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Effect of *Vernonia amygdalina* Del. on liver function in alloxan-induced hyperglycaemic rats

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Abstract

The protective effect of ethanolic leaf-extract of an anti-diabetic plant, *Vernonia amygdalina* Del. on hepatic cell architecture and some biochemical indices of liver function has been studied in alloxanized Wistar rats. Twenty-one rats (120-160g) were included in the study and assigned to 3 groups of 7 rats each. Groups 1 and 2, normal and diabetic controls received placebo treatment, whereas group 3 the experimentally diabetic rats administered the plant extract (400mg/kg body weight) by gastric intubation for 14 days. Serum aminotransferase activities, significantly raised ($P<0.05$) in diabetic control group (15.75 ± 1.75 and 37.81 ± 7.62 respective for alanine and aspartate aminotransferase) relative to their respective normal control values (5.02 ± 1.79 and 12.22 ± 1.24) were decreased significantly ($P<0.05$) following treatment with the extract (8.57 ± 2.69 and 18.90 ± 1.37) representing 45.59% and 50.01% decrease respectively. Alkaline phosphatase activity follow a similar trend: Diabetic extract treated values (307.06 ± 54.42) decreased significantly with respect to diabetic untreated (433.08 ± 29.96) a 19.75 %. Serum protein total and albumin levels were respectively increased (7.54 ± 0.60 and 3.18 ± 0.27) compared to their diabetic control values (6.45 ± 0.60 and 2.88 ± 0.88). However, the increase was only significant ($P<0.05$) for albumin. Histological studies reveal for the extract treated group a restoration of hepatocyte degeneration, cellular sequestration and disoriented architecture observed in diabetic control group. *Vernonia amygdalina* Del. therefore can protect against hyperglycemia induced hepatotoxicity, besides its hypoglycaemic action.

Keywords: *Hyperglycaemia, Vernonia amygdalina, liver function, liver histology.*

Introduction

In Africa and the third world countries in particular, the use of indigenous plants in the management of diabetes mellitus is now a common practice (Nimenibo-Uadia, 2003). Over 400 of such plants have been reported by Bailey and Day (1989). Two exhaustive reviews have also been published based on global literature survey on 150 plants (Handa *et al.*, 1989) and 343 plants (Rahman and Khurshidi, 1989) from different parts of the world. The World Health organization (WHO) Expert Committee on Diabetes on its part has recommended accordingly that this area warrants further investigation (WHO, 1980). Cited references by Jelodar *et al.* (2005) support and presupposed that these medicinal plants achieve their hypoglycemic action via a variety of mechanisms: insulin-like substances contained therein, inhibition

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of insulinase activity, interference with carbohydrate absorption, and/or increase in beta cell mass in the pancreas. A survey of available literature and update on diabetes mellitus show that research in medicinal plants has concentrated mainly on the screening of these plants for their blood-sugar lowering effect (hypoglycemic and anti-hyperglycemic action). Little or no research is focus on the effect of these glucose lowering medicinal plants on organs and tissues associated with glucose homeostasis, xenobiotics biotransformation and those organs on which complications of diabetes find expression. It is a necessary compliment of any treatment option that a drug impacts favourably, in a wholistic sense, the entire physiology and biochemistry of the patient, besides its primary action, in this case hypoglycemic/antihyperglycemic action.

The liver remains one of the key organs involved in central body metabolism and xenobiotics biotransformation and the target site of glucose homeostasis and diabetes complications. Chronic hyperglycaemia of diabetes mellitus has been strongly associated with damage, dysfunction and failure of several organs (Lyra *et al.*, 2006). More so, long term hyperglycaemia increases the generation of free radicals via glucose auto-oxidation (Ugochukwu *et al.*, 2003) and the increment in free radical load may lead to liver cell damage, hence increased liver enzyme activity in the plasma. It is of immense importance therefore to assess the impacts of any potential treatment option, particularly of traditional origin on the liver cell function.

The plant, *Vernonia amygdalina* Del. (Compositae) is widely distributed in the West Coast of Africa where it grows wild and as a domestic browse plant (Farombi, 2003). It is commonly known as "bitter leaf" in Nigeria because the leaves and the stem have an astringent bitter taste. It is a source of many local/ traditional medications and has a long history of use in folk medicine (Biser, 1998) particularly among the peoples of sub-Saharan Africa. The hypoglycemic and anti-hyperglycemic effects of this plant have been reported (Akah and Okafor, 1992; Nimenibo-Uadia, 2003; Akah *et al.*, 2004). The plant extract is believed to have some unique features such as beta cell protective and regenerative properties (Ebong *et al.*, 2006).

Nevertheless, there is a paucity of information on the safety of its use in the management of diabetes, i.e. how it impacts the overall physiology and biochemistry of the individual. Ibrahim *et al.*, (2000) have merely reported significant reduction in body weight, but normal histology of kidneys, hepatic and testicular architecture of normal rats upon chronic feeding with leaves of *Vernonia amygdalina* Del.

We therefore, in this study, assessed the impact of the ethanolic extract of the leaves of this anti-diabetic plant on the histology and biochemical indices of liver function in alloxan induced diabetic rats, with a view to ascertaining its protective effect or otherwise on the hepatocytes.

Experimental

Collection of plant and preparation of extract. Matured leaves of *Vernonia amygdalina* Del. obtained from the Endocrine Research Farm of the University of Calabar, Calabar were rinsed with distilled water and dried under shade. The dried leaves were ground into coarse powder, and 100g of the powdered leaves suspended in 600ml of ethanol (98.67%, BDH). The suspension was agitated with an electric blender for about 10 minutes then allowed in a Refrigerator (4°C). Twenty four hours later, the suspension was filtered with a chess cloth and the filtrate concentrated *in vacuo* to 10% of the original volume at 40°C. This concentrate was allowed in a water bath at 37°C for complete dryness, yielding 35.0g (35% yields) of the crude extract. This

extract was reconstituted to an appropriate concentration prior to administration.

Animal handling and experimental protocol. Twenty-one Wistar rats (120-160g) of both sexes obtained from the Animal House, Department of Human Anatomy, University of Calabar were used for this study after due permission from the College Animal Ethics Committee had been obtained. The animals, were allowed one week of acclimatization in Biochemistry departmental animal house facility, and thereafter reweighed and housed in standard cages (North Kent Co. Ltd). The animal house facility was maintained under standard environmental conditions of temperature (28 + 2°C); relative humidity (50± 5%) and 12 hour light/dark cycle with adequate ventilation. The animals were maintained throughout the experiment on commercial rat chow and tap water provided *ad libitum*. The animals were divided into 3 groups of 7 rats each, Groups I (NC) and II (DC) were respectively normal and diabetic controls and both treated with equivalent volume of vehicle, whereas group III (DT) comprised of the test rats administered with the extract in dose 400 mg/kg body weight. The oral administration was done twice per day (12-hour cycle – 7.00am and 7.00pm) for a 14-day period. Diabetes was induced in animals in groups II and III by intra-peritoneal injection of 150mg/kg body weight of alloxan monohydrate (Sigma, St. Louis, MO, USA) in distilled water after an overnight fast. The rats were rested for four days to facilitate development of hyperglycemia and blood glucose equilibration. Thereafter hyperglycemia was confirmed using a glucometer – Lifescan (One-Touch Basic). Only animals with Random Blood Glucose level ≥ 200mg/dl (11.1mmol/l) were considered diabetic and used for groups II and III.

Collection of samples for analyses. Twelve hours after last feeding and drug treatment (overnight fast) the animals were anaesthetized under chloroform vapour and dissected. Whole blood obtained by cardiac puncture into non- heparinized tubes were allowed to clot for about 2 hours, and thereafter centrifuged (at 4,000g for 10min) to remove cells and recover serum, which was used for the biochemical assays. The liver tissues were surgically removed, cleansed of blood with 0.25M sucrose solution and then fixed in 10% formaldehyde preparatory to histological processing

Biochemical assays. Assay kits used in biochemical assays were obtained from DIALAB Produktion und Vertrieb von chemisch-technischen Produkten und laborinstrumenten Gesellschaft m.b.H (GmbH); A-1160 Wien-Panikengasse 3-5: Serum glucose (Trinder, 1972) aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) (Thefeld *et al.*, 1974) serum protein total (TP) and albumin (Tietz, 1976).

Histopathological study. The fixed liver tissues were sectioned (5-micron thickness) and sections stained with Haematoxilin and Eosin (H&E) according to method of Conn, (1946). Photomicrographs (magnification: x 400) were then developed.

Statistical evaluation. The results are expressed as Mean ± SD: The Students unpaired t–test was used to evaluate the differences in mean changes of parameters in the test groups with respect to controls at $P<0.05$ confidence level.

Results

Table I: Serum glucose and some indices of liver function of alloxanized rats treated with ethanolic extract of *V. amygdalina* Del. for 14 days. The changes in blood glucose and selected indices of liver function: AST, ALT, ALP, TP and albumin in serum of alloxan–induced hyperglycemic rats following treatment with extract of *V. amygdalina* Del.

are shown in table I. Serum blood glucose (247.25 ± 4.83), ALT (15.73 ± 1.75), AST (37.81± 7.62) and ALP (433.08 ± 29.96) of the hyperglycemic rats were increased significantly (P<0.05) with respect to values of their respective normal controls (73.51±18.98, 5.02±1.79, 12.22±1.24 and 239.74±23.70). These however, decreased significantly (P<0.05) upon treatment with extract to: 144.14±25.83, 8.57±2.69, 18.90 ± 1.37 and 307.06±54.42 respectively. Serum total protein (6.45±0.60) and albumin (2.88±0.88) levels which were decreased in the hyperglycemic control animals with respect to normal control values become increased following treatment with extract (7.54 ± 0.60 and 3.18± 0.27). However, these increases were only significant (p<0.05) for albumin levels, whereas total protein changes were non-significant (p>0.05) relative to their respective diabetic control.

Changes in the histology of the liver tissues of the various treatment groups are shown above in plates I – III. Plate I, NC was most intensely stained, with no evidence of cell injury: Prominent central vein (V) from where hepatocytes (H) radiate outward in form of spokes in a wheel. The polygonal hepatocyes (H) showed distinct-singular and/or polynuclei (Nu) and cell outline. The cytoplasm stained eosinophilic and hepatic sinusoids (S) run in between sheets of the hepatocytes. The diabetic control histology (plate 2) revealed cell sequestration, indistinct cell nuclei (Nu) and outline: The sinusoid(S) were not radiating, but tend to be wider and intermittent/interrupted. The cells were degenerated, with reduction in number of nuclei. However, photomicrograph of diabetic treated rats (plate 3) showed a fairly non-distorted central vein (V) with sinusoid(S) radiating, though they appear slightly wider compared to normal control. The hepatocytes became well outlined and stained with no sign of degeneration when compared to diabetic control. There was also increase in the number of stained nuclei.

Discussion

Serum enzymes are the most commonly used and most sensitive biochemical tools for the assessment of hepatocellular injury and its resultant jaundice. The most commonly used enzymes are the aminotransaminases (ALT and AST), alkaline phosphatase (ALP) and α-glutamyltranspeptidase (GGT). Whereas, abnormal increases in aminotransferases specifically ALT generally reflect liver cell damage (hepatotoxicity), that of ALP is more specific for cholestasis- hepatobiliary damage (Nduka, 1997; Mayne, 1998). In this study AST, ALT and ALP levels were all significantly (p<0.05) elevated in serum of diabetic control animals, but later to be ameliorated in the diabetic group, treated with the extract. This observation is in line with the recent findings of Kim *et al.* (2006). The authors reported significant increases in serum activities of AST, ALP, LDH (lactate dehydrogenase) by 3.9, 2.6, and 27.1 times respectively in alloxan-induced diabetic rats.

Table 1: Serum glucose and some indices of liver function of alloxanized rats treated with ethanolic extract of *V. amygdalina* Del. for 14 days. (Values expressed as Mean ± SD, n = 7)

Group	Serum glucose (mg/dl)	ALT (IU/l)	AST (IU/l	ALP (IU/l)	TP (g/dl)	Albumin (g/dl)
Normal Control (0.2ml dist. water)	73.51 ± 18.98	5.02 ± 1.79	12.22 ± 1.24	239.74 ± 23.70	6.95 ± 0.27	3.12 ± 0.14
Diabetic Control (0.2ml dist. water)	247.25 ± 4.83*	15.73 ± 1.75*	37.81 ± 7.62*	433.08 ± 29.96*	6.45 ± 0.60	2.88 ± 0.88*
Diabetic Treated (400mg/kg of extract)	144.14 ± 25.83 **	8.57 ± 2.69 **	18.90 ± 1.37 **	307.06 ± 54.42 **	7.54 ± 0.60	3.18 ± 0.27**

* = significant (P<0.05) relative to normal control ** =significant (P<0.05) relative to diabetic control

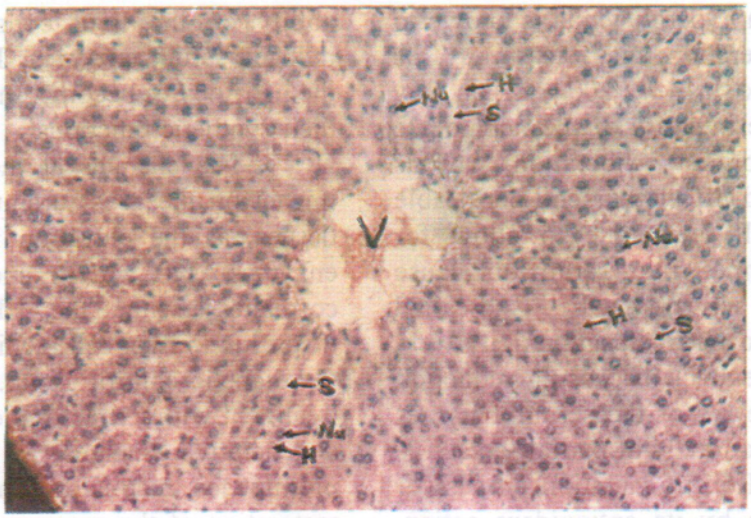

Plate 1: Photomicrograph(x 400) of liver tissue of normal control rat stained with H and E

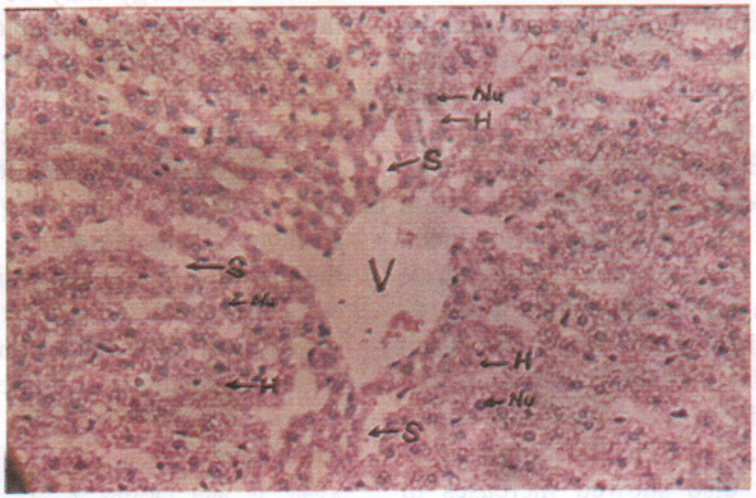

Plate II: Photomicrograph(x 400) of liver tissue of diabetic control rat stained with H and E

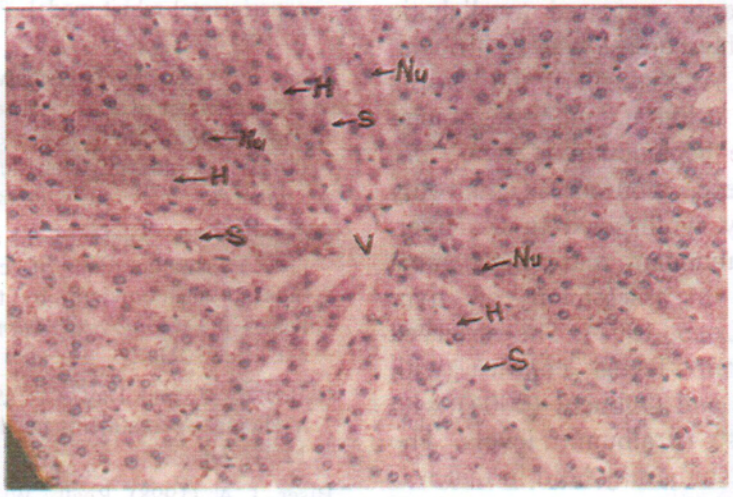

Plate III: Photomicrograph(x 400) of liver tissue of diabetic rat treated with plant extract stained with H and E

Long term hyperglycemia is known to increase the generation and release of free radicals (Ugochukwu *et al.*, 2003) which may interfere with the integrity of the hepatocyte membranes-membrane liberation. This interference could affect the semi-permeable status of the cell membrane to allow for leakage of enzymes into serum to cause increase in activity, the so-called hyperglycemia-induced hepatotoxicity. *V. amygdalina* leaf-extract in causing reduction in the elevated levels of the serum enzymes may have reversed totally or partially reactive oxygen species (ROS) generation process or mopped them up by its antioxidant action. Secondary metabolites and vitamin C and E present in the plant have been reported by Igile *et al.*, (1994) to exhibit antioxidant effect. The proposition here, is also strengthened by the fact that our report is consistent with Babalola *et al.* (2001) who had previously demonstrated the restoration of carbon tetrachloride-induced hepatotoxicity, (a classical case of free radical damage), using a terpenoid extract from *V. amygdalina*. In their study, they also used AST and ALT as the indicators of hepatotoxicity and compared the effect with a well known antioxidant from *Garcinia cola.*

Our study also showed increases in serum total protein and albumin levels in extract treated group compared to diabetic control, although the increase was significant, only for albumin. Almost all of the plasma proteins, save immunoglobulin, are synthesized in the hepatocytes (Gaw *et al.*, 1995) and of these, albumin is the only protein exclusively synthesized in the liver, hence the most abundant and single most important index in assessment of liver synthetic ability (Loeb, 1991). The observed increase in albumin levels contrast with results of Uhegbu and Ogbuehi (2004) who reported a decrease in albumin levels. The observation in our study may hold in that it is in conformity with the extract's protective ability on the liver cells, already seen in reduced serum enzymes. The more protected the hepatocytes become, the more the boost in their synthetic functions.

The changes at the molecular levels impact on the gross architecture of the liver tissues, as the elevation in liver diagnostic enzymes-ALT, AST and ALP, strongly correlates with the disorientation and distortion in liver cell architecture observed in the diabetic control group. Administration or treatment with extract remediated the hitherto degenerative changes, as the cells regained prominence, distinct outlines and nuclei. The restoration of the gross architectural changes in liver histology may be a consequence of the re established glucose control and not the direct effect of the extract on the liver cells themselves. Ibrahim *et al.* (2000) have shown that the leaves of this plant on their own have no ability to alter the histology of the liver.

Besides the hypoglycaemic and antihyperglycaemic action of *V. amygdalina*, the result of this investigation suggests that it can also protect against diabetes-induced hepatic cell injury as a compliment of its use in the management of diabetes.

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