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Full Length Research Paper

Hypoglycemic and Hypolipidemic Effects of Aqueous and Ethanolic Leaf Extracts of *Vitex doniana* (Verbenaceae) in Normoglycemic Albino Rats

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The effects of aqueous and ethanolic leaf extracts of *Vitex doniana* on blood glucose and lipid profile levels of Normoglycemic albino rats were investigated. The study was conducted with 25 albino rats (wistar strain), assigned into five groups of 5 rats each, and daily administration of aqueous and ethanolic leaf extracts of *V. doniana* for 21 days was done. Group 1 was the normal control, while group 2, 3, 4 and 5 were administered (100 mg/kg or 200 mg/kg body weight) extracts of *V. doniana* respectively. Significant ($p<0.01$) reduction in fasting blood glucose (FBG) levels relative to their initial values were observed for all treated group as compared with the normal control at the end of treatment. The FBG levels decrease by 23.01% and 21.9% for 100 mg/kg and 200mg/kg aqueous extracts respectively, and 19.31% and 20.19% for 100mg/kg and 200mg/kg ethanolic extracts respectively. The normal control rats maintained a stable FBG level (90.8±52 to 90.6±4.34 mg/dL). There was no significant ($p>0.05$) reduction on the levels of total cholesterol (TC), triacylglycerol (TAG) and low density lipoprotein-cholesterol (LDL-c), while the high density lipoprotein-cholesterol (HDL-c) concentration was increased for all the animal administered the extract compared to the control. Administration of the extracts shows no significant ($p>0.05$) difference in body weight change for the entire groups (except 100 mg/kg aqueous treated group). Evaluation of PCV of treated rats as compared to the normal control groups showed a slight variation which are within the normal range (from 44±1.6% to 48.5±0.6%). These findings may suggest the use of leaf extracts of *V. doniana* in the management of diabetes.

Keywords: *Vitex doniana*, hypoglycemic, hypolipidemic, body weight, PCV.

INTRODUCTION

The existence of experimental animal models of a disease aids not only the understanding of the pathophysiology of such disease, but also the development of drugs for its treatment. According to World Health Organization (WHO), there are approximately 160,000 diabetes worldwide, the number of diabetics has double in the last few years and is expected to double once again in the year 2025 (Beretta, 2001). Diabetes is a major degenerative disease in the world today, affecting at least 15 million people and having complications which include hypertension, atherosclerosis

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and microcirculatory disorders (Ogbonnia *et al*., 2008). The prevalence of diabetes is on the increase globally and in African communities due to the ageing of the population and drastic lifestyle changes accompanying urbanization and westernization (Sobngwi *et al.,* 2001). Also, studies from five West African communities in Nigeria and Ghana have identified genes within populations that create susceptibility to diabetes (Rotimi *et al*., 2001). Hence, it represents a growing burden of health care systems of African countries, most of which already face difficult economic conditions. The disease remains incurable and can only be controlled with drugs; hence, a scrupulous control is needed to help reduce hyperglycemia and the risk of long-term complications, which are known to be the major causes of morbidity and mortality (Rotimi *et al*., 2010).

In Nigeria, information available from the indigenous traditional healers indicates that, a decoction of the chopped stem barks and leaf of *V. doniana* is prepared and taken orally for treatment of diabetes and other disease conditions. The plant extracts have been used as medication for infertility, liver disease, anodyne, stiffness, hypertension, cancer, febrifuge, as tonic galactagogue to aid milk production in lactating mothers, sedative, digestive regulator and treatment of eye troubles, kidney troubles and as supplement for lack of vitamin A and B (Burkill, 2000; Sofowora, 1993). Research report by James *et al*., (2010) on the antihepatotoxic ability of aqueous leave and stem extract of *V. doniana* showed that it was effective against carbon tetrachloride induced liver injury in rats. The anti-hypertensive effect of extract of stem bark of *V. doniana* has been reported by Olusola *et al,* (1997), and shows that the extract exhibited a marked dose-related hypotensive effect in both normotensive and hypertensive rats. Extracts of stem bark of *V. doniana* have also demonstrated some level of *in vitro* trypanocidal activity against Trypanosoma *brucei brucei* (Atawodi, 2005). The aqueous and methanolic extracts has been reported to exhibited anti-diarrhea activity (Agunu *et al*., 2005).

This work was therefore designed to investigate the hypoglyceamic and hypolipidemic effects of the aqueous and ethanolic leaf extracts of *V. doniana* in Normoglycemic rats.

MATERIALS AND METHODS

Plant samples collection and identification

Fresh leaves of *Vitex doniana* were collected from Ankpa, Kogi State, Nigeria in the month of April 2011. The plant was identified and authenticated at the Herbarium unit, Biological Sciences Department, Ahmadu Bello University Zaria, Nigeria, where a voucher specimen number (900076) was deposited.

Experimental animals

Adult albino rats (140 – 220 g) of both sexes were obtained from the laboratory Animal house, Department of Pharmacology, ABU, Zaria. The animals were acclimatized for 2 weeks under the laboratory conditions (the temperature and humidity were maintained at 25^{o}C and 50% respectively. Dark and light cycle were maintained at 12 hrs each). They had access to grower's mash (Vital feed, Grand Cereal Plc, Bukuru, Jos, Plateau State) and water *ad libitum*.

Preparation of plant sample

The collected plant leaves were rinsed in clean water and shade dry at room temperature for two weeks. The dry plants sample was ground into powder using pestle and mortar. The powder obtained was then used to prepare the extracts.

Aqueous extraction

To 100 g of powdered plant material, 500 mL portion of distilled water was added and then boiled in a conical flask for 2hrs. After the set time, the suspension was filtered using cloth with fine pore, and the filtrates were then concentrated in a crucible using a water bath set at 45ºC and the weight of the sample taken. The concentrated extracts were then stored in an air tight sample bottle in a refrigerator until required for analysis.

Ethanolic extraction

500 g of the powdered plant material were soaked in 2.5 Liter of 70% ethanol at room temperature in a conical flask for 72hrs. After the set time, the suspension was filtered using cloth with fine pore, and the filtrates were then concentrated in a crucible using a water bath set at 45ºC and the weight of the sample taken. The concentrated extracts were then stored in an air tight sample bottle in a refrigerator until required for analysis.

Lethality (LD_{50}) test

The mean lethal dose (LD_{50}) of the aqueous and ethanolic extracts were determined in albino rat (weighing 150g-200g) using the method described by Lorke (1983).

Animal grouping and treatment

The study was conducted in albino rats using the plant

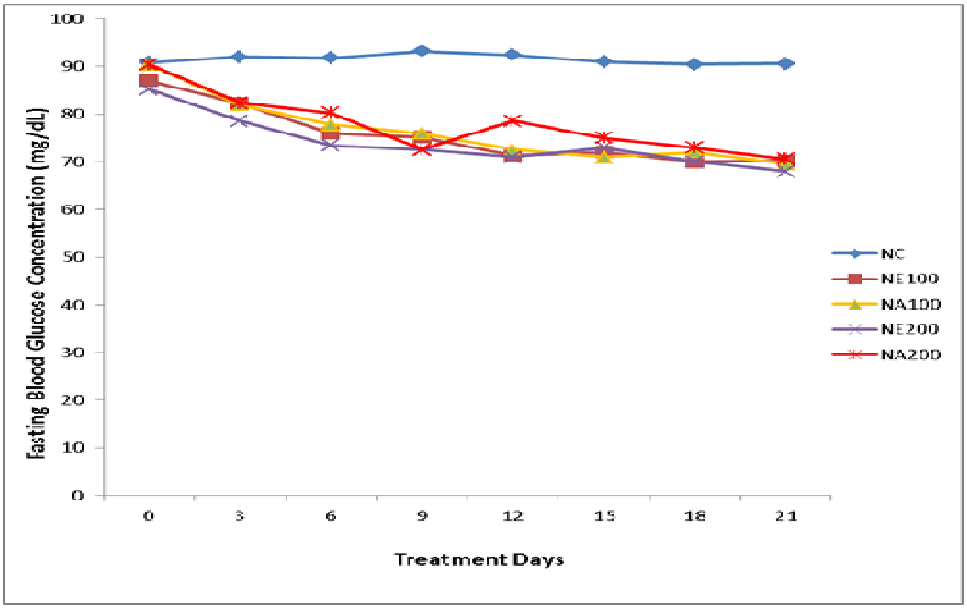

Figure 1. The fasting blood glucose levels of non-diabetic rats treated with different doses of aqueous and ethanolic leaf extracts of *V. doniana* for 21 days.

NC: Normal rats Control, NE_{100}: Normal rats + Ethanolic Extract (100mg/kg), NA_{100}: Normal rats + Aqueous Extract (100mg/kg), NE_{200}: Normal rats + Ethanolic Extract (200mg/kg), NA_{200}: Normal rats + Aqueous Extract (200mg/kg).

extracts at an increasing dosage of 100 mg/kg and 200 mg/kg body weight.

The groupings are:

Group 1: Normal control rats given vehicle of administration orally.

Group 2: Normal rats given 100 mg/kg bw/day ethanolic extract orally

Group 3: Normal rats given100 mg/kg bw/day aqueous extract orally

Group 4: Normal rats given 200 mg/kg bw/day ethanolic extract orally

Group 5: Normal rats given 200 mg/kg bw/day aqueous extract orally

Sub-chronic studies/ Collection and treatment of samples

The extracts were reconstituted in distilled water, and administered orally on daily basis by gastric intubation for 21 days. At the end of 21 days, the rats were weighed, fasting blood glucose level and PCV determined. The animals were anaesthetized using chloroform and bled by cardiac puncture 24 hrs after the last treatment. The blood sample was collected in specimen bottles, allowed to clot and the serum separated by centrifugation at 3000 rpm for 10 minute and then subjected to biochemical parameters analysis.

Biochemical Analysis

The fasting blood glucose levels were determined based on glucose oxidase/peroxidase principle, as described by Clark and Lyons (1962) using a digital glucometer (Accu-Chek Advantage II) after fasting the rats for 12 hrs. The Packed cell volume was assayed according to the method described by Schalm *et al., (*1975). The serum levels of total cholesterol, triacylglycerol and HDL-c were determined by enzymatic method described by Stein (1987), while the serum levels of LDL-c was measured according to protocol of Friedewald *et al*., (1972).

Statistical Analysis

Results are presented as mean ± Standard error of mean (SEM). Within groups comparisons were performed by the analysis of variance using ANOVA test (using SPSS 17.0 for windows Computer Software Package). Significant differences between groups were compared by Duncan's new Multiple Range test; a probability level of less than 5% ($P< 0.05$) was considered significant (Duncan, 1955).

Table 1. Percentage change(s) in fasting blood glucose of non-diabetic rats treated with aqueous and ethanolic leaf extracts of *V. doniana* for 21 days.

Groups (n=5)	Mean Initial FBG (mg/dL)	Mean Final FBG (mg/dL)	Change (mg/dL)	Value	% Change
NC	90.8±5.20[a]	90.6±4.34[a]	0.2±0.86		0.22
N+Eth$_{100}$	87.0±5.80[a]	70.2±6.25[b]	16.8±0.45	↓	19.31
N+Aq$_{100}$	90.4±3.20[a]	69.6±2.70[b]	20.8±0.50	↓	23.01
N+Eth$_{200}$	85.2±4.93[a]	68.0±4.35[b]	17.2±0.58	↓	20.19
N+Aq$_{200}$	90.4±2.73[a]	70.6±2.42[b]	19.8±0.31	↓	21.90

Values are means ± SEM. Values with different superscripts along the row are significantly different (P < 0.01).
↓ Decrease

NC: Normal rats Control, N+Eth$_{100}$: Normal rats + Ethanolic Extract (100mg/kg), N+Aq$_{100}$: Normal rats + Aqueous Extract (100mg/kg), N+Eth$_{200}$: Normal rats + Ethanolic Extract (200mg/kg), N+Aq$_{200}$: Normal rats + Aqueous Extract (200mg/kg). FBG: Fasting blood glucose

Table 2. The lipid profile of non-diabetic rats treated with different doses of aqueous and ethanolic leaf extracts of *V. doniana* for 21 days.

Groups (n=5)	Serum TC (mg/dl)	Serum TAG (mg/dl)	Serum HDL-c (mg/dl)	Serum LDL-c (mg/dl)
NC	76.09±2.42[a]	76.87±4.19[a]	27.32±4.87[a]	33.32±6.47[a]
N+Eth$_{100}$	57.66±6.06[ab]	66.09±8.04[a]	30.90±8.09[a]	26.04±3.74[a]
N+Aq$_{100}$	63.95±7.57[a]	57.09±2.01[a]	36.16±5.21[a]	21.38±5.54[a]
N+Eth$_{200}$	63.66±4.45[a]	58.54±9.40[a]	32.42±2.54[a]	19.54±2.71[ab]
N+Aq$_{200}$	72.66±10.55[a]	72.13±11.79[a]	29.47±3.95[a]	22.27±7.81[a]

Values are means ± SEM. Values with different superscripts down the column are statistically different (P < 0.05)

NC: Normal rats Control, N+Eth$_{100}$: Normal rats + Ethanolic Extract (100mg/kg), N+Aq$_{100}$: Normal rats + Aqueous Extract (100mg/kg), N+Eth$_{200}$: Normal rats + Ethanolic Extract (200mg/kg), N+Aq$_{200}$: Normal rats + Aqueous Extract (200mg/kg)

TC: Total cholesterol, TAG: Triacylglycerol. HDL-c: High density lipoprotein cholesterol, LDL-c: Low density Lipoprotein cholesterol**.**

RESULTS

Effect on glycemia

The effect of daily doses of aqueous and ethanolic leaf extract of *vitex doniana* on blood glucose levels of normoglycemic rats is presented in Figures 1. Daily oral administration of the plant extract to rats cause a significant ($p<0.01$) reduction in fasting blood glucose levels after 21 days. The FBG levels decrease by 23.01% and 21.9% for 100 mg/kg and 200mg/kg aqueous extracts respectively, and 19.31% and 20.19% for 100mg/kg and 200mg/kg ethanolic extracts respectively. The normal control rats maintained a stable FBG level (90.8±52 to 90.6±4.34 mg/dL).

Effect on lipids

The effect of sub-chronic administration of aqueous and ethanolic leaf extract of *V. doniana* on lipid profile of normoglycemic rats is shown in Table 2. There was no significant ($p>0.05$) reduction in the level of cholesterol (exception of 100 mg/kg ethanolic extract group) as compare to the normal control. The plant extracts also had no significant ($p>0.05$) decrease in the level of triacylglycerol and LDL-c (exception of 200 mg/kg ethanolic group). Furthermore, the extract caused no significant ($p>0.05$) increase in the HDL-c level with the 100 mg/kg aqueous extract causing the highest increase.

Effect on body weight

Table 3 shows the body weight change of normoglycemic rats administered with aqueous and ethanolic leaf extracts of *V. doniana* for 21 days. From the results, no significant ($p>0.05$) difference was observed in the body weight change of the treated groups before and after treatment. The normal control rats recorded an increase in body weight (from 131.8±4.14g to 142.8±3.85g). However, a significant ($p<0.05$) change was observed in body weight

Table 3. Mean body weight change non-diabetic rats treated with different doses of aqueous and ethanolic leaf extracts of *V. doniana* for 21 days.

Groups (n = 5)	Mean Initial Body Weight (g)	Mean Final Body Weight (g)	Change Value (g)
NC	131.8±3.24	142.8±3.85	11.0±2.95[a]
N+Eth$_{100}$	201.4±8.66	203.6±7.91	5.0±1.52[ab]
N+Aq$_{100}$	210.8±3.06	211±2.83	2.8±0.49[b]
N+Eth$_{200}$	191.8±13.7	187±15.02	6.8±2.42[ab]
N+Aq$_{200}$	182.2±17.96	183.8±16.3	6.8±1.24[ab]

Values are means ± SEM. Values with different superscripts down the column are statistically different (P < 0.05)

NC: Normal rats Control, N+Eth$_{100}$: Normal rats + Ethanolic Extract (100mg/kg), N+Aq$_{100}$: Normal rats + Aqueous Extract (100mg/kg), N+Eth$_{200}$: Normal rats + Ethanolic Extract (200mg/kg), N+Aq$_{200}$: Normal rats + Aqueous Extract (200mg/kg).

Table 4. Change in PCV of normoglycemic rats treated with different doses of aqueous and ethanolic leaf extracts of *V. doniana* for 21 days.

Groups (n=5)	Initial Mean PCV (%)	Final Mean PCV (%)	Change Value (%)
NC	46±1.08	45.8±1.8	4.00±0.91
N+Eth$_{100}$	47.4±1.29	46.4±2.1	4.80±1.75
N+Aq$_{100}$	46.3±2.73	46.8±1.7	4.75±1.31
N+Eth$_{200}$	46±1.68	46.6±1.9	2.00±0.40
N+Aq$_{200}$	45.8±1.98	48.5±0.6	3.75±1.11

Values are means ± SEM. Values down the column and along the row are not significantly different (P > 0.05)

change of 100 mg/kg aqueous extract group as compare with the normal control.

Effect on packed cell volume

The effects of the plant extract of *V. doniana* on packed cell volume (PCV) of normoglycemic rats is presented in Table 4. The extracts at a dose of 100 and 200 mg/kg body weight had no significant (p>0.05) effect on the PCV of normoglycemic rats over the period of the study.

DISCUSSION

The used of normal healthy animals is still a valid screening method for the testing of potential hypoglycemic agents (Williamson *et al.,* 1996). This method allows for the effect of the drug to be tested in the animal with intact pancreatic activity, in addition to diabetic animal models (Williamson *et al.,* 1996).

Normoglycemic rats in different groups were administered with extracts of *V. doniana* and the effects on certain biochemical parameters were studied. The results of the present study demonstrated that the different doses of aqueous and ethanolic leaf extracts of *V. doniana* significantly (p<0.01) reduced the fasting blood glucose (FBG) level of non-diabetic rats after treatment for 21 days, while the normal control showed no significant (p>0.01) change in FBG levels. The 23.01% and 21.9% reduction for 100 mg/kg and 200mg/kg aqueous extracts respectively was found to be similar with the 19.31% and 20.19% reduction for 100mg/kg and 200mg/kg ethanolic extracts respectively. Preliminary hypoglycemic screening of the aqueous and the ethanolic extracts (data not shown) on Streptozotocin-induced diabetic animals revealed that the extracts caused a significant reduction in the FBG levels. This result is in line with earlier reports by Gidado *et al*, (2008), that the aqueous and ethanolic extracts of *N. latifolia* significantly lowered the fasting blood glucose levels of non-diabetic rats.

The hypoglycemic action of medicinal plants may be of the following mechanism; inhibition of renal glucose reabsorption, enhanced secretion of insulin from β-cells of the pancreas, increased tissue uptake of glucose by enhancement of insulin sensitivity, regeneration/repair of the β-cells, stimulation of glycogenesis and hepatic glycolysis, increasing the size and number of cells in the Islets of Langerhans, protective effect on the destruction of the β-cells and/or prevention of oxidative stress that is

possibly involved in pancreatic β-cells destruction, as reported for other antidiabetic plants (Nandhini *et al*., 2004; Ogawa *et al.,* 2005; Kim *et al.,* 2006). Thus, in this study, the hypoglycemic action of *V. doniana* may be due to increase tissue uptake of glucose by enhancement of insulin sensitivity or stimulation of glycogenesis and hepatic glycolysis.

Furthermore, the aqueous and ethanolic extract of *V. doniana* has no significant ($p>0.05$) reduction on the level of total cholesterol (exception of 100 mg/kg ethanolic extract), triacylglycerol and low density lipoprotein. These observed non-significant reductions may be attributed to the gut intra-lumenal interactive effect of saponins. Saponins are known antinutritional factors which reduce the uptake of certain nutrients including glucose and lipid especially cholesterol at the gut through intra-lumena physicochemical interaction. Hence saponins have been reported to have hypocholesterolemic effect (Price *et al*., 1987). Saponin, among other secondary metabolites is reported to be present in the leaves of *V. doniana* (Egharevba *et al*., 2010). The low concentration of cholesterol may have contributed to the observed non-significant high serum HDL-cholesterol in the animals. About 30% of blood cholesterol is carried in the form of HDL and it is hypothesized that HDL-cholesterol can remove cholesterol from antheroma within arteries and transport it back to the liver for excretion or re-utilization, thus high level of HDL-C protect against cardiovascular disease. The observed non-significant ($p>0.05$) increase in HDL-cholesterol concentration upon administration of the extracts (100 and200 mg/kg bw) indicates that the extract does not have HDL-C boosting effect and it does not also have significant ($p>0.05$) lowering effect on LDL-cholesterol at those concentration in normoglycemic animals.

From the result obtained, the weight gain change of the rats treated with 100 mg/kg body weight of aqueous extract was significantly ($p<0.05$) lower compared to the normal control. The weight change difference may be as a result of change in feed intake (data not shown). The results also shows no significant ($p>0.05$) change in the PCV of treated animals as compare with the control group. The non-significant change in body weight (except 100 mg/kg aqueous extract) and PCV indicate relative safety of the extract in the experimental animals.

In conclusion, the result of this study has substantiated the traditional use of *V. doniana* in the management of diabetes. Research is going on to study the effect of administration of the extracts to Streptozotocin-induced diabetic rats and to also probe the nature of the Phytochemical (s) responsible for the hypoglycemic activity.

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