

The Effect of *Abelmoschus esculentus* in Alloxan-Induced Diabetic Wistar Rat

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Abstract: *The anti-hyperglycaemic activity of Abelmoschus esculentus fruits in alloxan-induced diabetic wistar rats was investigated. The animals were grouped into five (5); A, B, C, D, and E groups. Diabetic Mellitus was induced in groups B-E by single intravenous injection of alloxan, 65mg/kg body weight. Group A served as normal control (non-diabetic). Group C and D diabetic rats were administered with aqueous extracts and dried powdered form of Abelmoschus esculentus fruit respectively. Group E was administered with standard anti-diabetic drug [glibenclamide]. Treatment was for 14 days. Blood samples were taken and the fasting blood glucose levels measured at days 3, 7, and 14. The aqueous and dried powdered form of Abelmoschus esculentus significantly decreased ($p < 0.05$) blood glucose levels of the animals. The results support the therapeutic use of Abelmoschus esculentus as an anti diabetic plant.*

Keywords: Anti-hyperglycaemic, *Abelmoschus esculentus*, Alloxan, Glibenclamide

1. Introduction

Diabetes mellitus (DM) can be described as the increase of glucose in the blood. It is a metabolic disorder of multiple causes characterised by chronic hyperglycaemia, absolute or relative lack of insulin and late complications due to disturbance of carbohydrate, fat, and protein metabolism (WHO, 2011). It is a condition which results when the pancreas no longer produces enough insulin or when the cells stop responding to the insulin produced leading to impaired glucose uptake into the cells of the body. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. Under this condition of chronic hyperglycaemia, there is a non-enzymatic irreversible attachment of glucose molecules to N-terminal of haemoglobin molecules in the red blood cells, a process called glycation.

DM is a potentially morbid condition with high prevalence. Epidemiological studies in 2010 show that about 2.8% of the global population, an estimated 285 million people had diabetes (Beretta, 2012). The figure is believed to increase by the year 2035 and in sub-Saharan Africa; Nigeria will be leading in the figures. Due to this high prevalence and psychological state, diabetes is a major medical concern. The disease remains incurable and can only be controlled with drugs, and in some cases exercise and dietary recommendations (Macedo *et al.*, 2002)

In developing countries including Nigeria, most diabetic patients find it increasingly difficult to manage hyperglycaemic conditions, the major cause of the complications of DM, not only because of the high cost of the synthetic ant-diabetic drugs that may even be more devastating than the disease itself and can lead to death (Ichinose *et al.*, 2009). The management of DM without any side effects is still a challenge to the medical system as the treatment of DM is relatively limited by significant side effects. There is growing interest however in the use of natural health products as an alternative approach to current medications. Plant sources have become a target to explore new drugs and in searching a biologically active compound

(Yehet *et al.*, 2003). Ethnopharmacological surveys indicate that more than 1200 plants are used worldwide in traditional medicine for their alleged hypoglycaemic activity (Berreta, 2012).

Drug and chemical induced diabetes are commonly obtained from the use of an experimental animal model of diabetes mellitus. Alloxan induced DM appear to be the most reliable and easily reproducible method of inducing DM in experimental animals (Rees and Alcolado, 2005). Alloxan, a β -cytotoxin, induces diabetes by damaging the β -cells of islets of langerhans of the pancreas resulting in decrease endogenous insulin production and release, thereby causing hyperglycaemia within a short period.

The investigation of anti-diabetic agents of plant origin which are used in traditional medicine is thus of great significance. Some plant products used in traditional medicine have proven their potential as ant-diabetic agents and are from edible plants. This has added more interest in their study because of the duality of their role, as food and as medicine for the management of DM and other ailments. Some medicinal plants have been implicated in the management and control of hyperglycaemia. Available literature indicates that more than 800 plant species have hypoglycaemic activities (Rajaopal and Sakrikala, 2008).

Abelmoschus esculentus (hereafter referred to as *A. esculentus*) is known as lady's finger in many English-speaking countries. Okra, as it is known in Nigeria is a flowering plant in the mallow family (Chopra *et al.*, 1996), valued for its edible green seed pods is an important vegetable and widely distributed from Africa to Asia, Southern Europe etc. (Khomsug, 2010). *A. esculentus* is an annual herb that is coarse, erect and has branches 0.6-1.5m high in length and with a long petioled leaves, orbicular-ovate that has about 25cm or less in length. The fruit of this plant gives nutritional benefits like protein, niacin, riboflavin, phosphorus, zinc, copper, potassium, vitamins A, B, C and K, thiamine, magnesium, folate, calcium and manganese.

The superior fibre found in okra helps to stabilize the blood sugar by curbing the rate at which sugar is absorbed from the intestinal tract. Okra's mucilage binds also cholesterol and bile acids carrying toxins dumped into it by filtering liver. Okra helps lubricate the large intestines due to its bulk laxative qualities. The okra fibre absorbs water and ensures bulk in stools, which helps prevent and improve constipation. Previous literatures have reported few studies on the lipid lowering properties of okra in blood. This present study is to investigate antidiabetic properties of *Abelmoschus esculentus* as a model to see how the okra seed, which is widely eaten for its nutritional benefits can also be used as medicinal alternative in the treatment of diabetes. Also, the people who live in the villages have little knowledge of the many medicinal properties of the plant in their environment while the city dwellers for time rely heavily on conventional drugs, which are expensive and with side effects.

2. Justification for the Study

The quest for dietary based remedies for DM necessitates investigation of available and cheap edible natural foods of plant origin with fewer side effects in experimental animals. The aim of this study therefore was to evaluate the effects of *A. esculentus* extracts on the blood glucose levels of alloxan-induced hyperglycaemic rats as a new approach or alternative in the treatment of diabetes as its medicinal potential is yet to be fully exploited

3. Materials and Methods

Experimental Animals

Thirty male wistar albino rats weighing between 150-350g were obtained from the animal house of college of Health Sciences, University of Port Harcourt. The animals were placed in standard ventilated cages and maintained under standard laboratory conditions with free access to food and water and studies were carried out in strict guidelines for the care of laboratory animal.

Okra Collection and Identification

Fresh fruits of *A. esculentus* were bought from Mile 3 Market in Port Harcourt, Nigeria on the 2nd of June 2014. A curator in the Department of Applied and Environmental Biology, Rivers State University of Science and Technology did botanical identification.

Preparation of Aqueous extract

Fresh okra was washed and both ends were cut off and placed in a clean drinking water overnight. The fruits were removed and the aqueous extracts, put into water bottles. The extracts were prepared fresh each day.

Dried Powder Preparation

After washing, some of the fruits were sundried for days and grinded into powder and stored into airtight containers for use throughout the study.

Chemicals

Alloxan monohydrate and other chemicals used in this work were of good grades and were purchased from Ekoistic Laboratories, Port Harcourt, Nigeria.

Toxicity Studies

There was no toxicity observed in the aqueous extracts of *A. esculentus* up to 200mg/kg body weight. Toxicity was observed in the rats using alloxan monohydrate at a concentration range of 55-80mg/kg-body weight dissolved in normal saline. Each of the animals was injected with a specific concentration and observed for mortality within 7 days. Within the 7day period, the animals that received 80mg/kg of alloxan died due to hypoglycaemia while those that received 55-60mg/kg of alloxan did not attain the desired hyperglycaemic level but the animals that received alloxan at 65mg/kg body weight had a pronounced hyperglycaemia, survived over the period.

Induction of Diabetes Using Alloxan in Rats/Experimental Design

Healthy animals were fasted overnight. At the end of the of the fasting duration, they were weighed and the baseline glucose level was determined with blood drawn from the tail vein using Accucheck Active glucometer with slot number 995.

Twenty -five rats were divided into five groups of rats labelled A-E. Group A served as normal control (non-diabetic). Groups B-E were induced by injecting 65mg/kg body weight I.V with alloxan monohydrate prepared by dissolving the equivalent milligrams of alloxan in 0.5ml of normal saline in a dark environment to preserve the potency of the photo labile diabetogen. Group B served as diabetic control while group C was treated with aqueous extract (100mg/kg) of *A. esculentus*. Group D was treated with dried okra powder (100mg/kg) dissolved in distilled water and clean drinking water. Group E served as standard drug group treated with glibenclamide (5mg/kg). All treatments were given orally for 14 days. To forestall death of the rats due to hypoglycaemia, the rats received 10% dextrose water after 6hrs post alloxan induction. Induction of diabetes was verified after 72hrs by measuring blood glucose levels using Accucheck Active glucometer with slot number 995. Animals were allowed 7 days for the stabilization of blood glucose level and animals having a blood glucose level of up to 250mg/dl or above were considered diabetic. Estimation of blood glucose was done every week. On the 14th day, the animals were anaesthezied with chloroform pulled over their face and blood sample collected in the fluoride oxalate bottle for the estimation of blood glucose determined using the glucose oxidase method after plasma separation by centrifugation at 1500rpm for 3minutes.

Statistical Analysis

All statistical analysis was done using statistical package for social sciences (SPSS) version, 17.0 windows and differences in means were compared using the students' t-test and one-way analysis of variance (ANOVA). Error of probability or $P < 0.005$ was considered significant.

4. Results

In alloxan induced diabetic rats, there was marked hyperglycaemia (group C-E). The groups treated with an antihyperglycaemic agent and aqueous and dried okra extract showed steady decrease in glucose levels (table 1) significantly ($P < 0.05$) in the first and second week. Diabetes

was induced after the administration of alloxan. Alloxan induces a multiphasic blood glucose response when injected into an experimental animal and accompanied by corresponding inverse changes in the plasma insulin concentration, followed by sequential beta cell changes leading to necrotic cell death (Kliberet *et al.*, (1996). The first phase within few minutes after alloxan injection is the transient hypoglycaemic phase that lasts maximally for 30minutes, which has been noted to be the result of transient stimulation of insulin secretion confirmed by an increase of the plasma insulin concentration (Kliberet *et al.*, (1996). The underlying mechanism of the transient high insulin level may be attributed to a temporary rise in ATP availability due to inhibition of glucose phosphorylation through glucokinase inhibition (Wrenshaw *et al.*, 1996).

Table 1: Multiple Comparison of the Mean Glucose Levels (Mmol/L) of all the Groups in Day 3, Week 1 and Week 2.

GROUPS	DAY 3	WEEK 1	WEEK 2
GROUP A	4.86 ± 0.21 ^a	4.81 ± 0.28 ^a	4.57 ± 0.32 ^a
GROUP B	12.28 ± 1.69 ^c	12.70 ± 1.76 ^c	15.88 ± 2.84 ^d
GROUP C	16.76 ± 1.10 ^e	14.93 ± 1.20 ^d	10.15 ± 3.04 ^b
GROUP D	18.88 ± 1.76 ^e	17.22 ± 0.98 ^e	10.74 ± 2.17 ^b
GROUP E	11.26 ± 1.81 ^c	10.45 ± 0.98 ^b	7.69 ± 1.81 ^b

Values are mean ± Standard Deviation. n=5. Within and across rows, same alphabets represent no significant difference in mean value while different alphabets represents a statistically significant difference in mean (P<0.05).

In table 1, it was observed that the groups, which were induced with alloxan, had hyperglycaemia; of the induced groups, the ones that were treated with the standard antihyperglycaemic agents and aqueous and dried okra extract showed steady decrease in glucose levels over the two-week period. The diabetic controls however did not show any improvement but rather got increasingly hyperglycaemic.

Table 5 Multiple Comparison Tests For Different Periods In Group C, Group D, And Group E

Comparison		Mean Difference	T-Value	P-Value
GROUP C	DAY 3 VS WK 1	-1.826	2.403	P>0.05
	DAY 3 VS WK 2	-6.608	3.689	P<0.05
	WK 1 VS WK 2	-4.782	3.556	P<0.05
GROUP D	DAY 3 VS WK 1	-1.658	2.596	P>0.05
	DAY 3 VS WK 2	-8.140	5.563	P<0.05
	WK 1 VS WK 2	-6.482	5.178	P<0.05
GROUP E	DAY 3 VS WK 1	-0.810	0.719	P>0.05
	DAY 3 VS WK 2	-3.568	6.580	P<0.05
	WK 1 VS WK 2	-2.758	2.933	P<0.05

P<0.05 Represents A Significant Difference in Mean Values.

Table 5 is an illustration of a multiple comparison test for different periods in group C, D and E. The difference in mean observed in the table for all the groups (C,D and E) were negative indicating that there was a decrease in the glucose levels of the various groups over the two week period. This decrease was more pronounced between week 1 and week 2 than between Day 3 and week 1.

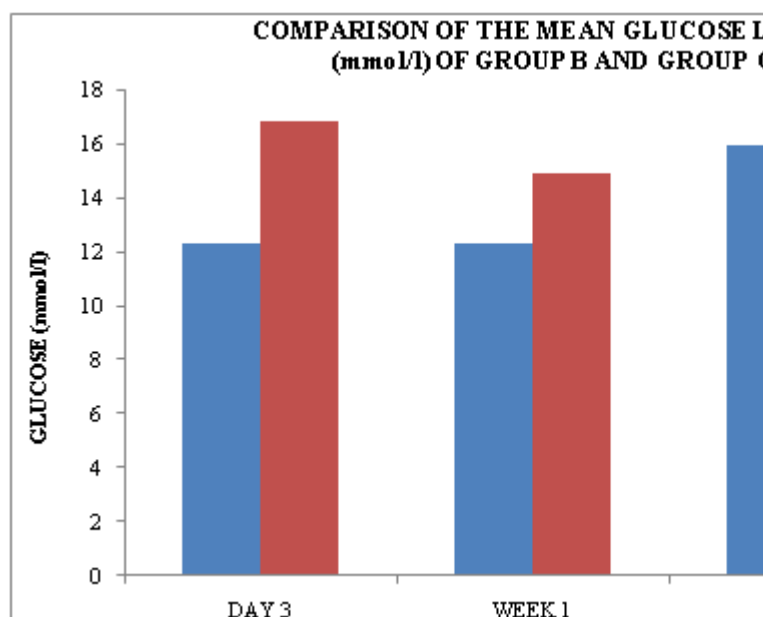


Figure 4.1: Comparisons of the Mean Glucose Levels (Mmol/L) of Group B And Group C in Day 3, Week 1, and Week 2.

5. Discussion

Plants are natural resources for human ailment. Folk medical uses are still implemented in this modern civilized era for the remedy of various complications. *Abelmoschus esculentus* revealed statistically significant hypoglycemic activity. Previous biological investigation showed that this plant contains secondary metabolites to reflect their hypoglycemic activity.

Aqueous extract and the dried powdered form of *Abelmoschus esculentus* were studied for their hypoglycemic effect and it was discovered that aqueous extract was showing maximum effect, using glibenclamide as standard.

There are many reports available to support the multiple mechanisms of anti diabetic plants to exert their blood glucose lowering effect, such as inhibition of carbohydrate metabolizing enzymes, enhancement of insulin sensitivity, regeneration of damaged pancreatic islets B-cells, and enhancement of insulin secretion and release (Moller and Nair, 2008). The aqueous extract and dried powder may exert blood glucose lowering activity possibly with the above mechanism(s) and the anti-hyperglycemic activity was comparable to that of glibenclamide.

In this study, the rats in group E had significantly lower glucose levels compared to that of group C. However, the difference in mean values between days 3 and week 2 of group C was significantly higher than that of group E meaning that Okra extract acts faster than the standard drug glibenclamide.

In the multiple comparison test for different periods in group C, D and E, the difference in mean observed in the table for all the 3 groups were negative indicating that there was a decrease in the glucose levels of the various group over the 2

week period. This decrease was more pronounced between day 3 and week 1 meaning that the extracts became more available in the animal system in the second week than in the first week showing that its effects gets better with continuous use.

6. Conclusion

Diabetes is a widely spread disease. The present study was done with an objective to explore the biological use of *Abelmoschus esculentus* (okra), which is a commonly used plant throughout the world. In the present study, anti-hyperglycaemic effect of aqueous extract and powdered form of *Abelmoschus esculentus* fruit was evaluated in alloxan induced diabetic rats. Okra is a natural product and it has anti-hyperglycemic activity and so the usage of the soaked okra extracts and powdered form of okra is not harmful to human health. *In vivo* experiments with Wistar rats shows a good result of anti-hyperglycemic activity, our experiment gives a preliminary information on this activity. Therefore the okra fruit can be used to treat/manage diabetes mellitus.

Future pharmacological investigations are suggested on the basis of the isolation principles and their mechanisms of anti-hyperglycemic effect.

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