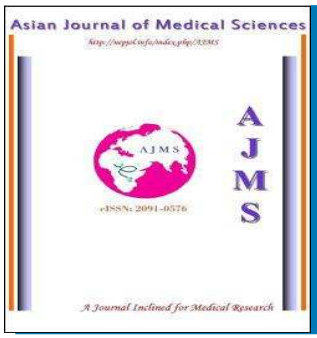

THE ANTI-DIABETIC ACTIVITIES OF THE METHANOL LEAF EXTRACT OF *PHYLLANTHUS AMARUS* IN SOME LABORATORY ANIMALS

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"The methanol extract of the Phyllanthus amarus caused a dose-related hypoglycaemia in rats. This effect is even higher than those of the reference drug, glibenclamide. The plant also appeared safe at the stated doses."

ABSTRACT

Background: *Phyllanthus amarus* is used in Nigeria and other parts of the world as a medicinal plant.

Aim and objective: The plant is being evaluated for antidiabetic potential because diabetes mellitus has assumed a worldwide dimension and plant with safe potential are being deployed as they are available all year round and are cheap for use by the rural populace.

Materials and Methods: The antidiabetic effect of the methanol extract (ME) of *Phyllanthus amarus* was evaluated in rats. Standard phytochemical methods were used to test for the presence of phytoactive compounds in the plant. Acute toxicity was carried out in mice to determine safe doses for this plant extract. The anti-diabetic activities of the ME of the plant were assessed using some standard tests as well as histological changes in liver, kidney and pancreas. Diabetes mellitus was induced in rats using alloxan while glibenclamide at 0.2mg/kg was the reference drug used in this study.

Results: The ME at 200 and 400mg/kg body weight caused a significant reduction of fasting blood glucose, significant change in the oral glucose tolerance test, marked effect in the hypoglycaemic activity test and pronounced reduction on the glucose level of diabetic rats. Histopathologically, there was no visible lesion seen in the liver, kidney and pancreas of extract-treated and glibenclamide-treated groups.

Conclusion: This study may have validated the traditional basis for the use of *Phyllanthus amarus* as an antidiabetic agent. At the doses used, ME also appeared safer than glibenclamide even though the latter is more potent.

Key words: *Phyllanthus amarus*, anti-diabetic agent, glibenclamide, hypoglycaemia, diabetes mellitus, normoglycaemia, rats, mice.

INTRODUCTION

Diabetes mellitus is possibly the world's largest growing metabolic disorder, and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases[1, 2]. Diabetes is a major health problem, affecting about 5% of the total population in the U.S. and 3% of the population worldwide. Over 90% of patients with diabetes have type 2 diabetes; the remainders have type 1 diabetes. Although the two types of diabetes have distinct pathogeneses, hyperglycemia and various life-threatening complications resulting from long term hyperglycemia are the most common features. Epidemiological studies[3, 4, 5] and clinical trials[6, 7] strongly support the notion that hyperglycemia is the principal cause of complications. Effective blood glucose control is the key to preventing or reversing diabetic complications and improving quality of life in patients with diabetes[8]. Thus, sustained reductions in hyperglycemia will decrease the risk of developing microvascular complications and most likely reduce the risk of macrovascular complications[9, 10].

Phyllanthus amarus belongs to the family Euphorbiaceae (the spurge family) of which the largest genus is the genus Euphorbia. The widespread usage of this herb has prompted several investigations[11]. The plant has a history of use in Ayurvedic medicine for over 2000 years as well as a wide variety of traditional applications[12]. This plant is gaining in popularity in many continents as an herbal remedy. Many of the active constituents are attributed to biologically active lignanes, glycosides, flavonoids, alkaloids, ellagitannins and phenylpropanoids found in the leaf, stem and root of the plant[13]. Five flavonoids have been identified: quercitin, astralgin, quercitrin, isoquercitrin and rutin[14].

Many studies have thus been carried out on the plant in various parts of the world but there is a resurgence of interest in this plant as antidiabetic agent. The present study was therefore undertaken to investigate the phytochemical constituents, anti-diabetic and safety potentials of the methanol leaf extract of *Phyllanthus amarus* Schum in experimental animals especially that this plant is used to treat diabetes in Nigeria.

MATERIALS AND METHODS

Plant material and preparation of extracts

Fresh leaves of *Phyllanthus amarus* Schum were collected from the campus of the University of Ibadan, Nigeria in April 2012. The leaves were identified by botanists and a voucher specimen (UIH ADE/005/2012) deposited at the herbarium of the Department of Botany, University of Ibadan. The ground leaves (200g) were dissolved in methanol (1 L) for 48 h on an orbital shaker at room temperature of 24°C. Extracts were filtered using a Buckner funnel and Whatman No 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40ºC using a rotary evaporator. The thick solution was used for pharmacological investigations. The extract yield was 12.68%.

Animals

The animals used in this study were male Wistar rats weighing between 100 and 200g as well as mice weighing between 15 and 30g. They were maintained at the Experimental Animal House of the Faculty of Veterinary Medicine, University of Ibadan in rat cages and fed on commercial rabbit cubes (Ladokun and Son Livestock Feeds, Nigeria Ltd). The animals were allowed free access to clean fresh water in bottles *ad libitum*. All experimental protocols were in compliance with University of Ibadan Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

Chemicals

Alloxan used in this was obtained from Sigma-Aldrich (Chemie Gmbh, Steinheim, Denmark). The

standard drug used in the various experiments was glibenclamide. The chemical and drug used were of analytical grade. Normal saline and distilled water were also used in this study.

Phytochemical Screening

The phytochemical analysis was performed in the powdered leaf of *P. amarus* for identification of the constituents such as alkaloids, tannins, saponins, anthraquinones, free glycosides and flavonoids[15].

Acute toxicity test

The acute toxicity of *P. amarus* aqueous was determined in rats according to the method of Hilaly *et al.* [16] with slight modifications. Rats fasted for 16 h were randomly divided into groups of six rats per group. Graded doses of the plant's extract (100, 200, 400, 800 and 1600 mg/kg p.o.) were separately administered to the rats in each of the groups by means of bulbed steel needle. All the rats in the groups were then allowed free access to food and water and observed over a period of 48 h for signs of acute toxicity. The number of deaths within this period of time was recorded.

Antidiabetic studies

Hypoglycaemic activity test

The hypoglycaemic effect of the methanol extract (ME) was studied in alloxan-induced diabetic rats. The rats were fasted for 8 hours but allowed free access to water. At the end of the fasting period, the basal fasting blood glucose (FBG) level of the rats was determined. Subsequently, diabetes was induced by single intraperitoneal injection of alloxan monohydrate (70 mg/kg)[17] and normal feeding maintained thereafter. Five days later, blood was drawn from each rat and the blood glucose level was measured to establish diabetes. Animals with blood glucose level ≥225mg/dl was considered to be diabetic and used for this study. The diabetic animals were randomly divided into four groups (n=5) and received oral administration of methanol extract (ME) (200 and 400 mg/kg), normal saline (3ml/kg) and Glibenclamide (0.2 mg/kg) respectively. ME was dissolved in normal saline. Blood glucose was then measured before (i.e. 0 h) and at 0.5, 1, 2 and 4 h after treatment.

Normoglycaemic activity

Animals fasted overnight were randomly divided into four groups (n=5) and received oral administration of the extract (200 and 400mg/kg), glibenclamide (0.2mg/kg) and vehicle control (3ml/kg) respectively. The blood glucose level of each animal was measured prior to (pretreatment) and at 0.5, 1, 2 and 4 h after extract and drug administration[18].

Oral glucose tolerance test

Animals that were fasted for 16 h but with free access to water were randomly divided into four groups (n=5) and received oral administration of the extract (200 and 400mg/kg), glibenclamide (0.2mg/kg) and vehicle control (3ml/kg) respectively. Ninety minutes later, the rats were fed with glucose (4g/kg). The blood glucose level of animals in each group was then measured before (0) and at 30, 60, 90, 120, 150, 180 min after glucose load[18].

Antidiabetic activity test

The antidiabetic effect of the plant extract 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 the rats was determined and diabetes was induced as described before. 25 diabetic rats with glucose level ≥225 were selected and used for the study. The rats were fasted for 8h but allowed free access to water[18]. They were then divided randomly into five groups (n=5) and received oral administration of extract (200 and 400mg/kg), glibenclamide (0.2mg/kg, diabetic control), extract (200mg/kg) and the vehicle (3ml/kg) both of which serve as non diabetic control. The treatment was administered orally to the animals once daily for 28 days. Blood glucose level was then measured as described before

commencement of the treatment. The body weight of each animal was also measured on these days.

Effects of the extract on lipid profile of diabetic rats

The effect of the extract 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 mins. The serum was collected and the total cholesterol and triglyceride levels of each sample were separately determined by enzymatic colorimetric method[19] 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 absorbance of each sample containing the reaction mixture with or without serum was read at 540nm in a UV spectrophotometer. Total cholesterol or triglyceride is calculated using the formula: Total cholesterol (mg/dl) = SAod/STod x 200, where SAod = optical density of test sample and STod = optical density of standard.

Effect of the extract on haemoglobin and cell counts of diabetic rats

The effect of chronic administration of the extracts 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 the commencement of treatment (Post-treatment). Cyanohaemoglobin method was used for estimation of haemoglobin[20] and the cell counts determined using the methods described by Cole[20.]

Histological studies on the pancreas of the extract-treated diabetic rats

The effect of the extracts 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 pancreas, kidney and liver from each animal were removed and placed in 10% formalin for histological studies.

Data analysis

Data was analyzed using graph pad prism 5 and the results expressed as mean ± SD. The results were further subjected to student's t-test for comparisons and differences between means were considered significant at $P< 0.05$.

RESULTS

Phytochemical screening

Phytochemical screening of the leaves of *P. amarus* showed the presence of alkaloids, tannin, flavonoids, saponin, anthraquinones and cardiac glycosides.

Acute toxicity test

No death was recorded in all the groups. All the mice appeared to be normal and none of them showed any visible signs of toxicity.

Normoglycaemic test

The ME caused a significant ($P<0.05$) dose related reduction in the fasting blood glucose (FBG) level of normoglycaemic rats. Maximum reduction occurred within 4 h post- treatment with 400mg/kg dose of the extract at 40.2% (Table 1).

Oral glucose tolerance test

Following oral administration of glucose, postpandrial blood glucose levels of the control rats increased to the peak at 60 min. Pre-treatment with ME (200 and 400 mg/kg) suppressed the rise in blood glucose by 18.0 and 28.7% (both within 3 h)

respectively. Methanol extract evoked a progressive dose-dependent decrease in blood glucose level up to 180 mins. At this time, the blood glucose level of ME-treated rats remained significantly below the basal levels compared to glibenclamide-treated (8.5%) and control rats (Table 2).

Hypoglycaemic test

The ME caused a significant (P<0.05) reduction in the blood glucose level in diabetic rats. The highest reduction (29.8%) is seen at 4 h post-treatment and at the dose of 200mg/kg (Table 3).

Table 1: Effects of methanol extract of *P. amarus* on blood glucose of normoglycaemic rats.

Treatment	Dose mg/kg	Fasting Blood Glucose level (mg/dl)				
		Pretreatment	0.5hr	1hr	2hr	4hr
Control	3ml/kg	66.8±8.7	66.4±9.0	64.4±9.0	62.4±7.7	62.0±7.1
Glibenclamide	0.2	70±4.9	61.8±4.3[b] (11.7)	53.0±6.1[ab] (24.3)	50.2±5.7[ab] (28.3)	47.8±5.5[ab] (31.7)
Methanol Extract	200	69.6±1.7	58.6±6.6[b] (15.8)	57.6±5.0[b] (17.2)	50.8±4.2[ab] (27.0)	47.4±4.2[ab] (31.9)
Methanol Extract	400	70.2±4.9	55.0±8.0[b] (21.7)	43.4±3.4[ab] (38.2)	45.2±1.7[ab] (35.6)	42.0±2.8[ab] (40.2)

N= 5; mean ± SD. [ab]P< 0.05 compared to control and pre-treatment values respectively (t-test). Superscripted items ([ab]) indicate significant values when compared to control and pre-treatment values respectively.
Values in parenthesis represent reduction (%) in fasting blood glucose levels of normoglycaemic rats calculated relative to pre-treatment values

Table 2: Effects of methanol extract of *P. amarus* on oral glucose tolerance in rats.

Treatment	Dose mg/kg	Blood Glucose level (mg/dl)						
		0 min	30min	60min	90min	120min	150min	180min
Control	3ml/kg	60.8±3.4	73.8±7.3 (21.4)	72.2±5.7 (18.8)	64.2±9.0 (5.6)	62.6±7.3 (3.0)	62.0±6.4 (2.0)	59.0±6.6 (3.0)
Glibenclamide	0.2	63.8±2.9	67.2±3.2 (5.3)	74.2±3.31[a] (16.3)	70.0±3.6[a] (9.7)	65.2±4.6 (2.2)	63.2±3.6 (0.9)	58.4±7.5 (8.5)
Methanol Extract	200	63.2±4.6	67.8±5.0 (7.3)	73.6±3.4[a] (16.5)	66.8±3.8 (5.7)	61.0±3.6 (3.5)	54.2±12.2 (14.2)	51.8±11.6 (18.0)
Methanol Extract	400	63.4±6.6	69.0±5.3 (8.8)	66.8±7.1 (5.4)	62.0±7.0 (2.2)	59.6±6.8 (6.0)	52.4±5.0[a] (17.4)	45.2±9.2[a] (28.7)

n= 5; mean± SD. [a]P< 0.05 compared to 0 minute values (t-Test). Superscripted items ([a]) indicate significant values when compared to 0 min values, Values in parenthesis represent change (%) in blood glucose level calculated relative to 0 min.

Table 3: Hypoglycaemic effects of methanol extract of *P. amarus* on diabetic rats.

Treatment	Dose Mg/kg	Blood Glucose level (mg/dl)				
		Pretreatment	0.5hr	1hr	2hr	4hr
Control	3ml/kg	338.0±31.2	346.0±33.8	324.8±31.6	321.2±32.8	318.0±31.2
Glibenclamide	0.2	308.0±21.4	294.0±18.6[a] (4.6)	278.0±19.1[ab] (9.7)	256.0±19.3[ab] (16.9)	233.0±12.5[ab] (24.4)
methanol Extract	200	314.0±40.1	301.2±33.8 (4.1)	282.4±33.8 (10.1)	266.0±36.8[a] (15.3)	230.0±38.0[ab] (29.8)
methanol Extract	400	286.0±26.5[a]	268.0±22.5[a] (6.3)	249.8±35.6[a] (12.7)	235.0±17.9[ab] (17.8)	218.0±13.3[ab] (23.8)

n= 5; mean± SD. [ab] P< 0.05 compared to control and pre-treatment values respectively (t-test). Superscripted items ([ab]) indicate significant values when compared to control and pre-treatment values respectively.

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

Effect of ME of P. amarus on blood glucose of diabetic rats

Chronic oral administration of ME caused a significant (P<0.05) dose-related reduction in blood glucose of diabetic rats. The extract at dose of 400mg/kg reduced the blood glucose of the treated rats better than glibenclamide i.e. 64 and 52.1% respectively; while the extract at 200mg/kg also exerted a higher effect (56.1%) than the glibenclamide on 28th day (Table 4).

Effect of ME of P. amarus on Cholesterol, Triglyceride and Haemoglobin levels of diabetic rats

Chronic administration of ME and glibenclamide caused a significant (P<0.05) reduction in total cholesterol level of treated diabetic rats. The magnitude of reduction was greater on day 28 than day 14 and it was in the order 400 (48.3%), 200 (39.7%) and glibenclamide (9.2%). The extract reduced triglyceride concentration of the diabetic rats and this reduction was significant (P<0.05). The 400mg/kg dose caused the highest reduction on day 28 i.e. 23.4%. The magnitude of reduction of triglyceride level by glibenclamide and the 200mg/kg dose of the extract was similar i.e. 17.1 and 17.9% respectively.

Also, there was a significant difference between the haemoglobin level of diabetic pre-treated and diabetic post-treated in the glibenclamide and 200mg/kg dose of the extract groups. While the haemoglobin level of the diabetic post-treatment values of these groups did not measure up to the diabetic pre-treated level, it was not so with the 400mg/kg dose group because there was no significant change between the diabetic pre-treated value and those of the diabetic post-treated value on day 28 (Table 5).

Effect of ME of P. amarus on the red and white blood cell counts of diabetic rats

The RBC count of all the animals was reduced on day 14 with all the groups showing significant difference except the 400mg/kg dose of the ME. Subsequently, there was increase in the RBC count on day 28 with the 200mg/kg dose group even showing significant difference. On the other hand, the white blood cell (WBC) count of the extract-treated and glibenclamide-treated animals experienced an increase on day 28 post-diabetic treatment relative to diabetic pre-treatment value. Likewise, there was subsequent increase in the control (both NDT and NDNT) groups and glibenclamide-treated group on day 28 with no

Table 4: Effect of methanol extract of *P. amarus* on blood glucose of diabetic rats

Treatment	Dose mg/kg	Blood glucose concentration (mg/dl)			
		Pre-Diabetic (Basal)	Diabetic (Pre-Rx)	Diabetic Post-Rx	
				Day 14	Day 28
Control (NDNT)	3ml/kg	57.6±7.4	56.8±7.7	71.8±4.8[a]	89.4±5.6[a]
Control (NDT)	200	53.4±10.6	58.6±11.5	72.2±2.8[a]	91.8±9.1[a]
Glibenclamide	0.2	55.8±14.6	286.0±26.5	147.0±16.0[a] (48.6)	137.0±17.2[a] (52.1)
Methanol Extract	200	54.2±11.3	314.0±40.3	176±34.4[a] (44.0)	138.0±24.8[a] (56.1)
Methanol Extract	400	59.4±33.0	286.0±26.5	146.0±21.5[a] (49.0)	103.0±10.8[a] (64.0)

n=5, mean± SD [a]P<0.05; compared to diabetic pre-treatment values (t-Test); Superscripted items ([a]) indicate significant values when compared to diabetic pre-treatment values. 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 (200mg/kg).

Value in parenthesis represents reduction (%) of blood glucose calculated for treatment groups relative to diabetic pre-treatment values.

Table 5. Effect of methanol leaf extract of *P. amarus* on cholesterol, triglycerides and haemoglobin levels of diabetic rats (n=5; mean ± SD.)

Treatment	Dose mg/kg	Parameters	Total Cholesterol (mg/dl); Triglycerides (mg/dl); Haemoglobin (g%)			
			PreDiabetic (Basal)	Diabetic (Pre-Rx)	Diabetic Post-Rx	
					Day 14	Day 28
Control (NDNT)	2ml/kg	Cholesterol Triglycerides Hb	121.4±6.3 113.0±4.2 14.4±1.0	121.0±5.7 114.0±5.1 13.2±1.2	123.0±6.9 (-1.7) 117.6±5.3 (-3.2) 13.8±0.7	122.0±6.0 (-0.8) 119.0±4.4 (-4.4) 13.5±0.6
Control (NDT)	200	Cholesterol Triglycerides Hb	124.6±3.4 103.2±11.0 15.7±1.4	119.4±4.7 95.6±6.4 14.1±1.2	114.6±4.3[a] 97.8±8.6 (-2.3) 15.2±1.0	109.2±7.3[ab] 95.2±8.0 (0.4) 14.9±1.0 (0.4)
Glibenclamide	0.2	Cholesterol Triglycerides Hb	121.0±9.7 97.6±10.7 14.5±0.9	130.0±7.1 118.0±2.9 13.5±0.5	123.6±17.3 (4.9) 107.8±4.8[b] (8.6) 12.7±0.8[a]	118.0±15.0 (9.2) 97.8±3.3[b] (17.1) 12.6±0.6[ab]
Methanol Extract	200	Cholesterol Triglycerides Hb	118.4±7.1 110.0±11.3 15.7±1.8	148.0±8.5 147.2±11.4 14.9±1.6	106.8±7.9[ab] (27.8) 137.8±8.2[a](6.4) 14.2±1.4	89.2±8.5[ab] (39.7) 120.8±6.6[b] (17.9) 13.4±1.0[a]
Methanol Extract	400	Cholesterol Triglycerides Hb	126.0±8.6 116.8±8.1 15.4±1.1	150.8±6.9 148.2±7.1 14.9±0.8	103.6±13.5[ab] (31.3) 126.4±12.4[b] (14.7) 14.1±1.1	78.0±7.2[ab] (48.3) 113.6±10.8[b] (23.4) 14.1±1.4

n=5, mean± SD. [ab]P<0.05 compared to Basal and Diabetic pre-treatment values respectively (t-Test); Superscripted items ([ab]) indicate significant values when compared to control and pre-treatment values respectively. 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 (200mg/kg).

respectively. 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 (200mg/kg).

significant difference (Table 6).

Effect of chronic administration of methanol extract of Phyllanthus amarus on body weight of diabetic rats

There was increase in the body weight of all the groups on day 14 and the increase continued on day 28 with the 400mg/kg dose of ME showing significant difference ($P<0.05$). The weight increase occurred most in the NDT control group followed by the NDNT control group and then the 400mg/kg dose of ME (Table 7).

Morphological and histopathological observation

There were changes in the pancreas, kidney and liver of the animals used in the study. However, the extract-treated animals recovered from the effect of alloxan more than rats treated with glibenclamide (Figures 1-6).

Table 6: Effect of methanol leaf extract of *P. amarus* on the Red Blood Cell (RBC) and White Blood Cell (WBC) counts of diabetic rats (n=5; mean ± SD.)

Treatment	Dose mg/kg	Parameters	RBC ($X10^6$/µL); WBC ($X10^3$/µL)			
			PreDiabetic (Basal)	Diabetic (Pre-Rx)	Diabetic Post-Rx	
					Day 14	Day 28
Control (NDNT)	2ml/kg	RBC WBC	5.1±0.1 4.7±0.3	4.9±0.1 4.7±0.3	4.6±0.1[ab] 4.8±0.3	7.0±0.8[ab] 4.8±0.3
Control (NDT)	200	RBC WBC	5.2±0.1 4.8±0.3	5.2±0.03 4.7±0.3	5.0±0.1[ab] 4.7±0.2	4.9±0.04[a] 4.9±0.03[ab]
Glibenclamide	0.2	RBC WBC	5.5±0.3 4.9±0.6	5.3±0.3 4.9±0.4	4.7±0.3[ab] 4.8±0.6	5.4±0.2 5.0±0.6
Methanol Extract	200	RBC WBC	5.2±0.1 4.8±0.3	5.1±0.1 4.8±0.3	4.8±0.1[ab] 4.9±0.3	5.4±0.2[ab] 5.0±0.3
Methanol Extract	400	RBC WBC	4.8±0.4 4.9±0.2	4.6±0.3 4.9±0.1	4.6±0.1 5.0±0.1	4.9±0.1 5.1±0.2[b]

[ab]$P<0.05$ compared to Basal and Diabetic pre-treatment values respectively (t-Test); Superscripted items ([ab]) indicate significant values when compared to control and pre-treatment values respectively. NDT = Non diabetic treated was a non diabetic control and received methanol extract (200mg/kg); NDNT = Non diabetic non treated was a non diabetic control and received the vehicle.

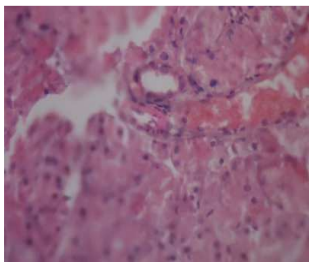

Figure 1: Pancreas of extract-treatec rat showing no histological changes

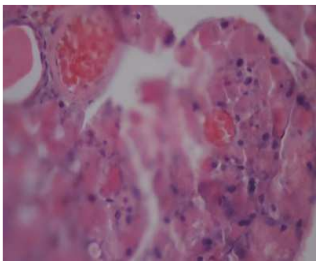

Figure 2: Pancreas of glibenclamide treated rat showing slight necrosis and enlargement

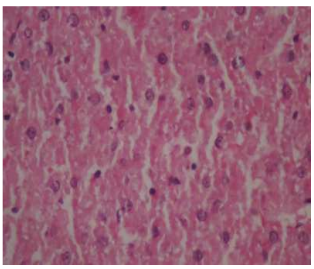

Figure 3: Liver of extract-treated rat showing no histological changes

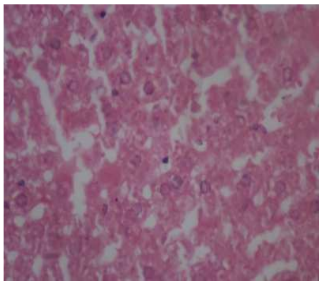

Figure 4: Liver of glibenclamide treated rat showing slight necrosis and enlargement

Table 7: Effect of chronic administration of Methanol extract of *P. amarus* on body weight of diabetic rats

Treatment	Dose mg/kg	Body weight (g)			
		Pre-Diabetic (Basal)	Diabetic (Pre-Rx)	Diabetic Post-Rx	
				Day 14	Day 28
Control (NDNT)	3ml/kg	204.1±29.6	197.2±30.9	218.6±14.5 (10.9)	224.0±12.4 (13.6)
Control (NDT)	200	220.5±20.4	212.9±23.0	222.6±20.7 (4.6)	225.0±19.9 (5.7)
Glibenclamide	0.2	211.8±15.2	206.8±17.1	212.0±15.1 (2.5)	217.0±15.5 (4.9)
Methanol Extract	200	228.2±25.2	223.4±30.8	236.0±17.9 (5.6)	239.0±17.7 (7.0)
Methanol Extract	400	168.4±20.7	163.4±18.5	174.0±19.2 (6.5)	175.0±17.3 (7.1)

n=5, mean± SD. NDT = Non diabetic treated was a non diabetic control and received ME (200mg/kg); NDNT = Non diabetic non treated was a non diabetic control and received the vehicle.
Value in parenthesis represents percentage increase (%) of body weight calculated for treatment groups relative to diabetic pre-treatment values.

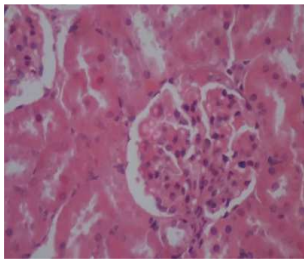

Figure 5: Kidney of extract-treated rat showing no histological changes

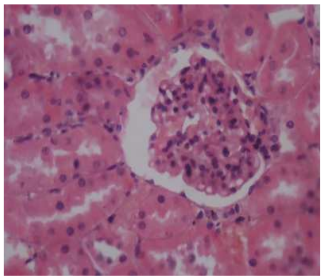

Figure 6: Kidney of glibenclamide treated rat showing no histological changes

DISCUSSION

Diabetes mellitus (DM) is a chronic disease with complex underlying etiologies. The incidence of diabetes mellitus is on the rise worldwide. Based on the World Health Organization (WHO) report, the number of diabetic patients is expected to increase from 171 million in year 2000 to 366 million or more by the year 2030[21]. Herbal medicine has been used as an antidiabetic therapy alone, along with insulin or other synthetic oral hypoglycemic agents. The use of synthetic agents, on the other hand, has shown several undesirable side effects and has failed to correct the fundamental biochemical lesion and diabetic complications[22, 23].

Phytochemical analysis of the leaves of *Phyllanthus amarus* showed the presence of alkaloids, anthraquinones, cardiac glycosides, tannins, saponins, and flavonoids. Flavonoids and tannins are phenolic compounds and plant phenolics are also a major group of compounds that act as primary antioxidants or free radical scavengers[24]. Tannins and saponins are also found to be effective antioxidants, antimicrobial, and anti-carcinogenic agents[25]. Polyphenolic compounds are ubiquitous in foods of plant origin, and thus they constitute an integral part of the human diet[26]. Interest in polyphenols has greatly increased recently because these phytochemicals are known to suppress rates of degenerative processes such as cardiovascular disorders and cancer[26, 27]. Some of these potential health

benefits of polyphenolic substances have been related to the action of these compounds as antioxidants, free radical scavengers, quenchers of singlet and triplet oxygen and inhibitors of peroxidation[28]. Thus as a group, phenolic compounds have been found to be strong antioxidants against free radicals and other reactive oxygen species, the major cause of many chronic human diseases[29].

The results of the acute oral administration of *Phyllanthus amarus* to mice indicated that the plant is non toxic even at the dose of 1600mg/kg body weight indicating that the LD_{50} is greater than 1600mg/kg. It thus showed that this plant is safe for medicinal use.

Experimental evaluation of the anti-diabetic potentials of *P. amarus* from this study 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. 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[30, 31]. 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 antidiabetic activity studies, daily oral administration of the extract for 28 days produced a gradual but sustained reduction in blood glucose levels in diabetic rats. 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 rats[32]. Experimentally, alloxan-induced diabetic hyperglycaemia is accompanied by increase in serum cholesterol and triglyceride levels[18, 33, 34] 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 dislipidaemia. The extract also produced better changes in the levels of haemoglobin, red and white blood cell counts relative to the reference drug. The extract also caused significant increase in body weight and this is important because an association exists between obesity and diabetes mellitus especially that poor glycaemic results in weight loss[35, 36]

Several factors such as oxidative stress[37], chronic hyperglycaemia[38] and autoimmune[39] or fibrocalculous[39] types of chronic pancreatitis damage the pancreas and impair insulin secretion and hence glycaemic control. Results of histological studies on pancreas isolated from treated diabetic rat showed that the extract may have repaired the pancreas damaged by alloxan. Alloxan causes diabetes by destruction of ß-cells of the islet[40] 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. Also, the extract was able to restore the damaged kidney and liver to their normal architecture. Glibenclamide used as a reference hypoglycemic agent did not cause such effect to the same extent as the extract (Figures 1-6). The precise mechanism of this tissue repair is not known but since the plant is rich in flavonoids, there are chances that this and other constituents present may be responsible for the hypoglycaemic effect noted in this study.

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