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### Studies on Some Biochemical Effects of *Vernonia amygdalina* in Rats

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**Abstract:** This research was undertaken to determine the effects of the extract of *Vernonia amygdalina* on some biochemical parameters in rat. The ethanol extract of leaf of *Vernonia amygdalina* was administered daily orally to four groups of rats at a dose of 100, 250, 500 and 1000 mg kg<sup>-1</sup> weight, respectively, for 28 days. On the 29th day blood was obtained from the hearts of the rats. The blood samples were centrifuged to obtain the blood serum. Serum levels of the following enzymes and biomolecules were evaluated: alanine and aspartate transaminases, alkaline phosphatase, glucose, creatinine, proteins, total and conjugated bilirubin, total cholesterol, triglycerides, high density lipoproteins, low density lipoproteins and very low density lipoproteins. Weights of the animals were taken weekly. The results of this study show that the extract reduced significantly the levels of glucose by 15.6, 23.4, 19.5 and 11.7% at 100, 250, 500 and 1000 mg kg<sup>-1</sup> doses, respectively. While only 250 mg kg<sup>-1</sup> of the extract also decreased the value of triglycerides (1.0331 mmol L<sup>-1</sup>) compared to control (1.687 mmol L<sup>-1</sup>). It can be concluded from these results that the leaf extract of *Vernonia amygdalina* is safe for use in ethnomedicine, especially in ethnotherapy of diabetes mellitus.

**Key words:** *Vernonia amygdalina*, biochemical parameters, hypoglycemic effect, Triglycerides, bitter leaf

### INTRODUCTION

*Vernonia amygdalina* Del (Compositae) is a shrub or small tree that grows throughout tropical Africa. It is popularly called bitter leaf because of its abundant bitter principles. It is cultivated in Nigeria mainly for its nutritional value (Igile *et al.*, 1995a). The plant (especially the leaf) has been found useful in the ethnotherapy of diabetes (Akah and Okafor *et al.*, 1992; Uhegbu and Ogbuehi, 2004; Nwajo, 2005), asthma, schistosomiasis, malaria (Masaba, 2000), measles, diarrhoea, tuberculosis, abdominal pain and fevers, cough (Akinpelu, 1999). The fresh leaves of the plant contains 82%, 5.3, 0.4, 10.0, 1.5 and 1.7 g of moisture, proteins, fat, carbohydrate, fibre and ash, respectively. It also contains 145 mg, 5.0 g and 51 mg of Ca, P, Fe and Vitamin C, respectively (Anne, 1979).

Phytochemicals contained in *Vernonia amygdalina* include saponins, sesquiterpenes, lactones and flavonoids. Steroid glucosides such as Vernoniosides A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, A<sub>4</sub>, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, D and E have been isolated from the plant (Ohigashi, 1994; Aregbeore *et al.*, 1997; Igile *et al.*, 1995b). But it is yet to be ascertained which of these are responsible for some observed biological effects of the plant.

In view of the wide use of the plant in both human nutrition and ethnomedicine, the present research was undertaken to evaluate the effect of daily, prolonged consumption of the plant on some important biochemical parameters. This will enable us ascertain the safety or otherwise of the plant.

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## **MATERIALS AND METHODS**

### **Plant Collection**

The fresh leaves of *Vernonia amygdalina* were collected in March 2005 from the medicinal garden of Faculty of Pharmacy, University of Uyo, Nigeria. They were identified in the Pharmacognosy Department of the same Faculty by Dr Kolawole Ajibesin (a pharmacognosist). The leaves were air dried before extraction.

### **Preparation of Extract**

The leaves (662.5 g) were macerated with 4 L ethanol (95%) for 72 h. The extract obtained was filtered, concentrated in vacuo in a rotary evaporator and dried in a desiccator containing Silica gel (self indicating). 14.5 g of extract was obtained, giving a yield of 2.18%.

### **Animals**

Wistar rats of both sexes obtained from the animal house of the University of Uyo, Nigeria, were used. They were bred and kept under standard laboratory conditions. They had free access to both food and water. They were kept in the care of experienced animal technicians. Animal work conformed to approved ethical standards of the University.

### **Administration of Extract**

Twenty five rats were divided into five equal groups A, B, C, D, and E. One mL<sup>-1</sup> of the extract of the plant was administered orally and once daily in the dose of 100, 250, 500 and 1000 mg kg<sup>-1</sup>, to groups A, B, C and D, respectively. Group E served as control and received 1 mL of the vehicle (i.e., water only).

### **Collection of Blood**

Blood sample was obtained on the 29th day from the hearts of the overnight fasted animals under chloroform anaesthesia. Blood samples were allowed to clot after which they were centrifuged at 7000 rpm for 10 min to obtain serum. Serum was stored in a refrigerator at -4°C until used for analyses.

### **Biochemical Parameters**

Appropriate commercial kits (Randox Laboratories) were used to evaluate the following enzymes and biomolecules.

#### **Glucose**

This was evaluated using the glucose oxidase method (Trinder, 1969).

#### **Alanine Transaminase (ALAT)**

The method involves the monitoring of the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine (Rietman and Frankel, 1957).

#### **Aspartate aminotransferase (ASAT)**

The principle of the method used involved monitoring the concentration of oxaloacetate hydrazone formed with 2, 4,-dinitrophenyl hydrazine (Rietman and Frankel, 1957).

#### **Alkaline Phosphatase (Phenolphthalein Monophosphate Method)**

This method is based on the principle that serum alkaline phosphatase hydrolyzes a colourless substrate of phenolphthalein that results in phosphoric acid and phenolphthalein at alkaline PH values. The pinkly coloured product is measured colorimetrically at 550 nm.

### **Triglycerides**

This involves the enzymatic colorimetric test of glycerol phosphate oxidase method (Zoppi and Fenili, 1976).

### **Total Cholesterol**

This was carried out by the enzymatic colorimetric chod-PAP method (Zoppi and Fenili, 1976).

### **HDL-Cholesterol**

High Density Lipoprotein (HDL) separated from chylomicrons. Very low density lipoproteins (VLDL) and Low Density Lipoproteins (LDL) by the addition of a phosphotungstic and magnesium chloride (precipitating reagent) to the serum. After centrifugation, the cholesterol content was determined by the enzymatic colorimetric method (Zoppi and Fenili, 1976).

### **LDL and VLDL Cholesterol**

These were calculated as recommended by Tietz (1999).

### **Total Protein**

This was done using the Biuret method.

### **Creatinine**

Modified Jaffe's method was used. Creatinine which is a hydride of creatine reacts with alkaline sodium picrate to form a red complex which can be determined photometrically (Jaffe, 1886).

### **Total and Conjugated Bilirubin**

This was based on colorimetric method of Jendrassik and Grof (1938).

### **Statistical Analysis**

Data were expressed as Mean $\pm$ SEM and were analysed by one way ANOVA and Scheffe's post test.  $p < 0.05$  was taken as significant.

## **RESULTS AND DISCUSSION**

Only the serum concentration of glucose (39.0, 35.4, 37.2 and 40.8 mg dL<sup>-1</sup>) was significantly different from the control (46.2 mg dL<sup>-1</sup>) at dose levels of 100, 250, 500 and 1000 mg dL<sup>-1</sup>, respectively (Table 1). The percentage reduction is 15.6, 23.4, 19.5 and 11.7%, respectively. While the extract reduced the levels of Triglycerides at all dose levels to some extent, only the 250 mg kg<sup>-1</sup> dose significantly reduced the concentration (1.0331 mmol L<sup>-1</sup>) at  $p < 0.05$  compared to control (1.687 mmol L<sup>-1</sup>). All the other biomolecules did not statistically differ at  $p < 0.05$  from the control.

However, the observed hypoglycemic activity of the extract was not dose dependent. 250 mg kg<sup>-1</sup> doses gave the highest percentage reduction.

The hypoglycaemic effect of *vernonia amygdalina* is well known. The hypoglycemic effect of all doses of the plant extract as observed in this work is therefore not surprising, it confirms the effectiveness of the leaves of *V. amygdalina* in the ethnotherapy of diabetes mellitus (Akah and Okafor, 1992). Alanine and aspartate transaminases and alkaline phosphatases are often present in high concentrations in the liver. They leak into the circulation when there is necrosis or hepatocellular injury. They are therefore employed in clinical diagnosis. Principally ASAT level is of use in diagnosis

Table 1: Effect of *Vernonia amygdalina* on some biochemical parameters in rats

Parameters	Control	100 mg kg <sup>-1</sup>	250 mg kg <sup>-1</sup>	500 mg kg <sup>-1</sup>	1000 mg kg <sup>-1</sup>
Glucose (mg dL <sup>-1</sup> )	46.2±1.94	39.0±6.54*	35.4±5.16*	37.2±3.76*	40.8±2.23*
Creatinine (mg kg <sup>-1</sup> )	99.6±6.41	95.4±2.24	88.2±9.41	101.4±12.78	84.0±6.84
ASAT (U/I)	14.4±2.06	14.6± 2.80	15.4±5.82	12.2±0.75	15.2 ±4.17
ALAT (U/I)	16.0±3.74	20.8±2.48	10.8±2.32	22.0±1.10	20.4±2.24
Total cholesterol (mmol L <sup>-1</sup> )	4.154±0.42	4.395±0.21	4.728±0.38	4.306±0.76	4.295±0.60
Triglycerides (mmol L <sup>-1</sup> )	1.687±0.119	1.671±0.04	1.0331±0.026*	1.396±0.132	1.57±0.116
High density lipoproteins (mmol L <sup>-1</sup> )	0.686±0.03	0.7332±0.022	0.73±46±0.04	0.785±0.172	0.767±0.44
Low density lipoproteins (Umol L <sup>-1</sup> )	3.8056±0.40	3.9274±0.283	3.3626±0.37	3.8254±0.62	3.845±0.48
Very low density lipoproteins (mmol L <sup>-1</sup> )	0.337±0.03	0.333±0.01	0.2694±0.01	0.304±0.03	0.3168±0.03
Total proteins (g L <sup>-1</sup> )	66.556±3.34	66.255±6.15	70.2684±7.02	66.0426±5.28	72.6006±8.08
Alkaline phosphatase (U L <sup>-1</sup> )	34.6192±1.91	29.3626±3.65	35.10±1.19	33.1326±4.26	29.9465±5.6
Total bilirubin (mg L <sup>-1</sup> )	5.94±0.46	6.0690±1.36	5.4864±0.49	6.1128±0.61	5.1192±0.48
Conjugated bilirubin (mg L <sup>-1</sup> )	3.5712±0.68	3.6288±0.89	4.7142±0.88	3.7152±0.59	3.5136±0.297

Mean±SEM, n = 5, \*p<0.05, n is the number of animals per group p is 95% significance level

of myocardial infarction. ALAT in viral hepatitis. Hyperbilirubinemia which may lead to jaundice is caused by over production of bilirubin, liver damage or obstruction to the excretory ducts of the liver. The values of both conjugated and total bilirubin, which were not significantly different from control in this study, further show that the extract did no harm to the liver. The results of this study therefore indicate that the extract did not injure either the heart or liver of the rats. The extract also did not generate any inflammatory conditions.

Lipid profile (which involves levels of total cholesterol, HDL, LDL and VLDL) serve as diagnostic indices in conditions such as chronic obstructive jaundice, hepatitis, coronary heart disease and atherosclerosis. Hyperlipidaemia is one of the risk factors for coronary heart disease while cholesterol is the major lipid constituent of atherosclerotic plaque. Extract of *v. amygdalina* did not affect lipid profile significantly, indicating that the use of the plant will not likely contribute to any disease associated with hyperlipidaemia. 250 mg kg<sup>-1</sup> of the extract significantly reduced the level of Triglycerides from 1.687 mmol L<sup>-1</sup> (control) to 1.0331 mmol L<sup>-1</sup>. It is not clear why the reduction in the level of Triglycerides at other dose levels were not significant. The plant extract also did not seem to have adverse effect on the kidney, since the creatinine levels were not significantly altered.

The present research show that there were no changes in most of the investigated parameters following the administration of the ethanolic extract of *V. amygdalina*. It confirms that *V. amygdalina* is useful in the ethnotherapy of diabetes mellitus. It could also be concluded that the plant is also safe for consumption as food or medicine, since there were no indication of toxicity judging from the values of the biomolecules evaluated. This research also seems to show that the dose of 250 mg kg<sup>-1</sup> of the extract is the optimal dose for the hypoglycemic and hypotriglyceridemic effects of the extract.

## REFERENCES

- Akah, P.A. and C.I. Okafor, 1992. Blood sugar lowering effect of *Vernonia amygdalina* Del. in experimental rabbit model. *Phytother. Res.*, 6: 171-173.
- Akinpelu, D.A., 1999. Antimicrobial activity of *Vernonia amygdalina* leaves. *Fitoterapia. J. Study Med. Plants*, 70: 432-435.
- Anne, R., 1979. The role of wild foliage plants in the diet. A case study of Lushota Tanzania. *Ecol. Food Nutr.*, 8: 87-93.
- Aregbeore, E.M., H.P. Makkar and S.K. Beeker, 1997. Chemical composition and Tannins in Leaves of some Browse plants from Delta (Central Nigeria) eaten by ruminants. *Proc. Soc. Nutr. Physiol.*, 5: 11-15.

- Igile, G., W. Oleszek, M. Jurzysta, R. Aquino, N. Tommas and C. Pizza, 1995a. Vernoniosides D and E, Two novel saponins from *Vernonia amygdalina* Del. *J. Natural Prod.*, 58: 1438-1443.
- Igile, G.O., W. Oleszek, S. Burda and M. Jurzysta, 1995b. Nutritional assessment of *Vernonia amygdalina* leaves in growing Mice. *J. Agric. Food Chem.*, 43: 2162-2166.
- Jaffe, M., 1886. Uber den Wiederschlag, Welchen Pikrinsaure in normalem Ham erzeugt und uber eine neue Reaktion des kreatinins. *Z. Physiol. Chem.*, 10: 391-400.
- Jendrassik, J. and P. Grof, 1938. Vereinfachte photometrische methoden Zur Bestimmung des Blutbilirubin. *Biochem. Z.*, 297: 81-89
- Masaba, S.C., 2000. The antimalarial activity of *Vernonia amygdalina* Del. (Composital). *Trans. Roy. Soc. Trop. Med. Hyg.*, 94: 694-695.
- Nwanjo, H.U., 2005. Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma lipoproteins and oxidative status in diabetic rat model. *Nig. J. Physiol. Sci.*, 20: 39-42.
- Ohigashi, M., 1994. Assessment of the antibacterial activity of some traditional Medicinal Plants. *Ethiopian J. Health Develop.*, 13: 211-216.
- Rietman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic-oxaloacetic acid and glutamic-pyruvic transaminases. *Am. J. Clin. Pathol.*, 28: 56-63.
- Tietz, N.W., 1999. *Textbook of Clinical Chem.* 3rd Edn. Philadelphia; W.B. Saunders, pp: 1204-1208.
- Trinder, P., 1969. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogen chromogen. *J. Clin. Pathol.*, 2: 158-161.
- Uhegbu, F.O., K.J. Ogbuehi, 2004. Effect of the aqueous extract (crude) of leaves of *Vernonia amygdalina* on blood glucose, serum cholesterol and serum albumin levels in alloxan induced diabetic albino rats. *Global J. Pure Applied Sci.*, 10: 189-194.
- Zoppi, F. and D. Fenili, 1976. Enzymatic determination of Total serum cholesterol with the Vickers D-300 Analyzer. *Clin. Chem.*, 22: 690-691.