

Evaluation of Anti-diabetic and some biochemical indices of Aqueous Ethanolic Leaf Extract of *Annona Muricata* in Alloxan-induced Diabetic Rats.

¹Ahmed A.G*, ²Zaruwa M. Z, ³Alexander, A. and ³Idris A.

¹Department of Science Laboratory Technology, Federal Polytechnic, Mubi Adamawa State, Nigeria.

²Department of Biochemistry, Adamawa State University, Mubi, Adamawa State, Nigeria.

³Department of Chemistry, Adamawa State University, Mubi, Adamawa State, Nigeria.

*Corresponding Author: Ahmed A.G +2348060735156

ABSTRACT

The ethanolic leaf extract of *Annona Muricata* sour sop (*Annonaceae*) was tested in alloxan induced diabetic rats alongside other biochemical parameters such as total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C and triglycerides (TG). The ethanolic leaf extract of *A. muricata* (LEAM) was administered to normoglycemic and alloxan induced diabetic Wister albino rats. The Biochemical parameters were estimated with lipid panel test strip device. The results showed significant increase ($p < 0.05$) in glucose concentration in diabetic rats compared to the normal control group because the action of alloxan monohydrate is known to induce the production of free radicals and causes β -cell injury leading to insulin deficiency. Oral administration of the extract significantly ($p < 0.05$) decreased the blood glucose levels. The results further showed insignificant differences ($p < 0.05$) in TC of treated rats compared to normal group. Significant differences ($p < 0.05$) were also observed in LDL-C and TG values of treated rats compared to normal group. The observations support the traditional use of LEAM as a hypoglycemic and hypolipidemic remedy. This research, recommend that LEAM should be subjected to further investigations like isolating novel hypoglycemic compounds responsible for this antidiabetic properties noticed in this work.

Keywords: *Annona Muricata*, Diabetes mellitus. Glucometer and hypolipidemia.

INTRODUCTION

Diabetes mellitus (DM) is an endocrine disorder that is characterized by an increase in glucose level called hyperglycemia. Primarily, DM occurs due to decrease in insulin production or insufficient insulin utilization, as well as aberration in intermediate metabolism of carbohydrate, protein and lipids. Persons living with DM are globally on the increase, most especially in developing countries due to population growth, aging, urbanization and life style of individuals [1]. According to the World Health Organization (WHO), it was estimated that in 2030, the number of people globally suffering from DM was estimated to reach about 171 million and by the year 2030, it would increase to 366 million patients. Relatively, in Africa it was estimated that in 2000 about 7.02 million people

suffered from DM and forecast estimates an increase to about 18.234 million by the end of 2030. In Nigeria however, the patient population may increase from 1.707 million to 4.835 million by 2030 [1].

Traditional believes, awareness and economic status has been shown to influence most patients' attitudes towards managing disease conditions especially DM. The use of herbal or traditional medicine for managing DM has been an old practice in African countries especially Nigeria. Plant or their parts such as *Anogeissusacuminata* [2], *Momordicacharantia L.* [3], *Anisopusmannii*, *Daniellaolivieri* [4], *Detariummacrocarpum*, *Leptedaniahastata* (Pers.) are common medicinal plants used in the treatment of DM which is becoming endemic in Northeastern Nigeria [5]. Some other plants which are not indigenous to the Northern parts of Nigeria were recently discovered to have potent hypoglycemic activity such as *Annonamuricata* or sour sop (*Annonaceae*) [1].

The *A. muricata* tree is a small straggly fruit tree growing up to 8 meters high. It originated from tropical America and West Indies [2]. However, it is now widely grown in the tropics of both hemispheres [4]. It is grown in a wide range of soils with good drainage and elevations of up to 1000 meters and requires warm humid climate [2]. The leaves are glossy, oval to lanceolate in shape and the fruits are oval or irregular, 15-30 cm long, with sparse soft green curved spines. The flesh is pulpy white, stringy and sour containing shiny black seeds [3]. It has a pleasant flavour and aroma.

The *A. muricata* is unrivaled for sherbets, soft drinks, ice-creams, syrups and nectars [4]. Soursop juice was reported to contain 19-23% sugar and 1.10-1.71% total protein when ripe. The fruit was reported to contain 12% sugar, mostly glucose and some fructose, pectin; other components are potassium, sodium, calcium, chloride and citrate [2]. *Annonamuricata* is also called prickly custard apple (English), in Fulfulde called it is *dukuu-je ladde*, *Habiwal* in Hausa and *Gawarii* in Yoruba languages, *Mamphal* (Indian), *Guanabana* (Spanish) and *Corossol* and *Sappadille* (French) [4], respectively.

This plant is used as a natural remedy for a variety of illnesses. Several studies demonstrated that the bark as well as the leaf had anti-hypertensive, vasodilator, anti-spasmodic (smooth muscle relaxant) and cardio depressant (slowing of heart rate) activities in animals [3]. Other properties of *A. muricata* are its use as anti-cancer, [4];[6], anti-bacterial, [7]; anti-fungal [3]; anti-malarial, anti-mutagenic (cellular protector), emetic (induce vomiting), anti-convulsant, sedative, insecticidal and uterine stimulant[5].

The objective of this study is to evaluate the anti-diabetic activity and some biochemical parameters of the aqueous leaf extract of ethanolic leaf extract of *A. muricata*. (LEAM)

Materials and Methods

Chemicals

DPPH (2, 2-diphenyl-2-picryl hydrazyl), Alloxan (Sigma, Chemical Company Ltd, USA), Thiobarbituric (TBAS), Gliberclamide (GIPLA Company), ethanol other reagents used were of analytical grade.

Equipment

Glucometer (Sigma, Chemical Company Ltd, USA), spectrophotometer (UV) (Global Napkin Machines India), Waterbath (Nand scientific and Laboratory Instrument India), PTS Panels Lipid Panel analyzer (Sigma, Chemical Company Ltd, USA), Thiobarbituric (TBAS)(TriveniInterchem PVT. Ltd India).

Plant Collection

Matured green leaves of *A. muricata* were collected from Imo state, South-Eastern Nigeria. The plant species was authenticated in the Adamawa State University Herbarium by Dr. Akesa, T.M. of the Department of Botany, Adamawa State University, Mubi. The plant specimen was kept in the University herbarium labeled Herbarium index number (*A. Muricata*) 15.

Animals

Matured (same age) albino rats weighing between (180-230g) was obtained from the Veterinary Research Institute Vom (Jos), Plateau State, Nigeria, and housed in a standard cages with free access to standard dry pellets and water *ad libitum*.

Methods

Preparation/Extraction of *A. muricata*

The plant leaves were thoroughly washed with tap water to remove dusts and other unwanted materials. The dust free leaves were dried under shade. The dried leaves were pulverized in a mortar with a pestle. A fine powder obtained after sieving (115 μ) the mass. The *A. muricata* powder (1000g) was weighed and soaked in a 500ml of ethanol in a 1000ml conical flask. The flask containing the leaf was corked, shaken and allowed to stand for 48hr at room temperature. After 48hr, the mixture was filtered using Watman Filter Paper No 1. The extracts was collected and concentrated to dryness with a Rotary evaporator [9].

INDUCTION OF DIABETES MELLITUS

The hypoglycemic activity of *A. muricata* was assessed using alloxan monohydrate induced diabetic rats. DM was induced in overnight fasted Wistar albino rats weighing 160 ± 10 g by single intraperitoneal injection of freshly prepared alloxan monohydrate at a dose of 40

mg/kg body weight in 0.1M citrate buffer (pH = 4.5). Seven (7) days after alloxan administration, blood was collected from tail vein and blood glucose level was determined using a glucometer. Rats with blood glucose level above 200 ± 10 mg/dl were considered diabetic and included in the study.

Acute toxicity study and dose selection

Healthy adult albino rats were used for this study. A pilot study was performed using three doses 500, 1000 and 2000 mg/kg body weight of the leaf extract of *A. Muricata*. The doses selected were 5, 10 and 20 times more than the conventional studies. The animals were observed continuously for 4 h and then occasionally for further 4 h and then finally overnight. The animals were observed for tremors, chronic convulsion, tonic extensions, catatonia, spasticity, ataxia, sedation, ptosis, and respiratory abnormality. After a period of 24 and 72 h, they were observed for any lethality or death. The result was used to determine the dose to be selected for further use.

Experimental design

In this experiment, a total of 30 rats were used; and only rats with fasting blood sugar (FBS) above 200 ± 10.00 mg/dl were selected. The rats were divided into six groups of five animals each as follows:

- (1) Normal control,
- (2) diabetic control,
- (3) diabetic treated with glibenclamide (600 μ g/kg),
- (4) diabetic treated with leaf extract of *A. muricata* 100 mg/kg (LEAM at 100/mg/kg),
- (5) diabetic treated with LEAM at 200/mg/kg,
- (6) diabetic treated with leaf extracts *A. Muricata* at 400/mg/kg, respectively.

Normal control and diabetic control rats were given normal diet *ad libitum* up to 28 days. All extracts/drugs were dissolved in distilled water and administered once daily (orally) in the morning between 9 and 10:00 am for 28 days. In all groups fasting blood glucose (FBS) levels was recorded before treatment and later on the 7th, 14th, 21st and 28th day. FBS levels were taken after overnight fasting. Body weights of animals were also recorded on 1st, 7th, 14th, 21st and 28th day in all groups.

Biochemical parameters

At the end of the experiment, the rats were sacrificed and blood was collected for the determination of total cholesterol (TC) level, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride (TG) using lipid panel test strip device and expressed as mg/dl. Low density lipoprotein cholesterol level was calculated using Friedewald's equation

and expressed in mg/dl. TC in serum was converted to glycerol and then estimated using glycerol kinase enzyme based kinetic method and expressed in mg/dl [9].

STATISTICAL ANALYSIS

For statistical analysis, data were analyzed by one-way analysis of variance (ANOVA) using Statistical Package of Social Sciences (SPSS) software version 16 for Windows. The results were expressed as mean \pm standard error (SE). P Value < 0.05 was considered to be significant.

RESULTS

Table 1. Weekly glucose concentration in alloxan induced diabetes in rats.

Group	Initial	Week 1	Week 2	Week 3	Week 4
1.(Normal Control)	95.20 \pm 1.88	98.20 \pm 3.14	100.80 \pm 2.24	90.00 \pm 1.74	94.80 \pm 7.74
2.(DM- Control)	209.60 \pm 0.93	218.00 \pm 0.89	193.20 \pm 1.66	193.80 \pm 6.69	218.80 \pm 4.58
3.(DM - Glib)	201.80 \pm 0.58223	80 \pm 0.58	185.60 \pm 8.82	190.40 \pm 1.11	168.00 \pm 9.33
4.(100 mg/kg)	212.80 \pm 1.59	231.00 \pm 0.63	199.20 \pm 3.40	112.40 \pm 4.20	99.60 \pm 1.21
5.(200 mg/kg)	204.80 \pm 0.58	213.60 \pm 0.75	193.20 \pm 2.30	95.80 \pm 4.41	88.40 \pm 3.67
6.(400 mg/kg)	218.00 \pm 2.28	221.00 \pm 0.45	202.00 \pm 4.91	220.40 \pm 5.37	197.40 \pm 1.29

Values are mean \pm standard error of mean at n = 5

The weekly glucose concentration in alloxan induced diabetic rats is presented in Table 1. All the induced rats at the end of week 1 were diabetic as seen by the increase in blood glucose levels. The blood glucose concentrations were high initially, while administration of the extract during week 2, 3 and 4 after caused reduction in the glucose level of the rats.

Table 2: Effect of *A. muricata* aqueous ethanolic leaf extract on lipid profile in alloxan-induced diabetes

Groups	TC	HDL	LDL	TG
1. (Normal control)	98.20 ±0.92	18.20 ±1.71	69.28 ^a ± 3.40	67.60 ^b ±7.52
2. (Diabetic control)	98.20 ±0.80	24.80 ±0.73	62.28 ^b ±0.71	69.80 ^b ±4.88
3. (DM - Glib)	97.80 ±0.97	23.60 ±1.72	59.40 ^c ±2.11	85.00 ^a ±1.20
4. (100 mg/kg bw)	97.00 ±1.26	26.20 ±4.75	65.40 ^b ±2.70	71.50 ^b ±7.00
5. (200 mg/kg bw)	96.00 ±0.71	23.80 ±2.40	55.24 ^c ±7.30	103.50 ^b ±2.31
6. (400 mg/kg bw)	96.20 ±0.49	23.80 ±2.40	48.92 ^c ±4.83	114.60 ^b ±1.11

Values are mean ± standard error of mean at n = 5. Difference in mean is significant p < 0.05

TC: Total cholesterol, HDL: High density lipoprotein cholesterol, LDL: Low density lipoprotein cholesterol, TG: Triglycerides

The biochemical parameters are shown in Table 2. The results showed that there were significant differences in LDL and TG values as compared to normal group shown in the table. In diabetic rats the serum lipid profiles of TC, TG, LDL was significantly ($P < 0.05$) increased above the normal control group.

DISCUSSION

A. Muricata was found to be safe at doses greater than 5000mg/kg body weight. No fatality was recorded and no visible sign of toxicity was observed after 72 hours of administration of the extract. The result in Table 1, showed significant increase ($p < 0.05$) in glucose concentration in diabetic rats when compared to normal control because alloxan monohydrate is known to induce the production of free radicals and causes β -cell injury leading to insulin deficiency [4]. Oral administration of the extract significantly ($p < 0.05$) decreased the blood glucose levels. The extract might have helped in regeneration of islet beta cells by restoration or increased insulin production [8], which stimulated the glucose uptake and utilization in cells. Various plants derived flavonoids, anthraquinones, saponins have been shown to have the ability to enhance β -cells regeneration leading to glucose

uptake by stimulating insulin release [9]. The results in Table 2, Showed that there is insignificant difference ($p < 0.05$) in total cholesterol of treated rats when compared to normal control. There was significant difference ($p < 0.05$) in LDL and TG values of treated rats compared to normal control as shown in the Table 4. In diabetic rats, the serum lipid profile (TC, LDL, and TG) significantly ($p < 0.05$) increased more than normal control which represents a risk factor for coronary heart disease. Deficiency in insulin is responsible for the re-arrangement in metabolic and regulatory mechanisms which causes accumulation of serum lipids [10]. The Impairment of insulin secretion enhances the lipid metabolism in adipose tissues to the plasma [11]. The administration of the extract and drug showed significant decrease in serum lipids due to the presence of flavonoids, saponins present in the extracts. This results showed that the LEAM enhances lipids metabolism in rats. This makes it a very good remedy for conditions such as hyperlipidemia and DM.

The overall results justified the use of *A. Muricata* decoction as a safe alternative in the management of DM by Alternative Medicine Practitioners. Further studies are recommended in the purification and isolation of pure compounds that will save as drugs for the management and treatment of DM.

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