EFFECTS OF AQUEOUS LEAF EXTRACT OF *VERNONIA AMYGDALINA* ON BLOOD GLUCOSE AND TRIGLYCERIDE LEVELS OF ALLOXAN–INDUCED DIABETIC RATS (*Rattus rattus*)

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ABSTRACT

The effect of Vernonia amygdalina aqueous leaf extract on serum glucose and triglyceride level of diabetic rats were investigated. The aqueous extract was administered to alloxan –diabetic rats. The blood glucose and serum triglyceride levels were estimated at time intervals post oral administration of the extract (80 mg/kg). The extract caused significant (P<0.05) and progressive time dependent reduction of blood glucose and serum triglyceride levels in both normoglyceamic and alloxan-induced diabetic rats, with similar time course of action. In conclusion, the significant reduction in blood glucose and serum triglyceride level observed in this study may help in alleviating some of the complications associated with diabetic conditions.

Keywords: *Vernonia amygdalina*, Leaf extract, Blood glucose, Serum triglyceride, Diabetic rats

INTRODUCTION

Differences in the lipid profile between diabetic and non-diabetic individuals are now apparent (Garg and Grundy, 1990; Siegel *et al.*, 1996) and lipid abnormalities are common in patients with diabetes mellitus (Siegel *et al.,* 1996). Dyslipidaemia has been identified as one of the major risk factors for macrovascular complications in diabetes mellitus (Stamler *et al,* 1984; Kannel, 1985; Stern and Haffner, 1991; ADACS, 1993). In Non-Insulin Dependent Diabetes Mellitus (NIDDM) the most common form of dyslipidaemia results in elevated plasma triglyceride levels, high low density lipoprotein cholesterol (LDL-C) total cholesterol and low high-density lipoprotein cholesterol (HDL-C) (Isseb *et al.,* 1996). The prevalence of dyslipidaemia in NIDDM varies among different populations (Stern et al 1989, Garg and Grundy 1990). Elevated serum lipids are associated with a higher risk of coronary heart disease (CHD) for patients with diabetes as they are for non-diabetes (Lehto *et al*, 1997). In addition hyperlipidaemia has been implicated in the progression of glomerulosclerosis and renal insufficiency (Stevenson and Kaysen, 1999; Philip *et al.,* 1999). There is a higher mortality rate in diabetics with hyperlipidaemia than those with hypolipidaemia. A significant association between hyperlipidaemia and poor glycaemic control has been reported (Akbar 2001). Coronary heart disease mobility is about four times higher in diabetics than in non-diabetics, and the mortality rate from CHD is up to 100 % higher in diabetic patients than in non-diabetics (Lehto *et al.,* 1997). There is evidence that cardiovascular complication rate associated with diabetes can be reduced considerably through adequate treatment of hyperlipidaemia (Pyorala *et al.,* 1997; Golderg *et al.,* 1998). Therefore, adequate treatment of diabetes dyslipidaemia through diet and weight control is critical in reducing these complications.

Vernonia amygdalina, Del (bitter leaf) is a common medium sized shrub with abundant bitter principle in every part of the plant. It is a widely used local plant in Nigeria for both therapeutic and nutritional purposes, where it serves as the main ingredient in 'bitter leaf soup'. Fresh extract of the leaf has been reported to contain alkaloids, saponins, tannins, flavonoids and proteins (Akah and Okafor 1992), as well as vitamins and minerals (Fafunso and Basir, 1977).

In addition to its numerous uses, as in malaria and stomach disorders (Dalziel, 1937; Bever, 1960), the leaf decoction of the plant is popular in traditional medicine as an antidiabetic remedy; the potency and safety of which has been documented (Iwu, 1980; Akah and Okafor, 1992; Akah *et al.,* 2002). The aim of this study was to determine the effect of *Vernonia amygdalina* Del. (Compositae) aqueous leaf extract on triglyceride level of diabetic rats and to relate this to its anti diabetic effect.

MATERIALS AND METHODS

Plant Material: Fresh leaves of *V. amygdalina* were collected in December 2000 from Umuahia, Abia State, Nigeria. Botanical identification was done by a curator, Botany Department, University of Nigeria, Nsukka and voucher specimen deposited in the University Herbarium.

Preparation of the Aqueous Extract: After washing, the leaves were sun-dried for seven days and milled to coarse powder using mortar and pestle. The powder (250 g) was soaked in 500 ml of distilled water. The mixture was allowed to stand for 24 hours with intermittent shaking. Following filtration, the filtrate was freeze-dried to afford a solid residue (48.7 g; 19.5 % yield). The extract was reconstituted in distilled water in appropriate concentration before administration.

Animals: Adult Wistar albino rats (*Rattus rattus*) (180 – 250 g) of either sex inbred and maintained in the Animal Unit of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka were used in the study. The animals were maintained under standard laboratory conditions and were allowed free access to food (grower mash Guinea Feed) and water before the beginning of the experiment.

Induction of Diabetes Using Alloxan: Normal healthy albino rats were fasted overnight with free access to water prior to and throughout the duration of the experiment. At the end of the fasting period, blood was withdrawn from the media canthus of the eyes by occipital puncture using heparinized capillary tube, and the fasting blood sugar (FBS) level determined using o-toludine method (Bauer *et al*., 1974). Serum triglyceride level was also estimated using the method of Onyesom and Atakuo (1998). Rats with FBS and serum triglyceride between 100 – 130 mg % and 150 – 200 mg % respectively were used. Forty mice were then divided into four groups of 10 rats each. Groups 1 and 2 were the normal (non-diabetic) rats. Groups 3 and 4 were given alloxan monohydrate (120 mg/kg ip), freshly prepared as a 10 % solution in distilled water, (Iwu 1980). The animals were allowed free access to food and water for 7 days. On the 8th day, the animals were fasted for 12 hours prior to the estimation of their blood glucose and serum triglyceride levels determined (0 hr). The treatment groups employed were;

Group 1 Normal (non-diabetic) control,
Group 2 Normal (non-diabetic) treated,
Group 3 Diabetic control
Group 4 Diabetic treated

Groups 2 and 4 were given *V. amygdalina* extract (80 mg/kg) as a single dose, (this dose was previously determined as the optimum anti-diabetic dose (Akah and Okafor, 1992); while groups 1 and 3 received distilled water (2 ml/kg). Blood glucose and serum triglyceride levels were determined for each rat in each group 2, 4, 8, and 24 hours post treatment.

Statistical Analysis: Values are expressed as means (mg %) $\pm$ SEM and two-way ANOVA and F-LSD was employed to test the significance of difference between means at $p = 0.05$.

RESULTS

Effect of the Extract on Blood Glucose Level: In alloxan-diabetic rats (Group 4) *V. amygdalina* extract (80 mg/kg) caused a significant ($P<0.05$) reduction in blood glucose level (Table 1). Blood sugar was reduced from an initial value of 291.1 $\pm$ 9.4 to 194.5 $\pm$ 2.0 in 8 hours, i.e. a 33.2 % reduction after 8 hr. The reduction, which became significant by the second hour persisted to the 8th hour. Similar pattern of effect was also observed in non-diabetic animals treated with the extract; thus there was a reduction from 125.4 $\pm$ 5.0 to 81.3 $\pm$ 6.1in 8hours, i.e. 35.6 % reduction after 8 hr.

Effect on Serum Triglyceride Level: The effect of the extract on serum triglyceride is shown in Table 2. The extract caused a prolonged and significant reduction ($P < 0.05$) in serum triglyceride level of diabetic rats. There was about 56 % reduction of serum triglyceride by the 8th hour in diabetic group. In non-diabetic rats, the extract also evinced significant ($P < 0.05$) reduction in serum triglyceride level. There was 29.4 % reduction in serum triglyceride by the 8th hour for the non-diabetic rats.

Table 1: Effect of *V. amygdalina* aqueous leaf extract on blood glucose of diabetic rats

Groups	Dose of extract	Blood glucose (mg %) at hr after treatment[a]					% Reduction after 8 hr
		0 hr	2 hr	4 hr	8 hr	24 hr	
Non-diabetic (control)	DW (2 ml/kg)	123.6±2.4	125.1±1.6	121.8±3.1	126.3±9.5	124.4±7.3	-2.0
Non-diabetic (treated)	80 mg/kg	125.4±5.0	114.5±4.4*	100.6±3.0*	81.3±6.1*	103.1±1.5*	35.6
Diabetic (control)	DW (2 ml/kg)	298.7±6.5	278.1±5.0	281.5±3.2	311.6±7.2	326.5±4.2	-4.3
Diabetic(treated)	80 mg/kg	291.1±9.4	265.0±8.6*	209.5±4.1*	194.5±2.0*	231.6±5.1*	33.2

[a] *Values are means ± SEM, (n=10), *P < 0.05; DW = distilled water*

Table 2: Effect of *V. amygdalina* aqueous leaf extract on serum triglyceride level of diabetic rats

Groups	Dose of extract	Serum triglyceride (mg %) at hr after treatment[a]					% Reduction after 8 hr
		0 hr	2 hr	4 hr	8 hr	24 hr	
Non-diabetic (control)	DW (2 ml/kg)	193.6±2.4	192.8±4.1	195.1±10.6	193.2±7.7	191.4±2.1	0
Non-diabetic (treated)	80 mg/kg	192.5±5.1	161.4±4.5*	150.7±5.4*	136.4±2.5*	160.5±1.6*	29.1
Diabetic (control)	DW (2 ml/kg)	386.9±6.5	407.1±10.2	404.8±5.2	381.7±4.2	403.8±17.8	1.3
Diabetic(treated)	80 mg/kg	355.5±6.0	333.5±5.3	188.3±4.2*	158.0±3.5*	231.6±5.7*	55.6

[a] *Values are means ± SEM; (n=10), *P < 0.05, DW = distilled water*

DISCUSSION

The hypoglycaemic potentials of medicinal plants have been documented (Akah *et al.,* 2002). The result of this study confirms our earlier report (Akah and Okafor, 1992) on the hypoglycaemic effect of the leaf extract of *V. amygdalina* in rabbits. Although several biologically active constituents were reported present in the extract (Fafunso and Basir, 1977; Akah and Okafor, 1992), it was not demonstrated which of the groups of phytochemical were responsible for the effect and the mechanism of action. The prompt and remarkable reduction in blood glucose in both the fasting normal rats and alloxan diabetic rats (with blood sugar levels comparable to total pancreatomy) point to a mechanism of action different from that of sulphonylureas, and unrelated to insulin secretion from pancreatic β-cells. In diabetes, the causes and sites of intervention in the biochemical process are diverse (Larner 1985), and high serum total triglyceride level has been implicated (Anaja 1995).

It is now widely believed that an important signal for insulin secretion may be the link between glucose and lipid metabolism; and long-term exposure of islet cells to high levels of fatty acids may result in β-cell dysfunction (lipotoxicity), and diminished glucose-stimulated insulin secretion (Krolewski *et al.*, 1994, Haffner *et al.,* 1998). It has been established that hyperlipidaemia does not only increase the risk of ischaemic heart disease (IHD) in diabetic patients, but also may impair glycaemic control, accelerates the progression of renal insufficiency and increases mortality (Akbar, 2001). Moreover, an alarming proportion of diabetic patients with dyslipidaemia is not aware of the problem and only a small fraction receives lipid-lowering therapy (Isseb *et al.,* 1996). Since dyslipidaemia occurs in most diabetic patients (Isseb *et al.,* 1996), the utilization of lipid-lowering drugs is now advocated for diabetic patients.

In the present study, *V. amygdalina* leaf extract evinced a potent lowering of serum triglyceride level in both normoglyceamic and alloxan-induced diabetic rats. The effect on blood glucose and serum triglyceride level followed the same time course and peaked by the 8th hour. Adequate treatment of diabetes dyslipidaemia through diet is critical in reducing risk and complications, and the role of medicinal plants in the treatment of diabetes is emerging.

A high fiber content that reduces insulin secretion was used in the management of hyperlipidaemia in diabetic patients (Paisey *et al.*, 1984). Furthermore, the effectiveness of the seed extract of *Trigonella foenum,* L, as a cholesterol-lowering agent has been reported (Sharma *et al.,* 1990). The seed was reported to contain soluble fibres, which decrease cholesterol absorption and bile acids reabsorption by disrupting the intraluminal formation of micelles (Sharma *et al.,* 1990). We suspect similar mechanism of action for *V. amygdalina* extract due to its high fibre content. *V. amygdalina* has been reported to decrease weight gain in rats (Ene-Obong and Amadi 1986). Although the mechanism of the weight reduction was not explored, it may be related to its lipid lowering effect as shown in the present investigation. The ability of the extract to reduce both blood glucose and serum triglyceride levels is remarkable. The effect of *V. amygdalina* on serum triglyceride is an added advantage towards effective glycaemic control. *V amygdalina* is very abundant and relatively cheap, thus recommended as dietary inclusion for diabetics.

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