

Investigation on the Anti-diabetic Activity of *Sphenostylis stenocarpa* Seed Milk Extract in Alloxan-induced Diabetes Rats

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Abstract- Diabetes mellitus is a chronic metabolic disease that is associated with abnormal metabolism of carbohydrates, proteins and fat. It is usually associated with excretion of excess sweet urine known as glycosuria. This study investigated the anti-diabetic activity of seed milk extract in Alloxan-induced diabetic rats. The seed milk extract at a concentration of 100, 200, 300 and 400 mg/kg body weight were orally administered to alloxan-induced diabetic rats for a period of fifteen (15) days. The oral glucose tolerance test was also carried out using animal experimental method. The phytochemical analysis of the milk extract revealed the presence of flavonoids, isoflavones, saponin, tannin, phytosterol, lignin and anthocyanidine at moderate concentrations. The acute toxicity test showed no lethality *Sphenostylis stenocarpa* seed milk up to a concentration of 5000 kg/body weight. In oral glucose tolerant test, the *S. Stenocarpa* seed milk extract exerted the highest response, similar to glibenclamide after 15 minutes and 30 minutes of administration compared with the control. The *S. Stenocarpa* seed milk extract recorded the highest blood glucose-lowering effect after day 15 of treatment ($p < 0.05$) compared with the diabetic rats that were administered normal saline and 0.3 mg/kg body weight of glibenclamide. The seed milk extract of *S. Stenocarpa* possessed anti-diabetic activity like the reference drug glibenclamide, and the results of this study revealed that the graded doses of the seed milk extract have blood glucose-lowering effect in a time and concentration-dependent manner.

Index Terms- glycosuria, diabetes, phytochemicals, metabolism, phytosterol, alloxan

I. INTRODUCTION

Diabetes mellitus is a metabolic disorder which is associated excess sweet urine known as glycosuria. The nature of diabetes attack and how it predisposes the body system to the attacks of other diseases made it to be known in medical parlance as “gate opener”. The world health organisation (WHO 2008) estimates that about 171 million people worldwide are suffering from diabetes mellitus and that this Figure will double

by the year 2030. Diabetes mellitus is characterized by recurrent/persistent hyperglycaemia (Suzanne *et al.*, 2010). Chemotherapy remained the only resort of diabetes control. However, most anti-diabetes drugs are not just costly, but always go with high levels of drug toxicity. The phobia generated by chemotherapeutic toxicity has paved way for alternative drugs of plant origin. Hence, the choice of *Sphenostylis stenocarpa* seed milk for anti-diabetes study. African yam bean (AYB) *Sphenostylis stenocarpa* is a tropical grain legume that Originated in Ethiopia, East Africa – (GRIN, 2009). Both the wild and cultivated types now occur in tropical Africa as far as Zimbabwe, throughout West Africa as from Guinea to Southern Nigeria. African yam bean is grown in West Africa particularly in Cameroun, Cote’d Ivore, Ghana, Nigeria and Togo (Potter, 1992). However, extensive cultivation of this legume has been reported in the Eastern Southern, and Western Nigeria (Abbey and Berezi, 1998; Ojiakor, *et al.*, 2010). African yam bean is an underutilized/orphan tropical climbing, vine-like tuberous legume (Obatule *et al.*, 2001), which has attracted research in recent times. This study was therefore designed to investigate the anti-diabetic effects of the seed milk extract of *Sphenostylis stenocarpa* and glibenclamide treatment on alloxan-induced diabetic rats.

II. MATERIALS AND METHODS

Seed Collection, Identification and Preparation

The harvested seeds of *Sphenostylis stenocarpa* (African yam bean) were purchased from Ogige Market, Nsukka Enugu State, Eastern Nigeria. The seeds were identified and authenticated by Mr. Alfred Ozioko and Prof. M.I. Uguru, both of Centre for Ethnomedicine Drug Development (BDGP) and Crop Agronomy Department of the University of Nigeria Nsukka respectively. The seeds were washed with normal saline and oven-dried. They were roasted at 30- 50°C, de-shelled for milk-making extraction.

Extraction of AYB Seed milk

Five hundred grammes (500 g) seeds of AYB was roasted using frying pan for 50 minutes at 30-50°C and were de-shelled when hot. The seed cotyledons were ground and sieved with fine-pored silk, and made in to milk by mixing and homogenizing in the ratio of 1:5 v/v of the flour/ de-ionized water. The prepared milk was used immediately for its storage always result to contamination and auto-oxidation of the labile substances.

The physicochemical properties of the milk were determined using both the sensory method and the use of instruments (AOAC, 2006). The colour and aroma of the oil were sensorily determined as described by Ihekoronye and Ngoddy, 1985. Chemical Properties

Physicochemical analysis of the Milk

Twenty panellists invited from the Department of Food Science and Technology, University of Nigeria, Nsukka conducted the sensory analysis of the extract. The chemical properties was conducted by titration for acid value, and pH with pH metre (Jenway, 3505) and the presence of minerals was carried out using Atomic absorption spectrometer (AAS)(NARICT, Zaria) Nigeria.

Proximate Analysis of the Milk

The crude lipid was extracted using petroleum ether as solvent in a Soxhlet apparatus and ash content (gravimetric by AOAC. The total carbohydrate was calculated by the difference method (sum of crude protein, ash, moisture and crude fat petroleum ether extract) minus the sum from 100). The moisture contents of the milk were determined after drying at 105 °C. The microkjeldahl method was also used to determine the total nitrogen and crude protein (m x 5.95). Nitrogen free extract (NEF) was calculated by difference as NFE total (CHO)_n – crude fibre.

Animal Protocol

Purchase of Animals

Thirty five (35) male albino rats weighing 100 – 180 g were used for the study, the rats were obtained from the College of Veterinary Medicine, University of Nigeria, Nsukka, Enugu state, Nigeria. They were acclimatized for Fourteen days in the animal house of Department of Biochemistry, and were given regular feed (grower' mash) Vital Feeds Nigeria Ltd, Jos, Nigeria and water *ad libitum*. This occurred under standard environmental conditions with a 12- hour light/dark cycle maintained.

Experimental Design

Thirty five (35) male albino rats weighing 180-220 grammes were used for the study; the rats were obtained from the faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. The rats were divided into seven groups with five animals per group, and different treatments administered to each group; Group I: Non- diabetic control (not induced).

Group II: Alloxan- induced diabetic rats administered 0.3 ml of normal saline.

Group III: Alloxan-induced diabetic rats administered with 0.3 mg/kg body weight of glibenclamide.

Group IV: Alloxan-induced diabetic rats administered 100 mg/kg body weight of *Sphenostylis stenocarpa* seed milk extract.

Group V: Alloxan-induced diabetic rats administered 200 mg/kg body weight of *Sphenostylis stenocarpa* seed milk extract.

Group VI: Alloxan-induced diabetic rats administered 300 mg/kg body weight of *Sphenostylis stenocarpa* seed milk extract.

Group VII: Alloxan-induced diabetic rats administered 400 mg/kg body weight of *Sphenostylis stenocarpa* seed milk extract.

Drug Preparation

Glibenclamide (Hovid, Ipoh Malaysia, Batch No. VUDIA 11 – 0, 5mg) was purchased from a pharmaceutical shop in Nsukka, Enugu State, Nigeria. The Tablets were finely powdered, suspended in a normal saline and was filtered using a Buchner funnel and Whatman no 1 filter paper at a concentration of 5mg/ml and was administered at 50mg/kg body weight.

Induction of Diabetes

Alloxan diabetic rat were prepared by adopting the method of Saidu *et al.*, (2011). All rats, except the Normal control group were intraperitoneally injected with 120 mg/kg body weight of the prepared Alloxan. After 6 hours of alloxan administration, rats in their cages were then allowed 10% glucose solution for the next 24 hours in order to prevent alloxan-induced hypoglycaemia. The animals were observed for polydipsia, polyuria, polyphagia as well as general reduction of body weight. Seventy two hours after the alloxan administration, the animals were fasted overnight and diabetes was confirmed by measuring fasting blood glucose level with the aid of a Glucometer (ACCU – Check, Active Roche Diagnostics). Only rats that have fasting blood glucose level > 7.0 mmol/l (126 mg/dl) were considered and included in the study (Saidu *et al.*, 2011).

Animal Treatment

The experimental animals were treated in four different groups for 15 days. Group I was treated with the standard drug (Glibenclamide) while groups IV, V, VI and VII were treated with African yam bean milk of 100 mg/kg b.w, 200 mg/kg b.w, 300 mg/kg b.w, and 400 mg/kg b.w. dosage respectively, twice daily. The mean blood glucose levels in the animals were measured 72 hours after the drug administration by tail tapping using Glucometer (ACCU–Check, Active Roche Diagnostics). The experimental animals were treated with the standard drugs and the AYB milk by oral administration for 15 days and their mean blood sugar were recorded group- wise.

Glucose Level Determination

Procedure

- The coding chip of the corresponding test strips to be used was inserted into the Accu–check glucometer.
- The area of the tail to be pricked was cleaned with swab containing methylated spirit and then pricked with a lancet.
- The next step is the insertion of the test strip in to the glucometer.
- A time, 2 – 4 minutes should be used for the activation of the strip in the glucometer after which, the blood sample was then dropped on the test area of the strip and the result displayed on the glucometer screen was recorded.

Glucose Profile Study

Glucose profile studies were conducted with alloxan-induced diabetic rats using the method of Atangwho *et al.*, (2013) with modification.

Oral Glucose Tolerance Test

The method, dosage of extract and the glibenclamide and animal groupings in this study were as described in the experimental design. Also, the rats had glucose administered orally at a concentration of (2 g/kg body weight) 30 minutes after dosing, and blood samples were obtained by tail puncture at time zero (0) before glucose dosing and at 15, 30, 45, 60, 90, and 120 minutes after glucose administration to measure the glucose level.

Acute Anti-hyperglycaemic Study

Glucose profile studies were conducted with diabetic rats, in the study, six groups of alloxan-induced diabetic rats were treated as follows: group 2 received 0.3 ml normal saline, group 3 received 0.3 mg/kg body weight of glibenclamide, group 3 received 100 mg/kg body weight of *Sphenostylis stenocarpa* seed milk extract, group 4 received 200 mg/kg body weight of *Sphenostylis stenocarpa* seed milk extract, group 5 received 300 mg/kg body weight of *Sphenostylis stenocarpa* seed milk extract, group 6 received 400 mg/kg body weight of *Sphenostylis stenocarpa* seed milk extract, and group 1 served as the normal control group. The normal saline and glibenclamide were administered in doses per day during the period of the study. Fasting blood glucose was measured on day 0 (baseline), 3, 6, 9, 12, and 15th day. At the end of the study, the animals were sacrificed and buried. The ACCU – Check, Active Roche Diagnostics glucometer was used to measure the glucose level with compatible strip.

III. RESULTS

The Seed milk extract of *Sphenostylis stenocarpa* was homogenized with de-ionized water in an electric blender (Nakai-462) China and used. The extract yield was observed to be 1.0 kg (33.3%).

In the experiment, there was no lethality or behavioural changes in the three groups of the mice that received 10, 100, and 1000 mg/kg body weight of the extract at the end of the first experiment. Based on this result, further increased doses of 1900, 2600 and 5000 mg/kg body weight of the extract showed that no death case was observed within 72 hours of administration. This result showed that the extract was safe at dose above 5000 kg body weight.

Physical Properties of African Yam Bean (AYB) Seed Milk (Table 1) shows that AYB was milk coloured, aroma 5.8 and overall acceptability was 6.8. Chemical Properties of AYB Seed Milk (Table 2) showed that AYB had a Titrable acidity of 25.24 and a pH of 4.76. The result of proximate analysis of AYB seed milk revealed the following: fibre (9.24 ± 0.18), carbohydrates (60.26 ± 1.02%), moisture (5.02 ± 2.04%), ash (3.40 ± 0.04%), crude protein (19.24 ± 0.06%), and lipids (2.84 ± 0.14%) (Table 3).

The qualitative phytochemical analysis as observed in Table 4 showed moderate presence of compounds such as isoflavones, flavonoids and saponin, while anthocyanidines, phytosterol, lignin and tannins were low in the sample.

The results of the effect of *Sphenostylis stenocarpa* seed milk extract and glibenclamide on oral glucose tolerance in non-diabetic rat was shown in Figure 1. The measured fasting blood glucose level reached its peak at 15 minutes after oral

administration of glucose. Animals administered 2 g/kg body weight of glucose and 0.3 mg/kg body weight of glibenclamide had the highest significant (p < 0.05) reduction of fasting blood glucose concentration and sustained throughout all the measured time compared to the glucose level of other treatment groups. The animals administered 2 g/kg body weight of glucose and 100 mg/kg body weight of *Sphenostylis stenocarpa* seed milk extract showed significant (p < 0.05) decrease in blood glucose level 30 minutes from treatment compared to glucose level after 15 minutes of treatment, and also showed significant (p < 0.05) reduction in glucose after 45, 60, 90 and 120 minutes respectively compared to glucose level after 30 minutes of treatment. The animals administered 2 g/kg body weight of glucose and 0.03 ml of normal saline showed significant (p < 0.05) decrease in glucose level after 30 minutes compared to glucose level after 15 minutes and showed significant (p < 0.05) increase in glucose level after 60 minutes from treatment. The effect of *Sphenostylis stenocarpa* seed milk extract on glucose level of alloxan induced diabetic rats is shown in Figure 2. All animals induced with 150 mg/kg body weight of alloxan monohydrate showed significant (p < 0.05) increase in blood glucose level on day 0. Animals induced and treated with normal saline showed significant (p < 0.05) reduction in blood glucose level on day 3, 6, 9, 12, and 15 respectively.

Table 1 Physical Properties of African Yam Bean (AYB) Seed Milk

Colour	milk colour
Aroma	5.8
Taste	6.4
Mouth feel	6.2
Overall acceptability	6.8

Table 2 Chemical Properties of AYB Seed Milk

Parameters	Results
Titration acidity	25.24
pH	4.76
Ca	0.12605 ppm
Fe	0.05605 ppm
Cu	0.0009 ppm
Pb and Cd	Not Detected

Table 3 Proximate Composition of AYB Milk

S/No.	Nutrient	Relative abundance (%)
1	Carbohydrates	60.26 ± 1.02
2	Crude Protein	19.94 ± 0.06
3	Lipids	2.84 ± 0.14
4	Crude Fibre	9.24 ± 0.18
5	Ash	3.0 ± 0.04

Table 4 Phytochemical Analysis of AYB Milk

Parameters	Relative abundance (mg/100ml)	abundance
Isoflavones	++	
Anthocyanides	+	
Flavonoids	++	
Saponins	++	
Phytosterol	+	
Lignin	+	
Tannins	+	

KEY: +: Low present: ++: moderate present.

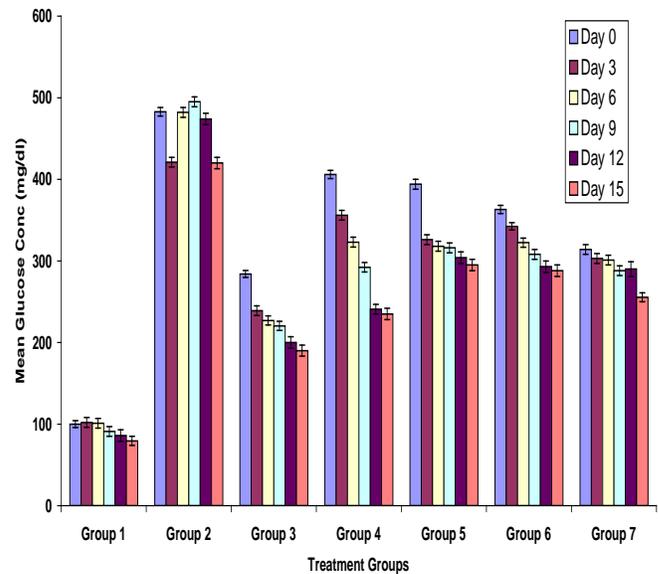
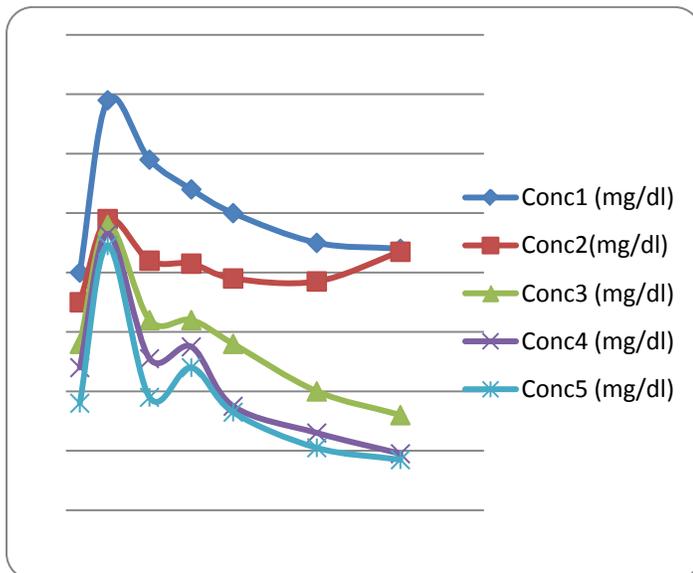


Fig 1: The effect of *Sphenostylis stenocarpa* milk extract on blood glucose concentration of alloxan-induced-diabetic rats



Group 1 = Normal control
 Group 2 = 0.3 ml of Normal saline
 Group 3 = 0.3 mg/kg of Glibenclamide
 Group 4 = 100 mg/kg b.w. of seed milk extract
 Group 5 = 200 mg/kg b.w. of seed milk extract
 Group 6 = 300 mg/kg b.w. of seed milk extract
 Group 7 = 400 mg/kg b.w. of seed milk extract

Group 1 = Normal control
 Group 2 = 0.3 ml of Normal saline
 Group 3 = 0.3 mg/kg of Glibenclamide
 Group 4 = 100 mg/kg b.w. of seed milk extract
 Group 5 = 200 mg/kg b.w. of seed milk extract
 Group 6 = 300 mg/kg b.w. of seed milk extract
 Group 7 = 400 mg/kg b.w. of seed milk extract

IV. DISCUSSION

The results of this study indicate that seed milk extract of *Sphenostylis stenocarpa* significantly reduced blood glucose levels in alloxan-induced diabetes animals. The physical properties showed that the colour was milk colour, and other parameters that were sensorily tested by 20 panelists invited from the Department of Food Science and Technology who scored the following; aroma- 5.80, taste -6.40, mouth feel-6.20 and overall acceptability-6.80. The results above was in agreement with work of Akubor, 1998, on physicochemical and sensory Characteristics of melon seed milk; Belewa, Belewa and Olatunji, 2005 on Soy- Coconut milk preparation; Mordi *et al.*, 2010 on physicochemical and sensory evaluation of Nigerian Tiger nuts and Nnam, 2003 who worked on the physicochemical properties of Tigernut-soy milk. Chemically, the titraTable acidity of the AYB milk is 25.244 and a pH of 4.76. These agree with the work of Nnam, 2003 who also worked on the physicochemical properties of Tigernut-Soy milk mixture. Also, the atomic absorption spectroscopy (AAS) gave us the results of Ca-0.1260 Ppm, Fe-0.05605 Ppm and Cu-0.0009 Ppm as the heavy metals Pb and Cd were not detected. Certainly, the above made the researchers convinced that the works of Uguru and Madukaife, 2001; Azeke *et al.*, 2005; Dhingra and Jood, 2005; as well as Uche, *et al.*, 2014 on the nutritional qualities of this legume seeds gave the seeds the novel properties that made the legume a potential but latent nutraceutical. The cultivation and uses of seeds and tubers of the African yam bean (AYB) was

worked on and reported by Klu *et al.*, 2001 ; Azeke, *et al.*, 2005; and Dhingra and Jood, 2005. Uguru and Madukaife, 2001 reported on the nutritional evaluation of AYB and proved it as a novel food. Abbey and Berezi, 1998 showed that if well processed, AYB's digestibility will become increased. The chemical composition of AYB were worked on and reported by Edem, Amugo and Eka, 1990, and by Ekpo, 2006, they collectively the abundance of novel amino acids, phytochemicals and good quality bioactive compounds. Also, Okigbo, (1973); Nwokolo, 1987 and Uguru and Madukaife, 2001, corroborated the work of Abbey and Berezi, 1998. Amoatey, 2001 reported on the processing of milk of AYB and in 2003, Nnam, reported that this legume is a good source of plant milk. The nutritional composition of AYB was also reported by Ene-Obong, in 1993, and in 2014, Uche *et al.*, reported the nutritional evaluation of this legume and suggested the prospect of using the legume to reduce the scourge of malnutrition in the Northern and Southern Nigeria, in some war-torn countries of sub-Saharan Africa and beyond. The rationale behind this is that *Sphenostylis stenocarpa* seed milk is anti-malnutrition. In Togo, Ghana and Nigeria, the lectin content of *Sphenostylis stenocarpa* seed is used as insecticide. The pastes made from the seeds are used as a cure for stomach ache/antacid. Also when this paste has water added to it, it becomes an anti-alcohol abuse (antabuse) which is natural unlike the drug disulfiram with its adverse drug reaction antecedents. (Asuzu, 1986). Enwere, 1998; Farinde and Omole, 2006 reported the use of *Sphenostylis stenocarpa* seed milk in the management of chronic diseases like diabetes mellitus, hypertension and other cardiovascular diseases because its dietary fibre content. Nwankwo and Ekeanyanwu, 2011 reported that the phytochemicals contained also in the *Sphenostylis stenocarpa* seed milk such as flavonoids, isoflavones, anthocyanides, saponins, phytosterols, and lignin have the potential health benefits functioning as anti-cancer, and heart disease, lower blood cholesterol, reduce risk of heart disease, anti-hypertensive, anti-diabetic, anti-osteoporosis as well as anti-inflammatory agent. Since *Sphenostylis stenocarpa* seed is a continental orphan legume which has the West Africans preferring the seeds to tubers, and the Easterners and Southerners relishing the tubers especially among the Bandudus, the Shabas and the tribe of Kinshasa in Democratic Republic of Congo. The FAO/WHO, (1991) have also reported the AYB amino acid profile to be higher than those in other legumes including soybean, and affirmed that this same amino acid profile compares favourably with whole hen's egg and met the organisations daily requirement of food. However, previous studies have shown that the phytochemicals like, polyphenols, flavonoids and tannins present in *Sphenostylis stenocarpa* seed milk are anti-diabetic Nwankwo and Ekeanyanwu, 2011. The result of this present study revealed that *Sphenostylis stenocarpa* seed milk has low glycaemic index, and may be the attribute that suggests its use in the management of diabetes mellitus.

V. Conclusion

The present study revealed that *Sphenostylis stenocarpa* seed milk has low glycaemic index, and may be the attribute that suggests its use in the management of diabetes mellitus.

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