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Original Research Article

Antidiabetic and Hypolipidemic Effects of Methanol Leaf Extract of *Napoleona vogelii* (*Lecythidaceae*) Hook & Planch on Alloxan–Induced Diabetes Mellitus in Rats

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Abstract

Purpose: *To evaluate the antihyperglycaemic and hypolipidemic effects of Napoleona vogelii Hook. & Planch (Lecythidaceae) methanol leaves extract in alloxan-induced diabetes in rats.*
Methods: *The leaves were extracted via cold maceration using 70 % methanol. Diabetes was then induced using alloxan (150 mg/kg I.P). Alloxan-induced diabetic rats were divided into 5 groups of 5 each and were treated with 100, 200 and 400 mg/kg of the methanol extract. The 4th and 5th diabetic groups were given glibenclamide (5 mg/kg) and distilled water respectively. The 6th group, normal rats were given distilled water. Treatment was continued daily and orally for 14 days. Blood glucose levels were monitored at 0, 2, 4, 8 and 24 h, and 14th days. The lipid profile was determined on the 14th day of administration. Oral acute toxicity and phytochemical screening were also carried out on the extract.*
Results: *The extract significantly ($p < 0.05$) reduce fasting blood glucose level at 100, 200 and 400 mg/kg notably from the 4th hour to the 14th day. The effect of the extract compared well with that of glibenclamide which also produced significant reduction ($p < 0.05$) in blood glucose level in the diabetic rats from the 4th hour onwards. The extract significantly increased and lowered ($p < 0.05$) High density lipoprotein (HDL)-cholesterol and Low density lipoprotein (LDL)-cholesterol + triglycerides), respectively, at 100 and 200 mg/kg doses. Total cholesterol was also significantly reduced ($p < 0.05$) at 200 and 400 mg/kg doses. Acute toxicity data revealed death at 4 g/kg dose. While phytochemical screening revealed the presence of flavonoids, saponins, glycosides, tannins and anthraquinones.*
Conclusion: *These data suggest that Napoleona vogelii leaves have potential hypoglycaemic effect and could be a useful source of an anti-diabetic agent.*

Keywords: *Napoleona vogelii, Lipid profile, Diabetes, Glibenclamide, Hypoglycemic effect*

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INTRODUCTION

Plants had been used for medicinal purposes long before recorded history. Researchers found that people in different parts of the world tended to use the same or similar plants for the same purpose [1].

Napoleona vogelii Hook. & Planch (*Lecythidaceae*), is known locally as *akpuruke, mkpodu*, or *odure* by the Ibos of South-Eastern Nigeria. The plant is found mostly in rain forest and alongside the seashores, extending from Sierra Leone to Nigeria. The methanol extract of *Napoleona vogelii* is used in the treatment of

peptic ulcer disease [2]. The leaves are also used in the treatment of cough and asthma and are said to possess hypoglycemic effect [3].

Diabetes mellitus is defined as an elevated blood glucose associated with absent or inadequate pancreatic insulin secretion, with or without concurrent impairment of insulin action. [4]. Many lipoprotein abnormalities are seen in the untreated, hyperglycemic diabetic patient. The non-insulin-dependent diabetic (NIDDM) patient with mild fasting hyperglycemia commonly has mild hypertriglyceridemia due to overproduction of TG-rich lipoproteins in the liver, associated with decreased high-density lipoprotein (HDL) cholesterol levels [5].

The management of diabetes mellitus usually involves the use of insulin and oral hypoglycemic drugs, but total recovery from this disease state has not been reported up to this date. Today, more people use dietary supplements and studies show that people with diabetes are more likely to use supplements than people without diabetes. From the foregoing, it is obvious that the importance of herbal medicines in the management of chronic illnesses, one of which is diabetes mellitus, cannot be overemphasized. Hence, this study was geared towards ascertaining the hypoglycemic effect of the leaves of *Napoleona vogelii* for the first time. Other studies previously carried out include its use in the treatment of peptic ulcer, asthma and cough [3].

EXPERIMENTAL

Plant material

The leaves of *Napoleona vogelii* were collected from Temboga in Ikpoba Okha Local Government Area of Edo State, Nigeria in February 2013. The plant was identified by Mr Sunny Nweke, of the Department of Pharmacognosy, University of Benin, Nigeria where a herbarium specimen exists. Botanical authentication of the plant was further confirmed at the Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria where a voucher specimen (FHI 109793) was deposited in the herbarium for future reference.

Extraction of plant material

The leaves of *Napoleona vogelii* were air-dried and ground into powder. About 523 g of the powered leaves was extracted via maceration for 72 h with 2.8L of absolute methanol. The extract was filtered using a funnel and a filter cloth, after which the filtrate was filtered again using cotton wool to ensure the extract, was devoid of any particle of the filtrate. The extract was evaporated to dryness using a water bath. The percentage yield was 2.7 %.

Drugs and chemicals

Alloxan monohydrate (Qualikens, laboratory reagent grade, China), glibenclamide (Hovid Berhad, Västernorrland County Sweden), methanol (Guandong Guanghua Sci-Tech Co Ltd, China) were used in the study. Others are the Randox kit for HDL, Total cholesterol and Triglycerides (United Kingdom) l grade.

Phytochemical analysis

The leaves of *Napoleona vogelii* were subjected to preliminary phytochemical screening for various constituents. The methods of analysis employed were those described by [6,7].

Experimental animals

Adult albino rats and mice of both sexes weighing between 195-245 g and 25-30 g respectively were used for the study. The animals were obtained from the Animal House of the Department of Pharmacology and Toxicology of the University of Benin, Benin City. They were kept in standard cages in a well-ventilated room, and fed with growers mash (Ewu feeds, Edo State) and water *ad libitum*. The animals were acclimatized for two weeks prior to experimentation.

Ethical approval

Ethical approval for the study was obtained from the Ethical Committee on the Use of Animals for Experiments, Faculty of Pharmacy, University of Benin, Benin City, Nigeria (approval letter dated January 2013. The animals were also handled according to the standard protocols for the use of laboratory animals [8].

Oral acute toxicity study

The oral acute toxicity was carried out using mice (n = 4-5) per group. The first group of mice were treated with 2 ml/kg of normal saline, this served as the control. The second, third and fourth groups were treated with the methanol extract of the plant: *Napoleona vogelii* at the dose of 1000 mg/kg, 2000 mg/kg and 4000 mg/kg respectively. All administrations were done orally using an

orogastric syringe. The mice were initially observed for 24 h for signs of toxicity and death and thereafter for the next 14 days for other signs of toxicity and death, while being administered water and fed daily [9].

Induction of diabetes

Thirty albino rats of both sexes were used. 25 rats were administered alloxan dissolved in normal saline at a dose of 150 mg/kg body weight intraperitoneally [10], while the remaining 5 were non-diabetic and served as control. After 3 days of alloxan administration, the rats with blood glucose levels greater than 200 mg/dl, were considered diabetic and hence were used in carrying out this research work. The blood glucose level of the rats was ascertained using the Accu-check glucometer [11].

Experimental protocol

The rats were divided into 6 groups of 5 rats each. The rats in each group were treated as follows: Group 1- Normal rats: received distilled water (2 ml/kg), Group 2- Diabetic rats: these were left untreated and received distilled water (2 ml/kg), Groups 3, 4 and 5- were all diabetic rats and received 100, 200 and 400 mg/kg of *N. vogelii* respectively. The sixth group, diabetic rats: received 5 mg/kg body weight of glibenclamide. All administrations were done orally for 14 days.

Determination of fasting blood glucose level

The animals were fasted overnight, though had access to water, which was however withdrawn during the actual determination. All blood samples used for the determination of fasting blood glucose levels were collected by cutting the tip of the tail of each rat at intervals of 0, 2, 4, 8, 24 hours and on the 14th day after drug/extract administration. The determination of blood glucose level was done using the Accu-check active glucometer. The results were recorded in mg/dl [12].

Determination of lipid profile

On the 14th day following drug/extract administration, the rats were sacrificed under chloroform anaesthesia. The blood samples were collected from the abdominal aorta and left ventricle of the heart and immediately introduced into lithium heparinized tubes to prevent clotting. This was used for the determination of total cholesterol, triglycerides, high density lipoproteins and the low density lipoprotein.

Statistical analysis

All data are expressed as mean ± SEM. Where applicable, the data were analysed statistically by Student's t-test using Graph Pad Instat, version 2.05a. $P < 0.05$ was considered significant.

RESULTS

Phytochemical profile

The results of the phytochemical analysis of the extract showed the presence of simple sugars, reducing sugars, cardiac glycosides, saponin glycosides, anthraquinone, alkaloids, tanins, and flavanoids, while cyanogenetic glycosides were absent.

Oral acute toxicity

The results of the oral acute toxicity study of the extract are presented in Table 1. From the study, it was seen that the extract produced no death except at the dose of 4 g/kg, where 20 % death was recorded. This point to the fact that the extract has a wide margin of safety and the LD_{50} could not be ascertained as no death was recorded at lower doses.

Table 1: Oral acute toxicity of the methanol extract of leaves of *Napoleona vogelii*

Treatment (g/kg)	Log-dose	Mortality (%)
Control	0	0
MENV (1)	3.000	0
MENV (2)	3.301	0
MENV (4)	3.602	20

Control animals received normal saline (2ml/kg); MENV = methanol extract of Napoleona vogelii

Anti-diabetic effect of *Napoleona vogelii* leaf extract

The effect of the methanol extract of *Napoleona vogelii* on alloxan-induced diabetic rats is as shown in Figs 1 - 5. The extract is seen to significantly ($p < 0.05$) reduce glucose level at all doses administered (100, 200 and 400 mg/kg). This reduction in the fasting blood glucose level is seen to be non-dose dependent.

At the 100 mg/kg dose there were reductions which were significant from the 2nd hour up to the 14th day ($p < 0.05$); at 200 mg/kg reductions were also noted, although significance was only observed from the 8th hour up to the 14th day,

while the 400 mg/kg dose produced significant reduction of the fasting blood glucose level from the 2nd hour and on the 14th day ($p < 0.05$). Comparisons were done with the untreated diabetic rats. The effect of the extract compares well with glibenclamide as evident from the results shown, where significant reduction ($p < 0.05$) in blood glucose level was observed in the diabetic rats from the 4th hour after administration.

On the 14th day, a significant reduction ($p < 0.05$) in blood glucose level was observed in the diabetic rats treated with the extract at all doses; and those treated with glibenclamide compared with the untreated diabetic rats.

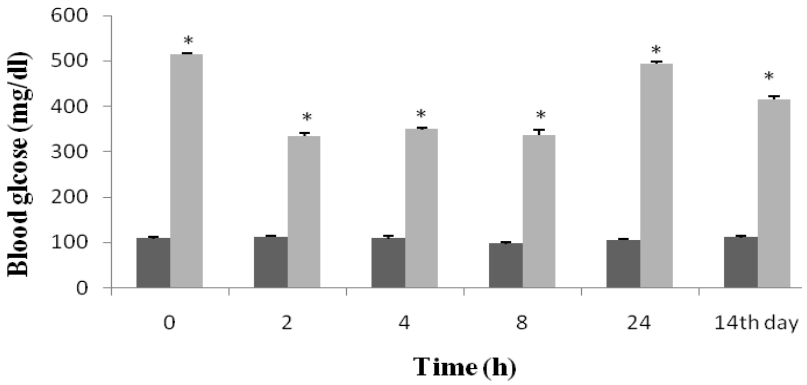

Fig 1: The blood glucose level of normal rats (dark bars) and untreated diabetic rats (grey bars). Values are mean ± SEM (n = 3 - 6); *$p < 0.05$ significantly different from the control

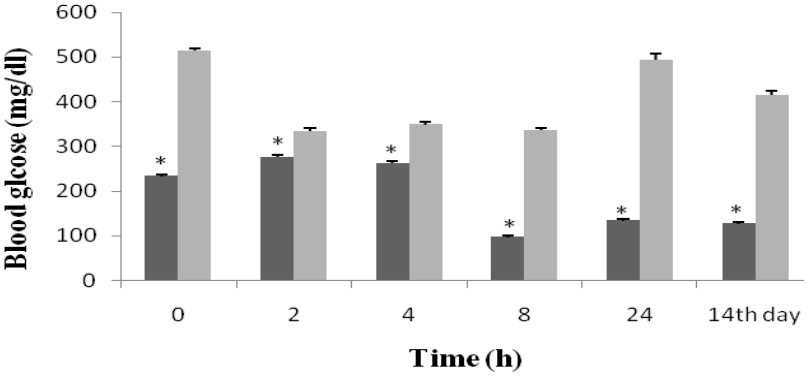

Fig 2: The Effect of the methanol leaf extract of *Napoleona vogelii* (100 mg/kg) on alloxan-induced diabetic rats; dark bars = treated; grey bars = untreated. Values are mean ± SEM (n = 3 - 6); *$p < 0.05$ significantly different from the untreated diabetic rats

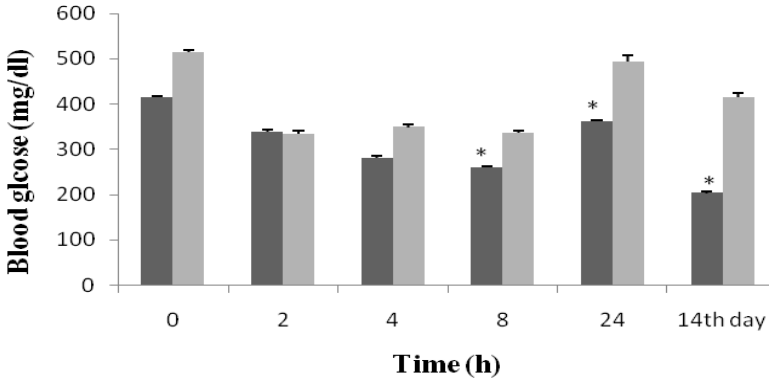

Fig 3: Effect of the methanol leaf extract of *Napoleona vogelii* (200 mg/kg) on alloxan induced diabetic rats; dark bars = treated; grey bars = untreated. Values are mean ± SEM (n = 3 - 6); *$p < 0.05$ significantly different from the untreated diabetic rats

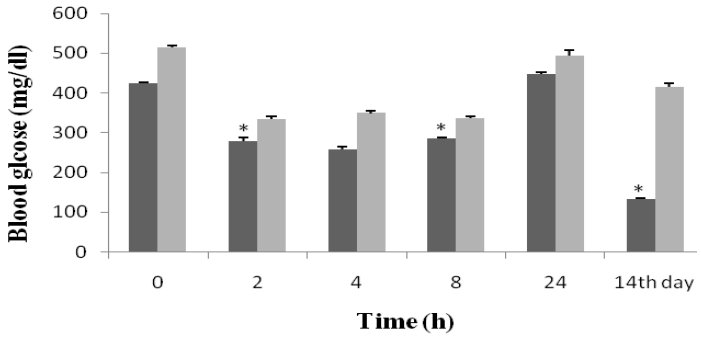

Fig 4: Effect of the methanol leaf extract of *Napoleona vogelii* (400 mg/kg) on alloxan induced diabetic rats; dark bars = treated; grey bars = untreated. Values are mean ± SEM (n = 3 - 6); *p < 0.05 significantly different from the untreated diabetic rats

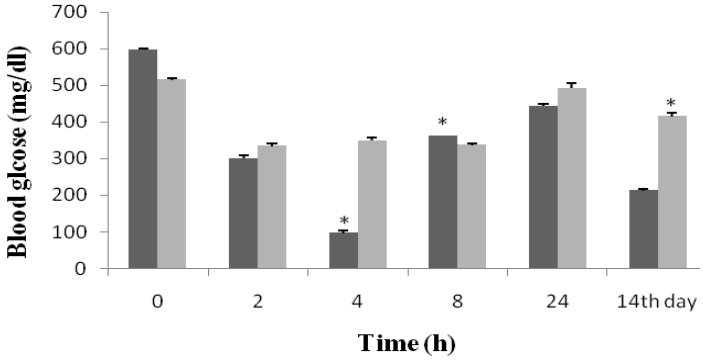

Fig 5: Effect of glibenclamide (5 mg/kg) on alloxan induced diabetic rats; dark bars = treated; grey bars = untreated. Values are mean ± SEM (n = 3 - 6); *p < 0.05 significantly different from the untreated diabetic rats

Effect of *Napoleona vogelii* leaf extract on plasma lipid profile

The plasma lipid profile of the rats is presented in Table 3. The extract produced a significant increase (p < 0.05) in the HDL-cholesterol concentration of the diabetic rats treated with 100 and 200 mg/kg of the extract when compared with the untreated diabetic rats, however the HDL-cholesterol concentration was insignificantly increased in diabetic rats treated with 400 mg/kg of the extract. This effect is similar to that seen in the diabetic rats treated with glibenclamide.

The extract also significantly reduced (p < 0.05) the total cholesterol concentration in the diabetic rats treated with 200 and 400 mg/kg. A decrease in triglyceride concentration was also noted, at all the doses administered, with significant reduction (p < 0.05) recorded at 200 mg/kg dose. The LDL-cholesterol concentration in the diabetic rats treated with the various doses of the extract was also reduced, with significant reduction (p < 0.05) observed at the 200 mg/kg dose. This effect is similar to that seen in the diabetic rats treated with glibenclamide.

Table 2: Effect of the methanol extract of *Napoleona vogelii* leaves on plasma lipid profile of alloxan-induced diabetic and normal rats

Group	Lipid profile (mg/dL)			
	TC	TG	HDL	LDL
C	63.6±4.0	60.3±5.8	49.9±3.4[a]	0.8±0.3
UTD	49.5± 5.3	71.5±12.2	21.5± 2.3	6.0± 1.3
D100	55.7± 5.8	64.2± 3.7	44.4± 4.2[a]	4.5± 1.9
D200	33.3± 5.1[a]	45.7±2.9[a]	33.1± 8.4	0.9±3.1[a]
D400	41.5±14.8[a]	67.0±3.1	25.8±5.4	5.1±7.2
DG	72.7± 0.9	83.4±11.4	43.0±3.5[a]	13.0±1.6[a]

Values are mean lipid levels ± SEM (n = 5, per group); [a]p < 0.05 significantly different from the untreated diabetic rats; C = control given normal saline; UTD = untreated diabetic rats; D100 = diabetic rats treated with 100 mg/kg of the extract; D200 = diabetic rats treated with 200 mg/kg of the extract; D400 = diabetic rats treated with 400 mg/kg of the extract; DG = diabetic rats treated with 5 mg/kg of glibenclamide

DISCUSSION

The use of alloxan for the induction of diabetes has been described as a useful experimental model in the study of the activity of hypoglycaemic agents. Alloxan has been shown to produce hyperglycaemia which may be due to partial or complete destruction of the beta cells [12].

The untreated diabetic rats had a significantly higher blood glucose level compared to the control. As clearly seen from results obtained, a significant reduction was observed at least from the second hour following administration of extract.

From the foregoing, based on results obtained and significant reduction of blood glucose levels produced by all doses of the extract, it is evident that the effect of the extract compares well with glibenclamide, which stimulates the secretion of insulin. Glibenclamide works by inhibiting the sulphonylurea receptor 1 (SUR1), the regulatory subunit of the ATP-sensitive potassium channels (KATP) [13] in pancreatic beta cells. This inhibition causes cell membrane depolarization opening voltage-dependent calcium channel. This results in an increase in intracellular calcium in the beta cell and subsequent stimulation of insulin release. The extract can therefore be said to have a similar mechanism for the stimulation of the release of insulin, as that of glibenclamide. Flavonoids, one of the secondary metabolites in the extract have also been reported to stimulate the secretion of insulin , saponins, another constituent in *Napoleona vogelii* has also been found to have hypercholesterolemic and antidiabetic properties[14].

Tannic acid stimulates glucose transport and inhibits adipocyte differentiation, phlorotannins possess some level of antidiabetic property [15,16].

Some anthraquinones have been found to possess mild antidiabetic effect. This is due to their marked glucose transport activity. They could play metabolic roles in the insulin-stimulated glucose transport pathway [14]. Based on these findings, the presence of flavonoids, saponins, anthraquinones and tannins in the methanol extract of *Napoleona vogelii* may account for the hypoglycaemic effect noticed. Hence, it can be said that their presence in the extract gives the extract a glibenclamide-like effect.

Acute toxicity describes the adverse effects of a substance that results either from a single exposure or from multiple exposures in a short space of time, usually less than 24 hours [17]. Acute toxicity study is used to determine the level of safety of drugs. The oral acute toxicity studies of the methanol extract of *Napoleona vogelii* revealed that the extract produced no death except at a dose of 4 g/kg. This therefore shows that the extract has a wide margin of safety.

Patients with untreated diabetes mellitus usually end up with many lipoprotein abnormalities. This is because diabetes mellitus is a metabolic disease associated with impaired glucose metabolism, [18], which adversely alters intermediary metabolism of lipids.

It is generally known that patients with diabetes mellitus usually have raised triglyceride, total cholesterol and LDL-cholesterol concentration. In this study, total cholesterol concentration of the diabetic rats treated with 200 and 400 mg/kg of the extract were lowered significantly compared to the untreated diabetic rats. Also, the triglyceride and LDL-cholesterol concentration of the diabetic rats treated with all doses of the extract was lowered, compared to the untreated diabetic rats, although significant reduction was only observed at the 200 mg/kg dose. A similar effect was seen in the diabetic rats treated with glibenclamide, as there was a significant reduction in the LDL-cholesterol concentration.

As seen from previous studies carried out [19], patients with type 2 diabetes have less HDL cholesterol than non-diabetics. In this research, the HDL-cholesterol concentration in the diabetic rats treated with all doses of the extract was increased compared to the untreated diabetic rats; although significance was only observed at 100 mg/kg dose, just as it was seen in the diabetic rats treated with glibenclamide. The effect of the extract on total cholesterol and LDL-cholesterol concentration was seen to be highest in the diabetic rats treated with 200 mg/kg.

According to American Heart Association in 2012, total cholesterol is made up of HDL-cholesterol, LDL-cholesterol and triglyceride. Elevated levels of triglyceride cause a buildup of fatty deposits in the artery walls (arthrosclerosis). Also, when too much LDL-cholesterol circulates in the blood, it can slowly build up in the inner walls of the arteries that feed the heart and brain. Together with other substances, it can form plaque that narrows the arteries and makes them less flexible, a condition known as arthrosclerosis, hence the need for a reduction in the concentration of triglyceride, LDL-cholesterol and total cholesterol in the blood. HDL-cholesterol helps to scavenge for LDL-

cholesterol and transports it to the liver, where it can be processed, hence the need for an increase in the concentration of HDL-cholesterol [20].

From the study carried out, the extract caused a decrease in the concentration of LDL-cholesterol, triglyceride and total cholesterol which probably may have been increased as a result of the diabetes mellitus. The extract also produced an increase in the HDL-cholesterol concentration. Thus, reasonably normalizing the lipid profile of the rats and reducing the risk of heart diseases such as atherosclerosis which could be a complication of uncontrolled diabetes mellitus.

CONCLUSION

This study has shown that the methanol extract of *Napoleona vogelii* leaves possess anti-diabetic properties in alloxan-induced diabetic rats. It also shows the presence of biologically active components in the extract of the leaves of the plant under study, notably flavonoids, saponins, tannins and anthraquinones, which could be responsible for the anti-diabetic effect observed. The methanol extract of *Napoleona vogelii* leaves has also be proven to reduce total cholesterol, triglyceride and LDL-cholesterol concentrations, while increasing HDL-cholesterol concentrations, in alloxan-induced diabetic rats.

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