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Anti-diabetic Activity of *Viscum album* (Guava Mistletoe) in Alloxan-induced Diabetic Rats

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Abstract: ***This study was carried out to investigate the antidiabetic activity of aqueous leaf extract of Viscum album (Guava mistletoe) and compare its antidiabetic efficacy against the oral antidiabetic drug metformin, using alloxan-induced rat models. Phytochemicals detected includes alkaloids, phenols, flavonoids, tannins, anthraquinones, cardiac glycosides and terpenoids. Biochemical analysis showed a dose-dependent significant decrease ($p<0.05$) in the levels of serum glucose in the rats when treated with Viscum album leaf extract after the induction of diabetic. This observation was vivid when Viscum album treated groups were compared to the diabetic control and metformin- treated groups. Similarly, there was a significant decrease in the levels of total cholesterol, triglycerides, and low density lipoproteins that were hitherto elevated before treatment with the leaf extract. A corresponding significant increase in the levels of high density lipoproteins was observed when compared with the diabetic control group. The plant extract significantly ($p<0.05$) lowered the serum activities of marker enzymes: Alanine and Aspartate aminotransferases. Similarly, a significant decrease ($p<0.05$) in creatinine and urea levels was observed in the extract and metformin treated groups when compared to the diabetic control group. In addition, the plant extract attenuated the weight loss observed in diabetic control group. Aqueous leaf extract of Viscum album exhibited high antidiabetic activity which compares fairly well with metformin and very effective in ameliorating other complications associated with diabetic mellitus.***

Keywords: *Viscum album*, mistletoe, antidiabetic, dose-dependent, aqueous leaf extract.

1. Introduction

Medicinal Plants

Medicinal plants have formed the basis of healthcare throughout the world since the earliest days of humanity and are still widely used with considerable importance in international trade (Kumar *et al.,* 2011). It is estimated that up to 90% of Africa's population still rely exclusively on plants as a source of medicines (Ozougwu, 2011). Effective health cannot be achieved in Africa, unless orthodox medicine is complemented with traditional medicine (Edem, 2009). As a consequence, the World Health Organization (WHO) had in one of its charters in Geneva recommended further investigation into this area, particularly as it concerns chronic and debilitating diseases such as diabetes mellitus (Budhwani *et al.,* 2010).

The use of herbal products for medicinal benefits has played an important role in nearly every culture on earth and for many years, the search for anti-diabetic products will continue to focus on plants and other natural resources. The ethnobotanical information reports about 800 plants that may possess anti-diabetic potential (Pulipaka *et al.,* 2012). The cost of administrating modern anti-diabetic drugs is beyond the reach of most people in the low income group and those living in the rural areas, hence the use of plants for the treatment of common diseases such as diabetes are very common (Osinubi *et al.,* 2006). The current shift to the use of herbal preparations could be due to presumed effectiveness and less side effects, relatively low cost and low toxicity (Nahar *et al.,* 2010).

Africa is a rich source of medicinal plants. Perhaps, the best known species is *Phytolacca dodecandra*. Extracts of the plant are used as an effective molluscicide to control schistosomiasis. Other notable examples are *Catharanthus roseus*, which yields anti-tumour agents such as vinblastine and vinvristine; and *Ricinus communis*, which yields the laxative--castor oil. In Botswana, Lesotho, Namibia and South Africa, *Harpagophytum procumbens* is produced as a crude drug for export. Similarly, *Hibiscus sabdariffa* is exported from Sudan and Egypt. Other exports are *Pausinystalia yohimbe* from Cameroon, Nigeria and Rwanda, which yields *yohimbine;* and *Rauwolfia vomitoria*, from Madagascar, Mozambique and Zaire, which is exploited to yield reserpine and ajmaline (Hoareau and DaSilva, 1999).

Viscum album (mistletoe) is a semi-parasitic woody perennial plant commonly found growing on oaks and other deciduous trees preferring those with soft bark like old apple trees, guava, cocoa, citrus and other trees (Ekhaise *et al.,* 2011). *Viscum album* is widely distributed in Africa, Europe, northwest Australia, central Asia and Japan. It is small, dioecious and shrubby, with oblong evergreen leathery entire leaves, clear dichasial branching and four-part flowers which form white sticky berries with a faint but characteristic odour and a bitter taste. Mistletoe is propagated by birds and is considered a semi parasitic plant because it synthesizes its own chlorophyll but depends on the host for its supply of water and minerals (Loeper, 1999).

Viscum album which belongs to the family Loranthaceae is commonly called 'Kauchi' in Hausa, 'Apari' in Igbo, 'Afomo' in Yoruba (Deeni and Sadiq, 2002), Tuchi and 'Pikko' in Wurkun. It represents one of the most mysterious magical and at the same time, sacred plants of the European folklore (Adodo, 2002). The scientific interest on mistletoe awakened in the 20th century when Gaultier (1907-1910) investigated the effect of oral applications of fresh *Viscum album* extract on the blood pressure of man and animals (Deeni and Sadiq, 2002).

Guava (*Psidium guajava*) on the other hand, is a member of the Myrtaceae family, which is native to tropical and subtropical countries. Its fruit is commonly used as food and

processed as juice and jam. The other common use of *Psidium guajava* is as folk medicine. Aside from these uses, Gutiérrez *et al*., 2008 have reviewed the potential pharmacologic activities of the extract from the fruit, leaf, bark or roots; these activities include antioxidant, hepatoprotective, anti-allergy, anti-microbial, anti-genotoxic, anti-plasmodial, cytotoxic, anti-spasmodic, cardioactive, anti-cough, anti-diabetic, anti-inflammatory and anti-nociceptive activities in vitro and/or in animal models (Deguchi and Miyazaki, 2010).

Diabetes Mellitus

Diabetes was first recognized by the ancient Egyptians, who considered it a rare condition associated with excess urine excretion and loss of weight. The term diabetes mellitus, reflecting the fact that the urine of those affected had a sweet taste, was first used by the Greeks. Diabetes mellitus is derived from the Greek words "dia" (through), and "bainein" (to go) and diabetes literally means pass through, while "mellitus" means "sweet" but there are several rarer conditions also named diabetes. The most common of these is diabetes insipidus (insipidus meaning "without taste" in Latin) in which the urine is not sweet (Polonsky, 2012; Vasudevan and Sreekumari, 2007).

Diabetes mellitus discovered between 700-200 BD, is one of the most common metabolic disorder with micro and macro vascular complications that results in significant mortality and morbidity (Philis-Tsimikas, 2009). Currently, 366 million have diabetes worldwide with 80% from developing countries which is likely to increase to 552 million by 2030 (WHO, 2011).

The disorder is characterized by symptoms such as thirst, polyuria, blurring of vision, and weight loss. It is also characterized by lipoprotein abnormalities (Hammadi and Nouman, 2009; Tenpe and Yeole, 2009), raised metabolic rate (Newata *et al*., 2004), hyperglycaemia (high blood sugar) (Hammadi and Nouman, 2009), defect in reactive oxygen species scavenging enzymes (Edem, 2009). In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made (Bastaki, 2005).

The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs. The long–term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular diseases (WHO, 1999; Mane *et al.*, 2012).

The use of orthodox drugs as oral hypoglycaemic agents such as sulphonyl ureas, biguanides, alpha glucosidase inhibitors and insulin result to severe hypoglycaemia and other complications in patients prompting the desire for alternative means of combating diabetes mellitus in Nigeria, Africa and the world-over (Onoagbe *et al.*, 1999; Pulipaka *et al.*, 2012). There are several forms of diabetes mellitus but it is often classified into three general categories namely: Type I, Type II and gestational diabetes mellitus (Bastaki, 2005).

Type I diabetes accounts for 5-10% of all diagnosed cases of diabetes (Polonsky, 2012), previously encompassed by the terms insulin–dependent diabetes (IDDM). Type 1 diabetes, or juvenile-onset diabetes, results from cellular-mediated autoimmune mediated destruction of the beta cells of the pancreas by CD4 and CD8 T cells and macrophages infiltrating the islets. The peak incidence of this form of Type 1 diabetes occurs in childhood and adolescence, but the onset may occur at any age, ranging from childhood to the ninth decade of life. There is a genetic predisposition to autoimmune destruction of beta cells, and it is also related to environmental factors that are still poorly defined (Verge *et al.*, 2006). Obese people are at increased risk of developing this type of diabetes. These patients may also have other autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, and Addison's disease (Betterle, 2003).

Type II diabetes is by far the most common form of diabetes, accounting for 85 to 95% of cases in developed nations and an even higher percentage in developing nations, according to the International Diabetes Federation (IDF, 2010). Diabetes mellitus of this type previously encompassed non– insulin–dependent diabetes mellitus (NIDDM), or adult–onset diabetes. It is a term used for individuals who have relative (rather than absolute) insulin deficiency. Patients with this type of diabetes frequently are resistant to the action of insulin (Stanley and Kohl, 2010; Ajayi *et al.*, 2010). At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive (Bastaki, 2005). This form of diabetes is frequently undiagnosed for many years because the hyperglycaemia is often not severe enough to provoke noticeable symptoms of diabetes (Stanley and Kohl, 2010). Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications. There are probably several different mechanisms which result in this form of diabetes, and it is likely that the number of people in this category will decrease in the future as identification of specific pathogenetic processes and genetic defects permits better differentiation and a more definitive classification with movement into "Other types" (Stanley and Kohl, 2010).

Although the specific aetiologies of this form of diabetes are not known, by definition autoimmune destruction of the pancreas does not occur and patients do not have other known specific causes of diabetes. Rates for type 2 diabetes rise sharply with age for both men and women and for members of all racial and ethnic groups. The prevalence of diagnosed diabetes is about seven times as high among adults aged 65 years or older as among those aged 20–44 years (CDC, 2012).

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2%–5% of all pregnancies and may improve

or disappear after delivery (CDC, 2012). It is characterized by carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy. It does not exclude the possibility that the glucose intolerance may antedate pregnancy but has been previously unrecognized. The definition applies irrespective of whether or not insulin is used for treatment or the condition persists after pregnancy (Betterle, 2003).

In the early part of pregnancy (e.g. first trimester and first half of second trimester) fasting and postprandial glucose concentrations are normally lower than in normal, non–pregnant women. Elevated fasting or postprandial plasma glucose levels at this time in pregnancy may well reflect the presence of diabetes which has antedated pregnancy, but criteria for designating abnormally high glucose concentrations at this time have not yet been established.

Alloxan

Alloxan (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetrone) was originally isolated in 1818 by Brugnatelli and named in 1838 by Wöhler and Liebig. "Alloxan" is coined from an amalgamation of the words "allantoin" and oxalic acid. Alloxan is a strong oxidizing agent forming a hemiacetal with its reduced reaction product dialuric acid (in which a Carbonyl group is reduced to a Hydroxyl group which is called alloxantin.

Mechanism of Action

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (that is beta cells) when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type I diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction. A study suggests that alloxan does not cause diabetes in humans while other studies report a significant difference in alloxan plasma levels in children with and without diabetes Type I (Dinz *et al.*, 2008).

Collection of Plant Material

Fresh leaves of *Viscum album* on guava plant was collected in Sangere, Girei Local Government Area of Adamawa State. The leaf sample was identified and authenticated in the department of Plant science, Modibbo Adama University of Technology, Yola.

The leaves were cleaned and air-dried at room temperature. The dried leaves were ground into powder and kept in air tight bottle for further analysis. About 200g of the fine powder was soaked in 1.5 litres of boiling water with stirring for 4 hours. The mixture was filtered and the filtrate concentrated in a rotary evaporator. This was preserved for further use.

Qualitative phytochemicals screening was carried out to detect the presence of tannins, flavonoids, saponins, alkaloids, glycosides, steroids, terpenoids, quinones, anthraquinones and phlobatannins as described by Trease and Evans (1985) and Sofowora (1993).

Twenty five (25) male albino Wistar rats weighing approximately 100-120g that were used in this experimental study were obtained from the animal house of the National Veterinary Research Institute (NVRI) Vom, Plateau State. They were kept under standard laboratory conditions with adequate access to food and water in the laboratory unit of the Biochemistry department of the university in well ventilated cages. The rats were randomly divided into five equal groups containing 5 rats each and were treated accordingly as shown below:

Induction of Diabetes Mellitus

The method of Osinubi *et al*., (2006) was used to induce diabetes in the rats. 80mg/kg body weight of alloxan was administered intraperitoneally to the experimental rats after overnight fast (access to water only). Diabetes mellitus was confirmed using glucose strip and only rats with serum glucose levels above 250mg/dl after seven days were used for the experiment.

Group 1: Were administered normal diet to serve as normal control.

Group 2: Were administered alloxan (80mg/kg body weight) in addition to normal diet to serve as diabetic control.

Group 3: Diabetic rats were administered 200mg/kg body weight of *Viscum album* aqueous extract for 21 days.

Group 4: Diabetic rats were administered 400mg/kg body weight of *Viscum album* aqueous extract for 21 days.

Group 5: Diabetic rats were administered 1.6mg/kg body weight of metformin orally per day to serve as drug control.

Glucose levels were measured at seven days interval and recorded accordingly before the rats were finally sacrificed at the end of 21 days of the experimental period.

Determination of Body Weight

The body weight of each rat in each group at three days interval for the period of the experiment was obtained using a weighing balance. The average weight of each group was taken and recorded carefully.

Biochemical Analysis

At the end of the experimental period, the animals were anaesthetised in chloroform vapour, dissected and blood samples collected by cardiac puncture into clean sample bottles. The blood was allowed to clot for few minutes. Serum was obtained by centrifugation at 3,000 rpm for five minutes using a bench top centrifuge.

Serum glucose concentration determination was carried out using the methods describe by Barham and Trinder, (1972). Principle of the method:

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator.

$$Glucose\ Conc.(mmol) = \frac{\text{Absorbance of Sample}}{\text{absorbance of STD}} \text{ x } Conc.of\ standard\ (5.5\text{mmol/l})$$

Total serum cholesterol concentration determination was carried out using the method described by Trinder, (1969). Cholesterol reacts with concentrated acids as a typical alcohol to produce coloured substances. In this method, acetic acid and acetic anhydride are used as solvents and dehydrating reagents, conc. H_2SO_4 is employed as a dehydrating agent. The final bluish green coloured obtained is read at 570nm.

$$\text{Cholesterol} + H_20 \xrightarrow{\text{estarases}} \text{cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{estarases}} \text{cholesterol-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + H_20$$

$$\text{Cnolesterol concentration} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ x Concentration of standard}$$

Determination of triglyceride was carried out according to the method describe by Trinder (1969) and Nagele *et al.*, (1984).

Principle of the method:
Triglyceride in the sample is hydrolysed to glycerol and fatty acids by lipoprotein lipase. Glycerine is then phosphorylated by glycerol kinase in the presence of ATP and Mg^{2+} ions. A coloured product which absorb well at 505nm is formed from hydrogen peroxide, 4-aminoantipyrine and phenol derivative in the presence of the peroxidase.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{lipases}} \text{Glycerol} + \text{fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPC}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{\text{POP}} \text{Quinoneimine} + HCl + 4H_2O$$

$$\text{Triglyceride concentration} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ x Concentration of standard.}$$

Principle of the method:
Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) are precipitated from serum by the action of polysaccharides in the presence of divalent cations. Then, high density cholesterol (HDL cholesterol) present in the supernatant is determined.

HDL = Absorbance of test x 5.43 (multiple factor given by reagent company).

Serum LDL was determined according to the method of Wieland and Seidel (1999). The LDL molecules are cholesterol rich lipoprotein molecules containing only apoB-100. Most of the LDL particles are derived from VLDL but a small part is directly released from the liver.

LDL (mmol/l) = total cholesterol- (TG/2.2) – HDL

Serum creatinine concentration determination was carried out using Jaffe reaction (Barthels and Bohmer, 1971).

Principle of the method
Creatinine present in serum or plasma directly reacts with alkaline picrate resulting in the formation of a red colour, the intensity of which is measured at 505nm/green filter. Protein interference is eliminated using sodium lauryl sulphate. A second absorbance reading after acidifying with 30% acetic acid corrects for non-specific chromogens in the samples.

$$\text{Serum creatinine} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{X14}$$

Principle of the method:
Urea in serum is hydolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction.

$$\text{Urea} + H_2O \longrightarrow 2NH_3 + CO_2$$

$$NH_3 + \text{Hypochlorite} + \text{Phenol} \longrightarrow \text{Iodophenol (blue compound)}$$

$$\text{Urea concentration} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{x Concentration of Standard}$$

The method of Reitman and Frankel (1957) was used for the determination of aspartate and alanine amintransferases.
Principle

The enzyme, GPT catalyzes the formation of pyruvate and glutamate from the reaction between alanine and ketoglutarate. The pyruvate formed now reacts with 2, 4-Dinitrophenylhydrazine to form 2, 4-Dinitrophenylhydrazone to yield the red colour and the activity of ALT is dependent on the intensity of the colour.

2. Results and Discussion

Qualitative phytochemicals screening of the leaf extract of *V. album* revealed the presence of alkaloids, phenols, flavonoids, tannins, anthraquinones, cardiac glycosides and terpenoids. Saponins and steroids were not detected. The summary of the result is shown in Table I:

Table I: Phytochemical components detected in the leaf extract of *Viscum album*

Phytochemicals	Inference
Alkaloids	+
Saponins	-
Phenols	+
Flavonoids	+
Tannins	+
Anthraquinones	+
Glycosides	+
Terpenoids	+
Steroids	-

Key:
+ = Presence of phytochemical component;
- = absence of phytochemical component.

Body Weights

The mean body weight of diabetic control group showed a significant decrease after the 9th day when compared with normal. The mean body weight of both extract treated and standard drug-treated (metformin) groups however showed a significant steady increase after the 6th day of extract administration as shown in the figure below.

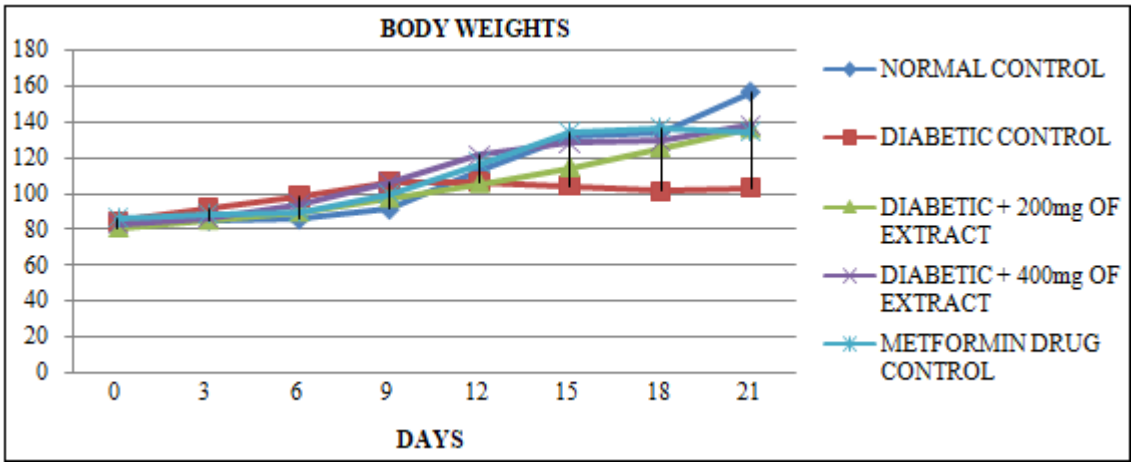

Figure I: Effect of *V. album* leaf extract on the Body Weight of alloxan-induced diabetic rats (g)

The induction of diabetes by alloxan (150mg/kgbwt) showed a marked rise in fasting blood glucose level in diabetic control as compared to normal control rats. However, at the end of the 21 days long treatment, a significant decrease in weekly fasting glucose level was observed with the leaf extract and metformin treated groups when compared to the diabetic control. A summary of the results is presented in Table II below:

Table II: Effects of *V. album* aqueous leaf extract on Weekly Blood Glucose level

Group	Fasting Blood Glucose level (mg/dl)			
	INITIAL	WEEK ONE	WEEK TWO	WEEK THREE
Normal Control	57.60±6.73	72.00±4.93	55.80±9.18	54.25±1.90
Diabetic control	273.60±11.93*	349.80±28.20*	453.60±6.73*	525.60±17.12*
200mg/kgbwt	280.80±22.48*	230.40±25.07$^{*\beta}$	205.20±13.77$^{*\beta}$	197.71±2.57$^{*\beta}$
400mg/kgbwt	309.60±43.87*	217.80±7.20$^{*\beta}$	180.00±5.69$^{*\beta}$	155.41±2.65$^{*\beta}$
Drug control (Metformin)	295.20±23.88*	203.40±11.94$^{*\beta}$	226.80±81.02$^{*\beta}$	143.82±0.64$^{*\beta}$

Results are expressed asMean±Standard error of mean (S.E.M); n=5
* Showed a significant increase compared with normal control at P<0.05
$^{\beta}$ Showed a significant decrease compared with diabetic control at P< 0.05

Total cholesterol, triglyceride and low density lipoprotein (LDL) levels were found to be significantly (P<0.05) increased and significant decrease in high density lipoprotein (HDL)-cholesterol levels in the diabetic control group in comparison with the normal control. Treatment with the extract and metformin significantly attenuated (P<0.05) the elevated total cholesterol, triglyceride and LDL levels and increased the HDL levels in comparison with the diabetic control as shown in Table III below:

Table III: Effects of *V. album* extract on serum Lipid Profiles of alloxan-induced diabetic rats

Group	**Serum Lipid Profile**			
	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	High Density Lipoprotein (mg/dl)	Low Density Lipoprotein (mg/dl)
Normal Control	98.94±1.45	70.32±0.30	53.70±1.04	31.17±0.44
Diabetic control	192.31±1.57*	153.09±1.53*	41.89±0.32	80.84±0.94*
200mg/kgbwt	141.46±2.49$^{*\beta}$	99.00±1.23$^{*\beta}$	61.33±0.76 $^{*\alpha}$	60.23±3.11$^{*\beta}$
400mg/kgbwt	122.24±2.49$^{*\beta}$	81.62±1.46$^{*\beta}$	63.30±0.81$^{*\alpha}$	42.61±1.50$^{*\beta}$
Drug control (Metformin)	109.88±0.89$^{*\beta}$	73.95±2.15$^{*\beta}$	64.18±0.00 $^{*\alpha}$	30.67±0.53$^{\beta}$

Results are expressed asMean± Standard error of mean (S.E.M); n=3
* Showed a significant increase compared with normal control at P< 0.05

$^{\beta}$ Showed a significant decrease compared with diabetic control at P< 0.05
$^{\alpha}$ Showed a significant increase compared with diabetic control at P< 0.05

Serum levels of creatinine and urea in the diabetic control group showed a significant increase when compared with the normal control group. However, treatment with the extract and metformin showed a dose dependent decrease in serum creatinine and urea in the treated groups when compared with the diabetic control group as shown in table IV below:

Table IV: Effect of *V. album* extract on serum levels of Creatinine and Urea

Group	Biochemical Parameters Creatinine (mg/dl)	Urea (mg/dl)
Normal control	0.65±0.03	21.87±0.52
Diabetic control	2.21±0.05*	70.08±0.65*
200mg/kgbwt	1.36±0.16$^{*\beta}$	31.36±2.15$^{*\beta}$
400mg/kgbwt	1.58±0.03$^{*\beta}$	26.92±0.62$^{*\beta}$
Drug control (Metformin)	1.45±0.10$^{*\beta}$	25.37±0.67$^{*\beta}$

Results are expressed asMean± Standard error of mean (S.E.M); n=3
* Significantly increased when compared with normal to normal control at P<0.05
$^{\beta}$ Significantly decreased when compared with diabetic control at P< 0.05

A significant elevation of ALT and AST activities was observed in the diabetic control group when compared with the normal control. On the other hand, the activities of AST and ALT in all the treatment groups significantly decreased when compared with the diabetic control group. This is presented in table V below:

Table V: Effect of *V. album* extract on serum ALT and AST activities

Group	Biochemical Parameters ALT (U/I)	AST (U/I)
Normal Control	28.50±1.89	31.00±7.64
Diabetic control	42.33±0.67*	56.67±2.33*
200mg/kgbwt	34.00±2.89$^{*\beta}$	35.00±6.11$^{*\beta}$
400mg/kgbwt	37.33±1.67$^{*\beta}$	31.33±2.60$^{\beta}$
Drug control (Metformin)	34.20±2.89$^{*\beta}$	31.33±2.60$^{\beta}$

Results are expressed as Mean± Standard error of mean (S.E.M); n=3
$^{\beta}$ Significantly decreased when compared with diabetic control at P< 0.05
*Significantly increased when compared with normal control at P<0.05

In this study, alloxan was used as diabetogenic agent in the experimental animals. Alloxan is known for its selective pancreatic islet β – cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals apart from streptozotocin (Ozougwu, 2011). It induces diabetes by the destruction of β-cells of the islets of langerhans via generation of O_2^-, hydrogen peroxide and hydroxyl radicals resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues. Insulin deficiency leads to various metabolic aberrations in animals including hyperglycemia, decreased protein content and increased levels of cholesterol and triglycerides (Tenpe *et al.*, 2009).

This study was conducted to evaluate the antidiabetic activity of *V. album* and compare its antidiabetic efficacy with metformin, a typical biguanide. Our investigations revealed that the intraperitoneally administered of alloxan (150mg/kg bwt) effectively induced diabetes mellitus in the rat models as shown by the significant elevation (P<0.05) in fasting blood glucose which was reduced in a dose dependent manner upon treatment with the extract and the standard drug, metformin. This is in agreement with earlier reports (Edem, 2009; Qamar *et al.*, 2011). It was also observed that the antidiabetic effects of *V. album* leaf extract compares fairly well with that of metformin which itself compares well with normal control. The significant antidiabetic activity of *V. album* may be due to the inhibition of free radical generation and subsequent tissue damage induced by alloxan or potentiating plasma insulin effect by increasing either pancreatic secretion of insulin from existing beta cells or its release from bound form as indicated by significant improvement in glucose level because insulin inhibit gluconeogenesis from protein (Nahar *et al*., 2010). In addition, several studies have shown that phytoconstituents like alkaloids inhibit alpha-glucosidase and decrease glucose transport through the intestinal epithelium. Flavonoids suppress the glucose level, reduce plasma cholesterol and triglycerides significantly and increase hepatic glucokinase activity probably by enhancing the insulin release from pancreatic islets (Bhushan, *et. al.*, 2010). These phytochemicals were detected in the leaf extract of *V. album.*

Furthermore, our findings agrees with the work of Dave and Katyare 2002; in that, a significant steady increase in the body weight of the alloxan-induced diabetic rats was observed after the second week of treatment with *V. album* leaf extract. *Viscum album per se* had no effect on body weight but attenuated the weight loss observed in alloxan-induced diabetic rats as shown in figure I.

Macrovascular complications engendered by altered lipoprotein metabolism are often evident in diabetes mellitus. This was observed in this study, as diabetic rats showed hypercholesterolemia and hypertriglyceridaemia and the treatment with plant extract and metformin significantly decreased (p<0.05) both cholesterol and triglyceride levels. This implies that aqueous extract of *V. album* leaf extract and the standard drug can prevent or be helpful in reducing the complications of lipid profile seen in some diabetics in whom hyperglycaemia and hypercholesterolemia coexist quite often. Similar results were reported by Jarald *et al.*, 2009 and Atangwho *et al.*, 2010.

Kidney function indicators, urea and creatinine were assessed to compare the effects of the two treatments on kidney function of the test animals. The treatments ameliorated/modulated the potential risk diabetes posed to the kidneys such as decreased urea levels, which rose in the untreated diabetic group. These results were consistent with earlier reports by Shahaboddin *et al.*, 2011.

The present study also evaluated serum markers of hepatotoxicity : – AST and ALT. Diabetic control rats showed significantly elevated ($p<0.05$) enzyme activities in serum compared to normal control. However, treatment with the extract and the drug showed a significant reduction ($p<0.05$) of both enzymes indicating the protective effect of the *V. album* leaf extract. The same results were previously reported (Ebong *et al.,* 2008). Interestingly, the 400mg/kgbwt treated group and the metformin treated group showed same decrease in AST activity (31.33±2.60 each).

In conclusion, this study has demonstrated that aqueous leaf extract of *V. album* tested for antidiabetic activity showed appreciable results in decreasing serum glucose level and other complications associated with alloxan - induced diabetes mellitus. This activity may be due to the flavonoids, tannins, alkaloids and other bioactive components present in the leaf extract. In addition, the antidiabetic activity of the aqueous extract of *V. album* is comparable to metformin, suggesting its high potential to be developed as alternative and/or complementary a plant-derived antidiabetic agent.

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