ANTIHYPERLIPIDEMIC EFFECTS OF ETHANOLIC EXTRACTS OF ALLIGATOR PEAR SEED (*PERSEA AMERICANA MILL)* IN ALLOXAN-INDUCED DIABETIC RATS.

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Summary

This study was designed to determine the effect of ethanolic extracts of alligator pear seed (*Persea Americana)* on serum lipid profile changes in experimentally-induced diabetic Wistar rats with view to elucidating its possible effects on cardiovascular diseases induced by hyperglycemia. Thirty adult Wistar rats were assigned into 6 groups of 5 rats each. One group (NC) was the normal control (which did not receive any extract), while another DC was the diabetic control (untreated diabetic group) which was not given any extract. Two test groups (DG-1 and DG-2) were made diabetic by intra-peritoneal injection of alloxan and treated with 450mg and 900mg/kg body weight of alligator pear seed extract. Two non-diabetic groups (NDG-1 and NDG-2) were also administered with 450mg and 900mg/kg body weight extract. The experimental period lasted for 14 days. Blood samples from all experimental rats were collected by cardiac puncture. The serum harvested was analyzed for triglyceride, total cholesterol (TC), high density lipoprotein cholesterol (HDLC) and non-HDLC, using scientific assay kits. The result of the serum lipid analysis showed a significant ($p < 0.05$) reduction in the serum concentrations of TC and non-HDLC, in addition to a significant ($p < 0.05$) increase in the serum HDLC concentrations of the NC and extract-treated groups when compared with the control.. Computed Antiatherogenic index (AAI) showed higher values for the extract-treated groups (161.93 - 183.07) when compared with the DC (70.21). However, these values were lower than those of the NC (219.19). The results of this study suggested that *P. americana* seed possesses anti-hyperlipidemic activities.

Keywords: Diabetes mellitus, alloxan, serum lipid profiles, hyperglycemia.

Introduction

Diabetes mellitus is basically a disease of glucose metabolism resulting from dysfunction of pancreatic ß-cells and insulin resistance. It is characterized by hyperglycemia, glucosuria and polyuria (among others), resulting from defects in insulin secretion, insulin action or both (1). The chronic hyperglycemia of DM is associated with long-term damage, and failure of various organs, (especially the pancreas, kidneys, eyes, hearts, nerves and blood vessels) in addition to causing glycation of body proteins (2). Diabetes mellitus often leads to microvascular or macrovascular complications, cardiomyopathy, retinopathy, neuropathy, encephalopathy and nephropathy (3, 4, 5, 6, 7). The hyperglycemia-induced oxidative stress in DM is believed to be the major cause of the development and progression of diabetic microvascular complications (8, 9). The antioxidant defense mechanisms are overwhelmed in diabetic patients (10, 11).

The number of people suffering from DM worldwide is estimated to be at least 150 million, and this number is likely to increase to more than 300 million by the year 2030 (12, 13). At later stages of the disease, lipid metabolism is also affected. Lipid profile, which is altered in the serum of diabetic patients, is a significant factor in the development of atherosclerosis Diabetic dyslipidemia is characterized by low levels of HDL- cholesterol and includes an increase in total cholesterol and non HDL- cholesterol levels [low density lipoprotein + very low density lipoprotein cholesterol] (14, 15, 16, 17, 18). Diabetes- induced hyperlipidemia is attributable to excess mobilization of fat from adipose tissues due to underutilization of glucose (19, 20, 21, 22).

No satisfactory effective therapy has been available (till date) in modern medicine to cure DM. There are several drawbacks like insulin resistance (23), anorexia nervosa, brain atrophy and fatty liver (24) after chronic treatment using insulin therapy for the management of DM. The use of amylin analogues, inhibitors of α- glycosidase, sulphonylureas and biguanides have certain effects like causing hypoglycemia at higher doses, liver problems, lactic acidosis and diarrhea (25). Due to the above side effects of currently-used drugs, there is a need for safe agents with minimal adverse effects, which can be consumed for long duration. The undesirable side effects, high cost and low availability of synthetic drugs have led to a strong preference for hypoglycemic drugs of plant origin, which are believed to be suitable for chronic treatments (21, 26).

Traditional plant remedies, relied upon by 80% of Africans for their basic health case needs (27, 28, 29, 30, 31, 32) have been experimented in the treatment of DM. One of these plants is the alligator pear plant *Persea americana* (33, 34). The alligator pear, which belongs to the family Lauraceae, is an ovoid or ellipsoid tropical fruit with green (or reddish purple or blackish) leathery rough skin and an edible yellowish green (or creamy-yellow) buttery flesh (pulp). The fruit pulp encloses a single large seed (35). The single seed is oblate, round, conical or ovoid, hard and heavy, ivory in colour, but enclosed in 2 thin brown, thin, papery seed coats (often adhering to the flesh cavity), while the seed slips out easily. While there are scanty reports on the extracts of the elliptic dark green leaves of *P. americana* (33, 34), the seeds which are normally discarded after consumption of the fruit has received very little scientific scrutiny (36). Thus this paper highlights the effects of ethanolic extracts of *P. americana* seed on lipidemia in rats challenged with alloxan-induced diabetes.

Methods

Animals: Thirty (30) male Wistar albino rats weighing 152 - 238g were obtained from the Animal House of the College of Health Sciences, University of Uyo, Uyo, Nigeria. The rats were kept in clean and only dry plastic cages, with 12hrs light-dark cycle at 25 $\pm$ 2^0C 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 animals 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 Health.
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 a stainless steel knife. They were then dried to constant weight in an oven at 55^0C. After drying, the seeds were ground into fine powder (which passed through a 30 – mesh sieve).
Preparation of Ethanolic Extract of Alligator Pear Seed: One hundred grams (100g) of the ground seeds were soaked in 1 litre of 99.9% ethanol for 24hrs for complete extraction. Thereafter, the mixture was filtered using cheesecloth and the extract obtained. One hundred milliliters (100 ml) aliquots of the extract were poured into separated beakers of known weight. The solutions were dried at 50^0C 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 100.0mg. The residue was dissolved in 1 ml of distilled water. Thus the concentration of the extract was 100.0 mg/ml and 0.82ml of the solution administered to 182g rat was equivalent to 450mg/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, St. Louis, USA) dissolved in sterile distilled water. The NC group was not treated with alloxan. Diabetes was confirmed 3 days after alloxan injection by determining the blood glucose concentration using One Touch Basic Glucometer. Then groups DG-1 and NDG-1 were administered with 450 mg/kg body weight of the extract, while groups DG-2 and NDC-2 were administered with 900 mg/kg body wt of the extract, daily for 14 days, by oral gavage. Groups DC and NC, which served as treatment controls, were gavaged with distilled water.
Collection and Treatment of Samples: After 14 days, animals were fasted for 12 hours and anaesthetized under chloroform vapour. Blood samples were obtained by cardiac puncture. The blood samples were centrifuged at 5000 rpm for 5 minutes to harvest the serum. All analyses were carried out within 24 hrs of blood collection.
Biochemical estimations: The serum levels of triglyceride (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDLC) were determined spectrophotometrically, using enzymatic colorimetric assay kits (Randox, Laboratories Ltd, Crumlin, Northern Ireland) while non-HDLC was calculated
Assay for triglycerides: 1000 µL of the reagent was added to 10µl each of the sample and standard. This was incubated for 10 minutes at 20-25°C and the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was measured against the reagent blank within 30 minutes.
TG concentration = $(A_{sample} / A_{standard}) \times 200$ mg dL^{-1}

Assay for Total Cholesterol (TC): 1000 µL of the reagent was added to 10µl each of the sample and standard. This was incubated for 10 minutes at 20-25°C and the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was measured against the reagent blank within 30 minutes.
Total cholesterol concentration = $(A_{sample} / A_{standard}) \times 200$ mg dL^{-1}

Assay for high-density lipoprotein cholesterol (HDLC): Low-density lipoproteins (low density lipoproteins + very low density lipoproteins cholesterol) and chylomicron fractions in the sample were precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature centrifuged for 10 minutes at 4000rpm. The supernatant represented the HDLC fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant, was determined.

Non-HDLC: The concentration of non HDLC was calculated (mg/dL) as stated below:
Non-HDLC = TC – HDLC.

Antiatherogenic Index (AAI): The antiatherogenic index was calculated according to the method of Guido and Joseph (37). AAI was calculated from total cholesterol and HDLC using the formula below: AAI = (HDLC / Non-HDLC) × 100. The values were expressed as a percentage.

Relative AAI: This was calculated as: $AAI_{experimental\ group} / AAI_{normal\ control}$.

Statistical Analysis: All values were presented as mean ± standard deviation. Student's t – test and a probability level of $p < 0.01$ were chosen as criterion of statistical significance.

Results

Table 1 illustrates the effects of *P. americana* seed extract on the levels of total cholesterol, triglycerides, HDLC, non-HDLC and AAI in the serum of experimentally induced diabetic rats.
The levels of total cholesterol, triglycerides and non-HDLC were significantly ($p < 0.01$) increased in diabetic rats whereas the level of HDLC and the percentage of AAI were significantly ($p < 0.01$) reduced in diabetic rats when compared to the control normal rats.
Administration of *P. americana* seed extract to diabetic rats restored all these changes to near normal levels by significant ($p < 0.01$) reduction of the level of total cholesterol, triglycerides and non-HDLC of diabetic rats and significant increase in the level of HDLC and percentage of AAI.

TABLE 1
Effects of Ethanolic Extracts of Alligator Pear Seed on Lipid Profile in rats *

Parameter mg / dL	Experimental Group					
	NC 0 mg/kg body wt	**DC** 0 mg /kg body wt	**DG- 1** 450 mg/kg body wt	**DG- 2** 900 mg/kg body wt	**NDG- 1** 450mg/kg body wt	**NDG- 2** 900 mg/kg body wt
Triglyceride	59.91 ± 3.62	59.54 ± 7.35	57.18 ± 3.98	54.61 ± 6.03	57.44 ± 5.20	56.81 ± 4.69
Total Cholesterol	182.12 ± 2.78 [a]	204.98 ± 11.50[b]	170.98 ± 8.27 [a]	175.75 ± 8.11[a]	173.31 ± 5.18 [a]	157.92 ± 3.85 [c]
HDL Cholesterol	125.07 ± 12.11[a]	84.55 ± 6.05[b]	105.71 ± 12.00[a]	107.98 ± 9.95[a]	112.08 ± 13.98[a]	99.59 ± 6.83 [a]
Non-HDL Cholesterol	57.06 ± 14.32 [a]	120.43 ± 11.07[b]	65.28 ± 17.92 [a]	67.72 ± 4.84 [a]	61.22 ± 10.05 [a]	58.33 ± 12.75 [a]
HDL / Total Cholesterol	68.67 %	41.25 %	61.83 %	61.44 %	64.69 %	63.06 %
AAI %	219.90	70.21	161.93	169.45	183.07	170.73
Relative AAI	1.00	0.32	0.74	0.77	0.83	0.78

*Values are means ± standard deviation (n = 5). Values in same row with different superscripts in a horizontal row represent means that are significantly different ($p < 0.01$).

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
HDL = High Density Lipoprotein AAI = Anti Atherogenic Index

Discussion

Diabetes mellitus is associated with profound alteration in the serum lipid and lipoprotein profile with an increased risk of coronary heart disease. Hyperlipidemia is a recognized complication of diabetes mellitus characterized by elevated levels of cholesterol, and changes in lipoprotein composition (38). The result of this present study clearly shows that *P. americana* has a lipid lowering effect on serum total cholesterol and non HDL cholesterol of alloxan- induced diabetic rats. *P. americana* treatment also increased the serum level of high-density lipoprotein cholesterol termed "good cholesterol". There is substantial evidence that lowering the total cholesterol, particularly low-density lipoprotein cholesterol + very low-density lipoprotein cholesterol levels, will lead to a reduction in the incidence of coronary heart disease which is still the leading cause of death in diabetic patients.

Reduced HDLC levels are the key characteristics of dyslipidemia in type 2 diabetes (39). The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids (FFA) from the peripheral depots, since insulin inhibits the hormone sensitive lipase (40). Serum FFA concentration are a result of the balance between the release from lipolysis, neosythesis and disposal and represent the major determinant of insulin effect on FFA oxidation and non-oxidative metabolism (41).

Hypercholesterolemia has been reported to occur in diabetic rats (40) and significant increases in total cholesterol and non HDLC observed in the present experiment were in accordance with these studies. Furthermore, increase in circulatory non HDLC is largely due to defective clearance of these particles from circulation (38). The increase and fall in the individual lipoprotein levels is a reflection of the serum total cholesterol levels i.e. the levels of HDLC and non HDLC increase or decrease with the levels of total serum cholesterol, and it is their ratio that determines the pathophysiology of lipoprotein metabolism.

As there is a close relationship between elevated serum total cholesterol level and occurrence of atherosclerosis, the ability of the *P. americana* in the selective reduction of total cholesterol through the reduction of non HDLC components could be beneficial in preventing atherosclerotic conditions and thereby reduce the possibilities of coronary heart disease in general. Considering the effect of extract of *P. americana* on serum HDL, the result of this study clearly show that the level of this lipoprotein fraction increased with this treatment.

Due to the fact that *P. americana* treatment increased the regeneration of β-cells of pancreatic islet of diabetic rats (36), it will also increase the insulin output from the pancreas of these rats. Insulin activates the enzyme lipoprotein lipase, which hydrolyses lipoprotein bound triglyceride. The strong antihyperlipidemic effect of *P. americana* extracts could also be through its control of hyperglycemia (42) as this is a major determinant of total cholesterol and non HDLC concentration (43). Administration of *P. americana* normalized these effects possibly by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues.

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