Hypoglycemic and Hypolipidemic Effects of Aqueous Leaf Extracts of *Vitex simplicifolia* in Alloxan - Induced Diabetic Wistar Rats

* [1]Salim MA, [2]Imam AA, [1]Dikko AAU, [3]Tanko Y, [3]Mohammed A

[1]Department of Human Physiology, Bayero University Kano, Nigeria
[2]Department of Biochemistry, Bayero University Kano, Nigeria
[3]Department of Human Physiology, Ahamadu Bello University, Zaria, Nigeria
*Corresponding Author: Salim MA, Department of Human Physiology, Bayero University Kano, Nigeria
Email: zafaralisalimm@gmail.com

Abstract

The effects of aqueous leaf extract of *Vitex simplicifolia* on blood glucose and lipid profile levels of alloxan; induced diabetic Wistar rats were investigated. The study was conducted with 30 Wistar rats, assigned into six groups of five rats each, and daily administration of aqueous leaf extract of *V. simplicifolia* for 21 days was done. Group 1 was the normal control; group 2 was the diabetic control, group 3 administered 10mg/kg of glibenclimide (positive control) and groups 4, 5, and 6 were administered 250, 500 and 1000 mg/kg body weight of leaf extract of *V. simplicifolia* leaf extracts. Significant ($p<0.01$) reduction in fasting blood glucose (FBG) levels relative to their initial values compared to the normal control was observed. The FBG levels decreased by 72, 75 and 81 in 250,500 and 1000mg/kg of aqueous extracts respectively. The normal control rats maintained a stable FBG level (102.8 ±40.0 to102.68 ±4.0) and the positive control decreased by 65%. This study may suggest the possible use of *V. simplicifolia* leaf in the management of hyperglycemia.

Keywords: *Vitex simplicifolia*, Hypoglycemia, Alloxan – Induced, Diabetic, Wistar Rats.

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 171 million diabetics worldwide, this number has double in the last few years and is expected to double once again in the year 2025.[1] 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.[2] 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.[3] Also, studies from five West African communities in Nigeria and Ghana have identified genes within populations that create susceptibility to diabetes.[4] The prevalence of diabetes mellitus in Nigeria is 1.9% with more than 1.5 million cases.[5] 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.[4] 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, kidney and as supplement for lack of vitamin A and B.[6,7]

Date received-07/09/2016 Date accepted- 10/12/2016

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 elucidating the hypoglycemic and hypolipidemic effects of aqueous leaf extract of *Vitex simplicifolia* on alloxan - induced diabetic Wistar 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 studies and scrutiny.[8]

Materials and Methods

Collection and Preparation of Plant Material

Fresh leaves of *Vitex Simplicifolia* were collected on 20th of January, 2015 from the Botanical Garden of Bayero University, Kano with the assistance of Herbarium keeper. The leaves were authenticated at the Department of Biological Sciences, Bayero University Kano (Herbarium number 242). The leaves were dried under room temperature and then ground using pestle and mortar to a semi powdered form.

Experimental Animals

Thirty (30) adult Wistar 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 guidelines were followed.[9]

Plant Extract

Vitex simplicifolia leaves (100g) were weighed into conical flask and distilled water (500ml) was poured into the flasks. The content of the flasks were shaken and the top was covered with aluminium foil and kept at room temperature for 48 hrs (2 days) after which the extracts were obtained by filteration using a Whatman No 1 filter paper. The extracts were concentrated using vacuum evaporator.

Lethality (LD_{50})

The mean lethal dose (LD_{50}) of the aqueous extract was determined in Wistar rats (weighing between 150 – 200g) using the method described by Lorke.[10] In the first phase, nine (9) Wistar rats were used. The nine animals were divided into three groups of three animals each. Each group was administered 10,100 and 1000mg/kg body weight of the extracts and then observed for 24 hours to monitor their behaviour and mortality. In the second phase two of the experiment, three animals were used; the animals were divided into three groups of one animal each. They were administered higher doses (1600, 2900 and 5000 mg/kg body weight) of the extracts and observed for behaviour as well as mortality. LD_{50} was calculated by the formula: $LD_{50} = \sqrt{(D_0 x D_{100})}$ where:

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

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.[11] Hyperglycaemia was confirmed by the elevated blood glucose levels, determined after 72 hrs 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).[12]

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 (Normal control)

Group 2 – Diabetic untreated rats (Diabetic control)

Group 3 – Diabetic rats administered 10 mg/kg of Glibenclamide.[13]

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

Sub-chronic Studies/ Collection and Treatment of Samples

The extracts were reconstituted in distilled water, and administered orally on daily basis for 21 days. At the end of 21 days, fasting blood glucose level was 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 3000 rpm for 10 minutes and then subjected to biochemical analysis.

Biochemical Analysis

The fasting blood glucose levels were determined based on glucose oxidase/peroxidase principle, as described by Clark and Lyons [14] using a digital glucometer (Accuchek) after fasting the rats for 12 hours. The serum levels of total cholesterol, triacylglycerol and HDL–C were determined by enzymatic method described by Lopes,[15] while the serum levels of LDL-C was measured according to protocol of Friedewald.[16]

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 Version 20 Software package for windows.

Results

Phytochemical screening

The result of both quantitative and qualitative screening of aqueous leaf extract of *Vitex simplicifolia* is presented in Table 1

Table 1: Results of phytochemical screening of the aqueous 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

Lethality (LD_{50})

The result of phase I and II acute toxicity studies is presented in Table 2- 3 below. The lethal dose (LD_{50}) determination was conducted using the method of Lorke (1983) through oral route in rats in two different phases. In both phases no signs of toxicity or mortality were observed after 24 hours of the administration.

Table 2: Phase I LD_{50}, of the Aqueous Leaf Extract of *Vitex simplicifolia*

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

Table 3: Phase II LD_{50} of the Aqueous Leaf Extract of *Vitex simplicifolia*

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

Effects of *Vitex simplicifolia* Leaf Extract on Glycemia

The effects of daily doses of aqueous 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 cause a significant ($p< 0.01$) reduction in fasting blood glucose levels after 21 days. The FBG levels decreased by 72, 75 and 81% in 250,500 and 1000 mg/kg aqueous leaf extract respectively and the rats administered with 10mg/kg of glibenclimade the FBG level decreased by 65%.

Table 4: Percentage Changes in Fasting Blood Glucose of Alloxan - Induced Diabetic Rats Treated with 250, 500 and 1000 Mg/Kg of Aqueous Leaf Extract of *Vitex Simplicifolia*.

Group	Mean Initial FBG(mg/dl)	Mean Final FBG(mg/dl)	Change (mg/dl)	% Change
Positive control 10 mg/kg GCLM	471.7 ± 20.0	165.1 ± 20.0	306.6.± 0.00	65
Diabetic+250mg/kg AGVSF	600.0 ± 0.00	174.0 ± 4.0	432.0 ± 4.0	72
Diabetic+500mg/kg AGVSF	557.0 ± 42.5	139.0 ± 4.0	418.0 ± 38.5	75
Diabetic+1000mg/kg AGVSF	557.5 ± 42.5	108.0 ± 4.0	449.5 ± 48.5	81

Key
AGVSF- Aqueous fraction of *Vitex simplicifolia* leaf GCLM- Glibenclimade

Effects of *Vitex simplicifolia* Leaf Extract on Lipid Profile

The effect of daily doses of aqueous leaf extract of *Vitex simplicifolia* on lipid profile of alloxan induced diabetic rats is presented in Table 5. There was no significant ($P > 0.05$) change in the level of total cholesterol (TC), triglycerol (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 Oral Administration of Aqueous Fraction of Leaf Extract of *Vitex simplicifolia* on Liver Glycogen Content and Lipid Profile on Alloxan - Induced Diabetic Rats.

Groups	Glycogen (mg/g)	Total Chol (mmol/l)	Triglyceride (mmol/l)	HDL (mmol/l)	LDL (mmol/l)
1	3.28 ± 0.09	0.24 ± 0.03	0.12 ± 0.05	0.20 ± 0.30	0.20 ± 0.04
2	1.96 ± 0.27	0.27 ± 0.01	0.11 ± 0.03	0.12 ± 0.02	0.10 ± 0.01
3	2.88 ± 0.02	0.14 ± 0.11	0.08 ± 0.01	0.13 ± 0.06	0.70 ± 0.04
4	2.27 ± 0.02	0.26 ± 0.06	0.19 ± 0.28	0.14 ± 0.28	0.15 ± 0.03
5	2.38 ± 0.46[b]	0.24 ± 0.47	0.14 ± 0.03	0.18 ± 0.06	0.08 ± 0.02
6	3.79 ± 0.29	0.23 ± 0.01	0.07 ± 0.02	0.68 ± 0.03	0.07 ± 0.01

Group 1: Normal control, **Group 2:** Diabetic control, **Group 3:** Positive control, **Groups 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. , [b] = significantly different ($P < 0.05$) from the Negative control

Discussion

The result of acute toxicity study indicated that the LD_{50} of the aqueous leaf extract of *Vitex simplicifolia* is greater than 5000 mg/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 study of Abdelmagid on essential oil of the leaves of *Vitex simplicifolia*.[17] Any chemical substance with LD_{50} estimate greater than 3000-5000 mg/kg (oral route) could be considered of low toxicity and safe.[18,19.20,21,22]

The use of plants in the treatment of diseases and in particular, diabetes mellitus, is as old as man.[6] This is because plants have shown to contain some potent bioactive compounds with antidiabetic properties.[23] 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. The result indicated that daily oral administration of reference drug and the aqueous extracts of *Vitex simplicifolia* for 21 days show significant reduction in fasting blood glucose showing 72, 75 and 81% at 250, 500 and 1000 mg/kg respectively. The extracts showed more potency than glibenclamide (reference drug). The observed anti diabetic effect of the aqueous leaf extracts of *Vitex simplicifolia* is an indication that the extracts contain bioactive phytochemicals with potent antidiabetic property. Antidiabetic activity of *Vitex simplicifolia* have never been reported to our knowledge, however, aqueous and methanolic extracts of *Vitex doniana* had been reported to have similar potent antidiabetic properties more potent than the reference drug (glibenclamide).[24] The hypoglycemic action of *Vitex simplicifolia* may be of the following mechanism; inhibition of renal glucose reabsorption, enhanced secretion of insulin from existing B-cells of the pancreas, increased tissue uptake of glucose by enhancement of insulin sensitivity, as reported for flavonoids and saponins. Flavonoids and saponins, among other secondary metabolites were found to be present in the leaves of *Vitex simplicifolia* in this study. [25,26]

In this study the aqueous leaf extract of *Vitex simplicifolia* showed no significant effect on lipid profile of the Wistar rats. This correspond with the reported study on methanolic and aqueous extract of *Vitex donniana*.[26] The aqueous extracts of *Vitex simplicifolia* leaf 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-lumena physicochemical interaction. Hence saponins have been reported to have hypocholestrolemic effect.[27] Saponin, among other secondary metabolites is found to be present in the leaves of *Vitex simplicifolia* in this study. 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.[26] The observed non – significant 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, aqueous extract of *Vitex simplicifolia* leaf was observed to possess a potent hypoglyceamic activity but have no hypolipidemic potential.

References

1. Beretta A .Campanha de prevencao e diagnostic do diabetes realizada pela UNIARARAS e prefeitura municipal na cidade de Araras. *Laes and Haes.* 2001; **22**(131):188-200.
2. Ogbonnia SO, Odimegwu JI, Enwuru VN. Evaluation of hypoglycaemic and hypolipidemic effects of aqueous ethanolic extracts of *Treculia africana Decne* and *Bryophyllum pinnatum Lam*. and their mixture on streptozotocin (STZ)-induced diabetic rats. *African Journal of Biotechnology*, 2008;**7**(15): 2535-2539.
3. Sobngwi E, Mauvais-Jarvis F, Vexiau P, Gautier JF. Diabetes in Africans: epidemiology and clinical specificities. *Diabetes Metab* (Paris). 2001;**27**: 628-34.
4. Rotimi SO, Olayiwola I, Ademuyiwa O, Adamson I. Inability of legumes to reverse diabetic-induced nephropathy in rats despite

improvement in blood glucose and antioxidant status. *Journal of Medicinal Food*, 2010;**13**:163-9.

5. International Diabetes Federation, (2015). Diabetes Atlas. 4th edn. The economic-impact of diabetes. Accessed on February 4, 2010.

6. Sofowora, A. Medicinal Plants and Traditional Medicine in Africa.2nd Ed., Spectrum Books, Nigeria, 1993;142-144.

7. Burkill HM. Useful Plants of West Tropical Africa. 2nd ed. Royal Botanic Garden Keiv. 2000:272-275.

8. Abdulrahaman, FI. Studies in natural products chemistry.The moraceae in African traditional medicine and management of psychiatry in Borno State.M.Sc Thesis. University of Maiduguri.Maiduguri. 2004.

9. National Institute of Health. Principles of animal care. National institute of health, Bethesda, Md.1985, 85-23.

10. Lorke DA. A new approach to practical acute toxicity testing. *Arch Toxicol* .1983; **54**:275–87.

11. Etuk E.Animal models for studying diabetes mellitus. *Agriculture and Biology Journal of North America*. 2010; 2151-7517.

12. Masiello P,Broca C,Gross R,Roye M,Manteghetti M,Hillare –Buys D,Novelli M, Ribes G . Expiremental NIDDM:development of a new model in adult rats administered with streptozotocin nicotinamide.*Diabetes.* 1998;**47**(2):224-9.

13. Prasanna R, Sivakumar V, Riyazullah M.S. Antidiabetic Potential of Aqueous and Ethanol Leaf Extracts of *Vitex negundo*. *International Journal of Pharmacognosy and Phytochemical Research.* 2012;**4(**2); 38-40.

14. Clark L and Lyons C. *Ann. NY Acad.Sci*.1962; 102–129.

15. Lopes MF, Stone P, Ellis S, Colwell JA. Cholesterol determination in high-density lipoproteins separated by three different methods. *Clinical Chemistry*, 1977; **23**(5):882-4.

16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of Low-density lipoprotein cholesterol in plasma, without use of the preparative Ultracentrifuge. *Clinical Chemistry*. 1972; **18**:499-502.

17. Abdel majid O, Noya S, Jean K, Sylvin O, Innocent P G. Acute toxicity and irritancy of the essential oil of the leaves of *Vitex simplicifolia Oliv.(Verbenaceae). Journal of Pharmacology and Toxicology*. 2014;**9** (1):62-67.

18. Bruce, R.D. A Confirmatory Study of up-and-down Method of Acute Oral Toxicity Testing.*Fundamental Applied Toxicology*. 1987; **8**: 97-100.

19. American Society for testing and Materials. Standard test method for estimating acute oral toxicity.OECD. 1987;**425**:1-8.

20. Aditya K and Ravi K. Toxicity studies of combined extracts of *Vitex pubescence,Vitex penducularis* and *Vitex agnucastus,Indian Journal of Research in pharmacy and Biotechnology,* 2014;**2**(2):1109 -1113

21. Kingsley C, Patrick I, Edidiong AO, Orish E. Evaluation of acute and sub-chronic toxicities of Vensestin Cleansers: A polyherbal supplement in female Wistar Albino rats, *Journal of Applied Pharmaceutical Science*; 2014;**4** (06): 074-078.

22. Ravichandran V, Deivam S, Anbu L, Reeta R. Acute and sub-acute toxicity studies of ethanolic poly-herbal extract in mice. *International Journal of Pharmacology & Toxicology*. 2014:**4**(2): 80-87.

23. Tanko Y, Sada NH,.Mohammed K.A, Jimoh M, Yerima, Mohammed A. Effect of ethanolic extract of Carallumadaizialli on serum electrolylytes levels on fructose-induced diabetes in wistar rats *Ann.Res*. 2013; **4**:157-161.

24. Nwogo AO,Kalu MK,Uchechukwu O, Glory O. Hypoglycemic effects of Aquesous and Methanolic Leaf extracts of vitex doniana on alloxan induced diabetic albino rats.*J.Med.Sci.* 2013;**13**(8):700 -707.

25. Kim C H. Expression of Extracellular Superoxide Dismutase Protein in Diabetes *Archives of Plastic Surgery*. 2013; **40**(5):517-521 .

26. Okpe O, Abdullahi SA, Nkeonye O, Ilechukwu CC, Nweke O, Ihuoma O. Hypoglycemic and Hypolipedimiceffects of aqueous and ehanalolic leaf extracts of vitexdoniana in normoglycemic Albino rats.*Global Advance Research journal of Microbiology*.2012;**1**(10)173-179.

27. Price KR, Johnson IT, Fenwick GR. The chemistry and biological significance of saponins in food and feeding stufs. *Critical Reviews in Food Science and Nutrition*, 1987;**26**: 27-133