Anti Hyperlipidemic Activities Of Annona Muricata (Linn)

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Citation

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Abstract

This study was designed to determine the effects of methanolic extracts of Annona muricata (Linn) on serum lipid profile changes in experimentally-induced diabetic Wistar rats with view to elucidate its possible effects on cardiovascular diseases induced by hyperglycemia. Thirty adult Wistar rats were randomly assigned into three groups (A, B and C) of ten rats each. Group A was the control, Group B was untreated diabetic group and group C was A. muricata-treated group. The changes in the serum lipid profiles were monitored in all the animals four weeks before the commencement of the experiment and throughout the experimental period. Diabetes mellitus was induced in groups B and C by a single intra-peritoneal injection of 80mg/kg streptozotocin dissolved in 0.1M citrate buffer. The control group was intraperitoneally injected with equivalent volume of citrate buffer and all the animals were monitored for another four weeks. Daily intra peritoneal injection of 100mg/kg A. muricata was administered to group C rats for two weeks and the animals were monitored for another two weeks. The data obtained were analyzed with descriptive and inferential statistics. The result of the serum lipid analysis showed a significant ($P < 0.05$) reduction in the serum total cholesterol, triglyceride, low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol; and a significant ($P < 0.05$) increase in the serum high-density lipoprotein cholesterol and antiatherogenic index of A. muricata-treated group when compared to untreated diabetic group of rats. The result of this study demonstrated that A. muricata possesses anti-hyperlipidemic activities.

INTRODUCTION

Diabetes mellitus (DM) is associated with an increased risk of thrombotic, atherosclerotic and cardiovascular disease. Hyperlipidemia is metabolic complication of both clinical and experimental diabetes[1]. Low-density lipoprotein in diabetic patients leads to abnormal metabolism and is associated with increase in very low-density lipoprotein (VLDL) secretion and impaired VLDL catabolism. Ultimately this leads to atherosclerotic plaque [2]. A number of known factors for coronary artery disease such as hypertension, obesity and dyslipidemia are more common in diabetics than in the general population. The World Health Organization (WHO) predicts that the number of cases worldwide for diabetes, now as of 171 million, will touch 366 million or more by the year 2030[3]. Patients with DM are more likely to develop microvascular and macrovascular complications than the non diabetic population [4]. Dyslipidemia is a frequent complication of DM and is characterized by low levels of high density lipoprotein-cholesterol (HDL-C) and high levels of low density lipoprotein-cholesterol (LDLC) and triglyceride (TG). Several groups of hypoglycemic drugs are currently available to treat DM.

Treatment of hyperlipidemia in diabetes involves improving glycemic control, exercise and the use of lipid lowering diets, drugs and hypoglycemic agents [5,6]. Mendez and Balderas [7] showed that non-glucidic nutrient such as L-arginine administration tended to normalize the glycemia, hyperlipidemia, and lipid peroxidation, which indicates non-glucidic nutrients exerted an inhibitory effect on lipid peroxidation and also improve the lipid profile, that may be relevant in preventing diabetic complications. The objective of this study was to investigate the anti hyperlipidemic properties of extracts of Annona muricata leaves

Annona muricata is a plant, which belongs to the family Annonaceae. It is a medicinal plant that has been used as a natural remedy for a variety of illnesses. Several studies by different researchers demonstrated that the bark as well as the leaves had anti-hypertensive, vasodilator, anti-spasmodic (smooth muscle relaxant) and cardio depressant (slowing of heart rate) activities in animals [8]. Researchers had re-verified A. muricata leaf's hypotensive properties in rats [9]. Other properties and actions of A. muricata documented by traditional uses include its use as anti-cancerous [10,11], anti-diabetes [12], anti-bacterial [13], anti-fungal [14], anti-malarial, anti-mutagenic (cellular protector), emetic (induce vomiting),

anti-convulsant [15], sedative, insecticidal and uterine stimulant. It is also believed to be a digestive stimulant, antiviral cardio tonic (tones, balances and strengthens the heart), febrifuge (cures fever), nerviness (balances/calms the nerves), vermifuge (expels worms), pediculocide (kills lice), and as an analgesic.

MATERIALS AND METHODS

PLANT MATERIAL

Annona muricata leaves were collected from Mowe, Ogun State, Nigeria in February 2006. The plant was identified by Dr. Folorunso of the Department of Botany, Obafemi Awolowo University, Ile Ife and a voucher specimen was deposited in the Herbarium of the Department

PREPARATION OF EXTRACT

A. muricata leaves were air dried at room temperature for four weeks. The air-dried leaves were powdered in a warring blender (Christy and Norris – 47362, England) at the Department of Pharmacognosy Obafemi Awolowo University, Ile Ife. A 600g of the powdered leaf leaves was soaked in 5 litres of 70% methanol for 72 hours at room temperature. The mixture was filtered and the filtrate was evaporated at 60°C using a vacuum rotary evaporator (RE 100B, Bibby Sterilin, United Kingdom). The wet residue was freeze-dried using a vacuum freeze drier (FT33-Armfield, England) and was stored until ready to use.

CARE AND MANAGEMENT OF ANIMALS

Thirty healthy adult Wistar rats (Rattus norvegicus) of both sexes, weighing between 150g and 250g were used for the experiment. The rats were bred in the animal holding of department of Anatomy and Cell Biology Obafemi Awolowo University Ile Ife, were maintained on standard rat pellets (Ladokun feeds, Ibadan, Nigeria), and were given water ad libitum. The animals were randomly assigned into three groups A, B, and C of ten rats each. Group A was the control, non-diabetic group of rats, group B was the experimentally induced diabetic group without A. muricata treatment while group C was the experimentally induced diabetic group treated with methanolic extracts of A. muricata. There was a pre-experimental period of four weeks during which the serum lipid profiles was monitored in the animals before the commencement of the experiment. The rats receive 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.

ACUTE TOXICITY TEST

Forty adult male Wistar rats (150 – 200g) were randomly assigned into six groups (T1, T2, T3, T4, T5 and T6) of eight animals in each group. T1 was treated with distilled water and is considered as control. The other five groups were treated with methanolic extract of A. muricata given intraperitoneally in increasing dosage of 25, 50, 100, 200, and 400mg/kg body weight. The extract was dissolved in distilled water and the average volume injected was 0.3ml. The control group was given equivalent volume of distilled water used in dissolving the extract. All the rats were returned to their cages and given free access to food and water. The mortality in each cage was assessed 24 hours, 48 hours and 72 hours after administration of extract. The percentage mortality in each group was calculated and plotted against the $\log_{10}$ of the extract dose. Regression line was fitted by method of least squares and confidence limits for the lethal dose (LD_{50}) values were calculated by method of Abdel-Barry et al [16].

ADMINISTRATION OF STREPTOZOTOCIN AND

Diabetes mellitus was experimentally induced in groups B and C by a single intraperitoneal injection of 80mg/kg body weight. streptozotocin (Sigma, St. Louis, USA) dissolved in 0.1M sodium citrate buffer pH 6.3. The control (group A animals) were injected intraperitoneally with equivalent volume of the citrate buffer. The rats were fasted overnight before STZ administration. The serum lipid profiles were monitored weekly in the animals for the next four weeks. After four weeks of experimental-induction of diabetes, group C rats were given daily intraperitoneal injection of 100mg/kg of extracts of A.muricata dissolved in distilled water for two weeks and the animals were monitored for another four weeks.

BIOCHEMICAL ESTIMATIONS

The serum levels of triglyceride (TGL), total cholesterol (TC) and high-density lipoprotein-cholesterol (HDLC) were determined spectrophotometrically, using enzymatic colorimetric assay kits (Randox, Northern Ireland) while low-density lipoprotein cholesterol (LDLC) and very low-density lipoprotein cholesterol (VLDLC) were calculated. Animals were fasted for 12-16 hours before blood samples were obtained. About two milliliters of blood was collected from the tail vein of each rat into an ice-cold centrifuge tubes. The blood samples were centrifuged in a Denley BS400 centrifuge (England) at 5000 R.P.M for 5-minutes. The supernatant (serum) collected was assayed for the serum

levels of TGL, TC and HDL-C using the Randox Biochemical kits while LDL-C and VLDL-C were calculated.

ASSAY FOR TRIGLYCERIDES

The serum level of TGL was determined by the method of Treitz [17]. 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.

Figure 1

$$TGL\ concentration = \frac{A\ sample}{A\ standard} \times 2.29 \frac{mmol}{L}$$

ASSAY FOR TOTAL CHOLESTEROL

The serum level of TC was determined after enzymatic hydrolysis and oxidation of the sample as described by Richmond [18] and Roeschlau et al., [19]. 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.

Figure 2

$$TC\ concentration = \frac{A\ sample}{A\ standard} \times 5.17 \frac{mmol}{L}$$

ASSAY FOR HDLC

Low-density lipoproteins (LDL and VLDL) 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 remains in the supernatant, was determined.

LOW DENSITY LIPOPROTEIN - CHOLESTEROL

The concentration of LDL cholesterol was calculated mmol/L using Friedewald's equation [20] as stated below.

Figure 3

$$LDLC = TC - \left(HDLC + \frac{TGL}{2.2}\right)$$

VERY LOW DENSITY LIPOPROTEIN - CHOLESTEROL

The concentration of VLDL cholesterol was calculated mmol/L using Friedewald's equation [20] as stated below.

Figure 4

$$VLDL = \frac{TGL}{2}.2$$

ANTIATHEROGENIC INDEX (AAI)

The antiatherogenic index was calculated according to the method of Guido and Joseph[21]. AAI was calculated from total cholesterol and HDL cholesterol using the formula below;

Figure 5

$$AAI = \frac{HDLC\ x\ 100}{TC - HDLC}$$

The values were expressed as a percentage

STATISTICAL ANALYSIS

The data were analysed using descriptive and inferential statistics. All values are presented as mean ± standard error of mean (SEM) for ten rats in each of the three group of rats. The significance of difference in the means of all parameters reported for the three groups of animals was determined using paired sample student t – test and a p – value of < 0.05 (two tailed) was considered as significant.

RESULTS

EFFECTS OF ON THE SERUM LIPID PROFILES

Tables 1 and 2 illustrate the effects of A. muricata on the levels of total cholesterol, triglycerides, HDLC, LDLC, VLDLC and AAI in the serum of experimentally induced diabetic rats. The levels of total cholesterol, triglycerides LDL-C and VLDL-C were significantly ($p < 0.05$) increased in diabetic rats whereas the level of HDL-C and the percentage of AAI (ratio of HDL to total cholesterol) were significantly ($p < 0.05$) reduced in diabetic rats when compared to the control normal rats. Administration of A. muricata to STZ diabetic rats restored all these changes to

near normal levels by significant ($p < 0.05$) reduction of the level of total cholesterol, triglycerides, LDLC and VLDLC of diabetic rats and significant increase in the level of HDL-C and percentage of AAI.

Figure 6

Table 1: Effects of on the Serum Total Cholesterol, Triglycerides and High Density Lipoprotein Cholesterol (HDLC) of Experimentally-Induced Diabetic Rats

Groups	Total Cholesterol (mmol / L)	Triglycerides (mmol / L)	HDL – C (mmol / L)
Group A (initial)	1.574 ± 0.025^a	0.843 ± 0.024^a	0.803 ± 0.022^a
Group A + Citrate Buffer	1.615 ± 0.025^a	0.876 ± 0.025^a	0.838 ± 0.015^a
Group B (initial)	1.568 ± 0.032^a	0.830 ± 0.010^a	0.813 ± 0.032^a
Group B + STZ	2.044 ± 0.048^b	1.650 ± 0.079^b	0.411 ± 0.007^b
Group C initial	1.585 ± 0.026^a	0.868 ± 0.017^a	0.815 ± 0.029^a
Group C + STZ	2.013 ± 0.015^b	1.656 ± 0.035^b	0.394 ± 0.012^b
Group C + STZ + *A. muricata*	1.649 ± 0.010^a	0.972 ± 0.024^a	0.807 ± 0.012^a

Values are given as mean ± SEM for ten rats in each group.

a, b within column signifies that means with different letters differs significantly at $P < 0.05$ (two tailed T-test) while means with the same letters does not differ significantly at $P < 0.05$ (two tailed T-test)

Figure 7

Table 2: Effects of on the Serum Low Density Lipoprotein Cholesterol (LDLC) Very Low Density Lipoprotein Cholesterol (VLDLC) and Antiartherogenic index (AAI) of Experimentally-Induced Diabetic Rats

Groups	LDL – C (mmol/L)	VLDL – C (mmol / L)	AAI (%)
Group A (initial)	0.387 ± 0.004^a	0.383 ± 0.004^a	104.46 ± 3.39^a
Group A + Citrate Buffer	0.381 ± 0.006^a	0.398 ± 0.011^a	107.78 ± 1.37^a
Group B (initial)	0.378 ± 0.009^a	0.377 ± 0.005^a	107.68 ± 4.51^a
Group B + STZ	0.883 ± 0.002^b	0.750 ± 0.035^b	25.31 ± 0.56^b
Group C initial	0.376 ± 0.010^a	0.395 ± 0.008^a	105.98 ± 4.30^a
Group C + STZ	0.867 ± 0.014^b	0.753 ± 0.016^b	24.41 ± 0.91^b
Group C + STZ + *A. muricata*	0.392 ± 0.005^a	0.442 ± 0.011^a	96.89 ± 2.20^a

Values are given as mean ± SEM for ten rats in each group.

a, b within column signifies that means with different letters differs significantly at $P < 0.05$ (two tailed T-test) while means with the same letters does not differ significantly at $P < 0.05$ (two tailed T-test

DISCUSSION

Diabetes mellitus is associated with profound alteration in the serum lipid and lipoprotein profile with an increased risk in coronary heart disease [5]. Hyperlipidemia is a recognized complication of Diabetes mellitus characterized by elevated levels of cholesterol, triglycerides and phospholipids; and changes in lipoprotein composition [22]. The result of this present study clearly shows that Annona muricata has a lipid lowering effects on serum triglycerides, total cholesterol and low-density lipoprotein cholesterol of STZ induced diabetic rats. A. muricata treatment also increase the serum level of High-density lipoprotein cholesterol termed “good cholesterol”. There is a substantial evidence that lowering the total cholesterol, particularly LDL-C level will lead to a reduction in the incidence of coronary heart disease which is still the leading cause of death in diabetic patients.

Increased triglycerides and reduced HDL-C levels are the key characteristics of dyslipidemia in type 2 diabetes [23]. Hypertriglyceridemia in type 2 diabetes can result from an increased hepatic very low-density lipoprotein (VLDL), overproduction and impaired catabolism of triglyceride-rich particles. The function of lipoprotein lipase, the key enzyme in removal and degradation of triglycerides is attenuated by both insulin deprivation and insulin resistance.

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 [24]. 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 free fatty and oxidation and non-oxidative metabolism [25].

Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats [26] and significant increase in total cholesterol and triglycerides observed in the present experiment was in accordance to these studies. Furthermore, increase in circulatory VLDL and their associated triglycerides are largely due to defective clearance of these particles from circulation [22]. The increase and fall in the individual lipoprotein levels is a reflection of the serum total cholesterol levels i.e. the levels of HDL-C, LDL-C and VLDL-C 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 A. muricata in the selective reduction of total cholesterol through the reduction of LDL and VLDL 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 A. muricata 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 A. muricata treatment increased the regeneration of -cells of pancreatic islet of diabetic rats [2728], it will also increase the insulin output from the pancreas of these rats. Insulin activates the enzyme lipoprotein lipase, which hydrolyses lipoprotein bound triglyceride [2930]. The strong antihyperlipidemic effect of A. muricata extracts could also be through its control of hyperglycemia [31] as this is a major determinant of total cholesterol, VLDL and triglyceride concentration [32]. Administration of A. muricata normalized these effects possibly by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues [32].

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