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Evaluation of antidiabetic potentials of *Phyllanthus niruri* in alloxan diabetic rats

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The antidiabetic potentials of methanol extract (ME) of aerial parts of *Phyllanthus niruri* L (Euphorbiaceae) was evaluated in normal and alloxan diabetic rats. The results showed that ME significantly ($P<0.05$) reduced fasting blood sugar in a dose-related manner and suppressed the postprandial rise in blood glucose after a heavy glucose meal in normoglycaemic rats. Chronic oral administration of ME caused a significant ($P<0.05$) dose-related reduction in blood glucose levels as well as total cholesterol and triglycerides levels in diabetic and normoglycaemic rats. Sub-chronic toxicity study showed that ME-treated rats had significant ($P<0.05$) reductions in haemoglobin (Hb) levels, red blood cell (RBC) and white blood cell (WBC) counts followed by a gradual rise which did not, however, attain basal levels; however, there was a progressive rise in the WBC of ME-treated diabetic rats. Also ME-treated and control rats had increases in weight throughout the study. Histological studies showed that ME-treated diabetic rats had the tissue architecture of their pancreas restored as against the control groups where there was evidence of necrosis. The acute toxicity and lethality test of ME in mice gave an oral LD₅₀ of 471.2 mg/kg. Results suggest that extract of aerial parts of *P. niruri* has great potentials as anti-diabetic remedy.

Key words: *Phyllanthus niruri*, antidiabetic, alloxan, pancreas, blood glucose, blood cells, toxicity.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia due to defective insulin action, insulin secretion or both. Several medicinal plants are used in the management of DM (Verspohl, 2002; Akah et al., 2002; De Sousa et al., 2004; Colca, 2006). One of such plants is *Phyllanthus niruri* L (Euphorbiaceae), an annual and field weed that is widespread in temperate and tropical climates (Izuka et al., 2006). *P. niruri* is a small erect annual herb growing up to 30 - 40 cm in height and indigenous to the Amazon rainforest and other tropical areas, including South East Asia, Southern India and China (Girach et al., 1994). The morphology of the plant has been described (Bagalkotkar et al., 2006). It is popularly used in Asia, Africa and South America (Mellinger et al., 2005) as a stomachic, aperitive, anti-hyperglycemic, antispasmodic, anti-hepatotoxic, anti-viral, antibacterial, laxative, diuretic, carminative, in the

management of diabetes, constipation, fever including malaria, jaundice, hepatitis B, dysentery, gonorrhoea, syphilis, tuberculosis, cough, influenza, diarrhoea, vaginitis, tumors and kidney stones (Syamasundar et al., 1985; Olive-Bever, 1986; Chopra et al., 1986; Unander et al., 1995; Paranjape, 2001; Lin et al., 2003). Studies on extracts from various parts of the plant have revealed the antioxidant (Tasaduq et al., 2003) and nitric oxide scavenging (Jagetia and Baliga, 2004), antimalarial (antiplasmodial) (Tona et al., 1999; Tona et al., 2001; Cimanga et al., 2004; Tona et al., 2004; Subeki et al., 2005; Mustofa et al., 2007), antihyperuricemic (Murugaiyah and Chan, 2006), antinociceptive/analgesic (Santos et al., 1994; Santos et al., 1995), diuretic, hypotensive, hypoglycaemic (Ramakrishnan et al., 1982), hepatoprotective (Syamasundar et al., 1985; Chatterjee and Sil, 2006; Bhattacharjee and Sil, 2006; Chatterjee et al., 2006; Sarkar and Sil., 2007; Bhattacharjee and Sil, 2007; Manjrekar et al., 2008), hepatocurative (Tasaduq et al., 2003), hypolipemic (Khanna et al., 2002), nematocidal (Shakil et al., 2008), platelet aggregation inhibitory

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(Iizuka et al., 2007), nephrolithiatic (Kieley et al., 2008), HIV-1 reverse transcriptase inhibitory (Ogata et al., 1992), HIV replication inhibitory (Naik and Juvekar, 2003), HIV/RRE binding inhibitory (Qian-Cutrone et al., 1996), urolithiatic (Campos and Schor, 1999; Freitas et al., 2002; Barros et al., 2003; Nishiura et al., 2004; Micali et al., 2006; Barros et al., 2006) and vasorelaxant (Iizuka et al., 2006) activities.

Phytochemical studies have led to the isolation of several constituents some of which have also been shown to be pharmacologically active. These include the alkaloids- 4-methoxy-securinine (Phyllanthine) and 4-methoxy-nor-securinine (Mulchandani and Hassarajani, 1984), arabinogalactan which stimulates superoxide anion production (Mellinger et al., 2005; Mellinger et al., 2008), ellagic acid, brevifolin carboxylic acid and ethyl brevifolin carboxylate with aldose reductase inhibitory effect (Shimizu et al., 1989), 1-O-galloyl-6-O-luteoyl-alpha-d-glucose with anti-babesial and antiplasmodial activities, beta-glucogallin, quercetin 3-O-beta-d-glucopyranosyl-(2-1)-O-beta-d-xylopyranoside, beta sitosterol, gallic acid (Subeki et al., 2005), the lignans- phyllanthin, hypophyllanthin, phylltetralin with antihyperuricemic (Murugaiyah and Chan, 2006) and antihepatotoxic effects (Syamasundar et al., 1985), cubebin dimethyl ether, urinatetralin (Elfahmi et al., 2006), niranthin (Murugaiyah and Chan, 2007), methyl brevifolin carboxylate with vasorelaxant (Iizuka et al., 2006) and platelet aggregation inhibitory (Iizuka et al., 2007) effects, niruriside with HIV/RRE binding inhibitory effect (Qian-Cutrone et al., 1996), the prenylated flavanones- 8-(3-Methyl-but-2-enyl)-2-phenylchroman-4-one and 2-(4-hydroxyphenyl)-8-(3-methyl-but-2-enyl)-chroman-4-one with nematocidal activity (Shakil et al., 2008), tricontanol and tricontanol with antihepatotoxic effect (Syamasundar et al., 1985) and xylans (Mellinger et al., 2005).

In continuation of the search for more potent anti-diabetic remedies from medicinal plants, we evaluated the antidiabetic potentials of *P. niruri*, a herb popularly used in eastern Nigeria for management of diabetes. Although the hypoglycaemic activity (Ramakrishnan et al., 1982) has been reported, experimental evaluation of the anti-diabetic potential is yet to be documented. This study evaluated the effect of daily oral administration of extract of the aerial parts of the plant on glycaemic control in normal and diabetic rats as well as indices of the diabetic disease such as lipid profile. The effect of the extract on the integrity of damaged pancreatic tissue was also studied.

MATERIALS AND METHODS

Animals

Adult albino rats (100-200 g) and mice (15-27 g) of either sex bred in the laboratory animal facility of the Department of Pharmacology

and Toxicology, University of Nigeria, Nsukka were used for the study. The animals were kept in steel cages within the facility and allowed free access to water and standard livestock pellets. All animal experiments were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85-23, revised 1985). On transfer to the research area, animals were allowed a 2 week acclimatization period before commencement of the experiments.

Preparation of extract

Fresh whole plant of *P. niruri* was collected from Orba town, Enugu State Nigeria in August, 2005. The plant was identified and authenticated by Mr. A. Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State. The roots were cut off and discarded, and the aerial parts sun-dried for 4 days and milled to coarse powder with an electric blender. The powdered plant material (2.4 kg) was subjected to continuous extraction in a soxhlet using methanol. The extract was concentrated in a rotary evaporator at 40 - 50 °C under reduced pressure to obtain 288 g of the methanol extract (ME; 12% w/w). Phytochemical analysis of ME for identification of constituents using procedures described by Trease and Evans (1983) and Harborne (1998) gave positive reactions for alkaloids, carbohydrates, flavonoids, glycosides, saponins, steroids and tannins.

Acute toxicity and lethality (LD₅₀) tests of ME

The acute toxicity and lethality (LD₅₀) of ME in mice (n = 13) was estimated using the method described by Lorke (1983). The study was carried out in two stages. In stage one, mice (n = 3) received oral administration of 10, 100, or 1000 mg/kg of ME (suspended in 20% Tween 80) and were observed for 24 h for number of deaths. At the end of 24 h, only the 1000 mg/kg dose caused death in treated mice. Consequently, a fresh batch of mice (n = 1) received 140, 225, 370 and 600 mg/kg of ME in the second stage of the test and were observed for 24 h for deaths. Death occurred only in the 600 mg/kg dose group. The LD₅₀ was calculated as the geometric mean of the highest non-lethal dose (370 mg/kg) and the lowest lethal dose (600 mg/kg) (Lorke, 1983).

Solvent-guided fractionation of ME and bioactivity-guided studies

The methanol extract (ME; 198 g) was subjected to solvent-guided fractionation in a silica gel (60-120 mesh size) column successively eluted with n-hexane, ethylacetate and methanol (100%) in order of increasing polarity. The fractions obtained were concentrated using rotary evaporator at 40-50 °C under reduced pressure to afford the n-hexane fraction (HF; 0.09 g; 0.045% w/w), ethylacetate fraction (EF; 37.4 g; 18.9% w/w) and methanol fraction (MF; 158 g; 79.8% w/w). HF was not tested further because of its very low yield. The ME, EF and MF were subjected to biological activity-guided studies using hypoglycaemic effect in alloxan-induced diabetic rats as activity-guide.

Hypoglycaemic activity test

The hypoglycaemic effect of the extract (ME) and its solvent fractions (EF and MF) was studied in alloxan-induced diabetic rats. The animals were fasted for 8 h but allowed free access to water. At the end of the fasting period, the basal fasting blood glucose (FBG) levels of the rats were determined using One touch®

glucometer kit (Lifescan, Johnson and Johnson Company, Milipitas, CA). Subsequently, diabetes was induced by single intraperitoneal injection of alloxan mono-hydrate (70 mg/kg) (Aruna et al., 1999) and normal feeding maintained thereafter. Five days later, blood was drawn from each rat by tail snipping and the blood glucose level measured to establish diabetes. Animals with blood glucose level ≥ 225 mg/dl were considered diabetic and used for the study. The diabetic animals were randomly divided into eight groups ($n = 5$) and received oral administration of ME (200 and 400 mg/kg), MF (200 and 400 mg/kg), EF (200 and 400 mg/kg), 20% v/v Tween 80 (5 ml/kg) and glibenclamide (0.2 mg/kg) respectively. Extract and fractions were suspended in Tween 80 (20% v/v). Blood glucose was measured before (0 h) and at 0.5, 1, 2 and 4 h after treatment. The results showed that ME exhibited greater hypoglycemic effect than the fractions. Consequently, ME was subjected to further studies without fractionation.

Effect of ME on normoglycaemic rats

Animals fasted overnight were randomly divided into four groups ($n = 5$) and received oral administration of ME (200 and 400 mg/kg), glibenclamide (0.2 mg/kg) and 20% v/v Tween 80 (5 ml/kg) respectively. The blood glucose level of each animal was measured prior to (pre-treatment) and at 0.5, 1, 2, and 4 h after extract administration.

Oral glucose tolerance test

Animals fasted for 16 h but with free access to water were randomly divided into four groups ($n = 5$) and received oral administration of ME (200 and 400 mg/kg), glibenclamide (0.2 mg/kg) and 20% v/v Tween 80 (5 ml/kg) respectively. Ninety minutes later, the rats were fed with glucose (4 g/kg). The blood glucose level of animals in each group was measured before (0) and at 30, 60, 90, 120, 150 and 180 min after glucose load.

Anti-diabetic activity test

The anti-diabetic effect of ME was studied by evaluating the effect of its chronic administration on the blood glucose level of alloxan-induced diabetic rats. The basal fasting blood glucose (FBG) of rats was determined and diabetes induced as described above. Twenty diabetic rats with glucose level ≥ 225 mg/dl were selected and used for the study. Rats were fasted for 8 h but allowed free access to water. They were randomly divided into four groups ($n = 5$). Groups I and II received 200 or 400 mg/kg of ME while groups III and IV were diabetic controls and received glibenclamide (0.2 mg/kg) or 20% v/v Tween 80 (5 ml/kg) respectively. Groups V and VI were non-diabetic controls and received ME (200 mg/kg) or 20% v/v Tween 80 (5 ml/kg). The treatments were administered orally to the animals once daily for 28 days. Blood glucose level was measured as earlier described before (pretreatment) and on days 14 and 28 after commencement of treatment. The body weight of each animal was also measured on these days.

Determination of effect of ME on lipid profile of diabetic rats

The effect of ME on the lipid profile of treated diabetic rats was studied by monitoring the cholesterol and triglyceride levels. Blood samples were collected by ocular puncture, transferred into test-tubes and centrifuged at 3000 rpm for 5 min. The serum was collected and the total cholesterol and triglyceride levels of each sample were separately determined by enzymatic colorimetric

method (Muller et al., 1977) using reagent kits. Lipid levels of diabetic animals were measured before (basal) and after the induction of diabetes (pre-treatment) as well as on days 14 and 28 after commencement of treatment. The cholesterol and triglyceride levels were determined using commercially available reagent kits (QCA, South Africa) following the manufacturer's instructions. The absorbance of each sample containing the reaction mixtures with or without serum was read at 540 nm in a UV spectrophotometer (UV-2102 PC Spectrophotometer, UNICO®, USA). Total cholesterol or triglyceride was calculated using the formula: Total cholesterol (mg/dl) = $SA_{O,D}/ST_{O,D} \times 200$, where $SA_{O,D}$ = optical density of test sample and $ST_{O,D}$ = optical density of standard.

Determination of effect of ME on haemoglobin and cell counts of diabetic rats

The effect of chronic administration of ME on haemoglobin (Hb) and cell counts [white blood cells (WBC) and red blood cells (RBC)] of diabetic rats was also determined. Blood samples were collected by ocular puncture using haematocrit tubes, transferred into EDTA-containing test-tubes and placed in a haematology analyzer (Abacus Junior®, Budapest- Hungary) for determination of the parameters. Measurements were taken before (basal) and after the induction of diabetes (pre-treatment) as well as on days 14 and 28 after commencement of treatment.

Histological studies on the pancreas of ME-treated diabetic rats

The effect of ME on tissue architecture of the pancreas of treated diabetic rats was evaluated by histological studies of tissue sections obtained from the animals. On day 28 of the experiment, one animal was randomly selected from the different groups and sacrificed by over-dose of chloroform anaesthesia. The whole pancreas from each animal was removed and placed in 10% formalin in normal saline for histological studies. The isolated organ was placed in an automatic tissue processor (Lecia®, Japan) for 24 h. After 24 h, the tissues were solidified in molten wax and sectioned using automatic tissue sectioner (Sorvall TC-2®, Sorvall Inc. Iwan). The tissue sections were then fixed on slides with haematoxylin and eosin. The stained slides were fixed with mountant (DPX®, Sigma), allowed to dry and viewed under the microscope ($\times 400$).

Statistical analysis

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

RESULTS

Acute toxicity and lethality (LD₅₀) test

The acute toxicity testing of the ME in mice gave an oral LD₅₀ of 471.2 mg/kg.

Hypoglycaemic effect of ME and fractions

The extract (ME) and fractions significantly ($P < 0.05$) reduced blood glucose level to varying extents in diabetic

Table 1. Hypoglycemic effect of ME and solvent fractions on diabetic rats.

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)				
		Pre-treatment	0.5 h	1 h	2 h	4 h
ME	200	359.6 ± 61.1	312.4 ± 58.8 (13.13)	293.4 ± 76.0 (18.41)	239.0 ± 74.2 ^a (33.54)	223.2 ± 70.6 ^a (37.93)
	400	403.4 ± 57.5	374.2 ± 45.4 (7.24)	316.2 ± 52.7 (21.62)	268.4 ± 31.0 ^b (33.47)	222.8 ± 28.0 ^{a,b} (44.77)
EF	200	315.2 ± 20.8	285.6 ± 5.1 (9.39)	260.2 ± 24.4 ^b (17.44)	275.6 ± 12.5 (12.56)	280.0 ± 7.3 (11.17)
	400	353.8 ± 49.2	348.8 ± 48.1 (1.41)	329.6 ± 34.7 (6.84)	295.8 ± 21.4 (16.39)	293.0 ± 20.6 (17.18)
MF	200	333.6 ± 17.8	304.0 ± 12.3 (8.87)	296.6 ± 11.7 (11.09)	278.8 ± 10.5 ^b (16.42)	252.8 ± 13.4 ^{a,b} (24.22)
	400	285.6 ± 4.2	281.6 ± 2.8 (1.40)	273.4 ± 5.5 (4.27)	270.2 ± 3.3 ^b (5.39)	261.2 ± 7.8 ^{a,b} (8.54)
Glibenclamide	0.2	303.0 ± 13.4	256.6 ± 27.0 ^a (15.31)	209.6 ± 31.9 ^{a,b} (30.83)	193.8 ± 32.2 ^{a,b} (36.04)	186.4 ± 29.9 ^{a,b} (38.48)
Control	-	366.8 ± 41.2	378.6 ± 58.9	356.8 ± 42.9	356.8 ± 44.4	360.4 ± 43.3

n = 5; ^{a,b}P < 0.05 compared to Control and pre-treatment values respectively (ANOVA; LSD post hoc); ME = methanol extract; EF = ethyl acetate fraction; MF = methanol fraction.

Values in parenthesis represent reduction (%) in blood glucose level calculated relative to pre-treatment values.

Table 2. Effect of ME on blood glucose level of normoglycaemic rats.

Treatment	Dose (mg/kg)	Fasting Blood Glucose level (mg/dl)				
		Pre-treatment	0.5 h	1 h	2 h	4 h
ME	200	75.6 ± 2.33	61.4 ± 2.6 ^{a,b} (18.78)	50.4 ± 3.4 ^{a,b} (33.33)	50.8 ± 2.82 ^{a,b} (32.80)	55.4 ± 2.66 ^{a,b} (26.72)
	400	74.8 ± 3.2	56.0 ± 2.17 ^{a,b} (25.13)	40.4 ± 1.02 ^{a,b} (45.99)	45.4 ± 2.56 ^{a,b} (39.30)	47.6 ± 2.37 ^{a,b} (36.36)
Glibenclamide	0.2	83.8 ± 7.3	72.0 ± 6.27 (14.08)	54.2 ± 4.8 ^{a,b} (35.32)	48.8 ± 2.82 ^{a,b} (41.77)	55.4 ± 2.66 ^{a,b} (33.89)
Control	-	80.4 ± 5.39	79.4 ± 5.42	78.2 ± 6.37	79.2 ± 5.34	78.0 ± 5.38

n = 5; ^{a,b}P < 0.05 compared to Control and pre-treatment values respectively (ANOVA; LSD post hoc); ME = methanol extract.

Values in parenthesis represent reduction (%) in fasting blood glucose levels of normoglycemic rats calculated relative to pre-treatment values.

rats. The hypoglycaemic effect was non-dose-related and of the order of magnitude of potency ME > EF > MF (Table 1).

Effect of ME on blood glucose level of normoglycaemic rats

The ME caused a significant (P < 0.05) dose-related reduction in the FBG of normoglycaemic rats. Maximum reduction occurred within 1 h post treatment (Table 2).

Effect of ME on oral glucose tolerance in rats

Following oral administration of glucose meal, post-prandial blood glucose levels of the control rats rose by 80% to a peak at 60 min. Pre-treatment with ME (200 and 400 mg/kg) suppressed the rise in blood glucose level by 73.93 and 12.72%, respectively. ME evoked a significant (P < 0.05) and progressive dose-dependent decrease in blood glucose level up to 180 min. At this time, the blood glucose level of ME-treated rats remained remarkably below basal levels compared to

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

Treatment	Dose (mg/kg)	Blood Glucose level (mg/dl)						
		0 min	30 min	60 min	90 min	120 min	150 min	180 min
ME	200	56.0 ± 1.64	85.8 ± 2.67 (53.21)	97.4 ± 4.33 (73.93)	58.4 ± 0.51 (4.29)	54.4 ± 2.37 (2.86)	50.2 ± 2.0 (10.36)	47.8 ± 2.31 ^a (14.64)
	400	67.6 ± 1.81	69.2 ± 2.67 (2.37)	76.2 ± 2.35 (12.72)	67.2 ± 2.56 (0.59)	61.6 ± 1.72 (8.88)	56.2 ± 3.7 ^a (16.86)	52.0 ± 3.65 ^a (23.08)
Glibenclamide	0.2	61.8 ± 2.0	68.8 ± 1.46 (11.33)	86.6 ± 1.72 (40.13)	66.2 ± 1.5 (7.12)	62.0 ± 2.26 (3.24)	61.8 ± 2.27 (0.0)	60.6 ± 2.42 (1.94)
Control	-	61.6 ± 2.73	80.4 ± 2.91 (30.52)	111.0 ± 5.2 (80.19)	93.8 ± 3.82 (52.27)	66.2 ± 2.27 (7.47)	63.4 ± 2.38 (2.92)	61.6 ± 2.84 (0.0)

n = 5; *P<0.05 compared to zero minute values (ANOVA; LSD post hoc); ME = methanol extract; EF = ethyl acetate fraction; MF = methanol fraction.

Values in parenthesis represent change (%) in blood glucose level calculated relative to 0 min.

Table 4. Effect of ME on blood glucose of diabetic rats.

Treatment	Dose (mg/kg)	Blood glucose concentration (mg/dl)			
		Pre-Diabetic (Basal)	Diabetic Pre-treatment	Diabetic Post-treatment	
				Day 14	Day 28
ME	200	58.6 ± 2.62	414.2 ± 60.43	241.2 ± 20.32* (41.77)	193.7 ± 31.5* (53.24)
	400	63.6 ± 7.76	324.0 ± 55.04	111.3 ± 10.35* (65.74)	107.7 ± 8.13* (66.76)
NDT	200	52.2 ± 0.97	56.6 ± 1.50	75.4 ± 7.98*	93.0 ± 6.43*
NDNT	-	55.6 ± 2.69	55.6 ± 2.87	76.8 ± 4.04*	97.0 ± 5.79*
Glibenclamide	0.2	62.4 ± 2.79	499.0 ± 15.57	235.8 ± 17.96* (52.75)	187.2 ± 3.17* (62.48)

n = 5; *P<0.05 compared to Diabetic Pre-treatment values (ANOVA; LSD post hoc); ME = Methanol extract; NDNT = Non-diabetic non-treated was a non-diabetic control and received the vehicle; NDT = Non-diabetic treated was a non-diabetic control and received ME (200 mg/kg).

Values in parenthesis represent reduction (%) of blood glucose calculated for treatment groups relative to Diabetic Pre-treatment values.

glibenclamide-treated and control rats (Table 3).

Effect of ME on blood glucose of diabetic rats

Chronic oral administration of ME caused a significant (P<0.05) dose-related reduction in blood glucose levels of diabetic, but not normoglycaemic rats. The extract reduced blood glucose of treated rats better than glibenclamide (Table 4) and prolonged the survival of diabetic rats beyond the period of the study. All the animals in the non-treated diabetic control group died by day 10 post induction of diabetes (data not shown).

Effect of ME on total cholesterol and triglyceride levels of diabetic rats

Chronic administration of ME reduced total cholesterol and triglycerides levels of diabetic rats. The ME caused a significant (P<0.05) dose-related reduction in total

cholesterol of treated diabetic and normoglycaemic rats (Table 5). Also, ME significantly (P<0.05) reduced serum triglyceride level of diabetic and normoglycaemic rats in a dose-related manner. The magnitude of reduction was greater in diabetic than normoglycaemic rats and also higher than that evoked by glibenclamide (Table 6).

Effect of ME on haemoglobin levels and cell counts of diabetic rats

The haemoglobin (Hb) levels of all the animals was significantly (P<0.05) reduced initially on day 14. Subsequently, on day 28, ME-treated diabetic rats had slight increase in Hb levels while the reduction continued in the non-extract treated groups (Table 7). The extract (ME) caused a similar effect on red blood cell (RBC) counts of diabetic rats except that the RBC counts of glibenclamide-treated rats increased from day 14 (Table 8). On white blood cells (WBC) counts, ME increased cell counts of diabetic and normoglycaemic rats. Also the

Table 5. Effect of ME on cholesterol level of diabetic rats.

Treatment	Dose (mg/kg)	Total Cholesterol (mg/dl)			
		Pre-Diabetic (Basal)	Diabetic Pre-treatment	Diabetic Post-treatment	
				Day 14	Day 28
ME	200	121.5 ± 5.3	180.7 ± 3.1	85.4 ± 35.1 ^b (52.74)	84.9 ± 34.9 ^b (53.02)
	400	120.0 ± 1.4	148.7 ± 4.8	70.0 ± 29.5 ^b (52.93)	67.6 ± 28.5 ^b (54.54)
NDT	200	128.6 ± 7.4	121.8 ± 3.2	103.8 ± 3.2 ^{a,b} (14.78)	103.5 ± 3.9 ^{a,b} (15.02)
NDNT	-	122.3 ± 5.8	123.0 ± 5.8	124.6 ± 5.3 (-1.30)	124.6 ± 5.2 (-1.30)
Glibenclamide	0.2	129.8 ± 20.8	152.6 ± 2.6	165.1 ± 2.4 ^b (-8.19)	156.5 ± 2.0 (-2.56)

n = 5; ^{a,b}P<0.05 compared to Basal and Diabetic Pre-treatment values respectively (ANOVA; LSD post hoc); ME = Methanol extract; NDNT = Non-diabetic non-treated was a non-diabetic control and received the vehicle; NDT = Non-diabetic treated was a non-diabetic control and received ME (200 mg/kg).

Values in parenthesis represent reduction (%) of total cholesterol calculated for treatment groups relative to Diabetic Pre-treatment values.

Table 6. Effect of ME on triglyceride level of diabetic rats.

Treatment	Dose (mg/kg)	Triglyceride concentration (mg/dl)			
		Pre-Diabetic (Basal)	Diabetic Pre-treatment	Diabetic Post-treatment	
				Day 14	Day 28
ME	200	116.36 ± 5.30	165.56 ± 6.50	142.42 ± 10.10 (13.98)	141.44 ± 9.67 (14.57)
	400	114.53 ± 7.53	148.59 ± 7.64	107.57 ± 1.66 ^a (27.61)	105.65 ± 2.15 ^a (28.90)
NDT	200	93.80 ± 4.36	89.96 ± 3.46	78.79 ± 2.21 ^a (12.42)	78.85 ± 2.03 ^a (12.35)
NDNT	-	104.55 ± 2.03	105.23 ± 1.73	108.18 ± 5.5 (-2.80)	109.38 ± 4.92 (-3.94)
Glibenclamide	0.2	90.47 ± 0.94	120.46 ± 4.21	112.0 ± 1.70 ^a (7.02)	99.72 ± 1.26 ^a (17.22)

n = 5; ^aP<0.05 compared to Diabetic Pre-treatment (ANOVA; LSD post hoc); ME = Methanol extract; NDNT = Non-diabetic non-treated was a non-diabetic control and received the vehicle; NDT = Non-diabetic treated was a non-diabetic control and received ME (200 mg/kg).

Values in parenthesis represent reduction (%) of triglyceride concentration calculated for treatment groups relative to Diabetic Pre-treatment values.

Table 7. Effect of ME on haemoglobin level of diabetic rats.

Treatment	Dose (mg/kg)	Haemoglobin level (g%)			
		Pre-Diabetic (Basal)	Diabetic Pre-treatment	Diabetic Post-treatment	
				Day 14	Day 28
ME	200	16.07 ± 0.06	15.56 ± 0.25	13.55 ± 0.24 ^{a,b}	13.78 ± 0.21 ^{a,b}
	400	15.94 ± 0.16	15.71 ± 0.19	13.74 ± 0.05 ^{a,b}	13.90 ± 0.08 ^{a,b}
NDT	200	16.13 ± 0.05	15.96 ± 0.03	14.80 ± 0.28 ^{a,b}	14.70 ± 0.23 ^{a,b}
NDNT	-	15.75 ± 0.26	15.65 ± 0.31	13.98 ± 0.23 ^{a,b}	13.56 ± 0.11 ^{a,b}
Glibenclamide	0.2	14.08 ± 0.08	14.03 ± 0.07	13.82 ± 0.07 ^a	13.66 ± 0.07 ^{a,b}

n = 5; ^{a,b}P<0.05 compared to Basal and Diabetic pre-treatment values respectively (ANOVA; LSD post hoc); ME = methanol extract; NDT = Non-diabetic treated was a non-diabetic control and received ME (200 mg/kg); NDNT = Non-diabetic non-treated was a non-diabetic control and received the vehicle.

WBC counts of the non-diabetic non-treated control as well as glibenclamide-treated groups were increased by day 28 (Table 9).

Effect of ME on body weight of treated diabetic rats

There was a significant (P<0.05) increase in the body

Table 8. Effect of ME on red blood cell count of diabetic rats.

Treatment	Dose (mg/kg)	Red Blood Cell count ($\times 10^6$) (mm^3)			
		Pre-Diabetic (Basal)	Diabetic Pre-treatment	Diabetic Post-treatment	
				Day 14	Day 28
ME	200	5.19 \pm 0.09	5.11 \pm 0.09	4.83 \pm 0.04	5.50 \pm 0.09
	400	4.98 \pm 0.10	4.87 \pm 0.09	4.69 \pm 0.05	4.95 \pm 0.08
NDT	200	5.24 \pm 0.09	5.15 \pm 0.10	4.90 \pm 0.12	4.81 \pm 0.11
NDNT	-	5.07 \pm 0.19	4.92 \pm 0.16	4.65 \pm 0.15	6.51 \pm 0.12
Glibenclamide	0.2	5.37 \pm 0.05	5.29 \pm 0.08	4.66 \pm 0.19	5.51 \pm 0.06

n = 5; ME = methanol extract; NDT = Non-diabetic treated was a non-diabetic control and received ME (200 mg/kg); NDNT = Non-diabetic non-treated was a non-diabetic control and received the vehicle.

Table 9. Effect of ME on white blood cell count of diabetic rats.

Treatment	Dose (mg/kg)	Total White Blood Cell count ($\times 10^6$) (mm^3)			
		Pre-Diabetic (Basal)	Diabetic Pre-treatment	Diabetic Post-treatment	
				Day 14	Day 28
ME	200	4.66 \pm 0.09	4.58 \pm 0.04	4.73 \pm 0.12	4.84 \pm 0.07
	400	4.86 \pm 0.16	4.78 \pm 0.14	4.95 \pm 0.11	5.12 \pm 0.10
NDT	200	4.83 \pm 0.12	4.75 \pm 0.15	4.47 \pm 0.13	4.75 \pm 0.14
NDNT	-	4.52 \pm 0.05	4.39 \pm 0.61	4.26 \pm 0.07	5.46 \pm 0.11
Glibenclamide	0.2	4.71 \pm 0.08	4.73 \pm 0.08	4.60 \pm 0.07	4.66 \pm 0.06

n = 5; ME = methanol extract; NDT = Non-diabetic treated was a non-diabetic control and received ME (200 mg/kg); NDNT = Non-diabetic non-treated was a non-diabetic control and received the vehicle.

Table 10. Effect of chronic administration of ME on body weight of diabetic rats.

Treatment	Dose (mg/kg)	Body weight (g)			
		Pre-Diabetic (Basal)	Diabetic Pre-treatment	Diabetic Post-treatment	
				Day 14	Day 28
ME	200	126.0 \pm 1.87	120.6 \pm 5.42	136.7 \pm 11.11 (13.34)	139.0 \pm 11.62 (15.26)
	400	120.0 \pm 5.24	120.0 \pm 5.24	160.0 \pm 5.5 ^a (33.33)	161.0 \pm 12.27 ^a (34.17)
NDT	200	124.0 \pm 8.28	123.6 \pm 8.57	181.6 \pm 11.51 ^a (46.93)	184.0 \pm 10.34 ^a (48.87)
NDNT	-	121.0 \pm 5.57	121.0 \pm 5.57	201.0 \pm 12.69 ^a (66.12)	202.6 \pm 13.31 ^a (67.44)
Glibenclamide	0.2	274.8 \pm 6.97	273.0 \pm 7.40	271.0 \pm 7.84 (NI)	275.0 \pm 6.38 (0.73)

n = 5; ^aP<0.05 compared to Diabetic Pre-treatment (ANOVA; LSD post hoc); ME = Methanol extract; NDNT = Non-diabetic non-treated was a non-diabetic control and received the vehicle; NDT = Non-diabetic treated was a non-diabetic control and received ME (200 mg/kg).

Values in parenthesis represent increase (%) in body weight calculated for treatment groups relative to Diabetic Pre-treatment values; NI = No increase.

weights of treated diabetic, non-treated non-diabetic and normoglycaemic animals. The weight increase occurred most in the non-diabetic non-treated group followed by non-diabetic treated rats. The ME-treated rats had modest increase in body weight while glibenclamide-treated rats had little or no remarkable change in weight (Table 10).

Effect of ME on pancreatic tissues of diabetic rats

Histopathological studies showed that no necrosis was seen in the non-diabetic non-treated group. The non-diabetic non-treated group did not show any microscopic lesion around the islet tissues and the alveolar cells (Figure 1). The diabetic non-treated group showed

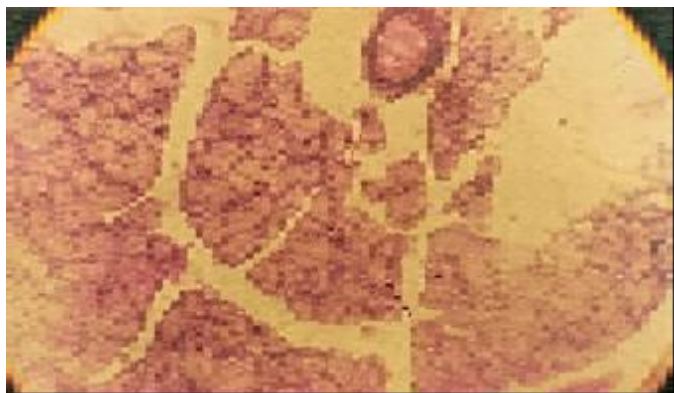


Figure 1. Pancreatic tissue from non-diabetic non -treated control.

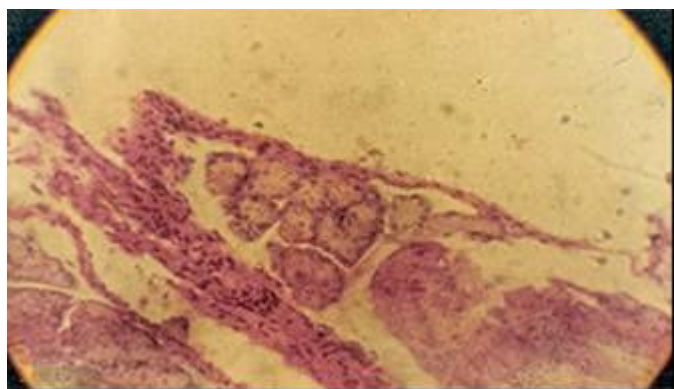


Figure 2. Pancreatic tissue from diabetic non - treated control.

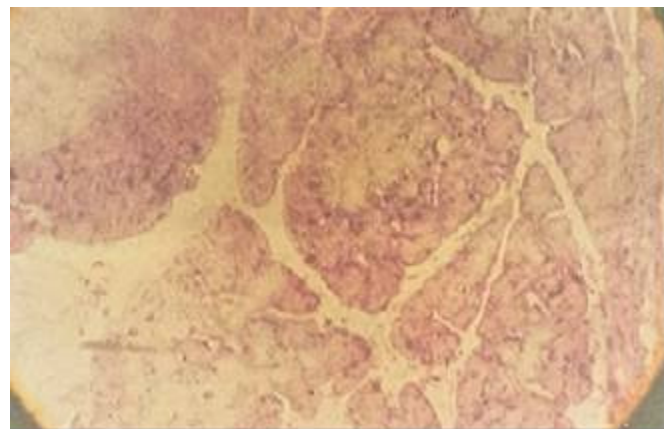


Figure 3. Pancreatic tissue from non-diabetic ME -treated control.

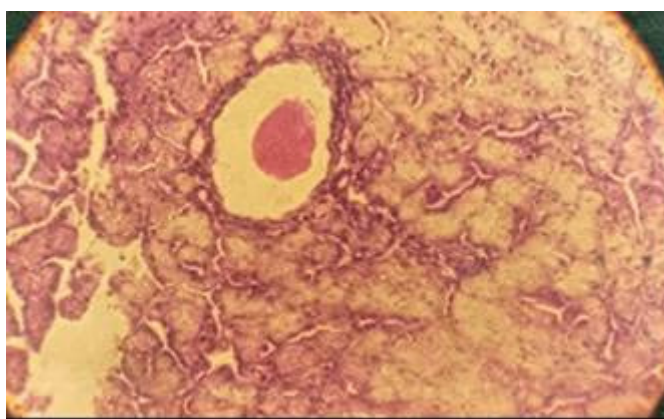


Figure 4. Effect of ME (200 mg/kg) on pancreatic tissue of alloxan-induced diabetic rats.

necrosis of the islet tissues with the alveolar cells moderately destroyed (Figure 2). In ME-treated animals, the architecture of the pancreas appeared intact. The characteristic interlobular and intralobular ducts as well as the alveolar granules were seen (Figures 3, 4 and 5). The glibenclamide treated group showed evidence of destruction of the alveolar cells with severe necrosis around the islet tissues (Figure 6).

DISCUSSION

DM has a significant impact on the health, quality of life and life expectancy of patients as well as healthcare expenditure. With increasing incidence and mortality from its complications, prompt and adequate glycaemic control in diabetes is paramount if management can meaningfully improve the quality of life and increase life expectancy. In this study, experimental evaluation of the anti-diabetic potentials of *P. niruri* has shown that single oral administration of the extract to normal rats reduced fasting blood glucose which suggests an inherent hypoglycaemic effect. The extract also suppressed the postprandial rise in blood glucose in normal rats

following a heavy glucose meal with maximum suppressive effect coinciding with the time of peak blood glucose level after the meal. The time-course of events also showed that the extract reduced glucose level to below basal in less than 2 h compared to the 2.5 h for glibenclamide to achieve a return to basal levels. Chronic hyperglycaemia in DM is a risk factor constantly fuelled by postprandial elevation of blood glucose. Control of postprandial hyperglycaemia in diabetes is of great importance due to its close relation to the risk of micro and macro-vascular complications and death (Balkau, 2000; Ceriello, 2005). Interestingly, in addition to hypoglycaemic effect, the extract may also suppress postprandial rise in blood glucose levels both of which are indices of effective glycaemic control.

In the anti-diabetic activity studies, daily oral administration of the extract for 28 days produced a gradual but sustained reduction in blood glucose levels in diabetic rats. Alloxan causes hyperglycaemia and glucose intolerance or syndromes similar to either type 1 or type 2 DM (Lenzen et al., 1996; Frode and Medeiros, 2008). Effec-

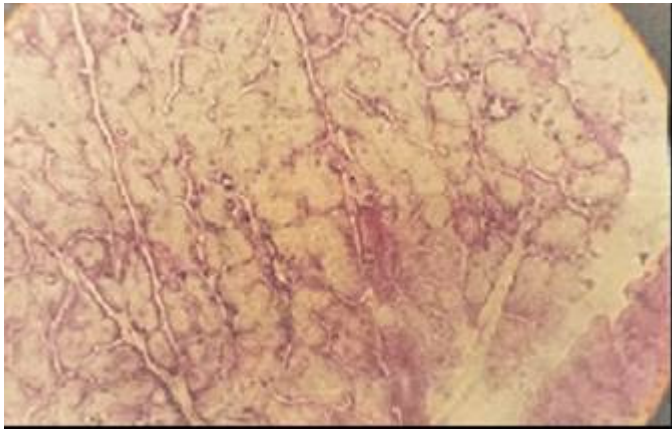


Figure 5. Effect of ME (400 mg/kg) on pancreatic tissue of alloxan-induced diabetic rats.

tive and sustained reduction in blood glucose levels of treated diabetic rats by the extract indicates that it may be useful in overt cases of DM. Treatment with the extract also reduced mortality of diabetic rats from hyperglycaemia and prolonged their survival. In this study, the diabetic non-treated control animals all died on day 10 post-induction of diabetes (data not shown) whereas the extract-treated group survived beyond the period of the experiment. Effective control of blood glucose level is a key step in preventing and reversing diabetic complications, and improving the quality of life of diabetic patients (The DCCT, 1993; Bavarva and Narasimhacharya, 2008). Hence, chronic administration of the extract may cause a progressively sustained reduction in hyperglycaemia known to reduce the risk of complications associated with the disease. Chronic oral administration of the extract also reduced total cholesterol and triglyceride levels in diabetic and normoglycaemic rats consistent with the hypolipidemic effect earlier reported (Khanna et al., 2002). Diabetic dyslipidaemia is marked by elevated triglycerides, cholesterol and low density lipoprotein (LDL) particles of altered composition and decreased high density lipoprotein (HDL), and constitutes an important cardiovascular risk factor in diabetics (Agrawal et al., 2006). Reduction in total cholesterol and triglycerides through dietary or drug therapy has been found beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetic patients (Brown et al., 1993; Ahmed et al., 2001). Experimentally, alloxan-induced diabetic hyperglycaemia is accompanied by increase in serum cholesterol and triglyceride levels (Choi et al., 1991; Platel et al., 1993; Sharma et al., 1997; Rao et al., 1999; Prince et al., 1999; Ahmed et al., 2001) and mimics overt diabetes disease. Thus, in addition to glycaemic control, extract of this plant may further reduce mortality from complications of the disease by ameliorating diabetes-induced dyslipidaemia.

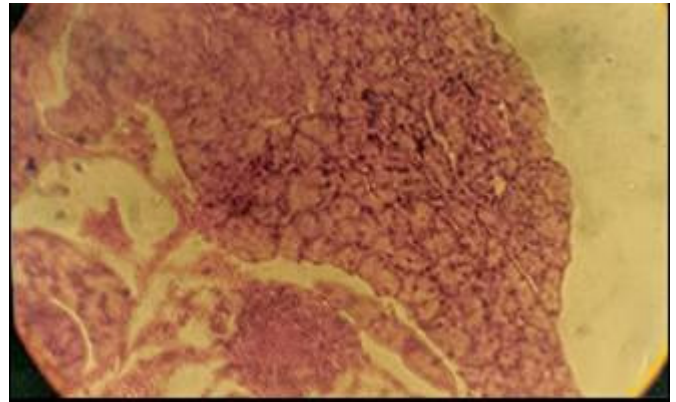


Figure 6. Effect of glibenclamide on pancreatic tissue of alloxan-induced diabetic rats.

Due to the association of obesity with DM, weight control is an important aspect of diabetes management. Poor glycaemic control usually results in weight loss. Our results showed that all the animals used gained weight during the study. The weight gain was highest in the nondiabetic treated control while glibenclamide-treated control had almost none. We had thought that this could mean that the extract could cause weight increase in treated diabetic animals. However, on a closer look at the body weights of these animals, it was evident that whereas animals in the glibenclamide group were mature adult rats, those in other groups were young adult rats in their active growth stage. As such, the increase in their body weights throughout the study period was likely due to ongoing natural growth process. Although adequate glycaemic control by some agents may lead to increase in body weight such as that observed with the thiazolidinediones (Monnier et al., 2003; Bhat et al., 2007), our current data is insufficient to draw any such conclusion with respect to the extract. That notwithstanding, it is important that chronic administration of the extract did not inhibit the natural growth process of these animals with or without diabetes. It is doubtful if extract of aerial parts of this plant may offer any additional benefit to obese diabetics in dire need of weight reduction therapy as adjunct to glycaemic control.

Assessment of the effect of chronic administration of the extract on Hb level as well as WBC and RBC counts revealed a slight increase following an initial reduction. It is not clear if this increase would progress to a return to basal levels and how long it may take. The extent of reduction in these parameters, however, did not appear to have posed any threat to the wellbeing and survival of treated animals.

Several factors such as oxidative stress (Hayden et al., 2005), chronic hyperglycemia (Leung and Leung, 2008) and autoimmune (Yoshida et al., 1995; Tanaka et al., 2000; Tanaka et al., 2001) or fibrocalculus (Mohan et al., 2008) types of chronic pancreatitis damage the

pancreas and impair insulin secretion and hence glycaemic control. Result of histological studies on pancreas isolated from treated diabetic rats showed that the extract may have repaired the pancreas damaged by alloxan. Alloxan owes its diabetogenic potential to destruction of β -cells of the islet (Szudelski, 2001; Fröde and Medeiros, 2008) which consequently impairs insulin secretion and gives rise to hyperglycemia. Treatment with the extract may have restored the integrity and perhaps, functions of the damaged pancreatic tissues. Glibenclamide used as a reference hypoglycemic agent did not cause any such effect. The precise mechanism of this tissue repair is not known. However, due to the large implication of oxidative stress (Hayden et al., 2005; Leung and Leung, 2008) in damage to the pancreas, it seems reasonable to suggest that the antioxidant (Tasaduq et al., 2003) and radical scavenging (Jagetiya and Baliga, 2004) effects of this plant may play a key role in protecting pancreatic tissues from oxidants including that generated by alloxan. Alloxan destroys insulin-producing pancreatic β -cells through the formation of reactive oxygen species that cause tissue damage (Lee et al., 2008). The hypoglycaemic effect of the extract may further protect the pancreas from the deleterious effect of chronic hyperglycaemia. Rather than possessing a direct tissue repair effect, it is likely that the extract, through antioxidant and hypoglycaemic effects, protected the already compromised pancreas from further assault or tissue damage which then allowed the natural repair processes to proceed and restore the tissues. However, it is not clear if the repaired tissues also had their functions fully or partially restored since the blood glucose level of the animals did not return to basal or pre-treatment levels as at the end of the experiment. A return to basal or pre-treatment levels would have indicated full restoration of insulin secretion by the repaired pancreatic tissues.

While the anti-diabetic effect of the extract may derive from its hypoglycaemic effect, the mechanisms of the hypoglycaemic effect are yet to be elucidated. The hypoglycaemic effect in normal and diabetic rats suggests an insulin-like effect probably mediated via peripheral glucose consumption (De Sousa et al., 2004; Zanatta et al., 2007). Also, postprandial hyperglycemia is related to postprandial hyperinsulinemia (Wang et al., 2004) and its suppression by the extract suggests an insulin-like effect. Phytochemical analysis of the extract revealed the presence of flavonoids, alkaloids, carbohydrates, glycosides and tannins which are typical plant constituents. Although there are chances that any of these constituents may possess anti-diabetic properties, extract of the whole plant may be more effective since the results of bioactivity-guided studies did not reveal any increase in magnitude of hypoglycaemic effect following solvent-guided fractionation. This observation precluded further fractionation of the extract in this study. It would thus be difficult at this stage to attribute the

anti-diabetic effect to any single or group of constituents.

Conclusion

The results of this study showed that aerial parts of *P. niruri* have great potentials as antidiabetic remedy due to the ability of its extract to lower blood glucose and lipid levels in diabetic rats and suppress postprandial rise in blood glucose levels. These effects reduce the risk of complications associated with diabetes. It may additionally protect the pancreas from further damage through antioxidant effect and effective glycaemic control. Chronic use may not cause any deleterious effect on haematological indices and body weight. The whole extract of the aerial parts may be more effective than the fractions although this does not preclude the isolation of hypoglycaemic constituents. Studies on the effect of the plant extract on peripheral glucose metabolism are ongoing.

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REFERENCES

- Agrawal RP, Sharma P, Pal M, Kochar A, Kochar DK (2006). Magnitude of dyslipidemia and its association with micro and macro vascular complications in type 2 diabetes: A hospital based study from Bikaner (Northwest India). *Diabetes Res. Clin. Pract.* 73: 211-214
- Ahmed I, Lakhani MS, Gillett M, John A, Raza H (2001). Hypotriglyceridemic and hypocholesterolemic effects of anti-diabetic *Momordica charantia* (karela) fruit extract in streptozotocin-induced diabetic rats. *Diabetes Res. Clin. Pract.* 51: 155-161.
- Akah PA, Okoli CO, Nwafor SV (2002). Phytotherapy in the management of diabetes mellitus. *J. Nat. Rem.* 2: 1-10.
- Aruna RV, Ramesh B, Kartha VN (1999). Effect of beta carotene on protein glycosylation in alloxan induced diabetic rats, *Indian J. Exp. Biol.* 32: 399-401.
- Bagalkotkar G, Sagineedu SR, Saad MS, Stanslas J (2006). Phytochemicals from *Phyllanthus niruri* Linn. and their pharmacological properties: a review. *J. Pharm. Pharmacol.* 58: 1559-1570.
- Balkau B (2000). The DECODE study, Diabetes epidemiology: collaborative analysis of diagnostic criteria in Europe. *Diabetes Metab.* 26: 282-286.
- Barros ME, Lima R, Mercuri LP, Matos JR, Schor N, Boim MA (2006). Effect of extract of *Phyllanthus niruri* on crystal deposition in experimental urolithiasis. *Urol. Res.* 34(6): 351-357.
- Barros ME, Schor N, Boim MA (2003). Effects of an aqueous extract from *Phyllanthus niruri* on calcium oxalate crystallization in vitro. *Urol. Res.* 30(6): 374-9.
- Bavarva JH, Narasimhacharya AVRL (2008). Antihyperglycemic and Hypolipidemic Effects of *Costus speciosus* in Alloxan induced Diabetic Rats. *Phytother. Res.* 22: 620-626.
- Bhat R, Bhansali A, Bhadada S, Sialy R (2007). Effect of pioglitazone therapy in lean type1 diabetes mellitus. *Diabetes Res. Clin. Pract.* 78: 349-354.

- Bhattacharjee R, Sil PC (2006). The Protein Fraction of *Phyllanthus niruri* Plays a Protective Role against Acetaminophen Induced Hepatic Disorder via its Antioxidant Properties. *Phytother. Res.* 20: 595-601.
- Bhattacharjee R, Sil PC (2007). Protein isolate from the herb, *Phyllanthus niruri* L. (Euphorbiaceae), plays hepatoprotective role against carbon tetrachloride induced liver damage via its antioxidant properties. *Food Chem. Toxicol.* 45(5): 817-26.
- Brown GB, Xue-Qiao Z, Sacco DE, Alberts JJ (1993). Lipid lowering and plaque regression. New insights into prevention of plaque disruption and clinical events in coronary disease. *Circulation*, 87: 1781-1791.
- Campos AH, Schor N (1999). *Phyllanthus niruri* inhibits calcium oxalate endocytosis by renal tubular cells: its role in urolithiasis. *Nephron*. 81(4): 393-397.
- Ceriello A (2005). Postprandial hyperglycemia and diabetes complications; is it time to treat? *Diabetes*, 54: 1-7.
- Chatterjee M, Sarkar K, Sil PC (2006). Herbal (*Phyllanthus niruri*) protein isolate protects liver from nimesulide induced oxidative stress. *Pathophysiology*, 13(2): 95-102.
- Chatterjee M, Sil PC (2006). Hepatoprotective effect of aqueous extract of *Phyllanthus niruri* on nimesulide-induced oxidative stress in vivo. *Indian J. Biochem. Biophys.* 43(5): 299-305.
- Choi JS, Yokozawa T, Oura H (1991). Improvement of hyperglycemia and hyperlipemia in streptozotocin-diabetic rats by a methanolic extract of *Prunus daidiana* stems and its main component, prunin. *Planta Med.* 57: 208-211.
- Chopra RN, Nayar SL, Chopra IC (1986). Glossary of Indian Medicinal Plants, CSIR, New Delhi Catholic Press, Ranchi, India.
- Cimanga RK, Tona L, Luyindula N, Mesia K, Lusakibanza M, Musuamba CT, Apers S, De Bruyne T, Van Miert S, Hermans N, Totté J, Pieters L, Vlietinck AJ (2004). *In vitro* antiplasmodial activity of callus culture extracts and fractions from fresh apical stems of *Phyllanthus niruri* L. (Euphorbiaceae): part 2. *J. Ethnopharmacol.* 95(2-3): 399-404.
- Colca JR (2006). Insulin sensitizers may prevent metabolic inflammation. *Biochem. Pharmacol.* 72: 125-131.
- De Sousa E, Zanatta L, Seifriz I, Creczynski-Pasa TB, Pizzolatti MG, Szpogancz B, Silva FRMB (2004). Hypoglycemic effect and antioxidant potential of kaempferol-3, 7-O-(β)-dirhamnoside from *Bauhinia forficata* leaves. *J. Nat. Prod.* 67: 829-832.
- Elfahmi, Batterman S, Koulman A, Hackl T, Bos R, Kayser O, Woerdenbag HJ, Quax WJ (2006) Lignans from cell suspension cultures of *Phyllanthus niruri*, an Indonesian medicinal plant. *J. Nat. Prod.* 69(1): 55-58.
- Freitas AM, Schor N, Boim MA (2002). The effect of *Phyllanthus niruri* on urinary inhibitors of calcium oxalate crystallization and other factors associated with renal stone formation. *BJU Int.* 89(9): 829-834.
- Fróde TS, Medeiros YS (2008). Animal models to test drugs with potential antidiabetic activity. *J. Ethnopharmacol.* 115: 173-183.
- Girach RD, Siddioui PA, Khan SA (1994). Traditional plant remedies among the kondh (Orissa), *Int. J. Pharmacol.* 32: 274-283.
- Harborne JB (1998). *Phytochemical Methods: A guide to modern technique of plant analysis*, 2nd ed., Chapman and Hall, London, p. 282.
- Hayden MR, Tyagi SC, Kerklo MM, Nicolls MR (2005). Type 2 Diabetes Mellitus as a Conformational Disease. *JOP. J. Pancreas (Online)*, 6(4): 287-302
- Iizuka T, Moriyama H, Nagai M (2006). Vasorelaxant effects of methyl brevilofincarboxylate from the leaves of *Phyllanthus niruri*. *Biol. Pharm. Bull.* 29(1): 177-179.
- Iizuka T, Nagai M, Taniguchi A, Moriyama H, Hoshi K (2007). Inhibitory effects of methyl brevilofincarboxylate isolated from *Phyllanthus niruri* L. on platelet aggregation. *Biol. Pharm. Bull.* 30(2): 382-384.
- Jagetia GC, Baliga MS (2004). The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants *in vitro*: a preliminary study. *J. Med. Food*, 7(3): 343-348.
- Khanna AK, Rizvi F, Chander R (2002). Lipid lowering activity of *Phyllanthus niruri* in hyperlipemic rats. *J. Ethnopharmacol.* 82: 19-22.
- Kieley S, Dwivedi R, Monga M (2008). Ayurvedic medicine and renal calculi. *J. Endourol.* 22(8): 1613-1616.
- Lee JH, Park JW, Kim JS, Park BH, Rho HW (2008). Protective Effect of Amomi Semen Extract on Alloxan-induced Pancreatic β -Cell Damage. *Phytother. Res.* 22: 86-90.
- Lenzen S, Tiedge M, Jorns A, Munday R (1996). Alloxan derivatives as a tool for the elucidation of the mechanism of the diabetogenic action of alloxan. In: Shafrir, E. (ed.) *Lessons from Animal Diabetes*, Birkhauser, Boston, pp. 113-122.
- Leung KK, Leung PS (2008). Effects of Hyperglycemia on the Angiotensin II Receptor Type 1 Expression and Insulin Secretion in an INS-1E Pancreatic Beta-Cell Line. *JOP. J. Pancreas (Online)* 9(3): 290-299.
- Lin TJ, Su CC, Lan CK, Jiang DD, Tsai JL, Tsai MS (2003). Acute poisonings with *Breynia officinalis*-an outbreak of hepatotoxicity. *J. Toxicol. Clin. Toxicol.* 41: 591-594.
- Lorke D (1983). A new approach to practical acute toxicity testing. *Arch. Toxicol.* 53: 275-289.
- Manjrekar AP, Jisha V, Bag PP, Adhikary B, Pai MM, Hegde A, Nandini M (2008). Effect of *Phyllanthus niruri* Linn. treatment on liver, kidney and testes in CCl₄ induced hepatotoxic rats. *Indian J. Exp. Biol.* 46(7): 514-520.
- Mellinger CG, Carbonero ER, Noletto GR, Cipriani TR, Oliveira MB, Gorin PA, Iacomini M (2005). Chemical and biological properties of an arabinogalactan from *Phyllanthus niruri*. *J. Nat. Prod.* 68(10): 1479-1483.
- Mellinger CG, Cipriani TR, Noletto GR, Carbonero ER, Oliveira MB, Gorin PA, Iacomini M (2008). Chemical and immunological modifications of an arabinogalactan present in tea preparations of *Phyllanthus niruri* after treatment with gastric fluid. *Int. J. Biol. Macromol.* 43(2): 115-120.
- Micali S, Sighinolfi MC, Celia A, De Stefani S, Grande M, Cicero AF, Bianchi G (2006). Can *Phyllanthus niruri* affect the efficacy of extracorporeal shock wave lithotripsy for renal stones? A randomized, prospective, long-term study. *J. Urol.* 176(3): 1020-1022.
- Mohan V, Farooq S, Deepa M (2008). Prevalence of Fibrocalculous Pancreatic Diabetes in Chennai in South India. *J. Pancreas (Online)* 9(4): 489-492.
- Monnier L, Lapinski H, Colette C (2003). Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type2 diabetes patients. *Diabetes Care*, 26: 881-885.
- Mulchandani NB, Hassarajani SA (1984). 4-Methoxy-nor-Securinine, a New Alkaloid from *Phyllanthus niruri*. *Planta Med.* 50(1): 104-105.
- Muller PH, Schmulling RM, Eggstein M (1977). A fully enzymatic triglyceride determination. *J. Clin. Chem.* 15: 457-504.
- Murugaiyah V, Chan KL (2006). Antihyperuricemic lignans from the leaves of *Phyllanthus niruri*. *Planta Med.* 72(14): 1262-1267.
- Murugaiyah V, Chan KL (2007). Determination of four lignans in *Phyllanthus niruri* L. by a simple high-performance liquid chromatography method with fluorescence detection. *J. Chromatogr. A.* 1154(1-2): 198-204.
- Mustofa, Sholikhah EN, Wahyuono S (2007). *In vitro* and *in vivo* antiplasmodial activity and cytotoxicity of extracts of *Phyllanthus niruri* L. herbs traditionally used to treat malaria in Indonesia. *Southeast Asian J. Trop. Med. Public Health*, 38(4): 609-615.
- Naik AD, Juvekar AR (2003). Effects of alkaloidal extract of *Phyllanthus niruri* on HIV replication. *Indian J. Med. Sci.* 57(9): 387-393.
- Nishiura JL, Campos AH, Boim MA, Heilberg IP, Schor N (2004). *Phyllanthus niruri* normalizes elevated urinary calcium levels in calcium stone forming (CSF) patients. *Urol. Res.* 32(5): 362-6.
- Ogata T, Higuchi H, Mochida S, Matsumoto H, Kato A, Endo T, Kaji A, Kaji H (1992). HIV-1 reverse transcriptase inhibitor from *Phyllanthus niruri*. *AIDS Res. Hum. Retroviruses*, 8(11): 1937-1944.
- Olive-Bever B (1986). *Medicinal plants in Tropical West Africa*. Cambridge University Press, Cambridge.
- Paranjape P (2001). *Indian Medicinal Plants: Forgotten Healers*. Chaukhamba Sanskrit Pratisthan, Delhi, p. 48.
- Platel K, Shurpalekar KS, Srinivasan K (1993). Effect of bitter gourd

- (*Momordica charantia*) on growth and blood constituents in albino rats. *Nahrung*, 37: 156-160.
- Prince PSM, Menon VP, Gunasekaran G (1999). Hypolipidaemic action of *Tinospora cordifolia* roots in alloxan diabetic rats. *J. Ethnopharmacol.* 64: 53-57.
- Qian-Cutrone J, Huang S, Trimble J, Li H, Lin PF, Alam M, Klohr SE, Kadow KF (1996). Niruriside, a new HIV REV/RRE binding inhibitor from *Phyllanthus niruri*. *J. Nat. Prod.* 59(2): 196-199.
- Ramakrishnan PN, Murugesan R, Palanichamy S (1982). Oral hypoglycaemic effect of *Phyllanthus niruri* leaves. *Indian J. Pharm. Sci.* 44: 10-12
- Rao BK, Kesavulu MM, Giri R, Rao CA (1999). Antidiabetic and hypolipidemic effects of *Momordica cymbalaria* Hook. fruit powder in alloxan-diabetic rats. *J. Ethnopharmacol.* 67: 103-109.
- Santos AR, Filho VC, Niero R, Viana AM, Moreno FN, Campos MM, Yunes RA, Calixto JB (1994). Analgesic effects of callus culture extracts from selected species of *Phyllanthus* in mice. *J. Pharm. Pharmacol.* 46(9): 755-759.
- Santos AR, Filho VC, Yunes RA, Calixto JB (1995). Analysis of the mechanisms underlying the antinociceptive effect of the extracts of plants from the genus *Phyllanthus*. *Gen. Pharmacol.* 26(7): 1499-1506.
- Sarkar MK, Sil PC (2007). Hepatocytes are protected by herb *Phyllanthus niruri* protein isolate against thioacetamide toxicity. *Pathophysiology*, 14(2): 113-120.
- Shakil NA, Pankaj, Kumar J, Pandey RK, Saxena DB (2008). Nematicidal prenylated flavanones from *Phyllanthus niruri*. *Phytochemistry*, 69(3): 759-764.
- Sharma SR, Dwivedi SK, Swarup D (1997). Hypoglycaemic, antihyperglycaemic and hypolipidemic activities of *Caesalpinia bonducella* seeds in rats. *J. Ethnopharmacol.* 58: 39-44.
- Shimizu M, Horie S, Terashima S, Ueno H, Hayashi T, Arisawa M, Suzuki S, Yoshizaki M, Morita N (1989). Studies on aldose reductase inhibitors from natural products. II. Active components of a Paraguayan crude drug Para-parai mi, *Phyllanthus niruri*. *Chem. Pharm. Bull. (Tokyo)*. 37(9): 2531-2532.
- Subeki S, Matsuura H, Takahashi K, Yamasaki M, Yamato O, Maede Y, Katakura K, Kobayashi S, Trimurningsih T, Chairul C, Yoshihara T (2005). Anti-babesial and anti-plasmodial compounds from *Phyllanthus niruri*. *J. Nat. Prod.* 68(4): 537-539.
- Syamasundar KV, Singh B, Thakur RS, Husain A, Kiso Y, Hikino H (1985). Antihepatotoxic principles of *Phyllanthus niruri* herb. *J. Ethnopharmacol.* 14: 41-44.
- Szudelski T (2001). The mechanism of alloxan and streptozotocin action in β cells of the rat pancreas. *Physiol. Res.* 50: 536-546.
- Tanaka S, Kobayashi T, Nakanishi K, Okubo M, Murase T, Hashimoto M (2001). Evidence of primary beta-cell destruction by T-cells and beta-cell differentiation from pancreatic ductal cells in diabetes associated with active autoimmune chronic pancreatitis. *Diabetes Care*, 24: 1661-1667.
- Tanaka S, Kobayashi T, Nakanishi K, Okubo M, Murase T, Hashimoto M, et al. (2000). Corticosteroid-responsive diabetes mellitus associated with autoimmune pancreatitis. *Lancet*, 356: 910-911.
- Tasaduq SA, Singh K, Sethi S, Sharma SC, Bedi KL, Singh J, Jaggi BS, Johri RK (2003). Hepatocurative and antioxidant profile of HP-1, a polyherbal phytomedicine. *Hum. Exp. Toxicol.* 22(12): 639-645.
- The Diabetes Control and Complications Trial Research Group (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 329: 977-986.
- Tona L, Cimanga RK, Mesia K, Musuamba CT, De Bruyne T, Apers TS, Hernans N, Van Miert S, Pieters L, Totté J, Vlietinck AJ (2004). *In vitro* antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. *J. Ethnopharmacol.* 93: 27-32
- Tona L, Mesia K, Ngimbi NP, Chrimwami B, Okond'Ahoka A, Cimanga K, De Bruyne T, Apers S, Hermans N, Totté J, Pieters L, Vlietinck AJ (2001). *In vivo* antimalarial activity of *Cassia occidentalis*, *Morinda morindoides* and *Phyllanthus nfruri*. *Ann. Trop. Med. Parasitol.* 65: 47-57.
- Tona L, Ngimbi NP, Tsakala M, Mesia K, Cimanga K, Apers S, De Bruyne T, Pieters L, Totté J, Vlietinck AJ (1999). Antimalarial activity of 20 crude extracts from nine African medicinal plants used in Kinshasa, Congo. *J. Ethnopharmacol.* 68: 193-203.
- Trease GE, Evans WC (1983). *Drugs of Biological origin*. In: *Pharmacognosy*, 12th ed., Balliere Tindall, United Kingdom, pp. 309-540.
- Unander DW, Webster GL, Blumberg BS (1995). Usage and bioassays in *Phyllanthus* (Euphorbiaceae). IV. Clustering of antiviral uses and other effects. *J. Ethnopharmacol.* 45: 1-18.
- Verspohl EJ (2002). Recommended testing in diabetes research. *Planta Medica*, 68: 581-590.
- Wang JJ, Qiao Q, Miettinen ME, Lappalainen J, Hu G, Tuomilehto J (2004). The metabolic syndrome defined by factor analysis and incident type 2 diabetes in a Chinese population with high postprandial glucose. *Diabetes Care*, 27: 2429-2437
- Yoshida K, Toki F, Takeuchi T, Watanabe S, Shiratori K, Hayashi N (1995). Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis. *Dig. Dis. Sci.* 40: 1561-1568.
- Zanatta L, De Sousa E, Cazarolli LH, Cunha Jr. A, Pizzolatti AMG, Szpoganicz B, Silva FRMB (2007). Effect of crude extract and fractions from *Vitex megapotamica* leaves on hyperglycemia in alloxan-diabetic rats. *J. Ethnopharmacol.* 109: 151-155.