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Effects of Aqueous and Ethanolic Extract of *Vernonia amygdalina* Leaf on the Plasma Lipid Profile and Liver Function Parameters of Normal Rats

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Abstract: Many plants with reported medicinal value have been demonstrated to exert a protective effect on the liver and some, with the ability of lowering the blood lipid level have also been elucidated. This study shows the effect of ethanolic and aqueous extracts from *Vernonia amygdalina* on the plasma lipid profile as well as on the level of the liver biomarker in the plasma of normal rats. The animals were distributed into two sets of four groups with five animals in each group. Each set had a control group while the other three groups were administered different concentrations of ethanolic and aqueous extracts from *V. amygdalina* leaf. The control groups were administered normal saline and the other groups' 100, 200 and 300 mg/kg of ethanolic and aqueous extracts respectively, twice daily for three weeks. The plasma Total Protein (TP), Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), Aspartate Transaminase (AST), Albumin (ALB) and Bilirubin (BIL) levels and plasma lipid profile of the rats were evaluated. The aqueous extract of the plant showed a significant increase in the plasmaconcentration of HDL-C with no significant difference in the plasma TC, LDL-C, VLDL-C and TG levels. Thus, these results suggested that the ethanolic extract from the leaves of *V. amygdalina* had a hepatoprotective effect while hypolipidemic effect can be suggested for the aqueous extracts.

Key words: Biomarkers, hepatoprotective, hypolipidemic, *Vernonia amygdalina*

INTRODUCTION

Medicinal plants have been used since the ancient time for preventive and curative measures for different ailments.Most primitive tribes possess expert knowledge of medicinal plants, which number at times in hundreds (Singerist, 1951). Although modern medicine may be available in developing countries, but the use of herbs fortreatment and management has often maintained popularity for historical and cultural reasons (Nwangwu *et al*., 2009). This practice has gained more grounds because of the ready availability of plants, the insignificant cost of preparation and the new crave to avoid the side effects of chemotherapy. One of such plant used is Vernonia amygdalina. Though several of its medicinal values had been ascertained, more work still need to be done for maximum value. The plant is widely distributed in Nigeria especially in the southeastern and southwestern parts of the country. It is a shrub of 2-5 m tall with petiolate leaves of about 6.0 mm wide (Ojiako and Nwanjo, 2006). The leaves of the plant are used in making soup while the juice serves as a tonic drink for the prevention of certain illnesses (Olatunde, 2003). It has found several uses in folk medicine including treatment of diabetes, laxatives and antimalaria. Many of its medicinal values had also been reported by researchers, incuding; hypoglycemic effect (Taiwo *et al*., 2009; Atangwho *et al*., 2010; Adikwu *et al*., 2010), antibacterial effect (Newbold *et al*., 1997; Kambizi and Afolayan, 2001; Cos *et al*., 2002); antiparasite activities (Hakizamungu *et al*., 1992); antimalaria (Madureira *et al*., 2002; Masaba, 2000; Tona *et al*., 2004). Antifungal activities had also been ascertained for the aqueous extract of the plant (Ogbebor *et al*., 2007). Other acclaimed medicinal properties include, anticancer (Izevbigie, 2003; Izevbigie *et al*., 2003; Izevbigie *et al*., 2004); and antiviral activities (Vlietinck *et al*., 1995). In this study the possible effect of both aqueous and ethanolic extracts from *V. amygdalina* leaves on plasma lipid and on the activity of the liver biomarkers in the plasma were investigated. The results obtained from the work may suggest the best extract for use in different
Diseased conditions

MATERIALS AND METHODS

Plant material: Fresh leaves of *Vernonia amygdalina* were harvested from a local farm in Onne, Rivers State, Nigeria and were identified at the Department of Botany, University of Port Harcourt, Port Harcourt, Rivers State,

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Nigeria. They were sorted, washed, air dried at room temperature and milled into powder. The solvent extraction was carried out using Soxhlet Extractor with water and ethanol as solvents. The extracts were concentrated to about 10% of the original volume using a rotary evaporator (BUCHI, type RE111, Rotavapor).

Experimental animals: The experimental animals (*Rattus norvegicus*), all male, which weighed between 100-160 g used, were kept at the animal house of the Department of Biochemistry, College of Health Sciences, Igbinedion University, Okada, at room temperature and 12 h light and 12 h dark cycle for the period of experiment. The animals were housed in well ventilated cages. They were given water and food *ad libitum* throughout the duration of the experiment. This study was conducted in 2010 in Biochemistry laboratory, Department of Biochemistry, College of Health Sciences, Igbinedion University, Okada.

Experimental design: The animals were randomly selected and grouped. There were a total of eight groups with five animals per group. The animals were distributed into two sets of four groups. Each set had one group which served as control while the other three groups in the two sets were administered different concentrations of the ethanolic and aqueous leaf extracts. The control groups were administered normal saline while the other groups were administered 100, 200, and 300 mg/kg of the ethanolic and aqueous extracts respectively twice daily for three weeks. The ethanolic and aqueous extracts were administered in normal saline orally. After 3 weeks of experimentation animals were sacrificed (fasted overnight). The experiments and procedures employed in this study where reviewed and approved by the Animal Care Committee of the College of Health Sciences, Igbinedion Univerisity, Okada, Edo State, Nigeria.

Biochemical assays: Total protein (TP), Alkaline phosphatase (ALP), Alanine Transaminase (ALT), Aspartate Transaminase (AST), Albumin (ALB) and Bilirubin levels in normal rats were evaluated using assay kits (Randox Laboratories LTD. United Kingdom BT29 4QY). The determination of serumTotal Cholesterol (TC) was by method of Searcy and Berquist (1960), High Density Lipoprotein Cholesterol (HDL-C) and Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C) were by Friedwald *et al*. (1972), while Triglyceride (TG) was by method of Tiez (1990).

Statistical analysis: The results obtained in the research work were expressed as mean±standard deviation. The difference between mean values was assessed for significance by student t-test at $p<0.05$ level of significance.

RESULTS

The result as shown in Table 1 shows a significant decrease in the activity of ALT in the plasma which did not depend on the concentration. The aqueous extract showed an increase in the plasma concentration of AST, TP and ALB which was not significant. However, there was a significant increase in the concentration of ALP. BIL level increased with 100 mg/Kg group but as the concentration of the extract increases the plasma BIL decreases. Table 2 showed the effect of the ethanolic extract of the plant on the plasma activity of ALT, AST, ALP, ALB, TP and BIL. There is a significant concentration dependent reduction in the plasma ALT and ALP levels. Although the extract caused a reduction in the levels of plasma AST, TP and BIL, it was not concentration dependent. An increase in the level of ALB was as well not concentration dependent. Table 3 represents the effect of the aqueous extract of the plant on the plasma lipid profile. In this table, the plasma level of HDL-C increases significantly with increase in concentration of the aqueous extract administered. The plasma concentration of VLDL-C and TG reduced with increase in concentration of the extract. There was an increase in TC levels as compared with the control but were not significant. The LDL-C decrease was concentration dependent but increased as the concentration of administered extract increased. Table 4 showed the effect of the ethanolic extract on the plasma lipid. TC and LDL-C showed an initial decreased in plasma concentration but as the concentrations increased the levels of the lipids increased. However, the plasma concentration of HDL-C decreases but not significant. There was a significant increase in the plasma VLDL-C and TG concentration of rats administered with ethanolic extract from the plant.

DISCUSSION AND CONCLUSION

The plasma concentration of ALT, AST, ALP, BIL, ALB and TP determines the functionality and cellular integrity of the liver (Shivaraj *et al*., 2009). ALT and AST are biomarkers of the hepatocytes. Under pathological conditions of the liver including, cirrhosis, adverse effects of some drugs (e.g., paracetamol), there is a leak of these enzymes in to the plasma, thus raising their activity (Nyblom *et al*., 2004). ALT is specific for the liver but AST is also found in other tissues including the red blood cells, the cardiac and the skeletal muscle. ALP is located in the biliary duct of the liver (Nyblom *et al*., 2006). Obstruction of this duct increases the level of the enzyme in the plasma.

Albumin and globulin constitutes the total plasma proteins and are in ratio 1:2. Thus, albumin constitutes the major component of the Total Plasma protein (TP).

Table 1: The effect of aqueous extracts of *Vernonia amygdalina* on plasma Total Protein (TP), Total Albumin (ALB), Bilirubin and serum enzymes activities

	Control	100 mg/kg	200 mg/kg	300 mg/kg
ALT (U/L)	29.12±2.66	24.00±2.66	14.76±4.25*	21.72±2.16*
AST (U/L)	12.70±3.93	15.74±3.69	15.9±6.73	20.95±1.32*
ALP (U/L)	94.60±29.67	124.4±27.10*	85.80±7.29	102.80±35.20
TP (g/dL)	04.40±3.24	06.81±1.48	06.04±0.82	06.01±0.97
ALB (g/dL)	02.85±7.12	04.45±0.13	04.31±0.09	04.53±0.24
BIL (g/dL)	00.15±0.67	00.30±0.36*	00.20±1.32	00.11±0.37

*: given as mean±standard deviation had significant differences, when compared with the control ($p<0.05$)

Table 2: The effect of ethanolic extracts of *Vernonia amygdalina* on plasma Total Protein (TP), Total Albumin (ALB), Bilirubin and serum enzymes activities

	Control	100 mg/kg	200 mg/kg	300 mg/kg
ALT (U/L)	28.43±1.55	33.80±1.55	18.40±4.08*	12.80±3.99*
AST (U/L)	13.30±1.70	11.50±0.52	13.20±1.39	12.70±2.86
ALP (U/L)	90.60±12.86	84.10±8.28	80.4±10.03	70.70±7.45*
TP (g/dL)	03.40±0.21	03.40±0.05	03.6±0.11	03.40±0.19
ALB (g/dL)	02.50±0.07	02.71±0.09	02.50±0.54	02.64±0.90
BIL (g/dL)	0.13±0.34	00.13±0.14	00.08±0.18	00.13±1.37

*: given as mean ± standard deviation had significant differences, when compared with the control ($p<0.05$)

Table 3: The effect of aqueous extracts of *Vernonia amygdalina* on plasma Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C) and Triglyceride (TG) on normal male rats

	Control	100 mg/kg	200 mg/kg	300 mg/kg
TC (mg/dL)	73.64±8.06	79.46±0.3	96.12±0.93	89.53±0.43
HDL-C (mg/dL)	06.98±1.19	15.12±0.54*	21.32±0.3*	04.26±12.01*
LDL-C (mg/dL)	65.24±0.27	62.97±0.39	73.54±0.65	84.00±0.41
VLDL-C (mg/dL)	01.42±0.27	01.31±0.13	01.26±0.22	01.27±0.18
TG(mg/dL)	07.08±4.68	06.55±0.12	06.28±0.13	06.37±0.16

*: given as mean±standard deviation had significant differences, when compared with the control ($p<0.05$)

Table 4: The effect of ethanolic extracts of *Vernonia amygdalina* on plasma Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C) and Triglyceride (TG) on normal male rats

	Control	100 mg/kg	200 mg/kg	300 mg/kg
TC (mg/dL)	65.89±3.49	50.39±1.98	77.52±0.83	86.82±3.99
HDL-C (mg/dL)	06.59±0.52	05.43 5.54	08.14±3.53	05.43±2.65
LDL-C (mg/dL)	57.60±2.62	43.22±0.77	66.48±0.72	78.17±3.90*
VLDL-C (mg/dL)	01.70±0.40	01.74±1.23	02.90±1.34	03.22±1.26*
TG (mg/dL)	08.50±3.49	08.70±1.20	14.50±1.74*	16.10±1.31*

*: given as mean±standard deviation had significant differences, when compared with the control ($p<0.05$)

It is synthesized in the liver and therefore a diagnostic tool for the determination of liver functionality. It has a half life of 120 days and its level is lowered in chronic liver disease such as cirrhosis and in poor diet or states of impaired protein catabolism. BIL is a catabolic intermediate of haem. High concentration of this molecule results in jaundice. However, the liver plays an important role in mopping bilirubin from the plasma. In the presence of liver disease or damage, the level of BIL increases Lipid profile is the collective term given to the estimation of, typically, TC, LDL-C, HDL-C and TG. This profile is used to access the risk of cardiovascular disease and is altered in the serum of various disease states as demonstrated in diabetes (Betteridge, 1994). LDL is one of the lipoprotein components of the blood. It transports cholesterol mainly to the arterial wall. This results in the buildup of insoluble lipid on the wall of the arteries thereby reducing blood flow and increases the pressure on the wall as well as the heart. The deposition of the cholesterol on the arterial wall results to a condition known as arteriosclerotic plaque which is the major cause of cardiovascular disease. Cardiovascular Diseases (CVD) are the leading cause of death in developing countries (Latunde-Dada, 1990). Hypercholesterolemia has been identified as a primary risk factor in the development of CVD. This implies that, preventing or reducing the serum levels is associated with reducing risk of CVD (Onyeneke *et al*., 2008). The ethanolic extract of *V. amygdalina* has hypolipidemic and hypocholesterolemia effects on alloxan induced diabetic rats (Igbakin and Oloyede, 2009).

In contrast, HDL binds to arterial cholesterol and transports it to the liver for metabolisms. People with higher levels of HDL-C seem to have fewer problems with CVD, while those with low HDL-C have increased rate of cardiovascular disease. Thus, substance that increase the plasma HDL-C level but decrease LDL-C

level will play an important role in reducing the risk of CVD. Therapeutic effects of plant foods have been the focus of many extensive dietary studies (Yokozawa *et al*., 2006).

The extract of *V. amygdalina* showed an effect of reducing the plasma and hepatic total cholesterol and triglyceride (Oluwatosin *et al*., 2008). HDL-C, LDL-C, and VLDL-C in the plasma form the total cholesterol level in the plasma. Though, the value of total cholesterol does not give the ideal arteriosclerotic status of an individual as the HDL-C might contribute to high TC level.

Cholesterol is synthesized from long chain fatty acid. These fatty acids are attached to the glycerol side chain of triglycerides. Thus, increase in the TG level often increases the synthesis of cholesterol from the liver. Consequence of this, high concentration of TG also increase the risk of cardiovascular diseases as high concentration of TG is linked to large size LDL-C (Richards *et al*., 1989; Kanter *et al*., 1985).

From this study, the aqueous extract from the leaves of *V. amygdalina* was able to increase the HDL-C level in a way that is concentration dependent. The increase in the HDL-C may have been influenced by the action of the extracts on the activities of Lecithin Cholesterol Acyl Transferase (LCAT), which plays a key role the maturation of HDL-C particles (Glomset, 1968). The LDL-C level only reduced in concentration in rats administered 100 mg/kg but later increased with increasing concentration. Triglycerides level decreased in rats administered the aqueous extract, with the highest level of reduction seen at 200 mg/kg group though not significant. The TG level of the ethanolic extract administered rats increased significantly in a concentration dependent pattern. This may be as a result of increased activitives of lipopropein lipase and triglyceride lipase that is associated with hypertriglyceridemia (Kanter *et al*., 1985; Richards *et al*., 1989).

In conclusion there was a significant decrease in the ALT level suggesting that the aqueous extract from *V. amygdalina* has the potential to resuscitate the hepatocytes. The ethanolic extract from *V. amygdalina* leaves suggests both increase in cellular integrity and functionality of the liver as demonstrated by the decrease in the level of ALT, AST, ALP and increase in ALB and TP and decrease in plasma bilirubin concentrations. This result agrees with the work of (Arhoghro *et al*., 2009; Iwalokun *et al*., 2006). From this study, the aqueous extract from the leaves of *V. amygdalina* was able to increase the HDL-C level in a way that is concentration dependent. Further work is also been encouraged on the effect of *V. amygdalina* on lipid profile.

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