

# Hypoglycaemic and Anti-hyperglycaemic Activity of Aqueous Extract of *Azanza garckeana* Leaves in Normal and Alloxan -induced Diabetic Rats

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## Abstract

The aqueous extract of *Azanza garckeana* (AG) leaves was tested for hypoglycaemic and anti-hyperglycaemic activities. The extract was administered to Wistar rats orally at 100 and 150 mg/kg doses in oral glucose tolerance test (OGTT), glucose baseline conditions and diabetic conditions. OGTT was tested with the administration of the aqueous extract to normal rats and blood glucose levels were measured for each animal at 30, 60 and 120 minutes after glucose loading. For the hypoglycaemic activity, the extract was administered to normal rats and blood glucose levels were measured for each animal, at 30, 60 and 120 minutes. Alloxan induced diabetic rats were used to determine the anti-hyperglycaemic activity. The extract was administered for 14 days and blood glucose levels were measured for each animal at a weekly interval for 2 weeks. The OGTT study in normal rats showed that administration of the extract at 150 mg/kg reduced blood glucose levels within 30 to 60 minutes after glucose administration, as was also observed with the standard drug (glibenclamide). For the hypoglycaemic study, fasting blood glucose test in normal rats showed that aqueous extract of AG leaf at 150 mg/kg, produced significant ( $P < 0.05$ ) blood glucose reduction at 60 minutes after administration while glibenclamide revealed significant reduction throughout the time as compared with normal control. 150 mg/kg was the most effective dose during the anti-hyperglycaemic study as it had a significant reduction in blood glucose levels after the 7<sup>th</sup> day. However glibenclamide had a more significant reduction when compared with treated groups. In conclusion, these results clearly indicate that aqueous leaf extract of *Azanza garckeana* possess hypoglycaemic and anti-hyperglycaemic effects in normal and diabetic rats and thus support its traditional use to treat diabetes. Therefore, a detailed mechanism-based study and isolation of bioactive compounds responsible for the observed activities of *Azanza garckeana* is recommended.

**Keywords:** *Azanza garckeana*, Hypoglycaemia, Oral Glucose Tolerance Test, Hyperglycaemia, Diabetes.

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## INTRODUCTION

Hyperglycaemia is the most common consequence of uncontrolled diabetes, which may over time lead to serious damage to vascular tissue and various organs (Saeedi *et al.*, 2019). In spite of the enormous advances that have been made during the last decades towards the control and treatment of diabetes, it has continued to be a serious health hazard. Many diabetic patients face significant challenges accessing diagnosis and treatment, which contributes to the high mortality and prevalence of the associated complications. A large number of anti-hyperglycaemic agents like insulin and glibenclamide etc. are used frequently in the treatment of diabetes mellitus but its prevalence still remains high (Hall *et al.*, 2011). A large number of plants have proved their efficacy in managing diabetes and especially hyperglycaemia (Tesfaye *et al.*, 2016).

Commonly referred to as tree hibiscus, Azanza and snot apple in English, *Azanza garckeana* is known as goron tula, morojwa and *Thespesia garckeana* in Nigeria, Botswana and South Africa respectively (Ochokwu *et al.*, 2015). It is a valuable edible indigenous fruit widely distributed in Tula, Kaltungo Local Government Area (LGA) of Gombe State and in Michika in Adamawa State (Orwa *et al.*, 2009; Mojeremane and Tshwenyane, 2004). It is also found in other African countries such as Sudan, Kenya, Mozambique, Namibia, Malawi, Tanzania, Zimbabwe, and Zambia. *A. garckeana* is widely distributed in the east, west and southern Africa (Maroyi 2017). Pharmacological studies on *A. garckeana* indicate that the species have a diverse range of pharmacological activities which include but not limited to anti-hyperglycaemic, antimalarial, antioxidant, antibacterial, antifungal, and iron absorption (Maroyi 2017). The leaf, root decoction and fruit pulp of *A. garckeana* is taken orally as remedy for diabetes, membrane rupture, infertility, edema, epilepsy and liver problems in DRC, Botswana, Kenya, Malawi, Nigeria (Amuri *et al.*, 2017; Esther *et al.*, 2017; Dikko *et al.*, 2016).

A few studies have reported on the ability of the fruit pulp to cause significant and progressive decreases in the fasting blood sugar (Lawal *et al.*, 2022; Alozieuwa *et al.*, 2022), however literature search showed only one study on the glucose lowering activity of the leaves of *A. garckeana* which reported appreciable base-line glucose lowering effect but an insignificant hypoglycaemic effect using a local strain of guinea pigs (*Cavia porcellus*).

The present study was aimed at evaluating the hypoglycaemic and anti-hyperglycaemic activities of *Azanza garckeana* aqueous leaf extract in normal and alloxan induced diabetic rats in order to further validate its use to treat or manage hyperglycemia and in extension diabetes mellitus.

## MATERIALS AND METHODS

### Drugs, Chemicals and Instruments

Alloxan and glibenclamide, were purchased from Sigma Chemicals Co., USA, accu-check analyzer was used to check the blood glucose level.

### Experimental Animals

Healthy adult male Wistar albino rats weighing 100 and 120 g were used in this study. The rats were obtained from the animal house, Faculty of Biological sciences, Bayero University Kano. The rats were housed in standard cages, kept under standard conditions, acclimatized for a period of 2 weeks and given a standard diet and clean water *ad libitum*. The experimental rats used in this research were handled in line with the guidelines of International Laboratory Animal use and Care (National Research Council, 2011).

### **Sample Collection and Identification**

*Azanza garckeana* (AG) leaves were collected from Kaltungo LGA, Gombe State of Nigeria. Identification was done at the herbarium/Botany unit, Biological Sciences Department, Bayero University Kano, Kano state, Nigeria, with voucher number BUKHAN 650. It was deposited in the herbarium unit.

### **Preparation of the Extract**

The AG leaves were air dried under shade and coarsely powdered (by using mortar and pestle) and macerated with cold water in glass flasks with occasional shaking. The liquid extract was separated from the marc by using muslin cloth and Whatman filter paper (No. 1). The extraction procedure was repeated thrice. Evaporation was done using water bath and concentrated extract was dried and stored in airtight opaque bottles.

### **Oral Glucose Tolerance Test (OGTT) in Normal Rats**

Oral glucose tolerance test (OGTT) was undertaken based on the method described by Chika and Bello (2010) with slight modification. Experimental rats that had fasted for 12 hours were carefully distributed into four groups of three rats each to have nearly same average weight. Two groups were administered 100 and 150 mg/kg of AG aqueous leaf extract. The other two groups served as negative (administered normal saline) and positive controls (administered 5 mg/kg of glibenclamide dissolved in normal saline). Thirty minutes following each administration, 2.5 g/kg of glucose solution was administered to each rat. Blood glucose levels were measured for each rat at 30, 60 and 120 minutes intervals following glucose administration.

### **Assessment of Hypoglycaemic Activity in Normal Healthy Rats**

Testing was carried out based on the method described by (Sai *et al.*, 2021) with slight modifications using different doses of the leaf extract in normal healthy male rats that had fasted overnight. The rats were divided into four groups (n=3). Control rats (group A) were given vehicle (distilled water), group B was given 2 mg/kg glibenclamide, while other groups C and D received aqueous leaf extract suspended in distilled water orally at doses 100 and 150 mg/kg respectively. Blood glucose levels were estimated before and after 30, 60, and 120 minutes of leaf extract administration. Blood glucose levels were monitored using accu-chek glucometer.

### **Assessment of Anti-hyperglycaemic Activity in Alloxan Induced Diabetic Rats**

The study was carried out based on the method described by (Sharma *et al.*, 2010) with slight modifications on five groups (A-E) of three rats each. Group A served as normal control, group B as diabetic control and groups C and D were orally treated with a dose of 100 and 150 mg/kg b.w. of leaf extract respectively suspended in distilled water and group E (2 mg/kg) glibenclamide daily for 2 weeks. Control rats (group A and B) were given vehicle (distilled water) only. Fasting blood glucose level was taken at the beginning, after 7<sup>th</sup> and 14<sup>th</sup> days of experiment.

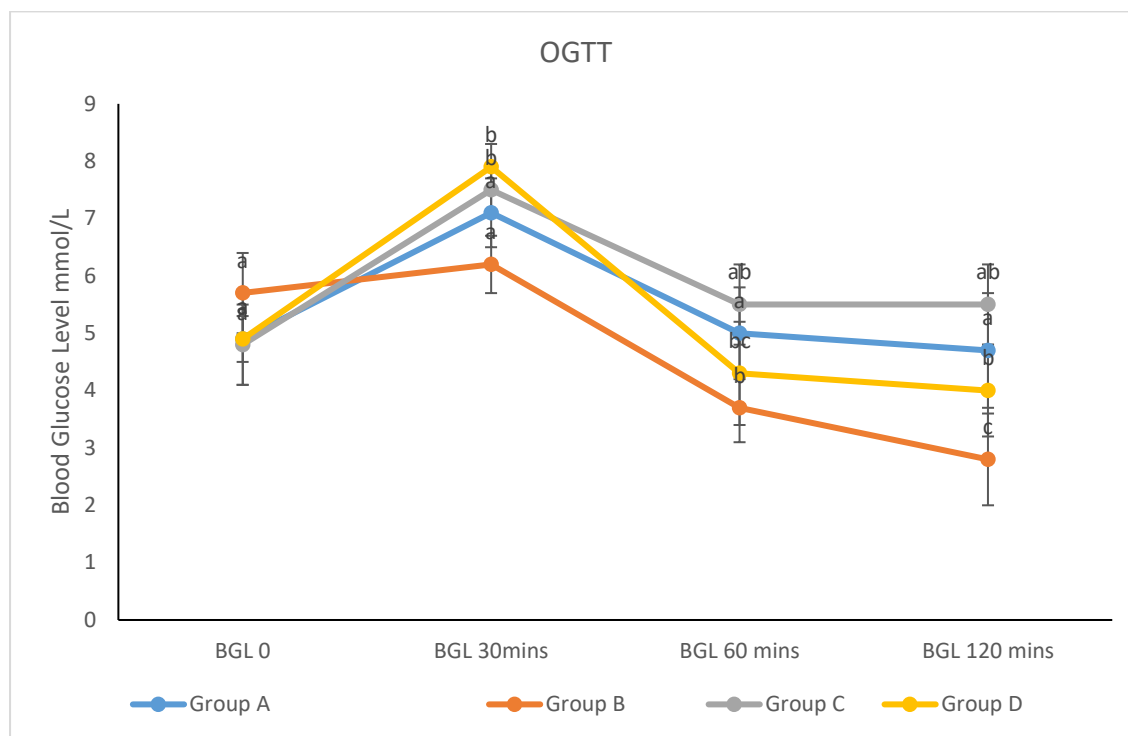
**Statistical Analysis.** Data obtained were expressed as mean  $\pm$  SEM. Analysis of Variance (ANOVA) was performed using the statistical package for social sciences (SPSS version 20, Chicago, IL, USA) on the Blood Glucose Levels.

## **RESULTS**

### **Oral Glucose Tolerance Test in Normal Rats**

Administration of glucose (2.5 g/kg) produced significant increase in blood glucose level of normal overnight fasted rats. Aqueous leaf extract of *Azanza garckeana* (100 and 150 mg/kg)

showed significant decrease in blood glucose levels ( $p < 0.05$ ) at 60 and 120 minutes after glucose loading though not as efficient as that of the control drug glibenclamide (Figure 1).

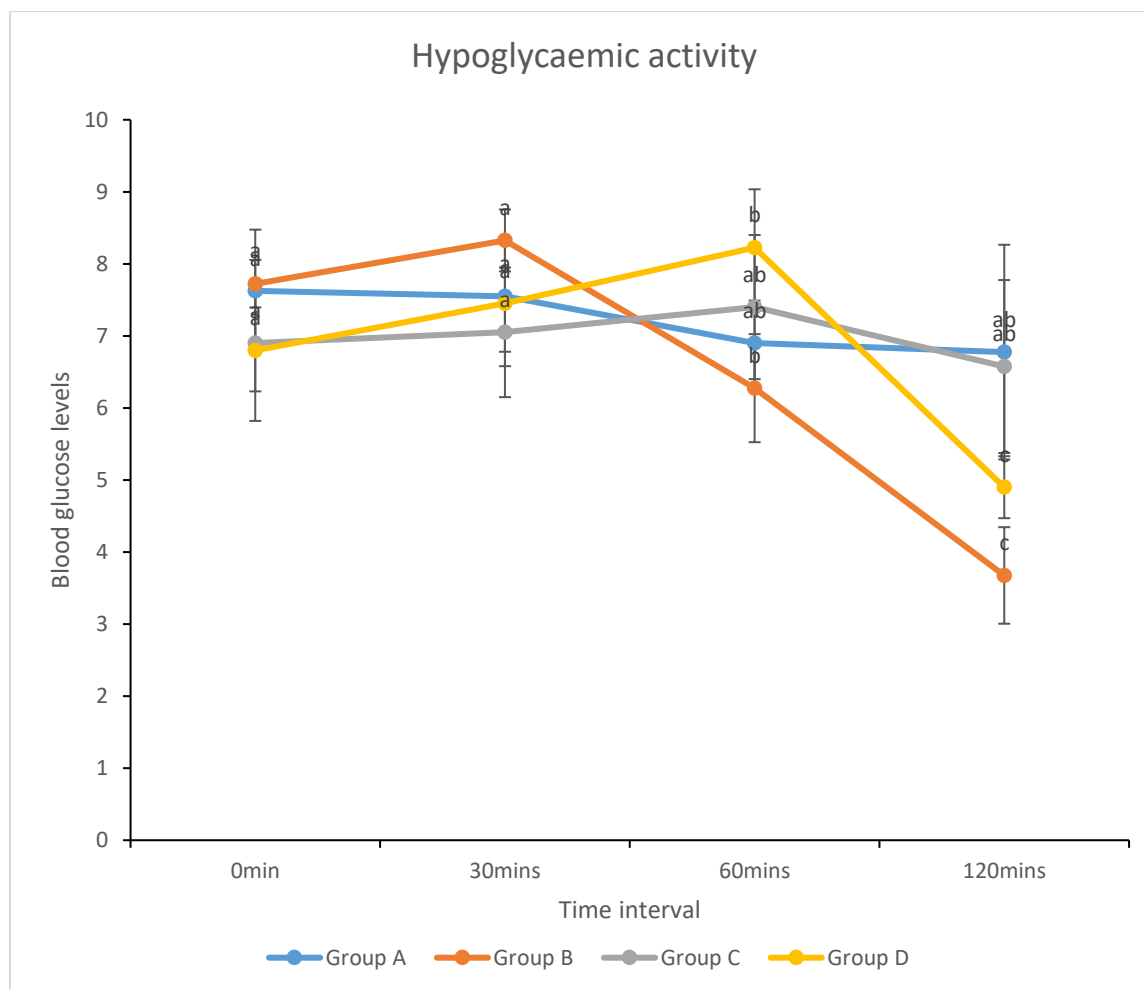


**Figure 1: Blood glucose levels of rats administered *Azanza garckeana* aqueous leaf extract (AGALE).**

Values are expressed as the mean  $\pm$  SEM ( $n=3$ ) values with different letters on the same line are significantly ( $p < 0.05$ ) different from each other. Group A: normal control, Group B: 5 mg/kg glibenclamide group, Group C were given 100 mg/kg AGALE group, Group D: 150 mg/kg AGALE group.

#### **Hypoglycaemic Activity of *Azanza garckeana* Extract in Fasting Normal Rats**

Fasting blood glucose test in normal rats showed that the aqueous leaf extract of *Azanza garckeana* (150 mg/kg) produced significant ( $P < 0.05$ ) blood glucose reduction 60 minutes after administration. Whereas glibenclamide (5 mg/kg) revealed significant reduction throughout the experimental period (Figure 2).



**Figure 2: Blood glucose levels of rats given *Azanza garckeana* aqueous leaf extracts**

Values are expressed as the mean  $\pm$  SEM (n=3), values with different letters on the same line are significantly ( $p < 0.05$ ) different from each other. Group A: normal control, Group B: 5 mg/kg glibenclamide group, Group C: 100 mg/kg AGALE group, Group D: 150 mg/kg AGALE.

### **Anti-hyperglycaemic Activity of *Azanza garckeana* Extract in Fasting Diabetic Rats**

The effect of aqueous leaf extract of *Azanza garckeana* leaf on fasting blood glucose level in diabetic animals is presented in Figure 3. A significant ( $P < 0.05$ ) reduction in blood glucose level was observed with aqueous leaf extract of *Azanza garckeana* (100 and 150 mg/kg) after the 7<sup>th</sup> day, however, the control drug had a more significant effect throughout the duration of the study as compared with the control.

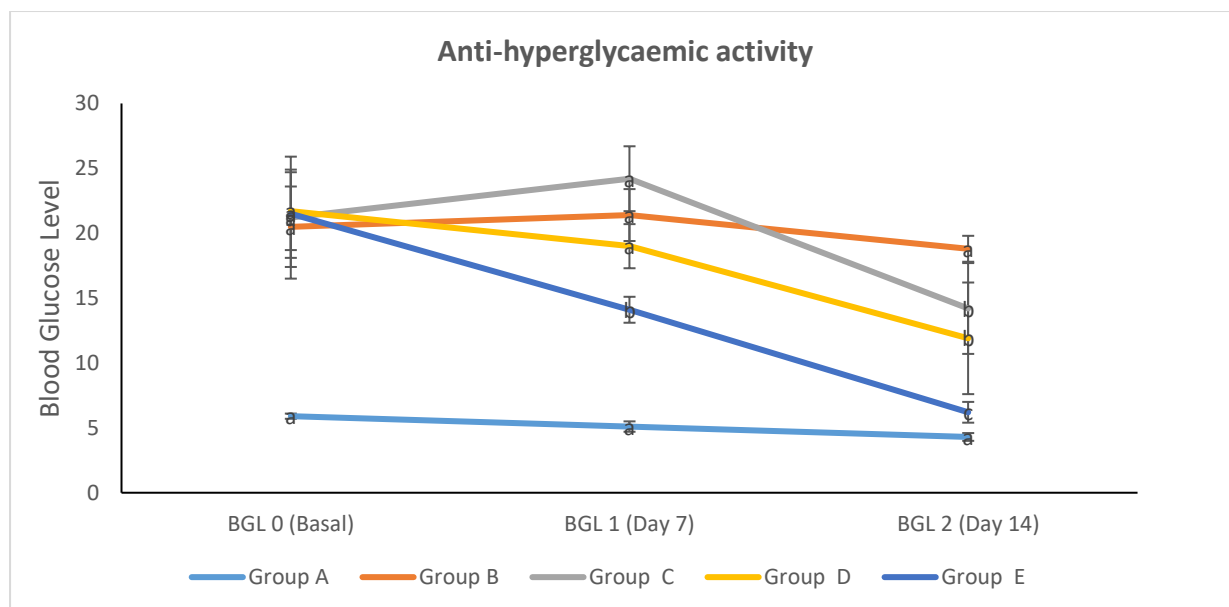


Figure 3: Blood glucose levels of rats given *Azanza garckeana* aqueous leaf extract

Values are expressed as the mean  $\pm$  SEM (n=3), values with different letters on the same line are significantly ( $p < 0.05$ ) different from each other. Group A: normal control, Group B: Positive control, Group C were given 100 mg/kg AGALE group, Group D: 150 mg/kg AGALE group, Group E: 2 mg/kg glibenclamide group.

## DISCUSSION

The oral glucose tolerance test on normal rats with doses of 100 and 150 mg/kg revealed that aqueous extract of *Azanza garckeana* has the capacity to lower blood glucose levels. The reduction in the blood glucose levels at 30 and 60 minutes after glucose loading indicates that the extract could improve glucose uptake and/or utilization. The action on glucose transporters has been suggested as a mechanism of action of some plant extracts in this case considering that glucose is a rapid absorption sugar that does not need the intervention of enzymes for its absorption (Amuri *et al.*, 2017). This finding is similar to the report of Amuri *et al.* (2017) who reported that *Azanza garckeana* aqueous leaf extract showed appreciable baseline glucose lowering effect using a local strain of guinea pigs (*Cavia porcellus*).

The aqueous leaf extract of *Azanza garckeana* at the dose of 150 mg/kg body weight showed significant hypoglycaemic activity in normal rats 60 minutes after its administration whereas glibenclamide (5 mg/kg) had significant reduction throughout the experimental time as compared with normal control. Lawal *et al.* (2022) demonstrated that crude methanol extract of *Azanza garckeana* pulp exhibited a dose-dependent hypoglycaemic effect of the extract in streptozotocin-induced experimental diabetic rats which they attributed to its high phenolic components. However, according to Amuri *et al.* (2017), *Azanza garckeana* aqueous leaf extract exhibited an insignificant hypoglycaemic effect using a local strain of guinea pigs (*Cavia porcellus*).

In this study, the extract also exhibited a marked anti-hyperglycaemic activity in alloxan induced diabetic rats. A significant reduction in blood glucose level was observed with the tested doses especially after the 7<sup>th</sup> day. Alozieuwa *et al.* (2022) reported that treatment of the Streptozotocin (STZ)-induced diabetic rats with diethyl ether extract of *T. garckeana* pulp caused significant and progressive decreases in the fasting blood sugar (FBS) levels in a dose-

related manner. Treatment with the crude methanol extract of *A. garckeana* pulp caused dose-dependent decrease in fasting blood glucose levels and led to the improvement in body weight of the Streptozotocin (STZ)-induced diabetic rats (Lawal *et al.*, 2022).

Phytochemicals such as alkaloids, phenols, polysaccharides, steroids and flavonoids all of which have been reported to be present in *Azanza garckeana* plant have been reported to have glucose lowering capacity through various mechanisms such as inhibition of alpha glucosidase, decrease glucose transport, stimulation of insulin secretion and sensitivity (Ezeonu and Ejikeme, 2016; Gaikwad *et al.*, 2014). This could explain the ability of the plant to lower blood glucose levels as witnessed in this study.

## CONCLUSION

Medicinal herbs have been used for the treatment and management of diabetes across the globe. *Azanza garckeana* aqueous leaf extract exhibited significant hypo- and anti-hyperglycaemic activities in the animal model employed in this study. These findings support the use of the plant for diabetes treatment in traditional medicine. Therefore, *Azanza garckeana* leaves may be a promising source of new anti-hyperglycaemic agent and may be a potential source of safe and effective antidiabetic drug. A detailed mechanism-based study and isolation of bioactive responsible for the observed activities of *Azanza garckeana* is recommended.

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