Effects of n-hexane Leaf Extracts of *Vitex simplicifolia* on Blood Glucose Level, Liver Glycogen Content, Lipid Profile and Antioxidant Vitamins in Alloxan–Induced Diabetic Wistar Rats

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Abstract
The effects of oral administration of n-hexane leaf extract of *Vitex simplicifolia* on blood glucose, liver glycogen content, lipid profile, superoxide dismutase ,vitamins A,E and C levels in alloxan-induced diabetic Wistar rats were investigated. The study was conducted with 30 Wistar rats, assigned into six groups of five rats each, (n = 5) and daily administration of n- hexane leaf extract of *V. simplicifolia* for 21 days was done orally. 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 served as the normal control, group 2 served as the diabetic control, group 3 was administered 10 mg/kg of glibenclimide (positive control) and groups 4, 5, and 6 were administered 250, 500 and 1000 mg/kg body weight of n-hexane leaf extracts of *V. simplicifolia* respectively. The result showed 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 decreased by 58 %, 71 % and 78% for 250, 500 and 1000 mg/kg of n- hexane extracts respectively. The normal control rats maintained a stable FBG level (102.8 ± 40.0 to102.68 ± 4.0 mg/ dL) and the positive control decreased by 51.5%. There were no significant changes ($P< 0.01$) in the levels of total cholesterol (TC), triglycerol (TAG), low density lipoprotein – cholesterol (LDL_{-C}), high density lipoprotein –cholesterol (HDL_{-C}), SOD. The result also showed no significant ($P< 0.05$) change in vitamins A, E and C for the animals administered with the extract compared with the control groups. However, oral administrations of n-hexane extracts of *Vitex Simplicifolia* for 21 days significantly increased ($P< 0.05$) liver glycogen content. The result of this study may suggest the possible use of *Vitex simplicifolia* leaf in the management of hyperglycaemia.

Keywords: *Vitex simplicifolia*, Blood glucose, Liver glycogen content, Lipid profile, Antioxidant Vitamins

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. [1] 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.[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] Studies from five West African communities in Nigeria and Ghana have identified genes within populations that increased the 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 hyperglycaemia and the risk of long-term complications, which are known to be the major causes of morbidity and

Date received-06/09/2016 Date accepted- 10/12/2016

mortality.[4] Antioxidant is a molecule that inhibits the oxidation of other molecules. It terminates chain reactions by removing free radical intermediates, and inhibits other oxidation reactions. Antioxidant defence mechanisms involve both enzymatic and non enzymatic strategies. Common non–enzymatic antioxidants include the vitamins A, C, and E, glutathione, and the enzymes include; superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase.[6] 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.[7] 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. [7,8]

Although parts of this plant are used by traditional healers various ailments, there is paucity of scientific evidence 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 hypoglycaemic, hypolipidemic and antioxidant effects of n-hexane 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 study and scrutiny.[9]

Materials and Methods

Experimental Animals

Thirty (30) adult Wister rats weighing between 160 –240 g were obtained from the Animal House of Department of Human Physiology, Bayero University, Kano, Nigeria 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.[10]

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 Batch number 242). The leaves were dried under room temperature and then ground using pestle and mortar to a semi powdered form.

Plant Extraction

Vitex simplicifolia leaves (500g) were soaked in 2.5 litres of n-hexane 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.

Phytochemical Screening

Test for anthraquinones was conducted using the method of Felgils [11] and Alkaloids and Terpenes were tested using the method of Sofowora [7] while testing for saponins, flavonoids and tannins were done using the method of Earl.[12]

Determination of Mean Lethal Dose`

The mean lethal dose (LD_{50}) of the n-hexane extract was determined in Wistar rats (weighing between 150 – 200g) using the method described by Lorke.[13]

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.[14] Hyperglycaemia was confirmed by the elevated blood glucose levels, determined after 72 hrs and then confirmed after 7 days after induction. Rats found with elevated glucose level of 400 mg/dL and above were used for the study.[15]

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) animals (n = 5) each. Extracts were administered orally using 1ml syringe.

Group 1 - Normal untreated rats (Negative control)
Group 2 – Diabetic untreated rats (Diabetic control)

Group 3 – Diabetic rats administered 10 mg/kg of Glibenclamide [16]
Groups 4, 5 and 6 – Diabetic rats administered 250, 500 and 1000 mg/kg body weight of n-hexane leaf extract of *V. Simplicifolia* respectively.

Collection and Treatment of Samples
The extracts were reconstituted in distilled water, and administered orally on daily basis by oral route 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 clotting and the serum separated by centrifugation at 3000 rpm for 10 minutes and then subjected to biochemical parameters analysis.

Biochemical Analyses
The fasting blood glucose levels were determined based on glucose oxidase/peroxidase principle, using a digital glucometer (Accuchek, USA) after fasting the rats for 12 hours.[17] The serum levels of total cholesterol, triacylglycerol and HDL–C were determined by enzymatic method described by Allian (1974) [18] while the serum levels of LDL-C was measured according to protocol of Friedewald.[19] Liver glycogen was determined using the destruction by Alkali method [20]. Vitamins A, C and E were determined by the methods of Bessey [21] and Fabianek [22] respectively, while SOD was determine using the method of Mc cord and Fridorich.[23]

Statistical Analysis
The results obtained are presented as Mean $\pm$ standard error of mean (SEM). One way analysis of variance (ANOVA) was used for the data analysis. Significant differences between groups were detected in the ANOVA using Bonferroni post hoc test at P values less than 0.05 and 0.001.All the analysis was done using SPSS 20 Software package for windows.

Results
Table 1 and 2 below present the result of acute toxicity studies showing the LD_{50} of n-hexane extract of *Vitex simplicifolia* leaf. The LD_{50} was observed to be greater than 5000 mg/kg body weight.

Table 1: Phase I LD_{50}, (Oral) of the n-Hexane Extract of *Vitex simplicifolia* Leaf Extract

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

Table 2: Phase Ii Ld_{50} (Oral) Of The n-Hexane 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

LD $\geq$ 5000

Table 3: Qualitative Phytochemical Screening of the n-Hexane 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 n-hexane 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 58, 71 and 77% for 250, 500 and 1000 mg/kg of n-hexane leaf extract respectively and the rats administered with 10 mg/kg of glibenclimade (reference drug) decrease by 51.5%.

Table 4: Percentage Changes in Fasting Blood Glucose of Alloxan - Induced Diabetic Rats Treated with 250, 500 And 1000 Mg/Kg Of n-Hexane Extracts of *Vitex Simplicifolia* Leaf.

Group	Mean Initial FBG(mg/dL)	Mean Final FBG(mg/dL)	Change (mg/dL)	Percentage Change
Positive control 10mg/kg GCLM	471.7 ± 20.0	229.0 ± 20.0	306.6.± 0.00	51.5
Diabetic+250mg/kg NHVSF	525.0 ± 43.3	221.0 ± 20.0	304.0± 23.3	58
Diabetic+500mg/kg NHVSF	541.0 ± 59.0	156.0 ± 20.0	385.0± 39.0	71
Diabetic+1000mg/kg NHVSF	541.0 ± 59.0	125.0 ± 20.0	416.0± 39.0	77

NHVSF- n-Hexane fraction of *Vitex simplicifolia* Leaf, GCLM- Glibenclimade

Effects on Lipid Profile and Liver Glycogen Content

The effect of daily doses of n-hexane leaf extract of *V. 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), trglyceride (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 liver glycogen content is significantly lower in the diabetic control compared to the normal control and significantly higher in the test groups compared to the control groups.

Table 5: The Effect of n-Hexane Extract of Leaf Extract of *Vitex Simplicifolia* on Glucose, 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	0.23 ± 0.26	0.12 ± 0.05	0.20 ± 0.03	0.24 ± 0.00
2	1.96 ± 0.24	0.27±0.01	0.11±0.03	0.12 ± 0.00	0.10 ± 0.00
3	2.88 ± 0.020	0.12 ± 0.11	0.08 ± 0.00	0.13 ± 0.06	0.07 ± 0.00
4	2.94 ± 0.01	0.24 ± 0.00	0.36 ± 0.27	0.32 ± 0.00^{bc}	0.12 ± 0.01^{a}
5	6.02± 0.50^{abc}	6.31 ± 0E-7	0.08 ± 0.01	0.04 ± 0E7^{a}	0.02 ± 0.01^{abc}
6	3.18 ± 0.00^{b}	0.23 ± 0.04	0.12 ± 0.014	0.39 ±0.04^{abc}	0.21 ± 0.00^{abc}

Group 1: Normal control, **Group 2:** Negative 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. ^{a} = significantly different ($P < 0.05$) from the Normal control, ^{b} = significantly different ($P < 0.05$) from the Negative control, ^{c} = significantly different ($P < 0.05$) from Positive control.

Effects on SOD and Antioxidant Vitamins

The effect of daily doses of n-hexane leaf extract of *Vitex simplicifolia* on SOD and Vitamins A, C and E in alloxan induced diabetics rats is presented in Table 6. There is significant decrease in vitamin A and no significant ($P < 0.05$) change in superoxide dismutase (SOD), Vitamin C and E for the animals administered with 250, 500 and 1000 mg/kg of the extract compared with the control groups

Table 6: Effect of Oral Administration of n-Hexane Fraction of Leaf Extract of *Vitex Simplicifolia* on the Activity of SOD and Level of Vitamin A, C, E On Alloxan Induced Diabetic Rats.

Group	SOD(µmole/min/mg protein)×10^5	VIT-A (mg/dl)	VIT-C (mg/dl)	VIT-E (mg/dl)
1	1.57±0.28	632.40±72.00	0.53±0.23	20.60±0.88
2	0.41±1.16	499.67±113.92	0.28±0.14	22.69±1.73
3	0.44±0.32	46.00±1.00	0.92±0.08	23.52±3.49
4	0.90±0.58	27.33±6.06^{ab}	0.97±0.03	21.67±0.60
5	0.23±0.23^{a}	771.00±0.00^{b}	1.00±0.57	20.00±0.00
6	1.22±0.29	72.00±26.00^{ab}	0.50±0.50	19.45±0.45

Group 1: Normal control, **Group 2:** Negative control, **Group 3**: Positive control, **Groups 4, 5 and 6** 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 Normal control, ^{b} = significantly different ($p<0.05$) from the Negative control, ^{c} = significantly different ($p<0.05$) from Positive control.

Discussion

The result of acute toxicity study indicated that the LD_{50} of the n-hexane leaf extract of *V.simplicifolia* is greater that 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 *V.simplicifolia* is relatively safe on acute oral exposure. It can therefore be concluded that *V.simplicifolia* leaf extract is non-toxic, which is in agreement with the report of Abdelmagid [24] on essential oil of the leaves of *Vitex simplicifolia* and others, [25,26,27,28,29] that any chemical substance with LD_{50} estimate greater than 3000-5000mg/kg (oral route) could be considered of low toxicity and safe.

The use of plants in the treatment of diseases and in particular diabetes mellitus is as old as man, [7] This

is because plants have shown to contain some potent bioactive compounds with antidiabetic properties. [30] 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 n-hexane extracts of *V.simplicifolia* for 21 days show significant reduction in fasting blood glucose showing 55%, 71% and 77% for 250,500 and 1000 mg/kg respectively. However extracts showed more potency then glibenclamide (reference drug).

The observed anti diabetic effect of the n-hexane leaf extracts of *V. simplicifolia* is an indication that the extracts contain bioactive phytochemicals with potent antidiabetic property. Anti diabetic activity of *V. 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),[31,32,33] The hypoglycemic action of medicinal plants may probably 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,[34,35,36] Flavonoids and saponins, among other secondary metabolites were found to be present in the leaves of *V. simplicifolia* in this study (Table 3).

In this study the n-hexane leaf extracts of *V.simplicifolia* showed no significant effect on lipid profile of the experimental animals. This corresponds with the reported study on methanolic and aqueous extract of *Vitex donniana.*[36] The n-hexane extract of *V.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-lumena physicochemical interaction. Hence saponins have been reported to have hypocholestrolemic effect.[37] Saponin, among other secondary metabolites is found to be present in the leaves of *Vitex simplicifolia* in this study (Table 3).

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 atheroma within arteries and transport it back to the liver.HDL–cholesterol protect against cardiovascular disease.[33] 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.

Oral administrations of n-hexane extract of *Vitex simplicifolia* for 21 days significantly increase liver glycogen content. The liver glycogen content is significantly lower in the diabetic control compared to the normal control. This finding does not appear to be related to dietary conditions, since all experimental animals were feed the same type of feed. It is possible that diabetic subjects have defects in liver and muscle glycogen synthesis.[38] The increase in liver glycogen content may be accounted for by reversal of the earlier mentioned mechanisms (defects in liver and glycogen synthesis).

Oral administration of n-hexane leaf extract of *V. simplicifolia* in this study showed a significant decrease in vitamin A and no significant ($P < 0.05$) change in superoxide dismutase (SOD), vitamin C and E. A decrease in SOD and vitamin A is an indication of lack of antioxidant activity. [39] Anti oxidant defence mechanism involves both enzymatic and non enzymatic strategies. Common antioxidants include vitamins A, C and E and superoxide dismutase. This antioxidants work in synergy with each to neutralize different types of free radicals.[6] There is paucity of data on the antioxidant activities of *V. Simplicifolia*.

In conclusion, the n- hexane leaf extract of *V. simplicifolia* is observed to have potent hypoglycaemic activity on alloxan- induced diabetic Wistar rats.

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