Chapter 5

The Antidiabetic Activities of the Aqueous Leaf Extract of Phyllanthus Amarus in some Laboratory Animals

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Additional information is available at the end of the chapter

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1. Introduction

Diabetes mellitus (DM), often simply referred to as diabetes, is a group of metabolic diseases in which a patient has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). It is characterized by hyperglycaemia due to defective insulin action, insulin secretion or both. Several medicinal plants are used in the management of diabetes mellitus (Akah et al., 2002). According to the World Health Organization (WHO), there are approximately 160,000 diabetics worldwide, the number of diabetics has double in the last few years and is expected to double once again in the year 2025 (Beretta, 2001). Due to its high prevalence and potential deleterious effect on a patient physical and psychological state, diabetes is a major medical concern (Macedo et al., 2002). The disease remains incurable and can only be controlled with drugs. The three main types of diabetes mellitus (DM) are: Type 1 DM results from the body's failure to produce insulin, and presently requires the administration of insulin for treatment (Lambert et al., 2002). It is also referred to as insulin-dependent diabetes mellitus (IDDM) or "juvenile" diabetes). Type 2 DM results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency (Boussageon et al., 2011). It is formerly referred to as noninsulin-dependent diabetes mellitus (NIDDM) or "adult-onset" diabetes). Gestational diabetes occurs when pregnant patient, who have never had diabetes before, have a high blood glucose level during pregnancy. It may precede development of type 2 DM (Sattar et al., 2010).

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Several plants have been used in folkloric medicine for the treatment and prevention of infectious and non-infectious diseases in man and his animals and this has led to renewed scientific interest in the use of plants for these purposes (Oridupa et al. 2011). There is global resurgence in the use of herbal preparations and in some developing countries like Nigeria; it is being gradually integrated into the primary and secondary health care systems. Nearly all societies have used herbal materials as sources of medicines and the development of these herbal medicines depended on local botanical flora (Adedapo et al., 2009).

Phyllanthus amarus belongs to the family Euphorbiaceae (the spurge family) of which the largest genus is the genus Euphorbia. The plant is known to originate from Malaysia. The species *Phyllanthus amarus* is a small tropical herb, which occurs widely as a rainy-season weed throughout the hotter parts of India (Bagchi et al., 1992). The widespread usage of this herb has prompted several investigations (Calixto et al., 1998; Odetola and Akojenu, 2000; Adeneye et al., 2006). The plant has a history of use in Ayurverdic medicine for over 2000 years as well as a wide variety of traditional applications. The plant is commonly found in Southern Nigeria, Sierra Leone and Equatorial Guinea. It also occurs in Ghana and other parts of tropical Africa (Irvine, 1930; Adedapo et al., 2005). It is a weed of cultivated land and in waste spaces, it is common to find it growing and spreading freely along the road sides, under flower beds and in many other places (Burkill, 1994). For this reason, grazing animals are prone to consuming this plant along with their feed particularly in drier tropical climates where lush green grass is not often available (Adedapo, 2002).

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 aqueous leaf extract of *Phyllanthus amarus* Schum in experimental animals especially that diabetes has assumed a global dimension as a non-communicable disease.

2. Material and methods

2.1. Plant material and preparation of extracts

Fresh leaves of *Phyllanthus amarus* Schum were collected from the campus of the University of Ibadan, Nigeria in March 2012. The leaves were identified by botanists and a voucher specimen (UIH ADE/003/2012) deposited at the herbarium of the Department of Botany, University of Ibadan. The ground plant material (200 g) was shaken in 1 L distilled water for 48 h on an orbital shaker at room temperature of 24°C. The extract was filtered using a Buckner funnel and Whatman No 1 filter paper. The filtrate was concentrated to dryness under reduced pressure at 40ºC using a rotary evaporator. The thick solution was lyophilized using freeze drying system for biological investigations. The extract yield was 13.85%.

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2.2. 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.

2.3. Chemicals

Alloxan used in this study 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.

2.4. Phytochemical screening

The phytochemical analysis was performed on the ground (powered) leaf of *P. amarus* for identification of the constituents. The constituents tested for were alkaloids, tannins, saponins, anthraquinones, cardiac glycosides and flavonoids as described by Trease and Evans (1983); Abate (1989); Shale et al., (1999); Evans, (2002); Moody et al., (2006) and Sawadogo et al., (2006).

2.5. Acute toxicity test

The acute toxicity of *P. amarus* aqueous was determined in mice according to the method of Hilaly *et al.* (2004) with slight modifications. Mice fasted for 16 h were randomly divided into groups of six mice per group. Graded doses of the plant's extract (100, 200, 400, 800 and 1600 mg/kg p.o.) were separately administered to the mice in each of the groups by means of bulbed steel needle. All the mice 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.

3. Antidiabetic studies

3.1. Hypoglycaemic activity test

The hypoglycaemic effect of the aqueous extract 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) (Aruna *et al.,* 1999) 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 aqueous extract (200 and 400 mg/kg), Distilled water (5ml/kg) and Glibenclamide (0.2 mg/kg) respectively. Aqueous extract was dissolved in distil water. Blood glucose was then measured before (i.e. 0 h) and at 0.5, 1, 2 and 4 h after treatment.

3.2. 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 (5ml/kg) respectively. The blood glucose level of each animal was measured prior to (pretreatment) and at 0.5, 1, 2 and 4hour after extract administration (Okoli et al., 2010).

3.3. Oral glucose tolerance test

Animals were fasted for 16h but with free access to water were randomly divided into four groups (n=5) and received oral administration of the aqueous extract (200 and 400mg/kg), glibenclamide (0.2mg/kg) and vehicle control (5ml/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 (Okoli et al., 2010).

3.4. 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 (Okoli et al., 2010). 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 (5ml/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 (pretreatment) and on days 14 and 28 after commencement of the treatment. The body weight of each animal was also measured on these days.

3.5. 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 (Muller *et al.,* 1977) using reagent kits. Lipid levels of diabetic animals were measured before (Basal) and after the induction of diabetes (pre-treat-

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ment) 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.

3.6. Effects of the extract on haemoglobin and cell counts of diabetic rats

The effect of chronic administration of the extract 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).

3.7. Estimation of haemoglobin

Cyanohaemoglobin method was used for this purpose (Coles, 1986). Four mls of Drabkin´s solution (diluents) was placed in a tube, 0.02ml of the collected blood sample was put in the tube using pipette and the pipette was rinsed for more than three times. The mixture was stirred well and allowed to stand for 10minutes. This was read in a colorimeter at 540nm wavelength. The equivalent haemoglobin was read from a calibration curve or table. The haemoglobin value of the blood sample was calculated as:

Photometer reading of unknown x g% Hb value of standard x DF/Photometer reading of standard

DF = dilution factor and it was calculated as:

Volume of the whole blood used + volume of diluent/volume of whole blood used.

It is expressed in gram percent.

3.8. Determination of erythrocyte (rbc) count

Erythrocyte diluting pipette marked 101 above the bulb was used to draw the blood sample up to exactly 0.5mark. The tip of the pipette was then inserted into the erythrocyte diluting fluid - Gower´s solution and through a steady suction; the pipette was filled with the fluid to the 101 line above the bulb, rotating it gently while filling. The pipette was brought to a horizontal position and finger tip was placed over the tip before removing the rubber tubing. The pipette was shaken for more than 30minutes in a mechanical shaker; the haemocytometer was then filled with the diluted blood and then allowed to stand for a few minutes for the cells to settle. The erythrocytes were then counted under microscope lens (x 40 objective) counting all the erythrocytes in the 5 of the 25 small squares in the central area. Each of the 5 small squares to be counted was bordered by double or triple lines and was divided into 16 smaller squares. A total of 80 of these small squares were counted. The cells were

counted beginning at the left of the top row of small squares, then from right to left for the next row and so on.

Calculation

Cells counted x 10(0.1mm depth) x 5(1/5 of sqmm) x 200(1:200) dilution = erythrocytes per cu mm.

OR

The sum of the cells in the five small squares multiplied by 10,000 = total erythrocytes per cu mm (Coles, 1986)

3.9. Determination of leucocyte (wbc) count

Leucocyte diluting pipetting was used to draw the blood sample to a point marked 0.5 and filled with leucocyte diluting fluid up to the 11mark above the bulb. The mixture was shaken for 3minutes until well mixed. Two to three drops from the pipette was discarded before filling the counting chamber of haemocytometer. The leucocytes were allowed to settle for 1minute. The leucocytes in the larger squares of haemocytometer chamber were counted and multiplied by 50 to obtain the total number of white blood cells (Coles, 1986).

Calculation:

Cells counted x 20 (1:20 dilution) x 10(0.1mm depth)/4 (no of sq mm counted)

= WBC/cubic mm

OR

The sum of the cell counted in the 4 corner squares multiplied by 50 = total leucocytes per cubic mm.

4. Histological studies on the liver, kidney and pancreas

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 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 for 24 hrs. After 24 hrs, the tissues were solidified in molten wax and sectioned using automatic tissue sectioner. The tissue sections were then fixed on slides with haematoxylin and eosin. The stained slides were fixed with mountant, allowed to dry and viewed under the microscope (x400). This procedure was repeated for the liver and kidney collected from the sacrificed animals.

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4.1. Statistical analysis

Data was analyzed using graph pad prism 5 and the results expressed as mean ± SD. The results were further subjected to one way ANOVA for comparisons and differences between means were considered significant at $P< 0.05$.

5. Results and discussion

Phytochemical screening of the leaves of *P. amarus* showed the presence of alkaloids, tannin, flavonoids, saponin, anthraquinones and cardiac glycosides. 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 (Adedapo et al., 2008a, 2008b, Ayoola et al., 2008). Tannins and saponins are also found to be effective antioxidants, antimicrobial, and anti-carcinogenic agents (Lai et al., 2010). Polyphenolic compounds are ubiquitous in foods of plant origin, and thus they constitute an integral part of the human diet (Bravo 1998). Interest in polyphenols has greatly increased recently because these phytochemicals are known to suppress rates of degenerative processes such as cardiovascular disorders and cancer (Bravo 1998, Duthie 2000, Huang et al., 2007; Jimoh et al., 2010). 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 (Li-Chen et al., 2005). 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 (Kyung-Hee et al., 2005, Chen and Yen 2007).

In the 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. Acute oral administration of *Phyllanthus amarus* to mice indicated that the plant is non toxic even at the dose of 1600mg/kg body weight. It thus showed that this plant is safe for medicinal use at this dose.

The aqueous extract caused a significant ($P<0.05$) dose related reduction in the fasting blood glucose (FBG) of normoglycaemic rats. Maximum reduction occurred within 2hr post- treatment with 400mg/kg dose of the extract (Table 1). In this study, experimental evaluation of the antidiabetic potentials of *P. amarus* has shown that single oral administration of the extract to normal rats reduced fasting blood glucose which suggests an inherent hypoglycaemic effect (Table 2). 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 (Table 3). 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).

Treatment	Dose mg/kg	Fasting Blood Glucose level (mg/dl)				
		Pretreatment	0.5hr	1hr	2hr	4hr
Control	2ml/kg	72.4±5.5	71.2±6.0	70.6±5.6	70±5.1	70±4.7
Glibenclamide	0.2	72.2±4.1	66.6±3.4[b] (7.7)	62.0±6.9[b] (14.1)	54.0±3.4[ab] (25.2)	49.0±3.9[ab] (32.1)
Aqueous Extract	200	73.0±4.0	69.6±4.0 (4.7)	64.0±4.2[b] (12.3)	56.0±4.9[ab] (23.3)	50.6±5.8[ab] (30.7)

[ab]P< 0.05 compared to control and pre-treatment values respectively (t-test). Values in parenthesis represent reduction (%) in fasting blood levels glucose levels of normoglycaemic rats calculated relative to pre-treatment values.

Table 1. Effects of Aqueous extract of *P. amarus* on blood glucose of normoglycaemic rats (n=5; mean ± SD.).

Treatment	Dose mg/kg	Blood Glucose level (mg/dl)						
		0 min	30min	60min	90min	120min	150min	180min
Control	2ml/kg	61.4±1.9	74.4±4.6 (21.2)	78.0±7.0 (27.0)	73.2±5.8 (19.2)	69.8±5.5 (13.7)	63.2±7.1 (2.9)	59.6±5.1 (2.9)
Glibenclamide	0.2	65.0±2.8	67.8±2.7 (4.3)	70.2±4.0[a] (8.00)	66.8±4.1 (2.8)	64.2±7.8(1.2)	62.4±1.5(4.00)	61.8±1.8(4.9)
Aqueous Extract	200	62.6±6.7	73.2±7.5[a] (16.9)	80.2±6.7[a] (28.1)	72.8±4.5[a] (16.3)	64.2±3.5 (2.6)	55.4±5.8 (11.5)	49.4±4.6[a] (21.1)
Aqueous Extract	400	62.0±9.0	65.4±6.2 (5.5)	67.0±5.4 (8.1)	63.4±4.7 (2.3)	59.6±3.8 (3.9)	54.0±2.3 (12.9)	50.6±2.2[a] (12.4)

[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 2. Effects of aqueous extract of *Phyllanthus amarus* on oral glucose tolerance in rats (n=5; mean ± SD.)

Treatment	Dose mg/kg	Blood Glucose level (mg/dl)				
		Pretreatment	0.5hr	1hr	2hr	4hr
Control	2ml/kg	311.0±33.8	298.0±34.3	282.0±31.2	268.0±24.8	256.0±24.2
Glibenclamide	0.2	290.0±26.1	268.0±27.7 (7.6)	252.0±27.1 (13.1)	228.0±17.2[ab] (21.4)	208.0±16.3[ab] (28.3)
Aqueous Extract	200	296.0±39.3	276.0±32.2 (6.8)	256.0±35.1 (13.5)	230.0±36.9[b] (22.3)	204.0±43.1[ab] (31.1)
Aqueous Extract	400	276.0±24.2	256.0±20.6[a] (7.3)	236.0±20.6[ab] (14.5)	217.0±24.4[ab] (21.4)	200.0±18.7[ab] (27.5)

[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.

Table 3. Hypoglycaemic effects of aqueous extract of *P. amarus* on diabetic rats (n=5; mean ± SD.)

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Following oral administration of glucose, postprandial blood glucose levels of the control rats increased to the peak at 60min. Pre-treatment with aqueous extract (200 and 400mg/kg) suppressed the rise in blood glucose by 28.1 and 8.1% respectively. The aqueous extract used in this study evoked a progressive dose-dependent decrease in blood glucose level up to 180mins. Chronic oral administration of aqueous extract 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; while the extract at 200mg/kg exerts almost the same effect as glibenclamide. The highest reduction in the blood glucose was 60.9% and this was obtained with the 400mg/kg on 28th day (Table 4). In this study, 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). Effective and sustained reduction in blood glucose levels of treated diabetic rats by the extract indicates that the plant may be useful in overt cases of DM. 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 (Bavarva and Narasimhacharya, 2008).

Treatment	Dose mg/kg	Blood glucose concentration (mg/dl)			
		Pre-Diabetic (Basal)	Diabetic (Pretreatment)	Diabetic Post-Rx	
				Day 14	Day 28
Control (NDNT)	2ml/kg	57.4±6.2	58.8±5.8	69.2±4.6[a]	86.6±4.6[a]
Control (NDT)	200	54.8±8.4	56.8±8.7	70.8±7.4[a]	90.2±5.5[a]
Glibenclamide	0.2	56.2±5.2	290±26.1	148.0±13.3a (49.0)	122.0±9.3a (57.9)
Aqueous Extract	200	55.0±5.0	296.0±32.3	140.0±20.7a (52.7)	126±19.9a (57.4)
Aqueous Extract	400	53.4±3.3	276.0±24.2	132.0±17.2a (52.2)	107.8±5.0a (60.9)

[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 AE (200mg/kg). Values in parenthesis represent reduction (%) in blood glucose level calculated for treatment groups relative to diabetic pre-treatment values.

Table 4. Effect of Aqueous extract of *P. amarus* on blood glucose 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	122.4±7.3 114.0±5.7 14.6±0.5	124.6±5.20 115.8±7.4 13.8±0.6	124.0±9.7 (0.5) 126.4±14.7 (-9.2) 14.0±1.3	126.2±4.6 (-1.3) 117.6±13 (-1.6) 13.3±1.2
Control (NDT)	200	Cholesterol Triglycerides Hb	117.2±7.8 106.4±9.0 14.4±1.1	111.0±9.9 109.6±6.5 14.0±0.9	109.6±8.5 (1.3) 92.6±11.6[b] (15.5) 15.5±1.1	105.8±7.9[a] (4.7) 85.4±9.5[ab] (22.1) 14.1±2.3
Glibenclamide	0.2	Cholesterol Triglycerides Hb	120.8±8.3 104.2±11.2 14.8±1.0	115.0±14.1 119.4±7.5 13.9±0.8	118.8±8.6 (-3.3) 111.2±5.0 (6.0) 15.4±1.0[b]	110.6±5.2[a] (3.8) 104.0±4.3[b] (12.9) 14.3±0.4
Aqueous Extract	200	Cholesterol Triglycerides Hb	118.6±4.7 114.2±6.1 15.4±2.0	128.0±2.8 142.0±9.0 14.8±1.8	95.0±7.1[ab] (25.8) 124.8±5.2ab (12.1) 15.4±0.7	88.4±7.1[ab] (30.9) 109.6±7.6[b] (22.8) 15.2±1.1
Aqueous Extract	400	Cholesterol Triglycerides Hb	115.0±7.1 114.0±9.2 15.8±1.5	123.2±9.1 121.0±8.8 14.6±1.8	100.0±7.1[ab] (18.8) 115.2±7.8 (4.8) 15.2±0.8	87.0±5.1[ab] (29.4) 105.6±9.4[b] (12.7) 14.7±0.8

[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 aqueous extract (200mg/kg). Values in parenthesis represent reduction (%) of total cholesterol and triglycerides calculated for treatment groups relative to diabetic pre-treatment values.

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

Chronic administration of aqueous extract reduced total cholesterol level of diabetic rats. The aqueous extract caused a significant (P<0.05) reduction in the total cholesterol of treated diabetic rats. The magnitude of reduction was greater than that evoked by glibenclamide. Chronic administration of aqueous extract reduced triglyceride concentration of the diabetic rats. The aqueous extract caused a significant (P<0.05) reduction in the triglyceride level of treated diabetic rats. The magnitude of reduction was greater than that evoked by glibenclamide. The haemoglobin level of all the animals was increased initially on day 14 with no significant difference except for the glibenclamide-treated animals. Subsequently, there was reduction in the haemoglobin level on day 28 (Table 5). 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;

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Ahmed et al., 2001; Okoli et al., 2010) 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 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 aqueous extract. Subsequently, there was increase in the RBC count on day 28 with all the groups showing significant difference with the exception of the 400mg/kg dose of the aqueous extract. The white blood cell (WBC) count of all the control and glibenclamide- treated animals was reduced initially on day 14 with no significant difference; while there was increase in the WBC count of the extract-treated group on day 14 and the increase continued on day 28 with the 400mg/kg dose of the extract showing significant difference. Likewise, there was subsequent increase in the control (both NDT and NDNT) groups and glibenclamide-treated group on day 28 with no significant difference (Table 6). Again, assessment of the effect of chronic administration of the extracts on haemoglobin level as well as white blood cell and red blood cell counts revealed an increase following an initial reduction in most cases of the experiment. It is not clear if it would progress to a return to basal levels and how long it may take.

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	5.2±0.1	4.9±0.1	4.6±0.1[ab]	6.8±0.7[ab]
		WBC	4.8±0.3	4.8±0.3	4.8±0.3	4.9±0.3
Control (NDT)	200	RBC	5.2±0.1	5.1±0.03	4.9±0.1[ab]	5.0±0.2a
		WBC	5.0±0.3	4.9±0.3	4.8±0.3	5.1±0.03
Glibenclamide	0.2	RBC	5.6±0.2	5.3±0.3	4.8±0.3ab	5.4±0.2
		WBC	4.7±0.6	5.0±0.5	4.7±0.2	4.8±0.1
Aqueous Extract	200	RBC	5.2±0.1	5.1±0.1	4.8±0.2ab	5.4±0.1ab
		WBC	4.8±0.3	4.8±0.3	5.0±0.3	5.1±0.3
Aqueous Extract	400	RBC	4.8±0.4	4.6±0.3	4.6±0.1	4.8±0.1
		WBC	4.9±0.2	4.8±0.2	5.0±0.1	5.2±0.1[ab]

[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 aqueous extract (200mg/kg); NDNT = Non diabetic non treated was a non diabetic control and received the vehicle.

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

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 aqueous extract 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 aqueous extract (Table 7). Due to the association of obesity with DM, weight control is an important aspect of diabetes manage-

ment. Poor glycaemic control usually results in weight loss. The 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 has modest weight gain. In some cases however, 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). The result showed that at 400mg/kg dose, aqueous extract showed a significant increase in body weight. It is also important to note that chronic administration of the extracts did not inhibit the natural growth process of these animals with or without diabetes.

Treatment	Dose mg/kg	Body weight (g)			
		Pre-Diabetic (Basal)	Diabetic (Pre-Rx)	Diabetic Post-Rx	
				Day 14	Day 28
Control (NDNT)	2ml/kg	214.0±11.2	209.0±14.1	226.6±10.4 (8.4)	231.8±7.68 (10.91)
Control (NDT)	200	135.0±36.9	137.0±35.9	150.0±37.5 (8.7)	157.0±38.43 (14.60)
Glibenclamide	0.2	191.0±34.6	188.0±37.9	196.0±33.9 (4.3)	200.0±32.6 (6.4)
Aqueous Extract	200	196.0±18.1	192.0±18.2	206.6±20.9 (7.6)	211.0±22.2 (9.9)
Aqueous Extract	400	214.0±15.0	212.0±13.0	228.0±7.8[b] (7.6)	234.0±5.8[ab] (10.4)

[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 AE (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.

Table 7. Effect of chronic administration of aqueous extract of *Phyllanthus amarus* on body weight of diabetic rats (n=5; mean ± SD.).

Histological examination of the pancreas shows the necrosis of the islet tissues with the alveolar cells moderately destroyed; there was also moderate congestion of the blood vessels (Figure 1) in the diabetic non-treated group. In the extract-treated group, the architecture of the pancreas appeared intact. The interlobular, intralobular and the alveolar granules were seen (Figure 2). There was slight necrosis of the pancreas around the islet tissues in the glibenclamide-treated group (Figure 3). Microscopical examination of liver section of diabetic non-treated group (Figure 4) showed various degrees of pathological changes such as centrilobular fatty degeneration, cloudy swelling, and vacuolar change of the hepatocytes as well as necrosis of hepatic cells. Microscopical examination of liver section of diabetic extract-treated control group (Figure 5) showed normal arrangement of hepatocytes with clear broad of central vein at portal layer. The histopathological study showed recovery of the damaged liver cells in the extract- treated group. The liver of the glibenclamide-treated group showed widespread vacuolar change of the hepatocytes (figure 6). Microscopical ex-

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amination of the kidney section of diabetic non-treated control showed moderate loss of renal tubules and congestion of renal blood vessels in the medulla (figure 7). There was no visible lesion seen in the extract-treated (figure 8). There was no visible lesion seen in the glibenclamide-treated group (Figure 9). Several factors such as oxidative stress (Hayden et al., 2005), chronic hyperglycaemia (Leung and Leung, 2008) and autoimmune (Yoshida et al., 1995) or fibrocalculous (Mohan et al., 2008) 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 (Szudelski, 2001; Frode 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. 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 1-9). 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 (Jagetia 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 ß-cells through the formation of reactive oxygen species that cause tissue damage (Lee et al., 2008).

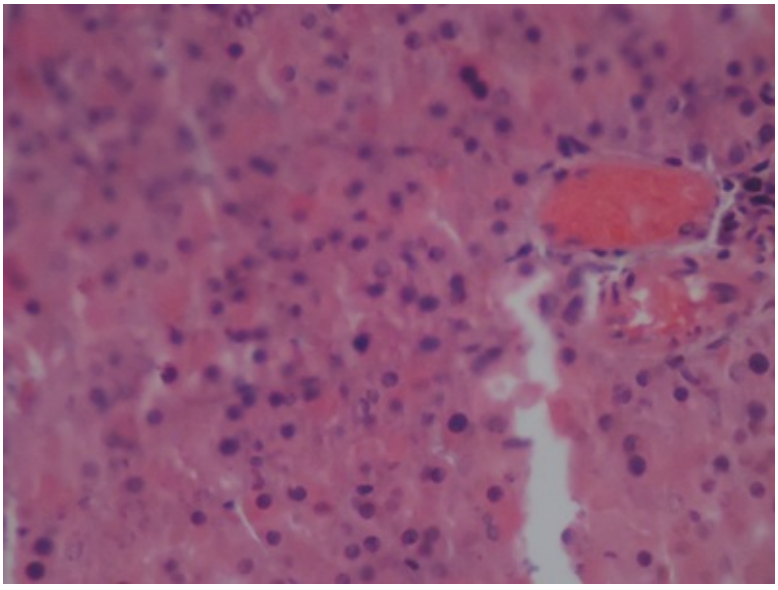


Figure 1. Pancreas of diabetic non-treated rat (X400)

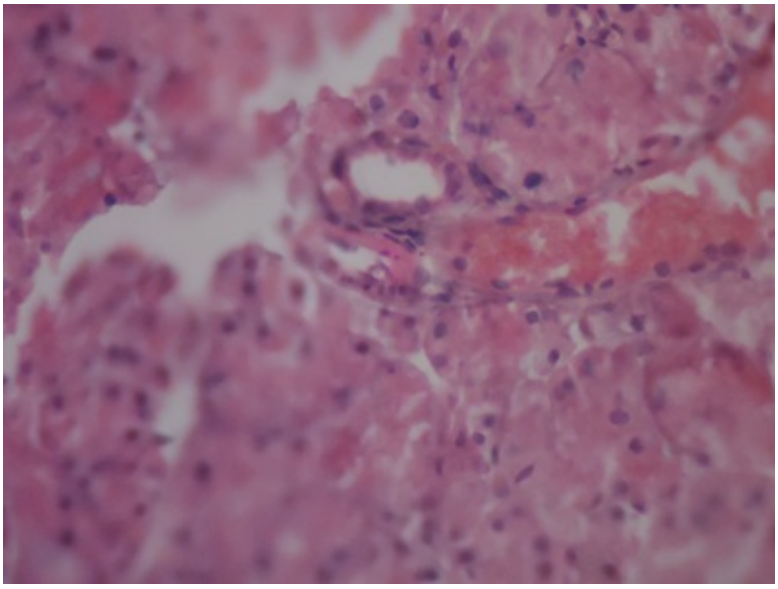


Figure 2. Effect of *P. amarus* extract on the pancreas of alloxan-induced diabetic rats (X400)

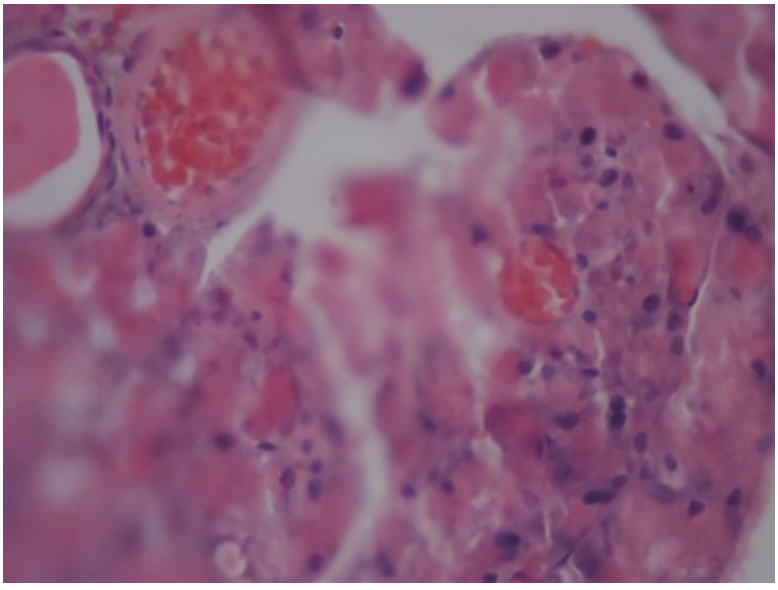


Figure 3. Effect of glibenclamide on the pancreas of alloxan-induced diabetic rat (X400)

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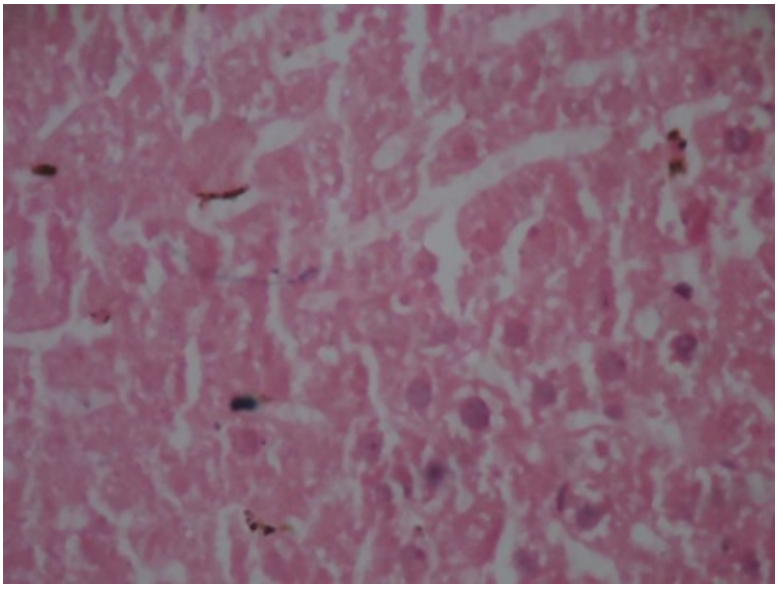


Figure 4. Liver section of diabetic non-treated group (X400)

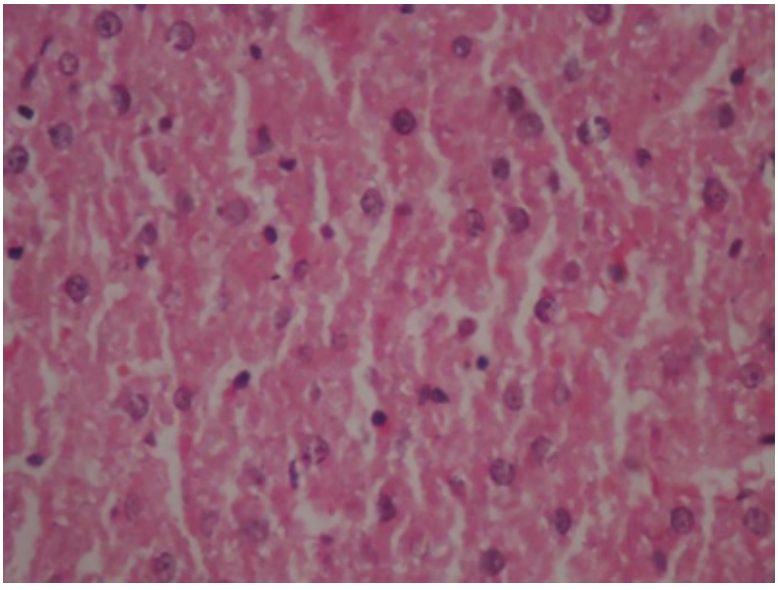


Figure 5. Effect of *P. amarus* extract on the liver of alloxan-induced diabetic rats (X400)

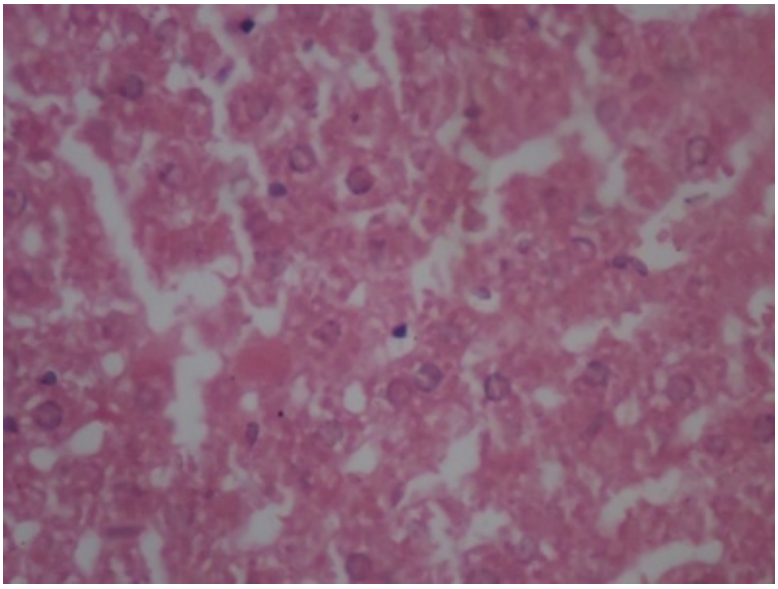


Figure 6. Effect of glibenclamide on the Liver of alloxan-induced diabetic rat (X400)

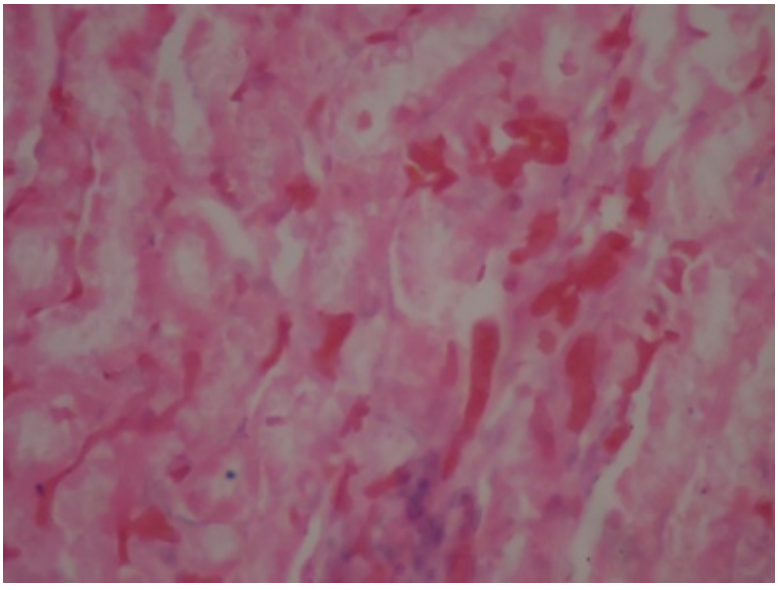


Figure 7. Kidney section of diabetic non-treated group (X400)

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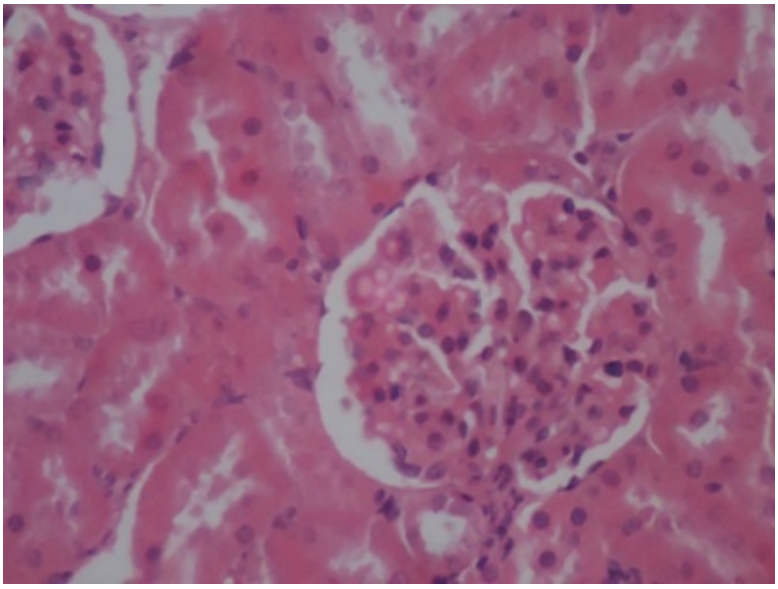


Figure 8. Effect of *P. amarus* extract on the kidney of alloxan-induced diabetic rats (X400)

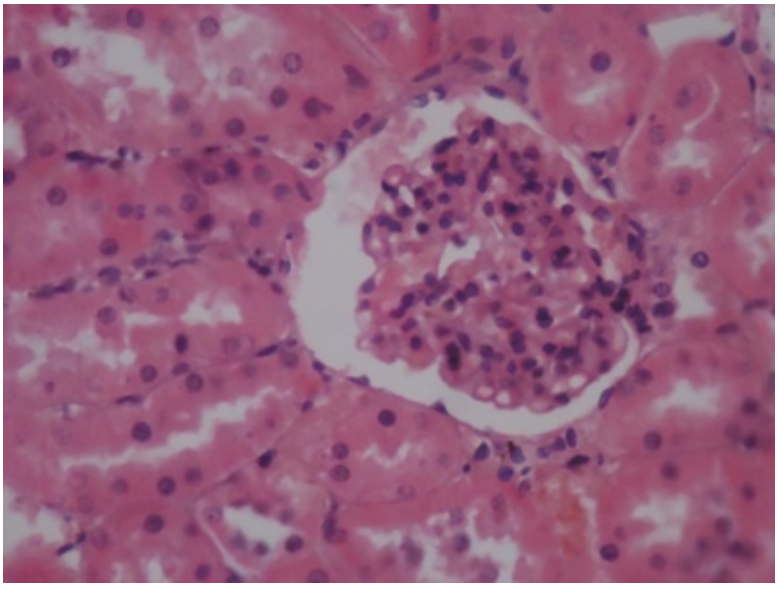


Figure 9. Effect of glibenclamide on the kidney of alloxan-induced diabetic rat (X400)

6. Conclusion

While the antidiabetic 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, glycosides and tannins which are typical plant constituents. Thus there are chances that any of these constituents may possess anti-diabetic properties. The histological studies also indicated that 0.2mg/kg of glibenclamide produced some histological changes whereas the 200 and 400 mg/kg doses of the plant extracts did not produce any histological change. This showed that this plant is not only effective as medicinal agent but also has high safety margin.

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The authors declare that we have no conflict of interest to declare

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