# EFFECT OF AQUEOUS EXTRACT (CRUDE) OF LEAVES OF *Vernonia Amygdalina* (DEL) ON BLOOD GLUCOSE, SERUM ALBUMIN AND CHOLESTEROL LEVELS IN DIABETIC ALBINO RATS.

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(Received 11 December, 2001; Revision accepted 8 May, 2002)

## ABSTRACT

Effect of crude aqueous extract of leaves of *Vernonia amygdalina* on blood glucose, serum albumin and cholesterol levels on alloxan induced diabetic albino rats was investigated. Blood glucose, serum albumin and cholesterol levels were significantly reduced. From $296.75 \pm 9.0$ mg/dl to $179.0 \pm 7.3$ mg/dl for blood glucose; $4.08 \pm 0.4$ mg/dl to $0.93 \pm 0.23$ mg/dl for serum albumin, while cholesterol level decreased from $280.33 \pm 4.65$ mg/dl to $170.45 \pm 4.52$ mg/dl.
The effect was dose dependent, as the reduction followed increase in dose given to the animals. The biochemical implication of our findings are discussed.

KEYWORDS: Blood glucose, serum albumin, Cholesterol, hypoglycemic, antilipolytic and lipogenic effects.

## INTRODUCTION

*Vernonia amygdalina* (bitter leaf) is a shrub or small tree of about 5 metres high, the leaves (5X15) are simple and entire (or minutely toothed), obovate oblanceolate, finely glandular, low and displaying few lateral nerves (Iwu, 1993). The species can be easily differentiated from the wild growing *vernonia colorate* by the hairy leaves of the latter (Iwu 1993). *Vernonia amygdalina* has abundant bitter principles in all its parts and it is a widely used local plant in Nigeria for both therapeutic and nutritional purposes. In folklore, the leaf decoction is traditionally employed as an antidiabetic remedy (Delziel 1937, Bever, 1960).

The leaves are reputed to be effective remedies for gastrointestinal disorders, and as a general tonic. The aqueous decoction of the leaves has been used for the treatment of fevers, while the dried leaves are chewed for the same purpose; and by pregnant women to check nausea. The fresh leaves are believed to be aboitifacient (Iwu, 1993).

The hypoglyeemic affect of the extract of *Vernonia amygdalina* has been demonstrated by Aka and Okafor (1992) while Iwu (1981) demonstrated the hypoglycemic effect activity of *Bridelia Farungimea* in fasting rats. In normal and hyperglycemic animals; *Salvica lavandolifolia* was found to reduce blood sugar levels independent of the effect of insulin (Jimenez, 1985).

According to White and Campbell (1992), diabetes mellitus is a chronic disease characterized by disorders in carbohydrate (and associated fat and protein) metabolism because of an absolute or relative deficiency in the action of the insulin and possibly abnormally high amounts of glucagon and other insulin antagonists like growth hormones and corticosteroids. In essence, insulin secretion in diabetes may be normal or entirely deficient. The plasma glucose level rarely exceed 120mg/dl in normal humans, but much higher levels are routinely found in patients with deficient insulin action. After a certain plasma glucose level is attained (generally more than 80mg/gl humans) the maximum level of renal tubular reasorption of glucose is exceeded and sugar is excreted in the urine (glycosuria) (Grammer, 1996). The urine volume is increased owing to osmotic diuresis and coincident obligatory water loss (polyuria), and this leads to dehydration (hyperosmolarity); increased thirst and excessive drinking (polydipsia). Very high levels of glucose in the blood can in turn result in a syndrome; diabetic non-ketotoxic hyperosmolar coma (Cing et al 1996)

Glycosuria causes a substantial loss of calories (4.1kcal) for every gram of glucose excreted); this loss when coupled with the loss of muscle and adipose tissue, results in severe weight loss in spite of increased appetite (polyphagia) and normal or increased caloric intake (Grammer 1996).

Protein synthesis diminishes in the absence of insulin, partly because the transport of amino acids into muscle is diminished (the amino acids serve as gluconeogenic substrates). Also,

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insulin-deficient patients are in a negative nitrogen balance. The antilipolytic action of insulin is lost as its lipogenic effect, hence plasma fatty acid level rises. In the long standing diabetes, general thickening of the capillary basement membrane may lead to the development of retinopathy and neuropathy (Haring and Obermaier-Kusser 1989).

Cholesterol, is a member of a larger family of lipids known as steroids, (Rawn 1989), and is an essential component of animal cell membrane. It is present in tissues and in plasma lipoproteins either as free cholesterol or combined with a long-chain fatty acid as cholesteryl

Regulation of cholesterol synthesis is exerted near the beginning of the pathway, at the HMG-CoA reductase stop (Murray et al 1990). There is a marked decrease in the activity of HMG-CoA reductase in fasting animals, which explains the reduced synthesis of cholesterol during fasting; cholesterol synthesis is also inhibited by LDL-cholesterol taken up via LDL-receptors. A diurnal relationship occurs both in cholesterol synthesis and reductase activity. Administration of insulin or thyroid hormone increases HMG-CoA reductase activity (Fielding and Fielding (1985).

Albumin is the major protein of human plasma (2.4-4.7g/dl) and makes up approximately 60% of total plasma protein (Rand et al 1906). Some 40% of albumin is present in the plasma, and the other 60% is present in extracellular space; the liver produces about 12g of albumin per day. The synthesis of albumin is depressed in a variety of diseases, particularly those of liver,. The plasma of patients with liver disease often show a decrease in the ratio of albumin to globulin. Severe hepatic cirrhosis leads to the decreased production of plasma albumin which can be manifested as hypoalbuminemia. Hypoalbuminemia also occurs in Kwashiorkor patients (Brown and Rand 1980), and in diabetes mellitus (Granner 1996).

In the rural areas of South Eastern Nigeria, Vernonia amydalina leaves, stem and roots are used in the management of diabetes, gastrointestinal disorders and fevers (Bever 1960). We therefore thought it necessary to collaborate or otherwise this belief experimentally on alloxan induced diabetic albino rats vis-à-vis variations of blood glucose levels, serum albumin and cholesterol levels.

## MATERIALS AND METHODS

### Plant Materials

Fresh leaves of Vernonia amygdalina were bought as sold in Uturu market and the specie identified and confirmed at the Botany Department, Abia State University Uturu.

### Preparation of aqueous plant extract

Fresh leaves of the plant were completely washed with distilled water and 2N sodium chloride solution to remove debris and contaminants. The leaves were then sun-dried for four days and milled to a coarse powder. About 10g of the powder was soaked in 100ml of distilled water, and the mixture allowed to stand for 24hrs with occasional shaking. The resulting mixture was filtered using a muslin cloth; and the resulting extract served as a 10% stock solution. From the stock solution, 6% solution was prepared by adding 6ml of the 10% solution to 4ml of distilled water, while 4% solution was prepared by adding 4ml of the 10% stock solution to 6% of distilled water. All the solutions were stored in a refrigerator at $4^0$C.

### Determination of Concentration of Extract

The concentration of the substance in the extract was determined by heating to dryness 1ml of the aqueous extract in hot oven at 50°C. 1ml of the aqueous extract left 290mg of the dry substance. Hence concentration of extract is 290mg/ml.

### Determination of Diabetes in the rats

96 hours after intraperitoneal administration of alloxan, blood was collected from the tails of the rats. The animals were held by their heads and xylene rubbed on their tails using cotton wool. Xylene causes vasodilation. Blood was collected by removing approximately 3mm tips of their tails with a pair of sharp scissors. The wounded tail ends were rubbed with absolute alcohol to prevent infection. About 0.1ml of serum was used for the blood glucose analysis using the O-toluidine method as described by Bauer and co-workers (1974).

### Plant Extract administration

Group 1 rats, as control received 1ml of distilled water; group 11 received 0.72mls of the 4% extract which is equivalent to 103. 95mg/ml. Group 111 received 0.80ml of the 6% solution, which represents 115. 6mg/ml of extract, while group IV received 1.10ml of the 10% solution or 159.9mg/ml of the extract. Mode of administration was by oral intubation, and the animals had access to only water after the administration of the extracts.

### Serum Collection

The animals were anasthesized using chloroform, the animals were placed on a dissecting slab and their limbs were pinned down

using dissecting pins, a longitudinal cut was first made abdominally to the rib cage, followed by a transverse cut to the limbs; blood was obtained by cardiac puncture, using a sterile needle to pierce the heart, and collecting the blood by the use of syringe, the blood was put in test tubes and allowed to clot, the serum was subsequently collected. The collection of blood was 6hrs after extract administration.

## Analysis

### Glucose estimation

Estimation of glucose was by 0-toluidine method as described by Bauer *et al* (1974). The method is based on the fact that glucose produces on heating with 0-tolidine in an acetic acid solution, a green colouration whose intensity is proportional to glucose concentration.

### Serum Albumin Estimation

The method employed was the Biuret method as described by Reinhold (1953). The principle is based on the fact that peptide bonds of proteins react with $Cu^{2+}$ ions in alkaline solution to form a coloured product whose absorbance is measured in the spectrophotometer at 560nm, and is proportional to the protein concentration.

### Estimation of Serum Cholesterol

Determination of serum cholesterol was by the Leiberman- Buchard method. The principal involves the reaction of hot acid solution with cholesterol, to produce a blue-green coloured complex which is measured at a wavelength of 570nm, comparing test with a known cholesterol standard.

### RESULTS:

The result in table 1 shows that there is a decrease in blood glucose levels in the diabetic animals treated with aqueous leaf extract of *Vernonia amygdalina* when compared with the control (Group 1) which did not receive the plant extract. The control group had a mean blood glucose level of 296.75±9.0mg/dl. The observed value for the rats treated with 4% (103.59mg/ml) of extract (group 11) was 277.0±19.70mg/dl, representing a decrease of 6% over the diabetic index of all the diabetic rats which was about 300mg/ dl. The mean blood glucose level for the rats treated with 6% (115.6mg/ml) of the extract was 204.5±6.70mg/dl, representing a decrease of 31.8% from the control group, while the mean blood glucose level of the rats in group IV treated with the 10% (159.5mg/ml) of the extract was 179.0±7.3mg/dl, representing a decrease of 40.3% over the control group. All the results obtained for the decrease in blood glucose level in the rats were statistically significant at ($p \leq 0.05$) level of significance.

Table II shows the variation of serum albumin levels after induction of diabetes and subsequent administration of the plant extracts. Results show that there is a decrease in the serum albmin levels in the diabetic animals treated with the aqueous extract of Vernonia amygdalina when compared with those of the control groups. The control group had a mean serum albumin level of 4.08±0.4g/dl, the observed value for group II rats treated with 4% (103.59mg/ml) was 2.60±5g/dl representing a 36.3% decrease from that of the control, for group III rats treated with 6% (115.6mg/ml) extract, the mean serum albumin levels was 1.68+0.13g/dl, which also represents a decrease of 58.8% over the control. The mean serum albumin levels observed for group IV rats treated with 10% (159.5mg/ml) of plant extract was 0.93±0.23g/dl, which also represents a decrease of 77.2% over that of the control. All the results obtained were statistically significant at ($p \leq 0.05$) level of significance.

Table 1: Blood glucose levels after induction of Diabetes and subsequent Administration of extract (mg/dl).

| Animal/ groups | Group II (control) | Group II | Group III | Group IV |
|---|---|---|---|---|
| Conc. of extract administered | 1ml distilled. Water | 4% Extract (103.59mg/ml) | 6% Extract (115.6mg/ml) | 10% Extract (159.5mg/ml) |
| Blood glucose (conc. mg/dl) | 296.75±9.0 | 277.0±19.70 | 204.5+6.701 | 179.0±7.3 |

Table 2: Serum albumin levels after Induction of Diabetes and subsequent administration of extract (mg/dl)

| Animal/ groups | Group I (control) | Group II | Group III | Group IV |
|---|---|---|---|---|
| Conc. of Extract administered | 1ml dist. Water | 4% Extract (103.9mg/ml) | 6% Extract (115.6mg/ml) | 159.5mg/ml |
| Serum albumin (conc. Mg/dl) | 4.08 ± 0.4 | 2.6±0.5 | 1.68±0.13 | 0.93±0.23 |

Table 3: Serum cholesterol level after induction of diabetes and subsequent administration of extract (mg/dl).

| Animal /group | Group I (control) | Group II | Group III | Group Iv |
|---|---|---|---|---|
| Conc. of extract administered. | 1ml dust water | 4% extract (103.59mg/ml) | 6%extract (115.6mg/ml) | 10% extract 159.5mg/ml) |
| Serum Cholesterol conc. (mg/dl) | 280.33±4.65 | 226.23±9.65 | 193.15±6.23 | 170.45±4.5 |

Result on table 3 shows that there is a decrease in the serum cholesterol levels in the diabetic animals treated with crude aqueous extract of the plant when compared with results of the control. The control group had a mean serum cholesterol level of 280.55±4.65 mg/dl, while group II animals treated with 4% (103.59mg/ml) of extract had 226.23±9.65 mg/dl; which represents a decrease of 19.38% over that of the control. Serum cholesterol levels for group III rats administratered 6% (115.6mg/ml) of plant extract was 193.15±6.23mg/dl and represents a decrease of 31.17% over that of the control, while the mean serum cholesterol levels for group IV rats treated with 10% (159.5mg/ml) of plant extract was 170.45± that of the control. All the results were statistically significant at (p ≤ 0.05) levels of significance.

## DISCUSSION

The mean blood glucose level for the control group (Table 1) was 296.75±9. 0mg/dl which was observed to be higher than the normal range of 65-110mg/dl (Kroop et al 1987). All the rats in groups I, II, III and IV which served as control and test groups had mean blood glucose level of about 300mg/dl after 96hrs of alloxan administration, and this level served as the hyperglycemic index in the rats. Table 1 shows that the plant extracts caused reduction in the blood glucose levels of the diabetic rats in groups II, III and IV. It is apparent from (Table 1) that the reduction of blood glucose levels were dose-dependent as 4% extract caused a reduction to 277.0±19.7 mg/dl which represents 6% decrease; the 6% extract caused reduction to 204.5±6.7 which represents a 31.8% reduction, while 10% extract caused a reduction to 179.0±7.3% which is the highest reduction representing 40.3% reduction. All the reduction of blood glucose levels in the groups were statistically significant at (p ≤ 0.05) level of significance.

These results are in agreement with those obtained by Akah and Okafor (1992), who reported the hypoglycemic properties of the aqueous leaf extract of Vernonia amygdalina administered to diabetic rabbits. These observations also lend credence to the report of

Dalziel (1937) and Bever (1960) that in folklore, the leaf decoction is employed as an antidiabetic remedy traditionally. Since alloxan destroys the beta cells of the pancreas leading to insulin deficiency, Akah and Okafor (1992) opined that it is probable that the mode of action of the aqueous extract of the leaf is not related to insulin secretion by the pancreatic beta cells.

Table 2 shows that the mean serum albumin levels for group II rats which received 4% (103.59mg/ml) of extract was 2.60±0.5g/dl; for group III rats given 6% (115.6mg/ml) of extract had 1.68+0.13g/dl; while the mean value for 10% (159.5mg/ml) extract was 0.93±0.23g/dl. These results suggest that the extract had hypolalbuminemic effects on the rats and hence could also posses hypoalbuminemic properties.

Although insulin deficiency is characterized by disorder in carbohydrate metabolism, and also associated with this are disorders in protein and fat metabolism (White and Campbell, 1992). From results on table 2, it is apparent that a longer period of diabetes is necessary to elicit an appreciable fall in the protein (e.g. serum albumin) levels. Kropp et al (1987) reported that the serum albumin level falls within a range of 3.5-5.5g/dl. The control groups had a mean serum albumin level of 4.08±0.4g/dl while the group II, III and IV rats had 2.6±0.5, 1.68±0.13 and 0.93±0.23g/dl respectively as a result of administration of the leaf extract. These reductions are significant at (p ≤ 0.05) level of significance.

Although, the desired result would have been for the extract to show the ability to increase the serum albumin levels because of the importance of albumin in the body metabolism which include: regulation of the osmatic pressure in the human plasma; binding and transport of calcium and several drugs in the plasma (Bowman and Rand 1980); binding and transport of tyroxine, cortisol and aldosterone (Granner, 1996); binding and transport of fatty acids (Guyton, 1996); binding and transport of bilirubin and copper (Murray et at 1990); it therefore follows that these biochemical processes might be impaired due to the low levels of the serum albumin as our result shows.

Granner (1996) reported that insulin has

antilipolytic and lipogenic effects; hence its deficiency as noticed in diabetes leads to a rise in the plasma fatty acid levels. This could lead to a rise in the levels of acetyl-CoA (which is the major oxidation product of fatty acids). Finally, this may also lead to elevated levels of cholesterol, since all carbon atoms in cholesterol are derived from acetyl CoA (Rawn 1989). Coppack (1987) also reported that insulin deficiency causes excessive mobilization of free-fatty acids and underutilization of chylomicrons and VLDL. Mayes (1990) confirmed this by observing that in this kind of state, there is an elevated level of VLDL and LDL, and consequently cholesterol in poorly controlled diabetes.

These facts about insulin deficiency and serum cholesterol were clearly evident in this study, as the levels of the serum cholesterol in the rats were also elevated. The mean serum cholesterol levels in the group 1 (control) rats is 280.6±4.65mg/dl as against normal level of 150.220mg/dl as reported by Kropp and Co-workers (1987).

The mean serum cholesterol levels in the group II rats which received 4% (103.59mg/ml) of the extract was 226.23+9.65mg/dl, while that in group III administered 6% (115.6mg/ml) of the extract was 193.15±6.23mg/dl and the value for group IV rats which were given 10% (159.6mg/ml) of the extract was 170.45±4.52mg/dl. Though the serum cholesterol levels in these diabetic rats were observed to be about 285mg/dl after 96hrs of alloxan administration, the activity of the leaf extract was nonetheless found to be dose-dependent in its reduction of serum cholesterol levels (table 3) which were all significant at (p $\leq$ 0.05) level of significance.

Although the serum cholesterol levels of the diabetic rats after 96hrs of alloxan administration was not excessively high at about 280mg/dl, it is nevertheless, desirable that the leaf extract significantly reduced the serum cholesterol levels, since a high serum cholesterol concentration in the form of LDL have been implicated as the most important causative factor in atherosclerosis (Levy et al 1988). This condition may eventually lead to a decreased cardiac output, damming of blood in the pulmonary or systemic vein with death resulting from edema, fibrillation of the heart and occasionally rapture of the heart (Guyton, 1996).

Akah and Okafor (1992), Igile et al (1995) reported that the leaves of *Vernonia amygdalina* contain saponins, sesquiterpenes, lactones, steroid glycosides. alkaloids, tannins and flavonoids; while Iwu (1993) reported that the plant extract is generally non-toxic, but that excessive consumption could be purgative. With these reports in view, it is advisable that the use of the plant extract or the leaves extract could reduce blood glucose, serum albumin and serum cholesterol levels appreciably as our findings show, and with bioaccumulation could endanger the health of the consumer amongst other physiological complications. But since the aqueous plant extract produced a great fall in albumin as well as cholesterol levels, it hypoglycemic properties could be ascribed to toxic or inhibitory effects, and therefore consumers may be exposed more to toxic substance rather than hypoglycemic substances.

## REFERENCE

Akah, P. A. and Okafor C. I.., 1992. Blood sugar lowering effect of Vernonia amygdalina (Del) in an experimental rabbit model. Phytotherapy Research. 6: 171-173.

Bauer, J. D., Ackerman, P. A. and Toro, G., 9174. Clinical Laboratory methods (8th edition) C. V. Mosby Company St. Louis, pp.383-386.

Bever, B. O., 1960. Medicinal plants of Nigeria. Nigerian College of Arts, Science and Technology, Ibadan, Nigeria. pp 17.

Bever, B. O., 1986. Medicinal plants of Tropical West Africa C. V. Mosby Company Cambridge pp 28.

Bowman, W. C and Rand. M. J., 1980. Textbook of Pharmacology, St. Louis Press UK. Pp. 20-23.

Bult. H., Vilictinck, A. J. and Herman, A. G., 1983. Isolation and pharmacological characterization of Vernolepin J. Nat. Prod. 46(2): 161-169.

Coppack, S. W., 1987. Effects of Insulin on human adipose tissue metabolism in vivo. Clin. Sci. 77: 663.

Dalziel. J. M., 1937. The useful plants of West tropical Africa. Crown Overseas agents for the Colonies London pp 65.

Fielding. C. J and Fielding. P. E., 1985. Metabolism of cholesterol and Lipoproteins. In: Biochemistry of Lipids and membrances. Vance D. E and Vance J. E. (eds). Benjamin/Cummings Press UK.

Granner, D. K., 1996. Glucose metabolism. In: Harper's biochemistry, 22nd ed. Appleton and Lange, Connecticut USA pp 576- 580.

Guyton, A. C. and Hall. J. E., 1996. Dietary fats. In: Textbook of medical physiology 9th ed. W. E. Saunders. Philadelphia.

Ohagashi, H., 1995. Medical use of plant diets by Chimpanzee in the wild. Nippon sholuhin Kagaku Kaishi 42 (10): 859-868.

Rand, M. L., Harfenist, E. J. and Murray R. K., 1996. Plasma proteins, immunogolbulins and Blood coagulation. In: Harpers biochemistry 24th ed. Appleton and Lange Connecticut. Pp 701-731.

Rawn, J. D., 1989. Cholesterol biosynthesis and regulation. In: Biochemistry International edition. Nei patterson Publishers, New York. Pp 555-564.

Reinhold, T., 1953. Practical clinical biochemistry. C. V. Mosby Company St. Louis Cambridge pp. 170.

White, J. R. and Campbell, R. K., 1992. Diabetes. In: Clinical Pharmacy and therapeutics 5th ed. Williams Publishers, London. Pp. 307-320.