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VERNONIA AMYGDALINA DEL: A POTENTIAL PROPHYLACTIC ANTI- DIABETIC AGENT IN LIPID COMPLICATION

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ABSTRACT

In a study to evaluate the potentials of some indigenous African herbs in ameliorating hyperlipidaemia, a complication of diabetes mellitus, aqueous extracts of *Vernonia amygdalina Del* (African bitter leaf), were administered enterally by orogastric intubation to 2 groups of rats (alloxan induced diabetic and non-diabetic groups) at a dose 400mg/kg body weight for a 21- day period. Both the diabetic and non-diabetic control groups received placebo treatment. Lipid fractions including TC, TG, HDL, VLDL and LDL were assayed in serum obtained from treated as well as control animals. Both diabetic and non-diabetic treatment groups showed significant reductions ($P<0.05$) in serum levels of TC, TG, VLDL and LDL but increase in HDL relative to their respective controls. However, treated non-diabetic animals showed a significant ($P<0.05$) decrease in weight, whereas diabetic treated animals gradually gained weight. The extract is both hypolipidaemic (prophylactic) and antihyperlipidaemic (curative). *Vernonia amygdalinaDel* could reduce body weight and circulating lipid fractions, risk factors in aetiology of type II DM as well as prevent or delay onset of complications of lipid metabolism in diabetes, through its antihyperlipdaemic effects.

KEYWORDS: *Vernonia amygdalina Del*, Hypolipidaemic, Antihyperlipidaemic, Diabetes mellitus.

INTRODUCTION

Cardiovascular disease (CVD) or complication resulting from artherosclerosis is the leading cause of death in diabetes (Choate, 1999). World Health Organization report that approximately 60% of diabetics (50% in industrial countries) die of vascular disease and 35% of coronary heart disease (WHO, 2004). Artherosclerosis, however derives/stems largely from abnormal lipid metabolism either of dietary or pathological origin.

Lipoprotein abnormalities including hypercholesterolemia and hypertriglyceridemia, are prominent features in chronic diabetes (Scoppola et al, 2001) due mainly to insulin deficiency or insensitivity in target tissues (Meyes, 2003). Hypercholesterolemia and hypertriglyceridemia have been identified as necessary indices for glucose intolerance usually observed in type II diabetes (Gaw et al, 1994). Additionally, the increased Advance Glycosylation End products (AGE) formed as a consequence of sustained hyperglycemia tend to lower High Density Lipoprotein (LDL) levels (protective molecule against artherosclerosis (Robinson and Johnston, 1997).

The prevailing excess triglycerides and cholesterol together with the Advanced Glycosylation Endproduct(AGEs) react with collagen in the intima of arteries to precipitate artherosclerosis and related cardiovascular complications (Bierman, 1992). The decreased protection against artherosclerosis due to decreased levels of HDL enhances the process. More so, diabetics have been reported to have increased platelet adhesiveness to the walls of the arteries, possibly due to increased thromboxane A_2 synthesis and reduced prostacyclin (Cotran et al, 1999).

Furthermore, most chemotherapeutics with hypoglycemic activity in common use in the management of diabetes mellitus, including sulfonylurea, meglitinides, thiazolidinediones and insulin aggravate this situation in patients. These treatments cause undue weight gain (Luna and Feinglos, 2001), perhaps via enhanced lipoprotein metabolism. Therapy with these agents does not only place the subject at increased risk of complications but also slows response to a long-term

This work therefore investigates the effect of whole leaves of *Vernonia amygdalina Del* used in the phytherapy of diabetes mellitus on hyperlipidemia of diabetic rats, with a view to ascertaining its contribution to artherogenesis and/or cardiovascular complications. *Veronia amygdalina* is an indigenous African herb with a high reputation as an anti diabetic plant in folk medicine Infact it is only second *Casia alata* of the several herbs used by traditional healer of Southern Nigeria in the management of diabetes (Abo et al., 2000).

MATERIAL AND METHODS

Plant Material

Whole fresh leaves of *Vernonia amygdalina Del* (100g), harvested from the Endocrine Research Farm, University of Calabar, Calabar – Nigeria were ground with a blender (National, France) in 400ml of distilled water and stored overnight (4^0c) The suspension was then filtered using chess clothe and the filtrate obtained

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after the residue is removed was used as the aqueous crude drug. The concentration of this crude extract was determined gravimetrically by estimating the weight of the residue in a known volume of drug after complete evaporation of the vehicle.

Animals and Animal Treatment

Twenty-four rats (male and females) of *Wistar* strain weighing between 100-165g obtained from the Animal House, Department of Biochemistry, University of Calabar were used for this study. They were allowed one week to acclimatize after which they were reweighed and housed in plastic cages with plastic bottom and wire mesh top (North Kent Co. Ltd) under standard conditions (28 ± 2^0c ambient temperature and 46% relative humidity with a 12 hour light – dark cycle).

The animal room was adequately ventilated. Animals were fed with commercial rat chow and tap water *ad libitum*.

Diabetes was experimentally induced by intraperitoneal injection of 150mg/kg body weight of alloxan monohydrate (Sigma, St. Louis, MO, USA) in distilled water as the vehicle(Nimenibo-Uadia,20030). Tail vein blood glucose (Random Blood Glucose, RBG) levels, were measured with a Glucometer analyzer (One – Touch, Basic) four days after. Animals with RBG levels ≥ 200mg/dl were assigned into the diabetic study groups. Normal non-diabetic animals were also assigned into study groups. In all, they were 4 groups of five rats each: Group 1 animals served as healthy control (NC), while those of group 3 were untreated diabetic control (DC) and both received placebo treatment (distilled water). Groups 2 and 4 respectively consisted of healthy and diabetic animals both gavaged with 400mg/kg body weight of the crude drug. Treatment lasted for 21days.

Twelve hours after last administration and feeding (overnight), the animals were anaesthetized under chloroform vapor and then dissected. Whole blood was collected by cardiac puncture from which serum was obtained and used for lipid profile and blood glucose assays.

Biochemical Assays

Serum glucose levels were assayed spectrophotometrically (Optima SP- . 300, Japan), using Dialab Diagnostic glucose oxidase kit. The method originally described by Barham and Trinder, (1972) is based on the oxidation of glucose in the presence of glucose oxidase to form gluconic acid and hydrogen peroxide. The H_2O_2 so formed reacts with phenol and 4 – aminophenozone in the presence of a peroxidase to form a quinoneimine dye whose colour intensity is proportional to the glucose concentration in the sample.

Triglycerides in serum were estimated by GPO – PAP with ATCS method (Cole *et al.*, 1997) as adapted in Dialab kits. The principle entails enzymatic hydrolysis of triglyceride. The glycerol formed goes through a reaction series to produce H_2O_2 which is quantified via an indicator system. The concentration of H_2O_2, and hence the colour intensity is in proportion to triglyceride concentration in the samples. Total cholesterol (TC) was evaluated by CHOD – PAP with ATCS method of Dialab kit based on Richmond, 1973; Thomas, 1984. In the method, cholesterol esters are hydrolyzed to fatty acids and free cholesterol. The latter, through a series of a reaction sequence produces H_2O_2 which is also quantified using a quinoneimine indicator

High density lipoprotein cholesterol (HDL-C) was estimated by direct – immunoinhibition method of Dialab kit. In the method, chylomicrons, VLDL and LDL are adsorbed by antibodies, leaving free HDL which is quantified using an enzymatic system.

VLDL and LDL – cholesterols were obtained by calculation using the empirical relationships of Friedewald *et al.*, (1972).

VLDL (mg/dl) = TG/5, and LDL (mg/dl) = TC - (HDL + VLDL)

The relationship is based on the fact that the ratio of VLDL and TG in serum is fixed relatively at 1 5 in fasting subjects with TG concentration not exceeding 400mg/dl (Burstein and Samaelle, 1960).

Statistical analysis

The results were analyzed by one way ANOVA test, and test group means compared with controls with the use of student's t-test. Difference was considered significant at $P<0.05$. All data are expressed as means ± SD.

RESULTS

Table 1. Effect of crude aqueous leaf-extract of *Vernonia amygdalina Del* on blood glucose, body weight and serum lipid profile of healthy and diabetic rats treated for 21 days.

Table 1: Effect of crude aqueous leaf-extract of *Vernonia amygdalina Del* on blood glucose.

Group	Weight gain (%)	Blood glucose (mg/dl)	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	High Density Lipoprotein (mg/dl)	VLDL (mg/dl)	LDL-C (mg/dl)
Normal control (NC) (0.5 distilled water)	18.59 ± 7.91	73.51 ±18.98	72.35± 10.07	102.71 ± 11..57	52.08 ±3.80	15.63 ± 1.85	30.43 ± 9.03
Normal Treated (NT) (400mg/kg of drug)	7.08 ± 4.13[a]	67.94 ± 13.88	60.55 ± 0.53[a]	77..93 ± 2.94	54.36 ± 9.14	5.59 ± 0.68[a]	14.73 ± 2.36[a]
Diabetic control (DC) (0.5ml distilled water)	14.53 ± 3.40	247.25 ±4.83	102.91 ± 5.65	179.55 ± 7.25	45.85 ± 2.28	23.45 ± 1.90	35.45 ± 13.16
Diabetic treated (DT) (400mg/kg drug)	10.14 ± 7.41	144.14 ± 25.83[b]	72.35 ± 7.75[b]	101.58 ± 16.01[b]	43.91 ± 1.70[b]	13.13 ± 037[b]	15.10 ± 2.53[b]

mean ± SD, n = 6 a = $P<0.05$ compared to NC b = $P<0.05$ compared to DC

To evaluate the effect of extracts *V. amygdalina* leaves on serum lipid profile and blood glucose, the extract was administered for 21 days to normal and diabetic rats. The results are as shown in table 1 above. Blood glucose level was significantly reduced (144.14 ± 25.83) in diabetic rats treated with the extract compared to the untreated diabetic control (247 25 + 4.83). However, the normal treated animals showed non significant (P>0.05) changes in blood glucose relative to the normal control. All experimental groups did show weight gain, but the gain in the treated normal animal was significantly smaller (P<0.05) compared to both controls

Results of serum lipid profile showed significant reductions (P<0.05) in TC, TG, VLDL and LDL of the normal and diabetic groups relative is their respective controls. Serum HDL levels were significantly decreased in the diabetic treated group compared to diabetic control. This change was however, not observed in HDL levels of normal treated normal. Hence non-significant (P>0.05).

DISCUSSION

In diabetic studies generally, weight changes and blood glucose remain the most valuable indicators of severity as well as the tools to monitor the response to treatment plan. In this study, though the animals in the diabetic groups had recorded slight decreases in weight after alloxan induction, upon treatment the animals gained weight at the end of the experiment. This observation is well in line with that of Nimenibo – Uadia (2003) who reported an appreciation in weight of diabetic animals after a two-week treatment with aqueous root extract of *V amygdalina Del* as the rats gradually ate more. Non-diabetic animals treated with the extract showed an increasingly smaller and smaller, weight gain. This also agrees with earlier reports of Ibrahim *et al.*, (2000) who recorded significant reductions in body weight of normal animals chronically fed with whole leaves of *V. amygdalina Del* for 2 months. That the extract tends to significantly decrease circulating lipid fractions in blood (principal fatty acid carriers) may suffice to explain this observation. The supply of fatty acids for lipid biosynthesis may have been reduced as a consequence, thereby leading to the observed weight reduction.

Diabetes induced rats that presented with a spectrum of severity with hyperglycemia, had blood glucose significantly reduced though the restoration was not complete (normoglycemia). This finding again agrees with that of Nimenibo – Uadia, (2003) and Uhegbu and Ogbuehi, (2004) who had earlier reported significant blood glucose reductions upon treatment with aqueous extract of *Vernonia amygdalina*. Alloxan used in the induction of diabetes is known to mediate the destruction of beta-cells of the pancreas by establishing a redox cycle, with the consequence of forming reactive oxygen species which set in place, chains of destructive oxidative processes, (Szkudelski, 2001). The *Vernonia amygdalina* extract could have started a reversal of this process by mopping up the free radicals and the destructive oxidative process by its antioxidant effects extensively studied by Igile *et al.*, (1994). Hence probably allowing a regeneration of the hitherto destroyed islet cells which produce insulin leading to tissue uptake of glucose, to cause the reduction in circulation. The inability of the extract to completely restore normoglycemia could have been due to the brief administration period as the previously destroyed islet cells may not have all been restored

Alteration/changes in lipoprotein metabolism typical of diabetes, due primarily to defects in insulin action (Robinson and Johnston, 1997) were also determined. Diabetic treated animals showed significant reductions in serum levels of TC, TG, and the lipoprotein cholesterols. Again this is consistent with the works of Uhegbu and Ogbuehi, (2004) and Nimenbo-Uadia, (2003). However, in this study significant reductions in serum lipid fractions (save HDL) was also observed in non-diabetic treated animals compared with the normal control. In the former (i.e. diabetic treated), the extract could have imparted positively on the pancreatic cells causing a gradual restoration in insulin production which would in turn affect lipid responsive hormone sensitive lipoprotein lipase, fatty acid synthetase and sterol regulatory element binding proteins -1 (STREBP - 1) These proteins were markedly increase in a study with streptozotocin – induced diabetes, and treatment with insulin prevented increased in their expression and hence accumulation of TG (Lijun *et al.*, 2002) Since TG constitute about 90% (Nduka, 1997) of VLDL and LDL, it's most probable that reduction in TG could concomitantly decrease VLDL and LDL too.

The rich saponin content of the leaves of *Vernonia amygdalina* (responsible for the cupious foaming) could likely be responsible for its hypolipidaemic effect in non-diabetic animals Saponins bind cholesterol in the intestinal lumen, making it less readily absorbed and / or bind bile acids causing their faecal excretion This excretion is off-set by an enhanced conversion of cholesterol to bile acids, consequently lowering cholesterol in plasma (Sidhu and Oakenful, 1986, Oakenfu and Sidhu, 1990). Because little of cholesterol and TG constitute HDL, the effect of the extract appears not to be felt in this parameter of non – diabetic treated animals

HDL levels were significantly reduced in diabetic treated animals The synthetic mechanism of the liver usually compromised in diabetes may not have completely been restored to produce apo-proteins, the richest component of HDL in required amount or quantity. Moreover, other constituents of the extract might have impacted differently on the HDL.

In conclusion, results from this study show that aqueous leaf-extract of *Vernonia amygdalina* possesses antihyperlydemia (curative) hypolipidaemic (prophylactic) and weight reduction activities (at least in rats) apart from its antihyperlycemic effect. However, histopathological analyses are in progress in our laboratory to confirm the involvement of the pancreas.

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