Antihyperglycaemic Activity of Piper Guineense in Diabetic Female Albino Wistar Rats

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ABSTRACT

This study was carried out to determine the effect of *Piper guineense* on diabetic female albino wistar rats. Forty-two albino wistar rats were used and divided into six groups of seven animals each. Group 1 was the Normal control and received water and feed *ad libitum*. Groups II to VI were induced with diabetes using Alloxan. Diabetes was confirmed after a period of 3 days in animals with Blood Glucose Level (BGL) more than 200 mg/dl. After which the animals were treated daily with the extract through oral administration for a period of 14days. Group II animals were treated with 40mg/kg of the extract (Low dose), Group III were treated with 80mg/kg of the extract (medium dose), Group IV were treated with 100mg/kg body weight of the extract while Group V animals which served as positive control were treated with 10mg/kg body weight of Glibenclamide (Anti diabetic drug) and Group VI were left untreated and served as Diabetic Control. Change in Blood Glucose Level was monitored after 1, 3, 5 and 7 hours for acute study and after 3, 5, 7 and 14 days for prolonged studies. The results showed that there was no significant reduction in the BGL of all groups within the first seven hours of therapy. However, the medium and high doses caused significant reduction of the BGL after 14 days, when compared with the control. The group treated with glibenclamide also showed reduction. Thus from the study, it was found that methanolic extract of *Piper guineense* has a delayed glucose lowering effect.

Key Words: *Diabetes, Piper Guineense, Alloxan, Antihyperglycaemia.*

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INTRODUCTION

Diabetics Mellitus is a metabolic disorder usually characterized by prolonged high level of sugar in the blood usually as a result of defects in insulin secretion, insulin action, or both insulin secretion and action. [1]

The American Diabetes Association reported that an estimate of 415 million people has diabetes all over the world as at the year 2015, out of which type 2 diabetes mellitus made up about 90% of the case. [2] Diabetes doubles a person's risk of death and the International Diabetes Federation has revealed that the number of people with diabetes is expected to rise to 592 million by 2035. This increasing number is due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity. [3]

This has brought about the use of plants and plant preparations with anti-diabetic properties. Tropical plants have elaborate diverse phytochemicals that are medicinally useful especially in the management of

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diabetes. [4][5] One of such plant is *Piper guineense.*

From time past and present, extracts and medicines from plants have made immense contributions to the overall health and wellbeing of human beings. [6]

Piper guineense, popularly known as African black pepper or hot leave is widely consumed in some part of West Africa especially Nigeria and Ghana on account of its nutritional and medicinal properties [7][8]. Leaves of *P. guineense* have been used by traditional medical practitioners for the treatment of respiratory diseases and correction of female infertility problems. However, not much has been reported on its effect on diabetes thus this work. [9]

MATERIALS AND METHODS

Preparation of piper guineense

Fresh leaves of *Piper* guineense were purchased from Choba Market in Port Harcourt and were identified by an agronomist in the Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt. The leaves were washed, air dried for 7 days and grounded into fine powder with an electric mill. The powdered leaves were soaked in methanol for 72h and were filtered with Watman filter paper and the filtrate evaporated to dryness using a rotary evaporator at 45^{o}C. 100g of the leaves produced 85g of the powder which resulted in 21.25g of the extract at a percentage yield of 25%. The dried powder was then stored in universal sample bottles for work use.

Animal models

Forty-two healthy adult female albino wistar rats weighing 160g – 220g were bred in the animal house of the Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt. The rats were housed in a wire-gauzed plastic cage lined with wood chip beddings, under standard conditions. The rats were given feed (Top feed Ltd., Nigeria) and water *ad libitum*. The use of animals received institutional approval by the ethics committee of the University of Port Harcourt. Experimental procedures in this study involving the animals and their care were conducted in conformity with the guiding principles for research involving animals. [10]

Experimental design

The animals were randomly divided into six groups of seven animals each: Group 1 (Non Diabetic Control) and Groups 2 – 6 (Diabetic Test Groups). The animals were allowed to acclimatize for 7 days after which their basal blood glucose level was determined by tail tipping method. The Blood was dropped on the dextrostix reagent pad, which was inserted into microprocessor digital blood glucometer and the readings recorded so as to ensure they were not diabetic (FBGL below 120mg/dl). At the commencement of treatment, their BGL was measured after 1, 3, 5 and 7 hours of administration of a single dose of the extract (acute study) and at the end of 1, 3, 5, 7 and 14 days (Prolonged Treatment).

Experimental induction of diabetes

The rats were subjected to a 12 h (overnight) fast and diabetes induced by a single intra-peritoneal injection of 150 mg/kg body weight alloxan monohydrate dissolved in normal saline. [11] The animals were given 2ml of 5% dextrose solution using orogastric tube immediately after induction to overcome the drug induced hypoglycemia. Diabetes was confirmed after three days (72h) in animals with FBGL above 200mg/dl which were selected for the experiment.[12] All treatments were carried out daily and lasted for a period of 14days with Group 1: Non Diabetic rats ; Group 2: Diabetic rats (Test 1) treated with 40mg/kg body weight of the extract which served as the low dose; Group 3: Diabetic rats (Test 2) treated with 80mg/kg body weight of the extract which served as the Medium dose; Group 4: Diabetic rats (Test 3) treated with 100mg/kg body weight of the extract which served as the High dose. Group 5: Diabetic rats (Positive Control) treated with 10mg/kg of Glibenclamide an anti-diabetic drug and Group 6: Diabetic rats (Diabetic Control) left untreated.

Statistical analysis

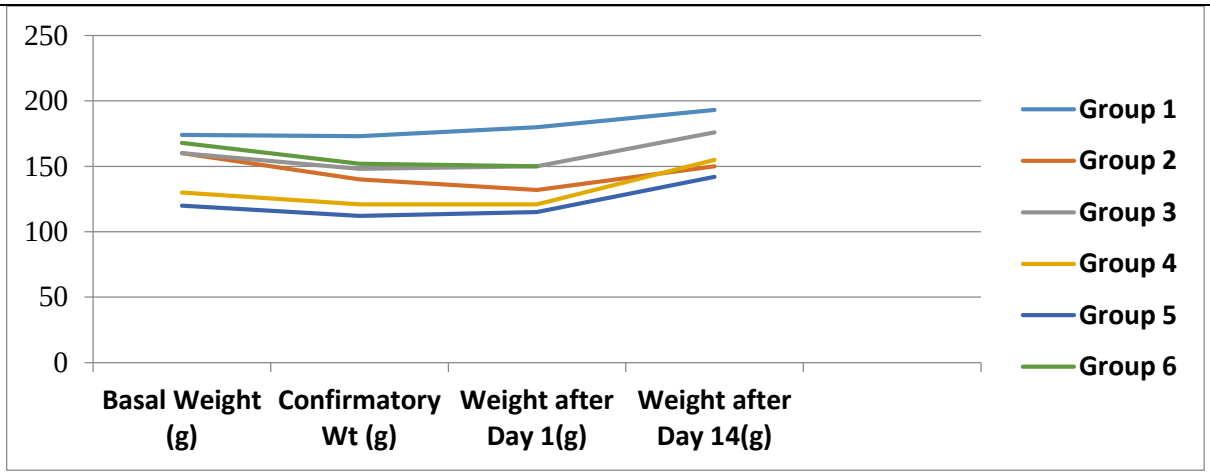
All data were collected and analyzed using the Statistical Package for Social Science (SPSS). Differences between the groups were examined by ANOVA with multiple comparisons and the significant differences determined at P≤ 0.05. The results were presented as mean ± SEM.

RESULTS

Mortality

There was no record of mortality after 7 hours of drug administration in all groups but mortality occurred in untreated Group 6 (Diabetic control) after two days due to the high BGL which remained at 600mg/dl (Table 2).

Graph 1: Chat showing the effect of P. *guineense* extract on the Body Weight of Diabetic Rats



Basal Weight= Weight before induction of diabetic, Confirmatory Weight= Weight after three days of induction, Day 1 Weight = Weight after day 1 of treatment, Day 14 Weight = Weight after day 14 of treatment.

Table 1: Effect of P. *guineense* extract on the Blood Glucose Level of Diabetic Rats

Grp	Basal FBGL (Mg/dl)	BLOOD GLUCOSE LEVEL (mg/dl)								
		Before Treatment (Confirmatory)	Treatment							
			Day 1				Day 3	Day 5	Day 7	Day 14
			1hour	3hrs	5hrs	7hrs				
1	84.14 ±3.05	82.83 ±3.05	82.83 ±34.86	82.00 ±12.23	107.67± 12.23	87.83 ±9.00	93.39±1 3.16	112.50± 4.49	129.67± 6.23	76.50 ±3.34
2	109.38 ±2.47	442.67 ±52.03	484.00 ±34.86	517.00 ±62.41	561.00 ±19.16	558.00 ±42.00	407.00 ±21.76	392.60 ±36.60	382.00 ±24.14	257.00 ±48.97
3	96.29 ±6.84	524.80 ±70.23	490.00 ±48.84	459.17 ±72.19	441.00 ±47.06	404.00 ±73.55	364.83 ±59.89	256.50 ±51.85	239.80*± 29.25	212.00* ±24.17
4	88.71 ±2.27	491.7.67 ±48.63	507.67 ±78.06	474.50 ±37.38	474.33 ±53.81	460.00 ±69.62	429.17 ±74.01	349.00 ±104.47	217.33*± 42.95	206.33* ±35.32
5	89.00 ±2.01	512.60 ±23.71	485.80 ±20.97	479.40 ±19.53	466.00 ±36.93	447.60 ±26.86	446.00 ±14.16	374.00 ±46.53	352.20± 31.35	247.25 ±54.26
6	116.17 ±5.76	578.20 ±12.05	578.20 ±12.05	578.20 ±12.05	600.00 ±36.93	600.00 ±36.93	-	-	-	-

Values are expressed as mean ± SEM; * significantly lowered at P< 0.05;

The results of the basal BGL reveals that though there was no significant difference in the BGL of the animals in Groups 2 - 6 when compared to Group 1 at (P< 0.05), with the highest sugar level of 116mg/dl and the lowest 84mg/dl, none of the animals were diabetic.

The confirmatory study revealed that there was a significant increase in the BGL of animals in Groups 2 - 6 when compared to Group 1 at (P< 0.05). Diabetics were confirmed in the BGL of the animals in Groups 2 to 6 with none having BGL less than 442mg/dl.

After seven hours of extract administration, there was no significant (P< 0.05) reduction within Groups 2-6 at (P<0.05), but after fourteen days of treatment, there was a significant reduction in the sugar levels of the diabetic rats in Groups 3 and 4 treated with 80mg/kg and 100mg/kg body weight of the extract respectively as their BGL was almost restored to normal. An insignificant (P< 0.05) reduction was equally observed in Group 2 animals treated with 40mg/kg body weight of the extract as well as group 5 animals treated with 10mg/kg body weight of glibenclamide.

DISCUSSION

The graph presented reveals an initial weight loss in the animals in groups 2 - 6 after a period of three days of diabetics' induction which is an indication of diabetics' mellitus with poor glycaemia control. [13] The appreciation in weight noticed in groups 2 – 5 after the commencement of treatment suggests that treatment with *P. guineense* (groups 2- 4) and glibenclamide (group 5) gradually reduced the blood glucose thus allowing the tissue to have access to glucose for energy and tissue building required for tissue growth. [14]

From the results presented, it is observed that after seven hours of acute study of *P. guineense* administration, there was no reduction in the BGL of the diabetic rats. The prolonged treatment however showed reduction in the blood glucose levels.

The significant reduction in the BGL after seven days of treatment may be due to the presence of phytochemicals such as alkaloids, flavonoid, saponin, tannin, resins and essential oils. [15] It has been reported that Alkaloids possess anti-hyperglycemic activities [16] a property peculiar to all *Piperine.* The presence of flavonoids has also been reported to be

responsible for the anti-diabetic properties of *C. nurvala* stem bark on alloxan induced albino wistar rats. [17] Phytochemicals such as flavonoids, steroids, alkaloids, terponoids and saponins as well as other glycosides has been connected with anti-diabetic activities. [12] It was reported that fibers of alkaloids and flavonoids such as quercetin, kaempferol and caffeoyl glucosides as well as saponins and glycosides have all demonstrated to inhibit hyperglycemia in animal models. [18] [19]

CONCLUSION

This study has revealed that prolonged consumption of *Piper guineense* has glucose lowering effect on diabetes mellitus due to its phytochemical constituents. Therefore, consumption of *Piper guineense* should be encouraged among diabetic or pre-diabetic persons. It should also be incorporated in the formulation of diabetic diets.

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