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Full Length Research Paper

Anti-diabetic activity of the seed extract and the morphological changes on pancreatic beta cells in alloxan induced-diabetic rabbits

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Anti-hyperglycaemic and anti-diabetic activity of petroleum ether seed extract of *Sphenocentrum jollyanum* (SJ) Pierre (Menispermaceae) was evaluated in hyperglycaemic and in alloxan diabetic rabbits. The seed extract of SJ and glibenclamide administered 30 min prior to the glucose load significantly ($p < 0.05$) reduced the peak values and the area under curve of blood glucose by 20.0 % and 43.8 % respectively compared to the untreated. In alloxan diabetic animals, treatment with the seed extract of SJ significantly ($p < 0.05$) lowered the blood glucose level in a dose dependent manner from day 3 of daily treatment and continued till the last day of the treatment. Further decrease was however recorded post-treatment with the extract doses of 300, 600 and 1200 mg/kg body weight (bw) showing maximum percentage decrease of 12.3, 29.2 and 32.7 respectively while glibenclamide group (10 mg/kg) recorded a decrease of 51.9 %. Tissue morphology of the extract treated diabetic rabbits showed necrotic beta cells with some viable cells at the periphery. In glibenclamide treated, considerable damage to the beta cells was equally recorded. The result indicates that the extract attenuated hyperglcaemia in alloxan diabetic rabbits.

Key word: Anti-hyperglycaemia, alloxan-diabetes, *Sphenocentrum jollyanum,* seed, rabbit.

INTRODUCTION

Diabetes mellitus (DM) is a clinical condition in which the metabolic activity of carbohydrate, protein and fat is seriously impaired. Available reports suggest that DM is a metabolic disorder with the highest rate of prevalence and mortality worldwide (Harris et al, 1998; Barcelo and Rajpathak, 2001). Within the last few decades, the frequency of its occurrence showed marked increase with nearly 10% of the world population said to be living with the ailment (Vetrichelvan et al, 2002). Consequently, it is projected to become one of the world's major health problems within the next 25 years with developing countries expected to be worst affected (Marx, 2002).

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The use of insulin and several oral hypoglycaemic drugs such as sulfonylureas and biguanids represent significant intervention for both type 1 and type 2 diabetes. But even with their effective control of blood glucose, they could not guarantee prevention of diabetic complications (Holman and Turner, 1991). Besides, they are known to have a number of limitations such as significant side effects (Xie et al. 2005) suggesting that alternative treatment strategies are needed.

The use of herbal remedy in the treatment of diseases has been a long time practice. Due to various reasons in recent times, their popularity has increased. The search for new pharmacologically active agents through screening of natural source of plant origin has led to the discovery of many clinically useful drugs that are of vital importance in treating various types of diseases including metabolic disorder (Pareek et al, 2009). Medicinal plants

used in the treatment of DM are of considerable interest because of their perceived little or no side effect thus making them to be more preferred to the synthetic therapeutic agents known to be associated with many side effects (Venkatesh et al, 2003).

Sphenocentrum jollyanum Pierre (Menispermaceae) is a rain forest plant that grows naturally along the west coast sub region of Africa with expanse from Cameroon across Nigeria to Sierra Leone. The plant is deep rooted with few branches. It bears fruit that is yellowish in colour when ripe and contains a single large oval shaped seed. SJ has been shown to display a wide spectrum of biological and pharmacological activities. Its medicinal importance was first reported by Dalziel (1955) in which it was noted that the leaves decoctions were used as vermifuge. It is reputed for use in dressing wounds particularly chronic wounds, feverish conditions, cough as well as being an aphrodisiac (Dalziel, 1955; Iwu, 1993). The plant is also used for treating jaundice, breast engorgement related to the menstrual cycle, tumours and inflammatory conditions (Iwu, 1993; Odugbemi, 2006). According to studies (Iwu, 1993; Nia et al, 2004), the leaves of the plant possessed significant anti-inflammatory, anti-angiogenic and analgesic properties. They have also been found to be potent against polio type-2 virus (Moody et al, 2006). Investigations equally showed that the leaves (Mbaka et al, 2008) and the roots (Mbaka et al, 2009) exhibited significant anti-diabetic effect.

Since previous findings showed the root and the leaf to possess significant anti-diabetic property, the present study was therefore undertaken to examine the possible anti-diabetic activity of the seed extract and the morphological changes on pancreatic beta cells in alloxan induced-diabetic rabbits.

MATERIALS AND METHODS

Plant material

The dried seeds of *Sphenocentrum jollyanum* were bought from Oja Ibode market in Ibadan, Oyo State, Nigeria. They were 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).

Preparation of plant seed petroleum ether extract

The dried seed were subjected to size reduction to a coarse powder with electric grinder. The powder (1240 g) was placed in a soxhlet extractor and extracted with petroleum ether in three cycles for about 60 h. The extracted material was filtered with Whatman filter paper No. 4. The filtrate obtained was concentrated *in vacuo* between 30-36 ^{O}C. The yield about 142 g was stored in a refrigerator (4 ^{O}C) till it was needed.

Animals

Healthy adult rabbits of either sex weighing between 1.5-1.9 kg 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 ^{O}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 use of the animals and the experimental protocol was in strict compliance with the standard guide-lines on the use and care of experimental animals.

Induction of experimental diabetes

Rabbits fasted overnight (18 h) were induced with a single intravenous injection (i.v.) of 150 g/kg of alloxan monohydrate (Khosla et al, 1995) (with modification). Hyperglycaemia was confirmed where elevated blood glucose level was ≥ 250 mg/dl after 72 h of injection (Olajide et al, 1999).

The animals weight were recorded before the diabetic induction (day 0) and on days; 3, 5, 10 and 15.

Evaluation of anti-hyperglycaemic activities

(i) Oral glucose tolerance test (OGTT)

The rabbits were fasted for 18 h 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 distilled water orally. Group 2 received 1 g/kg of the extract prepared using 2 % Tween 80 solution while group 3 received 0.01 g/kg of glibenclamide by gavages. Thirty minutes after distilled water, petroleum ether extract or glibenclamide administration, the rabbits in the three groups were given oral glucose load at 1 g/kg (Perfumi et al, 1991). Blood was collected from the animals at 0.5, 1, 2, 3 and 4 h after the oral glucose load for the blood glucose estimation (Moshi 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) orally; groups III, IV and V received graded doses of 300, 600 and 1200 mg/kg/day of the extract respectively by gavages. 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 et al, 1999).

Tissue histology

The pancreatic tissue was fixed in Bouin's fluid before being processed and stained with aldehyde fuschsin which colours the beta cells purple.

Phytochemical analysis

Phytochemical screening of the extract for the presence of secondary metabolites was performed using the following reagents

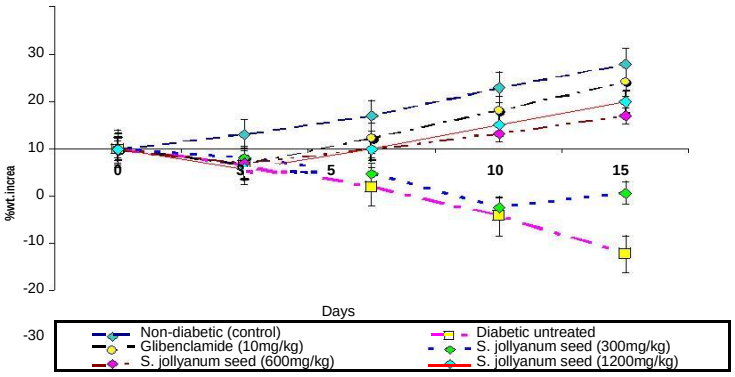

Figure 1. Percentage weight increase in the treated animals

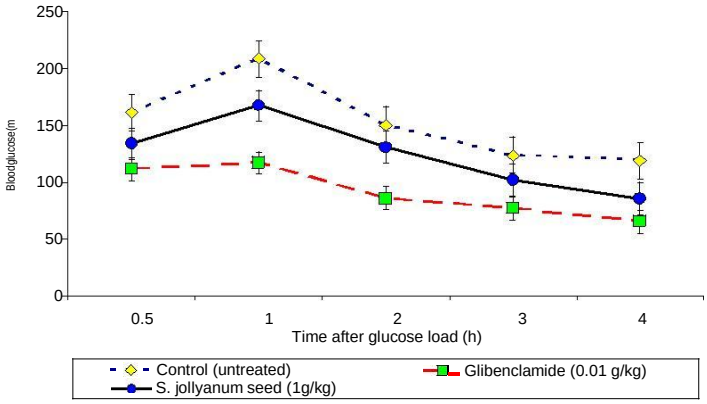

Figure 2. The effect of seed petroleum ether etract of spenocentrum jollyanum on OGTT. Values represent Mean±SEM (n=5).

and chemicals: alkaloids with Mayer's and Dragendorffs reagents **(**Farnsworth, 1966; Harborne, 1998), flavonoids with the use of Mg and HCl (Silva et al, 1993; Houghton and Raman, 1998), tannins with 1 % gelatin and 5 % ferric chloride solution, and saponins with ability to produce suds (Houghton and Raman, 1998). Liebermann-Buchard test consisting of a mixture of glacial acetic acid and sulphuric acid (19:1) was used to differentiate the terpenoids and steroidal compound (Farnsworth, 1966).

Acute toxicity

Mice randomly grouped (8 per group) received different doses (0.5, 1, 2, 4, 8 and 16 g/kg) of the extract administered by gavages. The doses were prepared by dispersing 20 g of the gel with 7 mL Tween 80 (2 %) solution in a 100 ml beaker and transferred to a 20 mL volumetric flask. The beaker was thoroughly rinsed with the Tween 80 solution; the content added to the volumetric flask and the volume made to mark with the Tween 80 solution. The animals were observed continuously for the first 4 hours and then for each hour for the next 12 hours, followed by 6 hourly intervals for the next 56 hours (72 hrs observations) to observe any death or changes in general behaviour and other physiological activities (Shah et al, 1997; Bürger et al, 2005).

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

Variation of body weight

The body weight changes of the control and the extract /glibenclamide groups are shown in Figure 1. A reduction in body weight was observed in the diabetic groups three days after alloxan administration. Gradual weight gain was however recorded from day 5 of the treatments that showed progressive increase to the end of the experiment with the exception of the group treated with 300 mg/kg of the extract that exhibited weight fluctuation.

The activities of the extract in hyperglycaemic rabbits

As shown in Figure 2, the untreated rabbits (vehicle group) exhibited hyperglycaemia after oral glucose load that failed to return to baseline glycaemia 4 h later. The seed extract of SJ and glibenclamide administered 30 min prior to the glucose load significantly ($p < 0.05$) reduced the peak values and the area under curve of

Table 1. Plasma glucose level of rabbits treated with SJ seed extract for 15 days

	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
Control (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*
S. jollyanum seed	300	331.5±7.4	331.7±5.9*	318.9±7.6*	308.1±8.5*	295.6±7.6*	290.3±4.2*	279.3±6.5*	279.6±4.6*
S. jollyanum seed	600	347.8±11.3	331.1±12*	313.0±10.8*	300.2±8.8*	289.2±8.3*	282.4±5.8*	271.7±6.8*	268.6±4.5*
S. jollyanum seed	1200	358.3±8.4	340.1±9.7	331.6±9.0*	313.1±8.2*	301.6±7.5*	291.2±7.8*	274.3±7.2*	267.9±3.6*

Table shows the plasma glucose concentration during 15 days of extract/glibenclamide administration or 100 mg/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 treated with SJ seed extract for 9 days

	Dose (mg/kg)	Plasma glucose levels (mg/100ml) post treatment with the extract				
		Day 0	Day 3	Day 5	Day 7	Day 9
Control (untreated)		410.7±10.7	450.3±13.4	473.0±14.2	499.8±21.1	525.1±24.6
Glibenclamide (10mg/kg)	10	244.7±2.7*	218.5±1.2*	197.9±4.0*	177.3±5.7*	158.5±5.0*
S.jollyanum seed (300mk/kg)	300	279.6±4.6*	284.1±7.4	286.6±11.1*	282.2±11.5*	290.6±9.7*
S. jollyanum seed(600mk/kg)	600	268.6±4.5*	251.3±5.1*	249.0±8.4*	241.5±10.4*	246.4±8.8*
*S.jollyanum*seed(1200mg/kg)	1200	267.9±3.6*	252.9±5.5*	249.7±6.0*	245.2±8.9*	241.0±9.3*

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).

blood glucose levels by 20.0 % and 43.8 % respectively compared to the untreated. Thus, glibenclamide exhibited more effective glucose tolerance having lowered the blood glucose level to 65.7±2.0 4h later compared to the extract treated which was 85.9±5.2 at the same time interval.

Activities in alloxan-hyperglycaemic rabbits

The administration of alloxan at the dose of 150 mg/kg intravenously (i.v) led to elevated level of fasting blood glucose (Table 1) in diabetic control which increased progressively over the period of the experiment. Treatment with the seed extract of SJ significantly (p < 0.05) lowered the blood glucose level in a dose dependent manner from day 3 of daily treatment. Decrease in glycaemia continued till the last day of treatment in the entire extract group with glibenclamide treated exerting more effective decrease. The study was extended to assess post-treatment effect in which further decrease in glycaemia was observed (Table 2). At day 9, the three extract groups showed maximum percentage decrease of 12.3, 29.2 and 32.7 respectively while glibenclamide group recorded a decrease of 51.9 %.

Histopathology of pancreatic tissue

The photomicrograph of vehicle group (Figure 3) showed the normal islet organization with compact beta cell arrangement. In diabetic control, extensive damage occurred at the islet cells (Figure 4). A shrunken mass of amorphous material formed a condensed crumb at the centre with a halo around it. The extract treated (Figure 5) showed necrotic beta cells which formed an aggregate shrunken amorphous mass with some viable cells at the periphery. In glibenclamide treated (Figure 6), beta cells necrosis was equally observed with considerable number of beta cells having normal appearance. On the other hand, the exocrine pancreatic acinar, ducts and connective tissue surrounding the islet cells showed normal appearance in all the groups of both treated and untreated animals.

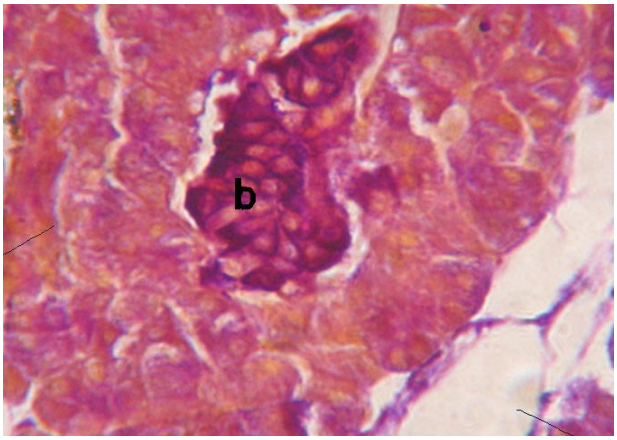

Figure 3. The histology of pancreatic tissue of a normal rabbit. The beta cells (b) of the islet formation are highlighted constituting deeply stained area. Stain: Gomori Aldehyde Fuchsin. Magnification x 400.

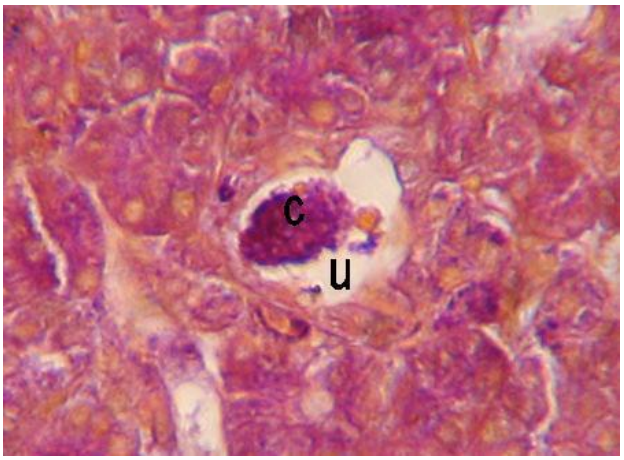

Figure 4. The histology of the pancreas of diabetic control rabbit showing distorted islet organization (c) and a hollow (u) around. Stain: Gomori Aldehyde Fuchsin. Magnification x 400

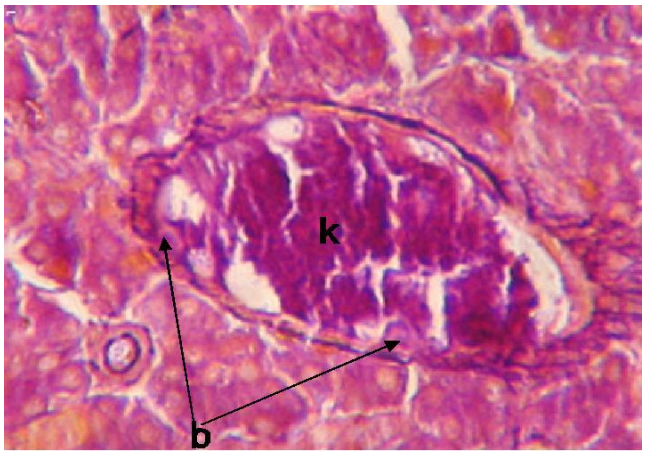

Figure 5. The histology of the cross section of pancreas of diabetic rabbit treated with the seed extract. Aggregate shrunken mass of cells (k) is observed with few survivor beta cells (b) at the periphery. Stain: Gomori Aldehyde Fuchsin. Magnification x 400.

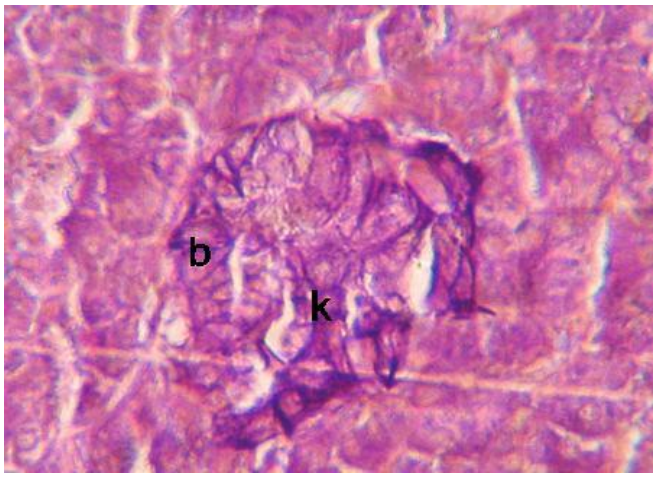

Figure 6. Cross section of pancreas of diabetic rabbit treated with glibenclamide, showing beta cells necrosis (k) with a few survivor cells (b). Stain: Gomori Aldehyde Fuchsin. Magnification x 400.

Phytochemical screening

The active compounds found in the extract include; saponins, terpenoids, anthraquinones, flavonoids and tannins.

Acute toxicity test

There was 100 % mortality at the extract dose of 16 g/kg body weight (bw) while no death occurred in the animals that received 4 g/kg bw and less. The median acute toxicity (LD_{50}) of the extract was determined to be 13g /kg bw

DISCUSSION

Alloxan, a beta cytotoxin, is widely used in animal models to induce chemical diabetes by damaging the pancreatic beta cells (Ho et al, 1999; Saravanan and Pari, 2005). Consequently, there is reduced secretion of insulin leading to clinical conditions such as hyperglycaemia, polyphagia, polydipsia, polyuria and weight loss (Braganca, 1996). Wide application of medicinal plant in the management of DM has been reported (Latha and Pari, 2004; Pareek et al, 2009; Bera et al, 2010).

In this study, decrease in body weight following alloxan administration was observed. This was however ameliorated in the course of treatment with the extract/glibenclamide that showed dose effect.

The extract exhibited significant anti-hyperglycaemic activity by improving glucose tolerance though considerably less compared to glibenclamide. Factors that could have necessitated the effect may be due to the presence of saponins, terpenoids and flavonoids known to be bioactive against diabetes (Abdel-hassan et al, 2000; Loew and Kaszkin, 2002). Although the extract pathway of activity was yet to be determined, it is speculated that these active substances may have triggered the beta cells to increase insulin production.

In alloxan-induced diabetic rabbits, decrease in glycaemia occurred following oral administration of the seed extract. However, the maximum percentage decrease of 32.7 of glucose level was suggestive of a severe damage to the beta cells by the diabetogenic agent resulting in decrease on the endogenous insulin production. This assumption was strengthened by tissue histology study that showed extensive damage to the pancreatic islet with few surviving beta cells at the periphery. The stimulation of insulin release by the survivor/residual beta cells may have been responsible for the moderate glycaemic decrease thereby alluding credence to the fact that the extract anti-diabetic activity was through the release of insulin from the pancreas. Similar effect was reported on the leaf and the root (Mbaka et al, 2008; 2009). A number of other anti-hyperglycaemic plants have also been reported to exhibit insulin stimulatory effects (Olajide et al. 1999; Saravanan and Pari, 2005; Pareek et al, 2009).

CONCLUSION

The result showed that petroleum ether seed extract of SJ significantly lowered blood glucose levels in hyperglcaemic and alloxan diabetic rabbits. The extract equally showed improvement in body weight. However due to its weak activity, it would be more useful in combination therapy.

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