



HISTOLOGICAL STUDIES OF PANCREATIC B-CELLS OF STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS TREATED WITH METHANOLIC EXTRACT OF *SPHENOCENTRUM JOLLYANUM*

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ABSTRACT

Diabetes mellitus is characterized by hyperglycaemia and destruction in the microarchitecture of the β -cells of the pancreatic islets of Langerhans. The search for drugs with low cost, more potential and with less adverse effects is continuous. This study investigated the effects of methanolic extract of *Sphenocentrum jollyanum* on hyperglycemia and the morphological changes on pancreatic β -cells in streptozotocin-induced diabetic rats and compared the extract efficacy with glibenclamide, a known antidiabetic drug. Twenty four adult Wistar rats randomly divided into four groups were used. Group A was the control, diabetes mellitus was induced in groups B, C and D by a single intraperitoneal injection of streptozotocin (80mg/kg body weight) dissolved in 0.1 M citrate buffer. Group B diabetic rats were untreated while groups C and D received extracts of *S. jollyanum* (200mg/kg) and glibenclamide (5mg/kg) once daily for two weeks respectively. The result showed a significant fall in blood glucose level with the extract and glibenclamide administration. Histological examination showed recovery of β -cells of the *S. jollyanum* and glibenclamide treated rats when compared with the diabetic group. There was no significant difference between the effects of the crude extract and the reference drug, glibenclamide.

Keywords: Diabetes mellitus, *Sphenocentrum jollyanum*, streptozotocin, hyperglycemia and microarchitecture.

INTRODUCTION

Recent studies have shown that against previous belief, the endocrine pancreas is a plastic organ and the β -cell has the significant capacity for adaptation to changes in insulin demand¹. Diabetes mellitus is the most common metabolic disorder with a global prevalence of about 2.8 %². Insulin and various oral anti-diabetic drugs are used as monotherapy or in combination to achieve better glycemic control, however, each of these drugs suffers a number of serious side effects^{3,4}. These drugs are known to manage the hyperglycemia caused by diabetes mellitus leaving the pathogenesis of this disorder i.e. the degeneration of the β -cells of the pancreatic islets of Langerhans. Thus, management of diabetes mellitus without any side effect is still a challenge to the medical system.

Interest in medicinal plants has been fuelled by the rising costs of prescription drugs in the maintenance of health and by the bio-prospecting of new plant-derived drugs. In spite of the presence of antidiabetic drugs, remedies from medicinal plants are considered to be free from side effects compared with synthetic ones⁵. Also, the management of diabetes mellitus depends on continuous hypoglycemic therapy, which may not be consistently adhered to by the patient.

Sphenocentrum jollyanum (SJ) is a deciduous shrub which belongs to the family, Menispermaceae (Linn). Several studies have shown that *S. jollyanum* has antihypertensive, antioxidant, antinociceptive, antiviral, and anti-angiogenic effects in animals^{6,7}. The plant is also documented for the treatment of different human diseases including chronic coughs, worms, and other inflammatory conditions as well as tumour^{8,9}. It is also believed to be an emetic and purgative; the sap is believed to relieve stomach ache and constipation, and to boost appetite and sexual drive¹⁰. In Cote d' Ivoire, pounded roots are taken against high blood pressure, while the boiled roots are given against epileptic fits. In Ghana, the pulped root is been applied to treat breast tumours¹¹. In Nigeria, the edible fruit is taken against fatigue and a

decoction of the root is applied to treat diabetes and tropical ulcer^{12,13}.

Streptozotocin (STZ) is widely used to induce experimental diabetes in various laboratory animals as it is particularly toxic to the pancreatic insulin-producing beta cells in mammals^{14,15}. STZ is similar enough to glucose to be transported into the cell by the glucose transport protein GLUT 2 but is not recognized by the other glucose transporters. This explains its relative toxicity to beta cells since these cells have high levels of GLUT 2¹⁶. Streptozotocin causes fragmentation of DNA in pancreatic beta cells of rats through the formation of free alkylating radicals leading to a reduction in the cellular levels of nucleotides and related compounds. This causes a rapid necrosis of the β -cell of pancreatic islets¹⁷.

MATERIALS AND METHODS

Animal care and management

The study was carried out on healthy male and female Wistar rats weighing between 150 to 250 g. The animals were housed in clean plastic cages under natural light and dark cycles at room temperature. Animals in all groups were fed normal laboratory chow *ad libitum* and allowed free access to water. The rats were randomly divided into four groups A, B, C and D of 6 rats each. The rats received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health¹⁸.

Extraction of *Sphenocentrum jollyanum*

The roots of *Sphenocentrum jollyanum* (Linn.) were collected from a farmland in Ikere Ekiti, Ekiti State. The plant was botanically identified by the Curator of the Department of Plant Science and Forestry, University of Ado Ekiti, Ekiti State. A voucher specimen (UHAE 044) was placed in the

Herbarium of the Department. The roots of *S. jollyanum* were air dried at room temperature for eight weeks. The air dried roots were pounded using a wooden mortar and pestle and milled into fine powder in an electric blender. 980 g of the powdered bark was extracted in methanol (100%) using a Soxhlet Extractor. The mixture was filtered and the filtrate evaporated at 60°C using a vacuum Rotary Evaporator. The wet residue was freeze-dried using a vacuum Freeze Drier and stored in a desiccator. An aliquot portion of the crude extract residue was dissolved in distilled water for use on each day of the experiment.

Animal treatment

There was a pre-treatment period of four weeks during which the body weight and blood glucose level was monitored in all the animals. This served as baseline conditions. Diabetes mellitus was induced in groups B, C and D animals by a single intraperitoneal injection of STZ (80 mg/kg body weight) freshly dissolved in 0.1 M citrate buffer¹⁹. The rats were fasted for 16 hrs before commencement of the experiment. The rats in group A (the control) were given equal volume of the citrate buffer used in dissolving STZ intraperitoneally. Twenty four hours after the injection of STZ, diabetes was confirmed in the animals with blood glucose level greater than 18 mmol/l. After four weeks of STZ induction of diabetes, the rats in group B were left untreated. Methanolic extract of *S. jollyanum* was administered orally to the rats in group C for two weeks at 200 mg/kg body weight¹³. Glibenclamide, a standard anti diabetic drug was dissolved in distilled water and administered to group D rats for the same period of time at a dosage of 5 mg/kg²⁰. The animals were monitored for another four weeks.

Determination of body weight and blood glucose level

The body weights of the animals were measured using a top loader weighing balance. Blood glucose concentrations were

determined with a digital glucometer (Accu-check® Active, Roche Diagnostic, Germany). After an overnight fast, blood samples were obtained from the tail vein of the rats by cutting the tip of the tail after sterilizing the tail with methylated spirit. A drop of blood was placed on the reagent strip. The strip was inserted into the microprocessor digital glucometer and the reading on the screen noted.

Sacrifice of animals

Animals were sacrificed by anaesthesia with chloroform and a mid-line incision was made through the anterior abdominal walls. The pancreas is located at the junction of the supracolic and infracolic compartments of the abdominal cavity as it extends transversely across the posterior abdominal wall between the duodenum on the right and the hilum of the spleen on the left; the pancreas was isolated from the surrounding organs.

Histological procedure

The splenic part of the pancreas was fixed in Bouin's fluid for 24 hours and processed via the paraffin wax embedding method of Drury and Wallington²¹. Paraffin-embedded sections were cut at 5 μ m and stained with haematoxylin and eosin (H&E) and Gomori aldehyde fuchsin for light microscopic examination of the pancreatic islets architecture. The sections were examined under a Leica research microscope (Leitz Wetzlar, Germany) with a Leica EC3 camera attached. Digital photomicrographs of the pancreatic sections were taken at various magnifications.

Statistical Analysis

One-way ANOVA was used to analyze data followed by Student Newman-Keuls (SNK) test for multiple comparisons. Primer for windows (McGraw-Hill, version 4.0.0.0) was the statistical package used to analyze data. Results were expressed as mean \pm standard error of mean. $P < 0.05$ was taken as accepted level of significant difference.

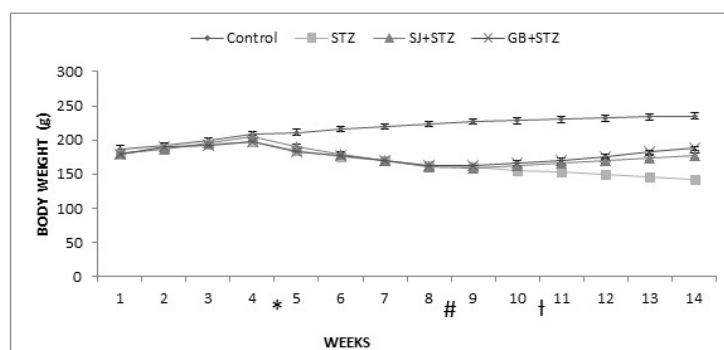


Fig. 1. Weekly changes in body weight of control, untreated diabetic, *S. jollyanum* extract (SJ)-treated diabetic rats and glibenclamide (GB) experimental animals. *STZ administration; #SJ administration ends; †GB administration begins.

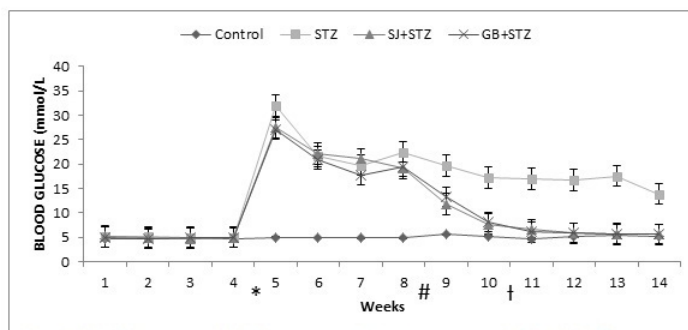


Fig. 2: Weekly changes in blood glucose level of control, untreated diabetic, *S. jollyanum* extract (SJ)-treated diabetic rats and glibenclamide (GB) experimental animals. *STZ administration; #SJ administration begins; †SJ administration ends

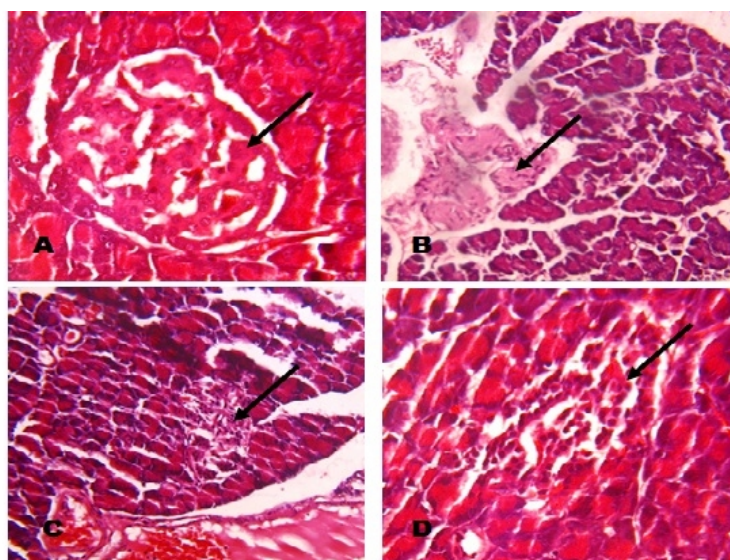


Fig. 3: H & E staining of pancreatic islets of normal (control), untreated diabetic, *S. jollyanum* treated diabetic and glibenclamide treated diabetic rats (A, B, C and D respectively). Observe islet cells with defined boundary (arrows) in A, C and D when compared with B. In B, there is severe vacuolation and degranulation. C and D shows more obvious islet pattern when compared with B x 400.

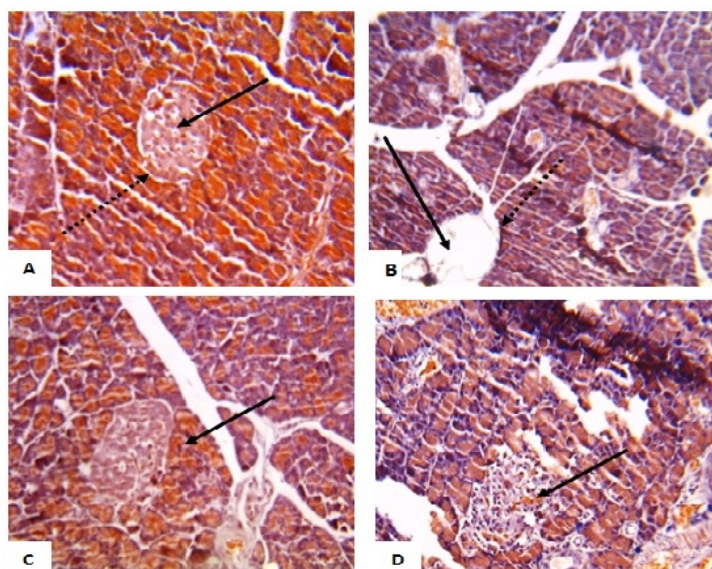


Fig 4: Gomori Aldehyde Fuchsin staining of pancreas for all groups (A, B, C and D). Observe the clusters of centrally placed beta-cells (straight arrow) and peripherally placed alpha-cells (broken arrow) in A, degranulation and vacuolation of the pancreatic islets of diabetic rats in B. Note the reduced areas of hemorrhage in C and D when compared with B x 160

RESULTS

Effects on body weight and other physical observations

The mean body weight as shown in figure 1 during the pre-experimental period indicated the following values: Group A=196.5 \pm 4.56g, Group B=189.8 \pm 3.84g, Group C=191.8 \pm 4.99g, Group D=189.5 \pm 3.80g. Comparing the data statistically using ANOVA, there was no significant difference at $P<0.05$. At the end of the first week following STZ administration there was reduction in body weights of the rats in groups B, C and D. This reduction continued till the end of the Post-STZ treatment period, where there was significantly ($P<0.05$) reduced body weight in groups B (173.2 \pm 4.73g), C (175 \pm 6.34g) and D (173.2 \pm 4.48g) when compared to the control (217.5 \pm 2.60g). Also, SNK test for multiple comparison showed that there was no significant difference among the experimental groups (B, C and D). At the end of week 11, the first week in the Post-GB/SJ treatment period, a rise in body weight was observed in groups C and D. The weight of the animals in group C and D gradually increased with treatment with S.J. extract/GB over the period of three weeks (Fig. 1). Also, at the end of the experiment, groups C and D significantly increased in body weight when compared to group B ($P<0.05$). The diabetic animals manifested alopecia and poly-urea, shown by marked wetness of the ventral body surface of the animals. There was an improvement in the physical outlook of the extract treated animals over time.

Effects on the fasting blood glucose level

During the pre-experimental period, prior to STZ administration there was no significant difference in the blood glucose level among the four groups of animals. At the end of the Post-STZ treatment period, there was significantly increased blood glucose level in groups B, C and D animals when compared to the control. Also, multiple comparisons showed that there was no significant difference among the experimental groups (B, C and D). At the end of week 11, the first week in the Post-GB/SJ treatment period, a fall in blood glucose level was observed in groups C and D animals. At the end of week 14, only group B animals were significantly higher than the control. Also, there was no significant difference between groups C and D (Figure 2).

Histopathological Examination of the Pancreas

The histological appearance of the pancreatic islet cells of the control was normal (Figure 3A and 4A). Microscopic examination of the pancreatic sections of the untreated diabetic group revealed a breakdown of micro-anatomical features including necrotic changes, β -cell degranulation, pycnotic β -cell nuclei, and severe vacuolation (Figure 3B and 4B) in the islet; though the pancreatic acinar epithelium, and ductal and connective tissues appeared normal. Comparison of the normal and diabetic groups clearly shows destruction of islet cells in diabetic rats as they were irregularly shaped and atrophic. Also, there was degeneration of the islets cells with varying degree of traversing tiny fibrosepta separating the tissues into compartments in the diabetic rats.

Figure 3C and 4C show the islets of SJ-treated rats while islets of GB-treated diabetic rats are shown in Figure 3D and 4D. Comparing these two groups with the untreated diabetic rats, there was evidence of recovery of β -cells of the islets, reduced fibrosepta and a more obvious islet pattern with well outlined boundaries but vacuolation was reduced or absent in many islets. Also, reduced areas of haemorrhage were

observed in the SJ and GB treated rats when compared with the diabetic group of rats. This indicates wound healing effects in areas of cellular injury. The morphology of the pancreas of SJ-treated diabetic rats revealed remarkable improvement in the islet of Langerhans than those of the GB-treated diabetic rats.

DISCUSSION

Streptozotocin (STZ) has been used to induce an animal model of diabetes mellitus for ages. STZ selectively destroys the pancreatic insulin secreting β -cells, leaving less active pancreatic islet cells and resulting in diabetes mellitus²². This well-established model is characterized by insulin deficiency associated with insulin resistance²³. Consequently, there is reduced secretion of insulin leading to clinical conditions such as hyperglycaemia, polyphagia, polydipsia, polyuria and weight loss²⁴. In this present study, all these conditions were observed in the STZ treated rats. The β -cells were also observed to be degenerated with varying degree of transversing tiny fibrosepta and distortion in tissue architecture. Also, a significant decrease in blood glucose and evidence of ameliorative effects on the β -cells was observed in the group of diabetic animals treated with extracts of *S. jollyanum* and glibenclamide.

A number of plants have been used traditionally to treat diabetes and some have been proven to have hypoglycemic effects. These studies have identified compounds like terpenes and tannins²⁵, alkaloids²⁶ and flavonoids²⁷ to be responsible for the hypoglycaemic properties. The chemical analyses of *S. jollyanum* showed the presence of terpenes, tannins, alkaloids and flavonoids among others²⁸. Although, the mode of action of this extract has not been documented, the observed hypoglycaemic effects could be due to the combined activity of these compounds. The comparable pattern of the hypoglycaemic activity of the extract under study with that of the reference drug, glibenclamide, demonstrated a possible similarity in their mechanism of action.

Results from this study showed a decrease in body weight in untreated diabetic animals. Generally, there is a decrease in the body weight of diabetic untreated animals due to the under utilization of glucose. Decrease in body weight of diabetic rats is due to catabolism of fats and protein²⁹. At the end of the experiment, a significant increase in body weight was observed in the groups of animals treated with *S. jollyanum* and glibenclamide when compared with the weight at the post-STZ treatment period. This may be attributed to the potent hypoglycaemic activity. The results of this study indicated that there was no significant difference between the effects of the crude extract and the reference drug, glibenclamide.


In conclusion, the findings from this study revealed the beneficial effects of the root extract of *S. jollyanum* on hyperglycemia in STZ induced diabetic rats. However, further studies are needed to investigate and elucidate the possible mechanism of action of the active ingredients and evaluate the potential value of the extract of *S. jollyanum* roots for the management of diabetes. This may be helpful in developing new drugs from this plant for the management of diabetes and associated complications.

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