



Acute Toxicity and Anti-Diabetic Studies of the Aqueous and Alcoholic Extracts of *Phyllanthus amarus* in Albino Rats

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ABSTRACT

This study was carried out on the acute toxicity and anti-diabetic effect of the extracts of *Phyllanthus amarus* on blood glucose concentration (BGC) of normal and alloxan-induced diabetic rats. The study was done in the extracts alone and in combination of the extracts with glibenclamide drug. The acute toxicity test of the plant extracts gave a lethal dose of 3400mg/kg in mice. The anti-diabetic effect of the plant extracts was dose-dependent. Ethanolic extract alone (300mg/kg and 600mg/kg) caused a reduction in BGC of 18% and 23.5% ($p < 0.05$) respectively in non-diabetic rats. Aqueous extract (300mg/kg) gave 23.6% and (600mg/kg) 25.8% ($p < 0.02$). Glibenclamide alone gave 37.5% reduction ($p < 0.01$). The simultaneous administration of the plant extracts 300mg/kg with 5mg/kg glibenclamide gave 34.4% reduction (ethanolic) and 36.5% (aqueous), 600mg/kg extracts caused reduction of 37.6% and 38.4% ethanolic and aqueous respectively on non-diabetic rats ($p < 0.01$). The percentage reductions in BGC in alloxan induced diabetic rats were 15.8% and 27.7% ($p < 0.01$) for 300mg/kg ethanolic and aqueous extracts alone respectively. The extracts alone 600mg/kg gave 26.7% ($p < 0.05$) ethanolic and 29.9% ($p < 0.01$) aqueous. The extracts 300mg/kg in combination with 5mg/kg glibenclamide gave a percentage reduction of 35.5% ($p < 0.01$) ethanolic, 37.4% ($p < 0.001$) aqueous while 600mg/kg gave 39.2% and 58.1% reduction for ethanolic and aqueous extracts respectively ($p < 0.001$). Glibenclamide administered alone on diabetic rats gave 43.8% reduction ($p < 0.01$). However, the higher percentage reduction were obtained with the dose of 600mg/kg, also aqueous extract in combination with 5mg/kg glibenclamide gave higher percentage reduction in BGC in alloxan induced diabetic rat.

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Introduction

The use of plants for medicinal purposes has been dated back to antiquity (Ogunyemi, 1979; Patlak, 2002). From creation plants have provided man with real and supposed means of healing (Etang, 1999). World Health Organization (WHO) (1991) has estimated that 80% of the inhabitants of the entire world rely mostly on traditional medicines for the treatment of various ailments, which in many cases, by trial and error, proved effective (Akerele, 1984, Soforowa, 1982). This may explain the current emphasis on the use, formulation and standardization of herbal medicine. Herbs are natural agents which are harmless in an ordinary dose as compared to man-made chemicals for prevention, treatment and control of diseases. Nature has been blessed with a whole store house of natural remedies to cure the ills of all mankind, it simply remained for man to discover them and to put them to good use (Henri, 1942).

Plants are known to contain some bioactive components and the use of these plants as drugs by ethno-medicine practitioners has raised the name medicinal plants. Medicinal plants and herbs contain substances known to modern and ancient civilization for their healing properties. Sofowora (1982) and Itah (1996), observed that the traditional medicine healers plays vital roles in the health care delivery system in Africa in general and Nigeria in particular.

Extracts from various plants and herbs have been used in the treatment of ailment ranging from fever to eczema, tumors, abdominal pains, hypertension, diabetes, mental disorder, malaria, sickle cell anemia and many others (Okide, *et al.*, 2000).

Diabetes mellitus is a metabolic hereditary diseases characterized by hyperglycaemia and disturbances of carbohydrate, fat and protein metabolism due to absolute or relative lack of insulin (Pamela, *et al.*, 2005; Emdex, 2006). There are three main forms of diabetes mellitus, these are Type 1, 2 and gestational diabetes (occurring during pregnancy). These have similar sign, symptoms and consequences, but different causes and population distribution.

Diabetes can cause many complications. These are acute complications (hypoglycaemia, ketoacidosis, or non-ketotic hyperosmolar coma) which may occur if the disease is not adequately controlled. Long term complication include cardiovascular disease, chronic renal failure retinal damage (which can lead to blindness), nerve damage and microvascular damage which may cause erectile dysfunction (impotence) and poor healing of wounds.

The increasing prevalence of *diabetes mellitus* among the countries in the world and specifically in our rural areas, calls for research into more herbs for the management of this disease and *Phyllanthus amarus* is being used presently in Akwa Ibom State and in Nigeria as a whole for the treatment. Hence the aim of this work is to determine the effect of aqueous and alcoholic

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(ethanolic) extracts of *Phyllanthus amarus* on diabetic rats, and to compare the effect of combination of the plant extract with commercial available anti-diabetic drugs (Glibenclamide).

Material and methods

Sample collections, preparations and preservation

The entire samples comprising the stem, root, leaves, fruit, seeds and flowers of the plant *P. amarus* was collected from cultivated farmland in Uyo Capital City, Akwa Ibom State. The plant was identified traditionally by the chief herbalist, Mr. Abia Williams in the Faculty of Pharmacy, scientifically by Mr. Bala Danladi and Mr Okon Etefia, technologists in the department of Pharmacognosy, Faculty of Pharmacy, University of Uyo. The plant was separated from other weeds and dirt, washed with ordinary water and rinsed with distilled water. It was sundried for 5 days and then ground with pestle and mortar into coarse powder and packed in an air tight plastic container pending extraction.

Preparation of the Plant Extracts

About 150g of the sample was weighed into a 2 litre conical flask and was extracted with 70% ethanol at room temperature (27°C) for 72 h with occasional shaking. Aqueous extract was obtained by extracting the weighed sample in distilled water directly. Fresh sample, crushed and uncrushed dried samples were also treated as above. Each extract was filtered using a clean muslin cloth and coloured filtrates were obtained. The filtrates were concentrated at temperature between 40 – 50 °C using water bath. The raw extracts were labeled accordingly, cooled and stored in a desiccator.

Acute Toxicity Test LD₅₀

The acute toxicity test (LD₅₀) was carried out using the method outlined by Lorke (1983). This method involves an initial dose-range determination stage in which twenty-one albino mice weighing between (14-20g) were used. The plant extracts were dissolved in distilled water and doses of 100, 500, 1000, 3000, 4000, 4500 and 5000 mg/kg were administered to the appropriate groups of mice. The extract was given by intraperitoneal injection and observed within twenty four (24) hours. The LD₅₀ was then calculated as the geometric mean of the dose killing none of the animals and the one killing all the animals. (Osadebe *et al.*, 2004).

Anti-diabetic Evaluation of *Phyllanthus amarus*

The anti-diabetic study was carried out using the method recommended by Ali, 1997; Osadebe *et al.*, 2004; Eseyin *et al.*, 2005.

Experimental Animals

More than 80 albino rats (120-220g), obtained from the animal house, University of Jos at a tender age and bred in the animal house of the University of Uyo, were used for the anti-diabetic evaluation. The rats were housed in standard cages and had free access to food and water. They were fasted for 24 hours before the experiment.

Induction of diabetes

The total number of 40 albino rats were made diabetic by intraperitoneal injection of alloxan-monohydrate, at dose 150mg/kg dissolved in distilled water. The experimental animals were fasted overnight before the alloxan injection. The alloxan induced animals were allowed to rest for 4 days for the stabilization of glucose level with free access to both food and water. All surviving animals with glucose level greater than 150mg/dl were considered diabetic.

Blood Collections

Blood samples (0.1ml) were collected from the tail vein of the rats for the glucose concentration determination before and after induction of diabetes with alloxan.

Effect of Administration of varying doses of the aqueous and ethanolic plant extracts and glibenclamide on blood glucose concentration (BGC) in non-diabetic albino rats, alone and in combination

Procedure: The method followed was as described by Ali (1997), Osadebe *et al.* (2004) and Eseyin *et al.*, (2005). Rats were divided into ten groups of 4 rats in each group and fasted overnight before and throughout the experiment. The doses were considered based on the result from the acute toxicity test (LD₅₀) and method described by Mainen *et al.*, (2001). A total number of 40 rats were used and were divided into 10 groups and treated as follows:

Group 1: Control 1ml distilled H₂O

Group 2: 300mg/kg ethanolic extract only

Group 3: 600mg/kg ethanolic extract only

Group 4: 300mg/kg aqueous extract only

Group 5: 600mg/kg aqueous extract only

Group 6: 5mg/kg glibenclamide only (Ali, 1997 and Osadebe *et al.*, 2004)

Group 7: 300mg/kg ethanolic extract + 5mg/kg glibenclamide

Group 8: 600mg/kg ethanolic extract and 5mg/kg glibenclamide

Group 9: 300mg/kg aqueous extract and 5mg/kg glibenclamide

Group 10: 600mg/kg aqueous extract and 5mg/kg glibenclamide

Before the start of the experiment, blood was collected by tail clipping from the rats to determine the initial blood glucose (zero time) and 1,2,4 and 24 hours after oral administration of the extracts and drugs.

Effect of administration of varying doses of aqueous and ethanolic plant extracts and glibenclamide on blood glucose concentration (BGC) in alloxan-induced diabetic albino rats, alone and in combination

The 30 surviving alloxan induced diabetic rats with blood glucose concentration greater than 150mg/dl were divided into ten groups of 3 rats in each group and the extracts and drug were administered orally as follows:

Group 1: Control -distilled water

Group 2: 300mg/kg ethanolic extract only

Group 3: 600mg/kg ethanolic extract only

Group 4: 300mg/kg aqueous extract only

Group 5: 600mg/kg aqueous extract only

Group 6: 5mg/kg glibenclamide only

Group 7: 300mg/kg ethanolic extract + 5mg/kg glibenclamide

Group 8: 600mg/kg ethanolic extract + 5mg/kg glibenclamide

Group 9: 300mg/kg aqueous extract + 5mg/kg glibenclamide

Group 10: 600mg/kg aqueous extract + 5mg/kg glibenclamide

Blood samples were collected from tail vein of the rats, for all the groups before treatment and after 1,2,4 and 24 hours. The glucose level was determined using glucometer (GX mode; Ames Incorporated Germany) with its test strip (one touch test strips).

The percentage glucose level reduction was calculated using the formula below.

$$\text{Change in glucose level (\%)} = \frac{G_x - G_0}{G_0} \times 100$$

Where G_0 = initial glucose concentration

G_x = Glucose concentration at 1, 2, 4 and 24 hours (Osadebe *et al.*, 2004)

Acute toxicity LD₅₀ Test Results

From Lorke's method of determination the LD₅₀ for *Phyllanthus amarus* 3400mg/kg and the dose administered to the rats were calculated based on this finding. The result for the acute toxicity test is shown in Table 1. The results indicated 4000mg/kg as the minimum dose that kill all the animals used. The LD₅₀ calculated from this by Lorke's (1983) method was

3400mg/kg and further calculation gave the doses administered to the animal in evaluating the anti-diabetic effect of the plant. *P. amarus*. The LD₅₀ falls within the non-toxic range given for some anti-diabetic plants (Mainen et al., 2001). According to Williamson et al., (1996) and Osadebe et al., (2004), mice and rats have similar basic anatomy and the use of either of them gave a good estimate of the acute toxicity. That is why mice were used in this study. These results indicated that the extract exhibited a good margin of safety between a dose of 100mg/kg and the lethal dose of 3400 mg/kg.

Table 1: Acute Toxicity LD₅₀ result of *Phyllanthus amarus* extracts on Mice

Group	No per group	Dose mg/kg	Log dose	No of death	% Death
A	3	100	2.000	0	0
B	3	500	2.699	0	0
C	3	1000	3.000	0	0
D	3	3000	3.477	0	0
E	3	4000	3.602	3	100
F	3	4500	3.653	3	100
G	3	5000	3.699	3	100

Effects of Administration of varying doses of aqueous and ethanolic extracts of *P. amarus* and glibenclamide on blood glucose concentration of non-diabetic albino rats alone and in combination

The results for the effect are shown in Table 2. The results indicated the reduction in blood glucose level after administration of the extracts alone and in combination with glibenclamide. Medicinal plants from ages are often used to treat different ailments including diabetes because of the firm belief on them by the users particularly in the rural areas in developing countries. The combination of the medicinal plant preparation and modern therapeutic agents is commonly practiced in most regions of the world including Tanzania, United Arab Emirates and many others (Ali, 1997; Jafri et al., 2000). The results in Table 2 indicated a moderate decrease in blood glucose level in normal albino rats after oral administration of the aqueous and ethanolic extract separately and in combination with glibenclamide at two different doses. The results indicated a dose-dependent effect since pronounced reduction was observed when the dose of 300mg/kg was doubled.

The 300mg/kg ethanolic extract caused a reduction in blood glucose concentration from 43.8±3.0mg/dl to 35.8±3.6 mg/dl after 4 hours administration, 300mg/kg aqueous extracts caused a reduction from 50.8±1.7mg/dl to 38.8±4.6mg/dl, 600mg/kg ethanolic extract alone decreased the blood glucose concentration from 42.5±4.2mg/dl to 32.5±3.7mg/dl and a decrease from 38.5±3.3mg/dl to 25.5±3.1mg/dl for aqueous extract. The 5mg/kg glibenclamide alone caused an expected decrease of blood glucose concentration in the rats from 52.8±3.4mg/dl to 33.0±1.6mg/dl. The extracts in combination with 5mg/kg glibenclamide decreased the blood glucose concentration from 55.5±7.5mg/dl (300mg/kg ethanolic) to 36.5±4.6mg/dl and from 59.5±6.6mg/dl to 38.8±2.2mg/dl (300mg/kg aqueous). The dose of 600 mg/kg with 5mg/kg glibenclamide caused a decrease from 51.3±5.6mg/dl to 32.0±3.5mg/dl (ethanolic) and 58.8±13.5mg/dl to 36.2±4.3mg/dl (aqueous). The results indicate that the blood glucose concentration of the normal rats decreased consistently in all the groups except in control group in which the blood glucose concentration was at increase. The highest decrease in blood glucose concentration was observed in group administered with 600mg/kg aqueous extract plus 5mg/kg glibenclamide. The rats were fasted for 24 hours before and throughout the

experiment until after the fourth hour's readings, then normal feeding was re-introduced to the animals and the animals were tested again at the twenty-fourth hour. The blood glucose concentration of the twenty-fourth hour was at an increase, this may be attributed to the diminishing effect of the drug administered after some hours and also to findings by Pamela et al., (2005) that some carbohydrate containing foods (like the animal feed) may either cause a rapid or gradual rise followed by steep fall or slow decline in blood glucose concentration. The results were statistically significant at P<0.05.

Percentage Reduction in blood glucose concentration in non-diabetic albino rats

The results for the percentage reduction in blood glucose concentration in non-diabetic albino rats are as shown in Table 3. This indicates the percentage reduction in blood glucose level after the administration of the plant extracts alone and in combination with glibenclamide. The 600mg/kg dose caused a percentage reduction of 23.5% for ethanolic extract alone and 25.8% for aqueous extract after 4 hours of administration 300mg/kg dose gave 18.2% (ethanolic) and 23.6% (aqueous). The extracts in combination with 5mg/kg glibenclamide gave a reduction of 34.4% and 36.5% (300mg/kg) respectively for ethanolic and aqueous extracts, also 37.6% and 38.4% (600mg/kg) ethanolic and aqueous respectively. Glibenclamide alone caused a percentage reduction of 37.5%. The highest percentage decrease was 38.4% obtained for the group in which 600mg/kg aqueous extract with 5mg/kg glibenclamide was administered.

Effect of Administration of varying doses of aqueous and ethanolic extracts of *Phyllanthus amarus* and glibenclamide on blood glucose concentration (BGC) in alloxan-induced diabetic albino rats, alone and in combination

The results of the effect of administration of varying doses of aqueous and ethanolic extracts of *Phyllanthus amarus* and glibenclamide on blood glucose concentration (BGC) in alloxan-induced diabetic albino rats, alone and in combination are as shown in Table 4. The results indicate that the plant extracts caused reduction in the BGC after the oral administration in fasted diabetic rats within the range of 1- 4h. It is also apparent from the result in Table 4, that the reductions are dose dependent as seen in non-diabetic rats treatment. 300mg/kg ethanolic extract caused a reduction in BGC from 393.3±4.2mg/dl to 331.0±4.8mg/dl in 4 hours after administration 500mg/kg caused a reduction from 189±11.5mg/dl to 138.7±6.0mg/dl in 4 hours after administration. These were statistically significant at P<0.01 and P<0.05 respectively. The oral administration of 300mg/kg and 600 mg/kg aqueous extract alone caused a reduction in blood glucose concentration from 281±6.8mg/dl to 203.3±4.4mg/dl and 255.3±5.9mg/dl to 178.3±4.0mg/dl respectively. These results were also statistically significant at P<0.01. Glibenclamide alone had an effect on glucose levels; this caused a reduction in BGC from 326.5±9.2mg/dl to 186.0±6.2mg/dl (P < 0.001). The simultaneous treatment of the diabetic rats with the glibenclamide and the extracts decreased the BGC from 275.8±6.7mg/dl to 177.8±6.8mg/dl (300mg/kg ethanolic), 167.8±6.5mg/dl to 106.3±6.9mg/dl (300mg/kg aqueous extract). These were statistically significant respectively at P<0.01 and P<0.001. The 600mg/kg extracts with glibenclamide increased the hypoglycaemic effect in an additive manner. A decrease in BGC from 324.7±3.3mg/dl to 197.3±5.0mg/dl (P<0.001) for ethanolic and from 218.0±3.5mg/dl to 91.3±3.9mg/dl (P<0.001) aqueous extract were observed.

Table 2: Effect of Administration of different doses of aqueous and ethanolic extracts of *Phyllanthus amarus* and glibenclamide on blood glucose concentration in non-diabetic albino rats: alone and in combination

Blood Glucose Concentration (mg/dl)										
Time	Control	300mg/kg Extracts		300mg/kg Extracts + 5mg/kg drug		5mg/kg drug	600mg/kg extracts		600mg/kg extracts + 5mg/kg drug	
Hour	Distilled H ₂ O	Ethanolic only	Aqueous only	Ethanolic + drug	Aqueous + drug	Glibenclamide only	Ethanolic only	Aqueous only	Ethanolic + 5mg/kg drug	Aqueous + 5mg/kg drug
0	50.8±2.8	43.8±3.0	50.8±1.7	55.5±7.5	59.5±6.6	52.8±3.4	42.5±4.2	38.5±3.3	51.3±5.6	58.8±3.5
1	54.3±3.9	41.3±3.0	48.8±4.0	48.5±6.4	51.3±4.6	44.3±3.0d	41.5±4.2	36.8±4.2	42.0±4.5 e	47.3±4.8 d
2	57.0±4.7	38.5±1.3 d+	41.8±4.9 d	40.5±4.9 d	45.5±2.3 d	35.8±3.0 b	35.8±3.4 e	31.8±3.3 e	35.8 ± 3.8 c	40.3 ±4.7 b
4	62.5±3.9	35.8±3.6 d	38.8±4.6 c	36.5±4.6 b	38.8±2.2 c	33.0±1.6 b	32.5±3.7d	25.5±3.1 c	32.0±3.5 b	36.2±4.3 b
24	68.8±4.6	55.8±3.3 d	63.5±4.7	62.0±5.0	65.5±5.2	60.3±3.0 e	52.0±11.2e	46.8±7.4 c	55.8±5.3 d	62.8±3.3 e

Key: values are expressed as mean ± SD (n = 4) and drug is glibenclamide
 Statistically significant at (a) P < 0.001; (b) P < 0.01, (c) P < 0.02; (d) P < 0.05 and (e) P < 0.01

Table 3: Percentage reduction in blood glucose concentration in non-diabetic albino rats

Percentage reduction BGC (%)										
Time	Control	300mg/kg Extracts		300mg/kg Extracts + 5mg/kg drug		5mg/kg drug	600mg/kg extracts		600mg/kg extracts + 5mg/kg drug	
Hour	Distilled H ₂ O	Ethanolic only	Aqueous only	Ethanolic + drug	Aqueous + drug	Glibenclamide only	Ethanolic only	Aqueous only	Ethanolic + 5mg/kg drug	Aqueous + 5mg/kg drug
1	+6.9	-5.9	-3.9	-12.6	-13.2	-16.1	-12.4	-4.4	-18.8	-19.6
2	+12.2	-12.1	-17.7	-27.9	-23.5	-32.3	-15.8	-18.2	-30.2	-31.5
4	+23.0	-18.2	-27.6	-34.4	-36.5	-37.5	-23.5	-25.8	-37.6	-38.4
24	+55.2	+27.4	+25.0	+11.7	+10.4	+14.2	+22.4	+21.7	+8.7	+6.8

Key: + = Increased BGC
 - = Decreased BG

Table 4: Effect of Administration of varying doses of aqueous and ethanolic extracts of *Phyllanthus amarus* and glibenclamide on blood glucose concentration in alloxan-induced diabetic albino rats: alone and in combination

Blood Glucose Concentration (mg/dl)										
Time	Control	300mg/kg Extracts		300mg/kg Extracts + 5mg/kg drug		5mg/kg drug	600mg/kg extracts		600mg/kg extracts + 5mg/kg drug	
Hour	Distilled H ₂ O	Ethanolic only	Aqueous only	Ethanolic + drug	Aqueous + drug	Glibenclamide only	Ethanolic only	Aqueous only	Ethanolic + 5mg/kg drug	Aqueous + 5mg/kg drug
0	201.0±6.	393.3±4.2	281.0±6.8	275.8±6.7	169.8±6.5	326.5±9.2	189.7±11.5	255.3±5.9	324.7±3.3	218.0±3.5
1	216.0±5.5	385.3±6.8	253.5±6.4d	294.3±3.3b	159.8±4.9	283.3±8.4d	184.3±7.4	226.0±4.7d	318.7±5.8	198.7±3.1 c
2	233.2±4.4	369.3±5.9d	218.8±6.7b	219.0±3.4b	126.5±4.3c	251.8±6.6a	145.0±6.2d	181.7±4.6b	235.0± 3.2a	149.8 ±3.9 b
4	242.5±4.8	331.0±4.8	203.3±4.4b	177.8±6.8 b	106.3±6.9a	186.0±6.2 a	138.7±6.0d	178.3±4.0b	197.3±5.0a	91.3±3.9a
24	285.0±5.1	388.0±6.1	300.3±6.4	344.0±6.7	197.5±5.9b	348.8±6.7	198.0±8.4b	267.0±6.5	340.7±6.0	228.7±6.1

Key: values are expressed as mean ± SD (n = 3) and drug is glibenclamide
 Statistically significant at (a) P < 0.001; (b) P < 0.01, (c) P < 0.02; (d) P < 0.05 and (e) P < 0.01

Table 5: Percentage reduction in blood glucose concentration in alloxan-Induced diabetic albino rats

Percentage reduction BGC (%)										
Time	Control	300mg/kg Extracts		300mg/kg Extracts + 5mg/kg drug		5mg/kg drug	600mg/kg extracts		600mg/kg extracts + 5mg/kg drug	
Hour	Distilled H ₂ O	Ethanolic only	Aqueous only	Ethanolic + drug	Aqueous + drug	Glibenclamide only	Ethanolic only	Aqueous only	Ethanolic + 5mg/kg drug	Aqueous + 5mg/kg drug
1	+7.5	-2.0	-9.8	-6.7	-5.9	13.2	-2.8	11.5	-1.8	-8.9
2	+16.6	-6.1	-22.4	-20.6	-25.5	-22.9	-23.6	-28.8	-27.6	-31.5
4	+20.6	-15.8	-27.7	-35.5	-37.4	-43.8	-26.7	-29.9	-39.2	-58.1
24	+41.8	-1.3	+6.9	+24.6	+18.9	+6.8	+4.4	+4.5	+4.9	+4.5

Key: + = Increased BGC
 - = Decreased BGC

The control group which received only distilled water showed an increase in BGC from 201.06.9mg/ dℓ to 285.0±5.1mg/dℓ.

Percentage reduction in BGC in alloxan-induced diabetic albino rats

The results for the percentage reduction in BGC in alloxan induced diabetic rats are as shown in Table 5. It is apparent from the result that the percentage reductions were dose dependent as in non-diabetic rats treatment. Ethanolic extract 300mg/kg alone caused 15.8% decrease and 600mg/kg caused 26.7% decrease in blood glucose concentration. The aqueous extracts gave a percentage reduction of 27.7% with 300mg/kg and 29.9% with 600mg/kg. There was 43.8% reduction when glibenclamide alone was administered. The results lend credence to the report of Ali (1997) and Osadebe et al., (2004) that glibenclamide caused substantial blood glucose reduction on normal and alloxan-induced diabetic rats. The simultaneous treatment of the diabetic rats with the glibenclamide and extracts of *P. amarus* at doses of 300mg/kg and 600mg/kg increased their hypoglycaemic effect in an additive manner. Percentage reductions of 35.5% and 39.2% were observed for 300mg/kg and 600mg/kg for ethanolic extract respectively in combination with 5mg/kg glibenclamide, 37.4% decreased for (300mg/kg) and 58.1% (600mg/kg) for aqueous extract. The control group showed 41.8% increase in blood glucose concentration. These reductions exceeded those induced by *P. amarus* extracts alone by 30% and 32% respectively for aqueous and ethanolic (600mg/kg) and 16% for 300mg/kg for both aqueous and ethanolic extract. The results obtained show that water was a better solvent than ethanol for extraction of this plant and for its consumption (Mainen et al., 2001).

Their results confirmed the claims and use of *P. amarus* in traditional medicine as an anti-diabetic agent in Nigeria, India, Tanzania and many other countries (Chhabra et al., 1994; Mahabir et al., 1997; Mainen et al., 2001; Etukudo, 2003). The extent of reduction in BGC was more prominent in alloxan-induced diabetic rats than in non-diabetic rats. The mechanism of these as suggested by Hardy et al., (1997) and Sharma et al. (1983) could be due to the suppression of hepatic gluconeogenesis, stimulation of glycolysis, inhibition of glucose absorption from the intestine, stimulation of insulin release by pancreas and inhibition of conversion of dietary disaccharide. It is also observed and suggested that the anti-diabetic effect of plant extracts could be due to possible increase in the peripheral utilization of glucose and/or potentiating of the biological effect of insulin. This suggestion is supported by the fact that despite pore-treatment of the rats with alloxan, which is known to cause permanent destruction of pancreatic β-cells (Williamson et al., 1996) blood glucose concentration reduction was still observed in alloxan-induced diabetic rats as well as in fasted non-diabetics albino rats. From the result obtained the observed anti-diabetic effect of the plant might be due to the presence of alkaloids, saponin, flavonoid, steroid, nutritive and elemental components of this plant (Ali, 1997; Jafri et al., 2000; Osadebe et al., 2004;), and the present of these component in this plant (*P. amarus*) have been reported in our previous work (Umoh, et al, 2013). These results are in agreement with those obtained by Moshi et al., (1997).

Conclusion

The acute toxicity test carried out on the aqueous and alcoholic extracts of *Phyllanthus amarus* confirms that the plant is safe for consumption. The study comparatively assessed the effect of the extracts of *P. amarus* of different solvents in normal and alloxan-induced diabetic rats and indicated water as the most appropriate solvent for the extraction, and for oral

administration. The plant extracts caused considerable percentage reduction in blood glucose level when administered to non-diabetic and alloxan-induced diabetic albino rats. The simultaneous treatment of normal and alloxan-induced diabetic rats with *Phyllanthus amarus* extracts and glibenclamide caused considerable reductions in BGC with the highest percentage reduction recorded for aqueous extracts in combination with glibenclamide, the percentage reduction was dose dependent. The whole plant of *Phyllanthus amarus* might be considered from the results as a good herbal drug for alternative and/or complementary medicine in the management of diabetes mellitus.

Finally, the plant extracts in combination with glibenclamide significantly reduced the blood glucose level of both non-diabetic and alloxan-induced diabetic rats. The entire results obtained in this work justified the use of *Phyllanthus amarus* in managing diabetes mellitus.

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