



# **The Impact of Aqueous Leaf Extract of Soursop on Glucose and Lipid Profile in Alloxan Induced Diabetic Albino Rats**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors PEE, JOE helped in Conceptualization and designed the study, authors PEE, JOE and JIO performed Methodology, did statistical analysis, wrote the protocol, and first draft of the manuscript. Author LSZ did data visualization; Authors PEE, JIO administered the project. All authors read and approved the final manuscript.*

## **Article Information**

DOI: 10.9734/JALSI/2021/v24i330225

Editor(s):

(1) Dr. Muhammad Kasib Khan, University of Agriculture, Pakistan.

Reviewers:

(1) Nazreen Binti Che Roslan, Universiti Teknologi MARA, Malaysia.

(2) Nicoleta Corina Predescu, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania  
Complete Peer review History: <http://www.sdiarticle4.com/review-history/70300>

**Original Research Article**

**Received 20 April 2021**

**Accepted 25 June 2021**

**Published 29 June 2021**

## **ABSTRACT**

The blood glucose, total serum cholesterol, High Density Lipoprotein (HDL) and Triglyceride (TRIG) levels of all rats in each group were determined before induction, post-induction with alloxan and post treatment with various concentrations of extract and standard drug. The alloxan diabetic rats treated with gliberclamide and aqueous leaf extract of *Annona muricata* showed average means of body weights as; (235.73±3.14<sup>a</sup>, 263.94±2.25<sup>a</sup> and 236.5±1.74<sup>a</sup>) respectively after 4 weeks of treatment. The Glucose level revealed; 168.43±5.06<sup>e</sup>, 65.29±4.57<sup>f</sup> and 57.86±3.93<sup>e</sup> respectively. Lipid profile raised significantly post exposure of diabetic rats to both standard drug and extract after 4 weeks of treatment. Therefore, *A. muricata* compared favorably with the standard drug in the context of diabetes management.

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**Keywords:** Blood glucose; diabetes; metabolic disorder.

## ABBREVIATIONS

TCHO	: Total Cholesterol
HDL	: High Density Lipoproteins
LDL	: Low Density Lipoproteins
TRIG	: Triglycerides
CHON	: Cholesterol Normal
CHO PI	: Cholesterol Post Induction
CHOTPI	: Cholesterol Treatment Post Induction
LDLN	: Low Density Lipoproteins Normal
LDLPI	: Low Density Lipoproteins Post Induction
LDLTPI	: Low Density Lipoproteins Treatment Post Induction
HDLN	: High Density Lipoproteins Normal
HDLPI	: High Density Lipoproteins Post Induction
HDLTPI	: High Density Lipoproteins Treatment Post Induction
TRIGN	: Triglyceride Normal
TRIGPI	: Triglyceride Post Induction
TRIGTPI	: Triglyceride Treatment Post Induction
TPI	: Treatment Post Induction
PI	: Post Induction

## 1. INTRODUCTION

Diabetes mellitus is viewed as a complex metabolic disorder characterized by hyperglycemia amounting from malfunction in insulin secretion and/or insulin action. It is recognized as the most common endocrine disease in the world affecting hundreds of millions and having incidence rate of about 1% in industrialized countries [1]. Diabetes is said to appear in three categories. Type 1 diabetes is usually diagnosed in childhood, hence referred to as juvenile onset diabetes. In this category, the body produces little or no insulin and so the remedy the situation, daily injection of insulin is often needed. There is no known cause but it is said to be linked to genetics, viruses, and autoimmune problems [2]. Symptoms of type 1 diabetes include, excessive thirst, fatigue increased urination, nausea, vomiting, and weight loss regardless of increased appetite [3]. Type II diabetes, the most common type and occurs in adulthood, but young people are increasingly being diagnosed with this challenge. Here, the pancreas fails to make enough insulin to keep the blood glucose levels normal, mostly because the body fails to respond promptly to insulin [4]. This type is associated with sedentary lifestyle, lack of exercise, and obesity and symptoms such as blurred vision, fatigue, increased appetite, increased thirst, and urination are common among this group [4]. The third is gestational diabetes which can occur at any time during pregnancy in non-diabetic women [2]. Diabetes mellitus is therefore a global crisis.

Current therapeutic measures to treat this disorder include the use of insulin and other synthetic agents such as amylin analogues, alpha glucosidase inhibitors and biguanides. These drugs are certainly not without some adverse effects as such causing hypoglycemia at higher doses, liver problems, lactic acidosis and diarrhea [5]. Apart from these options of the treatment that are available, many herbal medicines have been recommended for treatment of diabetes. Traditional herbal medicines are employed throughout the world for the prevention and management of different ailments. Herbal drugs are prescribed in low-middle income countries probably because of their effectiveness, lesser side effects and cost friendliness [6]. *Annona muricata* (Annonaceae) is a tropical plant species known for its edible fruit with known medicinal merits. Traditional applications of *Annona muricata* (Soursop) have been identified to treat diverse ailments such as fever, pain, respiratory and skin illness, internal and external parasites, bacterial infections due to the presence of alkaloids, which inhibited bacterial and fungal growth [7]. hypertension by inhibiting the activities of Angiotensin-I converting enzyme (ACE) due to the antioxidant ability of the phenolics [8], inflammation due to its ability to decrease the level of pro-inflammatory cytokines [9], diabetes due to the presence of polyphenol and flavonoid compounds thus protecting the  $\beta$ -cells of the pancreas by preventing oxidative damage [10] and cancer due to the presence of acetogenins which has

cytotoxic activity [11]. More than 200 phytochemical compounds have been identified and isolated from this plant; the most vital being alkaloids, phenols and acetogenins [12].

The aim of the study is to determine the anti-diabetic ability of the soursop by evaluating the effect on blood glucose level and total lipid profile.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

The leaves of soursop (*Annona muricata* L.) were harvested from a garden located in Rayfield Jos, Nigeria during the months of March & April, 2021. The leaves were air-dried under the shield at room temperature. The plant characterization and identification was carried out by a taxonomist in the department of Plant Science, University of Jos.

### 2.2 Aqueous Extraction

A known amount (1000g) of *Annona muricata* leaves was extracted with 4000ml of distilled water using cold maceration. It was subsequently filtered first with glass wool finally with Whatmann No. 4 filter paper.

### 2.3 Animal Materials

Thirty – five mature female albino rats weighing between 150 – 220g were purchase from the animal breeding unit of the University of Jos. Animals were allowed free access to water and standard feed.

### 2.4 Acute Toxicity and Lethality

Assessment on acute toxicity of the aqueous extract of test plant with estimation of the median lethal dose ( $LD_{50}$ ) was investigated using a slightly modified method [13].

Twenty-one experimental animals weighting (150g – 180g) were used. The groups of albino rats with three animals each were administered 100, 200, 400mg/kg respectively of the aqueous leaf extract orally. They were monitored closely for 24hrs for lethality or any obvious behavioral response. Based on the result obtained, increased doses of 600, 800, 1000mg/kg were given still orally to twelve other rats. They were observed for 24hrs for any mortality.

### 2.5 Phytochemical Examination of *Annona muricata* L.

The phytochemical analysis of aqueous extract was carried out [14]. Basic phytochemical screening was carried out using simple chemical test to detect the presence or absence of secondary metabolites of plant; some of the constituents checked for included; alkaloids, flavonoids, tannins, glycosides, carbohydrate, saponins and anthraquinones.

### 2.6 Experimental Protocol

The present study was performed according to the prescribed international guidelines regarding animal experiment. Five experimental groups of 7 rats each were used as follow:

- Group 1** (Control group): Non – diabetic control rats
- Group 2** (Diabetic group) without treatment
- Group 3** (Diabetic group) treated with glibenclamide for 4weeks
- Group 4** (Diabetic group) treated with 200mg/kg body weight of extract once daily via oral route for 4 weeks.
- Group 5** (Diabetic group) treated with 400mg/kg body weight of extract once daily by oral administration for 4 weeks.

### 2.7 Determination of Body Weight, Blood Glucose and Lipid Profile Levels

The body weights of the animals were measured using a digital weighting balance; before alloxan – induction, post-alloxan – induction and at every 7 days interval during treatment with extract. Blood sample was obtained from the tail vein of the animals and the fasting blood glucose level was determined using a digital glucometer (Accu-chek (R), Rode Diagnostic, Germany); before alloxan-induction, post-alloxan – induction and after every 7 days on treatment with extract. The lipid profile (TCHO, HDL, LDL. & TRIG) was estimated using cobas cIII.

### 2.8 Administration of Alloxan Monohydrate

Diabetes mellitus was experimentally induced in groups 2 – 5 by a single intraperitoneal injection of 150mg/kg b.w. with freshly prepared

physiological saline. Diabetic state of animals was monitor for two days after alloxan administration. On the second day, only animals with fasting blood glucose levels  $\geq 200\text{mg/dl}$  were selected as diabetic rats for the current study after establishing it as the safe dose. The study adopted the method as described by [15].

### 2.9 Statistical Analysis

The data was analysis using Graph Pad-Prism version 8.0. All values are presented as mean  $\pm$  standard deviation for 7 rats in each of the five

groups. The significant difference in the means of all parameters reported for 5 groups of animals was determined using ANOVA and a P-value of  $< 0.05$  was considered as significant.

### 3. RESULTS

The study revealed the presence of some phytochemical constituents; alkaloids, flavonoids, tannins, anthraquinones, carbohydrates as shown in (Table 1). The constituents revealed in this analysis are in agreement with the work of [16].

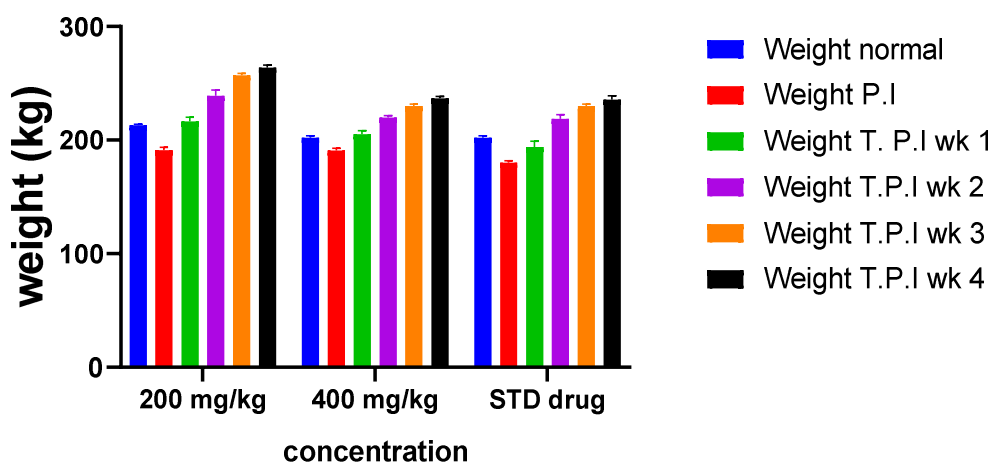


Fig. 1. Effect of *Annona muricata* on body weights of alloxan diabetic rats

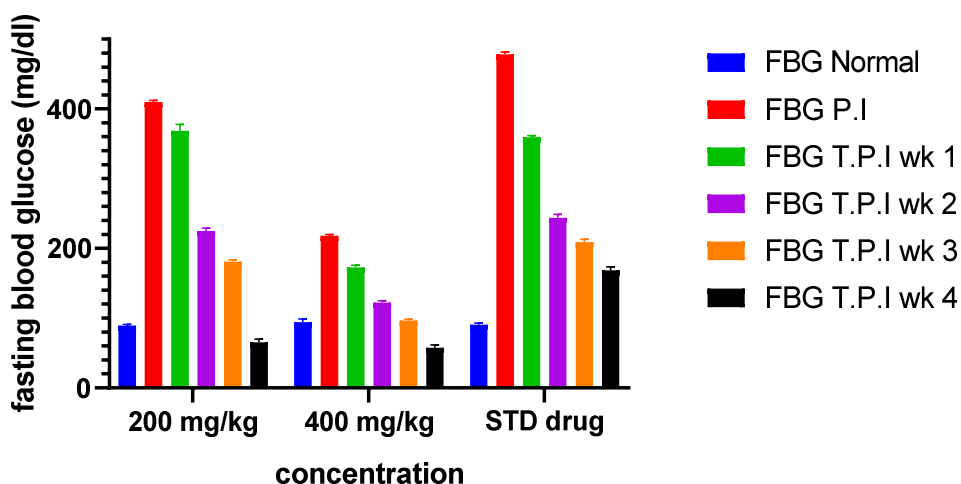


Fig. 2. Effect of aqueous leaf extract of *Annona muricata* on blood glucose of alloxan diabetic rats

**Table 1. Qualitative phytochemical screening of aqueous leaf extract of *Annona muricata* L**

S/N	Test	Inference
1.	Alkaloids	
	a. Mayers's test	++
	b. Wagner's test	++
	c. Diagendorff's	++
2.	Flavonoids	+++
3.	Carbohydrates	
	molisch's test	+
4.	Saponins froth test	+
5.	Tannins ferric chloride	-
6.	Anthraquinones	
	Ammonia test	+

**Table 2. Effect of *Annona muricata* on the lipid profile of alloxan induced diabetic rats**

Ss Conc.(mg/kg)	Lipid profile											
	CHON	CHO PI	CHOTPI	LDLN	LDLPI	LDLTPI	HDLN	HDLPI	HDLTPI	TRIGN	TRIGPI	TRIGTPI
200	1.66±0.0 1 <sup>b</sup>	2.51±0.0 2 <sup>a</sup>	1.25±0.0 1 <sup>c</sup>	0.79±0.0 1 <sup>a</sup>	- 1.47±0.0 4 <sup>c</sup>	0.04±0.0 2 <sup>b</sup>	0.77±0.0 1 <sup>c</sup>	0.91±0.0 1 <sup>b</sup>	0.97±0.0 1 <sup>a</sup>	0.22±0.0 1 <sup>c</sup>	6.75±0.0 1 <sup>a</sup>	0.63±0.0 3 <sup>b</sup>
400	1.82±0.0 5 <sup>a</sup>	1.81±0.0 1 <sup>a</sup>	1.71±0.0 1 <sup>b</sup>	1.80±0.0 4 <sup>b</sup>	- 2.63±0.0 1 <sup>c</sup>	0.40±0.0 1 <sup>b</sup>	0.81±0.0 1 <sup>c</sup>	0.96±0.0 1 <sup>b</sup>	0.98±0.0 2 <sup>a</sup>	0.42±0.0 1 <sup>c</sup>	7.68±0.0 0 <sup>a</sup>	0.73±0.0 1 <sup>b</sup>
std	1.39±0.0 2 <sup>c</sup>	1.80±0.0 2 <sup>b</sup>	2.12±0.0 1 <sup>a</sup>	0.22±0.0 2 <sup>b</sup>	- 2.66±0.0 3 <sup>c</sup>	1.45±0.0 2 <sup>a</sup>	0.77±0.0 1 <sup>b</sup>	0.98±0.0 1 <sup>a</sup>	0.22±0.0 2 <sup>c</sup>	0.86±0.0 1 <sup>a</sup>	7.67±0.0 1 <sup>a</sup>	0.98±0.0 1 <sup>b</sup>
L.S.D	0.02											
S.F	****											

Prior to alloxan treatment, there was no noticeable difference in average weights of all rats. But a significant reduction in weight was observed post alloxan-induction. However, the body weights of rats in groups 3-5 gradually increased on treatment with extract at different concentrations and standard drug a sign of recovery in body weight gains four weeks post extract treatment, ( $235.73 \pm 3.14^a$ ,  $263.94 \pm 2.25^a$  and  $236.5 \pm 1.74^a$ ) respectively. A significant ( $P \leq 0.05$ ) difference occurred between the extract treated groups and the control groups. Again, the blood glucose level was significantly ( $P \leq 0.05$ ) higher in groups 2-5 when compared to normal control group. The blood glucose level of animals in group 3-5 gradually decreased on treatment with extract of *Annona muricata* over a treatment period of 4 weeks. (Fig. 1). At the end of the experiment a significant ( $P \leq 0.05$ ) difference was noticed between group treated with extract and glibenclamide; as groups 3, 4, and 5 had average means of blood glucose levels as;  $168.43 \pm 5.06^e$ ,  $65.29 \pm 4.57^f$  and  $57.86 \pm 3.93^e$  respectively.

Diabetic rats showed a significant increase in TCHO, HDL and TRIG relative to the corresponding control. Treatment of diabetic rats with the extracts after 30 days revealed a significant reduction in the values of TCHO, LDL and TIRG but with significant increase in HDL values in diabetic rats as observed in Table 2

#### 4. DISCUSSION

Diabetes mellitus is a metabolic disorder that is characterized by hyperglycemia and increased lipid profile. This disorder could be related to the inability of insulin to trigger cellular uptake of glucose in the blood after digestion. With an increase in mortality due to the disease, it is however very important to develop more effective and less expensive drugs for its treatment and management. Several reports have recorded significant impact of the *A. muricata* on parameters that are involved in the occurrence of the diabetes mellitus. Studies have reported anti-hyperglycemic activities, increase in body weight, improved serum lipid profile by decreasing the TCHO, TRIG and LDL, VLDL, and increase in the level of HDL and the percentage of anti-atherogenic index (AAI) [17].

Elevated lipid profile is an established risk factor for cardiovascular diseases and affects patients with diabetes [18]. Results of our study showed that *A. muricata* extract significantly redeemed sera lipid profiles by reducing the values of

TCHO and LDL and elevating the HDL and TRIG of diabetic rats in a dose dependent manner. Furthermore, there was a significant reduction in blood glucose levels, LDL, TCHO, TRIG after 28 days of treatment with aqueous extract of *Annona muricata* [19], which was also observed in the present study. The ability to lower the glucose level in diabetic rats could be attributed to the presence of bioactive compounds in the plants. Studies have shown that the soursop has the ability to inhibit the  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes which are responsible for the breakdown of carbohydrates thus reducing the blood glucose concentration [20].

#### 5. CONCLUSION

The aqueous extract of the soursop showed significant control of the blood glucose level and lipid profile in the body and may be considered for the treatment of diabetes.

#### ETHICAL APPROVAL

Ethical clearance was obtained from the University of Jos Ethical Committee.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:  
The peer review history for this paper can be accessed here:  
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