



Weight Gain Reduction and Hypoglycemic Effects of *Xylopia aethiopica* Fruit Extract on Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author EOO conceptualized the study, Author PCU designed the study. Author INN managed the literature searches and managed the analyses of the study. Author UO wrote the protocol while author AIA performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

Editor(s):

(1) Dr. Alberto Olaya Vargas, Universidad Nacional Autonoma de México, México.

Reviewers:

(1) Fengyang Huang, Hospital Infantil de México Federico Gómez, México.

(2) Fernando Fabrizzi, Educational Foundation of Penápolis, Brazil.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/63917>

Original Research Article

**Received 12 October 2020
Accepted 18 December 2020
Published 04 January 2021**

ABSTRACT

Aim: This study is aimed at determining the effect of *Xylopia aethiopica* fruit on weight gain and blood sugar level of Wistar rats.

Methodology: The fruits of *Xylopia aethiopica* were obtained from new market in Aba, Abia State, Nigeria and were authenticated. They were air-dried and extracted using Soxhlet apparatus and ethanol as solvent. The median lethal dose (LD₅₀) of the extract was determined using standard method. Thirty Wistar rats were used for this study. They were acclimatized for seven days, weighed and divided into five groups of six rats each. Animals in group A were administered 129.62 mg/kg body weight (10% of LD₅₀) of *X. aethiopica* fruit extract, those in group B were administered 259.23 mg/kg body weight (20% of LD₅₀) of *X. aethiopica* fruit extract, those in group C were administered 388.85 mg/kg body weight (30% of LD₅₀) of *X. aethiopica* fruit extract, those

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in group D were administered 518.46 mg/kg body weight (40% of LD₅₀) of *X. aethiopica* fruit extract, while those in group E (control) received normal feeds and water only. The administration was done once daily for 28 days via oral route. At the end of 28 days treatment, animals were weighed and weights recorded, and were sacrificed under ether anaesthesia after an overnight fast. Organs were harvested and weighed. Blood glucose level was determined using glucometer.

Results: The physical signs of toxicity observed in the animals included excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma which was followed by death. The extract was observed to reduce weight gained by animals when compared with those in the control group at P<0.05. Similarly, a significant reduction was observed in the blood sugar level of animals administered extract of *X. aethiopica* fruit when compared with those in the control group. This reduction was dose-dependent.

Conclusion: The result of this study revealed that *X. aethiopica* fruit possesses hypoglycemic potential but highly toxic at high dosage.

Keywords: Blood sugar level; toxicity; weight gain; *X. aethiopica* fruit.

1. INTRODUCTION

Diabetes has been reported to be a complex metabolic disorder associated with developing insulin resistance, impaired insulin signaling and β -cell dysfunction, abnormal glucose and lipid metabolism, sub-clinical inflammation and increased oxidative stress [1]. These metabolic disorders has been reported to lead to long-term pathogenic situations including micro- and macro-vascular complications, neuropathy, retinopathy, nephropathy, and a consequent decrease in quality of life and an increase in the rate of mortality [2]. Among the various risk factors underlining the incidence and progression of diabetes, diet has been reported to be the main modifiable factor. Both experimental and epidemiological evidences have proved that the consumption of plant materials rich in variety of phenolic compounds and exhibits high antioxidant potential could have beneficial relationship with the incidence and prevalence of diabetes [3]. Dietary control remains one of the most effective methods in preventing and managing degenerative diseases like diabetes and cardiovascular diseases.

Africa, since ancient times is blessed with so many medicinal plants whose extract can be used in the treatment and management of diseases. Factors such as poverty and illiteracy still militate against availability and accessibility of conventional medical services. Large number of these tropical plants have shown beneficial therapeutic effects such as contraceptives, fertility enhancing capacities, antioxidant, anti-inflammatory, anticancer and antimicrobial potentials [4]. Amongst these plants with great therapeutic potential is *Xylopia aethiopica* which is commonly referred to as 'African guinea

pepper' or 'Ethiopian pepper' or locally known as 'Uda' in the south eastern part of Nigeria. It is an angiosperm of the Annonaceae family and grows up to 20metres in height, bearing aromatic seeds, predominantly in humid forest zones of West Africa [5]. The tree has a straight stem and smooth bark, and remains ever green with a constituent aroma [6]. It is found all over the lowland rainforest and savannah zones of Nigeria [7].

Xylopia aethiopica possesses great nutritional and medicinal values in traditional medicine [8]. The seeds have been reported to contain chemical constituents like alkaloids, glycosides, saponins, tannins, sterols, carbohydrates, proteins, free fatty acids, mucilages and acid compounds. These phytochemicals contain antioxidant and play vital roles in human health [9,10]. According to Okeke et al. [11], the fruit serves as spice, while its aqueous decoctions are used for its antiseptic properties. The powdered root is employed as a dressing and in local treatment of cancer [12]. Its antihypertensive and diuretic effects have been reported [13]. Extracts of the fruit are used in the treatment of cough, biliousness, bronchitis, rheumatism, dysentery, malaria, uterine fibroid and amenorrhoea [14,15]. This study is aimed at determining the effect of *X. aethiopica* fruit on weight gain and blood sugar level of Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Materials

The fruits of *Xylopia aethiopica* were obtained from new market in Aba, Abia State and were identified and authenticated by Prof. (Mrs)

Margaret Bassey of the Department of Botany and Ecological Studies, University of Uyo with the voucher number UU/PH/4e. The plant was deposited in the Herbarium of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Akwa-Ibom State, Nigeria.

2.2 Extraction of Plant Materials

The extraction was carried out in the Post-graduate Laboratory of Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Akwa-Ibom State, Nigeria. It was carried out according to the method described by Ogbuagu et al. [16]. The fruits were washed under running tap water to remove contaminant and air-dried. This plant material was then pulverized using laboratory blender to provide a greater surface area. The pulverized plant material was macerated in 250 mL of 99.8% ethanol (Sigma Aldrich) contained in round bottom flask, which was then attached to a Soxhlet extractor coupled with condenser and heating mantle (Isomantle). It was then loaded into the thimble, which is placed inside the Soxhlet extractor. The side arm is lagged with glass wool. The mixture was heated using the heating mantle (Isomantle) at 60°C and as the temperature increases it begins to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. This continues until it is exhaustively extracted. The process runs for a total of 13 hours. Once it was set up, it was left to run without interruption as long as water and power supply were not interrupted. The equipment was turned on and off and overnight running was not permitted, and the time split over a number of days. The extract was poured into 1000 mL beaker and concentrated to dryness in water bath (A3672- Graffin Student Water Bath) at 35°C. The total weight of the marc (residue) and the concentrated extract were recorded, these processes took several days. The dried extract was preserved in the refrigerator at 4°C for further analysis.

2.3 Determination of Median Lethal Dose (LD₅₀)

The median lethal dose (LD₅₀) of the extract was estimated using albino mice according to the method described by Airaodion et al. [17]. This method involves two phases:

In phase one, five groups containing five mice each weighing between 20 g and 27 g were fasted for 18 hours. They were respectively administered 1000 mg/kg, 2000 mg/kg, 3000 mg/kg, 4000 mg/kg and 5000 mg/kg body weight intraperitoneally (i.p) and were observed for physical signs of toxicity and mortality for 24 hours. 1000 mg/kg recorded 0% mortality while 2000 mg/kg, 3000 mg/kg 4000 mg/kg and 5000 mg/kg recorded 100% mortality within 24 hours. Based on the value of phase one, phase two was conducted.

In phase two, twenty albino mice weighing between 20 – 27 g were grouped into four of five mice per group and were fasted for 18 hours. Each group was administered 1200 mg/kg, 1400 mg/kg 1600 mg/kg and 1800 mg/kg body weight intraperitoneally (i.p) and was observed for physical signs of toxicity and mortality within 24 hours. 1200 mg/kg recorded 0% mortality while 1400 mg/kg, 1600 mg/kg and 1800 mg/kg recorded 100% mortality within 24 hours. The LD₅₀ was calculated as geometrical means of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b).

$$LD_{50} = \sqrt{ab}$$

2.4 Experimental Procedure

Thirty Wistar rats obtained from the University of Uyo, Nigeria were used for this study. They were acclimatized for seven days before the commencement of the experiment. They were weighed and divided into five groups of six rats each. Groups A, B, C, D served as the experimental groups, while group E served as the control. Animals in group A were administered 129.62 mg/kg body weight (10% of LD₅₀) of *X. aethiopica* fruit extract, those in group B were administered 259.23 mg/kg body weight (20% of LD₅₀) of *X. aethiopica* fruit extract, those in group C were administered 388.85 mg/kg body weight (30% of LD₅₀) of *X. aethiopica* fruit extract, those in group D were administered 518.46 mg/kg body weight (40% of LD₅₀) of *X. aethiopica* fruit extract, while those in group E (control) received normal feeds and water only. The administration was done once daily for 28 days via oral route. At the end of 28 days treatment, animals were weighed and recorded, and were sacrificed under ether anaesthesia in a desiccator after an overnight fast. Blood samples were collected via

cardiac puncture. Organs were harvested and weighed.

2.5 Determination of Relative and Absolute Organ Weights

Absolute organ weight is the actual weight of the organs.

Relative organ weight was determined in percentage as:

$$\text{Relative organ weight (\%)} = \frac{\text{Weight of Organ(absolute)}}{\text{Weight of Animal}} \times 100$$

2.6 Determination of Glucose Concentration

Glucose concentration was determined using glucose oxidase method with the aid of a glucometer (Accu-chek active).

Principle: It is based on the reaction of glucose and oxygen in the presence of glucose oxidase to yield gluconic acid and hydrogen peroxide. The hydrogen peroxide formed subsequently reacts under catalysis of peroxidase with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator. The hydrogen peroxide oxidizes the dyes in a reaction mediated by peroxidase producing a blue coloured product, with the intensity of the colour read from the accu-chek active glucometer. The colour intensity is proportional to the glucose concentration of the sample.

2.7 Statistical Analysis

Results are expressed as mean ± standard deviation. The levels of homogeneity among the

groups were assessed using One-way Analysis of Variance (ANOVA) followed by Tukey's test. All analyses were done using Graph Pad Prism Software Version 5.00 and P values < 0.05 were considered statistically significant.

3. RESULTS

The physical signs of toxicity observed in the animals included excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma which was followed by death. In the first phase of the median lethal dose determination, no mortality was recorded in the group treated with 1000 mg/kg body weight of *X. aethiopica* fruit extract. However, 100% mortality was recorded in the groups treated with 2000, 3000, 4000, and 5000 mg/kg body weight of *X. aethiopica* fruit extract respectively. Similarly, in the second phase of medial lethal dose determination, no mortality was recorded in the group treated with 1200 mg/kg body weight of *X. aethiopica* fruit extract while 100% mortality was recorded in the groups treated with 1400, 1600, and 1800 mg/kg body weight of *X. aethiopica* fruit extract respectively.

The median lethal dose (LD₅₀) was calculated as geometrical means of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b).

$$LD_{50} = \sqrt{ab}$$

Where

a = 1200 mg/kg

b = 1400 mg/kg

$$LD_{50} = 1296.15 \text{ mg/kg}$$

Table 1. The median lethal dose (LD₅₀) of *Xylopia aethiopica* fruit extract

Study Phase/ (Animal)	Dosage of Extract (mg/kg) b.w	No of Mice per Group	No. of Death Recorded	% Mortality
Phase One				
I	1000	5	0	0
II	2000	5	5	100
III	3000	5	5	100
IV	4000	5	5	100
V	5000	5	5	100
Phase Two				
I	1200	5	0	0
II	1400	5	5	100
III	1600	5	5	100
IV	1800	5	5	100

$$LD_{50} = 1296.15 \text{ mg/kg}$$

Table 2. Effect of *Xylopia aethiopica* fruit extract on animal body weight after 28 days of treatment

Weight	129.62 mg/kg Extract	259.23 mg/kg Extract	388.85 mg/kg Extract	518.46 mg/kg Extract	Control
Initial Weight (g)	123.78±13.69	131.43±9.01	131.73±16.39	133.08±13.19	125.10±11.82
Final Weight (g)	147.55±7.60	151.05±18.83	148.23±19.28	145.63±9.95	154.60±11.48
Weight Gain (g)	23.78±3.04 ^b	19.63±3.13 ^{bc}	16.50±4.58 ^{cd}	12.55±1.98 ^d	29.50±2.05 ^a

Values are presented as Mean±S.D, where n = 6. Values with different superscripts along the same row are significantly different at P<0.05

Table 3. Effect of *Xylopia aethiopica* fruit extract on relative organ weight (%) of animals after 28 days of treatment

Organs	129.62 mg/kg Extract	259.23 mg/kg Extract	388.85 mg/kg Extract	518.46 mg/kg Extract	Control
Heart	0.36±0.08 ^a	0.38±0.05 ^a	0.40±0.04 ^a	0.43±0.14 ^a	0.40±0.13 ^a
Liver	3.70±0.62 ^c	4.03±1.01 ^b	4.41±0.69 ^a	4.58±1.33 ^a	4.41±0.69 ^a
Kidney	0.65±0.10 ^c	0.67±0.16 ^c	0.71±0.07 ^b	0.91±0.46 ^a	0.77±0.11 ^b
Stomach	0.95±0.12 ^a	0.87±0.17 ^a	0.98±0.13 ^a	0.98±0.12 ^a	1.02±0.17 ^a
Pancreas	0.40±0.13 ^{ab}	0.36±0.11 ^b	0.47±0.08 ^a	0.47±0.20 ^a	0.36±0.10 ^b

Values are presented as Mean±S.D, where n = 6. Values with different superscripts along the same row are significantly different at P<0.05

Table 4. Effect of *Xylopia aethiopica* fruit extract on blood glucose level of animals after 28 days of treatment

Parameters	129.62 mg/kg Extract	259.23 mg/kg Extract	388.85 mg/kg Extract	518.46 mg/kg Extract	Control
Glucose Level (mg/dL)	121.5±5.80 ^b	119.75±4.27 ^b	101.75±2.87 ^c	92.75±3.10 ^d	133.25±1.71 ^a

Values are presented as Mean±S.D, where n = 6. Values with different superscripts along the same row are significantly different at P<0.05

4. DISCUSSION

The acute toxicity study of the plant extracts recorded 100% mortality at a dose of 1200 mg/kg bodyweight and above as presented in Table 1. This shows that the fruit of *X. aethiopica* is highly toxic. The physical signs of toxicity observed in the animals included excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma which was followed by death.

The effect of ethanolic extract of *X. aethiopica* fruit on body weight of rats used in this study is presented in Table 2. The results suggest that administration of extract of *X. aethiopica* fruit significantly decreases weight gain of Wistar rats when compared with those of the control group at P<0.05. Similar findings have been reported

by Chike and Adienbo [18], Woode et al. [5] and Eze [19] on weight. Specifically, Chike and Adienbo [18] reported that the decrease in weight gain was attributable to the active ingredients of the extract. According to Woode et al. [5], the reduction in body weight was due to the xylopic acid content of the extract. Considering also, the linkages between obesity, diabetics and hypolipidemia, a clue may be drawn [20,21]. Significant changes in body weights have been used as an indicator of adverse effects of drugs and chemicals. Nevertheless, the growth of an organism comprises many factors including physiological, biological and cellular processes [22].

Furthermore, *X. aethiopica* has also been reported to have anti-androgenic properties due

to the presence of xylopic acid and androgens which are known to possess anabolic properties [23]. This could also be responsible for the observed significant decrease in weight gain of animals used in this study. This corresponds to the findings of Obodo et al. [24], but contradicts those of Yusuf et al. [25] and Abaidoo et al. [26] who respectively reported a nonsignificant difference and a significant increase in body weight of animals treated with *Xylopic aethiopicum* fruit extract. However, the significant decrease in weight gain markedly influenced the relative weight of organs (except heart and stomach) of animals used in this study when compared with those of the control animals at $P < 0.05$.

The results of this study revealed that *X. aethiopicum* fruit extract caused a dose dependent decrease in the blood glucose level in treated rats when compared with the control. This might be attributed to the hypoglycemic property of the extract. This observation gives credence to the use of this plant product as a hypoglycemic agent [27]. The mechanism could be that *X. aethiopicum* decreases gluconeogenesis by decreasing the activities of key enzymes such as glucose-6-phosphatase, fructose-1,6-bisphosphate phosphoenol pyruvate carboxykinase [28,29]. Glucose-6-phosphatase is an important enzyme in homeostasis of blood glucose as it catalyzes the terminal step both in gluconeogenesis and glycogenolysis, while fructose-1,6-bisphosphatase is one of the key enzymes of gluconeogenic pathway [30,31]. Hence, the ability of this plant to decrease the activities of these enzymes probably makes it potential hypoglycemic agent. This result is similar to the hypoglycemic effect of *X. aethiopicum* reported by Nnodim et al. [6]. A number of other plant extracts have also been reported to have a hypoglycemic and an insulin-stimulatory effect [32,33]. Most of the plants with hypoglycemic properties have been found to contain metabolites such as glycosides, alkaloid and flavonoids [34,35]. Phytochemical analysis of *Xylopic aethiopicum* fruit has been shown to contain flavonoids, alkaloids, anthocyanins, coumarin, oxalate, saponins, steroid, glycoside, tannin and phenolic compounds [16]. It has also been proven to possess remarkable free radical scavenging ability [26]. Some of these chemical substances such as flavonoids, alkaloids and glycosides may be responsible for the hypoglycemic effect of *X. aethiopicum* fruit observed in this study. The blood glucose lowering effect of *X. aethiopicum* fruits may also

indicate that it possesses an antidiabetic agent which could control hyperglycemia. One therapeutic approach for treating early stage of diabetes is to decrease post-prandial hyperglycaemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes, α -amylase and α -glucosidase, in the digestive tract [36]. Consequently, inhibitors of these enzymes determine a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise [37]. Based on these findings, it could be suggested that *X. aethiopicum* fruit may inhibit platelet aggregation and promote vasodilatation, exerting an important protective role in the prevention of the development and progression of vascular complications caused by the hyperglycemic state. In fact, studies have shown that polyphenolic compounds present in some plant foods can inhibit the process of thrombus formation [38,39].

5. CONCLUSION

The result of this study revealed that *X. aethiopicum* fruit possesses hypoglycemic potential but highly toxic at high dosage.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the author.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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