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BIOACTIVE CONSTITUENTS OF SEED OF *SPHENOSTYLIS STENOCARPA* ATTENUATE BLOOD GLUCOSE LEVEL IN ALLOXAN-INDUCED DIABETES IN RAT

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ABSTRACT

Sphenostylis stenocarpa seeds are used locally to lower blood sugar level among diabetic patient in Southeastern Nigeria. The present study aimed at evaluating the effect of extract and fractions of *Sphenostylis stenocarpa* seeds on alloxan-induced diabetes in rat. The pulverized seed was soaked in methanol for 72h, filtered and concentrated to obtain the extract. The extract was subjected to Vacuum Liquid Chromatography (VLC) using gradient elution to afford n-hexane fraction, ethyl acetate fraction and methanol fraction respectively. Standard phytochemical method was used to screen the extract and fractions respectively. The method described by Lorke's was used to carry out the acute toxicity study. The alloxan induced diabetic model was used to evaluate the antidiabetic activity in the rats. The phytochemical constituents present in extract and fractions were alkaloids, flavonoids, terpenoids. The acute toxicity study indicated no mortality or any behavioral changes even at 5000mg/kg body weight. The extract and fraction at 200mg/kg and 400mg/kg exhibited antidiabetic activity in dose dependent manner. The 400mg/kg reduced blood glucose level significantly at $p < 0.05$ when compared to standard drug (glibenclamide). The *Sphenostylis stenocarpa* seeds have demonstrated an outstanding antidiabetic activity. The research has substantiated the use of the *S. stenocarpa* seeds to manage blood sugar level in folkloric medicine without record of adverse effect. This could be attributed to the present of flavonoids and terpenoids and alkaloids present in the samples with proven antidiabetic potentials. Further studies are required to isolate and characterize the bioactive compound responsible for the observed anti-diabetic activity.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia resulting from defects of insulin secretion and/or increased cellular resistance to insulin. There are three types of diabetes mellitus, among them type 2 DM is more prevalent in adult than type 1 DM

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while gestational diabetes occurs due to hormonal imbalance during pregnancy [1]. Chronic hyperglycemia and other metabolic disturbances of diabetes mellitus leads to long-term tissue and organ damage as well as dysfunction involving the eyes, kidneys, nervous and vascular systems leading to short lifespan. The prevalence of diabetes mellitus (DM) is increasing worldwide, and it is projected that by the year 2030 over 500 million adults will be affected by DM as a result of urbanization and aging of the population. Globally about 537 million people have diabetes in the world and 24 million people in the African Region with total cases of diabetes in adults over 3,623,500 million [2]. The conventional and easily available antidiabetics drug classes: insulin, biguanides and sulfonylureas are used to treat diabetes mellitus and they are relatively tolerated. Moreover, their side effect such as nausea, hypoglycemia, weigh gain, and deficiency of vitamin B12 is a major setback in diabetes management [1]. However, there is need to search medicinal plants that could serve as medicine to mitigate the alarming prevalence of diabetes globally. Plants have been proven to be a better source of medicine to manage glucose level as previously reported [3,4]. It has been established that bioactive constituents isolated from plants: phytosterols from *Aloe vera*, andrographenolide from *Andrographis paniculata*, methyl-4-hydroxybenzoate and agnucastoside from *Moringa oleifera*, apigenin-7-o-glucoside from *Psidium guajava* and vindoline-type alkaloids from *Catharanthus roseus* demonstrated significant antidiabetic property [5]. *Sphenostylis stenocarpa* popularly known as African yam beans (AYB) is among the neglected and underutilized leguminous crop cultivated extensively in West Africa. *Sphenostylis stenocarpa* is cultivated mainly for home consumption because of its nutritious seed and tuber among people of eastern Nigeria especially during food shortage or famine [6]. *Sphenostylis stenocarpa* seed is being increasingly consumed by locals of eastern Nigeria with challenges of diabetics, hypertensive and cardiovascular patients though there is no scientific reports to validate these claims. It was reported that the seeds can be eaten without harm, non-toxic to humans, suitable for consumption but must be roasted, fermented or soaked in water for about 12 h before being cooked or processed in order to soften the seed coat and reduce processing time and increase the nutritional content [7]. The physicochemical parameters reported showed that the dry matter comprises of protein, fat, total carbohydrate, amino acid, fiber and ash [8,9.10,11]. The preliminary phytochemical screening and GCMS profiling of *S. stenocarpa* seed constituents indicated the presence of alkaloids, flavonoids, tannins, steroids, phenolics, hydrocarbons and fatty acids esters as reported [12,13]. The pharmacological activity of *S. stenocarpa* seed in form of extract and processed form have been reported to demonstrated significant anti-inflammatory activity, anti-anemic, antioxidant, antidiabetic and heapto-renal activities

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[13,14,15,16]. Therefore, this study was designed to investigate the class of bioactive constituents responsible for the anti-diabetic potentials of *Sphenostylis stenocarpa* Seed. Moreover, suggesting a viable way to maximize its utilization in both food and pharmaceutical industries for management of diabetes and its complications.

MATERIALS AND METHODS

Collection and Preparation of Materials

Sphenostylis stenocarpa brown seeds were sourced from Eke market Agbani, Nkanu west local government area of Enugu State, Nigeria. It was authenticated by Mr. Patrick Obi, of the Department of Pharmacognosy, Enugu State University of Science and Technology, Enugu with the Herbarium number FP/Cog/06002. The seeds were properly hand selected to remove weevil infested and damaged seeds. The seeds were washed under a running tap water to remove soil particles and other contaminants. The seeds were air dried under a shade at room temperature for 5days thereafter pulverized to a fine powder using an electrical blender.

Experiment Animal

The mice were sourced at the animal house of the Department of Pharmacology, Enugu State University of Science and Technology Agbani, Enugu where the ethical clearance was gotten from the research and ethics committee with reference number ESUT/FPS/PHA/2023/024. The rats were housed in metal cages groups of 7 in a photoperiod cycle of 12h: 12h (Light and dark), at room temperature and fed with standard diet and tap water for a period of 7 days for acclimatization.

Extraction of Phytoconstituents of S. stenocarpa

The Extract was prepared by cold maceration method using methanol as solvent. The air dried, finely pulverized seeds 1 kg was soaked in 4.5 litres of methanol and left for 72 hours at room temperature with constant agitation. The mixture was filtered first, using a fine grade cloth filter and second filtration was done using funnel clogged with cotton wool. The filtrate was concentrated using rotary evaporator under reduced pressure and exposing under fan for evaporation to dryness to yield crude methanol extract. Then it was transferred into an air-tight container and stored at 4°± 2°C in a refrigerator until when needed.

Fractionation of Methanol extract of S. stenocarpa

The methanol extract 30g was fixed with silica gel of (70-230 mesh size). The vacuum liquid chromatography column measuring 4cm x 45 cm was dry packed with silica gel of 70-230 mesh size and then fixed extract introduced. The gradient elution using hexane (1200 ml), ethyl acetate (800 ml) and methanol (1500 ml) were employed. The individual fractions were concentrated with rotary evaporator set at 40°c and the resultant fractions were further dried at

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40°c in the water bath for 12hrs to yield n-hexane, ethyl acetate and methanol fractions respectively. The dried fractions were transferred into an airtight container and stored in a refrigerator.

Phytochemical Screening of Methanol Extract and Fractions

The tests used for the qualitative phytochemical analysis are standard methods of Trease and Evans [17].

Acute Toxicity Study

This was performed according to method describe by Lorke [18]. 12 mice were used after acclimatization. An initial investigation involving administering (10, 100 and 1000 mg/kg) of the plant extract to three different groups of three mice each. After 24 hrs and there was no death recorded. In this second stage, three dose levels were used (1600, 2900 & 5000 mg/kg). Physical observations were made up to 24 hrs for mortality and behavioral changes

Alloxan-Induced Diabetes Model

The baseline blood glucose levels of the rats and weight were determined before they were induced with diabetes by intraperitoneal (IP) injection of 120 mg/kg body weight of alloxan monohydrate solution. After 2 days, 5 days and 7 days, the glucose levels of the rats were checked, the rats had increased glucose level using an Acu-Answer glucometer, but 12 rats had died. The diabetic rats were regrouped into six groups table 1. The blood glucose level of the rats were checked after 5 days during the treatment period till the last day. The treatment lasted for 15 days after which the rats were sacrificed.

Table 1: Grouping of the rats and treatment

Group I	Naive rats un-induced and un-treated
Group II	Negative Control rats received no treatment.
Group III	Diabetic rats treated with 5mg/kg b.wt glibeclamide .
Group IV	Diabetic rats treated with 200mg/ kg. and 400mg/ kg. b.wt. methanol extract
Group V	Diabetic rats treated with 200mg/ kg. and 400mg/ kg. b.wt. methanol fraction

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Group VI	Diabetic rats treated with 200mg/ kg. and 400mg/ kg. b.wt. n-hexane fraction

Statistical Analysis

Numerical data obtained from the study were expressed as mean values ± Standard Error of Mean (N=5). Differences among means of control tested groups were determined using one-way Analysis of Variance (ANOVA) using IBM SPSS software followed by Turkeys comparison test for significance. A probability level of less than 5% ($p<0.05$) was considered significant. Graphical illustration was carried out using Microsoft excel, 2010.

RESULTS AND DISCUSSION

Phytochemical Screening Test

The phytochemical investigation of the methanol extract and fractions of *S. stenocarpa* seeds revealed the presence of bioactive secondary metabolite with established therapeutic property Table 2: Phytoconstituents of methanol extract and fractions of *S. stenocarpa* seeds.

Table 2: Results of phytochemical screening

Phytoconstituents	Samples		
	Methanol Extract	Methanol Fraction	N-Hexane Fraction
Flavonoids	+	+	-
Terpenoids	+	+	+
Saponins	-	-	-
Tannins	-	-	-
Alkaloids	+	+	+
Steroids	-	-	-

Key: + (present) ; - (absent)

Acute Toxicity Study

The acute toxicity study result obtained showed no mortality in both phase 1&2 of the study

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indicating the safe use of the *S. stenocarpa* seed as claimed by the traditional users.

Effect Of Extract and Fractions of S. stenocarpa seed on the Blood Glucose Level of Alloxan Induced Diabetic Rats

The extract at 200mg/kg and 400mg/kg exhibited anti-diabetics activity in dose dependent manner. 400mg/kg b. wt reduces blood glucose lowering effect significantly at $p < 0.05$ when compared to standard drug at 5mg/kg b. wt as presented in Table 5.

Table 3: phase 1

Samples	Dose	Death	Dose	Death	Dose	Death
Methanol extract	10mg/kg	0/3	100mg/kg	0/3	1000mg/kg	0/3

Table 4: phase 2

Samples	Dose	Death	Dose	Death	Dose	Death
Methanol extract	1600 mg/kg	0/3	2900 mg/kg	0/3	5000 mg/kg	0/3

Table 5: Mean ±SD of treatment group on blood glucose level response

TREATMENT	**DOSE**	**Fasting blood Glucose (mg/dl)**				
		Before induction	**After induction**	**5 day**	**10th day**	**15th day**
Naive rats	-	110.70±0.87	101.95±0.61	105.11±1.20*	107.81±1.15*[b]	112.22±0.65*[b]
Negative control	-	105.80±3.02	214.83±2.25	240.00±4.54	581.77±15.00[b]	600.00±0.00[b]
Positive control (Glibenclamide)	5mg/kg	111.27±12.60	337.58±40.95	205.90±2.64	186.55±2.20*	157.66±1.01*
Methanol extract	200mg/kg	110.80±5.52	572.40±0.31	341.52±14.65	124.01±0.47*[b]	123.12±0.61*[b]
Methanol extract	400mg/kg	112.65±9.16	482.71±29.44	263.45±27.50	134.25±0.68*[b]	120.20±0.96*[b]
N-hexane fraction	200mg/kg	114.35±8.98	367.00±81.04	155.12±0.29*	170.11±2.10*	163.72±1.31*[b]
N-hexane fraction	400mg/kg	100.87±0.78	472.00±9.14	300.10±7.72	197.34±2.40*	112.15±2.38*[b]
Methanol fraction	200mg/kg	102.52±6.51	373.70±50.00	208.81±2.67	103.70±0.20*[b]	102.91±0.40*[b]

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Methanol fraction 400mg/kg	107.92±4.44	328.12±29.00	184.21±1.92	108.55±2.42*[b]	80.24±1.40*[b]

*N = 4. Results are expressed as mean ± SEM. Significant difference is set at *p<0.05 level. One-way, ANOVA followed by Turkey HSD*
(*) Represent a group where there is a significant difference (reduction) in fasting blood glucose level when compared with the negative control.
([b]) Represent a group where there is a significant difference in fasting blood glucose level when compared with the positive group that received the standard drug.

Table 6: Mean ±SD of Weight Variation among the treatment groups

TREATMENT	**DOSE**	**Weight (grams)**				
		Before induction	**After induction**	**5 day**	**10th day**	**15th day**
Naive rats	-	105.73±1.10	116.87±1.40	126.23±2.57	128.22±1.69	130.21±2.08
Negative control	-	112.08±6.722	114.90±3.80	111.42±2.44	109.44±2.48	103.60±2.33
Positive control (Glibenclamide)	5mg/kg	76.95±12.99	103.10±7.56	114.40±3.10	109.29±6.55	106.41±3.71
Methanol extract	200mg/kg	96.08±3.40	110.25±3.99	89.75±4.02	89.33±4.12	90.22±4.02
Methanol extract	400mg/kg	125.81±18.41	147.95±19.59	114.75±13.00	110.39±12.38	105.56±11.16
N-hexane	200mg/kg	143.00±14.67	165.00±15.66	122.23±13.70	121.94±13.70	119.67±10.10
N-hexane	400mg/kg	121.97±1.32	146.25±2.63	118.44±1.70	115.41±1.37	108.65±3.34
Methanol fraction	200mg/kg	151.38±16.00	168.88±17.32	109.28±6.50	104.63±5.18	95.22±6.80
Methanol fraction	400mg/kg	165.84±1.80	186.18±3.10	106.23±0.15	101.27±41	99.45±1.01

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Discussion

This present research has shown that the methanol extract and fractions of *S. stenocarpa* seeds possess anti-diabetics activity due to its effect in lowering the blood glucose level of the alloxan induced diabetic rats. The phytochemical constituents of methanol extract and fractions as presented in table 2 are of medicinal importance. Some other researchers reported the presence of these phytoconstituents in the seed *S. stenocarpa* of substantiating our present study [12,13]. The result of acute toxicity study of methanol extract orally administered showed no mortality and behavioral changes even at doses less or equal to 5000mg/kg as presented in table 3 and 4. This suggests that the methanol extract is safe for ethno-pharmacological research and indicated prospect for phytomedicine utilization. Medicinal plants have been useful in lowering the blood glucose level via potentiation of insulin effect and also by increasing the pancreatic secretion of insulin from beta-cells mechanisms. The present study demonstrated that the methanol seeds extract and fractions of *Sphenostylis stenocarpa* seed significantly decreased the blood glucose level. At 400mg/kg body weight of the methanol fraction, there is a significant difference at $P< 0.05$ with best blood glucose effect when compared to standard drug as presented in table 5. It was observed that as the treatment progresses a significant decrease blood glucose level was recorded due to increase in the concentration of extract and fraction administered. Therefore, the anti-diabetic activity of the extract and fraction displayed a dose dependent effect. The significant decrease in blood glucose levels as shown in this study is an indication that the extract and fractions has blood glucose lowering property which could be attributed to the phytoconstituents: flavonoids, alkaloids and terpenoids detected. This is in agreement with previous researcher that reported terpenoids and polyphenols in *Sphenostylis stenocarpa* seed extracts being responsible for extended reduced blood glucose levels [16,19]. In most type 2 diabetic patient, management of weight is inevitable for effective blood glucose level control. As observed in the present study, there was a variation in the body weight of the treated diabetic rats as presented in table 6. The Positive control group had significant weight gain even though the Fasting glucose level decreased, but the weight increased significantly. The extract and fractions however, controlled the weight of the rats closely towards a healthier body weight while reducing the Fasting Blood Glucose level of the rats significantly. It was interesting to note that methanol extract and n-hexane fraction had a steady reduction after the normal wide drop in the blood glucose level after the first 5 days of treatment, which lasted throughout the 15 days study just within the normal range. Among the fractions, methanol fraction needed a short period of administration as it reduces the blood sugar levels of the rats with a wider margin at day 15. It suggested that after conclusion at day 15, most of the rats especially those administered 400 mg/kg

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of methanol fraction could developed hypotensive shock and died off. The best reduction at slightly above 100mg/dl of glucose level was obtained at day 10 for the methanol faction, so it can be inferred that blood glucose lowering effect within 10 days proved most efficient and should be discontinued immediately to prevent hypoglycemia and other possible complications. However, both the extract and fractions of *Sphenostylis stenocarpa* seed had a way better anti-diabetic activity than glibenclamide and shorter onset of action with better potency and efficacy.

CONCLUSION

The methanol extract and fractions of *Sphenostylis stenocarpa* seed demonstrated significant anti-diabetic activity. This was as a result of bioactive constituents with proven anti-diabetic activity present in the seed. The methanol fraction which is rich in flavonoids exhibited the best blood glucose lowering and weight management properties. These observations corroborate the traditional use of African yam bean in management of diabetes and other metabolic diseases. Further studies are required to study the mechanism of action, isolate and characterize the active ingredient responsible for the observed anti-diabetic activity of the seeds.

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