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Full Length Research Paper

Antihyperglycemic activities of methanolic leaf extract of *Anacardium occidentale* (Linn.) on the pancreas of streptozotocin-induced diabetic rats

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Anacardium occidentale **is a plant with reported antidiabetic and antioxidant properties. The stem, leave and bark extracts are known to contain saponin, phenolics, flavonoids, vitamins and selenium. Diabetes is a multisystemic disease characterised by defects in insulin secretion or inaction. In this study, forty male Wistar rats (*Rattus norvegicus*) were randomly divided into four experimental groups A, B, C and D. Hyperglycemia was induced by a single intraperitoneal injection of 70 mg/kg b.w streptozotocin (STZ). Hyperglycemia was confirmed 48 hours later. Five days after, the confirmation of hyperglycemia by using a glucometer (Roche(R)) and compatible glucose test strips, Groups A and B were treated with 300 mg/kg b.w of *A. occidentale* extract (AOE) and 1 I U/kg b.w insulin, respectively. Groups C and D served as hyperglycemia and normal controls and received 1 ml/kg b.w citrate buffer, respectively. After 16 days of treatment the animals were sacrificed and the pancreas was processed for histological staining. Data obtained were expressed as means of ten (10) replicates ± SEM and subjected to one way analysis of variance (ANOVA) and the Scheffe's post hoc test for multiple comparison. Findings showed that STZ-induced diabetes induced hyperglycemia and histopathological changes in the pancreas of untreated rats. Treatment with the methanolic extract of *Anacardium occidentale* resulted in reduction in hyperglycaemia and regeneration of beta cells.**

Key words: *Anacardium occidentale,* hyperglycemia, pancreas, streptozotocin, diabetes mellitus.

INTRODUCTION

The term diabetes mellitus describes a multi-systemic, metabolic disorder of multiple aetiology characteristic of carbohydrates, fats and protein metabolism due to defects in insulin secretion, insulin action or both. It has been described as a fasting venous plasma glucose concentration greater than 11 mmol/L or 200 mg/dl (Mayne, 1999). It is a syndrome associated with hyperglycemia, oxidative stress, polyurea, polyphagia, polydipsia, ketosis, nephropathy and cardiovascular disorders (Gandjbakhch et al., 2005).

Diabetes mellitus is the most prevalent metabolic disorder for all age-groups in the world today (Saladin, 2010). The prevalence for all age-groups was estimated to be 2.8% in 2000. As of 2000 at least 171 million people worldwide suffered from diabetes, or 2.8% of the population and projected to be 4.4% in 2030 (Wild et al., 2004). Type 2 diabetes is by far the most common, affecting 90 to 95% of the U.S. diabetic population. Africa has about 14 million people living with diabetes, with Nigeria having the highest number about 1,218,000

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(International Diabetic Foundation, 2006). It is projected that over 300 million people will be affected by the year 2025 (Ravid and Rachmani, 2005; Wild et al., 2004).

Present treatment procedures for diabetes mellitus include the use of oral hypoglycemics, exogenous insulin, transplantation and xenotranplantation. Current diabetes drug therapy does not provide sufficiently tight control of blood glucose to avoid diabetes complications (Serup et al., 2001). No satisfactory effective therapy is yet available to cure diabetes mellitus (Mallick et al., 2005).

The setbacks being encountered in present antidiabetic therapies call for innovative treatment therapies that are effective, less toxic, less expensive and with fewer side effects compared to synthetic drugs. Any innovative approach or novella therapy should target obesity, hyperglycemia and insulin resistance, dyslipidemia, Inflammation and hypertension (Iqbal, 2007).

Anacardium occidentale originated from Brazil (Paris et al., 1977) and is known to have a lot of medicinal values. The antimicrobial activities of *A. occidentale* extracts have been confirmed (Kubo, 1999; Laurens, 1982; Kudi, 1999; Akinpelu, 2001). Its fruits were shown to exhibit antibacterial activity against *Helicobacter pylori* that is commonly implicated in acute gastritis and stomach ulcers (Kubo, 1999). It has been found to also possess anti-viral (Gonclaves et al., 2005), anti-fungal (Schmourlo et al., 2005), anti-bacterial activities (Akinpelu, 2001).

In Nigeria, the decoction of root and stem is used as anti-inflammatory agent and antidiarrheal (Motal, 1985). The antidiabetic and anti-inflammatory properties of the leaf and bark extracts of the cashew plant have also been validated (Motal, 1985; Esimone et al., 2001; Kamtchoury, 1998). In Nigeria it is used to treat hypertension and diabetes (Esimone et al., 2001).

The stem-bark and leaves of *A. occidentale* have been reported to possess hypoglycemic activities (Ojewole, 2003) while radical scavenging activities has been reported for the shoot (Roach et al., 2003) and the leaves (Abas et al., 2006). Phytochemical study of the methanolic leave extract revealed the presence of phenolic, flavonoids, steroids and triterpenes (Fazali et al., 2011). *A. occidentale* also contains alkaloids, flavonoids, tannins, saponins, phenols, oxalate, quercetin-glycoside and phytate while the micronutrient composition included some vitamins (A, B, B2, B3 and C) and some minerals (Na, K, Ca, Mg, P, Fe, Cu and Se). Proximate composition revealed the presence of protein, carbohydrate, fat and fibre (Eliakim-Ikechukwu et al., 2010).

There is a dearth of literature on the antidiabetic effects of *A. occidentale* on pancreas morphology; thus this study was conducted to investigate the hypoglycemic activity of the methanolic leaf extract of *A. occidentale* and to determine if the extract has any differential effect on the histology of the pancreas of animals that vary in their blood glucose concentrations.

MATERIALS AND METHODS

Animal care

Forty (40) presumably healthy and normoglycemic adult male Wistar rats having fasting blood glucose level of 70 to 80 mg/dl and of average weight 162.5 g were used for this study. The animals were purchased from a commercial source. They were taken to the animal house of the Department of Anatomy, Bowen University where the rats were kept in iron cages at controlled room temperature of about 30°C and photo-periodicity of 12L: 12D. They were fed on rat pellet feed and water made available *ad libitum*.

Plant materials

Fresh leaves of *A. occidentale* were collected and identified by a plant taxonomist in the Department of Botany, University of Ilorin, Ilorin. The voucher specimen is deposited in the departmental herbarium (UIH/612).

Preparation of extract

The leaves of *A. occidentale* were washed and shaded and dried to constant weight and then ground into fine powder using a contact mill.1,977.7 g of the leave powder was macerated in 4,720 ml of methanol for 48 h and then filtered through Whatman filter paper at room temperature. The supernatant was concentrated under reduced pressure using a rotary evaporator (Laborato 4000, China). This was kept in a dessicator and the final product was a dark green sticky mass weighing 89.8 g, which represents a yield of 4.54%. This was refrigerated at 4°C before use.

Extract treatment

The extract was dissolved in 1% dimethyl sulfoxide (DMSO) water solution (v/v) before administration. Stock solution was prepared by dissolving 42 g of the extract in 265 ml of physiological saline to give a concentration of 0.16 g/ml.

Induction/determination of diabetes

Diabetes was induced in the rats by a single intraperitoneal injection of 70 mg/kg body weight of 0.1 M streptozotocin, STZ in 0.1 M citrate buffer (pH 4.5) (Ballester et al., 2004). 0.1 M citrate buffer was prepared by dissolving 2.1 g of citric acid and 2.94 g of sodium citrate in 100 ml of distilled water. The pH was adjusted to 4.5 by the proper addition of concentrated NaOH/HCL using a caliberated pH meter.

Accu-Check Glucometer (Roche) and compatible glucometer strips were used for the determination of blood glucose levels in over-night fasted rats 48 h after induction of diabetes. Blood samples were obtained from dorsal vein of the tail of conscious rats. Only rats with glucose level greater than 250 mg/dl were recruited into the study (Singh et al., 2007). Diabetes was allowed to stabilize for 5 days before the commencement of intervention.

Thereafter, the blood glucose level of all animals in each experimental group was assessed every other day. Animals were checked for clinical signs of drug toxicity such as tremors, diarrhoea, weakness, lethargy, poor wound healing, weight loss, hair loss, coma and death.

Table 1. Effects of methanolic extract of *Anacardium occidentale* and insulin on the body weight and blood glucose.

Treatment groups	Body weight (g)		Blood glucose (mg/dl)		Mean difference (%)
	Initial	Final	Initial	Final	
A	156.94 ± 3.69	145.32 ± 4.39[ab]	333.62 ± 15.19	102.78 ± 10.75[a]	69.20
B	167.36 ± 10.50	148.50 ±6.51[ab]	339.91 ± 9.99	112.89 ± 18.32[a]	66.79
C	174.88 ± 5.30	135.07 ± 8.97	358.64 ± 18.90	417.90 ± 33.39	14.18
D	148.77 ± 4.99	170.77 ± 10.16[a]	91.80 ± 3.81[b]	95.90 ± 4.16[a]	

Values are mean ± SEM; n=10 in each group. [a]Statistically different from Group C at P <0.05[c]. [b]Statistically different from Groups A,B and C at *P* <0.05.

Experimental design

The animals were divided into four groups of 10 rats each and treatment commenced for a period of 16 days after 7 days in the diabetic state: Group A were administered 300 mg/kg/day ethanolic leaf extract. Group B received 1 I U/kg/day insulin; Group C-diabetic control, received 1 ml/kg of citrate buffer (placebo) and Group D normal control, received 1 ml/kg of citrate buffer (placebo).

The body weight measurements were taken at two-two days interval and recorded, using a sensitive top loader balance (Adams Equipment, Belgium). All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the declaration of Helsinki and the guiding principles in the care and use of animals and were approved by the Departmental Committee on the use and care of animals (American Physiology Society, 2002).

After sacrifice by cervical dislocation, the animals were cut open by abdominopelvic incision and the pancreas were excised and fixed in Bouin's fluid. Tissues were processed histologically and stained with Gomory Aldehyde Fuchsin's stain (Culling, 1974).

RESULTS

Blood sugar

STZ induced hyperglycemia in all the experimental rats. Forty-eight (48) hours after injection with 0.1 M STZ Groups A, B and C had mean blood glucose of 365.54 **±** 12.64 mg/dl, 381.20 **±** 14.71mg/dl and 296.00 **±** 10.47 mg/dl respectively.Treatment with AOE produced significant (*P*<0.05) reduction in the blood glucose levels of treated rats compared to the untreated animals (102.78 ± 10.75 mg/dL v 417.89 ± 33.39 mg/dL). Treatment with insulin also resulted in significant reduction of glycemia at *P* > 0.05 (112.89 ± 18.32 mg/dL v 417.89 ± 33.39 mg/dL) (Table 1).

Mean body weight

Hyperglycemia was accompanied by weight loss in all treatment groups prior to intervention. AOE- and insulin-treated animals recorded a modest gain in body weight. Weight loss continued in the untreated hyperglycemic groups throughout the duration of the experiment. Final body weights of Groups A and B (145.32 ± 4.39 g and 148.50 ± 6.51 g) were significantly lower than D (170.77 ± 10.16 g) at *P*<0.05. However, both treated groups A and B showed final body weights that were higher than the untreated hyperglycemic group (145.32 ± 4.39 g; 148.50 ± 6.51g vs 135.07 ± 8.97 g) at *P* <0.05 (Table 1).

PANCREAS HISTOLOGY

The pancreas of control rats (Group D) showed enlarged islets of Langerhans, with abundant alpha and beta cells. The islets were surrounded by secretory acini containing centoracinar cells, excretory ducts and blood vessels (Figure 1 D). In the untreated hyperglycemic rats the pancreatic islets were diminished in sizes (Figure 1C) when compared to the pancreatic islets of control rats. Islets appeared to be reduced in cellular density compared to the control and extract-treated groups. There were prominent cellular lesions within and around the periphery of the islets. The border between the exocrine and endocrine pancreas appeared non-distinct (Figure 1 C). The pancreas of extract-treated (Group A) rats showed islets and acinar cells that were comparable to the control group. The islets showed a profusion of alpha and beta cells (Figure 1 A). Sections from the pancreas of insulin-treated (Group B) rats showed islets with a lot of alpha and beta cells. Slight lesions were observable in the periphery of the islets (Figure 1 B).

DISCUSSION

In this study, streptozotocin (STZ) treatment was associated with hyperglycemia and weight loss in untreated animals. Treatment with insulin and methanolic leaf extract of *A. occidentale* produced a significant reduction in blood glucose level in hyperglycemic rats. Though no precise mechanism of action has been postulated for the hypoglycemic activity of the extract, the blood sugar lowering effect of the plant is traceable to its constituents.

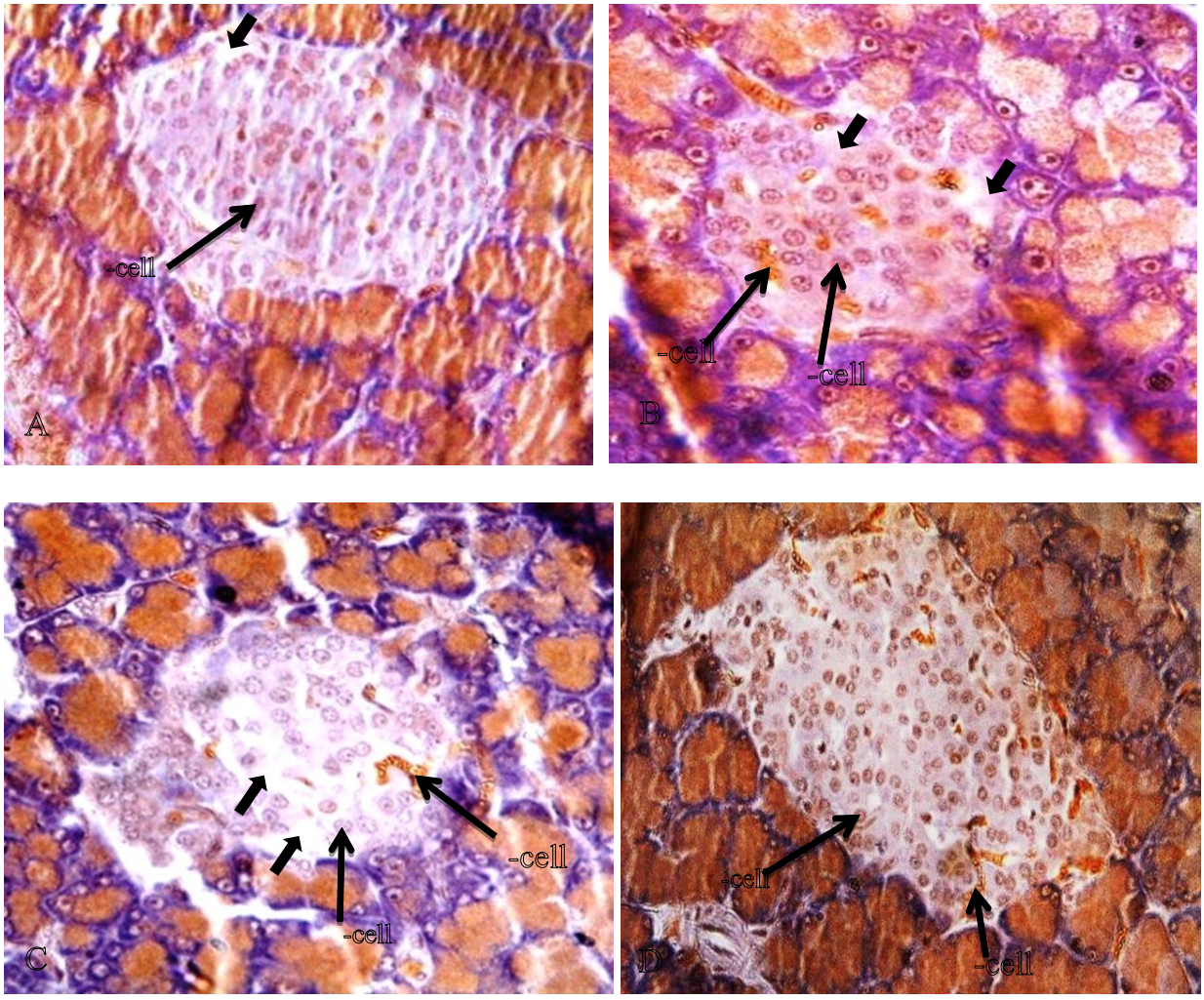

Figure 1. Islets of Langerhans and exocrine pancreas of Groups A (Extract-treated) showing mild lesions at islet periphery (block arrow) and abundant beta cells; B (insulin-treated) showing prominent alpha cells, fewer beta cells and slight lesions (arrow); C (Hyperglycemic) showing severe wide-spread lesions within the substance of the reduced islet and D(Normal rats) showing abundant beta cells. No evidence of islet lesions. Stain: modified Gomori's aldehyde-fuschin; Mag: ^{x}400.

Saponins are known to have anti-hyperglycemic effects (Soumyanath, 2006). Some saponin-containing plants with documented hypoglycemic effects are *Vernonia amygdalina* (Okolie et al., 2008); *Mormodica charantia* (Omar et al., 2007). The plant contains magnesium; studies suggest that magnesium deficiency disrupts insulin production in the pancreas and increase insulin resistance in body tissue, thus worsening the blood sugar control in Type II diabetes (Barbagallo et al., 2007).

A. occidentale is also known to contain α-glucosidase inhibitors (Toyomizu, 1993), kampferol, quercetol rthamnosides (Arya et al., 1989), and quercetin glycoside ((Eliakim-Ikechukwu et al., 2010*)*. α-glucosidase inhibitors inhibit α-glucosidase in the gastrointestinal tract, thereby inducing a reduction in hyperglycemia. Kampferol, quercetol rthamnosides are substances that stimulate insulin secretion (Ivorra et al., 1988; Sheehan et al., 1983).

In this study, STZ-induced diabetes produced marked loss in body weight. Diabetes is usually associated with weight loss; this is because the body switches to burning fatty acids due to insulin shortage. The process of gluconeogenesis also converts glycogen stores in liver and muscles to glucose in the diabetic state. However, increased body weights observed in the treated groups can be adduced to improved level of insulin in these groups. Insulin increases the activity of acetyl CoA carboxylase and provides glycerol for the esterification of

fatty acid to triacylglycerol; it is also responsible for the formation of glycogen in skeletal muscles (Cotran et al., 1999). Moreover, insulin is also an anabolic hormone that results in the synthesis of protein (Cotran et al., 1999).

In this study, islet lesions and destruction of beta cells were evident in the untreated hyperglycemic group. Our results agree with previous findings that STZ is injurious to the beta cells of the pancreas. STZ is similar enough to glucose to be transported into the cell by the glucose transport protein GLUT2, but is not recognized by the other glucose transporters. This explains its relative toxicity to beta cells, since these cells have relatively high levels of GLUT2 (Schnedl et al., 1994; Wang and Gleichmann, 1998). STZ damages the pancreas by generating reactive oxygen species and increasing oxidative stress (Kawada, 1992), mainly because the beta cells are low on free radical scavengers (Spinas, 1999). Streptozotocin selectively damages the pancreatic insulin secreting beta cells, leaving less active cells and resulting in a diabetic state (Junod et al., 1967).

Treatment with AOE resulted in the proliferation of beta cells and reversal of islet lesions. This will result in the upregulation of insulin secretion, inducing a reduction in hyperglycemia as recorded in treated animals. This suggests that the plant contains bioactive substances with anti-oxidant effects and capability to induce proliferation of beta cells and stimulation of insulin secretion as well as peripheral insulin utilisation, considering its effect on body weight. This is in agreement with previous reports on the anti-oxidant properties of the leaf of *A. occidentale* (Abas et al., 2006). Future studies on the anti-oxidant effects of *A. occidentale* leaf extract on pancreas homogenates are thus recommended.

We conclude that the methanolic extract of *A. occidentale* has hypoglycemic effect and protects the pancreas against STZ-induced diabetes. The results of this study justify the traditional use of the plant in the treatment of diabetes.

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