EFFECT OF AQUEOUS EXTRACTS OF ALLIGATOR PEAR SEED (*PERSEA AMERICANA MILL)* ON BLOOD GLUCOSE AND HISTOPATHOLOGY OF PANCREAS IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Effects of aqueous extract of alligator pear seed on normal and alloxan-induced diabetic rats were investigated in 6 groups of rats (5 rats per group). Test groups were made diabetic with intra-peritoneal injection of alloxan and treated with 300mg and 600mg/kg body weight of alligator pear seed extract. Two non-diabetic groups were also administered with 300mg and 600mg/kg body weight extract.

The levels of blood glucose were examined in all 6 experimental groups. In diabetic rats, blood glucose levels were significantly reduced ($p < 0.05$) by 73.26-78.24% on consumption of the extracts, with greater effect exhibited by the 600mg/kg extract. In normal rats, blood glucose levels were significantly reduced ($p < 0.05$) by 34.68-38.9% on consumption of the seed extract.

Histological studies showed a degenerative effect on the pancreatic islet cells of diabetic rats. The result suggested restorative (protective) effect of the extract on pancreatic islet cells.

Administration of aqueous extract of alligator pear seed may contribute significantly to the reduction of blood glucose levels and can be useful in the treatment of diabetes.

Keywords: Alligator pear, *Persia americana,* seed extract, hypoglycemic effects.

INTRODUCTION

Diabetes mellitus is a primary disorder of carbohydrate metabolism, which generally involves absolute or relative insulin deficiency and/or insulin resistance and ultimately leads to hyperglycemia. There has been increasing demand for the use of natural products with antidiabetic activity. The undesirable side effects of synthetic drugs, easier consumption or availability and the fact that they are not suitable for use during pregnancy, have been some of the factors leading to the strong desire to use hypoglycemic agents of plant origin (Berger, 1985; Jelodar *et al.*, 2007; Yadav *et al.*, 2008).

Some herbs and plant products have been shown to have antihyperglycemic action (Elder, 2004; Srinvasan, 2005; Badole *et al.*, 2006). Plants may act on blood glucose through different mechanisms. Some of them may have insulin-like substances (Collier *et al.*, 1987; Gray and Flatt, 1999); some may inhibit insulinase activity while others may increase beta cells in pancreas by activating regeneration of these cells (Shanmugasundaram *et al.*, 1990; Abdel *et al.*, 1997).

However, very few of the traditional treatments for diabetes have received scientific scrutiny. The aim of this study was to investigate the hypoglycemic effect of the seed of the alligator pear (*Persea americana*) used in traditional medicine in treatment of diabetes and its possible role on pancreatic tissue.

MATERIALS AND METHODS

Animals

Thirty (30) male Wistar albino rats weighing 180-200g were obtained from the Animal House of the Department of Biochemistry, University of Calabar, Nigeria. The rats were kept in clean and only dry plastic cages, with 12hrs light-dark cycle at 25 $\pm$ 2^{o}C and 45-55% relative humidity. The animals were fed with pelletized commercial rat feed (Pfizer Livestock Co. Ltd, Aba, Nigeria) and tap water ad libitum. The rats were assigned into 6 groups of 5 rats each. All animal were treated in accordance with the National Institutes of Health Guide for the Care and use of Laboratory Animals (NRC, 1985).

Sample collection

Samples of ripe alligator pear (*Persea americana*) were purchased from markets in Uyo metropolis of Akwa Ibom State in Nigeria. The plant material was authenticated by a taxonomist Dr (Mrs.) M.E. Bassey of the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. A voucher specimen with number 'Edem UUH 994' has been deposited in the herbarium of the University of Uyo. The samples were washed with clean tap water to remove dirt on the fruits. After the fruits were kept for 2 hrs for the water to dry off, a sharp stainless steel knife was used to cut open the fruits, in order to remove the seed. The seeds were chopped into very small pieces using the stainless steel knife. They were then dried to constant weight in an oven at 55^{o}C. After drying,

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the seeds were ground into fine powder (which passed through a 30 – mesh sieve).

Preparation of aqueous extract of Alligator pear seed

Sixty grams (60g) of the ground seeds were soaked in 1 litre of distilled water for 24 hrs for complete extraction. Thereafter, the mixture was filtered using cheesecloth and the extract obtained. One hundred milliliter (100 ml) aliquots of the extract were poured into separated beakers of known weight. The solutions were dried at 50^{o}C to constant weight using a rotary evaporator. The extract concentration was determined by gravimetric method. One milliliter (1 ml) of the extract was evaporated to obtain a residue of 60.0mg. Thus the concentration of the extract was 60.0 mg/ml and 0.90ml of the solution administered to 180g rat was equivalent to 300mg/kg body weight. Other doses per weight of rats were determined accordingly.

Animal treatments

The 6 group of rats were as follows: Diabetic group 1 (DG-1), Diabetic group 2 (DG-2), Diabetic control (DC), Non-diabetic group 1 (NDG-1), Non-diabetic group 2 (NDG-2) and Normal control (NC). DG-1, DG-2 and DC groups were made hyperglycemic by intra-peritoneal injection of 150 mg/kg body weight (wt) of alloxan monohydrate (Sigma) dissolved in sterile distilled water. The NC group was not treated with alloxan.

Diabetes was confirmed I week after alloxan injection by determining the blood glucose concentration using One Touch Basic Glucometer.

Then groups DG-1 and NDG-1 were administered with 300 mg/kg body weight of the extract, while groups DG-2 and NDC-2 were administered with 600 mg/kg body wt of the extract, daily for 21 days, by oral gavage. Groups DC and NC, which served as treatment controls, were gavaged with distilled water.

Collection and treatment of samples

After 21 days, the animals were anaesthetized under chloroform vapour. Blood samples were obtained by cardiac puncture. Aliquots of the blood were poured into screw-cap sample bottles containing fluoride/oxalate anticoagulant for blood glucose determination. All analyses were carried out within 24 hrs of blood collection.

Blood glucose analysis

Blood glucose concentration was estimated by glucose oxidase method, using a reagent kit from Randox Laboratory Ltd, UK.

Histopathological study

On the last day of experiment, the tail parts of the pancreas were removed and kept in 10% formaldehyde. Tissue processing was carried out by autotechnicon and the prepared 5μ thick sections were mounted on slides and stained with hematoxylin and eosin (H & E). Stained sections were morphologically evaluated.

STATISTICAL ANALYSIS

All data were expressed as means ± SD. Student's t-test was used to compare the mean values of test groups and control. Differences in mean values were considered significant at $p < 0.05$.

RESULTS

The effects of aqueous extract of seed of *Persea americana* (alligator pear) on blood glucose concentrations in NC and DC groups were 81.00mg/dl and 345.00mg/dl respectively after the experimental period. In comparison with the DC group, the other experimental groups had significantly lower mean blood sugar 47.83-102.24mg/dl ($p < 0.05$). No significant differences in blood sugar were observed between diabetic groups which received 300mg/kg body wt extract

Table 1: Hypoglycemic effects of aqueous extract of alligator pear seed*

Plasma Glucose Concen-trations	Experimental Group					
	NC 0 mg/kg body wt	DC 0 mg /kg body wt	DG- 1 300 mg/kg body wt	DG- 2 600 mg/kg body wt	NDG- 1 300mg/kg body wt	NDG- 2 600 mg/kg body wt
Initial (mg/dl)	84.00±5.61 [a]	357.00±22.00[b]	382.32±24.93[b]	390.24±33.87[b]	82.67±3.08 [a]	78.30±7.25
Final (mg/dl)	81.00±6.52 [a]	345.00±25.50[b]	102.24±10.15 [a]	84.92±1.65 [a]	54.00±2.16 [c]	47.83±3.87
% reduction	3.57	3.36	73.26	78.24	34.68	38.90

*Values are means ± standard deviation (n = 5). Values in same row with different superscripts in a horizontal row represent means that are significantly different ($p < 0.05$).
Legend: NC=Normal Control, DC = Diabetic Control, DG-1=Diabetic Group 1, DG-2=Diabetic Group 2, NDG-1=Non diabetic Group 1, NDG-2=Non diabetic Group 2

(102.24mg/dl) or 600mg/kg body wt extract (84.92mg/dl), in comparison with the normal control (NC) group ($p > 0.05$). However, the non-diabetic groups had significantly lower glucose concentrations (47.83-54.00mg/dl) when compared with the NC and diabetic groups ($p < 0.05$).

The results showed that treatment with 150mg/kg body wt alloxan after 7 days caused significant increases ($p < 0.05$) in blood glucose levels of rats (mg/dl) in groups DC (357.00), DG-1 (382.32) and DG-2 (390.24), when compared with the NC and NDG groups (78.30-84.00). Treatment of the hyperglycemic rats with 300mg/kg and 600mg/kg body wt of alligator pear seed extract resulted in significant reductions ($p < 0.05$) of blood glucose (by 73.26 % and 78.24 % respectively),when compared with the DC (a group without extract treatment), which had 3.36% reduction. The levels of blood glucose in the non-diabetic groups (78.30-82.67mg/dl) were significantly reduced ($p < 0.05$) after extract treatment (by 34.68-38.90 %), when compared with the controls (3.36-3.57 % reduction).

Effects of consumed extracts on histopathology of pancreas: Histomorphologic changes of pancreas

The cellular integrity and architecture were intact in the NC group (fig. 1). Pancreatic sections stained with hematoxylin and eosin (H & E) showed that alloxan caused severe necrotic changes of pancreatic islets, especially in the centre of islets. Nuclear changes, karyolysis, disappearance of nucleus and in some places, residue of destroyed cells were visible. Relative reduction of size and number of islets especially around the central vessel and severe reduction of beta cells were clearly seen (fig. 2).

Study of pancreas of treated diabetic groups (DG-1 and DG-2) showed increased size of islets and hyperchromic nucleus in sections stained with H & E. These was also a relative increase of granulated and normal beta cells in the diabetic group which consumed 600mg/kg body wt extract, when compared with the diabetic group which consumed 300mg/kg body wt extract (figs. 3 & 4 respectively). Pancreas of the non- diabetic group which consumed 300mg/kg body wt extract (fig. 5), showed close similarity to group NDG-2, which consumed 600mg/kg body wt extract (fig. 6), NC group (fig. 1) and DG-2 group (fig. 3).

DISCUSSION

The blood glucose concentration of the NC group (84.00mg/dl) is considered normoglycemic, while that of the DC group (357.00mg/dl) is considered hyperglycemic for the experiment (Mayne, 1994; Jelodar *et al*, 2007). There were significant reductions ($p < 0.05$) in blood glucose levels for the test groups (DG-1 and DG-2) and the non-diabetic groups that received extracts. Furthermore, the percentage reduction in glucose levels is high for all groups treated with extract. The results suggest both hypoglycemic and antihyperglycemic effects of the alligator pear seed extracts. The findings may indicate the presence of some hypoglycemic agents in the seed of alligator pear, which have been concentrated in the extracts. The hypoglycemic effects of plants may be due to the presence of insulin-like substance in plants (Collier *et al.*, 1987; Gray and Flatt, 1999), stimulation of ß cells to produce more insulin (Chang and Johnson, 1980; Khan *et al.*, 1990), increasing glucose metabolism (Broadhurst, 1997) or regenerative effect of plants on pancreatic tissue (Shanmugasundaram *et al.*, 1990).

In this study, the pancreatic ß cells were destroyed with the help of alloxan. Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin (Prince and Menon, 2000; Szkudelski, 2001; Ei-Soud *et al.*, 2007). Alloxan has a destructive effect on the beta cells of the pancreas (Jelodar *et al.,* 2007). The pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood. In response to elevated blood glucose, insulin is secreted. Histopathological study of diabetic untreated (DC) rats showed degeneration of pancreatic islet cells, which was due to alloxan used in this study. This probably gave rise to insulin deficiency. Insulin deficiency (or diabetes mellitus) causes excessive elevation of blood glucose and underutilization leading to hyperglycemia (Standl *et al.*, 2003). The histopathological study of diabetic treated group (DG-1 and DG-2) indicated increased volume density of islets and increased percentage of beta cells, in the diabetic rats that received the extracts, which may be a sign of regeneration. Signs of regeneration of ß cells, potentiation of insulin secretion from surviving ß cells of the islets of Langerhans and decrease of blood glucose have been reported following consumption of some plant extracts (Shanmugasundaram *et al.*, 1990; Abdel *et al.*, 1997; Ayber *et al.*, 2001; Suba *et al.*, 2004; Yadav *et al.*, 2008). Alligator pear seed may have some chemical components that exert regenerative effects on ß cells, stimulate these cells to produce more insulin (pancreatotrophic action) or may have some insulin-like substances. A higher dose of the extract has a greater restorative effect on the islet cells of diabetic rats than a lower dose of extract. There was no significant effect of the extract on the pancreas of normal rats.

CONCLUSION

The findings of this study indicate that consumption of the aqueous extract of alligator pear seed exerts significant hypoglycemic effect in diabetic rats. Histopathological studies of the pancreas of diabetic treated rat show evidence of signs of regeneration of ß

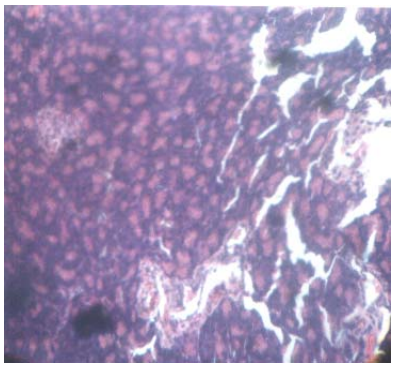

Fig. 1: NC (Normal control, 0mg/kg body wt extract) Pancreas of Normal Health Rat, H & E Staining (x 525)

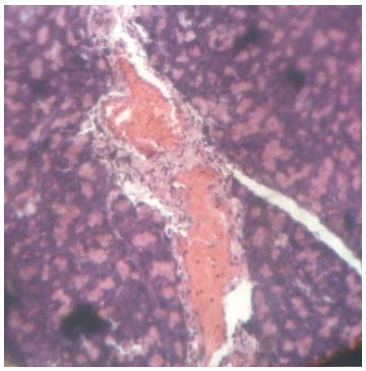

Fig. 2: DC (0mg/kg body wt extract) Pancreas of Diabetic Control Rat, H & E Staining (x 525)

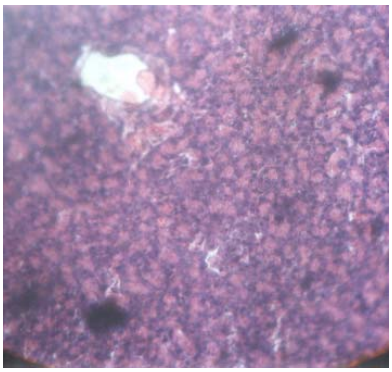

Fig. 3: DG-1 Pancreas of Diabetic Rat Treated with 300mg/kg body wt extract, H & E Staining (x 525)

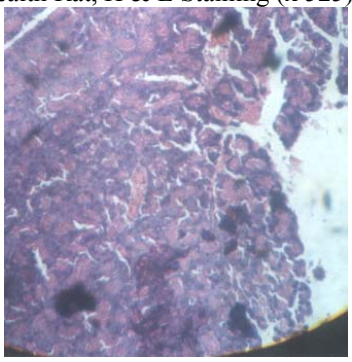

Fig. 4: DG-2 Pancreas of Diabetic Rat Treated with 600mg/kg body wt extract, H & E Staining (x 525)

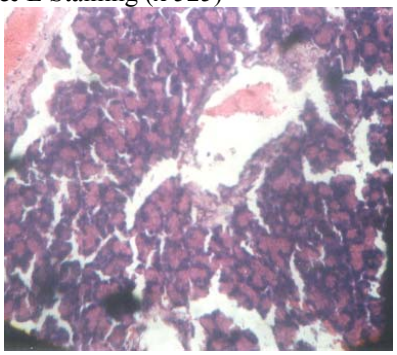

Fig. 5: NDG-1 Pancreas of Normal Rat Treated with 300mg/kg body wt extract, H & E Staining (x 525)

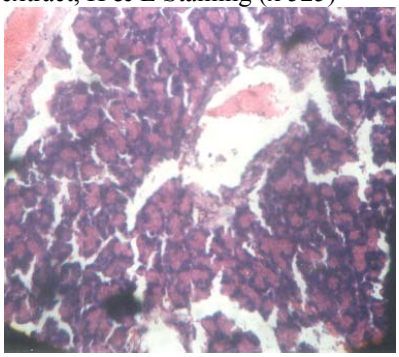

Fig. 6: NDG-2 Pancreas of Normal Rat Treated with 600mg/kg body wt extract, H & E Staining (x 525)

cells in groups receiving alligator pear seed extracts. These findings support the traditional use of alligator pear seed for controlling hyperglycemia in diabetics, in view of the restorative (protective) effects of the extract on pancreatic islet cells. Further investigation with longer period of higher dose may show clearer features of these findings.

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