogelii* root extract in diabetes may be due to its anti-oxidative potential [27]. Also, the presence of phenols and flavonoids in the extract may account for the observed hypoglycaemic effect since these bioactive compounds have been found to stimulate the secretion of insulin [28,25].

3.3 Effect of *A. vogelii* Ethanolic Root Extract and Fractions on Lipid Profile in STZ-induced Diabetic Rats

The ethanolic extract and fractions elicited a significant decrease ($P < 0.05$) in serum cholesterol, triglyceride and low density lipoprotein levels and an increase in high density lipoprotein level when compared with the control (Table 2).

More so, diabetes mellitus is usually associated with high level of serum lipids and such an increase causes a risk factor for coronary heart disease [29,30]. A variety of alterations in metabolic and regulatory mechanisms, due to insulin deficiency may be responsible for the observed accumulation of lipids [31,32]. Under normal physiological state, insulin activates the lipolytic hormones action on the peripheral fat depots which hydrolyses triglycerides and prevents mobilization of free fatty acids [33]. However, insulin deficiency inactivates the lipoprotein lipase which promotes liver conversion of free fatty acids into phospholipids and cholesterol and these lipids are finally discharged into the blood which results into elevated serum phospholipid level [34]. STZ-induced diabetic rats also developed hyperglycaemia which is in agreement with previous observations [35,36]. Estimation of lipd profiles is a well-accepted parameter used in the management and prognosis of diabetes [35]. In the present study, *A. vogelii* ethanolic root extract and fractions not only lowered serum cholesterol, triglyceride and low density lipoprotein level but also enhanced serum high density lipoprotein level in STZ-induced diabetic treated rats. The observed changes may be due to the indirect anti-hyperglycaemic potency and insulinotropic effects of *A. vogelii* ethanolic root extract and fractions in the diabetic treated rats. The result of serum lipid concentration suggests that the extract have the potential of reducing the risk of hypercholesterolemia that may lead to coronary atherosclerosis and other related cardiovascular diseases such as hypertension and congestive heart failure [37,38]. *A. vogelii* extracts exerted anti-diabetic and anti-hyperlipidemic activity may be by virtue of its anti-oxidant potential [38].

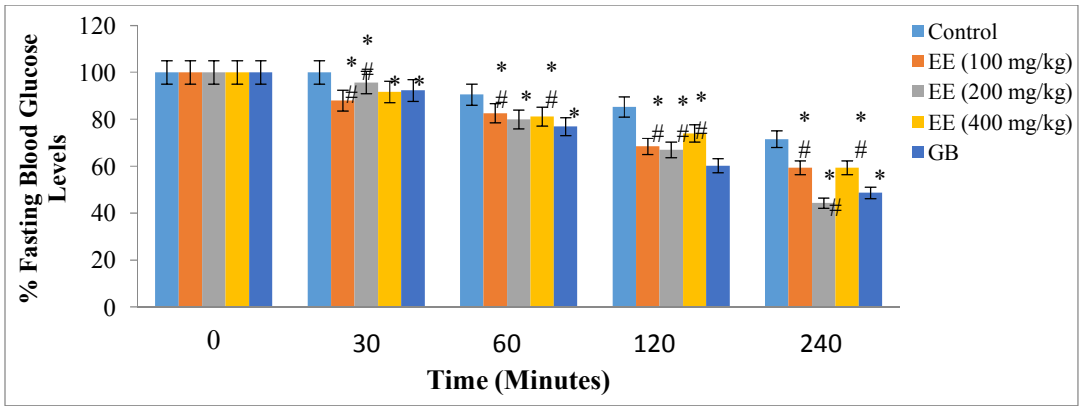

Fig. 1. Effect of *A. vogelii* ethanolic root extract on % fasting blood glucose level (FBGL) in glucose loaded rats

*Control: 10 mL/kg distilled water; EE: Ethanolic extract; GB: Glibenclamide; Values are given as Mean ± SEM; n = 5; Tt: Percentage FBGL at 30, 60, 120 and 240 minutes. *: P < 0.05 comparison of values vs that of control at Tt; #: P < 0.05 comparison of values vs that of glibenclamide at Tt*

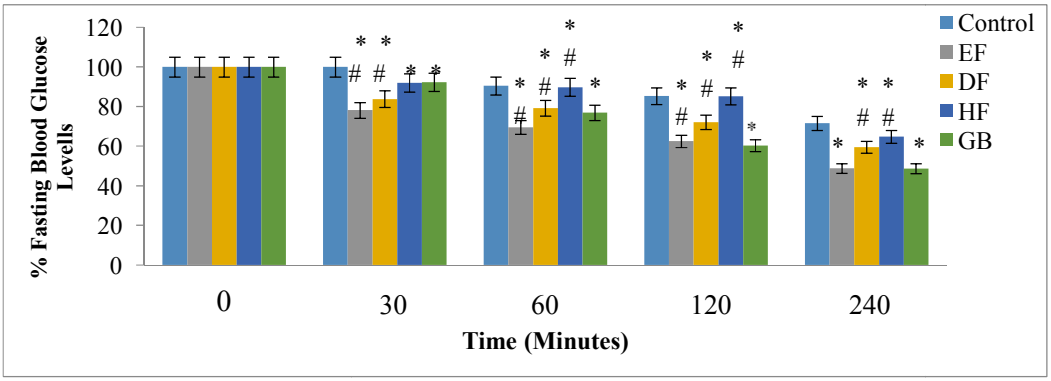

Fig. 2. Effect of *A. vogelii* ethanolic root extract fractions on % fasting blood glucose level (FBGL) in glucose loaded rats

*Control: 10 mL/kg distilled water; EF: Ethylacetate fraction; DF: Dichloromethane fraction; HF: n-hexane fraction; GB: Glibenclamide; Values are given as Mean ± SEM; n = 5; Tt: Percentage FBGL at 30, 60, 120 and 240 minutes. *: P < 0.05 comparison of values vs that of control at Tt; #: P < 0.05 comparison of values vs that of glibenclamide at Tt*

Table 1. Effect of *A. vogelii* ethanolic root extract and fractions on % FBGL in STZ-induced diabetic rats

Groups/Days	Day 1	Day 4	Day 7	Day 10	Day 14
Control	100.0 ± 1.3	111.0 ± 1.5	112.1 ± 1.7	115.0 ± 1.6	116.0 ± 1.7
Ethanolic extract (100 mg/kg)	100.0 ± 1.5	80.1 ± 1.2*	23.5 ± 0.3*#	22.3 ± 0.4*#	20.5 ± 0.4*
Ethanolic extract (200 mg/kg)	100.0 ± 0.6	76.5 ± 2.1*#	48.5 ± 2.4*	46.6 ± 2.3*#	41.7 ± 2.3*#
Ethanolic extract (400 mg/kg)	100.0 ± 1.2	70.9 ± 1.3*#	57.7 ± 1.9*#	51.0 ± 1.7*#	43.9 ± 1.4 *#
Ethyl acetate fraction (200 mg/kg)	100.0 ± 1.4	31.4 ± 1.5 *#	20.8 ± 0.4*#	16.4 ± 0.3*#	15.0 ± 0.3 *#
Dichloromethane fraction (200 mg/kg)	100.0 ± 0.9	79.7 ± 1.9*	75.1 ± 2.3*#	46.2 ± 2.1 *#	42.1 ± 2.0 *#
n-hexane fraction (200 mg/kg)	100.0 ± 0.5	77.8 ± 1.4 *#	56.3 ± 2.1 *#	33.5 ± 0.7 *	31.0 ± 0.6#*
Glibenclamide (5 mg/kg)	100.0 ± 1.2	85.0 ± 1.0 *	54.0 ± 1.5*	30.7 ± 0.1*	25.5 ± 0.4*

*Values are given as Mean ± SEM (n = 5); *: P < 0.05 comparison of values vs that of control at D_d; #: P < 0.05 comparison of values vs that of glibenclamide at D_d; D_d: Percentage FBGL on day 4, 7, 10 and 14*

Table 2. Effect of *A. vogelii* ethanolic root extract and fractions on lipid profile in STZ-induced diabetic rats

Groups/Lipid profile	CHOL (mmol/l)	TRIG (mmol/l)	HDL (mmol/l)	LDL (mmol/l)
Control	8.30 ± 0.27	3.12 ± 0.14	1.23 ± 0.24	3.74 ± 0.39
Ethanolic extract (100 mg/kg)	6.25 ± 0.51 *	0.50 ± 0.27 *	3.37 ± 0.21 *	2.38 ± 0.44
Ethanolic extract (200 mg/kg)	6.37 ± 0.15 *	0.63 ± 0.20 *	3.44 ± 0.16*	2.30 ± 0.20
Ethanolic extract (400 mg/kg)	6.33 ± 0.11 *	0.83 ± 0.12 *	3.64 ± 0.14 *	2.06 ± 0.26 *
Ethyl acetate fraction (200 mg/kg)	5.28 ± 0.55 *	1.25 ± 0.36 *	2.89 ± 0.24 *	0.99 ± 0.37 *
Dichloromethane fraction (200 mg/kg)	4.62 ± 0.17 *	0.24 ± 0.13 *	3.35 ± 0.31*	1.0 ± 0.29 *
n-hexane fraction (200 mg/kg)	4.97 ± 0.35 *	0.66 ± 0.15 *	3.47 ± 0.20 *	0.84 ± 0.30 *
Glibenclamide (5 mg/kg)	6.19 ± 0.46 *	0.47 ± 0.13*	3.59 ± 0.22 *	2.13 ± 0.41*

*Values are given as Mean ± SEM; n = 5; CHOL: Cholesterol; TRIG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein; *: Significantly different from control at P < 0.05*

3.4 Effect of *A. vogelii* Ethanolic Root Extract and Fractions on Serum Liver and Kidney Enzymes in STZ-induced Diabetic Rats

The ethanolic extract and fractions elicited a significant decrease in alanine aminotransferase, aspartate aminotransferase and creatinine levels when compared with the control and glibenclamide (Table 3).

In diabetic state, insulin deficiency also contributes to derangements of various metabolic and regulatory mechanisms in the body [32]. The present study also evaluated the effect of *A.vogelii* root extract on some liver marker enzymes. Several key enzymes of glucose metabolism are markedly altered to produce hyperglycemia, which leads to impaired insulin secretion and enhances the pathogenesis of diabetes complications [35]. As observed in this study, *A. vogelii* ethanolic root extract and its fractions exerted a significant decrease in alanine aminotransaminase (ALT), asparate aminotransaminase (AST) and creatinine (CRT) level in STZ-induced diabetic treated rats. Diabetic untreated rats (control) showed elevated levels of ALT, AST and CRT in serum and this may be due to leaking out of these enzymes from the liver and kidney; and thus, migrating into the circulation due to the adverse effect of STZ by free radical mechanism [39]. Reduced serum concentrations of these enzymes in the extract treated diabetic rats indicate that *A. vogelii* ethanolic root extract and fractions can reduce the hepatotoxicity induced by STZ. The result of serum liver and kidney enzymes suggests that the extracts have the potential of reducing the serum levels of these enzymes in diabetic conditions.

3.5 Effect of *A. vogelii* ethanolic Root Extract and Fractions on % Δ in Body Weight, Food and Water Intake in STZ-induced Diabetic Rats

The ethanolic root extract and fractions exerted no significant effect in the body weight of rats in week 1 and 2 when compared with the control (Table 4). The ethanolic extract and fractions exerted a significant decrease in food intake in week 2 when compared with the control (Table 5).

The ethanolic extract and fractions exerted a significant decrease in water intake in week 2 when compared with the control (Table 6).

High blood sugar levels in diabetic state also leads to increased thirst (polydipsia), increased hunger (polyphagia) and at times slight increase or decrease in body weight [40,41]. The ethanolic extract and fractions exerted a decrease in food and water intake in STZ-induced diabetic rats in week 2. Hence, the result of this study also suggests that *A. vogelii* ethanolic root extract and fractions have anti-diabetic potentials.

Table 3. Effect of *A. vogelii* ethanolic root extract and fractions on serum liver and kidney enzymes in STZ-induced diabetic rats

Groups	ALT (µl)	AST (µl)	CRT (µmol/l)
Control	37.46 ± 0.98	59.64 ± 1.80	50.0 ± 0.71
Ethanolic extract (100 mg/kg)	13.87 ± 0.71 *#	26.68 ± 1.33 *#	23.50 ± 1.01 *#
Ethanolic extract (200 mg/kg)	22.37 ± 1.27 *	35.98 ± 1.03 *	28.34 ± 0.70 *
Ethanolic extract (400 mg/kg)	21.38 ± 0.74*	35.65 ± 2.70*	29.20 ± 0.43 *
Ethyl acetate fraction (200 mg/kg)	21.09 ± 0.55 *	32.76 ± 0.88*	31.86 ± 0.50 *#
Dichloromethane fraction (200 mg/kg)	20.64 ± 0.61 *	32.98 ± 1.06 *	26.30 ± 0.35 *#
n-hexane fraction (200 mg/kg)	24.55 ± 2.45 *	33.66 ± 1.87 *	29.46 ± 0.58 *
Glibenclamide (5 mg/kg)	26.97 ± 1.14*	38.83 ± 1.48 *	29.30 ± 0.40*

*Values are given as Mean ± SEM; n =5; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CRT: Creatinine; * : Significantly different from control at p < 0.05; # : Significantly different from glibenclamide at p < 0.05*

Table 4. Effect of *A. vogelii* ethanolic root extract and fractions on % Δ in body weight in STZ-induced diabetic rats

Groups/Days	Week 0	Week 1	Week 2
Control	100.0 ± 7.0	97.7 ± 4.9	98.0 ± 6.2
Ethanolic extract (100 mg/kg)	100.0 ± 6.4	97.4 ± 7.5	93.4 ± 5.1
Ethanolic extract (200 mg/kg)	100.0 ± 5.8	97.4 ± 6.0	94.1 ± 7.2
Ethanolic extract (400 mg/kg)	100.0 ± 2.9	96.0 ± 1.9	83.3 ± 2.7
Ethyl acetate fraction (200 mg/kg)	100.0 ± 7.8	91.4 ± 7.8	91.4 ± 7.8
Dichloromethane fraction (200 mg/kg)	100.0 ± 3.2	96.3 ± 4.6	93.8 ± 3.4
n-hexane fraction (200 mg/kg)	100.0 ± 8.9	96.7 ± 7.4	84.8 ± 2.9
Glibenclamide (5 mg/kg)	100.0 ± 2.6	96.9 ± 4.9	93.8 ± 4.2

*Values are given as Mean ± SEM; n = 7; *: Significantly different from control at p < 0.05; #: Significantly different from glibenclamide at p < 0.05*

3.6 Effect of *A. vogelii* ethanolic Root Extract and Fractions on the Histology of the Pancreas of STZ-induced Diabetic Rats

The photomicrograph of the pancreatic tissues showed well aligned acini with zymogen cells in normoglyceamic rats (Plate 1a); general distortion of the pancreatic histoarchitecture in the control (Plate 1b); slight distortion of the acini with regenerating beta cells at 100, 200 and 400 mg/kg ethanolic extract (Plate 1c, 1d and 1e respectively). The photomicrograph of the pancreatic tissues showed regenerating beta cell and well aligned acini with regenerating beta cell at 200 mg/kg ethylacetate fraction (Plate 1f); histoarchitecture of the pancreas appeared normal with regenerating beta cells at 200 mg/kg dichloromethane fraction (Plate 1g); slight distortion of the acini with regenerating beta cells at 200 mg/kg *n*-hexane fraction (Plate 1h) and well aligned acini with regenerating beta cells at 5 mg/kg glibenclamide (Plate 1i). Several studies have provided evidence that loss of functional β-cell mass through apoptosis, impaired proliferation consequent to hyperglycaemia is central to the development of both type1 and type 2 diabetes mellitus [42]. Regulation of functional β-cell mass has been considered as a critical therapeutic challenge in patients with the disease [43]. The histopathology of the pancreas of diabetic treated rats in this study revealed that *A. vogelii* ethanolic root extract and fractions exerted β-cell regeneration, as well as liver and kidney cells in diabetic treated rats. The pancreatic tissue was able to regenerate to maintain or increase β-cell mass in response to metabolic demands as reported by [44]. The regeneration of β-cells may be responsible for the favourable changes observed in both the lipid profile parameters and the biochemical marker enzymes in the serum of *A. volgelii* treated rats. Other sudies have also demonstrated possible regeneration of β-cell islets following the administration of other crude medicinal plant extracts such as leaf extract of *Carica papaya* and fruit extract of *Terminalia catappa* in diabetic animal models [45]. The assessment of histopathology showed that *A. volgelii* extract has an effect on β-cell regeneration as compared to the control. This may be due to the direct regenerative potency of phytochemicals such as polyphenolic compounds and flavonoids present in *A vogelii* extract [46].

3.7 Effect of *A. vogelii* Ethanolic Root Extract and Fractions on the Histology of the Kidney of STZ-induced Diabetic Rats

The photomicrograph of the kidney tissues showed normal cortical architecture with normal glomerulus in normoglyceamic rats (Plate 2a); degenerating glomerulus with no glomerular space in the control (Plate 2b); normal cortical architecture with regenerating glomerulus at 100, 200 and 400 mg/kg ethanolic extract (Plate 2c, 2d and 2e respectively); normal cortical architecture with normal glomerulus at 200 mg/kg ethylacetate fraction (Plate 2f); normal cortical architecture with regenerating glomerulus at 200 mg/kg dichloromethane and *n*-hexane fractions (Plate 2g and 2h respectively) and regenerating glomerulus at 5 mg/kg glibenclamide (Plate 2i).

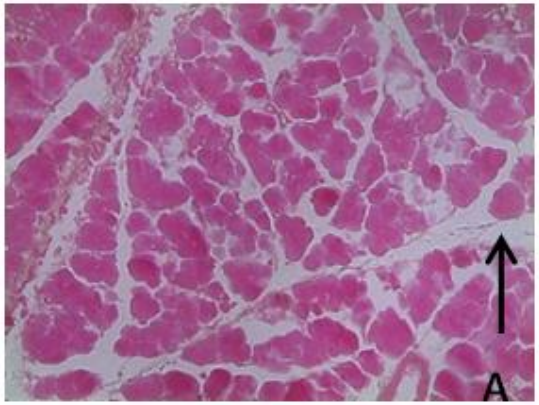

Plate 1a. Normoglyceamic rats

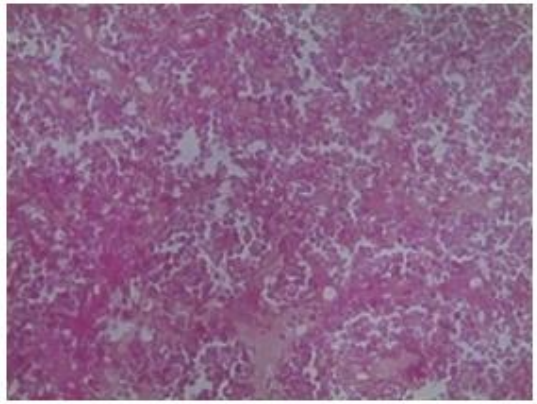

Plate 1b. Control

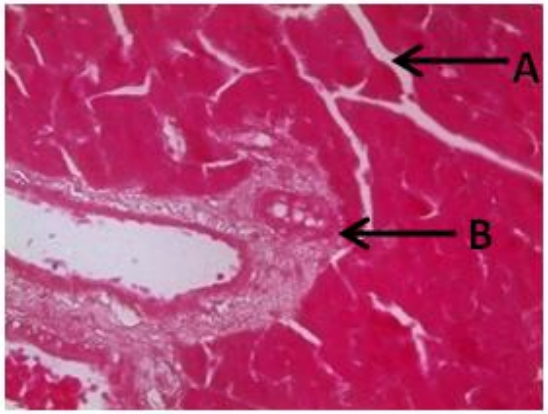

Plate 1c. EE (100 mg/kg)

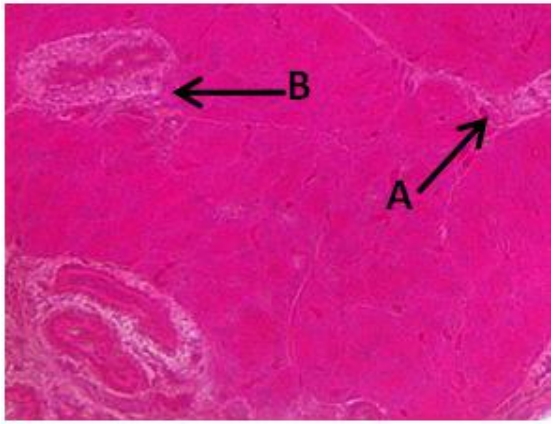

Plate 1d. EE (200 mg/kg)

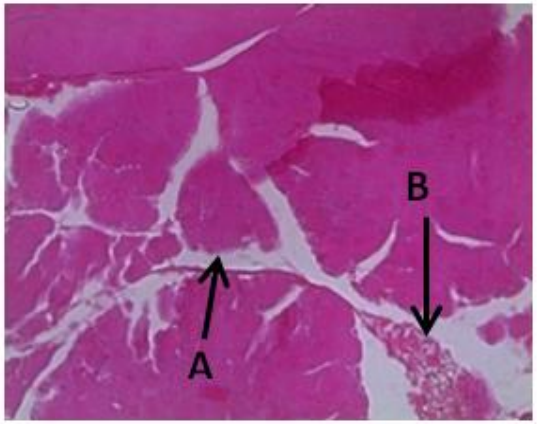

Plate 1e. EE (400 mg/kg)

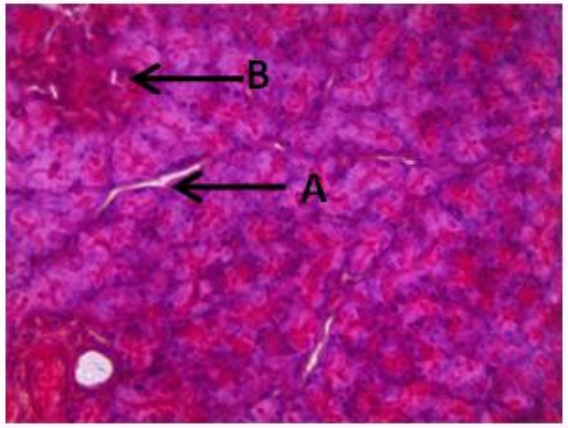

Plate 1f. EF

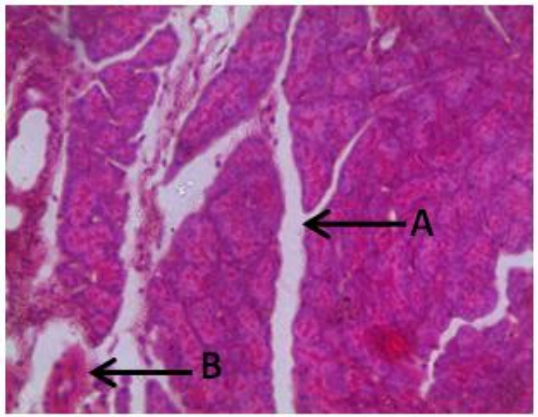

Plate 1g. DF

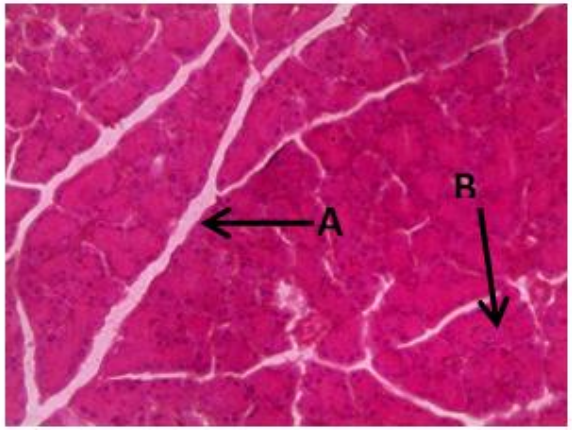

Plate 1h. HF

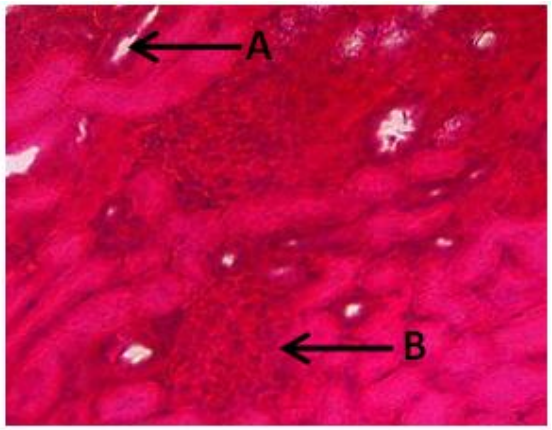

Plate 1i. GB

Plates 1a – 1i. Histopathological effect of *A. vogelii* ethanolic root extract and fractions on the pancreas of STZ-induced diabetic rats. EE: Ethanolic extract; EF: Ethylacetate fraction; DF: Dichloromethane fraction; HF: n-hexane fraction; GB: Glibenclamide; Magnification: X 400; A: Acini; B: Beta cells

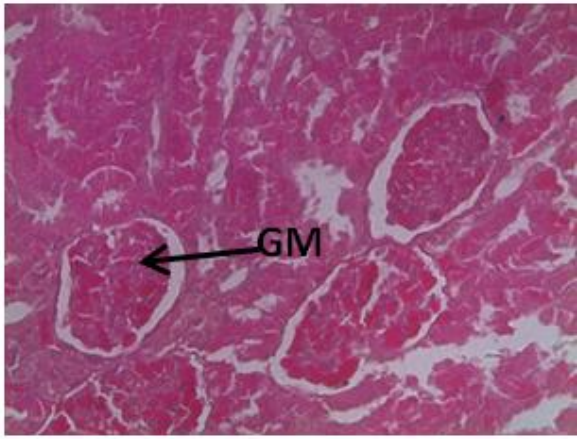

Plate 2a. Normoglyceamic rats

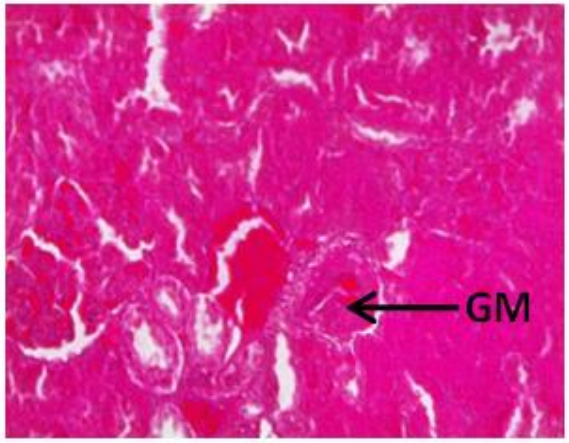

Plate 2b. Control

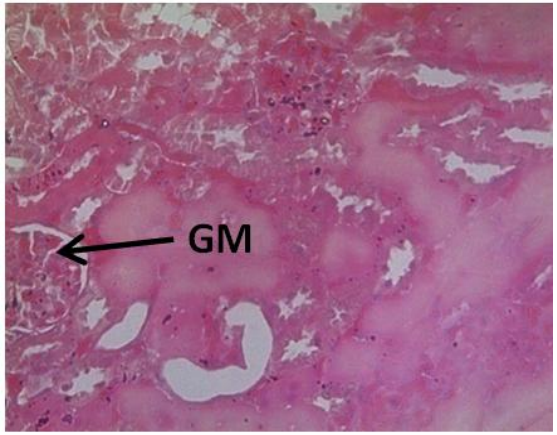

Plate 2c. EE (100 mg/kg)

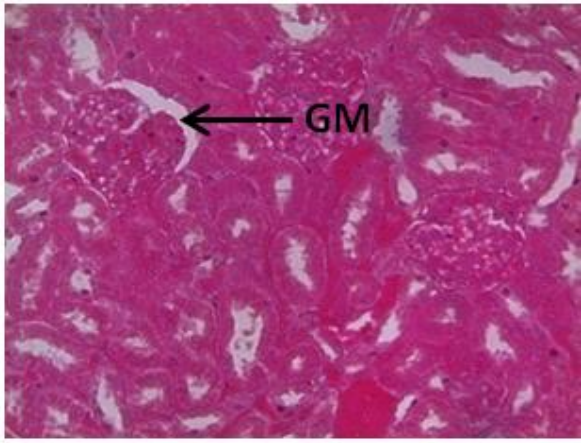

Plate 2d. EE (200 mg/kg)

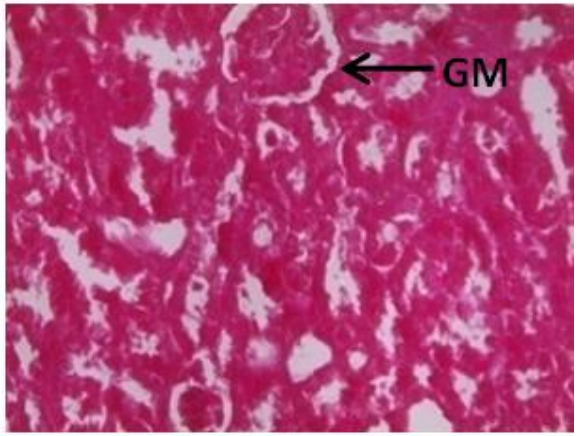

Plate 2e. EE (400 mg/kg)

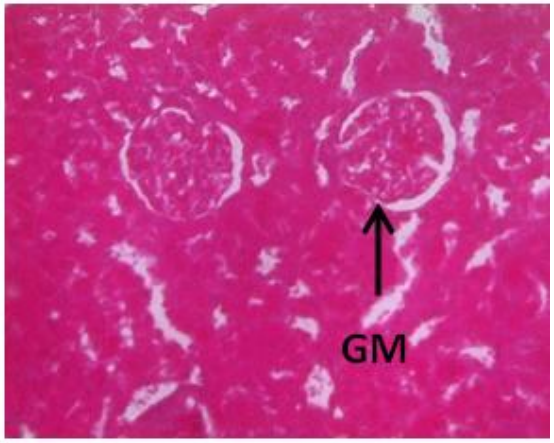

Plate 2f. EF (200 mg/kg)

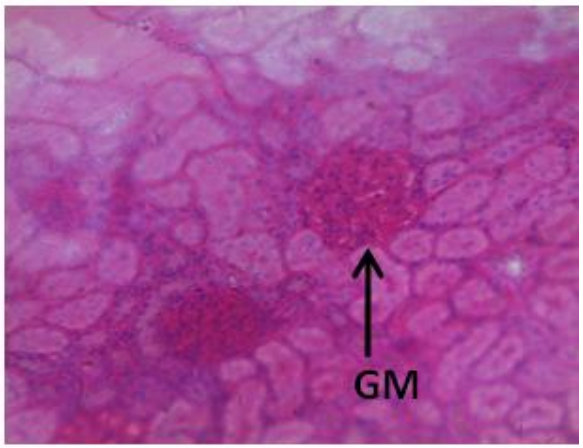

Plate 2g. DF

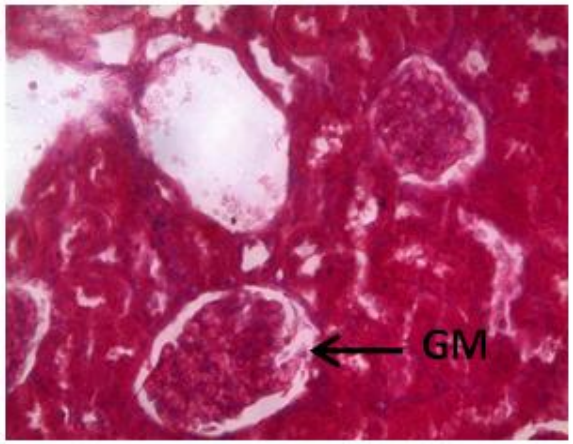

Plate 2h. HF

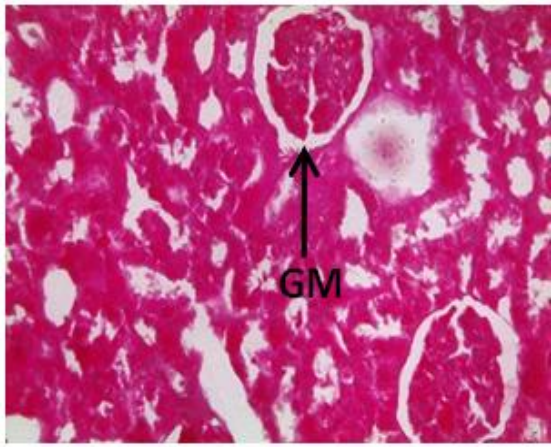

Plate 2i. GB

Plates 2a – 2i. Histopathological effect of *A. vogelii* ethanolic root extract and fractions on the kidney of STZ-induced diabetic rats. Normoglyceamic rats: non-diabetic; Control: 10 mL/kg distilled water; EE: Ethanolic extract; EF: Ethylacetate fraction; DF: Dichloromethane fraction; HF: *n*-hexane fraction; GB: Glibenclamide; Magnification: X 400; GM: Glomerulus

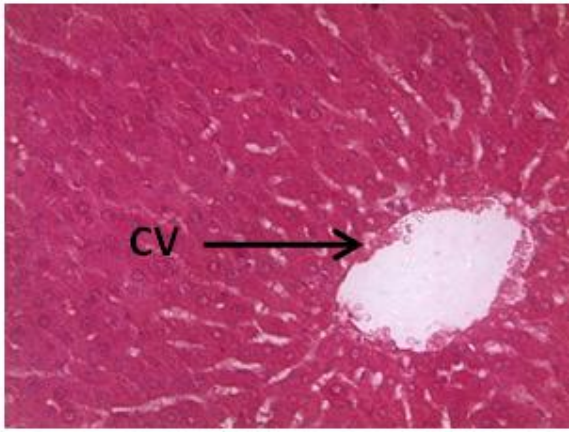

Plate 3a. Normoglyceamic rats

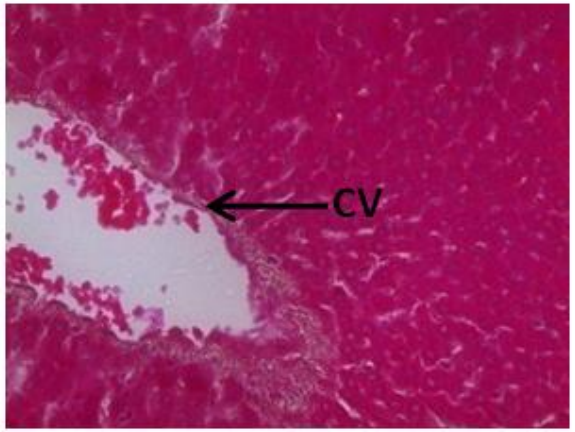

Plate 3b. Control

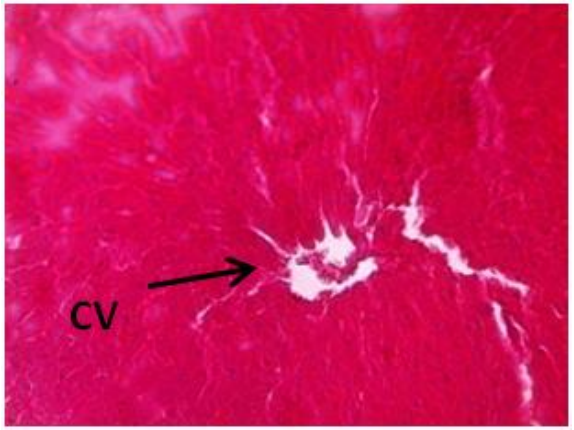

Plate 3c. EE (100 mg/kg)

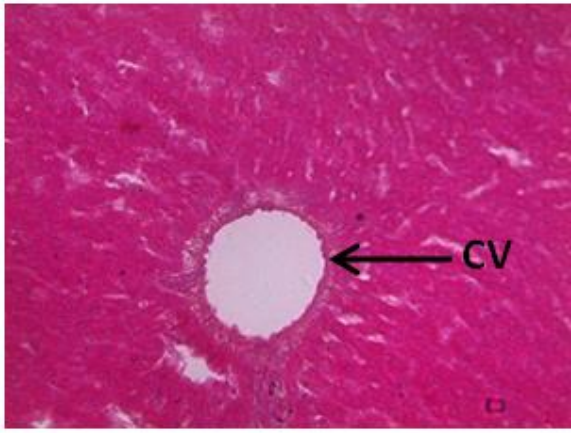

Plate 3d. EE (200 mg/kg)

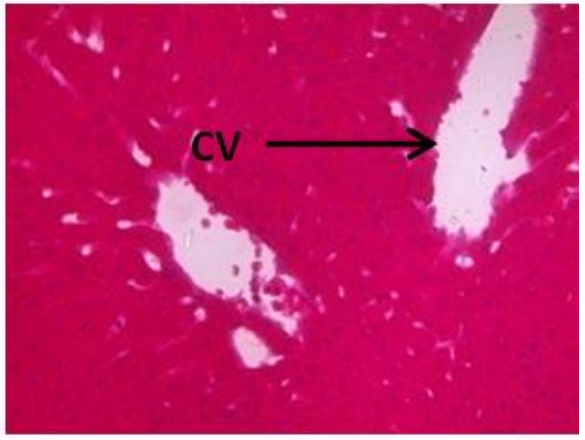

Plate 3e. EE (400 mg/kg)

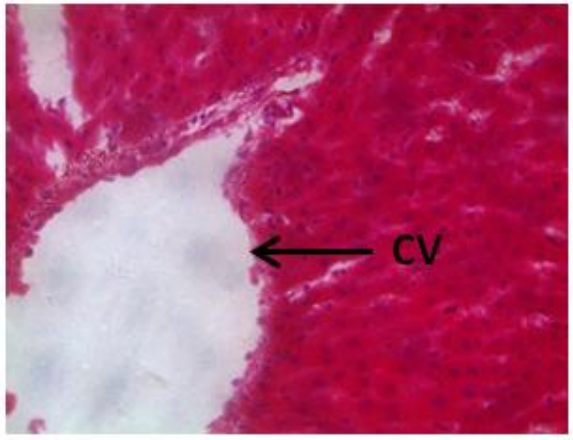

Plate 3f. EF

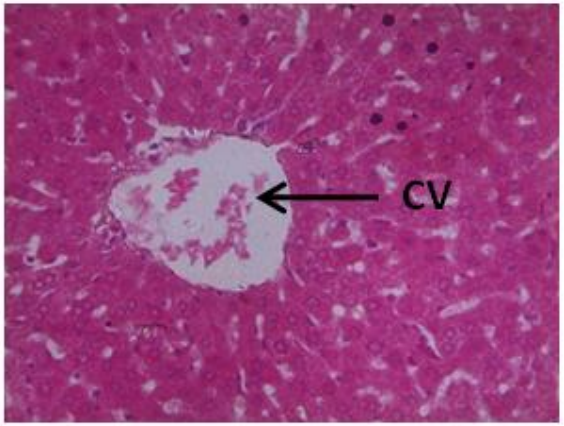

Plate 3g. DF

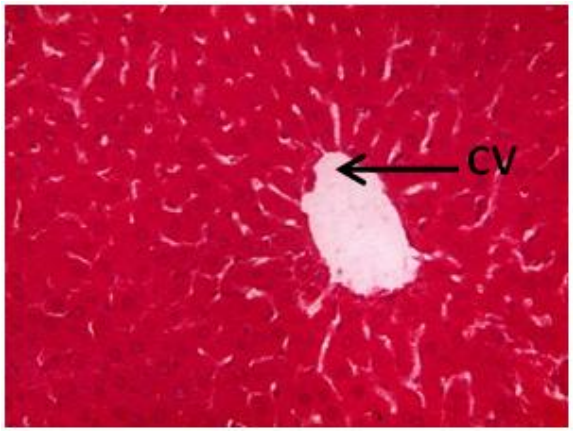

Plate 3h. HF

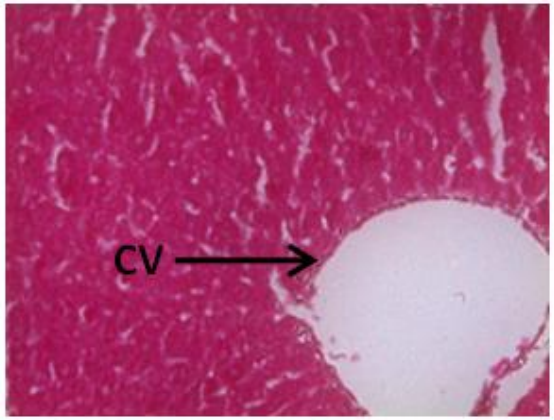

Plate 3i. GB

Plates 3a – 3i. Effect of *A. vogelii* ethanolic root extract and fractions on the liver of STZ-induced diabetic rats. Normoglyceamic rats; Control: 10 mL/kg distilled water; EE: Ethanolic extract; EF: Ethylacetate fraction; DF: Dichloromethane fraction; Magnification: X 400; CV: Central vein

Table 5. Effect of *A. vogelii* ethanolic root extract and fractions on weekly % food intake per body weight in STZ-induced diabetic rats

Groups/Weeks	Week 1	Week 2
Control	67.3 ± 3.2	50.2 ± 3.5
Ethanolic extract (100 mg/kg)	47.8 ± 2.1	46.3 ± 2.4*
Ethanolic extract (200 mg/kg)	44.9 ± 1.9	42.3 ± 1.8*
Ethanolic extract (400 mg/kg)	45.9 ± 3.2	44.7 ± 3.9*
Ethyl acetate fraction (200 mg/kg)	43.8 ± 2.2	41.1 ± 2.4*
Dichloromethane fraction (200 mg/kg)	42.9 ± 3.0	41.8 ± 3.3*
n-hexane fraction (200 mg/kg)	46.3 ± 2.1	45.3 ± 2.7*
Glibenclamide (5 mg/kg)	45.7 ± 2.7	42.4 ± 3.0*

*Values are given as Mean ± SEM; n =7; *: Significantly different from control at p < 0.05*

Table 6. Effect of *A. vogelii* ethanolic root extract and fractions on weekly % water intake per body weight in STZ-induced diabetic rats

Groups/Weeks	Week 2	Week 1
Control	191.2 ± 6.2	153.5 ± 6.8
Ethanolic extract (100 mg/kg)	140.9 ± 3.3	138.9 ± 3.0*
Ethanolic extract (200 mg/kg)	149.4 ± 2.8	131.3 ± 2.3*
Ethanolic extract (400 mg/kg)	155.1 ± 2.7	141.9 ± 2.1*
Ethyl acetate fraction (200 mg/kg)	142.8 ± 6.0	133.0 ± 6.6*
Dichloromethane fraction (200 mg/kg)	146.6 ± 3.4	144.8 ± 3.8*
n-hexane fraction (200 mg/kg)	143.6 ± 3.1	139.9 ± 3.2*
Glibenclamide (5 mg/kg)	143.8 ± 3.7	140.5 ± 4.6*

Values are given as Mean ± SEM; n =7
**: Significantly different from control at p < 0.05*

3.8 Effect of *A. vogelii* Ethanolic Root Extract and Fractions on the Histology of the Liver of STZ-induced Diabetic Rats

The photomicrograph of the liver tissues showed well aligned hepatic architecture around the central vein in normoglyceamic rat liver tissue (Plate 3a); disruption of hepatic architecture around a central vein in the control group (Plate 3b); regenerating hepatocytes around a central vein at 100 and 400 mg/kg ethanolic extract (Plate 3c and 3e respectively); apparently normal hepatocyte around a central vein at 200 mg/kg ethanolic extract (Plate 3d); apparently normal hepatocyte around a central vein at 200 mg/kg ethylacetate fraction (Plate 3f); regenerating hepatocytes around a central vein at 200 mg/kg dichloromethane and *n*-hexane fractions (Plate 3g and 3h respectively) and regenerating hepatocytes around a central vein at 5 mg/kg glibenclamide (Plate 3i).

4. CONCLUSION

In conclusion, *A. vogelii* ethanolic root extract and fractions are safe when administered acutely (p.o). Also, *A. vogelii* root ethanolic extract and fractions exerted potent anti-diabetic and anti-hyperlipideamic activity in diabetic treated rats. Furthermore, the qualitative data of histopathology indicates that *A. vogelii* extract was able to protect β-cell oxygen derived free radical-mediated destruction in the pancreas of diabetic rats. This gives credence to the use of the plant roots in ethno-medicine for the management of diabetes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The animal experiments were performed according to the approved guidelines of Obafemi Awolowo University research ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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