Original article

Anti-hyperglycaemic and hypoglycaemic effects of ethanol root extract of *Sphenocentrum jollyanum* in normal and alloxan-induced diabetic rabbits

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Abstract

Objective: To evaluate the anti-hyperglycaemic and hypoglyaemic effect of ethanol root extract of *Sphenocentrum jollyanum* (SJ) Pierre (Menispermaceae) in alloxan diabetic rabbits. Materials and methods: Alloxan diabetic rabbits (n = 5) were administered graded doses (50, 100 and 200 mg.kg^{-1}) of the extract or glibenclamide (10 mg.kg^{-1}) or vehicle (distilled water) for 15 days. Blood was collected i.v. from auricular vein on days 0, 3, 5, 7, 9, 11, 13, 15 for glucose estimation by oxidase method. In the oral glucose tolerance test (OGTT), rabbits received the extract (1 g.kg^{-1}) or glibenclamide (0.01 mg.kg^{-1}) or vehicle and 30 minutes later received oral glucose load (1 g.kg^{-1}). Glucose was estimated at 30 minutes, 1, 2, 3 and 4 hours. The hypoglycaemic activity was assessed on normoglycaemic rabbit that received extract at 100, 250 and 500 mg.kg^{-1} and estimated at 0, 4, 8 and 12 hours. Results: The extract significantly ($p < 0.05$) enhanced glucose reduction by decreasing the peak glycaemic level and the area under curve in OGTT compared to the untreated. In alloxan diabetic rabbit, the extract produced significant dose dependent decrease in glycaemia from day 3 of oral treatment with comparatively higher activity than glibenclamide treatment. It also exhibited marked hypoglycaemic effect at 500 mg.kg^{-1} with lowest glycaemic reduction of 54.5 ± 7.7 (30.1%). The pancreatic histology showed focal areas of beta cells necrosis with significant number of the cells normal, suggesting that the extract may have effectively attenuated the diabetogenic agent inf ammatory response. Conclusion: The ethanol root extract of SJ was very effective in lowering blood glucose.

Keywords: ant-diabetes, *Sphenocentrum jollyanum*, pancreas, histopathology.

1 Introduction

Diabetes mellitus is a metabolic disorder with the highest rate of prevalence in the world (HARRIS, FLEGAL, COWIE et al., 1998; BARCELO and RAJPATHAK, 2001). The disease which is multi-factoral is characterized by hyperglycaemia. It is one of the most common chronic diseases that has proved difficult to manage despite advances made in clinical sciences to understand its causes and complications. The major reason is the recurrent drawbacks encountered with currently available drugs (EARL, 2005). Because of these limitations, other therapeutic modalities have been introduced. The management of diabetes mellitus with oral hypoglycaemic agents has received a growing interest in which there have been intensified search for appropriate hypogycaemic therapy that would more effectively control the disease. This search appeared focused on indigenous natural source because of the lead provided by the traditional medicine that may be better treatment than the currently used drugs (RATES, 2001). The investigative outcome has consistently shown that several plant products exhibit unique hypoglycaemic activities in diabetic animal models (KUSANO and ABE, 2000). Furthermore, plant source is frequently considered to be less toxic and freer from side effects than synthetic drugs (BAILEY and DAY, 1989).

Sphenocentrum jollyanum Pierre is undergrowth of dense forest which grows up to 1.5 m high with few branches. The plant is distributed along the west coast of Africa from Sierra Leone to Nigeria (BURKILL, 1985; NIA, PAPER, ESSIEN et al., 2004). The root is bitter in taste but causes things eaten thereafter to taste sweet (MENNINGER, 1967). The plant belongs to the Menispermaceae family. A wide range of these plants are known for their biological activities that include remedy for diabetes and metabolic disorders in folkloric medicine.

In this study, the anti-hyperglycaemic and hypoglycaemic effects of the ethanol root extract of *Sphenocentrum jollyanum* were evaluated in normal and alloxan induced diabetic rabbits which were compared with those of glibenclamide, a known hypoglycaemic drug.

2 Material and methods

2.1 Plant material

Fresh roots of *S. jollyanum* were obtained from a farm land located in Ijebu-Oru community, Ogun State, Nigeria. The collection was in the month of November, washed with tap

water before dried under the sunlight. It was authenticated by a taxonomist, Dr. O. A. Ugbogu, Chief Research Officer at the Forest Research Institute of Nigeria (FRIN) where voucher specimen of the plant has been deposited in the herbarium (FHI/108203).

2.2 Preparation of plant ethanol extract

The dried roots having been chopped into pieces and dried (35-37 °C) were subjected to size reduction to a coarse powder with electric grinder. The powder (2280 g) was placed in a soxhlet extractor and extracted with absolute ethanol in three cycles for about 60 hours. 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 67 g was stored in a refrigerator (4 °C) till it was needed.

2.3 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 natural light and dark cycle at the temperature of 26 ± 5 °C 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.

2.4 Induction of experimental diabetes

Rabbits fasted overnight (18 hours) were induced with a single intravenous injection (i.v.) of 150 $g.kg^{-1}$ of alloxan monohydrate (KHOSLA, GUPTA and NAGPAL, 1995) (with modification). Hyperglycaemia was confirmed where elevated blood glucose level was ≥ 250 mg/dl after 72 hours of injection (OLAJIDE, AWE and MAKINDE, 1999).

2.5 Evaluation of anti-hyperglycaemic activities

(i) Oral glucose tolerance test (OGTT)

The rabbits were fasted for 18 hours and were randomized to 3 groups of 5 rabbits each. Blood was collected pre-treatment from each rabbit to determine fasting blood glucose. The rabbits in group 1 received 10 $mL.kg^{-1}$ distilled water orally. Group 2 received 1 $g.kg^{-1}$ of the ethanol extract while group 3 received 0.01 $g.kg^{-1}$ of glibenclamide by gavages. Thirty minutes after distilled water, ethanol extract or glibenclamide administration, the rabbits in the three groups were given oral glucose load at 1 $g.kg^{-1}$ (PERFUMI, ARNOLD and TACCONI, 1991). Blood was collected from the animals at 0.5, 1, 2, 3 and 4 hours after the oral glucose load for the blood glucose estimation (MOSHI, UISO, MAHUNNAH et al., 1997).

(ii) Alloxan- induced diabetic rabbits

The diabetic animals were randomized to the following groups of 5 rabbits each: group I was diabetic control; group II received glibenclamide (10 $mg.kg^{-1}$) orally; groups III, IV and V received 50, 100 and 200 $mg.kg^{-1}$ 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 (OLAJIDE, AWE and MAKINDE et al., 1999).

2.6 Evaluation of hypoglycaemic activity

Rabbits fasted for 18 hours were randomly divided into four groups of 5 per group. The first three groups (I, II and III) were administered by gastric gavage (single dose) with ethanol extract of SJ root dissolved in water at the concentration of 100, 250 and 500 $mg.kg^{-1}$ respectively (SHARMA, DWIVEDI and SWARUP, 1997). The fourth group (IV) which served as the control received distilled water (10 $mL.kg^{-1}$). Blood glucose level was determined at 0, 4, 8 and 12 hours later.

2.7 Acute toxicity

Rabbits were divided into five groups of 5 animals each. Different doses (500, 1000, 2000, 4000 and 8000 $mg.kg^{-1}$) of the alcohol extract was administered by gavage. The animals were observed for general toxicity signs. LD_{50} was determined by the method of Horn (HORN, 1956).

2.8 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.

3 Results

3.1 Activity in hyperglycaemic rabbits

Figure 1 summarized the result of the OGTT. Ethanol root extract of SJ significantly ($p < 0.05$) enhanced glucose reduction by decreasing the peak glycaemic level and the area under curve in an oral glucose load compared to the untreated. It produced reduction to baseline glycaemia 3 hours later which was comparable to the effect caused by glibenclamide. The extract and glibenclamide treatment showed maximum decrease of 74.6 ± 2.1 (38.3) and 65.7 ± 2.0 (41.0%) respectively 4 hours after glucose load.

3.2 Activity in alloxan hyperglycaemic rabbits

As shown in Table 1, the treatment of alloxan diabetic rabbits with graded doses of the root extract caused

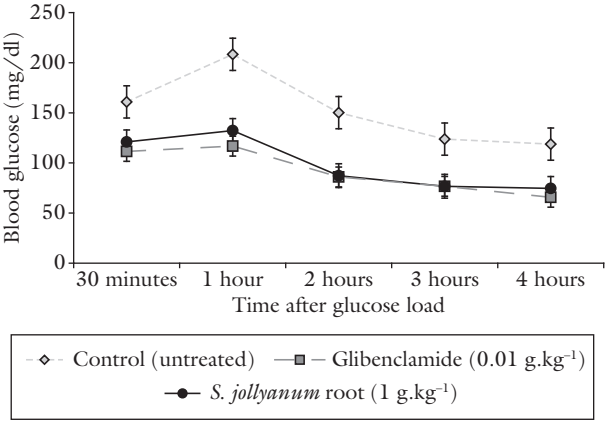

Figure 1. Ethanol root extract of *Sphenocentrum jollyanum* on OGTT. Values represent Mean ± SEM (n = 5).

significant decrease ($p < 0.05$) in blood sugar levels that were evident from the day 3 of oral dose and onward to the last day of the administration. The study was extended to assess post treatment effect in which further decrease in glycaemia was observed (Table 2). The extract exhibited dose dependent effect with maximum anti-hyperglycaemic activity of 57.2% (148.8 ± 7.6) observed in the group that received 200 mg.kg^{-1} per day of the extract compared to glibenclamide with 54.8% (158.5 ± 5.0).

3.3 Activity in normoglycaemic rabbits

Normal glucose levels did not change appreciably (Figure 2) in animals treated with 100 and 250 mg.kg^{-1} of the extract compared to the control. However, at 500 mg.kg^{-1}, significant ($p < 0.05$) decrease occurred after 4 hours of oral administration of the extract with the lowest reduction (30.1%) occurring 8 hours after. The effect produced at 12 hours was less.

3.4 Histopathology of pancreatic tissue

The photomicrograph (Figure 3) showed the normal islet organization with compact beta cell arrangement in vehicle treated rabbit. Extensive damage at the islet cells occurred in diabetic untreated (Figure 4). As shown, shrunken mass of amorphous material formed a condensed crumb at the centre with a halo around it whereas post diabetic treatment with the extract (Figure 5) showed only focal areas of beta cells necrosis with the majority having normal appearance. Treatment with glibenclamide exhibited less effectiveness in ameliorating the alloxan activity. As shown (Figure 6), there was more severe lesion and interspaced viable cells compared to the extract treatment.

3.5 Acute toxicity test

The extract administered orally up to the highest dose tested (8000 mg.kg^{-1}) produced no mortality. The animals did not manifest any sign of respiratory distress, restlessness, general irritation, coma or convulsion.

3.6 Discussion

In the light of the present study, the extract clearly exhibited effective glycaemic control by reversing high plasma glucose level to basal glycaemia following oral glucose load. The anti-hyperglycaemic effect of the plant may be due to a number of factors. SJ root is rich in ingredients that have been reported to possess anti-hyperglycaemic activities like saponins known to be bioactive against diabetes (ABDEL-HASSAN, ABDEL-BARRY and MOHAMMEDA, 2000). Tepenoids, flavonoids, glycosides and alkaloids frequently implicated with this activity (LOEW and KASZKIN, 2002) were

Table 1. Plasma glucose level of rabbits treated with SJ root extract.

	Dose (mg.kg^{-1})	Plasma glucose levels (mg.100 mL^{-1}) during treatment with the extract			
		Day 0	**Day 3**	**Day 5**	**Day 7**
Non-diabetic		78.2 ± 7.8	76.7 ± 5.5	72.7 ± 6.4	69.8 ± 5.0
Diabetic untreated		334.0 ± 8.5	360.1 ± 6.9	361.6 ± 7.3	368.0 ± 9.2
Glibenclamide	10	350.5 ± 8.8	326.3 ± 5.4*	304.4 ± 1.8*	288.3 ± 1.7*
S. jollyanum root	50	340.6 ± 18.4	320.4 ± 15.4*	305.8 ± 11.4*	286.1 ± 10.1*
S. jollyanum root	100	346.4 ± 18.5	321.7 ± 13.3*	306.7 ± 13.1*	288.3 ± 12.5*
S, jollyanum root	200	347.5 ± 10.4	323.2 ± 9.3*	299.8 ± 5.8*	286.7 ± 10.1*
		Day 9	**Day 11**	**Day 13**	**Day 15**
Non-diabetic		70.1 ± 5.0	70.4 ± 4.9	73.9 ± 4.2	72.3 ± 5.1
Diabetic untreated		373.8 ± 10.6	383.6 ± 10.6	399.3 ± 12.5	410.7 ± 10.7
Glibenclamide	10	279.9 ± 1.5*	267.9 ± 2.1*	259.4 ± 2.8*	244.7 ± 2.7*
S. jollyanum root	50	289.5 ± 6.7*	276.3 ± 7.4*	256.9 ± 5.5*	240.5 ± 5.7*
S. jollyanum root	100	285.7 ± 7.7*	267.7 ± 3.5*	247.8 ± 5.9*	222.9 ± 6.3*
S. jollyanum root	200	274.4 ± 4.5*	261.9 ± 5.9*	248.8 ± 5.6*	225.2 ± 6.9*

Table shows the plasma glucose concentration during 15 days of extract/glibenclamide administration or 10 mg.kg^{-1} 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^{-1})	Plasma glucose levels (mg.100 mL^{-1}) 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*
S. jollyanum root	50	240.5 ± 5.7*	219.0 ± 5.6*	207.6 ± 5.1*	194.6 ± 5.4*	185.1 ± 7.2*
S. jollyanum root	100	222.9 ± 6.3*	199.2 ± 7.0*	183.5 ± 7.3*	167.2 ± 8.1*	152.6 ± 10.0*
S. jollyanum root	200	225.2 ± 6.9*	201.9 ± 7.2*	183.6 ± 7.4*	167.1 ± 8.1*	148.8 ± 7.6*

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).

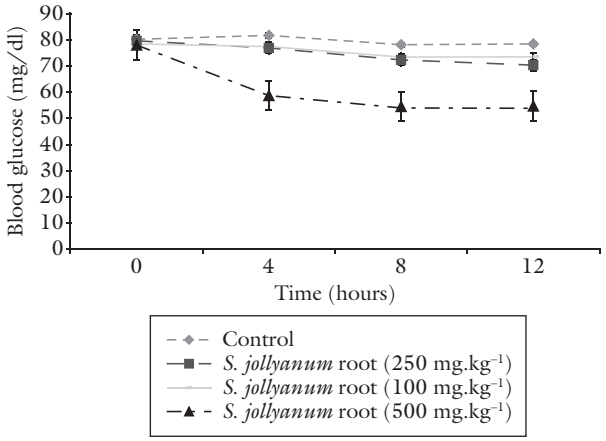

Figure 2. Hypoglycaemic activity of *Sphenocentrum jollyanum* root extract. Values represent Mean ± SEM (n = 5).

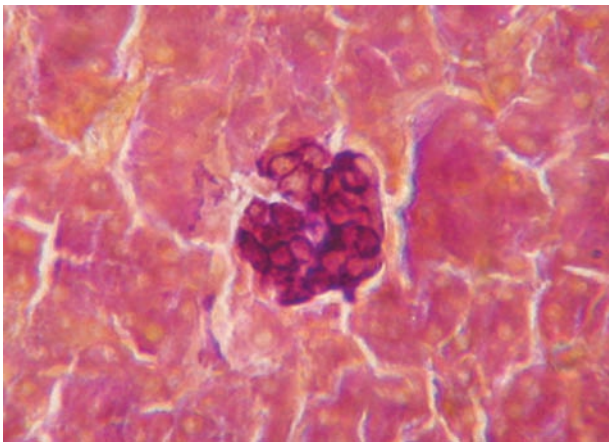

Figure 3. Normal pancreatic tissue highlighting the beta cells in the islet organization (Gomori aldehyde fuchsin stain) 400×.

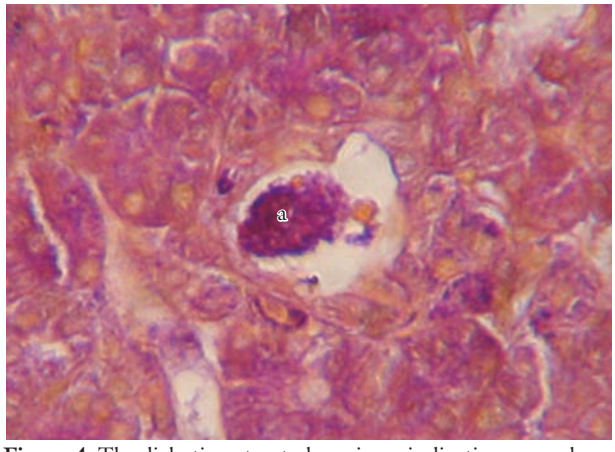

Figure 4. The diabetic untreated specimen indicating amorphous condensed mass (a) (Gomori aldehyde fuchsin stain) 400×.

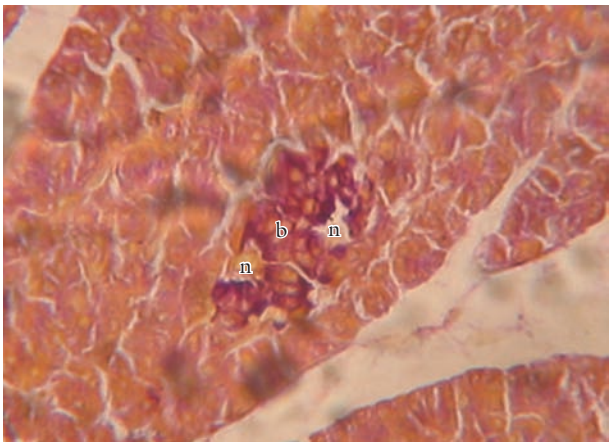

Figure 5. Diabetic study showing the survivor beta cells (b) and necrotic areas (n) after treatment with root extract (Gomori aldehyde fuchsin stain) 400×.

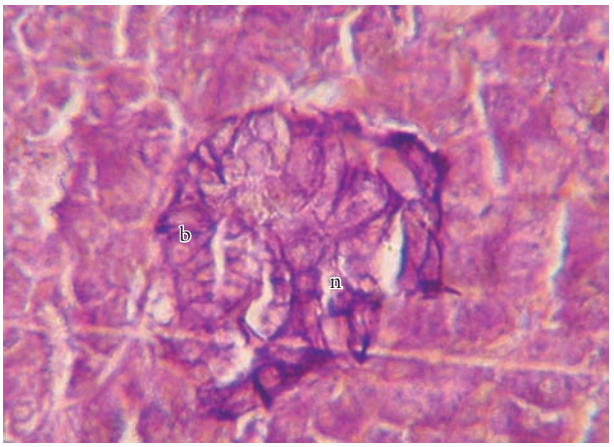

Figure 6. Diabetic study showing the survivor beta cells (b) and necrotic cells (n) after treatment with glibenclamide (Gomori aldehyde fuchsin stain) 400×.

also part of its active component. The activities of these substances may have triggered the beta cells to increase insulin production which promotes glucose uptake and utilization by other tissues. In alloxan induced diabetic rabbits, significant reduction in blood sugar level was also recorded. The fact that alloxan is known to destroy beta cells leaves two possible conjectures; the stimulation of survivor beta cells to enhance insulin production or activities that include extra-pancreatic pathway of action. However with maximum decreases of 45.6, 55.9 and 57.2% (50, 100 and 200 mg.kg^{-1}) considerably less than normal glycaemic index implied that the stimulation of the undamaged/survivor beta cells to facilitate insulin secretion appeared the likely mode. Similar explanation has been put forward on a number of plants believed to have anti-hyperglycaemic and insulin stimulatory effects (VENKATESWARAN and PARI, 2002; LATHA and PARI, 2003; LATHA and PARI, 2004).

Since diabetes mellitus is generally known to precipitate oxidative stress (MOHAMED, BIERHAUS, SCHIEKOFER et al., 1999), it is probable that the anti-oxidative activities (NIA, PAPER, ESSIEN et al., 2004) of the extract may have scavenged the free radicals liberated by alloxan as well as suppressed their further activation. Indication emerged from the result of the photomicrograph which showed only focal areas of beta cells necrosis unlike in the glibenclamide treatment with more severe lesion. It was obvious that the extract effectively attenuated the diabetogenic agent

inf ammatory response to enhance the activity of insulin producing beta cells.

The potentiation of insulin release from the beta cells may have equally accounted for the hypoglycaemic effect observed in oral dose of 500 $mg.kg^{-1}$ in normoglycaemic rabbits. This agrees with similar findings on some other plants (IVORRA, PAYA and VILLAR, 1988; SHARMA, DWIVEDI and SWARUP, 1997; SHALEV, 1999). Besides, saponins and glycosides found in the extract are strongly linked with hypoglycaemic activity with the saponins component speculated to act as direct hypoglycaemic agent (ABDEL-HASSAN, ABDEL-BARRY and MOHAMMEDA, 2000). It is not unlikely that this may have in part contributed to the root extract hypoglycaemic effect.

4 Conclusion

The result of this investigation clearly demonstrated that the ethanol root extract of SJ exhibited significant reduction in blood glucose level in hyperglycaemia and alloxan induced diabetic rabbit. The extract equally exhibited hypoglycaemic effect that was comparable to the standard oral hypoglycaemic agent used. It is apparent from these findings, that the root of SJ is a potential source that can be exploited for the development of new useful addition to available oral anti-diabetes drug.

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