

EFFICACY OF AQUEOUS LEAF EXTRACT OF *VERNONIA AMYGDALINA* ON PLASMA LIPOPROTEIN AND OXIDATIVE STATUS IN DIABETIC RAT MODELS.

H. U. NWANJO

Department of Medical Laboratory Sciences, Imo State University, Owerri

Summary: Many minor components of foods, such as secondary plant metabolites, have been shown to possess antioxidant activities, improving the effects of oxidative stress on diabetes and other disease conditions. This study evaluates the effect of aqueous extracts from *Vernonia amygdalina* leaves on lipid profiles and oxidative stress in streptozotocin induced diabetic rats. The results showed that the streptozotocin induced diabetic rats were subjected to oxidative stress as was shown by the extent of lipid peroxidation (high malondialdehyde levels) present in the plasma. The aqueous extract of *V. amygdalina* leaves possessed antioxidant activity as shown by decreases in malondialdehyde levels. High values of LDL-cholesterol and triglycerides levels, which are typical of the diabetic condition, were also found in streptozotocin induced diabetic rats. The aqueous extract also significantly reduced triglyceride levels and normalized cholesterol concentrations. This shows that the aqueous extract of *V. amygdalina* leaves have both hypolipidaemic and antioxidant properties.

Key Words: *Vernonia amygdalina*, lipoproteins, oxidative status, Diabetic rats.

Introduction

Diabetes mellitus has been defined by the world health organization (WHO), on the basis of laboratory findings, as a fasting venous plasma glucose concentration greater than 7.8 mmol/l (140mg/dl) or greater than 11.1 mmol/l (200mg/dl) two hours after a carbohydrate meal or two hours after an oral ingestion of the equivalent of 75g glucose, even if the fasting concentration is normal (Nwanjo, 2004). It is a metabolic disease characterized by hyperglycaemia and glycosuria due to absolute or relative lack of insulin (Aguwa, 1996).

Changes in lipid concentration and consequent disorders of lipid metabolism have been observed in diabetes mellitus (Ononogbu, 1988). With Ketosis of diabetes mellitus, hyperlipidaemia and hypercholesterolaemia may lead to increased level of lipid peroxidation. This enhances the oxidation of lipids and lipoproteins exposing a diabetic to dangers of atherosclerosis (Halliwell, 1990). Hence there may be an elevated lipid peroxidation in the plasma of diabetic patients. Supportive evidence to this approach was the finding of increased concentration of plasma lipid peroxides in diabetic patients with angiopathy (Sato and Hotta, 1979), hyperlipidaemic patients (Loeper *et al*, 1983) and patients with acute myocardial infarction (Loeper *et al*, 1987).

The non-pharmacological means (diet and exercise) and/or the pharmacological means (insulin and oral hypoglycaemics) may be used in the management of diabetes mellitus. The obvious limitations of these management methods necessitate a search for alternatives among the arsenal of herbs available to man. It was in this light that the World Health Assembly, in 1989, adopted among its resolutions, the support of national traditional medicine program, drawing attention to herbal medicines as being of great importance to the health of individuals and communities.

Many minor components of foods, such as secondary metabolites, have been shown to alter biological processes which may reduce the risk of chronic diseases in humans. *Vernonia amygdalina* (*compositae*) an edible rainforest plant native to the south Eastern part of Nigeria, has been widely used in folk medicine as anti-malaria, purgative, anti-parasitic, treatment of eczema and for maintaining healthy blood glucose levels (Nwanjo and Nwokoro, 2004).

In this study the possible hypocholesterolaemic potential and antioxidant property of *V. amygdalina* were investigated for its possible use for prophylaxis and treatment of atherosclerosis commonly found in diabetic diseases.

Materials and Method

Plant Materials

The fresh leaves of *V. amygdalina* were collected from the natural habitat in Owerri, Imo State, Nigeria in January 2005. Botanical identity was confirmed by Dr. C. Okeke (Head, Department of Plant Science and Biotechnology, Imo State University, Owerri). A voucher specimen of the plant is deposited in Imo State University, Owerri.

Preparation of Extract

Fresh leaves of *V. amygdalina* were collected and sorted to remove the dead ones, washed without squeezing to remove debris and dust particles. Large quantities of the leaves were collected and sun-dried for four days. The dried leaves were milled to get a course powder used for the extraction. 25g of the powder were macerated in a percolator with 250ml of distilled water. The mixture was allowed to stand for 24 hours after which it was filtered. The filtrate was then placed in an oven to evaporate and the solid residue (2.4g) referred to as extract. Appropriate concentrations of the extract were made in distilled water for experiments.

Acute Toxicity Test

The acute toxicity (LD₅₀) of the extract was estimated in 30 Wistar albino rats by the I.P. route as described by Miller and Tainter, (1944). In brief, the method involved the administration of 5 different doses of the extract to 5 groups of rats (6 rats/group). The number of deaths in each group within 24 hours was recorded. The LD₅₀ was estimated from the graph of percentage (%) mortality (converted to probit) against log-dose of the extract-probit 5 being 50%.

Phytochemical Tests

The chemical classes of constituent in the freshly prepared extract were detected using standard phytochemical reagents and procedures as described by Trease and Evans (1983). In general, tests for the presence or absence of phytochemical compound using the above methods involve the addition of an appropriate chemical agent to the crude material in a test tube. The mixture is then shaken vigorously or gently as the case may be. The presence or absence of saponins, flavonoids saponins, tannins, alkaloids etc. was observed.

Animals

Adult Wistar albino rats weighing 150-200g of either sex maintained at room temperature of 30°C in the animal house of

College Medicine and Health Sciences, Imo State University, Owerri, were used for this study. The animals were fed with standard diet (product of Pfizer Nigeria Ltd). The food was withheld 12 hours before the experiments, but there was free access to water.

Experimental Design

Eighteen rats included for this study were divided into 3 groups, each consisting of six animals. Out of the 3 groups, 2 were made diabetic by intraperitoneal injection of 65mg/kg body weight of streptozotocin (STZ) (Sigma, St. Louis, MO, USA) dissolved in citrate buffer (0.01M, pH 4.5). Diabetes was confirmed by determination of fasting blood glucose concentration on the third day post administration of STZ showing fasting blood glucose levels above 250mg/dl.

Body weight and fasting blood glucose levels of all the rats were determined before the start of the experiment. Rats were divided into the following groups.

Group 1: Control given only the citrate buffer (0.01M, pH 4.5)

Group 2: Streptozotocin induced diabetic, made with a single dose of streptozotocin (65mg/kg body weight) by intraperitoneal route.

Group 3: Diabetic rats treated with *V. amygdalina* 200mg/kg/twice a day,

Treatment was by oral compulsion. After 14 days of treatment the body weight and fasting blood glucose of the animals were again determined. Blood was collected and transferred on to a centrifuge tube for serum separation.

Analytical Procedure

Twelve hours after the last treatment and after the last food given, 6ml of blood was collected from all the rats. The blood collected was transferred on to a centrifuge tube and allowed for 30min to clot. The clotted blood was then centrifuged using Wisperfuge model 1384 centrifuge (Tamson, Holland) for 5min to facilitate separation. The serum thus obtained was used for malondialdehyde level MDA (product of lipid peroxidation), total cholesterol, HDL-cholesterol and triglycerides estimations.

Serum MDA was measured by a thiobarbituric acid assay procedure (Albro *et al*, 1986), which was calibrated using 1,1,3,3, -tetraethoxypropane (Sigma Chemicals, St. Louis, MO, USA.) as a standard. Results were expressed as nanomoles of MDA per millimeter of serum. Serum total cholesterol was estimated using method of Zak (1959), plasma triglyceride estimated by method of

Mendez *et al.*, (1975), HDL-C by Lopez-Vitrella *et al.*, (1975), LDL-C and VLDL-triglyceride values were calculated by a modification of the Friedewald formula (Sandkapm, 1990).

Statistical Analysis

All values were expressed as mean \pm SD. The statistical analysis was carried out using students' t-test to detect differences in the concentrations of serum MDA and lipoproteins between different groups. Tests with a probability value <0.05 were considered statistically significant.

Results

The results of the phytochemical analysis revealed the presence of alkaloids, carbohydrates, tannins, saponins, flavonoids and glycosides. Cyanogenic glycoside was however absent. Acute toxicity test in rats gave an LD₅₀ of 1265.22 \pm 56 mg/kg. From the results of the toxicity studies, convenient doses were chosen to preclude the lethal range.

Table I shows that there was a significant increase ($p<0.05$) in fasting blood glucose and decrease ($p<0.05$) in body weight in streptozotocin involved diabetic rats when compared with the normal control. The body weight slightly increased and the fasting blood glucose significantly decreased in diabetic rats when treated with aqueous leaf extract of *Vernonia amygdalina*.

In Table 2, the mean value of serum MDA levels (end-product of lipid peroxidation) in diabetic rat significantly increased when compared with the control but significantly decreased in diabetic rats treated with the aqueous leaf extract of *V. amygdalina*.

The mean values of triglycerides and LDL-cholesterol ($p<0.05$) were significantly higher in diabetic control rats compared to the treated diabetic and normal rats. Total cholesterol and HDL-cholesterol levels of diabetic rats treated with aqueous extract of *V. amygdalina* leaf were not significantly different when compared with the normal control rats and diabetic control rats.

Table 1: The mean values of body weight and blood glucose in both normal, diabetic and *V. amygdalina* treated diabetic rats. Values are expressed as mean \pm SD. (n = 6).

Body weight	Mean initial weight (g)	Mean final weight (g)	Mean weight gained (g)	Fasting Blood glucose (mg/dl)	
				Initial	After 4 weeks
Normal Control	135.4 \pm 12.1	171.5 \pm 10.	36.1 \pm 2.2	84.20 \pm 2.8	90.24 \pm 3.1
Diabetic control	137.2 \pm 9.9	124.9 \pm 13.0	-12.3 \pm 2.1*	251.88 \pm 4.6	268.24 \pm 4.8
Treated diabetic rats	136.9 \pm 10.5	130.1 \pm 11.1	-6.8 \pm 2.23**	253.82 \pm 3.8	128.62 \pm 4.6 [‡]

* Significantly different from normal control group ($p<0.05$). ** Significantly different from normal and diabetic controls ($p<0.05$). [‡] Significantly different from Initial Fasting Blood glucose ($p<0.05$).

Table 2: The mean values of lipid peroxide and vitamins E and C in both normal, diabetic and *V. amygdalina* treated diabetic rats. Values are expressed as mean \pm SD. (n = 6).

	Normal control	Diabetic control	Treated diabetic rats
MDA (nmol/ml)	3.28 \pm 0.26	*5.83 \pm 0.49	3.92 \pm 0.43
Total cholesterol (mg/dl)	105.40 \pm 0.36	115.0 \pm 0.36	101.52 \pm 1.25
HDL- cholesterol (mg/dl)	33.25 \pm 3.34	30.5 \pm 0.70	34.86 \pm 0.66
LDL- cholesterol (mg/dl)	51.45 \pm 1.22	**62.32 \pm 1.34	*47.76 \pm 1.65
Triglyceride (mg/dl)	91.0 \pm 0.36	**110.9 \pm 0.69	*94.5 \pm 0.43

* Significantly different from normal control group ($p<0.05$)

** Significantly different from normal control and treated diabetic rats ($p<0.05$)

Discussion

The results of this study clearly indicate that the administration of aqueous leaf extract of *V. amygdalina* produced both hypoglycaemic, hypolipidaemic and antioxidant effect. There are many bioactive constituents present in the extract and hence, at present, it is not certain, which of them is/are responsible for the observed effects. However, some reports have shown that flavonoids, tannins and saponins may play some roles in antioxidative and hypolipidaemic effect (Ezekwe and Obidoa, 2001). This study showed that extract produced a marked decrease in blood glucose of diabetic rats. These findings had been reported previously in our study (Nwanjo and Nwokoro, 2004).

The increase in serum triglycerides, LDL-cholesterol and serum MDA in diabetic controls are in conformation with previous reports documenting elevated serum triglyceride and lipid peroxide levels in diabetic subjects (Oberley, 1988). In this study administration of aqueous extract of *V. amygdalina* leaf significantly reduced triglyceride and suppressed free radical-induced oxidative damage.

These hypolipidaemic and lowering of oxidative stress may be due to decreased oxidative load. It may also act by either directly scavenging the reactive oxygen metabolites, due to the presence of various antioxidant compounds (Gupta *et al*, 2002), or by increasing the synthesis of antioxidant molecules. Consistent with the previous report that *V. amygdalina* has hypoglycaemic and hypolipidaemic effect, this study has shown that it suppresses oxidative stress. This warrants further study on the effect of *V. amygdalina* on other diseases that involve free-radical-induced oxidative damage.

Acknowledgement

The authors are grateful to Dr. C. Okeke, Head of Department of plant Science and Biotechnology, Imo State University, Owerri for the identification of the plants and Asso. Prof. E.A. Nwokoro, Head, Department of medical Laboratory Science of the same University reviewing this work.

References

- Aguwa, C.N. (1996). Diabetes mellitus. In: Therapeutic Basis of clinical pharmacy in the Tropics. Optimal Publishers, Enugu, Nigeria. Pp 1-453.
- Albro, P.W., Corbalt, J.T. and Schroeder, J.L. (1986). Application of the thiobarbiturate assay to the measurement of lipid products in microsomes. *Chem. Biol. Interact.* 86: 185-194.
- Ezekwe, C. I. and Obidoa, O. (2001). Biochemical effect of *Vernonia amygdalina* on rats liver microsomes. *Nigeria Journal of Biochemistry and Molecular Biology.* 16(3): 1745-1798.
- Gupta, S. K., Prakash, J. and Srivastava, S. (2002). Validation of traditional claim of Tulsi, *Ocimum sanctum* linn. as a medicinal plant. *Indian J. Experimental Biol.* 40:765-773.
- Halliwell, B. (1990). How to characterize a biological antioxidant. *Free Radical Res. Commun.* 9:1-32.
- Loeper, J. and Bedu, O. (1987). Lipid peroxidation and protective enzyme during the course of myocardial infarction. *Agents Actions* 22: 340 – 342.
- Loeper, J.; Emetit, J.; Groy J. and Bedu, O. (1993) Lipid peroxidation during human, atherosclerosis. *IRCS J. Med.* 11: 1034 – 1035.
- Lopez-Vitrella, M. F., Stone, P., Ellis, S., and Colwell, J. A. (1977). Cholesterol determination in high density lipoprotein, separated by three different methods. *Clin. Chem.* 23: 882-884.
- Mendez, A., Franklein J., and Slahegan, B. H. (1975). Simple manual method for determination of serum triglycerides. *Clin. Chem.* 21: 760-770.
- Miller, L. C. and Tainter, M. L. (1944). Estimation of EC50 and its error by means of log-probit graph paper. *Proc. Soc. Exp. Biol. Med.* 57: 261-269.
- Nwanjo, H.U. and Nwokoro, E.A. (2004). Antidiabetic and biochemical effects of aqueous extract of *Vernonia amygdalina* leaf in normoglycaemic and diabetic rats. *J. Innov. Life Sci.* 7: 6-10.
- Ononogbu, I. C. (1988). The role of lipid in the study and diagnosis of diabetes mellitus. *Proceedings of the 1st African Conference on Biochemistry of lipids 1:* 57 – 69.
- Sandkamp, M., Funke, H., Schuster, M., Kahlar, E. and Assman, G. (1990). Lipoprotein(a) is an independent risk factor for myocardial infarction at a young age. *Clin Chem.* 36: 20-23.
- Sato, Y. and Hotta-Nsoka, M. (1979). Lipid peroxide card in plasma of diabetic patients *Biochem. Med.* 21: 104 – 107
- Trease, G.E. and Evans, M.C. (1983). *Textbook of Pharmacognosy.* 12th edition Bailliere, Tindall, London, pp 343-383.
- Zak, B. (1959). Determination of total cholesterol using reaction with ferric chloride and sulphuric acid. *Am. J. Clin Path.* 27: 583-590.

Received: 24/9/2005

Accepted: 26/10/2005