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ANTIDIABETIC ACTIVITY OF *PHYSALIS ANGULATA* EXTRACTS AND FRACTIONS IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

The antidiabetic activity of aqueous methanolic and column fractions from whole plant of *Physalis angulata L.,* an edible plant used for treating various diseases, were evaluated in the oral glucose tolerance test and in alloxan induced diabetic rats and compared to effects of glibenclamide. A dose of the methanolic extract and its column fractions were administered to experimental diabetic rats and their blood glucose levels monitored over 7 days using glucometer. The extract exhibited significant blood sugar lowering effect in the glucose tolerance test and in the alloxan diabetic rats. Fraction F3, which produced improved activity compared to the crude extract, recorded significant reduction of blood glucose levels on day 7 of treatment (58.6% decrease; $p < 0.05$) at concentration of 500mg/kg. The hypoglycaemic effect was more pronounced in the hyperglycaemic rats than in normoglycaemic conditions. This plant holds potential for therapeutic application.

Keywords: *Physalis angulata L.,* Alloxan induced diabetes, hypoglycaemic activity

1. INTRODUCTION

Diabetes mellitus is considered the commonest endocrine disorder [1]. Inspite of the extensive use of hypoglycaemic agents, diabetes and associated complications continue to be a major health concern [2]. It is estimated that diabetes in adults is over 170 million worldwide and its prevalence is likely to increase to over 300 million by the year 2025 [3, 4]. About two thirds of these live in Asia and Africa [5]. Furthermore, it is known that the use of existing hypoglycaemic agents (mainly sulfonylureas and biguanides) is associated with undesirable side effects, secondary failure rates, cardiovascular disorders and coma [6-10]. For these reasons, the search for safe and effective antidiabetic agents continues to be an important area of research.

Physalis angulata Linn. (Family Solanaceae) commonly called "*Koropo*" (Yoruba) [11] is a small tropical annual herb. It bears cream-coloured flowers and small edible orange-yellow fruits [12]. It is used in ethnomedicine for treating sexually transmitted diseases [13], gastro-intestinal disorders [14], diabetes and leprosy [13].

Various physalins have been reported in *P. angulata as* molluscicides [15], cytotoxic and anti-tumour agents *in vitro* [16-20]. We became interested in this plant because it is used in herbal medicine to treat diabetes. We report the hypoglycaemic activities of whole plant extracts and fractions of *P. angulata* on normoglycaemic and alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1. Plant Material

Whole plant of *Physalis angulata L.* (Solanaceae) was collected at Onikanga village along Ibadan-Eruwa road, Oyo State and authenticated by Mr. Ariwaodo at the Forest Herbarium where herbarium specimen (Voucher No. FHI, 108409) has been deposited. The plant was air-dried and powdered for analysis.

2.2. Extraction

1kg of the powdered whole plant of *P. angulata* was macerated with 80% MeOH for five days and the combined filtrate was evaporated *en vacuo* in a rotary evaporator at 40^0C and weighed. The aqueous MeOH extract was tested on glucose – loaded hyperglycaemic and alloxan diabetic rats as described below.

2.3. Phytochemical analysis and fractionation of the aqueous methanol extract

Microchemical tests were performed on the aq. MeOH extract following standard procedures [21, 22]. The bioactive aq. MeOH extract (4g) was fractionated on column chromatography packed with silica gel G (70-230 mesh) eluting with gradient mixtures of hexane, ethylacetate and methanol. The fractions were monitored on analytical TLC (Silica gel GF_{254}) developing in hexane/diethylether (1:1:1) as

mobile phase. Four fractions (F1-F4) were obtained and each fraction tested on the alloxanised rats as describe below.

2.4. Biological assays

2.4.1. Experimental animals

Albino Wistar rats of both sexes weighing 180-230g were obtained from the Central Animal House of the College of Medicine, University of Ibadan. They were kept in metabolic cages in a well-ventillated room, fed on standard feed (Ladokun Feed Ltd, Ibadan) and water *ad libitum.*

2.4.2. Glucose-induced hyperglycaemia

The rats were divided into four groups (cages 1-4) of five rats each. The rats were fasted overnight but allowed free access to water. Initial blood glucose level (zero time) was determined before assay. Rats in groups B-D were made hyperglycaemic by oral administration of 1g/kg glucose solution. Rats in group A were neither glucose neither loaded nor treated with extract. Details of the glucose tolerance test have been previously reported [23, 24].

2.4.3. Alloxan- induced diabetes

Rats were divided into four groups (n=5), fasted overnight and then given a single intraperitoneal injection of alloxan monohydrate (80mg/kg, Sigma USA) in isotonic saline. They were allowed to rest for three days to stabilize blood glucose levels. Group A consisted of normal (untreated) rats; group B consisted of untreated diabetic control rats while rats in group C were diabetic but treated with appropriate extracts (1g/kg for crude aq. MeOH extract and 500mg/kg for column fractions). Group D were diabetic rats but treated with glibenclamide (2.5mg/kg). The rats were fasted for 18 hours and basal blood glucose level (zero time) determined prior to oral treatment. The aq. MeOH extract, column fractions and glibenclamide were separately administered immediately to rats in appropriate cages using treatment protocol previously described [25, 26]. Blood glucose levels were determined daily for seven days using a glucometer (Johnson and Johnson Company, California) following standard method [27-29].

2.4.4. Statistical analysis

Data are presented as Mean ± SEM. The significance of the differences between test and control rats was established by the students't-test.

3. RESULTS AND DISCUSSION

Diabetes mellitus is an endocrine and metabolic disease. It is a major public health problem causing considerable mortality. Prevalence of diabetes is increasing worldwide, particularly in developing countries. Rate of increase is expected to be 42% in developed countries and 70% in developing countries by the year 2025 [30-32]. Management of diabetes without side effects is still a challenge, hence the growing interest in evaluation of plant and herbal remedies which are considered less toxic with minimal side effects [33-35].

Phytotherapy is an important aspect of traditional medical practices in Africa. For centuries, herbal remedies have served as important source of medicines for prevention and treatment of diseases [2, 36-38]. Plant and plant products still represent source of new drugs to complement the action of oral hypoglycaemic agents and as dietary adjuncts to existing therapies [33, 35].

Table 1.Effect of aqueous methanolic extract of Physalis angulata (whole plant) and glibenclamide on blood sugar level of glucose loaded rats

	Blood Glucose Levels (mg/dl ± SEM)						
	Groups	**0 min**	**30 mins**	**60 mins**	**90 mins**	**120 mins**	**180 mins**
A.	Normal rats	58.0±1.30	60.2±1.16	63.6±0.93	61.2±2.33	60.4±1.88	57.8±0.97
B	Hyperglycaemic (untreated, control)	65.4±1.96	109.0±1.18	129.2±2.92	105.6±2.06	92.5±2.25	62.0±1.18
C	Hyperglycaemic (Treated with extract 1g/kg)	60.8±2.82	103.4±3.72 (5.1)	114.6±2.75 (11.3)	84.0±3.39 (19.5)*	71.8±1.80 (22.3)*	44.6±1.86 (26.1)*
D	Hyperglycaemic (Treated with glibenclamide 2.5mg/kg)	62.0±2.78	98.4±2.13 (8.4)	111.2±1.36 (12.9)*	79.1±1.97 (24.8)*	66.8±2.97 (27.7)*	40.2±2.08 (34.3)*

Values are Mean ± SEM; n = 5; Figures in parenthesis indicate % decrease in blood glucose level.

** Significantly different from control at $p < 0.05$*

Oral administration of the aqueous methanolic (Aq. MeOH) extract of whole plant of *P. angulata* at 1g/kg produced significant ($P < 0.05$) hypoglycaemic effect in the glucose tolerance test at 90, 120 and 180 minutes (Table 1). The most pronounced effect being recorded at 120 and 180 min with 22.3% and 26.6% reduction in blood glucose levels respectively. The rats treated with the reference drug (glibenclamide) at 2.5mg/kg exhibited significant reduction of blood glucose level after 60min. Peak decrease of 27.7% and 34.3% was observed after 120 and 180min of treatment. In the

alloxan-induced diabetic rats, the extract significantly lowered blood glucose levels of the animals when compared with the untreated control group (Tables 2 and 3). Peak reduction of 21.4% and 31.4% which was significant at $p < 0.05$ was observed after 120 and 180 mins respectively during the first day of treatment. A sustained reduction of blood glucose levels which peaked (29.7% $p < 0.05$) on day 6 of treatment was observed at concentration of 1g/kg of extract (Table 3). However, glibenclamide produced better reduction throughout the duration of study with peak decrease of 71.3% on day 7.

Table 2. Effect of aqueous methanolic extract of P. angulata and glibenclamide on blood glucose level of alloxanised rats during Day 1 of treatment

		Blood Glucose Levels (mg/dl ± SEM)			
	Groups	**0 min**	**60 mins**	**120 mins**	**180 mins**
A.	Normal rats	101±3.3	73.8±7.3	81.0±7.3	73.8±9.5
B	Diabetic (untreated control)	331.8±10.4	334.8±9.0	339.7±15.1	352.7±4.5
C	Diabetic (treated with extract (1g/kg)	421.8±10.2	275.4±12.0 (17.7)*	267±13.0 (21.4)*	241.8±10.6 (31.4)*
D	Diabetic (Treated with glibenclamide 2.5mg/kg)	344.4±7.9	306.6±10.6 (8.4)	257.2±8.8 (19.0)*	187.8±5.7 (46.8)*

Values are Mean ± SEM; n = 5; figures in parenthesis indicate % decrease in blood glucose level.
** Significantly different from control at $p < 0.05$*

Table 3.Effect of aqueous methanolic extract of P. angulata on blood glucose level of alloxan-diabetic rats treated daily

		Blood Glucose Levels (mg/dl ± SEM)					
	Groups	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
A.	Normal rats	73.8±7.30	99.8±4.41	99.8±4.40	95.6±5.73	72.6±10.6	81.0±1.70
B	Diabetic (untreated)	346.2±4.60	374.4±3.80	331.2±1.32	413.7±4.80	339.6±1.60	437.0±7.13
C	Diabetic (Treated with extract, 1g/kg)	252.6±1.50 (27.0)*	291.6±4.32 (22.1)*	274.2±3.70 (17.2)*	356.6±7.04 (14.0)*	238.9±4.72 (29.7)*	427.8±8.50 (2.2)
D	Diabetic (Treated with glibenclamide, 2.5mg/kg)	99.0±7.70 (57.7)*	270.0±9.51 (27.9)*	342.0±3.60 (21.8)*	99.6±1.53 (75.9)*	131.4±2.71 (63.3)*	98.4 ± 1.92 (71.3)*

Values are Mean ± SEM; n = 5; Figures in parenthesis indicate % decrease in blood glucose level.
** Significantly different from control at $p < 0.05$.*

Table 4. Effect of column fractions (F1-F4) from aqueus methanolic extract from P. angulata on blood glucose level of alloxanised rats during Day 1 of treatment

		Blood Glucose Levels (mg/dl ± SEM)			
	Groups	**0 min**	**60 mins**	**120 mins**	**180 mins**
A.	Normal rats	36.2±3.90	43.8±3.80	52.2±3.91	57.6±3.95
B	Diabetic (untreated control)	351.6±11.20	361.2±11.32	369.0±10.61	378.0±10.70
C	Diabetic (treated with column fraction (500mg/kg)				
	F1	355.8±4.31	360.6±10.24	370.1±5.63	385.5±4.85
	F2	200.4±6.70 (43.0)*	192.6±7.10 (46.7)*	186.6±6.40 (49.5)*	178.3±6.70 (52.8)*
	F3	194.4±6.20 (44.7)*	189.0±7.10 (47.7)*	184.8±6.60 (49.9)*	183±6.43 (51.6)*
	F4	340.0±8.61 (3.2)	333.0±8.4 (7.8)	337.2±8.10 (8.6)	315.6±7.80 (16.5)
D.	Diabetic (Treated with glibenclamide, 2.5mg/kg)	175.2±4.90 (50.1)*	171.0±4.42 (52.7)*	175.2 ±3.51 (52.6)*	168.6±4.34 (55.4)*

Values are Mean ± SEM; n = 5; figures in parenthesis indicate % decrease in blood glucose level;
** Significantly different from control at $p < 0.05$.*

Tables 4 and 5 show performance of the column fractions (F1-F4) on the bioassay model. Column fractions F1 and F4 were inactive throughout the seven days of treatment. Anti-diabetic activity resided in fractions F2 and F3 which exhibited improved reduction of blood glucose levels than the aq. MeOH extract. Significant reductions were recorded for column fraction F2 (52.8% on Day 1; 58.6% on Day 7, $p < 0.05$) and F3 (51.6% on Day 1; 58.6% on Day 7, $p < 0.05$) at concentration of 500mg/kg. There was no significant difference between reduction in blood glucose levels of the column fractions-treated and the glibenclamide-treated rats (55.4% on Day 1, 61.1% on Day 7, $p < 0.05$) (Table 5).

Table 5. Effect of column fractions (F1-F4) from aqueous methanolic extract of P. angulata on blood glucose levels of alloxanised rats treated daily

	Blood Glucose Levels (mg/dl ± SEM)						
	Groups	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
A.	Normal rats	79.2±2.87	79.2±3.69	80.0±3.09	85.2±2.35	94.8±2.41	66.6±3.84
B	Diabetic (untreated)	269.0±8.18	428.4±5.43	402.6±7.81	383.4±6.19	345.6±8.51	417.6±8.37
C	Diabetic (Treated with column fractions, 500mg/kg)						
	F1	260.7±6.40	421±7.28	403.0±3.84	390.6±2.73	339.2±7.15	425.1±10.30
	F2	240.0±7.63 (10.6)	271±8.90 (36.7)*	298.8±8.16 (25.8)*	280.8±8.18 (26.8)*	228.0±7.54 (34.1)*	213.6±7.17 (48.9)*
	F3	224.4±4.44 (16.7)*	251.4±7.13 (41.3)*	230.4±42.8 (42.8)*	235.2±5.96 (38.7)*	210.6±6.00 (39.1)*	172.8±6.22 (58.6)*
	F4	256.8±7.58 (4.7)	390.0±9.28 (8.9)	372.0±8.60 (7.6)	343.2±8.11 (10.7)	281.4±8.09 (18.6)*	379.2±8.33 (9.2)
D	Diabetic, (Treated with glibenclamide 2.5mg/kg)	208.2±6.46 (22.7)*	192.6±6.50 (55.0)*	193.2±6.33 (52.0)*	188.4±6.18 (50.9)*	172.8±5.98 (50.0)*	152.6 ± 5.86 (61.1)*

Values are Mean ± SEM; n = 5; Figures in parenthesis indicate % decrease in blood glucose level.
** Significantly different from control at $p < 0.05$.*

It was noted that the untreated diabetic rats eventually died within two weeks. Diabetic rats treated with fractions F2 and F3 gradually regained weight and fur regeneration during period of treatment.

Phytochemical screening of the aq.MeOH extract indicated abundance of saponins, tannins and flavonoids; traces of alkaloids; no anthraquinone derivatives and cardiac glycosides. The column fractions (F2 and F3) reacted intensively to ferric chloride spray reagent which implies presence of phenolics possibly flavonoids. A further study is in progress to identify the compound(s) responsible for the antidiabetic activities.

Diabetes is a chronic illness that requires continuous treatment and monitoring. Effective preventive natural remedies would be most beneficial in controlling the physical and financial burden on patients and to prevent acute side effects. Possible beneficial effects of extracts from *Spondias mombin*. [26, 39], *Parkia biglobosa* [40, 41] and *Gladiolus psittascinus* [32] have been reported. This study showed that extract and fractions from whole plant of *P. angulata* significantly lowered blood sugar levels of the alloxanised diabetic rats and the hypoglycaemic effect is more pronounced in hyperglycaemic than normoglycaemic conditions in rats, producing effect similar to the reference drug. Ritcher (1998) [42] reported that a water extract of root of *P. angulata* given intragastrically to mice showed weak hypoglycaemic activity. We report significant antidiabetic activity of aq.MeoH extract and fractions from whole plant of *P. angulata* in rats.

Alloxan causes massive reduction in insulin release by destroying the β-cells of islets of langerhans and thereby inducing hyperglycaemia which is the main cause of complications associated with coronary artery disease, renal failure, neurological complications, blindness and premature death [43-45]. The exact mode of action of *P. angulata* is unknown as very little is known about the antidiabetic effect of this plant. It is however possible that the hypoglycaemic effect may be due to pancreatic and/or extrapancreatic activity. This could be by stimulation of residual β-cells to release more insulin or due to an increased peripheral utilization of glucose. Further studies will be necessary to clarify the postulations.

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