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PRELIMINARY PHYTOCHEMICAL AND HYPOGLYCEMIC STUDIES ON *PHYLLANTHUS NIRURI* (EUPHORBIACEAE) LEAF EXTRACT

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Abstract

The hypoglycemic effect of the methanol leaf extract of *Phyllanthus niruri* (Euphorbiaceae) was evaluated on normoglycemic and hyperglycemic rats. Diabetes was induced in rats using alloxan (80 mg/kg body weight intraperitoneally). Normoglycemic and hyperglycemic rats were administered with two different doses (100 and 200 mg/kg) of the extract of *Phyllanthus niruri (P. niruri*), 10 mg/kg of Glibenclamide (Positive control) and 2 ml /kg of 3 % Tween 20 (Negative control). The blood sugar level was determined using a Glucometer. The classes of chemical components and the acute toxicity of *P. niruri* were also investigated. The leaf extract produced a dose dependent reduction ($P<0.05$) in blood sugar levels of both normoglycemic and hyperglycemic rats. When doses of 100 and 200 mg/kg of the extract were administered intraperitoneally to alloxan induced diabetic rats, significant decrease in blood sugar level occurred (25.49 and 52.91 % respectively) comparable to the sugar lowering effect of glibenclamide (60.71 %). In normal rats, the extract (100 and 200 mg/kg) exhibited a significant reduction of the blood sugar level of 17.28 % and 36.84 % respectively while glibenclamide caused a 42.80 % reduction. Phytochemical tests on *P. niruri* showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, reducing sugars, resins, steroids, tannins and terpenoids. Acute toxicity test carried out in mice using Lorke's methods showed that the extract was safe, since no death was recorded at the dose of 5000 mg/kg. The study shows that the extract of *P. niruri* possessed a significant and dose dependent hypoglycemic activity in normoglycemic and alloxan-induced diabetic rats and comparable to glibenclamide, the standard drug. This also supports its use in folklore management of diabetes.

Key words: diabetes, hyperglycemic activity, *Phyllanthus niruri*, Euphorbiaceae, toxicity.

Diabetes mellitus, a heterogeneous disorder responsible for a disruption of glucose homeostasis is characterized by an elevation of blood glucose caused by a relative or absolute deficiency of insulin (Mycek *et al.*, 2000). Alteration in metabolisms of lipids and proteins, and an increased risk of complication from vascular diseases are other characteristics of diabetes. It can also be defined as a chronic hereditary, endocrine, metabolic diseases characterized by hyperglycemia and eventual glycosuria causing considerable morbidity and mortality. Indigenous cultures around the world recognize diabetes mellitus and have plant based medicines to treat this disease. More than 400 traditional plant treatments for diabetes mellitus were reported seven centuries ago (Barley *et al.*, 1989), although these were abandoned on the discoveries of synthetic drugs. Herbs have therefore,

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been known as the first art of treatment available to man (Kafaru, 1994). The side effects of the synthetic drugs and their inability to give a permanent cure for diabetes have given a new resurgence of interest in the search for alternatives from plants. A good number of Nigerian plants and plants all over the world have been screened for hypoglycemic activity and includes *Shenostylis sternocarpa* (Odoh *et al.*, 2007), *Detarium microcarpum Gull and Perr* (Odoh *et al.,* 2005), *Prosopis africana* (Odoh *et al.*, 2003), *Mucuna slonaei* (Ezugwu *et al.*, 2005) and Corn silk (Ajali *et al.,* 2007).

P. niruri (Euphorbiaceae) is indigenous to the rainforests of the Amazon and other tropical areas throughout the world, including the Bahamas, Central and southern India and China. It grows as a weed worldwide. In Latin-American rainforest (the Amazon) this plant is known as chanca piedra or quebra pedra or stone breaker. In India, this plant is called bhumy amalaki. It is also known as *enyikwonwa* in Ibo language of the South Eastern Nigeria. A lot of researchers (Ramakrishnan *et al*., 1982; Sivaprakasan *et al*., 1995) who worked on *Phyllanthus niruri* confirmed that it has hypoglycemic properties. In Brazil, *P. niruri,* is used for urinary and bladder infections and blockages, liver ailments, painful joints, cystitis, prostate disorders, kidney disorders, hepatitis B, diabetes and as an antispasmodic and muscle relaxant (Paranjpe, 2001). In many parts of India especially in deserts, the roots mixed with *Commiphora mukul* are given to camel to cure indigestion. The decoction of leaves and stem are used for dyeing cotton black (Singh *et al.,* 1996). The plant is of medicinal importance for numerous ailments like dysentery, diuretics, kidney stones, influenza, antibacterial, antihyperglycemic and antiviral (Chopra *et al*., 1986). A lot of researchers have shown that *P. niruri*, has protective action on the different body organs especially the liver and the kidney, and no form of toxicity has been associated with the usage of this plant extract (Tabasum *et al*., 2005; Baros, 2003; Nishiura *et al.*, 2005). Thus *P. niruri* has been used for so many purposes in herbal medicine. The active compounds phyllanthin and hypophyllanthin have been isolated from the leaves and recently lignasniranthin, nirtetralin and phyltetralin have been isolated from the leaves (Rostogi and Mehrotra, 1991). It is a major component of many popular liver tonics in India including Liv-52. Fresh juice and powder of dried plant are used most frequently in Ayurvedic preparations (Sastry and Karatherka, 1991). The objective of this work is to evaluate the hypoglycemic activity of methanol extract of *P. niruri* leaves.

MATERIALS AND METHODS

Plant material

The *P. niruri* leaves used in this experiment were collected from Nsukka, Enugu State, Nigeria and identified in June, 2008 by Mr. Ozioko of Bioresources and, Conservation Programme, Nsukka, Enugu State. The voucher specimen (UNPCOG/08/395) was kept at the Herbarium of the Department of Pharmacognosy, University of Nigeria, Nsukka.

Chemicals and Equipment

The reagents were sourced commercially and were used as supplied. Methanol, hydrochloric acid, alloxan monohydrates, chloroform and Tween 20 (Sigma, USA), glibenclamide (Merck, Germany) silicia gel GF254, ethyl acetate and petroleum ether, n- hexane (Hopkin and Williams, England) and Accuchek Glucometer and Accuchek Strips (Manesty, England) were used in the present investigation.

Animals

Albino rats weighing 110-160 g and mice weighing 14-21 g were used in this experiment. They were obtained from the Animal House of Zoology Department of University of Nigeria, Nsukka. The animals were housed in white metallic cages and kept in a room where a 12-hour light/dark cycle was maintained with free access to water and food for seven days to acclimatize to the laboratory conditions. They were given free access to food and water before the experiment and after the induction of diabetes.

Extraction

A 500 g quantity of the powdered leaf material was exhaustively extracted in soxhlet extractor using 70 % methanol for 42 h. The extract was concentrated under reduced pressure using a rotary evaporator to yield a dark mass. The extract was then placed in a refrigerator until when needed for analysis.

Phytochemical studies

The chemical classes of constituents of the methanol extract of the leaf were investigated using standard methods (Trease and Evans, 1994; Harbourne, 1984). The chemical classes of constituents tested for include alkaloids, glycosides, cyanogenetic glycosides, cardiac glycosides, carbohydrates, reducing sugars, saponins, tannins, flavonoids and steroids.

Acute toxicity test (LD_{50})

The LD_{50} of the *P. niruri* leaf extract was determined using the method of Lorke (1983). Nine mice were used for preliminary test. They were divided into three groups of three mice each. The three groups were given 10, 100, 1000 mg/kg of the extract prepared in 5% (v/v) Tween 20 in water, intraperitoneally. The mice were observed for lethal effects for 24h and number of death was recorded. Other doses corresponding to 2000, 3000, 4000 and 5000 mg/kg were given to four mice respectively. The mice were observed for 24 h and number of death was recorded.

Hypoglycemic evaluation

Determination of hypoglycemic effect on normoglycemic rats

Twenty healthy rats weighing 110-160 g were used for this experiment. The animals were divided into four groups each containing five animals. They were fasted overnight for 12 h.

At the end of the fasting period, different doses of the extracts were given to the animals intraperitoneally. Group I received 100 mg/kg of the extract, Group 2 received 200 mg/kg of the extract, Group 3 received 10 mg/kg glibenclamide as a positive control and group 4 received 2 ml/kg of 5 % (v/v) Tween 20 (negative control). At 0, 1, 3 and 6 h, blood samples were withdrawn from the tail vein of each rat and the blood sugar level determined using the glucometer.

Determination of hypoglycemic effect on hyperglycemic rats

Twenty healthy albino rats weighing 110 - 200-g were used for the experiments. Albino rats were weighed and fasted for 12 hours. Hyperglycemia was induced by intravenous administration of 80 mg/kg of alloxan monohydrates prepared in distilled water. The animals were allowed free access to water and food for 7 days. On the eight day, rats whose blood glucose level were above 150 mg/kg were selected and divided into four groups (n=5). Group 1 received 100 mg/kg of the extracts, Group 2 received 200 mg/kg of the extract, Group 3 received 2 ml/kg of 5 % (v/v) Tween 20, and Group 4 received 10 mg/kg of glibenclamide. The blood samples were collected from the tail veins of

rats at 0, 1, 3 and 6 h respectively after treatment and the blood sugar levels were determined.

Statistical analysis

The results obtained were analyzed statistically and were reported as ± S.E.M .The results were compared using student's t- test (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The methanol extraction of the leaves gave 8.40 % (w/w) yield. From the results of acute toxicity test of the extract in mice it shows that it was non- toxic, as at the dose of 5000 mg/kg, no death was recorded and can be used at the studied doses in management of ailments. Administration of the methanol extract of *P. niruri* at a dose of 100 and 200 mg/kg body weight ($P < 0.05$) showed that the extract exhibited a significant and dose dependent reduction in the blood sugar level in both the normoglycemic and hyperglycemic rats (Table 1and 2).

Table 1: Mean blood sugar level of the hypoglycemic activity of leaf extract of *Phyllanthus niruri,* glibenclamide and Tween 20 on normal rats.

Atment	Dose (mg/kg)	Mean Blood sugar (MBS) Levels (mg/dl)				Percentage Maximum Reduction (%)
		0 h	1 h	3 h	6 h	
Extract	100	81.00 ± 12.03	80.00 ±13.36	71.00 ± 9.55*	67.00 ± 8.03*	17.28
	200	88.66 ± 12.03	86.33 ± 9.85	73.00 ± 3.52*	56.00 ± 3.06*	36.84
Gliben-clamide	10	78.67 ± 8.18	69.33 ± 10.36	51.00± 11.07*	45.00 ± 1.07*	42.80
3 % Tween 20 (2ml/kg)	_	68.33 ±0.33	68.33 ± 0.33	67.00 ± 0.58	67.00 ± 0.58	1.95

Values are expressed as Mean ± SEM; *P<0.05; n=5

In the normal rats, the doses of 100 and 200 mg/kg of the extract caused a percentage maximum reduction of 17.28 and 36.84 % respectively after 6 h while in the alloxan induced diabetic rats, the doses of 100 and 200 mg/kg of the extract caused a percentage maximum reduction of 25.49 and 52. 91 % respectively after 6 h post treatment.

From the results of phytochemical analysis, it was found that the plant extract contains many constituents. These constituents includes; alkaloids, carbohydrates, flavonoids, glycosides, reducing sugars, resins, steroids, tannins and terpenoids. Some plants that contain alkaloids have been reported to have hypoglycemic activity (Bever and Zahad, 1979). So the hypoglycemic activity associated with extract of this plant may be attributed to the presence of this alkaloids and flavonoids. These are mainly phenolic compounds, which have been reported to have anti-diabetic effects (Farjou *et al*, 1987). The precise mechanism of which this leaf extract lowers blood glucose is however not clear. The hypoglycemic action of the extract could be due to the possible enhancement of the peripheral utilization of glucose or by increasing the pancreatic secretion of insulin from the cells of islets of langerhans or its

Table 2: Mean blood sugar level of the hypoglycemic activity of leaf extract of *Phyllanthus niruri*, glibenclamide, and Tween 20 on diabetic rats.

Treatment	Dose (mg/kg)	Mean Blood sugar (MBS) Levels (mg/dl)				Percentage Maximum Reduction (%)
		0 h	1 h	3 h	6 h	
Extract	100	170.0± 5.78	164.67± 7.13	151.0± 10.23*	126.67± 8.83*	25.49
	200	179.0± 7.58	173.33± 6.37	152.33± 6.75*	84.30± 3.61*	52.91
Glibendamide	10	168.0± 3.79	159.0± 5.51*	118.67± 5.82*	66.00± 9.68*	60.71
3 % Tween 20 (2 ml/kg)	–	155.33± 9.85	155.33± 9.85	155.00±10.03	154.67± 10.18	0.42

Values are expressed as mean ± SEM; *P < 0.05; n = 5

release from bound insulin (Farjou *et al*., 1987; Pari and Amarnath, 2004). This suggestion is supported by the fact that despite pre-treatment of the rats with alloxan, which is known to cause permanent destruction of pancreatic β- cells (Zarrow *et al*., 1964), hypoglycemic effect was still observed in alloxan-induced diabetic rats. This insulin inhibits conversion of triglycerides of free fatty acids and the conversion of glycogen to glucose. It is also believed that some of these hypoglycemic plants perform this function by removing the insulin-inactivating compounds through the SH groups in these inactivating compounds. Similarly, other hypoglycemic plants containing anthocyanosides appear to act by improving vascularization of the pancreas. Others act by blocking oxidative enzyme of the Kerb cycle (succinic dehydrogenase and cytochrome oxidase), thus increasing anaerobic glycosides and decreasing gluconeogenesis and entailing on increased rate of transfer of glucose from the blood to the tissue (Bever and Zahad, 1979).

CONCLUSION

The leaf of *Phyllanthus niruri* contains some hypoglycemic principles responsible for its hypoglycemic activities in normal and alloxan induced diabetic rats. Further work can be done to isolate and characterize the bio-active principle responsible for this activity. This study justifies the use of this plant by traditional healers in the management of diabetes mellitus.

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