

Anti-Hyperglycaemic Effects of Ethanol Leaf Extract of *Sphenocentrum jollyanum* in Normal and Alloxan-Induced Diabetic Rabbits

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Abstract: The anti-hyperglycaemic activity of *Sphenocentrum jollyanum* (SJ) Pierre (Menispermaceae) was evaluated on alloxan-induced diabetic rabbits. The single extract dose in an oral tolerance test enhanced clearance of plasma glucose with decrease in the area under OGTT curve by 26.9% ($P < 0.05$) compared to the untreated. In the alloxan-induced hyperglycaemic rabbits, the extract exerted significant blood glucose reduction in a dose dependent manner from day 3 of continuous oral administration. The maximum anti-hyperglycaemic effect was observed in the group that received 200 mg/kg with plasma glucose level of 200.2 ± 2.6 mg/kg (42.8%) compared to glibenclamide (10 mg/kg) treated that showed maximum decrease of 158.5 ± 5.0 mg/kg (54.8%). The photomicrograph of the extract treatment showed amorphous material resulting from necrosis with few surviving beta cells spotted at the periphery. It is apparent that the tissue histology indicated no evidence of beta cells regeneration. The decrease in glycaemia was therefore believed to have occurred through the extract potentiation of the survivor beta cells to facilitate insulin production.

Key words: Anti-hyperglycaemia, *Sphenocentrum jollyanum*, Leaf, Alloxan-diabetes, Rabbit

INTRODUCTION

Diabetes mellitus is a chronic disease of endocrine pancreas characterized by the derangement of blood glucose homeostasis. The history of diabetes dates back to the ancient times and has remained a significant threat to life till date. The number of people living with the disease is reported to be increasing rapidly and huge amount of resources are spent by government all over the world to combat the menace [1]. Management of diabetes is usually very tasking. Insulin therapy afforded effective glycaemic control but with a number of shortcomings that includes ineffectiveness on oral administration [2] made the search for alternative therapy more compelling. Consequently, the introduction of oral hypoglycaemic agents like sulfonylureas was a welcomed development that stimulated great interest. However, the use of this medication, though have been a cornerstone in type 2 diabetic treatment it appeared insufficient to manage diabetic complications [3, 4].

The treatment of diabetes with plant parts has been in practice over the years. Because of the successes recorded overtime and based on WHO recommendation [5], greater awareness has been garnered of plant importance in the management of diabetes and diabetic complications. Over the last 10 years, the use of herbs in diabetic treatment has more than tripled [6]. The current shift may be due to the belief that plant parts are less toxic and freer from side effects than synthetic drugs [7-9].

Sphenocentrum jollyanum (SJ) is a shrub that grows along the west coast of Africa, extending from Sierra Leone to Cameroun through Nigeria. The leaf is up to 20 cm in length with the breadth of 5-12 cm. It is elliptically shaped with both surfaces smooth [10]. It belongs to the Menispermaceae family. SJ is a plant that has wide therapeutic value in folk medicine. Preliminary screening revealed the aerial part to have anti-diabetic potential. This necessitated the anti-hyperglycaemic study on alloxan-induced diabetic rabbits.

MATERIALS AND METHODS

Plant Material: Fresh leaves of *S. jollyanum* were collected from a farm land in Ijebu-Oru community, Ogun State, Nigeria. The collection was in the month of November and dried under the sunlight. The plant authentication was by a botanist at the Forest Research Institute of Nigeria (FRIN) where voucher specimens were deposited (FHI/108203).

Preparation of the Plant Ethanol Extract: The fresh leaves were air dried (36-39°C). The dried leaves were ground to a coarse powder with grinder. The powder (726 g) was placed in a Soxhlet extractor and extracted with ethanol in three cycles for about 60 h. The extracted material was filtered with Whatman filter paper No. 4. The filtrate obtained was dried *in vacuo* between 30-36°C. The yield about 24 g was stored at 4°C in the refrigerator till needed.

Animals: Healthy adult rabbits of either sex weighing between 1.5-1.8 kg⁻¹ were obtained from the Animal House of the University of Ibadan, Oyo State, Nigeria. Having certified their health conditions, were kept in aluminum cages under standard conditions in the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria. They were fed standard rabbit pellets from Livestock Feeds PLC, Lagos and water *Ad libitum*. The ethical use of the animals was sought and obtained from the animal department of College of Medicine, University of Lagos, Nigeria.

Induction of Diabetes: Rabbits fasted overnight (18 h) were induced with a single intravenous injection of 150 mg/kg alloxan monohydrate [11]. Hyperglycaemia was confirmed where elevated blood glucose level was = 250 mg/dl after 72 h of injection [12].

Effect of the Ethanol Extract on Oral Glucose Tolerance (OGTT): The rabbits were fasted for 18 h and were randomized to three groups of five rabbits each. Blood was collected pre-treatment from each rabbit to determine fasting blood glucose. The rabbits in group one received 2 ml/kg⁻¹ distilled water orally. Group two received 1 g/kg⁻¹ of the ethanol leaves extract while group three received 0.01 g/kg⁻¹ of glibenclamide by gavages. Thirty minutes after distilled water, aqueous extract or glibenclamide administration, the rabbits in the three

groups were given oral glucose load at 1 g/kg⁻¹ [13]. Blood was collected from the animals at 30 min, 1, 2, 3 and 4h after the oral glucose load for the blood glucose estimation [14].

Effect of the Ethanol Extract on Alloxan-Induced Diabetic

Rabbits: The diabetic animals were randomized to the following groups of five rabbits each: group one was diabetic control; group two received glibenclamide (10 mg/kg) orally; groups three, four and five received 50, 100 and 200 mg/kg of the extract respectively orally. Treatment was continued for 15 days. Before the treatment (day 0) and after the treatments (days 3, 5, 7, 9, 11, 13 and 15), plasma glucose levels were estimated by glucose oxidase method [12].

Acute Toxicity: Rabbits were divided into five groups of five animals each. Different doses (500, 1000, 2000, 4000 and 8000 mg/kg) of the ethanol extract was administered by gavage. The animals were observed for general toxicity signs. LD₅₀ was determined by the method of Horn [15].

Statistical Analysis: All values were expressed as mean ± standard error of mean and the statistical significance between treated and control groups were analyzed by means of Student's t-test. P<0.05 was considered significant.

RESULTS

Oral Glucose Tolerance Test (OGTT): As shown in Fig. 1, the untreated rabbits (vehicle group) demonstrated hyperglycaemia after oral glucose load that failed to return to baseline 4h later. The single extract dose administered 30 min prior to the glucose load significantly (P<0.05) enhanced the clearance of plasma glucose by reducing the peak value of glucose concentration as compared to the untreated. The activity of the extract was comparable to glibenclamide though to a lesser extent as demonstrated in the decrease of area under OGTT curve by both treatments with values of 26.9 and 43.8% respectively.

Effect of Ethanol Extract on Alloxan-induced Diabetes:

The administration of alloxan at 150 mg/kg (i.v.) (Table 1) led to elevation of fasting blood glucose that was maintained (diabetic control) over the period of the experiment. Treatment with the leaf extract (50, 100 and

Table 1: Plasma glucose level of rabbits treated with SJ ethanol leaf extract

	Dose (mg/kg)	Plasma glucose levels (mg/100ml) during treatment with the extract							
		Day 0	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15
Non-diabetic		78.2±7.8	76.7±5.5	72.7±6.4	69.8±5.0	70.1±5.0	70.4±4.9	73.9±4.2	72.3±5.1
Diabetic untreated		334.0±8.5	360.1±6.9	361.6±7.3	368.0±9.2	373.8±10.6	383.6±10.6	399.3±12.5	410.7±10.7
Glibenclamide	10	329.5±8.8	309.3±5.4*	294.4±1.8*	281.3±1.7*	273.9±1.5*	264.9±2.1*	258.4±2.8*	244.7±2.7*
<i>S. jollyanum</i> leaf	50	351.2±6.2	328.2±5.4*	302.5±4.4*	285.2±4.0*	280.3±4.3*	271.1±3.2*	266.2±5.1*	260.5±2.6*
<i>S. jollyanum</i> leaf	100	359.3±9.2*	331.4±5.8*	310.2±3.2*	295.0±3.2*	283.8±2.1*	269.6±4.2*	261.2±3.7*	257.0±2.6*
<i>S. jollyanum</i> leaf	200	350.2±8.1	324.2±3.1*	310.0±1.3*	292.1±3.5*	279.6±3.6*	265.6±4.6*	258.8±2.3*	255.0±2.2*

Table shows the plasma glucose concentration during 15 days of extract/glibenclamide administration or 10mg/kg distilled water (control). Values are Mean±SEM; n=5, *p<0.05 compared to control (Student's t-test)

Table 2: Plasma glucose level of rabbits' recovery post-treated with SJ leaf extract

	Dose (mg/kg)	Plasma glucose levels (mg/100ml) post treated with the extract				
		Day 0	Day 3	Day 5	Day 7	Day 9
Non-diabetic		74.2±4.7	70.7±3.9	75.0±1.4	68.2±0.4	71.2±3.0
Diabetic untreated		410.7±10.7	450.3±13.4	473.0±14.2	499.8±21.1	525.1±24.6
Glibenclamide	10	244.7±2.7*	218.5±1.2*	197.9±4.0*	177.3±5.7*	158.5±5.0*
<i>S. jollyanum</i> leaf	50	260.5±2.6*	242.2±4.3*	227.9±4.5*	218.8±5.0*	208.0±5.3*
<i>S. jollyanum</i> leaf	100	257.0±2.6*	240.2±3.1*	225.4±2.1*	212.2±1.7*	201.6±2.6*
<i>S. jollyanum</i> leaf	200	255.0±2.2*	239.7±3.8*	223.8±2.2*	210.2±2.8*	200.2±2.6*

Table shows the plasma glucose concentration during 9 days post extract/glibenclamide administration. Values are Mean±SEM; n=5, *P<0.05 compared to control (Student's t-test)

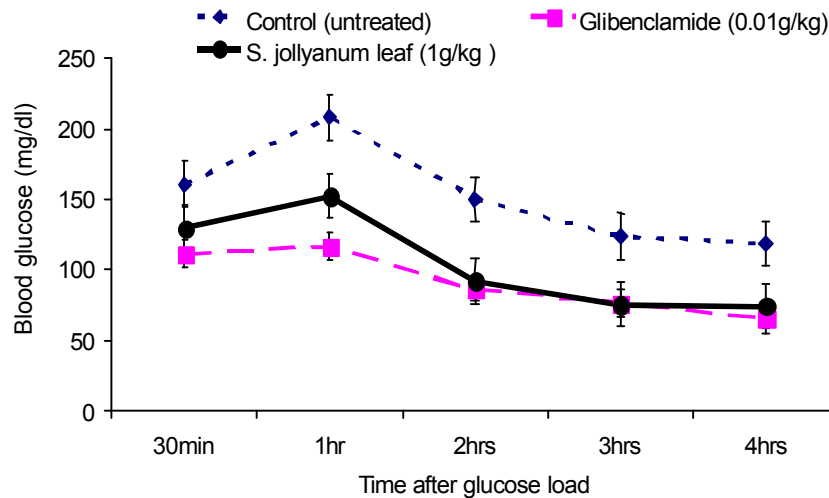


Fig. 1: Ethanol leaf extract of *S phenocentrum jollyanum* on OGTT. Values represent Mean±SEM (n=5)

200 mg/kg) of SJ significantly (P<0.05) lowered the blood glucose level in a dose dependent manner from day 3 of continuous oral administration to the last day of treatment. Further decrease in glycaemia occurred in all the treated groups as shown in Table 2. The maximum

anti-hyperglycaemic effect was observed in the group that received 200 mg/kg with plasma glucose level of 200.2±2.6 mg/dl (42.8%) compared to glibenclamide treated (10 mg/kg) with maximum decrease of 158.5±5.0 mg/dl (54.8%).

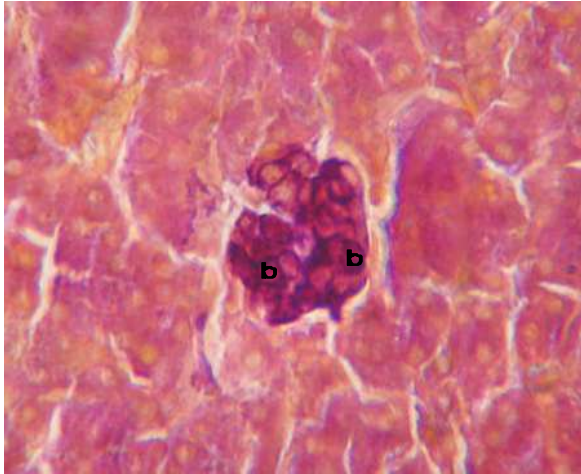


Fig. 2: Normal pancreatic tissue showing the beta cells (b) (Gomori aldehyde fuchsin stain) x400

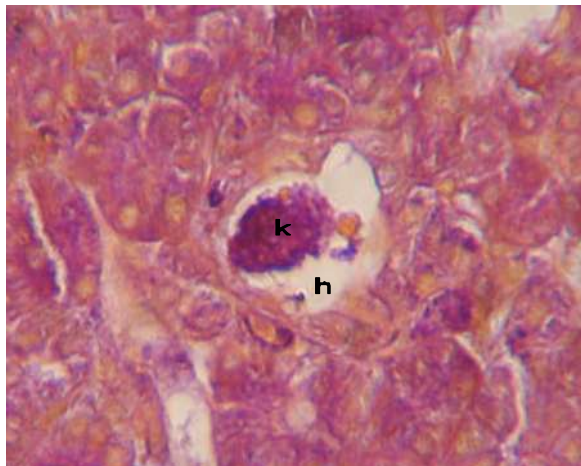


Fig. 3: The diabetic untreated specimen indicating amorphous crumb of necrotic cells (k) and a halo (h) around (Gomori aldehyde fuchsin stain) x400

Histopathology of Pancreatic Tissue: The photomicrograph (Fig. 2) showed the normal islet organization in vehicle treated rabbit. The beta cells devoid of deep nuclei staining were compactly arrangement. In diabetic untreated (Fig. 3), extensive damage occurred in the islet cells. As shown, shrunken mass of material formed a condensed crumb at the centre with a halo around it. In leaf extract treatment (Fig. 4), amorphous material due to necrosis was observed with few surviving beta cells spotted at the periphery while glibenclamide (Fig. 5) treated showed less severe lesion as more viable cells could be observed.

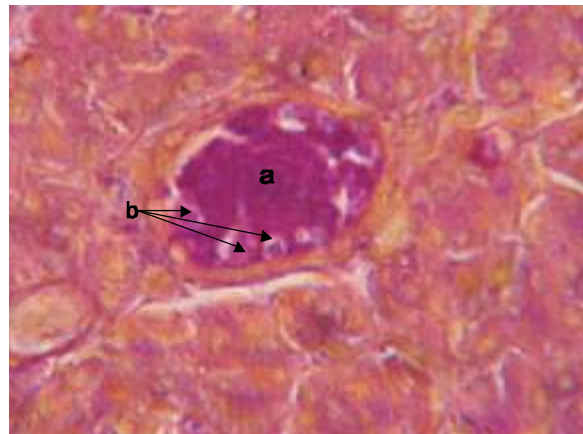


Fig. 4: Diabetic study showing arrowed, the survivor beta cells (b) and amorphous area (a) after treatment with the leaf extract (Gomori aldehyde fuchsin stain) x400

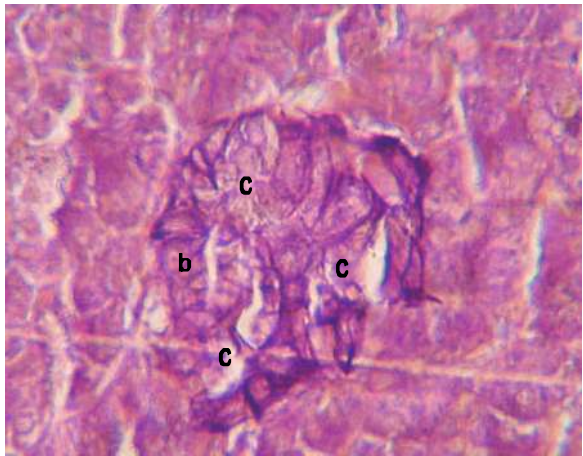


Fig. 5: Diabetic study showing, diffuse area (c) of necrotic cells and survivor beta cells (b) after treatment with glibenclamide (Gomori aldehyde fuchsin stain) x400

Acute Toxicity: The extract administered orally up to the highest dose tested (8000 mg/kg) produced no mortality. The animals did not manifest any sign of respiratory distress, restlessness, general irritation, coma or convulsion.

DISCUSSION

This study was undertaken to investigate the anti-hyperglycaemic activity of SJ ethanol leaf extract in hyperglycaemic normal and alloxan-induced diabetic rabbits. The results of the finding clearly indicated that ethanol extract of the leaves possesses

anti-hyperglycaemic property. The extract (Fig. 1) exhibited effective glycaemic control by decreasing the peak blood glucose concentration and the area under OGTT curve. The return to baseline glycaemia after 3 h in OGTT was indicative of an enhanced glucose utilization triggered by insulin production from the beta cells.

In alloxan-induced diabetic rabbits, dose dependent decrease in glycaemia occurred following oral administration of the extract. However, with the maximum percentage decrease of 42.8%, it was obvious that the beta cells destruction affected the activity of the extract. This implied that the leaf extract anti-hyperglycaemic effect was dependent upon the beta cells for insulin production. In endogenous insulin production the number of surviving beta cells in the pancreatic islet is of decisive importance. As shown in the photomicrograph (Fig. 4), extensive area of amorphous material was observed in the islets due to necrosis with few surviving beta cells at the periphery.

Although the mode of action of SJ leaf was yet to be determined, it is believed that the plant antioxidant defenses [10] may have suppressed further oxidative damage by alloxan and through the stimulatory action of the surviving/undamaged beta cells increased insulin production. This appears consistent with the explanation put forward on a number of plants reported to have anti-hyperglycaemic and insulin stimulatory effects [16-18]. It is however pertinent to note that the tissue histology indicated no evidence of beta cells regeneration (Fig. 4) as was reported with some other plants [19-22]. The further decrease in glycaemia that occurred post treatment (Table 2) with the extract/glibenclamide may have been due to the residual effect of the respective treatments.

CONCLUSION

The present investigation clearly shows that the ethanol leaf extract of SJ plant has effective blood glucose lowering potential in hyperglycaemic and alloxan-induced diabetic rabbits. The moderate glycaemic reduction that occurred in alloxan diabetic rabbit was believed to have resulted from potentiation of insulin release by the survivor beta cells. The ability of the plant extract to promote insulin release is therefore a potential discovery that if exploited could be of value in the management of diabetes mellitus.

ACKNOWLEDGMENT

The authors wish to sincerely thank Chief Adeyemi Adebambo, who assisted with the collection of the plant.

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