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HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECTS OF ETHYL ACETATE LEAF EXTRACT OF *Vitex simplicifolia* IN ALLOXAN INDUCED DIABETIC WISTER RATS

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ABSTRACT

The effects of oral administration ethyl acetate leaf extract of *Vitex simplicifolia* on blood glucose and lipid profile levels of alloxan induced diabetic Wister rats were investigated. The study was conducted with 30 Wister rats, assigned into six groups of five rats each, and daily administration of ethyl acetate leaf extract of *Vitex simplicifolia* for 21 days was done.; Diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection (i.p) of 150 mg/kg body weight of Alloxan monohydrate. Group 1 was the normal control group 2 was the diabetic control, group 3 was administered 10mg/kg of glibenclimide (positive control) and groups 4, 5, and 6 were administered 250,500 and 1000mg/kg body weight of methanolic of *Vitex simplicifolia* leaf extracts respectively. There was significant ($p<0.01$) reduction in fasting blood glucose (FBG) levels relative to their initial values compared to the normal and positive control. The FBG levels decrease by 8, 16 and 17% for 250,500 and 1000mg/kg of ethyl acetate extracts respectively. The normal control rats maintained a stable FBG level (102.8 ± 4.0 to 102.68 ± 4.0) and the positive control decreased by 65%. There were no significant changes ($p<0.01$) in the levels of total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein – cholesterol (LDL_c) and high density lipoprotein – cholesterol (HDL_c) for the animals administered with the extract compared with the control groups. The result of this study may suggest the possible use of *Vitex simplicifolia* leaf in the management of hyperglycemia.

Keywords: *Vitex simplicifolia*, hyperglycemic, hypolipidemic

INTRODUCTION

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycaemia and characterized by elevated blood glucose concentration caused by insulin deficiency, often combined with insulin resistance. According to World Health Organization (WHO), there are approximately 171 million diabetics worldwide, this number has double in the last few years and is expected to doubled once again in the year 2025 (Beretta, 2001). Diabetes is a major degenerative disease in the world today, affecting at least 150 million people and having complications which include hypertension, atherosclerosis 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). The prevalence of diabetes mellitus in Nigeria is 1.9% with more than 1.5 million cases (IDF, 2015). Hence, it represents a growing burden on 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 *Vitex simplicifolia* 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 (Sofowora, 1993; Burkill, 2000).

Although parts of this plant are used by traditional healers of various ailments, there is paucity of scientific study to establish the scientific basis of its use and like many other herbal remedy, there is little or no information about its possible side effects or toxicities.

This study, therefore, was aimed at determining` the hypoglycemic and hypolipidemic effects of aqueous leaf extract of *Vitex simplicifolia* on alloxan induced diabetic wister rats. This is important since science requires the validation of drugs by medicinal practitioners and drug regulatory authorities demand that all potential drugs should pass through a rigorous series of study and scrutiny (Abdulrahman, 2004).

MATERIALS AND METHODS

Collection and preparation of plant material

Fresh leaves of *Vitex simplicifolia* were collected from the botanical garden of Bayero University, Kano with the assistance of Herbarium keeper. The leaves were authenticated in the Department of Biological Sciences, Bayero University Kano (Herbarium number 242). The leaves were dried under room temperature and then grounded using pestle and mortar to a semi powdered form.

Experimental animals

Thirty (30) adult Wister rats weighing between 160 – 240g were obtained from the animal house of Department of Physiology, Bayero University, Kano and kept in cages at a room temperature for two (2) weeks to acclimatize and allowed access to food and water *ad libitum*. The principles of laboratory animal care (NIH publication number 85 – 23, revised, 1985) guidelines were followed.

Plant extract

Vitex simplicifolia leaves (500g) were soaked in 2.5 litres of ethyl acetate at room temperature in a conical flask. The content of the flasks were shaken and the top was covered with aluminium foil and kept at room temperature for 48h (2 days) after which the extracts were obtained by filtration using a Whatman No 1 filter paper. The extracts were concentrated using vacuum evaporator.

Determination of mean lethal dose`

The mean lethal dose (LD₅₀) of the ethyl acetate extract was determined in Wister rats (weighing between 150 – 200g) using the method described by Lorke (1983)

Induction of diabetes mellitus

Diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection (i.p) of 150 mg/kg body weight of Alloxan monohydrate (Etuk *et al.*, 2010). Hyperglycaemia was confirmed by the elevated blood glucose levels, determined after 72hrs and then confirmed after 7th day of injection. The rats found with elevated glucose level of 400 mg/dl and above were used for the study (Masiello *et al.*, 1998).

Experimental design

A total of thirty (30) Wister rats were used for the study. The rats were divided into Six (6) groups of five (5) each. Extracts were administered orally using 1ml syringe.

Group 1 - Normal untreated rats (Animal control)

Group 2 – Diabetic untreated rats (Diabetic control)

Group 3 – Diabetic rats administered 10 mg/kg of Glibenclamide (Prasanna *et al.*, 2012)

Groups 4,5 and 6 – Diabetic rats administered 250, 500 and 1000 mg/kg body weight of ethyl acetate leaf extract of *Vitex Simplicifolia* respectively.

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, fasting blood glucose level determined. The animals were anaesthetized using chloroform and bled by cardiac puncture 24 hrs after the last treatment. The blood samples were collected in specimen bottles, allowed to clot and the serum separated by centrifugation at 3000rpm for 10 minutes 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(Accuchek,USA) after fasting the rats for 12 hours. 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).

Phytochemical screening

Test for anthraquinones was conducted using the method of Felgils(1975) and Test for alkaloids and Terpenes using the method of Sofowora (1979) while Test for saponins,flavonoids and tannins were done using the method of Earl(1961)

Statistical Analysis

The results obtained are presented as Mean± standard error of mean (SEM). A one way analysis of variance (ANOVA) was used for the data analysis. Significant differences between groups were detected in the ANOVA using Bonferini test at P values less than 0.05 and 0.001.Using SPSS 20 Soft ware package for windows.

RESULTS

Tables 1 and 2 below indicate the result of acute toxicity studies showing the LD₅₀ of ethyl acetate extract of *Vitex simplicifolia* leaf is greater than 5000 mg/kg body weight.

Table 1: Phase I LD₅₀, (Oral) of the ethyl acetate extract of *Vitex simplicifolia* leaf extract

Group	No. of Animals	Doses (g/Kg)	No. of Death
1	3	10	0
2	3	100	0
3	3	1000	0

Table 2: Phase II LD₅₀ (Oral) of the ethyl acetate extract of *Vitex simplicifolia* leaf extract

Group	No. of Animals	Doses (mg/Kg)	No. of Death
1	1	1600	0
2	1	2900	0
3	1	5000	0

Table 3: Results of phytochemical screening of the ethyl acetate extract of the *Vitex simplicifolia* leaf

Phytochemicals	Qualitative
Alkaloids	Present
Cardiac glycosides	Present
Phenols	Absent
Flavonoids	Present
Saponins	Present
Tannins	Present
Steroids	Absent
Terpenoids	Absent
Phytates	Present
Oxalates	Present
Cyanates	Present

Effects on glycemia

The effects of daily doses of ethyl acetate leaf extract of *Vitex simplicifolia* on blood glucose levels of alloxan induced diabetic rats is presented in Table 4. Daily administration of the plant extract to the diabetic rats caused a significant ($p < 0.05$) reduction in fasting

blood glucose levels after 21 days. The FBG levels decreased significantly ($p < 0.05$) by 54, 70 and 71% for 250, 500 and 1000 mg/kg of ethyl acetate leaf extract respectively and the rats administered with 10 mg/kg of glibenclamide (reference drug) decrease by 51.5%.

Table 4: Percentage changes in fasting blood glucose of alloxan induced diabetic rats treated with 250, 500 and 1000mg/kg of ethyl acetate extracts of *Vitex Simplicifolia* leaf.

Group	Mean Initial FBG(mg/dl)	Mean Final FBG(mg/dl)	Change (mg/dl)	% Change
Positive control	471.7±20.0	229.0±20.0	242.7±0.00	51.5
10mg/kg GCLM				
Diabetic+250mg/kg ETVSF	553.0±21.0	254.0±2.0	299.0±19.0	54 ^a
Diabetic+500mg/kg ETVSF	435.0 ±16.5	131.0 ±2.0	304.0 ±14.5	70 ^a
Diabetic+1000mg/kg ETVSF	435.0 ±165.5	127.0 ±2.0	308.0±163.5	71 ^a

Grp 1: Positive control, Grps 2, 3 and 4 received 250, 500 and 1000mg/kg of extract, respectively. Values are presented as mean ± standard error of mean. ^a = significantly different ($p < 0.05$) from the positive control.

Key: ETVSF- Ethyl acetate extract of *Vitex simplicifolia* leaf
GCLM- Glibenclamide

Effects on lipid profile

The effect of daily doses of ethyl acetate leaf extract of *Vitex simplicifolia* on lipid profile of alloxan induced diabetics rats is presented in Table 5. There was no significant ($p < 0.05$) change in the serum level of total cholesterol (TC), triglyceride (TAG), low density

lipoprotein cholesterol (LDL_c) and high density lipoprotein – Cholesterol (HDL_c) for the animals administered with the extract compared with the control groups

Table 5: The effect of ethyl acetate extract of *Vitex simplicifolia* leaf on lipid profile in alloxan induced diabetic rats.

Groups	Total Chol (mmol/l)	Triglyceride (mmol/l)	HDL (mmol/l)	LDL (mmol/l)
1	0.24±0.01	0.12±0.05	0.20±0.03	0.02±0.03
2	0.27±0.01	0.11±0.03	0.12±0.02	0.10±0.01
3	0.14±0.11	0.08±0.03	0.13±0.06	0.07±0.04
4	0.12±0.01	0.06±0.012	0.06±0.012	0.21±0.02
5	0.12±0E-7	0.11±0.001	0.04±0.01	0.21±0.02
6	0.07±0.05	0.13±0.04	0.09±0.01	0.23±0.02

Grp 1: Normal control, Grp 2: Negative control, Grp 3: Positive control, Grps 4, 5 and 6 received 250, 500 and 1000mg/kg of extract, respectively. Total chol = Total cholesterol. Values are presented as mean ± standard error of mean. ^a = significantly different ($p < 0.05$) from the Normal control, ^b = significantly different ($p < 0.05$) from the Negative control.

DISCUSSION

The result of acute toxicity study indicated that the LD₅₀ of the methanolic leaf extract of *Vitex simplicifolia* is greater than 5000mg/kg body weight.

Thus, the non-lethal effects produced with the high dose of this extract are an indication that the leaf extracts of *Vitex simplicifolia* is relatively safe on acute oral exposure.

It can therefore be concluded that *Vitex simplicifolia* leaf extract is non-toxic, which is in agreement with the report of Abdelmagid (2014) on essential oil of the leaves of *Vitex simplicifolia* and with Bruce (1987), American Society for Testing and Materials (1987), Aditya and Ravi (2014), Kingsley *et al* (2014) and Ravichandra *et al* (2014), that any chemical substance with LD₅₀ estimate greater than 3000-5000mg/kg (oral route) could be considered of low toxicity and safe.

The use of plants in the treatment of disease and in particular diabetes mellitus is as old as man (Sofowora, 1993). This is because plants have shown to contain some potent bioactive compounds with antidiabetic properties (Tanko *et al.*, 2013). In this study, diabetes established on the basis of fasting blood glucose concentration in the alloxan treated rats on 5th day of the experimental period formed the baseline values (Table 4). The result indicated that daily oral administration of reference drug and the ethyl acetate extracts of *Vitex simplicifolia* for 21 days show significant reduction in fasting blood glucose showing 54, 70 and 71% at 250, 500 and 1000 mg/kg respectively. However extracts showed more potency than glibenclamide (reference drug). The observed anti diabetic effect of the ethyl acetate leaf extracts of *Vitex simplicifolia* is an indication that the extracts contain bioactive phytochemicals with potent antidiabetic property. Anti diabetic activity of *Vitex simplicifolia* have never been reported to our knowledge, however, the aqueous and methanolic extracts of *Vitex doniana* had been reported to have potent anti diabetic properties more potent than the reference drug (glibenclamide) by Nwogo *et al.*, (2013), Ibrahim *et al.*, (2014) and Muhammed *et al* (2015) reported similar hypoglycaemic activity of chloroform and n-hexane fractions of *Ceiba pentandra* leaf. The hypoglycaemic action of medicinal plants may be of the following mechanism; inhibition of renal glucose reabsorption, enhanced secretion of insulin from B-cells of the pancreas, increased tissue uptake of glucose by enhancement of insulin sensitivity, regeneration/repair of the B-cells and/or

prevention of oxidative stress that is possibly involve in pancreatic B-cells destruction as reported for other antidiabetic plants (Kim *et al.*, 2006; Okpe *et al.*, 2012).

In this study the ethyl acetate leaf extracts of *Vitex simplicifolia* showed no significant effect on lipid profile of the experimental animals. This correspond with the reported study on methanolic and aqueous extract of *Vitex doniana* by Okpe *et al.*, (2012). The ethylacetate extract of *Vitex simplicifolia* have no significant effect on the level of total cholesterol and low density lipoprotein. These observations may be attributed to the gut intra-luminal interactive effect of saponin. Saponins are known anti nutritional factors which reduce the uptake of certain nutrients including glucose and lipid especially cholesterol at the gut through intra-lumina physicochemical interaction. Hence saponins have been reported to have hypocholesterolemic effect (Price *et al.*, 1987). Saponin, among other secondary metabolites is found to be present in the leaves of *Vitex simplicifolia* in this study (Table 3) and corresponds with reported presence of saponin in *Vitex doniana* (Eghareuba *et al.*, 2010).

The low concentration of cholesterol may have contributed to the observed non-significant high serum HDL-cholesterol in the experimental 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. HDL-cholesterol protect against cardiovascular disease (Okpe *et al.*, 2012). The observed non-significant (P>0.05) increase in HDL-cholesterol concentration after administration of the extracts (250, 500 and 1000 mg/kg bw) indicates that the extract does not have HDL-cholesterol boosting effect and it does not also have significant (P>0.05) LDL-cholesterol lowering effect at these concentrations in induced diabetic experimental animals. In conclusion, the ethyl acetate leaf extract of *Vitex simplicifolia* is observed to have potent hypoglycaemic activity on alloxan induced diabetic wister rats.

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