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HYPOGLYCAEMIC ACTIVITY OF *NAUCLEA LATIFOLIA* SM. (RUBIACEAE) IN EXPERIMENTAL ANIMALS.

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Abstract

Aqueous, ethanolic and hexane extracts of the leaves of *Nauclea latifolia* (Rubiaceae) were assessed for their fasting blood glucose lowering effect in normoglycaemic and streptozotocin – diabetic rats. Wistar strain albino rats were given different doses of the extracts after 18 hrs fast and their blood glucose measured at 0,1,2,4 and 6 hours after treatment. The aqueous and ethanolic extracts significantly lowered the fasting blood glucose levels of the STZ–diabetic rats in a dose–dependent manner. The highest dose administered (400mg/kg) lowered the fasting blood glucose of the diabetic rats by 31.7% (aqueous) and 36.1% (ethanolic) extracts. The aqueous extract did not significantly lower the glucose levels of normoglycaemic rats (maximum 6.6%), nor was any significant decrease seen in the rats administered with the hexane (maximum of 4.0% for normoglycaemic and 2.4% for diabetics) extract. The hypoglycaemic and antihyperglycaemic potentials of the aqueous and ethanolic extracts were comparable to that of glibenclamide (1mg/kg).These results further support the traditional use of the plant in the treatment of diabetes mellitus.

Key words: *Nauclea latifolia*; Diabetes mellitus; Hypoglycaemia; Streptozotocin – induced diabetes.

Introduction

Diabetes mellitus (DM) is a common metabolic disorder affecting people in both developed and developing countries. Diabetics can be divided into two main groups based on their requirements for insulin: insulin dependent diabetes mellitus (Type 1) and non – insulin dependent diabetes mellitus (Type 2). The disease is characterized by chronic hyperglycaemia, which if not effectively controlled can cause blindness, kidney failure or nerve damage. Type 2 diabetes is the most prevalent, and accounts for approximately 90% of cases of diabetes mellitus worldwide (WHO 2002), and is characterized by a combination of defects in insulin secretion and insulin sensitivity. Glycaemic control is one of the targets for managing DM. Studies have confirmed that for the type 2 diabetes, effective control of blood glucose substantially decrease the risk of developing complications of diabetes (Ohkubo et al., 1995; UKPDS, 1997).

The many side effects of insulin therapy and oral hypoglycaemic agents (Holman and Turner, 1991; Rao et al., 1997) necessitated the search for more effective and safer antidiabetic drugs. Scientific search for hypoglycaemic agents from medicinal plants as recommended by the World Health Organisation (The WHO expert committee, 1980) has become even more important. In the last few decades over 500 herbal medicines have been reported to possess antidiabetic property (Handa et al., 1989; Ivorra et al., 1989; Jia et al., 2003).

Nauclea latifolia Smith (Rubiaceae) is a straggling shrub or small tree native to tropical Africa and Asia. It is reported to be used in the treatment of malaria (kokwaro, 1976; Akubue and Mittal, 1982; Boye, 1990), GIT disorders (Madubunyi, 1995), sleeping sickness (Kerharo, 1974), prolong menstrual flow (Elujoba, 1995) and hypertension (Akubue and Mittal, 1982). Different parts of *N. latifolia* are commonly prescribed traditionally as a remedy for diabetes mellitus. The veracity of these claims has not been established scientifically. Recently, we reported the antidiabetic property of the aqueous extract of the leaves of the plant (Gidado et al., 2005). In this study, we compare the fasting blood glucose lowering effect of different doses of aqueous, ethanolic and hexane extracts of the leaves of *N. latifolia* on both normal and streptozotocin-induced diabetic rats.

Materials and Methods

Plant material

Fresh leaves of *N. latifolia* were collected from Ahmadu Bello University main campus. It was identified and authenticated at the herbarium unit of Biological Sciences, Department, A.B.U. Zaria. It was identical with the voucher specimen (No. 1268) previously deposited at the herbarium. The leaves were dried under the shade and ground into powder with pestle and mortar.

Extract preparation

The different extracts were prepared by soaking 200g of the powder of *N. latifolia* leaves in 1 liter of water, 95% ethanol or hexane in different glass Jars for 2 days at room temperature. The extract was filtered and the process repeated three times. The extracts were concentrated at a lower temperature (<50^{o}C) under reduced pressure on a rotary evaporator. The percentage yields of the extracts were 13.4, 16.7 and 3.2% w / w respectively for water, ethanol and hexane.

Animals

Wistar strain albino rats (150 – 200g) bred in the Department of Pharmacology and Clinical Pharmacy, faculty of Pharmaceutical Sciences A.B.U. Zaria, were used for the study. The animals were fed *ad libitum* with pellet diet (Vital feeds, Jos, Nigeria) and water. They were also kept and maintained under laboratory conditions of temperature, humidity and light (24 ± 1 ^{0}C, 65% and 12 h light/dark cycle) respectively. We followed the Guide for the Care and Use of Laboratory Animals, 1985, issued by the US Department of Health and Human Services, Public Health Service, National Institute of Health, NIH Publication No. 86-23.

Induction of diabetes

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) (45mg / kg body weight) in 0.1M citrate buffer (pH 4.5) in a volume of 1ml / kg (Rakieten et al., 1963). Diabetes was confirmed in the STZ - treated rats by measuring the fasting blood glucose concentration 48 hours after STZ injection. Rats with fasting blood glucose of more than 200mg/dl were considered diabetic and included in the study after a stabilization period of 7 days.

Experimental design

To study the glucose–lowering effect of the different extracts, the rats were divided into nine groups of six animals each:

Group 1- Normal – untreated rats, Group 2- Diabetic- untreated rats, Group 3- Normal rats administered 100mg/kg bw of plant extract, Group 4- Normal rats administered 200mg/kg bw of plant extract, Group 5- Normal rats administered 400mg/kg bw of plant extract, Group 6- Diabetic rats administered 100mg/kg bw of plant extract, Group 7- Diabetic rats administered 200mg/kg bw of plant extract, Group 8- Diabetic rats administered 400mg/kg bw of plant extract, Group 9- Diabetic rats administered 1mg/kg bw of glibenclamide.

The rats were fasted for 18 hrs prior to extract administration. The extract suspended in distilled water was administered orally by intubation using a feeding tube (BMI, feeding tube, size 8). Groups 1and 2 were fed distilled water alone. Blood Samples for glucose estimation were collected in fluoridated eppendorff tubes from the rats' tail

vein at 0,1,2,4 and 6 hours after administering the extract, water or glibenclamide. Serum glucose concentration was determined based on the principle of Trinder (1969), using glucose oxidase kit (Randox, UK).

Statistical Analysis

The results are presented as mean ± standard error of the mean (S.E.M.). Mean blood glucose values at 0,1,2,4 and 6 hours were compared using one way analysis of variance (one-way ANOVA). Differences between two means was analysed by the student's t – test.

Results

Effects of the aqueous extract on fasting blood glucose.

The effect of different doses of the aqueous fraction of *N. latifolia* leaves on fasting blood glucose of normoglycaemic and STZ–diabetic rats is shown in Table 1. The three different doses administered to normal rats produced slight insignificant decreases in fasting blood glucose. In diabetic rats, the extract significantly ($P<0.05$) lowered their fasting blood glucose with the group administered 400mg / kg body weight showing the maximum reduction of 31.7% 4 hrs after treatment.

Effects of the ethanolic extract on fasting blood glucose

The effect of different doses of the ethanolic extract of *N. latifolia* leaves on fasting blood glucose of normoglycaemic and STZ –diabetic rats is shown in Table 2. The ethanolic extract significantly ($P<0.05$) lowered fasting blood glucose of both normal and diabetic rats. The 400mg/kg dose produced the maximal fall of 30.9% in normal and 36.1% in diabetic rats.

Effects of the hexane extract on fasting blood glucose.

The effect of different doses of the hexane extract of *N. latifolia* leaves on fasting blood glucose of normoglycaemic and STZ–diabetic rats is shown in Table 3. Statistically ($P>0.05$) the hexane extract showed no lowering effect on blood glucose. A maximum of 4.0% and 2.4% decreases were observed in normal and diabetic rats respectively. STZ – diabetic rats treated with glibenclamide showed 35.0% fall in fasting blood glucose which is comparable to the diabetic rats administered 400mg/kg extracts of the aqueous and ethanolic extracts.

Discussion and conclusion

In this study streptozotocin (STZ) was used for the induction of diabetes mellitus. Streptozotocin is well known for its selective pancreatic β-cell cytotoxicity and has been widely used to induce diabetes mellitus in experimental rats (Aderibigbe et al., 2001; Ojewole, 2003; Malalavidhane et al., 2003). The persistent hyperglycaemia in the STZ-diabetic rats also indicates partial β-cells destruction. Treatment of normal and STZ-diabetic rats with different doses of the leaves of *N. latifolia* extracted with water, 95% ethanol and hexane produced marked hypoglycaemic effects with the aqueous and ethanolic extracts (Tables 1 and 2). The ethanolic extract lowered the fasting blood glucose of both normal and STZ-diabetic rats. The 400mg/kg dose group showed the highest decrease in fasting blood glucose of both normal (30.9%) and diabetic (36.9%) rats.

The 36.9% reduction of fasting blood glucose of STZ-diabetic rats treated with 400mg/kg dose of ethanolic extract was found to be comparable with the 35.5% reduction observed with diabetic group treated with glibenclamide. Glibenclamide is often used as a standard drug in STZ-induced moderate diabetic models to compare antidiabetic properties of a variety of compounds (Andra-Cetto et al., 2000; Arulselvan and Subramanian, 2007).

The exact chemical constituent(s) of the plant extract that is / are responsible for the hypoglycaemic effects are not known at present. Preliminary Phytochemical screening of the extracts (data not shown) revealed that the aqueous and the ethanolic extracts contain alkaloids, flavonoids, steroids, saponins, glycosides, coumarins and carbohydrates. Coumarins, flavonoids, terpenoids and some other secondary plant metabolites have been reported to possess hypoglycaemic effect (Marles and Farnsworth, 1995; Ojewole, 2002).

Table 1: Effect of different doses of aqueous fraction of the leaves of *Nauclea latifolia* on fasting blood glucose levels (mg/dl) of normal and diabetic rats (Mean ± SEM). n=6

Treatment	Time (Hrs)				
	0	1	2	4	6
Normal Control	73.59± 2.21	73.16± 2.06	74.29± 2.24	71.90± 2.36	71.47± 1.54
Diabetic Control	265.51± 6.78	270.51± 8.36	265.13± 7.75	264.74± 7.59	266.28± 6.85
Normal + 100mg / kg	68.47± 1.74	68.75± 2.28	67.22± 2.89 (1.8%)	64.72± 2.17 (5.5%)	64.86± 0.79 (5.3%)
Normal + 200mg / kg	74.53± 2.14	73.87± 1.92	74.67± 2.58	69.60± 2.01 (6.6%)	71.731± 1.44 (3.8%)
Normal + 400 mg / kg	76.88± 1.28	73.44± 2.45 (4.6%)	75.49± 1.22 (1.9%)	72.84± 2.29 (5.4%)	73.57± 1.30 (4.4%)
Diabetic + 100 mg / kg	237.60± 2.10	230.67± 3.32 (2.9%)	209.20± 4.21** (12%)	183.47± 7.07*** (22.8%)	235.60± 2.98 (0.8%)
Diabetic + 200mg / kg	270.93±14.23	248.53± 12.34 (8.3%)	213.73±8.29** (21.1%)	188.93± 12.21** (30.3%)	218.93±9.63* (19.2%)
Diabetic + 400mg / kg	303.26± 10.72	266.48± 10.69* (12.1%)	234.61± 13.08** (22.6%)	207.13± 14.62*** (31.7%)	249.62±10.64** (17.7%)
Diabetic + Glibenclamide (1mg / kg)	274.03± 13.89	249.11± 12.55 (9.1%)	228.87± 12.30* (16.5%)	178.10± 10.28*** (35.0%)	206.35± 11.88** (24.7%)

Values in parenthesis are percentage of decreases in blood glucose

* P <0.05 compared with the initial level of blood glucose (0 h) in the respective group

** P <0.01 compared with the initial level of blood glucose (0 h) in the respective group

*** P < 0.001 compared with the initial level of blood glucose (0 h) in the respective grou

Table 2: Effect of different doses of ethanolic fraction of the leaves of *Nauclea latifolia* on fasting blood glucose levels (mg/dl) of normal and diabetic rats (Mean ± SEM). n=6

Treatment	Time (Hrs)				
	0	1	2	4	6
Normal Control	73.59± 2.21	73.16± 2.06	74.29± 2.24	71.90± 2.36	71.47± 1.54
Diabetic Control	265.51± 6.78	270.51± 8.36	265.13± 7.75	264.74± 7.59	266.28± 6.85
Normal + 100mg / kg	77.04± 1.76	74.93± 1.44 (2.7%)	68.56± 1.52** (11.0%)	62.44± 0.71*** (19.0%)	70.60± 1.56* (8.4%)
Normal + 200mg / kg	73.75± 1.78	70.83± 2.16(4.0%)	64.58± 1.34**(12.4%)	55.00± 0.78*** (25.4%)	56.53± 1.64*** (23.3%)
Normal + 400 mg / kg	77.78± 2.92	70.00± 2.55 (10.0%)	59.31± 1.30*** (23.7%)	53.75±1.53*** (30.9%)	62.78± 1.99** (19.3%)
Diabetic + 100 mg / kg	253.33± 4.91	243.07± 4.34 (4.1%)	228.53± 2.85** (9.8%)	216.13± 6.91** (14.7%)	223.60± 7.86** (11.7%)
Diabetic + 200mg / kg	276.87±3.99	256.40± 5.75* (7.4%)	233.60±2.56*** (15.6%)	212.13± 0.34*** (23.4%)	186.27±7.57*** (32.7%)
Diabetic + 400mg / kg	360.39± 7.22	335.26± 9.37 (7.0%)	296.54± 8.39*** (17.7%)	251.92± 7.27*** (30.1%)	230.39±4.65*** (36.9%)
Diabetic + Glibenclamide (1mg / kg)	274.03± 13.89	249.11± 12.55 (9.1%)	228.87± 12.30* (16.5%)	178.10± 10.28*** (35.0%)	206.35± 11.88** (24.7%)

Values in parenthesis are percentage of decreases in blood glucose

* P <0.05 compared with the initial level of blood glucose (0 h) in the respective group

** P <0.01 compared with the initial level of blood glucose (0 h) in the respective group

*** P < 0.001 compared with the initial level of blood glucose (0 h) in the respective group

Table 3: Effect of different doses of hexane fraction of the leaves of *Nauclea latifolia* on fasting blood glucose levels (mg/dl) of normal and diabetic rats (Mean ± SEM). n=6

Treatment	Time (Hrs)				
	0	1	2	4	6
Normal Control	73.59± 2.21	73.16± 2.06	74.29± 2.24	71.90± 2.36	71.47± 1.54
Diabetic Control	265.51± 6.78	270.51± 8.36	265.13± 7.75	264.74± 7.59	266.28± 6.85
Normal + 100mg / kg	74.39± 1.65	73.17± 1.48	73.04± 0.95	72.49± 1.53	72.36± 1.48 (2.7%)
Normal + 200mg / kg	74.58± 1.96	75.14± 1.85	72.60± 1.36	72.32± 2.42	71.61± 2.00 (4.0%)
Normal + 400 mg / kg	75.52± 2.10	73.81± 1.95	75.49± 2.37	72.97± 2.86	72.83± 2.63 (3.6%)
Diabetic + 100 mg / kg	243.14± 7.66	249.44± 5.33	250.00± 5.04	245.52± 6.02	247.06± 7.64
Diabetic + 200mg / kg	272.31±11.86	278.85± 13.41	272.05±12.47	273.46± 10.74	266.80±12.29 (2.0%)
Diabetic + 400mg / kg	264.49± 10.97	268.85± 10.83	261.79± 12.18	258.34± 11.64	258.21±11.02 (2.4%)
Diabetic + Glibenclamide (1mg / kg)	274.03± 13.89	249.11± 12.55 (9.1%)	228.87± 12.30* (16.5%)	178.10± 10.28*** (35.0%)	206.35± 11.88** (24.7%)

Values in parenthesis are percentage of decreases in blood glucose

* P <0.05 compared with the initial level of blood glucose (0 h) in the respective group

** P <0.01 compared with the initial level of blood glucose (0 h) in the respective group

*** P < 0.001 compared with the initial level of blood glucose (0 h) in the respective group

The hypoglycaemic and antihyperglycaemic activity of the extracts of *N. latifolia* leaves could arise through stimulation of insulin secretion from β-cells, increased availability of insulin or inhibition of intestinal absorption of glucose. The exact mechanism of the hypoglycaemic action of the plant extracts remains unknown at present but that it produces results similar to that of glibenclamide, supposes at least in part that a certain pancreatic activity is possible (Subramoniam et al.,1996; Prince et al., 1999).

Results of this study support the antidiabetic property of *N. latifolia* and thus substantiate the traditional use of the plant as a remedy for diabetes mellitus. Research is going on to study the effect of long term administration of the extracts to diabetic rats and to also probe the nature of the Phytochemical (s) responsible for the hypoglycaemic activity.

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