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RESEARCH ARTICLE

ANTIDIABETIC POTENTIAL OF DIETS CONTAINING V*ERNONIA AMYGDALINA* LEAVES IN STREPTOZOTOCIN- INDUCED DIABETIC WISTAR RATS

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ABSTRACT

This study determines antidiabetic potential of diets containing Vernonia amygdalina leaves in Streptozotocin- induced diabetic rat model. Fifty albino wistar rats were divided into five groups with 10 rats in each group**.** Group 1 and Group 2 (normal and diabetic control respectively) were fed with control diet; Group 3 and 4 (diabetic) were fed with diets containing *Vernonia amygdalina* leaves at 5% and 7.5%, respectively. Group 5 (diabetic) was fed with control diet and administered insulin. Feed and water were given *ad- libitum* for 28 days. Fasting blood glucose, plasma glucose, insulin, c-peptide, hemoglobin and glycosylated hemoglobin were determined using standard methods. Results *showed* that diabetic rats consuming *Vernonia amygdalina* leaves had significant (P<0.5) increase in plasma insulin, c-peptide and hemoglobin concentrations relative to the diabetic control. Diabetic rats consuming *Vernonia amygdalina* had significant (P < 0.5) reduction in the level of plasma glucose and glycosylated hemoglobin concentrations relative to the diabetic control. The results for *Vernonia amygdalina* diets were similar to insulin on the measured parameters except for plasma glucose concentration and their levels were not significantly different (P >0.5) when compared to the normal control. It was concluded that diets containing *Vernonia amygdalina* leaves are potential antidiabetic agents.

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INTRODUCTION

Diabetes mellitus is one of the fastest growing diseases in the world (WHO, 2008). The number of people affected by diabetes mellitus will soon reach 366 million worldwide by 2030 (WHO, 2008). Diet plays a prominent role in the management of diabetes mellitus. The use of diets to treat diabetes mellitus dates back to 3,500 B.C in Egypt and more than 2000 years ago in India (Ramachandran and Viswanathan, 1997; Murray and Pizzorno, 1990). In the eighteenth century it was observed that calorie restriction in the diabetic diet could reduce glycosuria in diabetes, and in the pre- insulin era, it was recommended that people with diabetes ate only a low-caloric diet to prevent ketoacidosis (Govindi and Myers, 1995). Over the years there have been tremendous improvements in the diets for diabetic people. The American Diabetes Association has made several recommendations regarding the dietary treatment of diabetes.

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These emphasize the importance of minimizing macrovascular and microvascular complications in people with diabetes. Four types of diets were reviewed for their effects on diabetes: the Mediterranean diet, a low-carbohydrate/high-protein diet, a vegan diet and a vegetarian diet. Each of the four types of diet has been shown to improve metabolic conditions. The degree of improvement however, varies from patient to patient (Khazrai *et al*., 2014), and this was attributed probably to patient's patho-physiological characteristics (Khazrai *et al*., 2014). However, most of these diets do not guarantee diabetic patients, flexibility in their eating habit (Khazrai *et al*., 2014) and it is now thought that failure to meet desired blood glucose level may be due in part to patience failure to cope with restricted dietary habit. It is important therefore, to provide a more flexible dietary regimen wherever possible in order to maximize the efficacy of the diet on reducing diabetes symptoms and to encourage patient adherence (Khazrai *et al*., 2014). Furthermore, there is need to reduce the multitude of diets currently being recommended and focus on a shorter list of useful regimens (Khazrai *et al*., 2014).

Many plants and herbs are consumed by man. Many of them have medicinal values and information is available on those that are used in folkloric treatment of diabetes mellitus. Scientific studies support most of these plants and herbs as potent antidiabetic agents. However, studies on medicinal plants have concentrated on plant extracts. The possible end-result of this innovation is the production of anti-diabetic drugs of plant origin, which may be expensive and out of reach of a good population of diabetics. Extracts are also found to have severe side effects. Since most medicinal plants are vegetables which have been used in the preparation of diets man has lived on over the years, it is suspected that consumption of such medicinal plant in diet may have prophylactic or therapeutic effects and may be well tolerated and devoid of side effects. Reports are limited on the prophylactic or therapeutic efficacy of such dietary preparations, which appear to present a household, available and accessible prophylactic and therapeutic options for diabetic treatment, especially in Africa, where most households eat such vegetables in diets and can easily prepare them. This study assessed the effect of consumption of diets containing *Vernonia amygdalina leaves* on blood glucose, hemoglobin, glycosylated hemoglobin, insulin, and c-peptide concentrations of Streptozotocin induced experimental diabetic Wistar rats, with the view to evaluating its antidiabetic potentials. Vernonia amygdalina and other medicinal plants contain some bioactive agents which are responsible for their antidiabetic effects. Consumption of such plants in diet may add these bioactive agents to the diet. This might provide a kind of "medicinal diet" that when eaten by people, prevents or treats their diabetes mellitus. This might allow patients to eat whatever they like provided such plant parts are added, and therefore provide more flexibility in the dietary habits of diabetics. This research is significant because it could help diabetics, physicians and nutritionists to improve clinical outcome and quality of life.

Vernonia amygdalina Del is popularly known as bitter leaf in most parts of Africa, due to its characteristic bitter and astringent taste (Akpan and Usoh, 2015). It is a species of vernonia and belongs to the family of compositae *(Asteraceace)* (Akpan and Usoh, 2015). There are more than 1500 species of vernonia occurring as herbs, shrubs or small trees in tropical America, Africa, Madagascar and Asia (Johri and Singh, 1997). *Vernonia amygdalina* has wide reputation in the folkloric treatment of diabetes mellitus. An ethno-botanical survey of plants used in the management of diabetes in South Western Nigeria, revealed that out of the 22 plants identified and documented, leaves and roots of *Vernonia amygdalina* were the ones more frequently used by traditional attendants (34% and 64% respectively) compared to other plants (Abo *et al*., 2000; Akpan and Usoh, 2015)). Scientific studies on the effect of extracts of *Vernonia amygdalina* in the management of diabetes mellitus have been reported by many authors. The extract of the leaves of the plant has both hypoglyceamic (Gyang *et al*., 2004) and antihyperglycemic effect on alloxan-induced diabetic rats (Akah *et al*., 2004). Akah *et al*. (2004) showed that the extract had positive impact on the lipid fragment in blood of the diabetic animals. Nimenido-Uadia (2003) reported that aqueous extract was significant in reducing fasting plasma glucose, serum lipid fractions and ketone bodies in alloxan-induced diabetic rats. The effect of the aqueous leaf extract on body weight and serum lipid management both in diabetic and non-diabetic rats was reported by Atangwho *et al*. (2007a) and Ekaidem *et al.* (2008). The leaf extract showed antihyperlipidemic and hypolipidemic effects. The effect of the leaf extract of *Vernonia amygdalina* in the management of macrovascular complications was compared to those of *Caranthus roseus* and chlorpropamide (Eteng *et al*., 2008). Furthermore, the ability of the leaf extract of *Vernonia amygdalina* to protect against internal tissue damage due to hyperglycemia has been widely investigated. Extracts from the leaves of *Vernonia amygdalina* were shown to protect the hepatocyte (Atangwho *et al*., 2007b) and Kidney (Atangwho *et al*., 2007c) against hyperglycemia induced damaged. Histology of the tissues corroborated results of biochemical indices. Nwanjo (2005) in his studies demonstrated the hepatocyte protective effect of the leaf extract of *Vernonia amygdalina* using malondialdehyde as a marker. The effect of dosage of the extract of *Vernonia amygdalina* in the management of diabetes mellitus has also been studied (Ekaidem *et al.,* 2008). Ekiadem *et al*. (2008) reported a non-dose dependent effect on blood glucose level but a dose dependent effect on HDL-cholesterol level. At higher dose, Atangwho *et al*. (2007a) demonstrated that the leaf extracts tend to precipitate hyponatremia (dilutional). Furthermore, the antidiabetic efficacy of a combination of extract of *Vernonia amygdalina* and those of *Azadirachta indica* (polyherbal therapy) was demonstrated by Ebong *et al*. (2008). In the study, it was shown that extracts from the two plants when combined produced a better gylcemic control and protection of the tissues, particularly the liver against damage, compared to the monotherapy. So far work done on *Vernonia amygdalina* Del in the management of diabetes have concentrated on the use of leaves and roots extracts. There are limited reports on the role of dietary intake of the leaves on diabetes mellitus. Akpan and Usoh (2015) recently investigated the role of diets containing the leaves of Vernonia amygdalina in the management of hematological and immunological derangement in diabetic rats.

MATERIALS AND METHODS

Collection and processing of plant materials

Fresh but matured leaves of *Vernonia amygdalina Del* were collected from the Endocrine Research Farm, University of Calabar, and from University of Calabar staff village, Calabar in March, 2011. These leaves were authenticated by a Taxonomist and Voucher Specimens were deposited in the herbarium in the Department of Botany, University of Calabar. The leaves were processed as in Akpan and Usoh (2015).

Formulation of Experimental Diets

Experimental diets were produced as in Akpan and Usoh (2015). Feed ingredients include: leaf powder, soybean meal, maize meal, Garri, mineral premix, vitamin premix, L-lysine L-methionine and corn oil. These feed ingredients were purchased from Victory Livestock ltd, an accredited Livestock feeds/ vaccines/drug dealers, located at 79, Aka road, Uyo, Akwa Ibom State. Standard rat chows (growers) were formulated according to the nutritional reguirement of rat (N.R.C., 1999) (Table 1). Three (3) different diets were

formulated namely: Control, VA-5%; VA-7.5%. Control diet differed from the other two diets because it did not contain leaf powder, but had all the other feed ingredients contained in the other diets. The other two diets contained leaf powder at five (5%) and seven and a half (7.5%) percent respectively. Diets were isocaloric and isonitrogenous. The percentage composition and nutrient analysis of the experimental diets are shown in Table 1.

Table 1. Percentage composition of experimental diets

Feed ingredients	Diets		
	Control	VA-5%	VA-7.5%
Soyabean meal	33.78	31.03	30.53
Garri	26	25	25
Maize meal	38	37	35
L- lysine	0.18	0.18	0.18
L-methionine	0.17	0.17	0.17
Min/vitamin	0.25	0.25	0.25
DCP	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00
Corn oil	0.25	0.25	0.25
V. amygdalina	-	5.00	5.00
Nutrient analysis:			
Cprotein	18.40	18.31	18.47
Cfat	4.30	4.01	3.97
Cfibre	3.71	4.21	4.58
ME(kcal/kg)	3219	3214	3213

Composition of premix:(nutrient in Amount in 2.5kg)Vit A (I.U) 12,000,000,vit D3 (I.U) 2,500,000, Vit E(mg) 20,000,vit K3(mg) 2,000,vit B1(mg) 2,000,vit B1 (mg) 5,000,Vit B6(mg) 4,000,vit B12(mg) 15,niacin(mg0 30,000,Pantotheic acid (mg) 11,000,Folic acid(mg) 1,500, Biotin(mg) 60,Choline chloride(mg)220,000, Antioxidant(mg) 1,250, Manganase (mg) 50,000, Zinc(mg) 40,000, Iron(mg) 20,000,Copper,(mg) 3,000,Iodine (mg) 1,000,Selenium (mg) 200,Cobalt(mg) 200

Animals

Seventy (70) albino rats of Wistar strain (female only) weighing between 83-121g were purchased from the animal house of the Faculty of Basic Medical Science, University of Uyo, Uyo, and transported in well ventilated cages to the animal house of the Department of Biochemistry University of Calabar, Cross River State, where they were kept throughout the duration of the experiment. The animals were allowed to acclimatize for two weeks. They were housed in well ventilated cages (wooden bottom and wire mesh top) and kept under controlled environmental conditions of temperature (25 ±5°C), relative humidity (50±5%) and twelve hour light/dark cycle. The animals were kept under the care of a trained animal technician and cared for according to Canadian Council on Animal Care (1993): Guide to the care and use of experimental animals. Animals were allowed free access to water and chow over a two weeks adaptation period. All animal experiments were performed in the laboratory according to the ethical guidelines suggested by the Institutional Animal Ethics Committee.

Experimental Design and Induction of Experimental Diabetes Mellitus

The design consisted of fifty (50) female rats divided into 4 groups of diabetic and 1 groups of normal rats with 10 animals in each group. Diabetic rats were obtained by subjecting some rats to an overnight fast (12 hrs) prior to administration of Streptozotocin. The weight of individual rats were measured and noted. Diabetes mellitus was induced in the diabetic groups by intraperitoneal injection of 55mg/kg body weight of Streptozotocin (STZ), (sigma St. Louis, MO. USA) reconstituted in 0.1% M sodium citrate buffer. The pH of the buffer was adjusted to 4.5. Rats whose fasting blood glucose concentration were higher or equal to 200 mg/dl three days after the induction were confirmed diabetic and recruited in the study. Group 1 (normal control, NC) was fed with control diet; Group 2 (diabetic control, DC) was fed with control diet; Group 3 (diabetic treated with 5%,VA, 5%,VA) was fed with 5% *Vernonia* amygdalina (VA) diet; Group 4 (diabetic treated with 7.5%, VA, 7.5% VA) was fed with 7.5% *Vernonia amygdalina (VA*) diet; Group 5 (diabetic treated with insulin, INSULIN) was fed with control diet and treated with insulin, a standard therapeutic agent, which was introduced for comparison. Insulin dose used was 5 U/kg body weight (b.w), given subcutaneously (s.c) according to Sonia and Scrinivasan (1999). It was given once per day at 4.00 pm. Treatment lasted for 28 days. During this period, blood glucose concentration was monitored every three days and noted.

Collection of sample for analysis

At the end of the 28 days, food and water were withdrawn. The rats fasted overnight. The following day, the rats were euthanized under chloroform vapor and sacrificed. Whole blood was collected via cardiac puncture using sterile syringes and needles into heparinized tubes and plasma was separated by centrifugation and used for biochemical assays.

Biochemical assays

Blood glucose was determined with assay kit (GDOPAP method) based on Barham and Trinder (1972), serum insulin by Anderson (1993), c-peptide concentration using microplate ELISA Kit of Diagnostic automation based on Ashby and Frier (1981**,** hemoglobin by Drabkin and Austin (1932), and glycosylated hemoglobin by Sudhakar and Pattabiraman (1981), proximate composition of feedstuffs and diets were determined by A.O.A.C. (2000).

Statistical analysis

The results were analyzed for statistical significance by one-way ANOVA using the SPSS statistical program and least square test (LSD) between group using MS excel programme. All data were expressed as mean + SEM. P value <0.05 was considered significant.

RESULTS

The blood glucose concentration monitored every three days during the 28-days of the experiment is shown in Figure 1. The results of the fasting plasma glucose, insulin, c-peptide, hemoglobin and glycosylated hemoglobin concentrations measured after 28 days of the experiment is shown in Table 2. The diabetic rats recorded fasting blood glucose concentrations that were higher than 200mg/dl three days after STZ administration. The rats in the diabetic control group gradually

increased their blood glucose level over the 28 days (pink line- Figure 1). There was 30 % mortality in the diabetic control group. Diabetic rats that were treated with *Vernonia amygdalina* diets maintained their high level of blood glucose for about 7 days, after which, their blood glucose level began to fall (yellow and brown lines- Figure 1). The blood glucose level of diabetic rats treated with insulin began to fall from the second day following insulin administration (blue line- Figure 1). The blood glucose level of normal rats was below the blood glucose level of the diabetic rats during the first seven days of commencement of the experiment (black line), but this line almost intercepted the diabetic treated group with 5% VA (yellow line) towards the last days of the experiment. The blood glucose level of the diabetic rats treated with insulin fell below those of normal control mid-way in the treatment. No mortality was recorded in the diabetic treated groups.

(P<0.05) when compared to those of the normal control (17.45±1.34 µU/ml, 17.05±0.46 ng/ml and13.40±0.04g/dL respectively) (Table 2). Treatment with the diets and insulin significantly decreased (P<0.05) the glycosylated hemoglobin and glucose concentration but significantly (P<0.05) increased the hemoglobin, insulin and c-peptide concentrations of the diabetic treated rats compared to the diabetic control rats (Table 2).

Comparisons of groups that received dietary treatments with insulin group on the measured parameters did not show any significant difference (P<0.05), except for glucose concentration which was significantly lower (p<0.05) for the insulin group tending towards hypoglycaemia.

Table 2. Effect of diets containing *Vernonia amygdalina* leaves on plasma glucose, insulin, c-peptide, hemoglobin and glycosylated hemoglobin

Treatment	Glucose(mg/dl)	Insulin((µU/ml))	c-peptide(ng/ml)	Total Hemoglobin(g/dl)	Glycosylated hemoglobin(mg/gHb)
Normal control	86.35 ± 4.23^{a}	17.45 ±1.34^{a}	17.50 ±0.46^{a}	13.40 ±0.04^{a}	3.54 ± 0.20^{a}
Diabetic control	289.35 ±6.21^{b}	8.79 ±0.65^{b}	4.39 ±1.24^{b}	8.76 ±0.27^{b}	11.54 ±0.12^{b}
VA- 5%	89.53 ±5.67^{a}	15.57 ±1.43^{a}	8.23 ±2.15^{c}	14.30 ±0.27^{a}	3.67 ±0.18^{a}
VA-7.55	98.43 ±4.21^{a}	15.34 ±1.34^{a}	9.12 ±0.93^{c}	13.10 ±0.13^{a}	3.21 ±0.05^{a}
Insulin	74.98 ±2.34^{c}	17.89 ±4.21^{a}	10.46 ±3.54^{c}	12.43 ±0.56^{a}	3.21 ±0.15^{a}

Results are expressed as mean ± S.D. for ten rats in each group. Values with different superscript (a-c) differ significantly with each other (P<0.05).

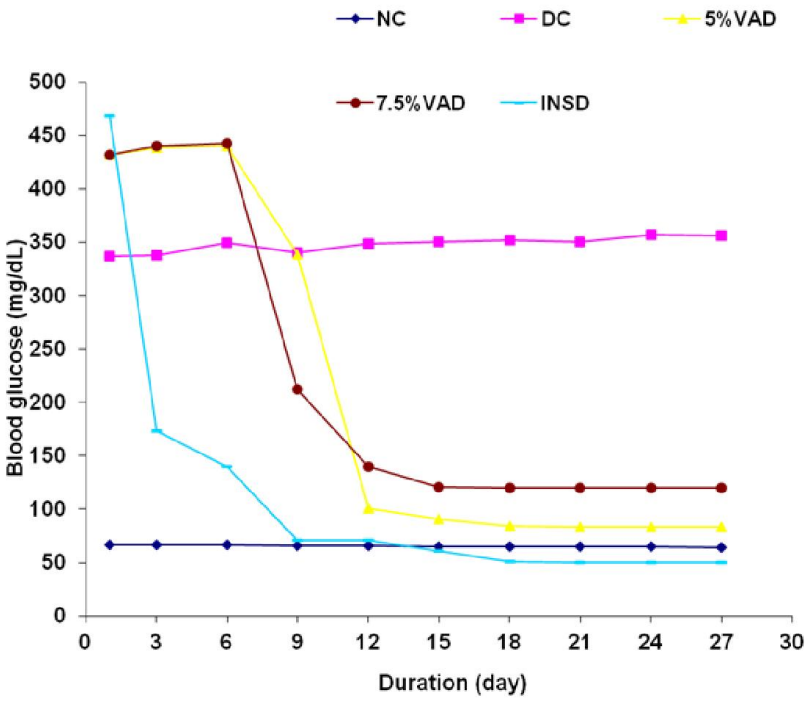

Fig. 1. Daily blood glucose levels of normal and diabetic rats. Mean values are expressed

Table 2 shows that the diabetic control rats had plasma glucose and glycosylated hemoglobin concentrations (289.35± 6.21mg/dL and 11.54±0.12mg/gHb respectively) that were significantly higher (P<0.05) when compared to the normal control (86.35±4.35mg/dL and 3.54±0.20mg/gHb). The diabetic control rats also had plasma insulin, c-peptide and hemoglobin concentrations (8.79±0.65µU/ml, 4.39±1.24ng/ml and 8.76±0.27g/dL respectively) that were significantly lower

DISCUSSION

The present study investigated the effect of consumption of diets containing *Vernonia amygdalina* leaves on plasma glucose, insulin, c-peptide, hemoglobin and glycosylated hemoglobin concentrations in diabetic wistar rats so as to evaluate the antidiabetic potential of the diets. The diabetic control rats in this study exhibited a significant increase in

blood glucose level compared to the normal rats. This result is in consistent with other such studies in rats (Al. Attar and Zari, 2007). Streptozotocin (STZ) is a pancreatic beta cell specific agent (Rodrigues, *et al*., 1999) that specifically destroys the beta cells via DNA alkylation, or increased free radical generation or nitric oxide production, or combination of any of the three mechanisms (Szkudelski, 2001) to induce a prototype of type-1 diabetes. The consequence of this was an increased blood glucose concentration above 200 mg/dl confirmed after 3 days of administration of STZ to the rats. The increased glucose level is due to the reduction in the level of insulin release due to the destruction of pancreatic β-cells by STZ. Numerous studies have demonstrated the antidiabetic efficacy of variety of plant extracts in STZ induced diabetic rats (Rajasekaran *et al*., 2005) but limited reports were available concerning such plants diets. We have observed a significant decrease in glucose level in diabetic rats consuming diets containing *Vernonia amygdalina* leaves when compared with the diabetic control (non-treated diabetic rats). Blood glucose lowering effect of the diets was not immediately after treatment as obtained with insulin, but started to fall after 7 days and progressed gradually until normal level of glucose was attained in another 7 days and beyond. It is unclear why the diets became effective at the 7 day, but we are thinking that it takes this time to obtain appreciable healing of the damaged pancreatic beta cells resulting in an improvement of pancreatic secretions.

Insulin and C-peptide are secreted into circulation in equal concentrations. The measurement of both insulin and C-peptide levels provides a better index for assessing insulin secretion and function rather than measuring insulin level alone (Doda, 1996). C-peptide and insulin levels were significantly decreased in the diabetic control rats because of the destruction of β-cells of pancreas by STZ. The significant increased in the levels of serum insulin and C-Peptide in the diabetic rats consuming *Vernonia amygdalina* diets compared to diabetic control rats shows that the diets stimulated the secretion of insulin from β-cells of pancreas. This could be possible only, if the cells are healed. The ability of medicinal plants to heal damaged cells of pancreas due to diabetes has been reported by several co-authors and our report is consistent with their findings (Ekaidem *et al*., 2008; Ebong, 2008).

However, *Vernonia amygdalina* contained a lot of biological active compounds that might act as insulin mimetics (Ezaki, 1990; Baker, 2001; Batell *et al*., 1999; Verma *et al*., 1998)). It is possible also that, the diets may act through this mimetics to directly increase glucose uptake. Our findings did support this possibility because of the duration it requires for the diet treatment to be effective (7 days). Except that the insulin mimetics needed time to bio-accumulate, it is more likely that this time was needed to heal the damaged pancreas. The improvement in the level of insulin and c-peptide secreted in the diet treated groups supports the healing of the damaged pancreas. Further work is needed to ascertain this. The possible mechanism of the antidiabetic effect of the diets is through healing of the damaged pancreas resulting in improvement in pancreatic secretion of insulin from beta cells of islets and enhancement of the transport of blood glucose to the peripheral tissue.

The significant decreased in the level of total hemoglobin observed in diabetic control rats might be due to the increased formation of glycosylated hemoglobin. Glycosylated hemoglobin level increase in uncontrolled diabetes in direct proportion to the fasting blood glucose level (Sen *et al*., 2005). Therefore, measurement of glycosylated hemoglobin is used as a standard biomarker for assessing glycemic control in patients with diabetes mellitus Fonseca, 2003). Consumption of diets containing *Vernonia amygdalina* reduced the formation of glycosylated hemoglobin in the diabetic treated rats compared to control. Since the level of glycosylated hemoglobin is the standard index for assessing blood glucose concentration in diabetes mellitus, the decreased level of glycosylated hemoglobin and the increased level of hemoglobin in the diets treated diabetic rats demonstrate the antidiabetic potential of diets containing *Vernonia amygdalina* leaves in diabetic wistar rats. In conclusion, consumption of diets containing Vernonia amygdalina leaves have been shown to be antidiabetic, antihyperglycemic, and normoglycemic in diabetic wistar rats and may play a significant role in the management of diabetes mellitus and diets for diabetic patients. Adding 5% or 7.5% of *Vernonia amygdalina* leaves to diabetic patient diets might allow diabetics to eat whatever they like. This is however subject to further studies. We provided these diets continuously; further studies are needed to examine the effect on diabetic rats, if the rats were to consume the diets occasionally or the effect on the blood glucose level when the diets are withdrawn for some time.

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