EFFECT OF AQUEOUS EXTRACT OF *Vernonia amygdalina* LEAF ON LIVER WEIGHT AND SERUM ENZYME ACTIVITIES IN ALLOXAN INDUCED DIABETIC RATS

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***[1]OPARA, R. N., [1]EBONG, P. E., [1]EYONG, E. U., [1]ATANGWHO, I. J., AND [2]IWUALA C. C.**
[1]Department Of Biochemistry, University of Calabar, Calabar, Nigeria
[2]Department of Public Health Technology, Federal University of Technology, Owerri
* Author Correspondence

ABSTRACT: The effect of *Vernonia amygdalina* aqueous extracts on the liver weight and some liver enzymes were determined in serum of alloxan induced diabetic rats. The enzymes assayed were the aminotransaminases (ALT and AST), alkaline phosphatase (ALP) and Glutathione peroxidase (GPX). The 21 day cumulative study showed a significant ($P<0.05$) increase in liver weight of animals in the diabetic control group (0.48IU/L) and the tested DT, DT_2 and DT_3 (4.91 IU/L ± 0.52, 5.10 IU/L ± 0.16 and 4.73 IU/L ± 0.43 respectively) compared to the negative control 3.94 IU/L ± 0.43. Alkaline phosphatase (ALP) activity (IU/L) in the serum were significantly raised ($P<0.05$) in the diabetic control group (833.08 IU/L ± 39.96) with respect to the negative control group (439.74 l/L ± 33.70). There were decreases in activities in the treated diabetic groups DT_1, DT_2 and DT_3 (787.06 IU/L ± 74.42, 484.32 IU/L ± 33.55 and 308.79 IU/L ± 5.20 respectively compared to the diabetic control. Gluthathione peroxidase (GPX) activity in liver tissues were significantly higher in diabetic group (2.12mu/ml ± 0.63) compared to the negative control (1.47 mu/ml ± 0.04). There were decreases in activities in treated diabetic group DT_1 and DT_2 (1.99mu/ml ± 0.41 and 1.73 ± 0.12) compared to the diabetic group. There were significant decrease in GPX and ALP activities (1.47mu/ml ± 0.04, 322.57lu/L ± 18.20) compared to the negative group. The diabetic control consistently showed significant ($P<0.05$) elevations in liver weight, AST, ALT, ALP and GPX activities compared to the respective controls.

INTRODUCTION

Vernonia amygdalina Del is used as a traditional treatment for diabetes mellitus. This plant *Vernonia amygdalina* Del (composite) 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 leaf and stem have bitter taste. In Nigeria, it is a major vegetable used for better leaf soup and has a long history of use in folk medicine (Bisser, 1998) particularly among the people of the sub-Sahara Africa. In an ethnobotanical survey which identified and documented 22 plants in the south western Nigeria used by traditional healers in management of diabetes mellitus. *Vernonia amygdalina* came second only to *cassia alata* as most frequently used Abo and Adediwora (2000). Scientific study have also confirmed it's antihyperglycemic (Alkah et al., 2004; Nimenbo 2003, action in diabetic and non diabetic rats respective. Winlemann (1989) in his report indicated a strong correlation between the presence of flavoinoid glycosides and phytosterols of plants with hypoglycemic and antihyperglycemic activities respectively. Generally, plants are known to exert their beneficial effect on diabetes via various mechanism – manipulating carbohydrate,. Lipid metabolism in liver (via key

enzymes), influence on the beta cell integrity and insulin releasing activity, aldose reductase and antioxidant defense system manipulation and glucose uptake and utilization Tiwari and Rao, (2002).

This study was aimed at determining the effect of aqueous extract of *Vernonia amygdalina* leaf on the activities of the liver enzymes ALT, ALP, AST and GPX in the serum of alloxan induced diabetic rats.

MATERIALS AND METHOD

Sample collection and preparation

1. **Plant:** matured leaves of *Vernonia amygdalina* Del. were harvested from the Endocrine Research Farm, University of Calabar. The leaves were ground into a gel-like paste and macerated in distilled water and stored overnight at 4°C in a refrigerator. The filtrate obtained after the residue was removed and was used as the aqueous extract. The concentration of this extract was determined by evaporating to complete dryness, and aliquot from the stock and the weight of the residue was considered the actual ingredients contained in a 1 ml of the extract. The aqueous extract concentrate were stored in the refrigerator.
2. **Animals:** Thirty male and female wistar rats weighing 120 – 165g were used. The animals were housed in metabolic cage in the animal house, department of biochemistry, University of Calabar under controlled environmental conditions of temperature (28 ± 2°c) and relative humidity (461 ± 41) and a 12 hour light/dark cycle. The animals were allowed to acclimatize for 7 days and were maintained on a regular commercial rat feed and tap water *ad litum* for 21 days.

Animal grouping and experimental design

The animals were randomly assigned on the basis of weight and diabetic status into 6 groups of five rats each, and treated accordingly.

Group 1 non-diabetic (negative control) NC received 0.2ml distilled water
Group 2 non diabetic (positive control) PC received 400mg/kg extract
Group 3 diabetic control (DC) received 0.2ml distilled water
Group 4 diabetic test I (DT_1) received 200mg/kg of extract
Group 5 diabetic test II (DT_2) received 400mg/kg of extract
Group 6 diabetic test III (DT_3) received 600mg/kg of extract

Administration was by oral gavage. Body weight of animals and RBG were monitored after every 2 days, throughout the administration period, using a beam balance and a glucometer analyzer (one-touch basic) respectively.

Induction of diabetes

Diabetes was induced by intraperitoneal injection of 150mg/kg body weight of alloxan monohydrate (sigma, St. Louis, MO, USA) using distilled water as the vehicle. Animals with random blood glucose (RBG) level ≥ 200mg/dl or 11.1 mol/L after 7 days were considered diabetic (Mayfield, 1998) and used for further experiment.

3. **Collection of samples:** At the end of the 21 days, the animals after an overnight fast were sacrificed and blood samples for analysis were collected by cardiac puncture. The liver and pancreas were equally removed surgically rinsed in normal saline and put into sample tubes containing 10% buffered formalin. The blood was divided into two fractions, a quarter into EDTA tubes for haematological analysis and three quarter into non-heparinsed tubes. Blood in non-heparinsed tubes was allowed to clot for 2 hours after which the tubes were centrifuged (1,000 rpm for 10 minutes) and serum removed with pasteur pipettes. The serum was used for biochemical assays within 2 days with appropriate refrigeration.

ASSAY OF AST, ALT, ALP AND GPX

1. **AST, ALT:** AST and ALT activities were assayed by modified IFCC method using kits obtained Dialab enzyme kits based on Thefeld *et al* (1974).
2. **ALP Activities:** ALP activity was assayed by the optimized DUKC method, using Dialab enzyme Kit, TIetz (1976).
3. **GPX activity:** GPX activity was assayed by colometric endpoint using Bioxytech kit based on Ursini *et al.* (1995).

Histopathological studies

Liver tissue blocks were sectioned at 5 micron with a microtome and sections were stained with haematoxylin and eosin (H and Et) as ascribed by Conn (1946) and photomicrographs developed (x400).

STATISTICAL ANALYSIS

Data are expressed as mean ± SD. Analysis of variance and students t-test were used for the test of significance of the data values of were regarded as significant.

RESULT

Result obtained showed that there were significant ($P<0.05$) increases in liver weight of animals in diabetic control group (5.67 IU/L ± 0.48) and the test groups DT_1, DT_2 and DT_3 (4.91 IU/L ± 0.52, 5.10 IU/L ± 0.16 and 4.73 IU/L ± 0.42 respectively) compared to the negative control (3.94 IU/L ± 0.43) when tested against the positive control (4.00 IU/L ± 0.61) only the diabetic control group and the group treated with the medium dose of the extract increased significantly ($P<0.05$) DT_1 and DT_3 showed increases but were not significant ($P>0.05$).

All experimental groups including DC, DT_1, DT_2, DT_3 and PC had ALT activity significantly raised compared to negative control. Again on comparing to PC only, groups DC and DT_2 showed significant ($P<0.05$) elevation. Groups treated with the low doses DT_1 increased slightly, where as the high dose recipient (DT_3) reduced mildly. Both of these changes were not-signiciant ($P>0.05$) more like ALT, all groups – DC, DT_1, DT_2, DT_3 and PC (also showed significant ($p<0.05$) elevation in ASTs activity with respect to negative control. When compared to the diabetic control groups all groups treated with extract showed reduction in this enzyme activity but significant ($P<0.05$) only in groups PC and DT_2. A comparison with the positive, only the test group treated with medium dose of the extract and the diabetic control group increased significantly ($P<0.05$), though others increased too, but non-0significantly ($P>0.05$). Alkaline phosphatase (ALP) activity (IU/L) in serum were significantly raise ($P<0.05$) in the diabetic control group (833.08 IU/L ± 39.96) with respect to negative control (439.74 IU/L ± 33.70). There were decreases in activities in the treated diabetic groups DT_1, DT_2 and DT_3 compared to the diabetic control unlike ALT and AST, the decrease were dose dependent but only significant in groups DT_2 and DT_3. Also unlike ALT, AST and liver weight PC showed significant ($P<0.05$) decrease in ALP activities (322.57 IU/L ± 18.20) compared to negative control.

Glutathione peroxidase (GPX) activity in liver tissue was significantly higher in diabetic control groups (2.12 mu/ml ± 0.63) compared to the negative control (1.47 mu/ml ± 0.04). There were decreases in activities in the treated diabetic group DT_1 and DT_2 (1.99 mu/ml ± 0.41 and 1.73 ± 0.12) compared to the diabetic group. There was a significant decrease in GPX activities (1.47 mu/ml ± 0.04) compared to the negative group (1.549 ± 0.50). The diabetic control consistently showed significant ($P<0.05$) elevations in liver weight AST, ALT, ALP and GPX activities compared to the respective controls.

HISTOMOPHOLOGY OF LIVER TISSUES

There were no evidence of liver cell injury for the negative and positive controls as the central vein is seen in each liver lobule from where the heptocytes radiate outward in the form spoke in a wheel cells were polygonal having distinct nuclei and cell outline, with the cytoplasm staining eosinoplulic as well as the hepatic sinusoids running in between sheets of the cells (plate 1 and plate 2) diabetic control histology revealed liver cell sequestration, indistinct cell nuclei and outline. Cytoplasm stained basophilic just like the nucleus (plate 3) in the diabetic treated groups sequestration of cells and improved and the liver cell. Architecture gradually returned to normal in a dose-dependent fashion. For instance in group that receive 60-0mg/kg body weight, the cell histology became same as for group1 (plate 4) except that the cytoplasm stained less eosinophilic.

Table 1**:** Liver weight and serum liver enzymes activities in rats treated with aqueous extract of *Vernonia amydalina*

GROUP	LIVER WEIGHT	ALT IU/L	AST IU/L	ALP IU/L	GPX (MU/ML)
Negative control (NC)	3.94 ± 0.43	5.02 ± 1.79	12.22 ± 1.79	439.74 ± 33.70	1.57 ± 0.50
Positive control (PC)	4.00 ± 0.61	9.74 ± 0.45a	21.90 ± 2.36 a,c	322.57 ± 18.70a	1.47 ± 0.04
Diabetic control (DC)	5.67 ± 0.98a,b	15.73 ± 1.75a,b	37.81 ± 7.62	833.08 ± 39.96a,b	2.12 ± 0.63
Diabetic treated (DT_1)	4.91 ± 0.52a	11.43 ± 2.69a,c	25.23 ± 1.37a,b	767.06 ± 74.42	1.99 ± 0.41
Diabetic treated II (DT_2)	5.10 ± 0.16a,b	13.33 ± 2.00a,b	29.51 ± 2.63	484.32 ± 55.88a,b,c	1.74 ± 0.12
Diabetic treated III (DT_3)	4.73 ± 0.42a,c	8.81 ± 0.01a,c	26.51 ± 3.26	308.79 ± 5.20a,c	3.60 ± 1.47

Values expressed as mean ± SD, n = 5
a = $p<0.05$ compared to the negative control
b = $p< 0.05$ compared to the positive control
c = $p<0.05$ compared to the diabetic control

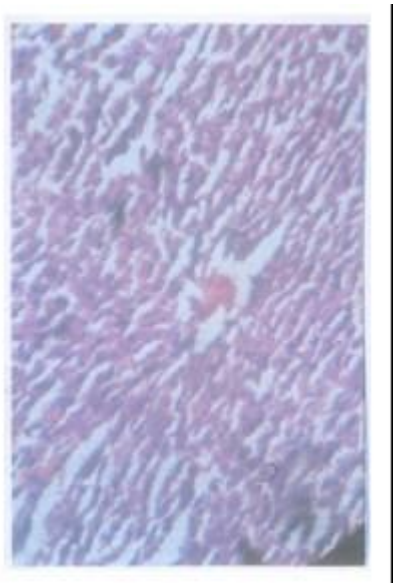

Fig. 1. Photomicrograph of experimental rats showing normal liver tissue

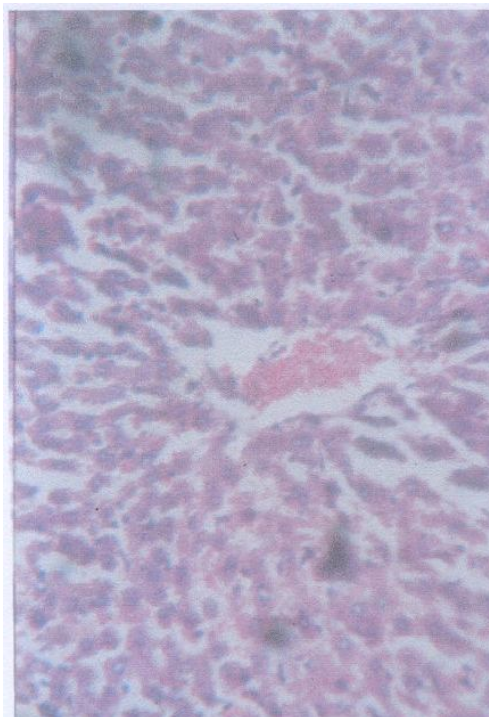

Fig 2. Photomicrograph of experimental rats showing d normal liver tissue (treated with 400mg/kg of extract)

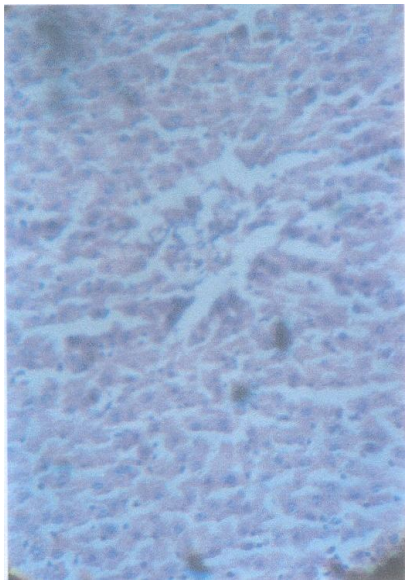

Fig. 3. Photomicrograph of experimental rats showing diabetic control liver tissue

Fig. 4. Photomicrograph of experimental rats showing diabetic liver tissue (treated with 200mg/kg)

DISCUSSION

Serum enzyme levels are most commonly used biochemical tools for the assessment of heptocellular injury and its resultant jaundice including the aminotransferases (ALT and AST) and alkaline phosphatase (ALP) and gamma-glutamyltranspeptidase (αGT) whereas increases in amino transaminases generally reflect liver cell damage, that of ALP is more specific for cholestasis hepatobillary damage (Nduka, 1997). Elevations in ALP could also result from damage in other isoenzymes sites – bone and kidney. Impaired glucose metabolism leads to oxidative stress (ceriello *et al.,* 1992) and protein glycation produces free radicals (Wolf *et al*., 1991). Therefore the decrease in GPX activities in diabetic control rats compared to rats treated with the highest dose of the crude extract from *Vernonia amygdalina* leaves could at least in part result from inactivation of enzymes by H_2+O_2 or by glycation, which are known to occur during diabetes (Sozmen, *et al*., 2001; Hoyson and Fridovich, 1995; Searle and Wilson, 1980). This study revealed elevation in diabetic groups of all the assayed enzymes, these elevations were later to be ameliorated by administration of the *Vernonia amygdalina* extract in a partial dose dependent fashion.

It is possible that the long standing hyperglycaemia could have caused the release of free radicals to interfere with the integrity of the cell membranes - membrane liberation (Ugochukwu and Babady, 2003). This interference could render the cell membranes porous enough to allow for leakage of enzymes into serum to cause increase in activity.

Vernonia amygdalina leaf in causing reduction in these raised levels may have reversed the above process by its antioxidant effect (Igile *et al*., 1994). This proposition is strengthened by the fact that Babalola *et al.,* (2001) previously demonstrated the restoration of carbontetrachloride-induced hepatotoxicity, (a classical case of free radical damage), using extract from *Vernonia amygdalina*. In their study, they also used AST, ALT and ornithine carbomyol transferase as the indicator compared the effect with a well known anti-oxidant from *Garcinai cola.*

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