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Effects of *Vernonia amygdalina* Del. (Asteraceae) on Glycemia and Mating Behavior of Male Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author JGG designed, performed the experiment, wrote the manuscript and analyzed the data. Author DSG performed the experiment and proof read the manuscript. Author SM performed experiment, analyzed data, provided technical assistance in writing the manuscript and proof read the manuscript. Author FKO conceived and supervised the experiment, provided technical assistance in writing the manuscript and proof read the manuscript. All the authors read and approved the manuscript to be sent for publication.

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Original Research Article

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ABSTRACT

Aims: To determine the effect of oral administration of *Vernonia amygdalina* ethanolic leaves extracts on blood glucose concentration of alloxan-induced hyperglycemic albino rats and the effect of the extract on the mating behavior of male albino rats.
Study Design and Methodology: Normoglycemic and alloxan-induced hyperglycemic rats divided into 6 groups of 6 rats each were treated with 400 mg/kg of 70% ethanolic leaves extracts, chlorpropamide 50 mg/kg and distilled water 10 ml/kg respectively once daily for 14 days. The blood glucose concentration and weight were monitored. Oral glucose tolerance test was carried out using another set of rats according to the same grouping. Another set of rats were pretreated with the extract for 7 days and then challenged with alloxan. Sexual function parameters of mount, intromission and ejaculation were evaluated in a fourth set of rats which were administered with 100, 200 and 400 mg/kg of the extract. Rats treated with 5 mg/kg sildenafil citrate and 10 ml/kg distilled water served as the positive and negative controls respectively.

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Results: The results obtained showed that the extract significantly reduced blood glucose concentration of alloxan-induced hyperglycemic rats and also improved glucose tolerance. However, in rats pretreated with 400 mg/kg extract for 7 days and then challenged with alloxan, there was a significant increase in blood glucose concentration. No significant changes were observed in the sexual function parameters of rats treated with the extract.
Conclusion: The 70% ethanolic leaves extract of *Vernonia amygdalina* possesses blood glucose lowering activity in alloxan-induced hyperglycemia and also improves glucose tolerance. However, it did not have aphrodisiac activity at the tested doses and duration of treatment.

Keywords: Vermonia amygdalina; diabetes; mating behaviour; rats; traditional medicine.

ABBREVIATIONS

VAOH (Vernonia amygdalina 70% ethanolic aqueous leaves extract); ND (Normoglycemic rats); D (Hyperglycemic rats); ED (Erectile dysfunction); NRC (National Research Council); OECD (Organization for Economic Cooperation and Development).

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disease and its prevalence continues to increase rapidly. In 2011, the International Diabetes Federation put the number of people suffering from the disease globally at 366 million [1]. The prevalence in 2016 was 422 million [2]. This translates to an increase of 56 million within 5 years [2]. The disease was reported to be responsible for 1.5 million deaths in 2012 which makes it the eighth leading cause of death worldwide [2]. Though the burden of diabetes is currently higher in developed countries, the demography is rapidly changing with Africa and Asia accounting for most new cases [3]. It is therefore estimated that by 2030, these continents will account for the majority of cases [3]. Dietary changes from traditional, highly nutritive and low-calorie diets to high-calorie low nutrient “western diets” is suspected to be the leading cause of the increasing diabetes burden [2,3].

Diabetes mellitus is usually associated with erectile dysfunction (ED) [4,5,6]. This is possibly because of some complications of diabetes mellitus such as vascular disease and neuropathy. ED is a long-term complication of diabetes mellitus and it has a complex pathophysiology. The causes of ED could be of neurogenic, vascular, hormonal or psychogenic origin [7]. Out of the four possible causes of ED, vascular disease and neuropathy appear to be the most frequent [7].

Many plants which are indigenous to Africa and Asia such as the mushroom *Hericium erinaceus* [8] *Citrus odorantum* [9], *Alium sativum* [10], *Alium cepa* [11] and *Vernonia amygdalina* [12,13,14,15] have been shown to be useful in the management of diabetes mellitus. In Nigeria and Cameroon, *Vernonia amygdalina* is commonly known as bitter leaf and the leaves are widely used as vegetable in soups. The leaves are also used for their medicinal importance. Several extracts which were obtained by treatment of *Vernonia amygdalina* leaves with solvents of varying polarities yielded some fractions with medicinal potential. The plant is used in many cultures for the treatment of malaria [16,17,18]. It has also been shown to have hepatoprotective [19,20], anticancer [21,22,23] and hypoglycaemic [12,13,14,15] activities. The chloroform extract of the leaves administered orally was shown to reduce glycemia in alloxan induced hyperglycemic rats [12]. Consumption of the raw leaves or drinking the juice obtained by washing the fresh leaves was shown to significantly decrease postprandial blood glucose concentration in non-diabetic humans [13]. Oral administration of the aqueous extract was also shown to reduce glycemia in streptozotocin-induced hyperglycemic rats [14]. In some communities in Nigeria, hydroalcoholic extract of the leaves is used in the management of different ailments including diabetes mellitus.

Considering the potential efficacy of *Vernonia amygdalina* in the management of diabetes mellitus, this work was carried out to determine the efficacy of 70% ethanolic extract of the leaves on alloxan induced hyperglycemia in rats. This is to mimic the traditional use of the plant in the management of diabetes mellitus. In addition, during an observational study where the ethanolic extract was administered to diabetic men, there were reports of improvement in erectile function by some participants (Okwuasaba 2011, Personal communication).

Hence the effect of the extract on mating behavior of normoglycemic male albino rats was also determined to ascertain whether Vernonia amygdalina has aphrodisiac effect in normal male rats.

2. MATERIALS AND METHODS

2.1 Plant Collection and Preparation

Fresh leaves of *Vernonia amygdalina* Del. Family Asteraceae were collected between November and January in Kuru, Jos South Local Government area, Plateau State. The plant was identified at the Department of Medicinal Plant Research and Traditional Medicine of the National Institute for Pharmaceutical Research and Development, Abuja Nigeria. Voucher specimen was prepared in the Department of Pharmacognosy, University of Jos and deposited in the herbarium with voucher specimen number AD-PHARM-00141. The leaves were washed with distilled water to remove any sand and unwanted debris. After washing, 3.5 kg of the fresh leaves were dried under the shade and 585 g of dried leaves was obtained. Dried leaves were pounded using pestle and mortar into a coarse powder and kept in an air tight container before extraction.

2.2 Plant Extraction

About 500 g of the coarse leaves powder was weighed and poured into a 10 liter glass tank and 2.5 liters of 70% ethanol (Sigma) in water was poured into it. The mixture was stirred thoroughly at regular intervals for 72 hours after which it was successively sieved through sieves (Impact Laboratory Test Sieves) with 800, 300 and 150 µm aperture to remove the exhausted chaff. The liquid portion was filtered using Whatman No. 1 filter paper to obtain a clear filtrate. The filtrate was poured into stainless steel trays and placed in an oven (Memmert) at 40^{o}C to dry. The dried extract was scrapped, transferred into a Duran bottle (Schott) and kept in a refrigerator at 4-8^{o}C pending use.

2.3 Phytochemical Screening

Qualitative phytochemical analysis was carried out according to the method described by Sofowora [24].

2.4 Housing and Feeding

Male Wistar strain albino rats weighing between 114 and 230g used in the study were obtained from National Institute for Trypanosomiasis Research Vom. They were fed with standard rat pellet of percent proximate nutritional composition (Moisture 6.30, Crude Protein 29.02, Crude Fiber 23.70, Fats 0.50, Ash 7.70, Carbohydrate 32.72, Calcium 1.05, Phosphorus 0.92%) obtained from Dagwom farm mill National Veterinary Research Institute Vom and tap water *ad libitum*. The rats were kept in plastic cages with stainless steel mesh top. The dimensions of the cages L X B X H was 17" X 11" X 8". They were housed in the cages according to their groups i.e. rats taking the same treatment were housed in one cage. Wood shavings were used as the bedding and lighting was 12 h light and 12 hour dark cycle. The temperature in the experimental room was 22^{o}C± 3^{o}C. A total of 145 rats were used for the entire study but only data from 120 is reported because more than the required number of rats were treated with alloxan in order to avoid a shortage of hyperglycemic rats in a situation where hyperglycemia was not achieved in some rats.

2.5 Preparation of Hyperglycemic Rats

After acclimatization for one week, the rats were fasted overnight and weighed using a top loading weighing balance (Mettler). Their fasting blood glucose concentrations were measured using the On Call plus® glucose meter and strip by piercing the tip of the tail with a needle and applying the blood onto the test strip. Alloxan (Sigma) dissolved in cold normal saline (+4^{o}C) was then administered intraperitoneally using a 1ml sterile syringe and needle (Easy Ject I) at a dose of 120 mg/kg body weight of rat. The concentration of the alloxan solution was prepared such that no rat was injected with more than 1 ml of the solution. After 72 hours, the fasting blood glucose concentrations were measured and rats with fasting glucose concentration above 179 mg/dl were allocated to the hyperglycemic groups. In all, 95 male rats were used for the experiment to test the effect of the extract on glycemia.

2.6 Experimental Design

2.6.1 Repeated dose study

The rats were divided into six groups consisting of six rats each. Groups 1, 2 and 3 were normoglycemic rats treated with 10 ml/kg distilled water, *Vernonia amygdalina* extract (VAOH) 400 mg/kg, and 100 mg/kg chlorpropamide (Diabenese®) respectively. While groups 4, 5 and 6 were hyperglycemic rats treated with 10

ml/kg distilled water, VAOH 400 mg/kg and chlorpropamide 100 mg/kg respectively. The normoglycemic rats were randomly allocated to their respective groups by picking from a bigger cage that contained all the rats to be used while the hyperglycemic rats were allocated based on their glucose concentration such that the mean concentrations will not differ significantly between the groups. All animals were treated by once-daily oral administration between 800hrs and 900hrs for 14 days. Blood glucose concentration and body weight were measured on days 1, 3, 7 and 14.

On the 15th day, all the rats were euthanized by light chloroform anesthesia, the blood collected by incision of the jugular vein and the serum was analyzed for cholesterol, triglycerides and high-density lipoproteins.

2.6.2 Oral glucose tolerance test (OGTT)

A different set of rats were used for the oral glucose tolerance test. The grouping of rats for the oral glucose tolerance test experiment was the same as that for the repeated dose study i.e. six groups each containing six rats. Glucose 2 g/kg was administered orally to each rat. Blood samples were taken from the tail vein at time 0 (just before glucose administration), 30, 60, 90, 150 and 270 minutes after glucose administration to determine the blood glucose concentration.

2.6.3 Effect of pretreatment with VAOH on alloxan diabetogenicity

Five normoglycemic rats were pretreated with VAOH 400 mg/kg daily for 7 days after which alloxan 120 mg/kg was administered. The fasting blood glucose concentration was measured 72 hours after alloxan administration.

2.6.4 Evaluation of mating behaviour

The physical methods of mounting and mating behavior as described by Yakubu et al. (2007) [25] were used to test for the aphrodisiac potential of the extract. Apparently healthy and sexually experienced female rats weighing between 150 – 180g were artificially brought to estrous by sequential administration of 100 µg/100 g ethinylestradiol suspension orally and 1 mg/100 g progesterone (SkinPharm) subcutaneously 48 and 6 hours respectively before mating. This is because female rats are receptive to the males for mating only during estrous. The receptivity of the females was tested by using male rats other than the ones used in the experiment. Apparently healthy and sexually experienced male rats weighing between 180 and 250 g that showed brisk sexual activity were used to mate the female rats. Before commencing the experiment, the rats were brought to the testing laboratory under dim light at 1800 hours to 2200 hours daily for 4 days to make them familiar with the testing conditions.

VAOH was administered at doses of 100, 200 and 400 mg/kg to rats in groups 1, 2 and 3 respectively while groups 4 and 5 rats were treated with 10 mg/kg distilled water and 5 mg/kg sildenafil citrate (Vigra®) respectively. The rats were mated in a ratio of 1 male to 1 female and the observation of mating behavior was done for the first two mating series. The occurrence of events and phases of mating were called out and recorded using a recordable phone (NOKIA 3210).

The parameters of male sexual behavior monitored were:

(i) Mount frequency (MF) - Mount is defined as the male assuming the corpulatory position but failing to achieve intromission. Mount frequency is the number of mounts without intromission from the time of introduction of the female until ejaculation [26].
(ii) Intromission frequency (IF) - Intromission is the introduction of the penis into the vagina. Intromission frequency is the number of intromissions from the time of introduction of the female until ejaculation. It is characterized by pelvic thrusting and a springing dismount [26]
(iii) Mount latency (ML) - Mount latency is defined as the time interval between the introduction of the female and the first mount by the male [26].
(iv) Intromission latency (IL) - Intromission latency is defined as the time interval between the introduction of the female and the first intromission;
(v) Ejaculatory latency (EL) - Ejaculation is the act of ejecting semen brought about by a reflex action that occurs as a result of sexual stimulation. Ejaculatory latency is defined as the time interval between the first intromission and ejaculation. This is characterized by a longer deeper pelvic thrusting and a slow dismount followed by a period of inactivity or reduced activity [26]
(vi) Post ejaculatory interval (PEI); Post ejaculatory interval is the time interval between ejaculation and the first intromission of the following series.

From the observed male sexual behavior parameters, other parameters were computed thus:

% Mounted = (No. mounted/No paired) x 100

% Intromitted = (No. Intromitted / Number paired) x 100

Intromission ratio = (No. of Intromission) / (No of mounts + No of intromissions)

% Ejaculated = (No. Ejaculated / No paired) x 100

A total of 25 male and 25 female rats were used for the evaluation of the aphrodisiac activity.

2.7 Ethical Consideration

All treatments were administered via the oral route using oral gavage needles and syringe. Extract and standard drugs were reconstituted in the solvent (distilled water) to a concentration such that the volume administered to any rate did not exceed the maximum convenient volume (2 ml/100g) for rodents as prescribed by the Organization for Economic Cooperation and Development (OECD) guideline 423 [27]. All experiments were conducted in accordance with the Principles and Guide for the care and use of laboratory animals of the National Research Council (1996) [28] and approved by the Ethics Committee of Faculty of Pharmaceutical Sciences, University of Jos, Nigeria.

2.8 Statistical Analysis

Data were expressed as mean±SEM. The repeated dose study and oral glucose tolerance test data were analyzed by two-way repeated measures ANOVA with Bonferroni's post-test. Data for the test of the effect of pre-treatment with VAOH on alloxan diabetogenicity was analyzed using students paired t-test and the test for aphrodisiac activity was analyzed by one-way ANOVA with Tukey's post-tests. All analyses were carried out using GraphPad Prism version 4.03 for windows; GraphPad software, San Diego California USA; www.graphpad.com.

P values < 0.05 were considered significant.

3. RESULTS

3.1 Phytochemical Analysis

The result of the preliminary qualitative phytochemical analysis showed that the extract contained alkaloids, saponins, resins, glycosides and flavonoids while tannins were not detected.

3.2 Effect of VAOH on Body Weight

The mean body weight of normoglycemic rats treated with distilled water 10 ml/kg and 100 mg/kg chlorpropamide increased by 17.67 g and 41.33 g respectively. While that of normoglycaemic rats treated with VAOH (400 mg/kg) increased by 1 g.

The mean body weight of diabetic rats treated with VAOH 400 mg/kg decreased by 8.66 g, that of diabetic rats treated with chlorpropamide 100 mg/kg decreased by 58.00 g while the weight of diabetic rats treated with distilled water 10 ml/kg decreased by 23.67 g (Table 1).

3.3 Effect of VAOH on Blood Glucose Concentration of Rats

There was no significant change in the blood glucose concentration of normoglycemic rats treated with VAOH. Mean fasting blood glucose levels in rats during the period of treatment is shown on Table 2.

Table 1. Body weight of rats treated with VAOH 400 mg/kg orally for 14 days

Treatments	Body weight (g)					
	Day 0	Day 3	Day 7	Day 14	Loss/ Gain	%Loss/ Gain
ND+Water	152.00±6.00	140.00±1.00	149.33±2.03	169.67±5.93	17.67	11.63
ND+VAOH	219.33±11.62	221.67±11.89	226.33±11.87	220.33±17.30	1.00	0.46
ND+Chlorpropamide	114.00±1.00	136.33±8.17	154.67±8.37	155.33±11.47	41.33	36.25
D+Water	185.00±18.58	159.00±15.87	148.67±11.41	161.33±11.84	-23.67	-12.80
D+VAOH	163.33±15.30	140.00±11.00	146.33±11.61	154.67±16.19	-8.66	-5.30
D+Chlorpropamide	174.67±8.95	122.00±20.66	110.00±28.01	116.67±33.38	-58.00	-33.21

ND=Normoglycemic rats, D=Hyperglycemic rats, VAOH=Vernonia amygdalina 400 mg/kg. n= 6 rats per group
Day 0 is 72 hours after intraperitoneal administration of alloxan

For the diabetic rats, there was significant reduction in blood glucose concentration in rats treated with VAOH 400 mg/kg, chlorpropamide 100 mg/kg and distilled water treated rats on the 7th day of treatment though the VAOH and chlorpropamide treated rats had significantly lower blood glucose concentrations when compared to the rats treated with distilled water. By the 14th day, the hyperglycemic rats treated with VAOH and chlorpropamide had blood glucose concentrations comparable to that of normoglycemic rats while that of the hyperglycemic rats treated with distilled water was significantly higher (p<0.05).

The results of serum lipids analysis showed that there was no significant difference (p>0.05) in the concentrations of cholesterol, triglycerides and HDL of rats in all the groups Table 3.

The result obtained from the oral glucose tolerance test is shown on Table 4.

The blood glucose concentration of normoglycemic rats reached peak concentration 30 minutes after administration of 2 g/kg glucose. But the peak concentration was not significantly (p>0.05) higher than the pretreatment blood glucose concentration for all the normoglycemic groups. The hyperglycemic peak and area under the glucose tolerance curve were comparable in all the groups though the chlorpropamide treated group had lower glucose levels.

The blood glucose concentration in diabetic rats reached a peak 30 minutes after administration of glucose (2 g/kg) and decreased over the period of monitoring. The blood glucose concentration of hyperglycemic rats treated with 10 ml/kg distilled water was significantly (p<0.05) higher than those of rats treated with VAOH (400 mg/kg) and chlorpropamide (100 mg/kg) after 90 and 270 minutes of treatment.

The result of pretreatment of rats with VAOH on alloxan diabetogenicity is shown on Table 5.

It shows that after pretreatment with VAOH 400 mg/kg, administration of alloxan to rats resulted in significantly higher blood glucose concentration.

3.4 Effect of VAOH on the Mating Behavior of Male Rats

The result showed no significant changes in mating parameters (mount, intromission and ejaculation) of rats treated with the extract when compared to rats treated with distilled water. Rats treated with sildenafil 5 mg/kg showed significant (p<0.05) changes in these parameters when compared to rats treated with the extract and distilled water (Table 6).

Table 2. Effect of daily administration of VAOH on blood glucose concentration of rats

Treatments	Days			
	Day 0	Day 3	Day 7	Day 14
ND+Water	63.33±2.78	63.50±2.17	64.40±1.82	61.50±3.39
ND+VAOH (400 mg/kg)	62.00±2.82	61.83±2.94	73.50±3.66	54.00±1.48
ND+Chlorpropamide	50.67±4.03	82.33±2.59	54.33±7.04	77.00±7.3.86
D+water	300.00±70.07***	273.25±42.22***	140.00±31.78**	168.75±52.72**
D+VAOH (400 mg/kg)	317.16±58.79***	257.50±82.26***	97.67±26.38	66.50±3.53
D+Chlorpropamide	437.00±70.36***	254.75±67.79***	56.25±12.36	70.00±7.91

*n= 6 rats, ND=Normoglycemic rats, D=Hyperglycemic rats, **=p<0.01, ***=p<0.001, Significantly different from Normoglycemic control by two-way ANOVA, Day 0 is 72 hours after intra peritoneal administration of alloxan*

Table 3. Effect of VAOH on serum lipids (Cholesterol, Triglycerides and HDL) of rats

Treatments	Lipid parameter		
	Cholesterol mmol/lit.	Triglycerides mmol/lit.	HDL mmol/lit
ND+Water	1.90±0.29	0.63±0.08	0.43±0.07
ND+VAOH	1.83±0.38	0.87±0.13	0.47±0.09
ND+Chlorpropamide	1.70±0.30	0.60±0.12	0.50±0.10
D+Water	1.93±0.29	0.70±0.06	0.47±0.07
D+VAOH	2.00±0.23	0.77±0.15	0.50±0.10
D+Chlorpropamide	1.90±0.29	0.70±0.15	0.57±0.09

N=Non Diabetic, D=Diabetic, HDL=High Density Lipoproteins

Table 4. Effect of VAOH administration on oral glucose tolerance in Normoglycemic and Alloxan-induced hyperglycemic rats

Treatments	Blood Glucose Concentration (mg/dl) at various time intervals Time (Minutes)					
	0	**30**	**60**	**90**	**150**	**270**
ND+Water	84±4.72	99.7±4.3	82.3±6.7	84.67±6.89	69±4.5	67.7±6.7
ND+VAOH	80.67±3.3	108.7±9.3	101±12.2	90.33±9.21	75.7±6.6	69.3±5.9
ND+Chlorpropamide	78.67±3.6	100±3.3	70±3.3	72±5.19	59.3±1.0	58.33±1.9
D+Water	243.3±13.6***	367±12.1***	335.3±14.2***	324.3±3.4***	247.7±20***	238.7±31.5***
D+VAOH	207.7±24.5***	313.3±53.3**	289.7±40.5***	238±35.3***	185±3.9***	148±21.2*
D+Chlorpropamide	222.3±4.7***	310±2.3***	268.7±11***	230.3±11.6***	180.3±13.5***	128.3±5.3

*n= 6 rats, ND=Normoglycemic rats, D=Hyperglycemic rats, *=P<0.05 **=P<0.01, ***=P<0.001, Significantly different from Normoglycemic control by two-way ANOVA*

Table 5. Effect of Pre-treatment with VAOH on alloxan diabetogenicity in rats

BGC (mg/dl) Before VAOH administration	BGC (mg/dl) After daily administration of VAOH for 7 days	BGC (mg/dl) 72 hours after alloxan administration
68±4.93	72.33±2.84	258.33±51.49**

*N = 5 rats **=p<0.01 by t-test*

Table 6. Effect of VAOH on Mount, Intromission and Ejaculation in rats

Treatments	Male sexual behavior parameters					
	Mount Latency (secs)	**Mount Frequency**	**Intromission Latency (secs)**	**Intromission Frequency**	**Ejaculatory Latency (secs)**	**PEI (secs)**
VAOH (100 mg/kg)	66±7	6	845	1	1200	609
VAOH 200 (mg/kg)	30±2	6	905	2	1200	424
VAOH 400 (mg/kg)	240±5	-	-	-	-	-
Sildenafil (5 mg/kg)	3±0.4***	3.8±0.3	4±1***	5.2±0.4	240±1.2**	189±5**
Distilled water 10 ml/kg	183±67	2	449	6.5±0.9	786	305

PEI=Post Ejaculatory Interval

Table 7. Effect of VAOH on computed male sexual behavior parameters

Treatments	% Mounted	% Intromitted	Intromission ratio	%Ejaculated
VAOH 100 mg/kg	16.67	16.67	0.10	16.67
VAOH 200 mg/kg	16.67	16.67	0.18	16.67
VAOH 400 mg/kg	16.67	0.00	0.00	0.00
Sildenafil 5 mg/kg	100.00	83.33	0.59	83.33
Distilled water 10 ml/kg	33.33	16.67	0.33	16.67

4. DISCUSSION

The significant reduction in blood glucose concentration of hyperglycemic rats treated with VAOH by the 7th day of treatment when compared to hyperglycemic rats treated with distilled water indicates that the extract possesses blood-glucose-lowering effect. The chloroform [12] and water [13,27] extracts of *Vernonia amygdalina* have been shown to have anti-diabetic activity. Many plants have been shown to possess blood glucose lowering effect. Metformin which is used in clinical practice for the management of diabetes mellitus was derived from the plant *Galega officinalis* [10].

The weight loss of rats in the hyperglycemic groups is expected because diabetes mellitus is usually characterized by excessive weight loss [29]. The marginal increase in weight of normoglycemic rats treated with VAOH compared to rats treated with distilled water and chlorpropamide showed that the extract may possess weight gain regulating activity. Moderation of weight can be beneficial since obesity is a predisposing factor to diabetes mellitus and obesity was observed in 55% of patients diagnosed with type-2 diabetes [30]. The higher percentage weight gain of normoglycaemic rats treated with chlorpropamide may be because the drug acts by stimulating the release of insulin from the beta cells of the pancreas. Insulin has anabolic effects and it controls the uptake, use and storage of glucose, amino acids and fatty acids and inhibits catabolic processes such as breakdown of glycogen, fat and protein [31]. The marginal weight gain of rats treated with VAOH compared to that of normoglycemic rats treated with chlorpropamide and distilled water may be an indication that the extract lowers blood glucose at least in part by a mechanism other than enhancing insulin release.

Oral glucose tolerance test measures the body's ability to utilize glucose as a source of energy. In this test, the significantly higher glycemic peak and wider glucose tolerance curve of hyperglycemic rats treated with distilled water when compared to that of rats treated with VAOH is an indication that the extract enhances utilization of glucose.

In the normoglycemic groups, the slightly lower blood glucose concentration of rats treated with chlorpropamide compared to that of rats treated with VAOH may be an indication of the propensity of chlorpropamide to cause hypoglycemia; an effect that is usually associated with sulfonyl ureas and other insulin secretagogues [31]. This may also be an indication that VAOH is euglycemic and further strengthens the hypothesis that it may be acting at least in part via a mechanism other than secretion of insulin. The biguanides, for instance, are known to act by increasing the sensitivity of insulin receptors to insulin while alpha-glucosidase inhibitors decrease the rate of absorption of glucose into the circulation by inhibiting the alpha glucosidase catalyzed breakdown of complex carbohydrates [32].

Dyslipidaemia is a complication of diabetes mellitus [33]. The insignificant changes in serum lipids may be because of the short duration of observation (14 days) and the model used to induce hyperglycemia in the rats (alloxan induced hyperglycemia). The hyperglycemic effect of alloxan is caused by hydroxyl radical attack on beta cells [34] which leads to reduced insulin secretion. Impaired lipid metabolism in non insulin dependent diabetes mellitus (NIDDM) is usually caused by insulin resistance while that in insulin dependent diabetes mellitus is usually caused by absolute lack of insulin ultimately resulting in the inhibition of insulin's antilipolytic effect [33]. Normally, far less insulin is needed to inhibit lipolysis than is needed to stimulate glucose uptake [35]. Therefore hyperglycaemia may occur without dyslipidaemia.

The phytochemicals detected in the extract of VAOH may be responsible for the observed effects on blood glucose concentration. These phytochemicals are bioactive substances which

are responsible for the medicinal properties of plants. Alkaloids of *Morus alba* have been shown to possess alpha glucosidase inhibitory activity [36]. Total flavonoids of *Polygonatum odorantum* have been shown to possess antidiabetic activity due to their alpha glucosidase and alpha amylase inhibitory activities [37]. Saponins from fenugreek fed to alloxan diabetic dogs have also shown anti diabetic activity [38].

The hyperglycemia observed in rats pre-treated with VAOH for 7 days before administration of alloxan shows that though VAOH reduced glycemia in alloxan induced hyperglycemic rats, it does not inhibit the ability of alloxan to cause hyperglycemia. This may be expected when the glucokinase inhibition mechanism of alloxan action is considered. Glucokinase couples changes in blood glucose concentration and insulin secretion [39,40]. Alloxan, on the other hand, inhibits glucokinase and prevents this coupling [41]. Administration of D-glucose and mannose have been shown to protect glucose induced insulin secretion and pancreatic beta cell glucokinase against inhibition by alloxan [42,43]. Because there is competition between glucose and alloxan for the active site of the enzyme, high alloxan concentration can overcome glucose protection of the pancreatic islet [44]. VAOH whose antihyperglycemic activity has been shown will not be a good protector of the pancreatic B-cells against alloxan-induced diabetogenicity. Some agents such as dimethyl sulphoxide [45], dimethyl urea [46], Ethanol [47] and amygdalin [48] have been shown to protect against the diabetogenic actions of alloxan. These agents have high reactivity with the hydroxyl radical (.OH) which is a potent oxidant that is thought to be generated and partly responsible for the diabetogenic actions of alloxan [34].

The comparable mating behavior parameters of male rats treated with the extract and distilled water and the significant difference in these parameters of rats treated with sildenafil citrate 5 mg/kg showed that VAOH extract has no inherent aphrodisiac activity at the tested doses. Any medicinal plant with aphrodisiac tendencies should produce a statistically significant increase in the indices of the sexual vigor of mount and intromission frequencies and a significant decrease in mount, intromission and ejaculatory latencies which are indicators of stimulation of sexual arousability, motivation and vigor [49]. Therefore the improvement in erectile function reported by participants in the earlier mentioned observational study may be as a result of glycemic control of *V. amygdalina* not an inherent aphrodisiac activity of the extract.

5. CONCLUSION

VAOH possesses blood glucose lowering effect which may be mediated at least in part via a mechanism other than increased insulin secretion. Therefore, it can be considered for further studies and development for the management of diabetes mellitus in humans. Also, VAOH does not have aphrodisiac activity at the tested doses and duration of administration.

There is a need for further studies to identify the active compound(s) of VAOH. The exact mechanism of action also needs to be elucidated. Here, the effect of VAOH on starch and glucose metabolizing enzymes and expression of GLUT-4 gene needs to be determined. The measurement of blood insulin levels and morphometry of the pancreas will also give further insight into the mechanism of action. Administration of VAOH for a longer period is recommended to ascertain the sub chronic and chronic effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The research was approved by the Research Ethics Committee of Faculty of Pharmaceutical Sciences, University of Jos, Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Whitting DR, Guariguata L, Weil C, and Shaw J. IDF diabetes atlas: Global estimates of the prevalence of diabetes for

2011 and 2030. Diabetes Research and Clinical Practice. 2011;94:311-21.

2. World Health Organization, Global Report on Diabetes. Geneva; 2016.
(Accessed 30 August 2016)
3. Wild S, Roglic G, Green A, Sicree R, and King H. Global prevalence of diabetes: Estimates for the year 2000 and projection for 2030. Diabetes Care. 2004;27(Suppl 5):1047-53.
4. Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, et al. Impotence and its medical and psychological correlates: Results of the Massachusetts male aging study. Journal of Urology. 1994;151:51-61.
5. Snow JS. Erectile dysfunction in patients with diabetes mellitus – advances in treatment with phosphodiesterase type 5 inhibitors. The British Journal of Diabetes and Vascular Disease. 2002;2:282–7.
6. Sairam K, Kulinskaya E, Boustead GB, et al. Prevalence of undiagnosed diabetes mellitus in male erectile dysfunction. British Journal of Urology International. 2001;88: 68-71.
7. Lee WH, Kim YC, Choi HK. Psychogenic versus primary organic impotence. International Journal of Impotence Research. 1994;6:94-7.
8. Jinn CW, Shu HH, Jih TW, Ker SC, Yi CC. Hypoglycemic effect of extract of *Hericeum erinaceus.* Journal of Science food and Agriculture. 2005;85:641-6.
9. Joan IAC, Alicia M, Per M. Lipid lowering effect of traditional Nigerian anti-diabetic infusion of *Rauwolfia vomitoria* foliage and *Citrus aurantium* fruit. Journal of Ethnopharmacology. 2006;104:379-86.
10. Grover JK, Yadav S, Vats, V. Medicinal plants of India with anti-diabetic potential. Journal of Ethnopharmacology. 2002;81: 81-100.
11. Kumari K, Mathew BC, Augusti KT. Antidiabetic and hypolipidemic effects of S-methyl cystein sulfoxide isolated from *Allium cepa* Linn. Indian Journal of Biochem and Biophysics. 1995;32(Suppl 1):49-54.
12. Gyang SS, Nyam DD, Sokomba EN. Hypoglycemic activity of *Vernonia amygdalina* (Chloroform extract) in normoglycemic and alloxan induced hyperglycemic rats. Journal of Pharmacy and Bioresources. 2004;1:10-5.
13. Okolie UV, Okeke CE, Oli JM, Ehiemere, OI. Hypoglycemic indices of *Vernonia amygdalina* on postprandial blood glucose concentration of healthy humans. African Journal of Biotechnology. 2008;7(Suppl 24):4581-5.
14. Taiwo IA, Odeigah PGC, Ogunkanmi LA. The glycemic effects of *Vernonia amygdalina* and *Vernonia tenoreana* with Tolbutamide in rats and implications for the treatment of diabetes mellitus. Journal of Science Research and Development. 2008;2:122-30.
15. Akah PA, Okafor, CL. Blood sugar lowering effect of *Vernonia amygdalina* Del. In an Experimental Rabbit Model. Phytotherapy Research. 1992;6:171-3.
16. Tona C, Cimanga RK, Mesia K, Musuamba CT, De Bruyne T, Apers S, Hernans N, Van Miert S, Pieters L, Tottle J, Vlietenck AJ. *In vitro* antiplasmodic activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. Journal of Ethnopharmacology. 2004;93:694-5.
17. Masaba SC. The antimalarial activity of *Vernonia amygdalina* Del. (Compositae) Trans R Soc Tropical Medicine and Hygiene. 2000;94:694-5.
18. Iwalokun BA. Enhanced antimalarial effects of chloroquine by aqueous *Vernonia amygdalina* leaf extract in mice infected with chloroquine resistant and sensitive *Plasmodium berghei* strains. African Health Sciences. 2008;8(Suppl 1):25-35.
19. Adaramoye OA, Akintayo O, Achem J, Fafunso MA. Lipid lowering effect of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. Vascular Health and Risk Management, 2008;4(Suppl 1):235-41.
20. Nwanjo HU, Ojiako OA. Is *Vernonia amygdalina* hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats. African Journal of Biotechnology. 2006;5 (Suppl 18):1648-51.
21. Izevbigie EB. Discovery of water soluble anticancer agents (Edotides) from a vegetable found in Benin City Nigeria. Experimental Biology and Medicine. 2003;228:293-8.
22. Izevbigie EB, Bryant JL, Walker A. A novel natural inhibitor of extracellular signal

regulated kinases and human breast cancer cell growth. Experimental Biology and Medicine. 2004;229:163-9.

23. Yedjou C, Izevbigie EB, Tchounwu P. Preclinical Assessment of *Vernonia amygdalina* leaf extracts as DNA damaging anticancer agent in the management of breast cancer. International Journal of Environmental Research Public Health. 2008;5(Suppl 5):337-41.
24. Sofowora A. Screening plants for bioactive agents. In: Sofowora A. editor Medicinal Plants and Traditional Medicine in Africa, 2nd edition Ibadan Spectrum Books Ltd. 1983;134-56.
25. Yakubu MT, Akanji MA, Oladiji AT. Male sexual dysfunction and methods used in assessing medicinal plants with aphrodisiac potentials. Pharmacognosy Reviews. 2007;1(Suppl 1):49-56.
26. Gauthaman K, Adaikan PG, Prasad RNV. Aphrodisiac properties of *Tribulus terrestris* extract (protodioscin) in normal and castrated rats. Life Sciences. 2002;71: 1385-96.
27. OECD, 2001. Guidelines for the Testing of Chemicals, 420. Acute Oral Toxicity-Fixed Dose Procedure. Organisation for Economic Cooperation and Development, Paris.
28. NRC. Guide for the Care and Use of Laboratory Animals. National Research Council (NRC), Academic Press, Washington DC, USA; 1996.
29. Swanston FSK, Day C, Flatt PR, Gould BJ, Bailey CJ. Glycemic effects of traditional European plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. Diabetes Research. 1989; 10:69-73.
30. Eberhart MS, Ogden C, Engelgan M, Cadwell B, Hedley AA, Saydah, SH. Prevalence of overweight and obesity among adults with diagnosed diabetes in United states 1988-1994 and 1999-2002, Morbidity Mortality Weekly Report. 2004; 53(Suppl 45):1066-8.
31. Davis SN. Insulin, Oral hypoglycaemics agents and pharmacology of the endocrine pancreas In: Brunton LL Lazo JS and Parker KL, editors. Goodman and Gilman's The Pharmacological basis of Therapeutics. Eleventh Edition. 2005; 1613-45.
32. Dabhi AS Bhatt NR and Shah MJ. Voglibose: An alpha glucosidase inhibitor. Journal of Clinical and Diagnostic Research. 2013;7(Suppl 12):3023-7.
33. Howard BV. Lipoprotein metabolism in diabetes mellitus. Journal of Lipid Research. 1987;28:613-28.
34. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001;50(Suppl 6):537-46.
35. Bohannon NJV. Lipid metabolism in type II diabetes mellitus. Postgraduate Medicine. 1992;92:2.
36. Asano N, Oseki K, Tomioka P, Kizu H, Matsui K. Nitrogen containing sugars from *Morus alba* and their glucosidase inhibitory activities. Carbohydrate Research. 1994; 259(Suppl 2):243-55.
37. Xiao-Shun S, Jin-Hai L, Jun T, Guo,ML, Huai-Den L, Ning M. Antihyperglycemic effects of total flavonoids from *Polygonatum odorantum* in STZ and alloxan induced diabetic rats. Journal of Ethnopharmacology. 2009;125:539-543.
38. Ribes G, Sauvaire Y, Da Costa C, Baccon JC, Loubatieres MMM. Antidiabetic effects of sub fractions from fenugreek seeds in diabetic dogs. Proceedings of the society of Experimental Biology and Medicine, 1986;182(Suppl 2):159-66.
39. Lenzen S, Panten U. Signal recognition by pancreatic B-cells. Biochem Pharmacol. 1988a;37:371-78.
40. Meglasson MD, Matschinsky FM. New perspectives on pancreatic islet glucokinase. Am J. Physiol. 1984;246:E1-E13.
41. Meglasson MD, Burch PT, Berner DK, Najafi H, Matschinsky FM. Identification of glucokinase as an alloxan-sensitive glucose sensor of the pancreatic B-cell. Diabetes. 1986;35:1163-73.
42. Lenzen S, Paten U. Alloxan: History and mechanism of action. Diabetologia. 1988b; 31:337-42.
43. Lenzen S, Tiedge M, Panten U. Glucokinase in pancreatic B-cells and its inhibition by alloxan. Acta Endocrinol. 1987;115:21-29.
44. Zawalich W, Beidler LM. Glucose and alloxan interactions in the pancreatic islets. American Journal of Physiology. 1973;224: 963-66.

45. Richard EH. The Prevention of alloxan-induced diabetes in mice by dimethyl sulphoxide. European Journal of Pharmacology. 1977;44(Suppl 2):191-93.
46. Richard EH, Felicitas SC. Protection against alloxan induced diabetes in mice by the hydroxyl radical scavenger dimethylurea. European Journal of Pharmacology. 1978;52(Suppl 1):57-60.
47. Richard EH, Hebert B, Gerald C. Prevention of alloxan-induced diabetes by ethanol administration. Journal of Pharmacology and Experimental Therapeutics. 1974;190:501-06.
48. Richard EH, Felicitas SC. The Prevention of alloxan-induced diabetes by amygdalin. Life Sciences. 1980;28:659-62.
49. Tajjudin A, Shamshad A, Abdul L, Iqbal AQ. Aphrodisiac activity of 50% ethanolic extract of *Myristica fragrans* Houtt. (Nutmeg) and *Syzigium aromaticum* (L) Merr. And Perry. (Clove) in male mice a comparative study. BMC Complementary and Alternative Medicine. 2003;3:6.

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