ANTI-DIABETIC EFFECT OF *ANTHOCLEISTA VOGELII* AQUEOUS ROOT EXTRACT IN STREPTOZOTOCIN INDUCED DIABETIC RATS.

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ABSTRACT

Anthocleista vogelii is one of the major constituents of herbal preparations traditionally used in the management of diabetes mellitus in the South western part Nigeria. This study was carried out to evaluate the potential anti-diabetic effect of *Anthocleista vogelii* aqueous root extract in streptozotocin-induced diabetic rats with a view of scientifically validating its ethno-medicinal properties. Albino rats of both sexes were randomly divided into five groups in glucose loaded (GL) rats (10 g/kg glucose p.o); Group 1 (control) diabetic untreated rats (10 ml/kg distilled water), group 2-4 diabetic treated rats (100, 200 and 400 mg/kg *A. vogelii* aqueous root extract [AVR]) and group 5 (5 mg/kg glibenclamide [GB]) while in streptozotocin (STZ)-induced diabetic rats (60 mg/kg; i.p.), Albino rats were randomly divided into three groups; Group 1 (control) diabetic untreated rats (10 ml/kg distilled water), group 2 diabetic treated rats (200 mg/kg AVR) and group 3 diabetic treated rats (5 mg/kg GB). Fasting blood glucose levels (FBGL) of the diabetic rats were determined at intervals of 30, 60, 120 and 240 minutes in GL rats and on days 4, 7, 10 and 14 in STZ-induced diabetic rats. After two weeks, the levels of serum cholesterol (CHOL), triglyceride (TRIG), high density lipoprotein (HDL), low density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine (CRT) of STZ-induced diabetic rats were analyzed. The LD_{50} of AVR was ≥ 5000 mg/kg in rats (p.o.). 200 mg/kg exerted a more significant reduction in FBGL in GL rats when compared with the control; hence only 200 mg/kg of AVR was used in STZ-induced diabetic rats. The extract exerted a significant ($P<0.05$) reduction in FBGL, CHOL, TRIG, LDL, ALT, AST and CRT levels and an increase in serum HDL when compared to the control in STZ-induced diabetic rats. The photomicrograph of the pancreatic tissues of the control group showed general distortion of the pancreatic histoarchitecture while in the treatment group the photomicrograph showed interlobular connective tissue septa with normal serous acini and zymogen cells. The study concluded that AVR is safe when administered orally. It has anti-diabetic and anti-hyperlipideamic effect when administered for fourteen days in STZ-induced diabetic rats.

Keywords: *Anthocleista vogelii*, Diabetes, Streptozotocin, Root.

INTRODUCTION

Diabetes mellitus is a metabolic disorder, that has to do with high fasting blood glucose level (hyperglyceamia), either because the beta cells of the islet of langerhans found in the pancreas do not produce insulin, or the beta cells do not produce enough insulin or because the cells in the body are not sensitive to the insulin that is being produced by the beta cells (Murray *et al*., 2003). This metabolic disorder is common in developed and developing countries, affecting both the young and old. A number of medicinal plants have been used traditionally in various herbal preparations for the management of diabetes mellitus. Despite considerable progress in the treatment of diabetes with synthetic hypoglyceamic agents, search for plants with hypoglyceamic and anti-hyperlipideamic properties is an area that draws attention of researchers. Studies have shown that medicinal plants are potential sources of hypoglycaemic drugs and plant derived compounds (herbs) have been used in the treatment of diabetes (Katerere and Eloff, 2005; Balde *et al*., 2006). *A. vogelii* is a medicinal plant used in ethno-medicine for the treatment of syphilis, chest pain, fever and diabetes (Noumi and Eloumou, 2011; Jegede *et al*., 2011; Igoli *et al.,* 2005; Soladoye *et al.,* 2012). Hence, this study evaluates the anti-diabetic property of the aqueous root extract of *A. vogelii* in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

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

Plant Collection and Extraction

A. vogelii root was collected from the premises of National Biotechnology Development Agency, Bioresources Centre, Ogbomosho, Nigeria and were authenticated at IFE-Herbarium where a voucher specimen (No. 17399) was deposited. *A. vogelii* root was washed, oven dried at 40 °C, pulverized and macerated in distilled water for 72 hours before undergoing filtration using muslin cloth and cotton wool in funnel. The filtrate was then concentrated into a solid paste *in vacuo* at 45 °C using a rotary evaporator (Okokon and Nwafor, 2009) and then freeze dried in a freeze drier. The dried extract was stored in a refrigerator at 4 °C till when it was needed for use.

Animals

Albino rats (both sexes) weighing between 150-200g were obtained from the 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 feeds and were given water *ad libitum*. The rats were allowed to acclimatize with the environment at ambient temperature (36 °C) under natural day light/night conditions for two weeks before the start of the experiment.

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.) using the method of Lorke (1983).

Glucose Loading

10 g/kg body weight of glucose was administered (p.o.) to Albino rats that were fasted overnight. After 30 minutes of glucose administration, the blood glucose level was checked using glucometre and glucose strip [Accu-Check Active Glucometer, model: GC0088, Mannheim Germany] (Etuk, 2010). Rats with blood glucose level above 7.0 mmol/L were taken for the test.

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 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 (Lenzen and Munday, 1991; Etuk, 2010).

Administration of Dose

In glucose loaded rats; Group 1 (control) diabetic untreated rats (10 ml/kg distilled water), group 2-4 diabetic treated rats (100, 200 and 400 mg/kg *A. vogelii* aqueous root extract) and group 5 (5 mg/kg glibenclamide). 200 mg/kg exerted a more significant reduction in FBGL in glucose-loaded rats when compared with the control; hence only 200 mg/kg of AVR was used in STZ-induced diabetic rats. In STZ-induced diabetic rats; Group 1 (control) diabetic untreated rats (10 ml/kg distilled water), Group 2 diabetic treated rats (200 mg/kg *A. vogelii* aqueous root extract) and group 3 diabetic treated rats (5 mg/kg glibenclamide). Administration was once in glucose loaded rats and daily for 14 days in STZ-induced diabetic rats.

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.

Determination of Biochemical Parameters

The FBGL was measured at 0, 30, 60, 120 and 240 minutes in glucose-loaded rats and on day 1, 4, 7, 10 and 14 in STZ-induced diabetic rats (Adebajo *et al.,* 2007; Okokon and Nwafor, 2009) 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. The blood samples were centrifuged at 2500 rpm for 25 minutes and the serum was used for biochemical analysis these include, CHOL (Abbel*et al.,* 1952, Richmond, 1973, Roeschlau *et al.,* 1974 and Trinder, 1969), TRIG (Tietz, 1990; Trinder, 1969 and Koditschek *et al.,* 1969), HDL (Jacobs *et al.,* 1990), CRT (Bartels and Bohmer, 1972 and Schirmeister *et al.,* 1964), AST and ALT (Reitman and Frankel, 1957, Schmidt and Schmidt, 1963).

Histopathology

The sections of the liver, kidney and pancreas 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.

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 using Primer (version 3.01).

RESULTS

Acute Toxicity Results of *A. vogelii A*queous Root Extract

The $LD_{50} \geq 5000$ mg/kg body weight *A. vogelii* aqueous root extract (p.o.).

Effect of *A. vogelii A*queous Root Extract on % FBGL

The aqueous extract and glibenclamide exerted a significant decrease in FBGL in both glucose loaded (Table 1) and STZ-induced diabetic rats (Table 2) when compared with the control.

Effect of Aqueous Root Extract of *A. vogelii* on Lipid Profile in Streptozotocin-induced Diabetic Rats

The aqueous extract and glibenclamide elicited a significant ($P < 0.05$) decrease in CHOL, TRIG and LDL levels and an increase in HDL level when compared with the control (Figure 1).

Effect of Aqueous Root Extract of *A. vogelii* on Biochemical Parameters in Streptozotocin-induced Diabetic Rats

The aqueous extract and glibenclamide also elicited a significant ($P < 0.05$) decrease in ALT, AST and CRT levels when compared with the control (Figure 2).

Effect of Aqueous Root Extract of *A. vogelii* on the Histology of the Pancreas of Streptozotocin-induced Diabetic Rats

The photomicrograph of the pancreatic tissues of normoglyceamic rats (Plate 1A) shows well aligned acini with zymogen cells; the control group (Plate 1B) shows general distortion of the pancreatic histoarchitecture; 200 mg/kg of *A. vogelli* aqueous extract group (Plate 1C) and in glibenclamide group (Plate 1D) shows interlobular connective tissue septa (black arrow) with normal serous acini and zymogen cells.

Effect of Aqueous Root Extract of *A. vogelii* on the Histology of the Kidney of Streptozotocin-induced Diabetic Rats

The photomicrograph of the kidney tissues of normoglyceamic rats (Plate 2A) shows normal cortical architecture with normal glomerulus (GM); the control group (Plate 2B) shows degenerating glomerulus with no glomerular space; 200 mg/kg aqueous extract group (Plate 2D) and glibenclamide group (Plate 2C) shows regenerating cortical architecture with regenerating glomerulus.

Effect of Aqueous Root Extract of *A. vogelii* on the Histology of the Liver of Streptozotocin-induced Diabetic Rats

The photomicrograph of normoglyceamic rats liver tissue (Plate 3A) shows well aligned hepatic architecture around the central vein (CV); the control group (Plate 3B) shows general disruption of hepatic architecture around a central vein; 200 mg/kg of *A. vogelli* aqueous extract group (Plate 3C) and glibenclamide group (Plate 3D) shows regenerating hepatocytes around a central vein.

Table 1: Effect of *A. vogelii* aqueous root extract on % FBGL in glucose loaded rats.

Groups/Time	0 minute	30 minutes	60 minutes	120 minutes	240 minutes
Control	100.0 ± 1.0	100.0 ± 0.8	90.5 ± 0.5	85.3 ± 0.3	71.6 ± 0.4
100 mg/kg Extract	100.0 ± 0.8	88.1 ± 0.6$^{*\#}$	88.1 ± 0.3$^{*\#}$	82.1 ± 0.4$^{*\#}$	70.6 ± 0.6$^{\#}$
200 mg/kg Extract	100.0 ± 0.7	93.9 ± 0.3*	87.8 ± 0.7$^{*\#}$	74.4 ± 0.9$^{*\#}$	48.9 ± 0.3*
400 mg/kg Extract	100.0 ± 0.1	90.8 ± 0.3*	89.1 ± 0.3$^{\#}$	85.5 ± 0.3$^{\#}$	76.3 ± 0.4$^{\#*}$
Glibenclamide (5 mg/kg)	100.0 ± 0.4	92.3 ± 0.4*	76.9 ± 0.3*	60.3 ± 0.1*	48.7 ± 0.1*

Values are given as Mean ± SEM (n = 5); *: P < 0.05 comparison of values vs that of control at T; #: P < 0.05 comparison of values vs that of glibenclamide at T; T: Percentage of FBGL at 0, 30, 60, 120 and 240 minutes.

Table 2: Effect of aqueous root extract of *Anthocleista vogelii* on % FBGL in streptozotocin-induced diabetic rats.

Groups/Days	DAY 1	DAY 4	DAY 7	DAY 10	DAY 14
Control	100.0 ± 1.4	111.9 ± 1.6	112.4 ± 1.8	114.9 ± 1.8	116.3 ± 1.8
200 mg/kg Extract	100.0 ± 2.2	80.2 ± 3.3*	69.8 ± 2.1$^{*\#}$	49.5 ± 2.8$^{*\#}$	42.9 ± 2.6$^{*\#}$
Glibenclamide (5 mg/kg)	100.0 ± 1.4	85.3 ± 1.0*	53.9 ± 1.5*	30.9 ± 0.2*	25.7 ± 0.5*

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

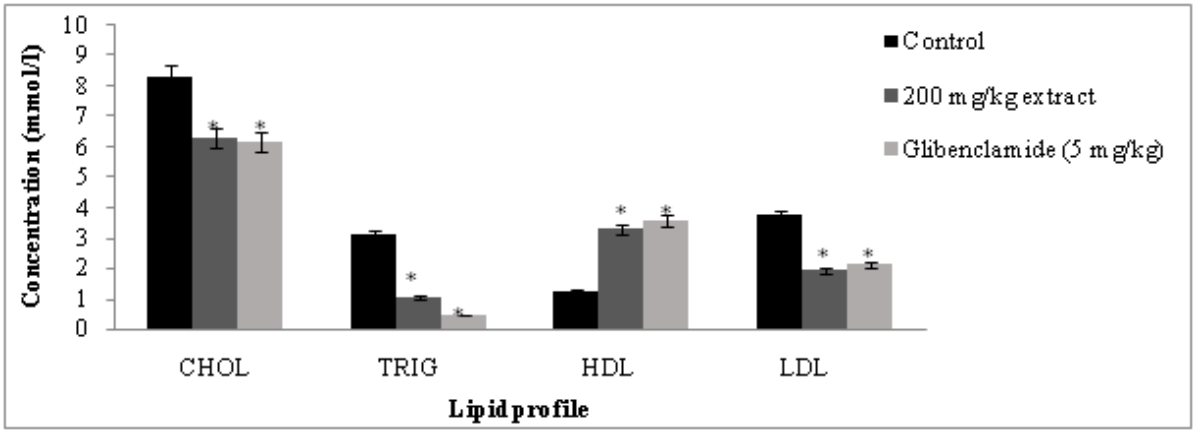

Figure 1: Effect of aqueous root extract of *A. vogelii* on lipid profile in streptozotocin-induced diabetic rats.

Values are given as Mean ± SEM (n = 5); *: P < 0.05 comparison of values vs that of control; #: P < 0.05 comparison of values vs that of glibenclamide; CHOL: Cholesterol; TRIG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein.

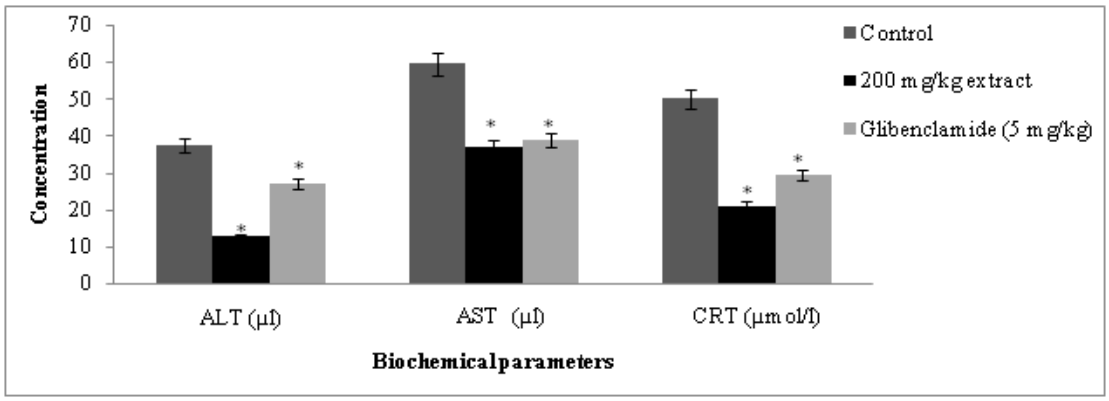

Figure 2: Effect of aqueous root extract of *A. vogelii* on biochemical parameters in streptozotocin-induced diabetic rats.

Values are given as Mean ± SEM (n =5); *: P < 0.05 comparison of values vs that of control; #: P < 0.05 comparison of values vs that of glibenclamide; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CRT: Creatinine

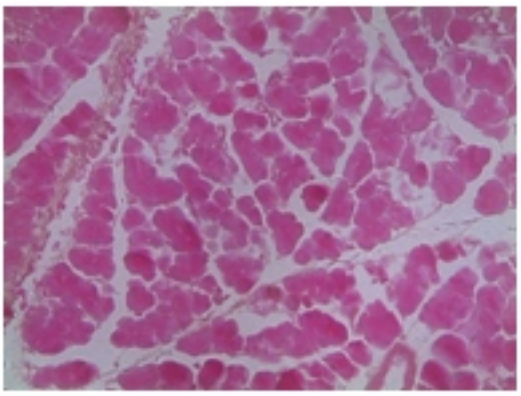

Plate 1A: Normoglyceamic rat

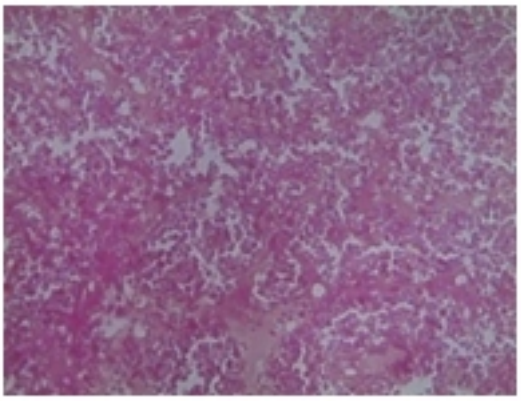

Plate 1B: Control

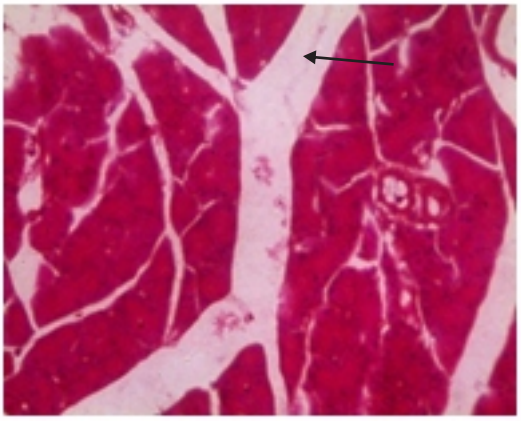

Plate 1C: 200 mg/kg extract

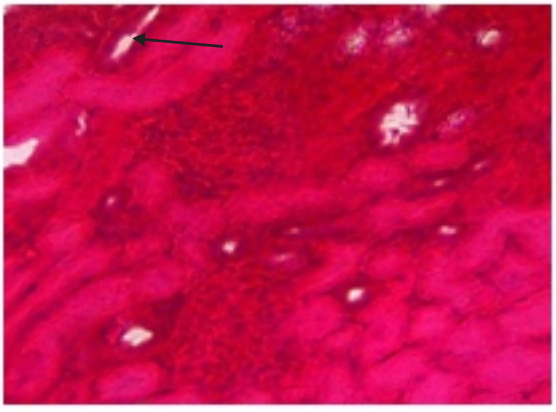

Plate 1D: Glibenclamide (5 mg/kg)

Effect of Aqueous Root Extract of *A. vogelii* on the Histology of the Pancreas of Streptozotocin-induced Diabetic Rats

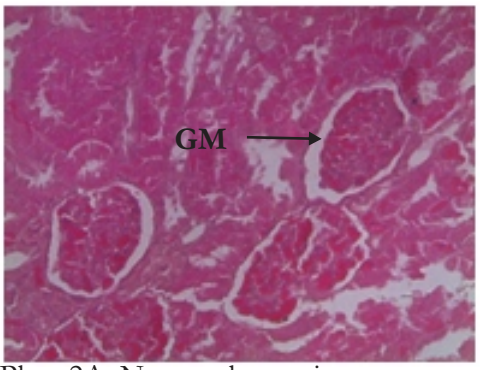

Plate 2A: Normoglyceamic rats

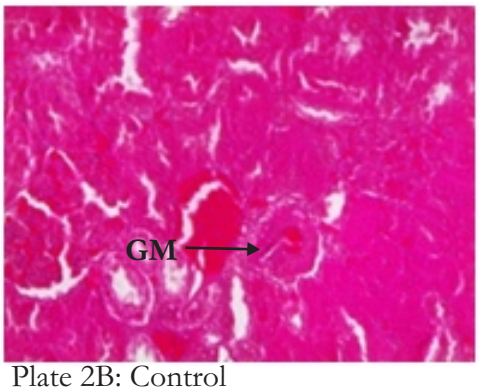

Plate 2B: Control

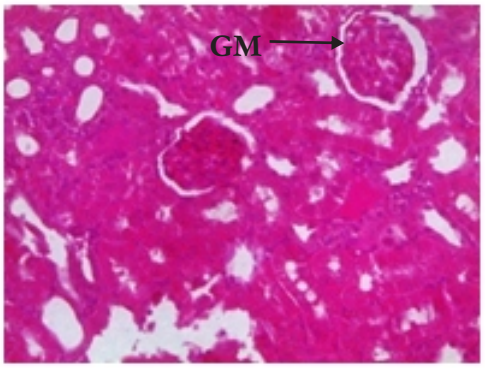

Plate 2C: 200 mg/kg extract

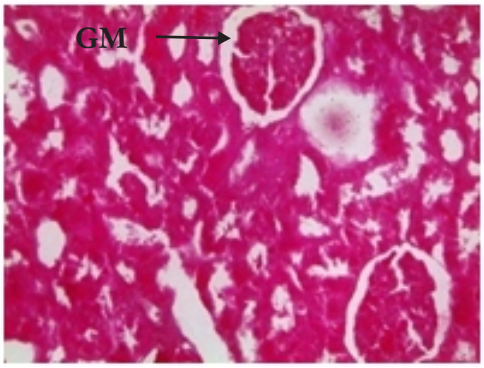

Plate 2D: Glibenclamide (5 mg/kg)

Effect of Aqueous Root Extract of *A. vogelii* on the Histology of the Kidney of Streptozotocin-induced Diabetic Rats

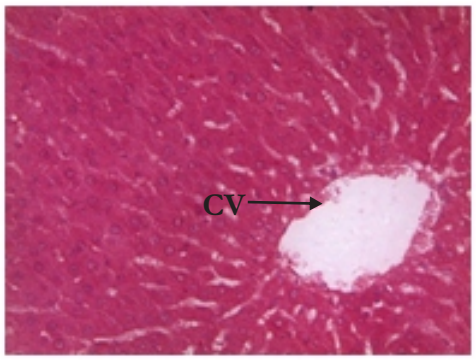

Plate 3A: Normoglyceamic rat

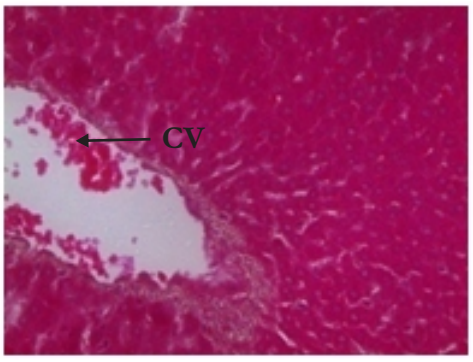

Plate 3B: Control

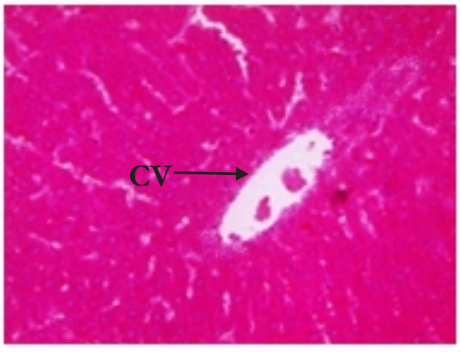

Plate 3C: 200 mg/kg extract

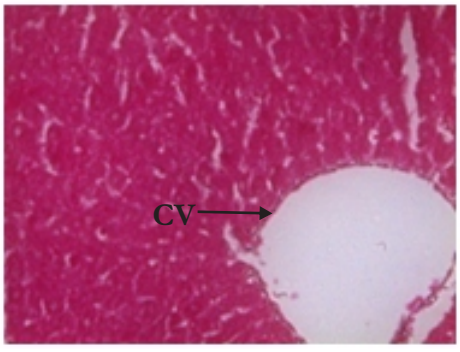

Plate 3D: Glibenclamide (5 mg/kg)

Effect of Aqueous Root Extract of *A. vogelii* on the Histology of the Liver of Streptozotocin-induced Diabetic Rats

DISCUSSION

Investigation of the acute toxicity is the first step in the investigation of an unknown substance in order to determine a safe dose for administration. The LD_{50} (p.o.) result shows that *A. vogelii* aqueous root extract is safe.

Diabetes mellitus arises from a deficient production of insulin by the beta cells of the pancreatic islets which lead to the complete or relative insufficiency of insulin secretion and or insulin action (Balkau *et al*., 2000). Several animal models have been developed for testing anti-diabetic agents. In this study, two models were employed in the induction of hyperglyceamia; these models include oral glucose loading (physiological induction of diabetes mellitus) and streptozotocin-induced diabetes model (chemical induction of diabetes mellitus) (Etuk, 2010). Oral glucose loading model has to do with the induction of hyperglycaemia in the presence of intact pancreas while streptozotocin is a beta cytotoxin, which induces diabetes in a wide variety of animal species by damaging the insulin-secreting pancreatic beta cells resulting in a decrease in endogenous insulin release which paves the way for the decreased utilization of glucose by the tissues (Yamamoto *et al*., 1981). Streptozotocin not only destroys the pancreatic beta cells, it also causes significant renal and hepatic toxicity (Rerup, 1970; Weiss, 1982). Glibenclamide has been widely accepted as a standard drug in diabetic animal experiments. Glibenclamide produces anti-diabetic effects through secretion of insulin associated with mild or moderate hyperglycaemia (Sokolovska *et al*., 2012). The FBGL of *A. vogelii* treated diabetic rats was reduced significantly at 30 minutes in glucose loaded rats and on the 4th day onwards all through the period of the experiment in streptozotocin-induced diabetic rats. There have been reports that *A. vogelii* contain pytochemicals such as alkaloid, saponin, tannin, steroid flavonoids and cardiac glycosides (Anyanwu *et al.,* 2013; Jegede *et al.,* 2011). Studies have shown that the presence of flavonoids in plants helps in the reduction of fasting blood glucose levels since flavonoids have been found to stimulate the secretion of insulin (Owolabi *et al*., 2011).

Diabetes mellitus is usually associated with high levels of serum lipids and such an increase causes a risk factor for coronary heart disease (Nathan *et al.,* 2005). Streptozotocin-induced diabetic rats also developed hyperglycaemia which is in agreement with previous observations (Fatima, *et al*., 2012). In diabetic state, insulin deficiency also contributes to derangements of various metabolic and regulatory mechanisms in the body. The result of this study reveals that the administration of *A. vogelii* aqueous extract not only lowered serum CHOL, TRIG, LDL, CRT, ALT and AST level, but also enhanced serum HDL level. This may be due to the anti-hyperglyceamic potency of the extract in diabetic 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 (Alabi *et al*., 2013).

The possible mechanism of action in relation to reduction of FBGL might be that it; stimulates the pancreatic beta cells to secrete insulin, improves insulin sensitivity (Bosenberg and van Zyl, 2008), slows down absorption of carbohydrate and hence slows down glucose production (Kruger and Gloster, 2004) or it slows down gastric emptying and increases satiety (VanDeKoppel *et al*., 2008). *A. vogelii* ethanolic root extract exerted anti-diabetic effect may be by virtue of the phytochemicals found present in it.

CONCLUSION

The study concluded that *A. vogelii* aqueous root extract is safe when administered acutely (p.o.), it has anti-diabetic, anti-hyperlipideamic activities and has no toxic effect on biochemical parameters (ALT, AST and CRT) when administered for fourteen days in streptozotocin-induced diabetic rats. This justifies the use of the plant roots in ethno-medicine for the treatment of diabetes.

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