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RESEARCH ARTICLE

DIETS CONTAINING VERNONIA AMYGDALINA LEAVES ARE ANTIHYPERLIPIDEMIC AND ANTIATHEROGENIC IN STREPTOZOTOCIN INDUCED DIABETIC WISTAR RATS

***Akpan, H. D., Usoh, I. F. and Ewere, E. G.**

Department of Biochemistry, Faculty of Basic Medical Sciences, University of Uyo, Akwa Ibom State, Nigeria, P.M.B. 1017, Uyo, Nigeria

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ABSTRACT

The effect of consumption of diets containing Vernonia amygdalina (VA) leaves on lipid profile of diabetic wistar rats was investigated. Fifty (50) female wistar rats were randomly distributed into five groups of ten rats each. Group 1 (normal control) was fed with normal rat feed; Group 2 (diabetic control) was also fed with normal rat feed; Groups 3 and 4 (diabetic, diet-treated) were fed with diets containing 5% and 7.5% Vernonia amygdalina (VA) leaves respectively; Group 5 (diabetic, insulin-treated) was fed with normal feed and treated with insulin. The study lasted for a period of 28 days. Results revealed that treatment with VA-containing diets caused significant ($P<0.05$) increase in serum concentration of high density lipoprotein cholesterol (HDLC) when compared with the diabetic control. VA-containing diets, but not treatment with insulin significantly ($P<0.05$) reduced TC, TG, VLDLC, and LDLc. VA-containing diets also decreased atherogenic index (log (HDLc/TC) in the diet treated groups compared to the diabetic control. The VA-containing diets were more effective than insulin and showed no significant differences in all the lipid parameters when compared to those of the normal control. It could be concluded that diets containing Vernonia amygdalina leaves are antihyperlipidemic and antiatherogenic and i potential nutritional strategy against lipid abnormalities and cardiovascular diseases associated with diabetes mellitus.

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INTRODUCTION

Diabetes mellitus is a metabolic disease condition in which the body is incapable of synthesizing adequate or responding properly to insulin, a peptide hormone synthesized by the beta cells of the islet of langerhans of the pancreas. Insulin helps to absorb glucose from blood into cells for production of energy or storage as glycogen or fat. Absence or deficiency of insulin results in accumulation of glucose in blood (hyperglycemia). In diabetes mellitus, the body system either fails to respond to insulin, or is incapable of producing enough insulin or both. As a consequence, there is an abnormal increase in blood glucose level which often leads to various complications (Tierney *et al.,* 2002, Rother, 2007). Two main types of diabetes mellitus are type 1 diabetes (Insulin-dependent diabetes) and type 2 diabetes (Non-insulin-dependent diabetes). If not properly managed**,** chronic hyperglycemia of diabetes mellitus lead to hyperlipidemia that causes long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (Nabel, 2003; Nagappa *et al.,* 2003).

****Corresponding author: Akpan, H. D.***
Department of Biochemistry, Faculty of Basic Medical Sciences, University of Uyo, Akwa Ibom State, Nigeria, P.M.B. 1017, Uyo, Nigeria.

Hyperglycemia lead to hypertriglyceridemia due to overproduction of TG-rich lipoproteins in the liver, associated with decreased high-density lipoprotein (HDL) cholesterol levels, and decreased activities of adipose tissue and muscle lipoprotein lipase (Nabel, 2003). Reports have shown that cardiovascular disease is a cause of morbidity and mortality in patients with diabetes mellitus owing to disturbances in lipoproteins viz a viz high serum triglycerides (TC) level, high serum cholesterol level, high low-density lipoprotein (LDL) level, and low high density lipoprotein (HDL) level (Khan *et al.,* 2008; Gadi *et al*., 2007). Therefore treatment of diabetes mellitus has shifted from focusing on the treatment for hyperglyceamia alone to that incorporating treatment for dyslipidemia.

The use of conventional drugs for the management of diabetes mellitus has been criticized for various reasons such as high cost, availability and side effects prompting researches into other sources of medicament. Several reports have been made in recent years showing that vegetable intake combats the onset of diabetes mellitus and improves the plasma glucose control in diabetic patients. Akpan and Dan (2015) have reported the antidiabetic potential of diets containing leaves of *Vernonia amygdalina* in streptozotocin (STZ) induced diabetic

rats. Diets containing *Vernonia amygdalina* leaves (Akpan and Etim, 2015) and diets containing *Gongronema latifolium* leaves (Akpan and Ekpo, 2015) were found to be protective against oxidative stress and liver damage in STZ induced diabetic rats. Diets containing the leaves of these two plants were also found to be beneficial against hematological and immunological disturbances usually associated with diabetes mellitus (Akpan and Effiong, 2015, Akpan and Usoh, 2015). The effect of diets containing *Vernonia amygdalina* leaves on serum lipid profile of diabetic rats has not been previously reported. The present study was therefore designed to determine the effect of consumption of diets containing *Vernonia amygdalina* leaves on lipid profile of streptozotocin-induced diabetic rats with the view to evaluating the involvement of the diets in the management of dyslipidemia and cardiovascular diseases common among diabetics. This research is significant because it could help diabetics, physicians and nutritionists to improve clinical outcome and quality of life.

Vernonia amygdalina (VA) commonly called bitter leaf is a shrub of 2 – 5 m belonging to the family *Asteraceae.* The leaves are petiolated and are about 6 mm in diameter with elliptic shape. The leaves are greenish and have a characteristic odour and a bitter taste (Singha, 1996). The leaves are used as vegetable for soup making and most people macerate and wash the leaves before eating to get rid of the bitter taste. They are used as vegetable in meals to stimulate the digestive system, and as a treatment for fever. The leaves have been reported to contain flavonoids (Igile *et al.,* 1994; Udensi *et al.,* 2002; Tona *et al.,* 2004), oxalates, phytates, and tannins (Udensi *et al.,* 2002; Ejoh *et al.,* 2007; Eleyinmi *et al.,* 2008). The efficacy and safety of decoction of the leaf is used in traditional medicine as an antidiabetic remedy (Akah and Okafor, 1992; Akah *et al*., 2002**)**. The antidiabetic, antimalerial, antihelminthic and antibiotic properties of the extracts of this plant have been reported.

MATERIALS AND METHODS

Collection and Processing of Plant Materials

Fresh and matured leaves of *Vernonia amygdalina* were collected from the Endocrine Research Farm, University of Calabar in March 2011. They were authenticated in the herbarium of Botany Department, University of Calabar by a Taxonomist and Voucher Specimens were deposited. The leaves were processed to powder using the method of Akpan and Ekaidem, 2015 and stored in a properly labeled amber container in the refrigerator at temperature of 2-8^{o}C prior to its use for the preparation of rat chow.

Formulation of Experimental Diets

Standard rat chows (growers) were formulated according to rat nutritional requirements (National Research Council, 1995) as shown in Table 1 below. Three (3) different diets were formulated viz: normal control, 5% VA (diet containing 5% *Vernonia amygdalina* leaves) and 7.5% VA (diet containing 7.5% *Vernonia amygdalina* leaves). All diets were of equal calorie and nitrogen value. The percentage composition and nutrient analysis of the experimental diets are shown in Table 1 below.

Table 1. Percentage Composition and Nutrient Analysis of Diets

Feed Ingredients	Control	5%VA	7.5%VA
Soybean 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	1.00	1.00
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	7.5
Analysis			
CP	18.40	18.40	18.46
CFAT	4.30	4.14	4.16
CFIBRE	3.71	4.15	4.41
ME	3219	3218	3218

Experimental Animals

Albino Wistar rats (female only) of weights between 83-121g were acquired from the animal house of the Faculty of Basic Medical Sciences, University of Uyo, Uyo. The animals were allowed to acclimatize for two weeks according to the method of Akpan and Ekaidem (2015). Approval was obtained from the Ethics committee of the College of Basic Medical Sciences, University of Calabar and the animals were cared for according to the Canadian Council on Animal Care: Guide to the care and use of experimental animals, 1993 under the care of a trained animal technician. The experimental animals were fed with water and chow *ad libitum* over a two week adaptation period and closely monitored.

Experimental design and induction of experimental Diabetes Mellitus

The method of Akpan and Ekaidem (2015) was adopted for the experimental design and induction of diabetes mellitus. Fifty (50) female wistar rats were randomly selected for induction of diabetes mellitus. A day prior to induction, the rats were fasted overnight (12hrs) and the following day, the weights of individual rats were measured and noted. Induction of diabetes mellitus was done by intraperitoneal injection of 55mg/kg body weight of streptozotocin (STZ) (Sigma St. Louis, MO. U.S.A) 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 greater than or equal to 200mg/dl three days after the induction were confirmed diabetic and used for the study.

Experimental groups and treatments

The experimental groups and treatments are shown in Table 2.

There were ten animals (n=10) in each group. Insulin is a standard therapeutic agent for diabetes mellitus and was introduced for comparison. The dose of Insulin used was 5 U/Kg body weight (b.w), given subcutaneously (s.c) according to Sonia and Srinivasan (1999). It was given once per day by 4.00pm. Treatment lasted for 28days.

Table 2. Experimental groups and treatments

Groups	Nomenclature	Treatment
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% *Vernonia amygdalina* diet, 5% VA	was fed with 5% *Vernonia amygdalina* diet (VA) diet
Group 4	diabetic treated with 7.5% *Vernonia amygdalina* diet, 7.5% VA	was fed with 7.5% *Vernonia amygdalina* (VA) diet
Group 5	diabetic treated with insulin, INSD	diabetic treated with insulin, INSD

Table 3. Effect of Consumption of diets containing *Vernonia amygdalina leaves* on serum lipid profile of diabetic rats

Treatment	TC(mg/dl)	HDLc(mg/dl)	LOG TG/HDLc	TG(mg/dl)	VLDLc(mg/dl)	LDLc(mg/dl)
NC	37.45±7.26^a	77.70 ±17.64^a	0.27±0.40^a	145±7.10^a	65.95 ±5.76^a	106.15±3.45^a
DC	47.07±0.70^b	45.35 ±10.10^b	0.69±7.60^b	227±76.76^b	103.18 ± 10.21^b	123.40±7.09^b
5%VAD	43.48±3.18^a	77.78 ±9.12^a	0.21±3.40^a	128±10.85^a	58.18 ±5.78^a	92.48 ±2.10^c
7.5%VAD	43.48±3.18^a	60.00 ±0.00^c	0.28±0.01^a	116±32.41^a	57.27 ±23.65^a	63.37 ±6.74^c
INSD	38.98±5.13^a	60.81 ±0.68^a	0.38±0.01^a	146±90.75^a	91.81±7.45^a	103.64±2.31^a

Sample Collection for Analysis

At the end of the 28 days treatment, food and water were withdrawn and the rats were fasted overnight. The following morning, the rats were euthanized under chloroform vapour and sacrificed. Whole blood was collected via cardiac puncture using sterile syringes and needles. The blood was emptied into plain tubes under septic condition and allowed to clot for about two hours. The clotted blood was thereafter centrifuged at 3,000rpm for 10 minutes to recover serum from clotted cells. Serum was separated with sterile syrings and needles and stored frozen at -20^{0}C until used for biochemical analysis of high density lipoprotein cholesterol (HDLc), total cholesterol (TC), triglyceride (TG).

Biochemical assays

Total cholesterol was measured by enzymatic methods of Allain *et al* (1974) with randox cholesterol kit (Randox England). Triglyceride concentrations were determined by enzymatic colourimetric assay using reagent kits from Dialab Production, France. High density lipoprotein cholesterol (HDLc)- was determined by HDLc precipitant method of Lopes *et al* (1977). LDLc was calculated by the formula of Friedewald (1972). VLDL- cholesterol was calculated using appropriate relationship VLDLc=TG/2.2. Blood glucose concentration was measured using one touch Glucometer (Lifescan, Inc. 1995, Milpas, California, U.S.A) and by the glucose oxidase method of Barham and Trinder (1972).

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 effect of consumption of diets containing *Vernonia amygdalina* leaves on serum lipid profile of the experimental rats is shown in Table 3.

Table 3 shows that rats in the diabetic control had significantly (P<0.05) lower level of HDL_C (45.35 ±10.10mg/dl) compared to normal control (77.70 ±7.64mg/dl). Total cholesterol concentration was significantly higher (P<0.05) for the diabetic rats (47.07 ±0.70mg/dl) compared to the normal control (37.45 ±7.26mg/dl). Log TG/HDL_C (atherogenic index) was significantly higher for the diabetic control rats (0.69 ±7.60) compared to the normal control (0.27 ±0.40). TG, VLDLc and LDLc were significantly higher (P<0.05) for the diabetic control rats (227.00 ±76.76, 103.18 ±10.21 and123.40 ±7.09mg/dl respectively) compared to the normal rats (145.00 ±7.10, 65.95 ±5.76, and 106.15 ±3.45mg/dl respectively).

Diabetic rats placed on diet containing 5% *Vernonia amygdalina* had significantly increased (p<0.05) in the HDL_C level (77.78 ± 9.12mg/dl) relative to the diabetic control (45.35 ±10.10mg/dl). Diabetic rats consuming diet containing 5% *Vernonia amygdalina* and 7.5% *Vernonia amygdalina* as well as diabetic rats on insulin treatment had significantly (P<0.05) reduction in the TC, TG, $VLDL_C$, and LDLc levels compared to the diabetic control. The effect of diets on the measured lipid parameters was not different compared to the insulin except for the LDLc which shows that treatment with the diets had greater impact than insulin.

DISCUSSION

The present study was designed to investigate the effect of adding *Vernonia amygdalina* leaves at 5% and 7.5% to diet of diabetic rats on the lipid profile of diabetic rats so as to evaluate its involvement in the management of dyslipidemia and cardiovascular conditions associated with diabetes mellitus. Accelerated astherosclerosis among diabetics is a major pathologic cause of macrovascular complications resulting in increased risk of myocardial infarction, stroke and lower extremity gangrene. Experimental and clinical evidences suggest that these complications are promoted by dyslipidemia (Goldberg, 2001). In this study the serum lipids of diabetic control rats (without consuming *Vernonia amygdalina* diets) were compared to those receiving *Vernonia amygdalina* diets at 5%VA and 7.5%VA) inclusion level, insulin, and normal

rats (NC). Table 3 shows that the diabetic control rats had significantly ($p<0.05$) lower serum concentration of HDL_C, higher serum concentration of TG, TC, $VLDL_C$ and LDL_C. The atherogenic index, ratio of $logTG/HDL_C$ was significantly higher ($p<0.05$) for the diabetic control rats compared to the treated groups. This shows a derangement in lipid profile which is due to derangement in metabolic activities as a result of hyperglycaemia of diabetes mellitus. The diabetic control rats were at risk of coronary artery diseases in view of well established association between cardiovascular risk and high level of serum LDLc (Gastelli, 1988; Igweh *et al.,* 2005), TG (Nwagha and Igweh, 2005) and low level of HDLc (Gastelli, 1988; Igweh *et al.,* 2005).

It was interesting to observe that diabetic rats that were placed on chronic consumption of the leaves both at 5% and 7.5% inclusion level did not show any derangement in the lipid profile. Following 28 days of dietary consumption of *Vernonia amygdalina leaves*, significant decreases were obtained for TG, TC, $VLDL_C$, and LDL_C concentrations and for the atherogenic index but a significant increase for the HDL_C concentration of the diabetic rats on the diets. The results show that diets containing 5% and 7.5% *Vernonia amygdalina* leaves are antihyperlipidemic and antiatherogenic. This study supports other findings that consumption of vegetables may be beneficial in the management of derangement of lipid profile in diabetes mellitus. Serum lipid abnormalities are an increasing concern and reason for medication use in diabetes. Certain principles like viscous fibers, proteins and sterols that are components of most vegetables are reported to be responsible for improving the blood lipid profile. It is possible that *Vernonia amygdalina* leaves may contain some factors that exhibit this significant metabolic benefit on blood lipid profile.

Conclusion

From the findings of the present study, it could be concluded that diets containing *Vernonia amygdalina* leaves at 5% and 7.5% inclusion level possess antihyperlipidemic and antiatherogenic effects that may be beneficial in the management of lipid profile perturbations with attendant cardiovascular diseases associated with diabetes mellitus.

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