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RESEARCH ARTICLE

Studies on the possible mechanisms of antidiabetic activity of extract of aerial parts of *Phyllanthus niruri*

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Abstract

Context/objectives: The effects of methanol extract of aerial parts of *Phyllanthus niruri* L. (Euphorbiaceae), an antidiabetic herb, on glucose absorption and storage in diabetes were studied to elucidate the mechanisms of blood glucose lowering and glycemic control in diabetes.

Methods: The effect of chronic oral administration of the extract on glycemic control was evaluated in alloxan diabetic rats using blood glucose lowering and post-prandial glucose suppression activities as well as effects on hemoglobin glycation and body weight. Effects on glucose mobilization and storage were assessed using the weight and glycogen content of liver isolated from treated diabetic rats, while *in vitro* inhibition of α-amylase and α-glucosidase enzyme activities were used as indices of effect on glucose absorption.

Results: Results showed that the extract lowered blood glucose, suppressed postprandial rise in blood glucose following a glucose meal, reduced hemoglobin glycation and increased absolute and relative weights as well as glycogen content of liver in diabetic rats. Treatment with the extract also ameliorated the decrease in body weights caused by the diabetic disease. *In vitro*, the extract inhibited α-amylase (IC_{50}: 2.15 ± 0.1 mg/mL) and α-glucosidase (IC_{50}: 0.2 ± 0.02 mg/mL) activities.

Discussion and conclusion: These findings suggest that aerial parts of *P. niruri* may owe their blood glucose lowering properties to inhibition of glucose absorption and enhancement of glucose storage.

Keywords: Antidiabetic, α-amylase, α-glucosidase, Phyllanthus niruri

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Introduction

Several medicinal plants spread across different families have been screened for antidiabetic activity with promising results (Mohamed et al., 2006). One such plant is *Phyllanthus niruri* Linn. (Euphorbiaceae), a small annual herb indigenous to rain forests, tropical and sub-tropical areas of the world (Damle, 2008) and popularly employed in traditional medicine practice in different parts of the world for the treatment of a variety of disorders. It is popularly used in Asia, Africa and South America (Mellinger et al., 2005) as a stomachic, aperitive, anti-hyperglycemic, antispasmodic, antihepatotoxic, antiviral, antibacterial, laxative, diuretic, carminative, in the management of diabetes, constipation, fever, malaria, jaundice, hepatitis B, dysentery, gonorrhea, syphilis, tuberculosis, cough, influenza, diarrhea, vaginitis, tumors and kidney stones (Syamasundar et al., 1985; Oliver-Bever, 1986; Chopra et al., 1986; Unander et al., 1995; Paranjape, 2001; Lin et al., 2003). In South America, it is used for the treatment of fever (Singh, 1986) venereal diseases and diarrhea (Holdsworth et al., 1989; Holdsworth & Balun, 1992). In Ayurveda, a decoction of the plant is administered orally for the treatment of asthma and diabetes (Jain & Sharma, 1967).

Pharmacologically, the HIV replication inhibitory (Naik & Juvekar, 2003), hepatoprotective (Shimizu, 1989), lipid lowering (Chandra, 2000), antimalarial (Neraliya & Gaur, 2004) and antidiabetic (Halim & Ali, 2002; Bavarva & Narasimhacharya, 2007) activities are some of the documented properties.

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Although previous studies have established the antidiabetic effect of this plant, the mechanisms underlying this activity are largely unknown. This study documents the effect of extract of aerial parts of this plant on indices of glucose absorption, mobilization and storage as mechanisms of its blood glucose lowering effect and glycemic control in diabetes.

Methods

Chemicals and reagents

Alloxan monohydrate, yeast α-glucosidase (EC 3.2.1.20, type 1 from baker's yeast), porcine pancreatic α-amylase (EC 232-565-6), soluble potato starch, 3,5-dinitrosalicylic acid and *p*-nitrophenyl-α-D-glucopyranoside were purchased from Sigma-Aldrich, UK. Acarbose (Glucobay®, Bayer), glibenclamide tablets (Daonil®, Aventis, batch number: 20375A) were purchased locally. Other chemicals used were of analytical grade.

Animals

Adult albino rats (150-300 g) of both sexes obtained from the Laboratory Animal Facility of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, were used. The animals were kept in steel cages within the facility with free access to potable water and standard pellet diet. All animal experiments were in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (1985).

Plant material

Fresh whole plants of *P. niruri* were collected in June 2008 from Orba, Enugu State, Nigeria. The plant material was identified and authenticated by A. Ozioko of the International Centre for Ethno medicines and Drug Development (InterCEDD) Nsukka, Enugu State, Nigeria, where a voucher specimen is maintained. The roots of the plant were cut off and the aerial parts dried under the sun for one week then pulverized to coarse powder in an electric hammer mill. The powdered plant material (900 g) was extracted with methanol (98% v/v) by cold maceration for 48 h with occasional mechanical shaking (4 h/day). At the end of 48 h, the mixture was filtered using ashless filter paper (size 110 mm, Whatman, USA) and the plant material washed repeatedly with fresh portions of methanol (98% v/v) and filtered. The filtrates were pooled together and concentrated under reduced pressure using a rotary evaporator to produce 98 g of the methanol extract (ME).

Phytochemical characterization/finger-printing of ME

The methanol extract was finger-printed using thin layer chromatography on pre-coated silica gel K5 glass plates (30 × 100 mm; Whatman) eluted with a mixture of hexane: ethyl acetate (3:2) to obtain six spots. The spots were visualized under daylight and UV light (Λ = 365 nm) before derivatization using 1% (w/v) vanillin sulfuric acid with heating for 2 min at 110°C.

Induction of experimental diabetes

Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared alloxan monohydrate (150 mg/kg) in distilled water. Thereafter, rats were allowed free access to food and water. On day 4, glucose level of rat blood drawn by tail snipping, was measured using a glucometer (One touch® Basic, Lifescan, Johnson and Johnson, USA). Rats with hyperglycemia (blood glucose range >200 mg/dL) were considered diabetic and selected for the study.

Experimental design

On day 0, forty rats were divided into eight groups (n = 5) and treated as follows: Groups I-III were diabetic rats and received 100, 200 or 400 mg/kg of ME respectively. Groups IV and V were normoglycemic rats and received 200 or 400 mg/kg of ME respectively. Group VI served as the positive control and received glibenclamide (0.5 mg/ kg). Groups VII and VIII were the normoglycemic and diabetic controls respectively and received the aqueous vehicle (5 mL/kg); a 1:1 binary mixture of tragacanth and low molecular weight sodium carboxymethylcellulose (0.1% w/v). Extract was administered orally once daily for 25 days and random blood glucose level and body weight of treated rats measured on days 3, 10, 17 and 24 during the experimental period.

Assessment of glucose tolerance in treated diabetic rats

On day 21 of treatment the rats were fasted overnight but allowed free access to water. On day 22, 30 min after extract administration, animals received an oral glucose load (1.5 g/kg). Blood glucose level was measured before and at 30, 60, 90 and 120 min later (Njike et al., 2005) as earlier described.

Determination of liver weight and hepatic glycogen

On day 26, the rats were euthanized by chloroform inhalation (NIH, 1985) and the liver of each rat excised, weighed, and the glycogen content determined by the method of Rajesh et al. (2004). Briefly, a portion (1 g) of the liver was placed in a glass test tube containing 2 mL of hot potassium hydroxide (30% w/v) and warmed in a hot water bath for 20 min, cooled in cold water before the addition of saturated sodium sulfate (0.2 mL) and absolute ethanol (5 mL), and centrifuging at 2000 rpm for 1 min. Dissolution of the off-white precipitate from the supernatant in 10 mL of distilled water with shaking on a vortex mixer was followed by addition of 1.2 M hydrochloric acid (1 mL) and boiling in a water bath for 50 min. After cooling, one drop of phenol red indicator solution was added to the hydrolysates and the solutions were neutralized with 0.5 M sodium hydroxide solution. Glucose from hydrolyzed glycogen was determined directly with *O*-toluidine (Morales et al., 1973) and the absorbance measured at 680 nm. The concentration of glucose was determined from a standard calibration plot and glycogen content expressed as glucose/g of liver tissue.

Estimation of hemoglobin glycosylation

The phenol-sulfuric acid method described by Nayak & Pattabiraman (1980) was used. Whole blood samples from euthanized rats in EDTA tubes were at 1000 centrifuged rpm for 10 min, and the plasma decanted. The packed erythrocytes were washed twice in normal saline and stored at –20°C for 14 days (Standefer & Eaton, 1983). Just prior to the assay, the samples were allowed to thaw at room temperature (30°C) and 100 μL vortex-mixed with 1.4 mL distilled water in a glass test tube for 2 min before the addition of 0.5 mL of 0.5 M oxalic acid and heating for 2 h in a boiling water bath. The tubes were cooled in cold water for 10 min, then 1 mL of ice cold 40% TCA added, vortex-mixed for 2 min and centrifuged at 2000 rpm for 1 min. The clear supernatant (1 mL) was treated with concentrated sulfuric acid (3 mL) and 80% phenol (0.05 mL), left to stand for 30 min for color development and the absorbance of the 5-hydroxylmethylfurfural (5-HMF) generated measured at 480 nm (Shimadzu UV-160 spectrophotometer, Kyoto, Japan).

α-Glucosidase inhibition assay

The chromogenic method described by Babu et al. (2004) was used in this study. Briefly, ME (320 μL of 1.25 mg/ mL) was incubated for 5 min with 1.6 mL of 100 mM phosphate buffer solution (pH 6.8) and 800 μL of enzyme solution (0.76 unit/mL). The mixture was further incubated for 15 min after the addition of 800 μL of *p*-nitrophenyl-α-D-glucopyranoside (5 mM). The reaction was stopped by the addition of 320 μL of 200 mM sodium carbonate and the *p*-nitrophenol generated measured at 400 nm (Shimadzu UV-160 spectrophotometer, Kyoto, Japan). Test incubations were carried out for ME (0.3125, 0.625 and 1.25 mg/mL) and acarbose (0.05 mg/mL). For each, blank and control incubations were prepared by replacing enzyme and extract solution with 800 μL buffer solution and 320 μL DMSO in blank and control incubations respectively. All the tests were run in triplicate. The IC_{50} was calculated from the range of concentrations of ME used while the level of enzyme inhibition (%) was calculated using the relation:

$$\text{Enzyme inhibition}\ (\%) = (1 - B/A) \times 100 \qquad (1)$$

where A represents the absorbance of the control without test samples, and

$$B\,(\text{net absorbance}) = A_{400nm}\text{Test} - A_{400nm}\text{Blank} \qquad (2)$$

α-Amylase inhibition assay

Assay of α-amylase inhibition was performed according to the chromogenic non-pre-incubation method described by Ali et al. (2006). Briefly, 120 μL of ME (20 mg/ mL in DMSO) was mixed with 480 μL of distilled water and 1.2 mL of 0.5% w/v soluble potato starch in 20 mM phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride in a test tube. The reaction was started (0 min) by the addition of 600 μL of the enzyme solution (4 units/mL in distilled water) and at 1 min intervals, 600 μL of the mixture was withdrawn into separate test tubes containing 300 μL DNS color reagent (1 g of 3,5-dinitrosalicylic acid, 30 g of sodium potassium tartrate and 20 mL of 2 N sodium hydroxide to a final volume of 100 mL in distilled water) and transferred to a hot water bath maintained at 85–90°C for 15 min. Afterwards, the mixture in each tube was diluted with 2.7 mL distilled water and the absorbance measured at 540 nm (Shimadzu UV-160 spectrophotometer, Kyoto, Japan). Test incubations were also prepared for 2.5, 5 and 10 mg/mL of ME to study the concentration dependent enzyme inhibition and to calculate the IC_{50} value (concentration required to inhibit 50% enzyme activity). For each concentration, blank incubations were prepared by replacing the enzyme solution with 600 μL in distilled water at the start of the reaction. Control incubations, representing 100% enzyme activity were conducted in a similar manner, replacing ME with 120 μL DMSO. All the tests were run in triplicate. Net absorbance (A) due to the maltose generated was calculated as:

$$A_{540nm}\text{ME} = A_{540nm}\text{Test} - A_{540nm}\text{Blank} \qquad (3)$$

From the value obtained, the percentage (w/v) of maltose generated was calculated from the equation obtained from the maltose standard calibration curve (0–0.1% w/v maltose). The level of inhibition (%) was calculated as:

$$100 - \%\ \text{reaction}\,(\text{at } t = 3\ \text{min}) \qquad (4)$$

where

$$\%\ \text{reaction} = \text{Mean maltose in sample} \times 100/\text{Mean maltose in control} \qquad (5)$$

The IC_{50} was also calculated from the range of concentrations of ME used.

Statistical analysis

Data was analyzed using one way ANOVA and the results expressed as mean ± SEM. The data was further subjected to LSD post hoc test for multiple comparisons and differences between means accepted significant at $P < 0.05$.

Results

Finger-printing of ME

TLC-finger printing of ME revealed six component spots with retention factor (R_f) values ranging from 0.52–0.92 (Table 1).

Effect of ME on blood glucose level of diabetic rats

Intraperitoneal injection of alloxan monohydrate caused a profound increase in mean blood glucose (>200 mg/ dL) of diabetic rats. Chronic administration of ME to the diabetic rats lowered blood glucose in a non-dose-dependent manner, provoking inhibition as high as 45.25%. However, the extract did not greatly alter blood glucose levels in non-diabetic rats (Table 2).

Effect of ME on oral glucose tolerance in diabetic rats

Oral administration of glucose load rapidly increased blood glucose levels in all the groups within 30 min and remained high over the next 120 min in diabetic control rats. Treatment with ME (200 and 400 mg/kg) caused a dose-related suppression of elevation in blood glucose level with 49.79% inhibition at 120 min. The ME also inhibited blood glucose rise in non-diabetic control rats (Table 3).

Table 1. Thin layer chromatography finger-print of ME.

Spot number	Color	Retention factor (R_f)	Detection
1	Dark brown	0.92	H_2SO_4/Vanillin
2	Purple	0.78	UV_{365}
3	Purple	0.72	UV_{365}
4	Purple	0.66	Daylight, UV_{365}
5	Purple	0.57	UV_{365}
6	Dark brown	0.52	H_2SO_4/Vanillin

Mobile phase = Hex/EtOAc (3:2).

Effect of ME on alpha amylase activity

Incubation of the enzyme with the substrate led to generation of maltose which the addition of ME significantly (P <0.05) inhibited in a concentration-related manner (IC_{50}: 2.15 ± 0.1 mg/mL). The lowest concentration (2.5 mg/mL) caused 58.95% inhibition while the highest concentration (20 mg/mL) evoked 86.32% (Table 4).

Effect of ME on alpha glucosidase enzyme activity

The extract caused a significant (P <0.05) non-concentration-related inhibition of α-glucosidase

Table 2. Effect of ME on blood glucose of treated diabetic rats.

		Blood glucose level (mg/dL)					
Treatment	Dose (mg/kg)	Basal	Day 0	Day 3	Day 10	Day 17	Day 24
Control	-	62.0 ± 2.74	378.0 ± 75.8	448.4 ± 89.19 (NR)	367.6 ± 48.43 (2.75)	398.2 ± 90.34 (NR)	285.2 ± 80.88 (24.55)
ME	100	59.0 ± 6.42	418.0 ± 78.02	348.0 ± 100.2 (16.75)	294.75 ± 89.86 (29.49)	284.25 ± 101.7 (32)	264.25 ± 111.4 (36.78)
	200	76.0 ± 5.21	332.0 ± 56.75	443.75 ± 125.2 (NR)	411.25 ± 111.5 (NR)	348.25 ± 106.2 (NR)	254.5 ± 66.35 (23.34)
	400	67.33 ± 12.33	375.67 ± 121.2	340.0 ± 122.02 (9.50)	350.67 ± 115.2 (6.65)	209.33 ± 100.1 (44.28)	205.67 ± 107.2 (45.25)
Glibenclamide	0.5	61.00 ± 2.92	391.40 ± 80.50	383.8 ± 95.22 (1.94)	341.4 ± 83.07 (12.78)	313.6 ± 85.69 (19.88)	320.2 ± 78.94 (18.19)
NDNT	-	58.4 ± 5.49	58.4 ± 5.49	59.0 ± 3.13 (NR)	81.8 ± 3.77 (NR)	61.8 ± 3.44 (NR)	65.6 ± 4.76 (NR)
NDT	200	70.0 ± 5.23	70.0 ± 5.23	63.0 ± 6.23 (10)	76.0 ± 5.52 (NR)	75.2 ± 8.11 (NR)	74.0 ± 4.2 (NR)
	400	75.75 ± 4.89	75.75 ± 4.89	64.25 ± 3.07 (15.18)	73.75 ± 4.91 (2.64)	83.75 ± 10.09 (NR)	75.5 ± 5.91 (3.3)

n = 5; ME, methanol extract; NDNT, non-diabetic non-treated; NDT, non-diabetic treated with ME.
Values of blood glucose shown are Mean ± SEM; Values in parenthesis represent %reduction in blood glucose calculated relative to day 4; NR, no reduction.

Table 3. Effect of ME on oral glucose tolerance in treated diabetic rats.

		Blood glucose level (mg/dL)				
Treatment	Dose (mg/kg)	0 min	30 min	60 min	90 min	120 min
Control	-	304.8 ± 86.34	322.2 ± 81.92	316.0 ± 89.75 (1.92)	274.0 ± 88.86 (14.96)	326.2 ± 104.16 (NI)
ME	100	233.5 ± 97.95	253.0 ± 109.65	229.5 ± 102.36 (9.29)	272.0 ± 109.78 (NI)	295.0 ± 114.22 (NI)
	200	259.75 ± 85.91	312.0 ± 85.39	248.25 ± 56.89 (20.43)	232.0 ± 78.83 (25.64)	167.25 ± 58.36 (46.39)
	400	248.67 ± 101.47	310.67 ± 99.62	278.67 ± 126.24 (10.3)	175.33 ± 44.05 (43.56)	156.0 ± 37.31 (49.79)
Glibenclamide	0.5	234.2 ± 90.06	335.8 ± 88.5	315.2 ± 77.01 (6.14)	269.6 ± 88.34 (19.71)	243.6 ± 74.15 (27.46)
NDNT	-	62.0 ± 3.21	82.6 ± 5.00	76.2 ± 2.85 (7.75)	68.4 ± 4.16 (17.19)	70.0 ± 3.67 (15.25)
NDT	200	60.8 ± 6.18	96.8 ± 3.81	91.4 ± 6.45 (5.58)	75.0 ± 4.6 (22.52)	64.0 ± 4.24 (33.88)
	400	51.0 ± 1.92	91.5 ± 5.72	90.5 ± 5.17 (1.09)	76.0 ± 7.01 (16.94)	66.0 ± 2.35 (27.87)

n = 5; ME, methanol extract; NDNT, non-diabetic non-treated; NDT, non-diabetic treated with ME.
Values of blood glucose shown are Mean ± SEM; Values in parenthesis represent % inhibition of elevation in blood glucose calculated relative to 30 min; NI, no inhibition.

Table 4. Effect of ME on α-amylase activity.

Treatment	Concentration (mg/mL)	Concentration of maltose generated (mg/mL) 0 min	1 min	2 min	3 min	Inhibition (%)
Control	-	0.46 ± 0.07	0.55 ± 0.09	0.63 ± 0.18	0.95 ± 0.03	-
ME	2.5	0.16 ± 0.01*	0.27 ± 0.01*	0.34 ± 0.03*	0.4 ± 0.01*	58.95 ± 1.22
	5	0.07 ± 0.03*	0.42 ± 0.04	0.29 ± 0.01*	0.29 ± 0.02*	69.47 ± 2.43
	10	0.20 ± 0.02*	0.18 ± 0.05*	0.27 ± 0.02*	0.21 ± 0.05*	77.54 ± 4.56
	20	−0.08 ± 0.03*	−0.13 ± 0.1*	−0.0 ± 0.01*	0.13 ± 0.11*	86.32 ± 11.39

*P <0.05 compared to control (ANOVA; LSD post hoc); ME, methanol extract. Concentration of generated maltose shown are Mean ± SEM; Inhibition (%) was calculated relative to control; IC_{50} = 2.15 ± 0.1 mg/mL.

enzyme activity (IC_{50}: 0.2 ± 0.02 mg/mL). The highest concentration (1.25 mg/mL) caused 89.52% inhibition while 91.11% was evoked by the lowest concentration (0.3125 mg/mL) (Table 5).

Table 5. Effect of ME on α-glucosidase activity.

Treatment	Concentration (mg/mL)	α-Glucosidase inhibition: p-Nitrophenol (absorbance)	Inhibition (%)
Control	-	0.99 ± 0.1	-
ME	0.3125	0.088 ± 0.02*	91.11 ± 1.54
	0.625	0.094 ± 0.01*	90.47 ± 0.51
	1.25	0.103 ± 0.01*	89.52 ± 0.81
Acarbose	0.05	0.76 ± 0.02	23.23 ± 0.84

*P <0.05 compared to control (ANOVA; LSD post hoc); ME, methanol extract.
Values of absorbance and inhibition (%) shown are Mean ± SEM; Inhibition (%) was calculated relative to control; IC_{50} = 0.2 ± 0.02 mg/mL.

Effect of ME on hemoglobin glycation in diabetic rats

The concentration of carbohydrate bound to hemoglobin was increased in the diabetic group. However, chronic treatment with ME caused a significant (P <0.05) reduction in the absorbance of 5-hydroxymethyl furfural (5-HMF) generated from hemoglobin-bound sugar in diabetic rats (Table 6).

Table 6. Effect of ME on hemoglobin glycosylation in treated diabetic rats.

Treatment	Dose (mg/kg)	Hemoglobin glycation (Absorbance of 5-HMF)	Inhibition (%)
Control	-	1.38 ± 0.44	-
ME	100	1.15 ± 0.48	16.67
	200	1.47 ± 0.47	NI
	400	0.86 ± 0.62	37.68
Glibenclamide	0.5	0.88 ± 0.39	36.23
NDNT	-	0.64 ± 0.54	NC
NDT	200	0.96 ± 0.37	NC
	400	0.47 ± 0.36	NC

n = 5; ME, methanol extract; NDNT, non-diabetic non-treated; NDT, non-diabetic treated with ME.
Inhibition (%) of hemoglobin glycation was calculated relative to control; Values of hemoglobin glycation (absorbance of 5-HMF) shown are Mean ± SEM; NI, no inhibition; NC, not calculated.

Effect of ME on liver weight and liver glycogen of diabetic rats

Chronic oral administration of ME significantly (P <0.05) increased absolute and relative weights as well as glycogen content of liver in diabetic rats. In non-diabetic rats, ME also increased liver glycogen but did not significantly increase absolute and relative weights of the liver (Table 7).

Effect of ME on body weight of diabetic rats

Induction of diabetes caused a decline in mean body weight of diabetic rats as measured on day 4. However, the body weight of ME-treated diabetic rats slightly increased from day 7 except for the 400 mg/kg dose. By the end of the study (day 24), the body weight of diabetic rats that received 100 and 200 mg/kg had increased to values beyond the body weight on day 4 (Table 8).

Discussion

Several mechanisms such as inhibition of carbohydrate metabolizing enzymes (Matsui et al., 2001), enhancement of glycogen regulatory enzymes expression in the liver (Kumar et al., 2006) and glucose uptake by tissues and adipocytes (Ghosh et al., 2004) as well as stimulation of pancreatic insulin release (Xu et al., 2008) have been associated with the antihyperglycemic effect of antidiabetic medicinal plants. In experimentally induced diabetes, alloxan selectively damages insulin secretory β-cells to impair insulin secretion and function (Lenzen, 2008). This leads to the formation of free radicals by oxidation of excessive glucose, the non-enzymatic glycation of proteins and subsequent oxidative degradation of glycation proteins (Bunn et al., 1978) which also occur in human forms of diabetes.

In this study, chronic oral administration of the extract lowered blood glucose level, enhanced oral glucose tolerance by suppressing postprandial rise in blood glucose level and slightly ameliorated the negative effect of diabetes on body weight by increasing the body weights of treated diabetic rats which are indices of effective glycemic control in diabetes. Adequate glycemic control in diabetes is largely dependent on effective management of postprandial glucose (Parkin & Brooks, 2002). Thus, the effectiveness and hence relevance of extract of this plant in diabetes may derive not just from its hypoglycemic effect, but potent suppression of postprandial elevations in blood glucose. However, postprandial blood

Table 7. Effect of ME on liver weight and liver glycogen in treated diabetic rats.

Treatment	Dose (mg/kg)	Liver weight (g) Absolute	Liver weight (g) Relative	Liver glycogen(glucose g/g of wet tissue)
Control	-	6.38 ± 0.18	3.43 ± 0.36	0.35 ± 0.12
ME	100	8.32 ± 1.02* (30.41)	3.77 ± 0.06 (9.91)	0.49 ± 0.13 (40)
	200	7.88 ± 0.55 (23.51)	3.84 ± 0.15 (11.95)	0.48 ± 0.06 (37.14)
	400	7.01 ± 0.4 (9.87)	4.23 ± 0.56 (23.32)	0.55 ± 0.1 (57.14)
Glibenclamide	0.5	6.29 ± 0.8 (NI)	3.5 ± 0.29 (2.04)	0.36 ± 0.09 (2.86)
NDNT	-	6.01 ± 0.54 (NI)	3.26 ± 0.2 (NI)	0.44 ± 0.1 (25.71)
NDT	200	5.8 ± 0.21 (NI)	3.08 ± 0.13 (NI)	0.48 ± 0.08 (37.14)
	400	6.89 ± 0.26 (7.99)	3.28 ± 0.21 (NI)	0.46 ± 0.12 (31.43)

n = 5; *P <0.05 compared to control (ANOVA; LSD post hoc); ME, methanol extract; NDNT, non-diabetic non-treated; NDT, non-diabetic treated with ME.
Values of liver weight and liver glycogen shown are Mean ± SEM; Values in parenthesis represent % increase in liver weight and liver glycogen calculated relative to control; NI, no increase.

Table 8. Effect of ME on body weight of treated diabetic rats.

Treatment	Dose (mg/kg)	Body weight (g) Basal	Day 0	Day 3	Day 10	Day 17	Day 24
Control	-	201.0 ± 8.84	181.6 ± 7.14	182.2 ± 8.39 (0.33)	190.6 ± 10.58 (4.96)	187.4 ± 13.46 (3.19)	191.8 ± 14.11 (5.62)
ME	100	218.25 ± 27.37	205.75 ± 28.22	204.0 ± 27.74 (NI)	217.5 ± 28.34 (5.71)	212.0 ± 25.03 (3.04)	220.25 ± 26.43 (7.05)
	200	191.5 ± 11.85	177.5 ± 7.19	184.25 ± 8.98 (3.8)	188.75 ± 8.21 (6.34)	187.75 ± 11.24 (5.77)	196.75 ± 10.77 (10.85)
	400	185.0 ± 8.15	167.0 ± 5.03	162.66 ± 7.54 (NI)	170.67 ± 11.1 (2.2)	149.0 ± 10.54* (NI)	166.0 ± 13.45 (NI)
Glibenclamide	0.5	187.4 ± 22.74	178.6 ± 21.27	183.0 ± 20.88 (2.46)	190.0 ± 22.96 (6.38)	188.0 ± 23.54 (5.26)	190.8 ± 26.94 (6.83)
NDNT	-	195.6 ± 13.67	195.6 ± 13.67	192.4 ± 13.77 (NI)	200.0 ± 15.66 (2.25)	202.0 ± 15.18 (3.27)	205.0 ± 15.21 (4.81)
NDT	200	188.8 ± 14.69	188.8 ± 14.69	183.8 ± 13.76 (NI)	184.6 ± 13.24 (NI)	179.8 ± 11.8 (NI)	189.4 ± 11.39 (0.32)
	400	193.5 ± 8.97	193.5 ± 8.97	193.5 ± 9.22 (NI)	195.0 ± 10.65 (0.78)	183.5 ± 11.02 (NI)	210.5 ± 9.22 (8.79)

n = 5; *P <0.05 compared to day 4 (ANOVA; LSD post hoc); ME, methanol extract; NDNT, non-diabetic non-treated; NDT, non-diabetic treated with ME.
Values of body weight shown are Mean ± SEM; Values in parenthesis represent % increase in body weight calculated relative to day 4; NI, no increase.

glucose elevation is dependent on the extent of glucose absorption after a meal and also likely glucose clearance by the liver for glycogen synthesis and storage, which are secondary effects to the release and action of insulin (Stalmans et al., 1997). Interestingly, results of our study showed that the extract may reduce the extent of carbohydrate absorption by inhibiting the enzymatic actions of α-amylase and α-glucosidase. α-Amylase and membrane-bound α-glucosidase in the lumen of the small intestine aid the production of glucose from the catabolism of complex starches and oligosaccharides. α-Amylase hydrolyzes complex starches to oligosaccharides, while α-glucosidase hydrolyzes oligosaccharides to glucose and other monosaccharides. Inhibition of these enzymes produces a postprandial antihyperglycemic effect by reducing the rate and extent of glucose absorption from the small intestine (Rhabasa-Lhoret & Chaisson, 2004) with modest reduction of hemoglobin glycosylation in diabetes (Kim et al., 2005). Thus, the enhanced glucose tolerant effect caused by the extract in treated diabetic animals is likely due to inhibition of glucose absorption. The potency of postprandial antihyperglycemic and glucose tolerant effects of the extract may derive from synergism due to concurrent inhibition of α-amylase and α-glucosidase. Inhibition of postprandial carbohydrate metabolism has a short-term blood glucose lowering effect and long-term effect evidenced by small reductions in glycated hemoglobin

(Lebovitz et al., 2001; Philips et al., 2001), which may be an additional mechanism of anti-diabetic activity of the extract.

Assessment of the effect of the extract on hemoglobin glycosylation in diabetic rats showed that treatment reduced glycation of hemoglobin as evidenced by reduced levels of hemoglobin-bound sugar. Glycation was increased in the diabetic control animals, probably as a result of chronic hyperglycemia. Reduction in hemoglobin glycation, which results from hypoglycemic and postprandial hyperglycemia suppressive effects of the extract, provide further evidence for its effective glycemic control in diabetes. The induction of chemical diabetes increases glycated hemoglobin levels in the long term (Koenig et al., 1976). Evidence has also shown that glycation can cause the generation of oxygen-derived free radicals in diabetes (Gupta et al., 1997) which is capable of worsening the insulin-secreting ability of the compromised pancreas.

Chronic administration of the extract also stimulated glycogen synthesis and storage as shown by increased liver weight and liver glycogen content. The liver plays a central role in maintaining glucose homeostasis by regulating its metabolism. At the dose levels used, the extract increased absolute and relative liver weight of treated diabetic rats as well as the liver glycogen content. It is undoubtedly clear that the increase in liver weight may be directly related to enhanced glycogen storage in the liver. Thus, it is reasonable to suggest that enhanced glucose clearance may contribute to the hypoglycemic effect of this plant.

Finger-printing of the extract was performed to establish a phytochemical identification marker for the extract. Although the anti-diabetic constituents of this plant are yet to be identified, tannins and polyphenolic principles from extracts of other plants have been shown to inhibit the amylase and glucosidase enzymes (McDougall et al., 2005). The presence of tannins, alkaloids and flavonoids which are polyphenolic compounds have been reported in extracts of this plant (Damle, 2008).

Conclusions

In conclusion, findings from this study showed that aerial parts of *P. niruri* may owe its blood glucose lowering properties to inhibition of glucose absorption and enhancement of glucose storage. The effect of the extract on insulin release remains to be investigated.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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