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ANTI HYPERGLYCEMIC ACTIVITIES OF *ANNONA MURICATA* (LINN)

Adeyemi, David Olawale^{1*}, Komolafe, Omobola Aderibigbe¹, Adewole, Olarinde Stephen¹, Obuotor Efer Martins², Adenowo Thomas Kehinde³

¹Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile Ife, Nigeria

²Department of Biochemistry, Obafemi Awolowo University, Ile Ife, Nigeria, ³Department of Anatomy, Olabisi Onabanjo University, Ikenne, Ogun State, Nigeria.

*E-mail: waledavade@yahoo.com

Abstract

This study was designed to determine the effects of methanolic extracts of *Annona muricata* (Linn) on the blood glucose level of streptozotocin-induced diabetic Wistar rats. 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 hyperglycemic group and group C was *A. muricata*-treated group. Hyperglycemia was induced in groups B and C by a single intraperitoneal 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 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 showed a mean body weight of $206 \pm 7.74\text{g}$, $173.29 \pm 5.13\text{g}$ and $197 \pm 5.62\text{g}$ respectively for the control, untreated diabetic and *A. muricata*-treated diabetic group, and a mean blood glucose concentration of $3.78 \pm 0.190\text{ mmol/L}$, $21.64 \pm 2.229\text{mmol/L}$ and $4.22 \pm 0.151\text{mmol/L}$ for the control, untreated diabetic and treated diabetic groups respectively. A significant difference exists between the blood glucose concentrations of treated and untreated hyperglycemic groups of rats. The result of this study demonstrated that *A. muricata* possesses anti-hyperglycemic activities.

Key words: *Annona muricata*, Diabetes mellitus, Streptozotocin, Blood glucose level, hyperglycemia

Introduction

Diabetes mellitus, characterized by hyperglycemia, is a genetically and clinically heterogeneous group of disorders with common feature of glucose intolerance (WHO, 1994). Based on WHO recommendation, diabetes mellitus is classified into four major subtypes: type I (insulin dependent diabetes mellitus, IDDM), type II (non-insulin dependent diabetes mellitus, NIDDM) other specific types and gestational diabetes mellitus. IDDM or Juvenile-onset diabetes results from a cellular mediated autoimmune destruction of the β -cells of the pancreas (Atkinson and Maclaren, 1994, Takeshi et al., 2002). However, NIDDM or adult-onset diabetes results from the development of insulin resistance and the affected individuals usually have insulin deficiency (De Fronzo et al., 1997). In modern medicine, the beneficial effects of standard medications on glycemic levels are well documented; the preventive activity of medications against the progressive nature of diabetes and its complications was the modest but not always effective. Insulin therapy affords glycemic control in type 1 diabetes, yet its shortcomings such as ineffectiveness on oral administration, short shelf life, the requirement of constant refrigeration, fatal hypoglycemia in event of excess dosage, reluctance of patients to take insulin injection and above all the resistance

due to prolonged administration limits its usage (Kasiviswanath et al., 2005). Similarly, treatment of type 2 diabetes patients with sulfonylureas and biguanides is always associated with side effects (Grandhipuram et al., 2006). Hence, search for a drug with low cost, more potential and without adverse side effect is being pursued in several laboratories around the world.

The bark, roots and leaves of *Annona muricata* has been reported to be used as anti-diabetes in the Peruvian Amazon (Vasquez, 1990). This investigation is therefore designed to confirm the effects of methanolic extracts of *A. muricata* leaves on glycemic control in streptozotocin-induced diabetic Wistar rats.

Annona muricata is a plant which belongs to the family *Annonaceae* (Taylor, 1996). 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 (Feng, 1962). Researchers had re-verified *A. muricata* leaf's hypotensive properties in rats (Carbajal et al., 1991). Other properties and actions of *A. muricata* documented by traditional uses include its use as anti-cancerous, (Oberlies et al., 1995; Tormo et al., 2003), anti-diabetes (Vasquez, 1990); anti-bacterial, (Takahashi et al., 2006); anti-fungal (Heinrich et al., 1992); anti-malarial, anti-mutagenic (cellular protector), emetic (induce vomiting), anti-convulsant (N'gouemo, 1997), 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. Padma et al., (1998) confirmed the anti-viral activity of ethanolic extracts of *A. muricata* against *Herpes simplex* virus. Extracts of *A. muricata* have been shown to have anti-parasitic (Bories et al., 1991), anti-rheumatic, astringent, (dos Santos and Sant'Ana, 2000), anti-leishmanial and cytotoxic effects (Jaramillo et al., 2000; Liaw et al. 2002). *A. muricata* has also been shown to be effective against multi-drug resistant (MDR) cancer cell lines (Oberlies et al., 1997; Liaw et al., 2002). Extracts of *A. muricata* were also shown to be effective against the cancer cell line U973 (Jaramillo et al., 2000), and hematoma cell lines *in-vitro* (Chen et al., 2000). Extracts were also shown to be lethal to the fresh water mollusk, *Biomphalaria glabrata*, which act as a host for the parasitic worm *Schistosoma mansoni* (dos Santos and Sant'Ana, 2000; Luna et al., 2006).

Materials and Methods

Plant Material

Annona muricata leaves were collected from Mowe, Ogun State, Nigeria in February 2006. The plant was identified by Dr. Foloruso of the Department of Botany, Obafemi Awolowo University, Ile Ife and a voucher specimen (IFE5) was deposited in the Herbarium of the Department

Preparation of Extracts

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 body weight and blood glucose level were 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. (1997)

Administration of Streptozotocin and *Annona muricata*

Diabetes mellitus was experimentally induced in groups B and C by a single intraperitoneal injection of 80mg/kg b.w. 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 blood glucose level was 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.

Determination of Body Weight and Blood Glucose Level

The body weights of the animals were measured using a top loader weighing balance. Blood sample was obtained from the tail vein of the animals and their fasting blood glucose level was determined in mmol/L using a digital glucometer (Accu-chek® Advantage, Roche Diagnostic, Germany). The animals were fasted for a period of 16 hours before their blood glucose level was measured.

Statistical Analysis

The data were analysed using descriptive and inferential statistics. All values are presented as mean \pm 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 *Annona muricata* on the Weight:

Prior to STZ administration, there was no significant ($p < 0.05$) difference in the average weights of the three groups of experimental animals. By the end of the first week after diabetes mellitus was experimentally induced, the weights of Groups B and C animals was significantly ($p < 0.05$) reduced despite the increase in food and fluid intake in these animals. This weight loss continued for four week after STZ administration (Figure 1). However, the weight of animals in group C gradually increased on treatment with extracts of *A. muricata* over the period of five weeks as shown in Figure 1. At the end of the experiment, there was a significant ($p < 0.05$) difference in the weights of groups B and C animals while there was no statistically significant ($p < 0.05$) difference between weights of animals in groups A and C (Figure 1, Table 1.)

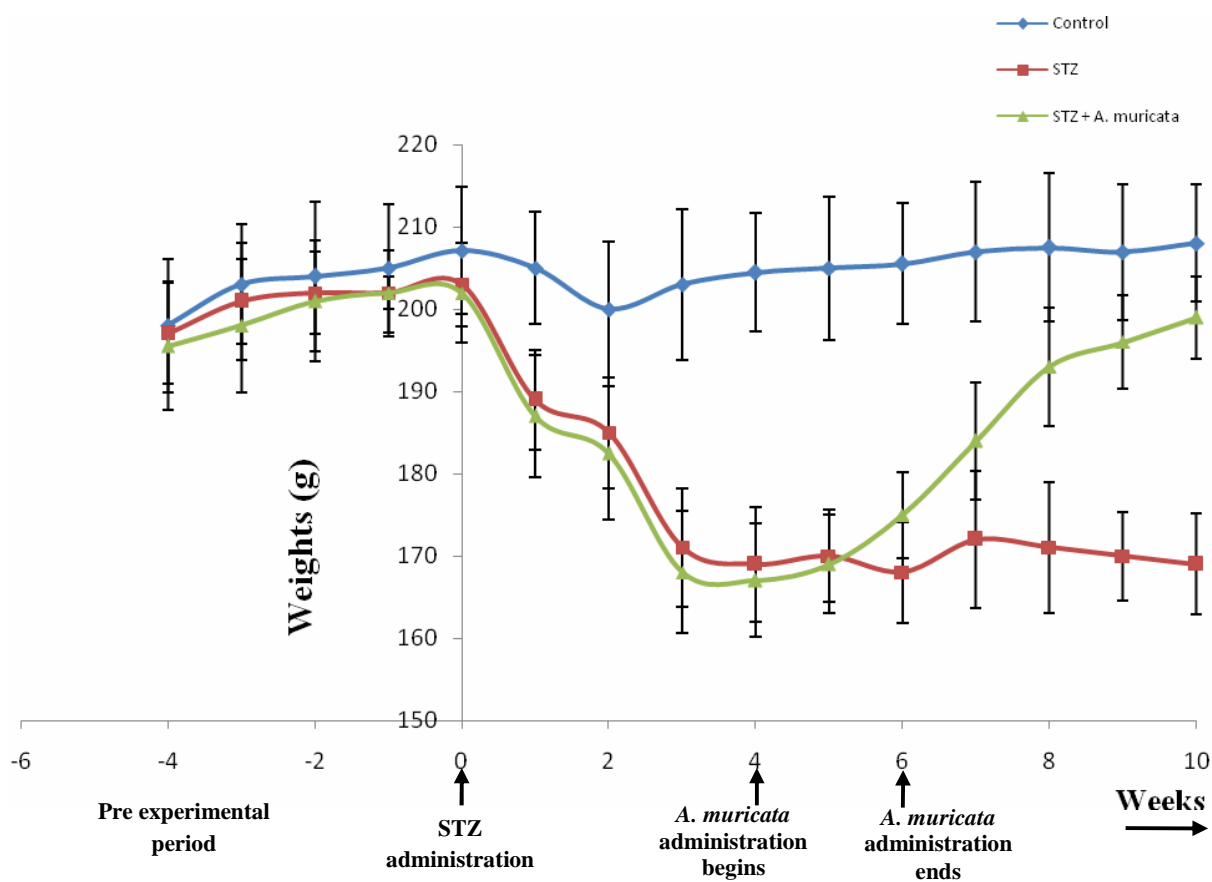


Figure 1: Weekly changes in the body weight of the rats after STZ and *A. Muricata* Administration

Figure 1 show that, in contrast to control rats, there was a significant ($p < 0.05$) weight loss among the group B and C rats, one week after STZ administration. However, *A. muricata* -treated rats showed sign of recovery in body weight gains four weeks after treatments ($197.70 \pm 5.62\text{g}$) compared to the diabetic control rats ($173.29 \pm 5.13\text{ g}$).

Effects of *Annona Muricata* on the Blood Glucose Level

Prior to STZ administration, the fasting blood glucose level did not differ significantly ($p < 0.05$) between the three groups of experimental animals. 24 hours after administration of STZ, the blood glucose level was significantly ($p < 0.05$) higher in groups B and C animals. The blood glucose level of animals in group C gradually decreased on treatment with extracts of *A. muricata* over the period of five weeks (fig. 2). Control rats treated with citrate buffer were euglycemic throughout the period of experiment. At the end of the experiment, there was a significant ($p < 0.05$) reduction in the blood glucose level of groups C rats compared to that of group B rats. In addition, there exist no significant ($p < 0.05$) difference between blood glucose level of rats in groups A and C (Fig. 2, Table 1) This shows that extracts of *A. muricata* demonstrate a potent antihyperglycemic activity.

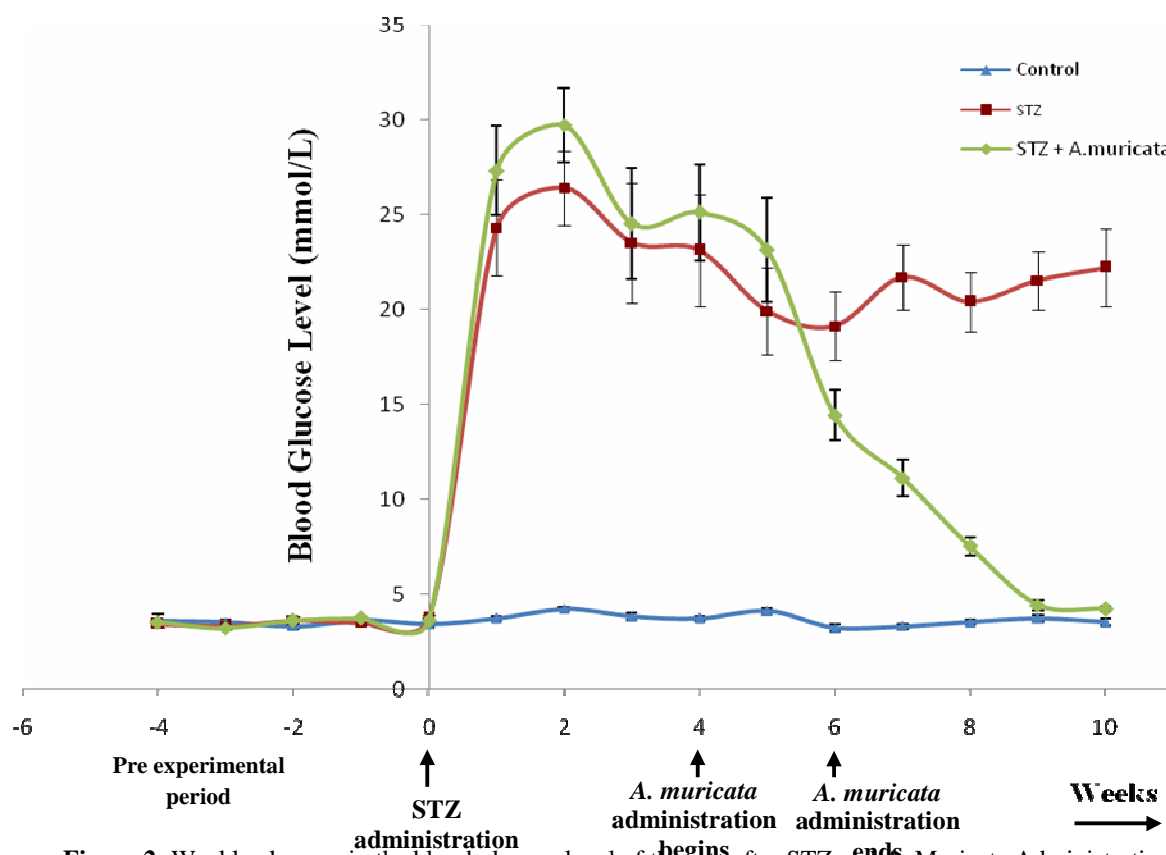


Figure 2: Weekly changes in the blood glucose level of the rats after STZ and A. Muricata Administration

Table 1: Changes in the Body Weight and Blood Glucose Level of Control and Experimental Group of Rats after STZ and *Annona muricata* Administration

Group	Body Weight (g)	Blood glucose level (mmol/L)
Group A (initial)	207.65 ± 9.31 ^a	3.60 ± 0.127 ^a
Group A + Citrate buffer	206.00 ± 7.74 ^a	3.78 ± 0.190 ^a
Group B (initial)	203.46 ± 7.99 ^a	3.28 ± 0.182 ^a
Group B + STZ	173.29 ± 5.13 ^b	21.64 ± 2.229 ^b
Group C (initial)	201.00 ± 7.26 ^a	3.57 ± 0.121 ^a
Group C + STZ	170.60 ± 5.23 ^b	24.74 ± 2.923 ^b
Group C + STZ + <i>A. muricata</i>	197.70 ± 5.62 ^a	4.22 ± 0.151 ^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 2 shows that prior to the extract administration, there was no significant ($p < 0.05$) difference between the blood glucose levels of the control and the two diabetic groups of animals. The blood glucose level of the two diabetic groups of rats significantly ($p < 0.05$) increased twenty-four hours after STZ administration, and there was no significant ($p < 0.05$) difference between the blood glucose levels of these two groups of diabetic rats for a period of four weeks. After 4 weeks of treatment with extracts of *A. muricata*, the blood glucose levels of the treated diabetic rats does not differ significantly ($p < 0.05$) from that of the control (4.22 ± 0.151 and 3.78 ± 0.190 mmol/L, respectively). In contrast, the blood glucose level of the untreated diabetic rat remained elevated throughout the experimental period (21.64 ± 2.229 mmol/L). The blood glucose level of the control remained unchanged throughout the period of the investigation.

Discussion

Streptozotocin-induced hyperglycemia in rats is considered a good model for the preliminary screening of agents active against type II diabetes (Ivorra et al., 1989) and is widely used. Generally, destruction of β -cells starts three days after STZ administration and reaches its peak at three to four weeks in rats (Adeghate and Ponery, 2002). Streptozotocin-induced diabetes in laboratory animals has been widely used for research on diabetes and its long-term complications. Control animals in these studies are usually injected with citrate buffer solution. However, STZ is known to possess pharmacological effects other than its diabetogenic properties (Schein et al., 1974), and extra pancreatic actions of streptozotocin cannot be excluded. The presence of GLUT2 in liver and kidney might explain the long-term complications seen with hepatic and renal tumors in rats treated with streptozotocin (Delahunt et al., 1995). Because of the extra pancreatic effects of streptozotocin, it may be difficult to distinguish effect secondary to diabetes from those secondary to streptozotocin *per se*.

Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration caused by insulin deficiency, often combined with insulin resistance. Diabetes mellitus is also major cause of disability and hospitalization and it results in significant financial burden (Vats et al., 2002). By the year 2010, the total number of people worldwide with diabetes mellitus is projected to reach 239 million. Region with greatest interest are Asia and Africa, where diabetes rates could rise to 2-3 folds than the present rates (American Diabetes Association, 1997).

Many traditional plant treatments for diabetes mellitus are used throughout the world (Marles and Farnsworth, 1995). Management of diabetes without any side effect is still a challenge to the medical system. This has led to an increasing demand for natural products with anti-diabetic activity and fewer side effects (Kameswara et al., 1999). Many herbs and plant products have been shown to have hypoglycemic action. Various morphological parts of *Annona muricata* have been reported to be useful as effective remedies against diabetes, hypertension, headache, dizziness, constipation, catarrh, liver problems, neuralgia, rheumatism and arthritis pain (de Almeida, 1993).

It has been suggested that bioactive compounds from plants sources having anti-hyperglycemic activities might act by several mechanisms such as stimulating insulin secretion, increasing repair or proliferation of β -cells and enhancing the effects of insulin and adrenalin (Fayed et al., 1998). The result of this present study indicated that there was a significant reduction in the blood glucose concentration of diabetic rats by *A. muricata* treatment. The present study also shows that daily intraperitoneal administration of 100mg/kg of extracts of *A. muricata* to diabetic rats for 15 consecutive days caused a statistically significant increase in the body weight of diabetic animals despite the decrease in food and fluid intake observed in these animals. This could be the result of improved glycemic control produced by *A. muricata* extracts.

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