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Histomorphological Effect of Ethanolic Extract of Vernonia Amygdalina Leaves on the Liver, Kidney and Pancreas of Alloxan Induced Diabetic Female Wistar Rats.

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ABSTRACT

Vernonia amygdalina is used as nutraceutical and in many cultures as edible vegetable, and phytomedicine in the management or treatment of a myriad of ailments including malaria, breast cancer and hypertension. This study was aimed at determining its histomorphological effect on the liver, kidney and pancreas of alloxan-induced diabetic rats. Fifteen female Wistar rats divided into 3 groups of 5 rats each were used for the study. Treatment was through oral route and lasted for two weeks. Diabetic test group was treated with 200mg/kg body weight extract, diabetic control group was treated with 0.2 ml distilled water and the non-diabetic (normal control group) was treated with 0.2 ml distilled water. Diabetic control animals showed significantly ($p<0.05$) raised blood levels of glucose (387.40±8.67mg/dl) while in the diabetic rats treated with the extract, the level of blood glucose was reduced significantly ($p<0.05$) to 266.32±5.00mg/dl when compared to the non-diabetic control animals. Histomorphological studies revealed severe diffuse degeneration of hepatocytes and marked congestion of portal areas, deposition of eosinophilic hyaline materials in the glomerular tufts and degeneration of islet cells in diabetic control group. Diabetic rats treated with 200mg/kg of extract revealed moderate diffuse fatty degeneration of hepatocytes, mild congestion of portal areas, eosinophilic hyaline deposits in some glomeruli and no significant lesion in pancreatic islets. The normal control rats showed no histomorphological changes in the sections of their liver, kidney or pancreas. It is possible that the antidiabetic action of *Vernoniaamygdalina* is probably mediated via insulin production from the regeneration of pancreatic beta cells in diabetic subjects and it may be a potential source of recovery for degeneration of tissue owing to oxidative injuries from chronic diseases like diabetes mellitus.

Keywords: Histomorphology, Vernonia amygdalina, Diabetes mellitus, Alloxan, Liver, Kidney, Pancreas

INTRODUCTION

Diabetes mellitus remains one of the age – long chronic diseases of the human race and its frontiers are expanding by the day. This became so real that in 2004, the World Health Organization identified it as an epidemic underway, since about 191 million persons worldwide were afflicted in 2000 and a possible increase to 366 million by 2030 was projected[1].

In diabetes, lipid abnormalities, anaemia, alteration of liver and kidney functional indices have been implicated as major risk factors to the progression of microvascular and macro vascular complications[2]. Different types of antidiabetic drugs such as biguanide, sulphonylurea, along with insulin have been employed for the treatment of diabetes. Still none of these drugs were able to cure the diseases without adverse reaction[3, 4]. Several conventional treatment options for Diabetes mellitus are available but none has proven the most efficacious in the management of this condition[5].

At present, there is a growing interest in herbal remedies due to the side effects associated with oral hypoglycaemic agent[6]. This situation has led to the consideration of other non-conventional options to manage this condition and notably the herbal oral hypoglycaemic concoctions[7].

Vernonia amygdalina is popularly known as bitter leaf or etidot in Efik language. It is a shrub of 2-5 m tall with petiolate green leaves of about 6 mm diameter. The leaves are characteristically bitter but the bitterness can be abated by boiling or by soaking in several changes of clean water[8]. In Nigeria, the divested stem and root bark are used as chewing sticks. The leaves are a very popular soup vegetable and have even been reported to be consumed by goats in some parts of Nigeria[9]. All parts of the plant are pharmacologically useful. The roots and the leaves are used in ethnomedicine to treat fever, hiccups, kidney problems and stomach discomfort among several other uses[8, 10]. Both aqueous and alcoholic extracts of the stem, bark, roots and leaves are reported to be extensively used as a purgative,

antimalarial and in the treatment of eczema[11].
The plant has acquired special relevance recently, having been proved in human medicine to possess potent antimalarial and anthelminthic properties[12] as well as antitumorigenic properties[13]. It is also known to have an amazing antiparasitic efficacy in zoopharmacognosy as it is easily recognized and used for self-medication by parasitized chimpanzees[14].

The plant *Vernoniaamygdalina* (bitter leaf) is a shrub growing under a range of ecological zones in Africa. It is widely consumed as food (bitter leaf soup) in Nigeria and also has a long history of folk medicine particularly among the people of sub-Saharan Africa[15].

Vernonia amygdalina leaf has been shown to be effective in the treatment of scurvy, rheumatism, pile, indigestion and blood sugar control[16, 17]. The effect of *Vernoniaamygdalina* in alloxan-induced diabetic albino rats has been shown to be hypoglycaemic and hence its use even in humans to treat diabetes mellitus [1, 15, 18, 19]

This study is therefore aimed at determining the effects of oral treatment of diabetic rats with extract of Vernonia amygdalina on blood glucose and histomorphological changes in the pancreas, liver and kidney.

MATERIALS AND METHODS

Collection and preparation of plant extract: The fresh leaves of *Vernoniaamygdalina* were collected in June from the natural habitat of School of Forestry, Jos in Plateau State. The plant was identified and deposited in the department of Pharmacognosy, University of Jos with a Voucher number of UJ/PCG/HSP/95C16. The chemical classes of the constituents in the freshly prepared extract were detected using standard phytochemical reagents and procedures as described by Trease and Evans[20]. Sixty (60) grams of the powdered leaf was weighed and packed in a Soxhlet extractor with 250 ml of 70% ethanol. The solution obtained after 72 hours was then transferred into a rotatory evaporator and concentrated to dryness using low heat of 40-55^{o}C. A yield of 31 % was obtained and the extract reconstituted in distilled water to an appropriate concentration before administration. The acute toxicity (LD_{50}) of the extract was determined using the method of Miller and Tainter[21].

Animals and animal grouping: Fifteen female rats of Wistar strain used for the study were obtained from the Experimental animal house of the University of Jos and acclimatized for two weeks with photoperiodicity of 12-hour light and l2-hour darkness, temperature ranging between 26-30 ^{o}C and relative humidity of 45-55%. They were fed with Growers mash Vital Feed from Grand Cereals and Oil Mills Ltd, Jos and water *ad libitum* until they weighed 150 -200g.

The design consisted of 3 groups of 5 rats each
Group1: Induced Diabetic (test group), treated with 200mg/kg body weight extract.
Group 2: Induced Diabetic (diabetic control), treated with 0.2 ml distilled water.
Group 3: Non diabetic (normal control), treated with 0.2 ml distilled water.
Treatment of the animals was done orally in a 12-hourly cycle daily for two weeks.

Induction of experimental diabetes: Diabetes was induced (groups 1 and 2) by intra-peritoneal injection of 150mg/kg body weight of alloxan monohydrate using distilled water as a vehicle after being deprived of food for 18 hours. Five days later, diabetes was confirmed in the alloxan treated animals with a Glucometer.

Measurement of blood glucose: The blood glucose levels in the animals were measured 72 hours after the drug administration through tail tipping using an On-Call Glucometer (Accu - Chek, Active, Roche Diagnostic's 9115 Hague road, Indiianopolis, 46256 Lo No 115764). Diabetes was confirmed in the alloxan treated animals with Random Blood Glucose (RBG) level of $\geq$ 200mg/dl (11.1mmol/L).

Sample collection for histomorphological analysis: Twelve hours after feeding and administration (overnight fast) for two weeks, the animals were euthanized using chloroform inhalation method and later dissected. The kidneys, pancreas and liver were surgically removed and de-capsulated then cleaned of blood with 0.25M sucrose solution and later fixed in 10% formol saline for routine histological procedure.

Histomorphological study: The fixed liver, kidney and pancreas tissues were sectioned with a rotatory microtome (Chadwell Health, E55Ex, England) at thickness of 5 micrometers and the sections stained with Haematoxylin and Eosin according to Conn[22] procedure. Sections were viewed under a microscope (Olympus KHSNA – 123, China) and photomicrographs developed with a camera (Scope Photo, CVQP – K87, China).

RESULTS

Analysis of blood glucose level of the animal groups is shown in table 1 while Histomorphological study is shown in plates 1 to 9. All data are expressed as mean ± standard deviation. Comparison between test and control groups was done using the student t – test. Differences between groups were considered significant at $p<0.005$.

Table 1: Effect of ethanolic extract of Vernonia amygdalina on blood glucose of alloxan induced diabetic Wistar rats

	Non Diabetic Control (0.2 ml distilled water)	Diabetic Control (0.2 ml distilled water)	Diabetic Treated (200mg/kg of extract)
Mean	105.240	387.400	266.3200
Std. Deviation	8.6705	4.5722	4.49856

Diabetic control animals showed significantly ($p<0.05$) raised blood levels of glucose (387.4±8.67 mg/dl) while in the diabetic rats treated with the extract, the level of blood glucose was reduced significantly ($p<0.05$) to 266.32±5.0 mg/dl as compared to their non-diabetic control animals.

The Non Diabetic Control rats showed no Histomorphological changes in the sections of their liver, kidney or pancreas respectively (plates 1, 2 and 3).

Histomorphological studies conducted on the liver, kidney and pancreas of diabetic control rats, revealed severe diffuse degeneration of hepatocytes with the presence of large vacuoles within the hepatocytes throughout the parenchyma and a marked congestion of portal triad (plate 4). The kidney showed deposition of eosinophilic hyaline materials in the glomerular tufts of a few glomeruli (plate 5). Degeneration of islet cells and deposition of eosinophilic hyaline materials in the surrounding tissues of the pancreas was also noted (plate 6).

Section of the liver of diabetic rats treated with 200mg/kg of the plant extract revealed moderate diffuse fatty degeneration of hepatocytes throughout the liver parenchyma and mild congestion of portal areas (plate 7) while section of the kidney revealed the presence of eosinophilic hyaline deposits in some glomerular mesangial cells within the renal cortex (plate 8). No marked lesion was however observed in the pancreatic islets (plate 9).

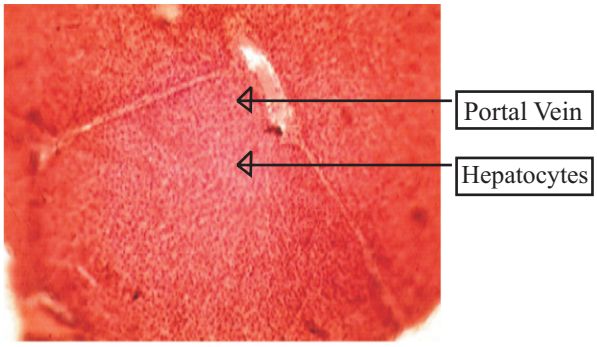

Plate 1: Normal histomorphology of the liver (H & E X 100)

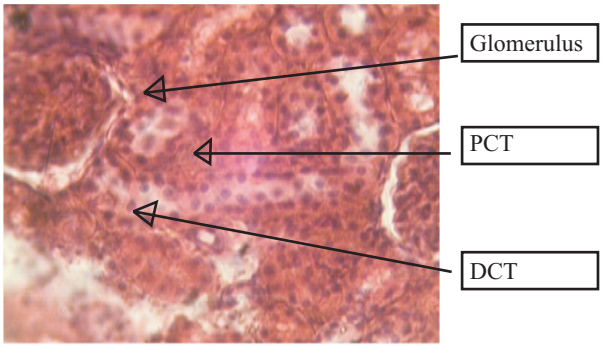

Plate 2: Normal histomorphology of the kidney (H & E X 400),

PCT: proximal convoluted tubule, DCT: distal convoluted tubule

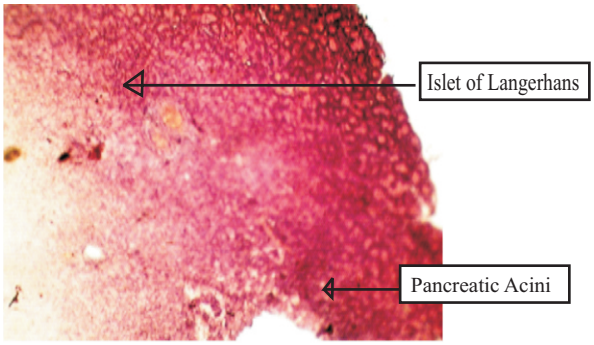

Plate 3: Normal histomorphology of the pancreas (H & E X 400)

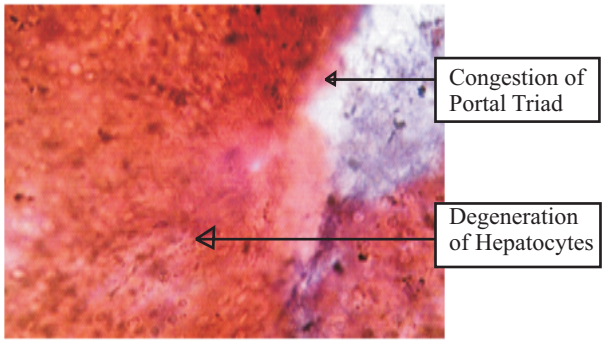

Plate 4: severe diffuse degeneration of hepatocytes with large vacuoles within the hepatocytes and marked congestion of portal triad (H & E X 400)

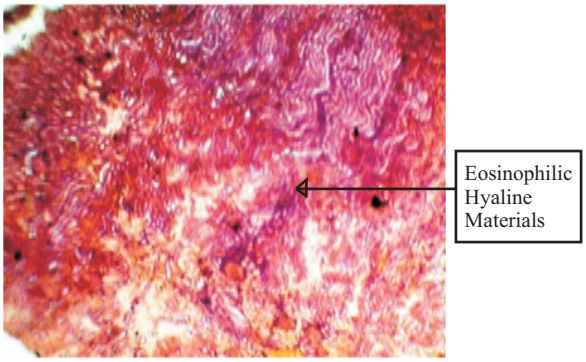

Plate 5: Deposition of eosinophilic hyaline materials in the glomerular tufts of a few glomeruli (H & E X 400)

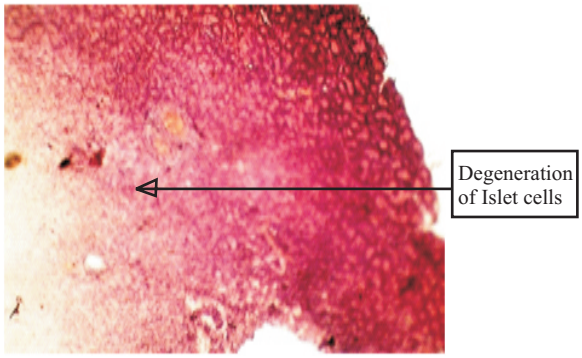

Plate 6: Degeneration of islet cells and deposition of eosinophilic hyaline materials in surrounding tissues of the pancreas (H & E X 400)

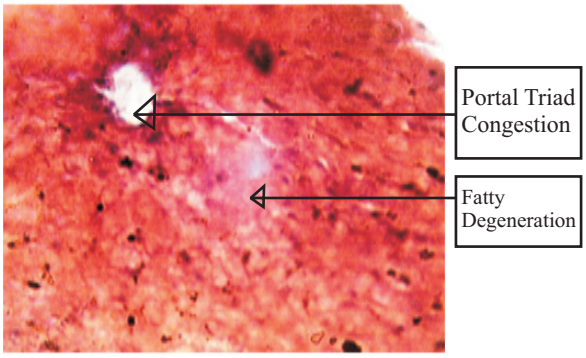

Plate 7: Moderate diffuse fatty degeneration of hepatocytes and mild congestion of portal triad (H & E X 400)

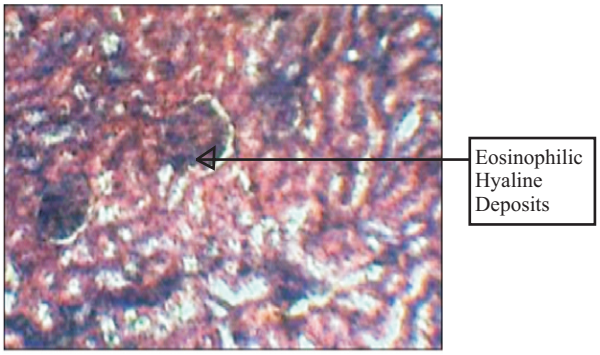

Plate 8: Eosinophilic hyaline deposits in some glomerular mesangial cells within the renal cortex (H & E X 400)

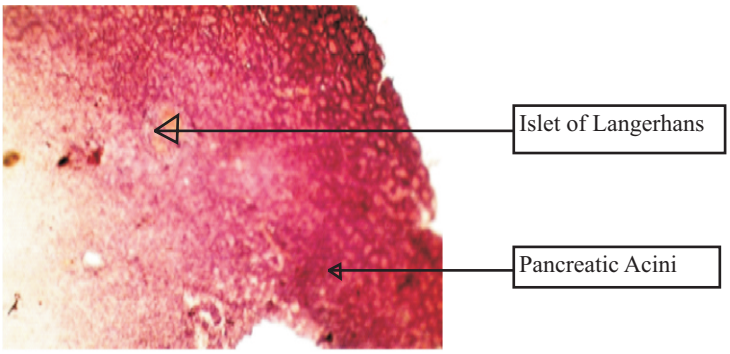

Plate 9: No significant lesion in pancreatic islets (H & E X 400)

DISCUSSION

This study showed significant reduction in blood glucose level of the alloxan - induced diabetic rats following treatment with extract of *Vernoniaamygdalina*. This agrees with earlier reportswhich also showed the antidiabetic action of Vernonia amygdalina[1, 18].

The severe Histomorphological changes seen in the liver, kidney and pancreas of untreated diabetic rats clearly indicated the induced oxidative stress associated with diabetes mellitus induced by Alloxan which is known to mediate beta – cell destruction via reactive oxygen species[23]. It can be suggested that there may be partial restoration of the histomorphological architecture of the studied tissues of diabetic rats treated with the extract of Vernonia amygdalina; however the restoration may be only to a partial extent. It can be hypothesized therefore, that extract from *Vernonia amygdalina* induces pancreatic function recovery by regenerating hitherto destroyed beta cellsand indeed of the kidney and pancreas[1].

Phytochemical analysis of the leaves of Vernonia amygdalina demonstrated the presence of flavonoids, polyphenols, steroids moderately and the slight presence of tannins, glycosides, saponins and alkaloids. Secondary metabolites of plants such as these may possess some alpha glycosidase inhibitors and competitively inhibit intestinal brush border enzymes with an eventual reduction in digestion and absorption of carbohydrates from the gut postprandial hyperglycemia, hence an effective glucose control[24].
However, this second approach, on its own would be insufficient to establish an effective glucose control in experimentally induced type 1 diabetes, requiring insulin. It is opined therefore that this can only act in synergy with the regenerated beta cells for effective glucose homeostasis in diabetes[1].

CONCLUSION

It is possible that the antidiabetic action of *Vernoniaamygdalina* is probably mediated via insulin production from the regeneration of pancreatic beta cells in diabetic subjects and that it is a potential source of recovery for degeneration of tissue owing to oxidative injuries from chronic diseases like diabetes mellitus.

REFERENCES

1. Atangwho IJ, Ebong PE, Eteng MU, Eyong EU, Obi AU. *Effect of Vernonia amygdalina Del Leaf on Kidney Function of Diabetic Rats*. Int. J. Pharmacol., 2007; 3(2): 143 – 148.
2. Mandade R and Sreenivas SA. *Anti-diabetic effects of aqueous ethanolic extract of Hibiscus rosasinensis L. On streptozotocin- induced diabetic rats and the possible morphologic changes in the liver and kidney*. Int. J. Pharmacol. 2007; 7:363-369.
3. Tolman KG and Chandramouli J. *Hepatotoxicity of the thiazolidinediones*. Clinics in Liver Disease. 2003; 7: 369–379.
4. Anees M, Jawad A and Hashmi I. *Distribution of ABO and RH blood group alleles in Mandi Bahauddin District of Punjab, Pakistan*. Proc. Pak. Acad. Sci. 2007; 44(4):289-294.
5. Robinson G and Johnson DE. *Metabolic Disorder: Diabetes. In: Mechanism of Disease. An Introduction to Clinical Science.* 1st Ed., Cambridge: Cambridge University Press.
6. Kim SH, Hyun SH, Choung SY. *Anti-diabetic effect of cinnamon extract on blood glucose in diabetic mice*. J. Ethnopharmacol. 2006; 104:119–123.
7. Bailey CJ and Dey C. *Traditional Treatment for diabetes*. Diabetes care, 1989; 12: 553 – 564.
8. Burkill HM. *The Useful Plants of West Tropical Africa: Review.* Dalziel's J.M Royal Botanic Gardens, Kew. 2nd ed. 1985.
9. Aregheore EMK, Makkar HPS, Becker K. *Feed value of some browse plants from the central zone of Delta State, Nig*. Trop. Sci. 1998; 38(2): 97-104.
10. Hamowia AM and Saffaf AM. *Pharmacological studies on Vernonia amygdalina (Del) and Tithonia diversifolia (Gray)*. Vet. Med. J. Giza 1994; 2: 91-97.
11. Kupcham SM. *Drugs from Natural products: Plant source in drugs discovery, science and development*. Am. Chem. Soc.1971; 6: 311- 318.
12. Abosi AO and Raseroka BH. *In vivo antimalarial activity of Vernonia amygdalina*. Br. J. Biomedical Sci. 2003; 60(2): 89-91
13. Izevbigie EB, Bryant JL, Walker A. *A novel natural inhibitor of extracellular signal-regulated kinases and human breast cancer cell growth*. Experimental Biol. Med. (Maywood). 2004; 229(2):163-169.
14. Huffman MA. *Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants*. Proceedings of the Nutr. Society. 2003; 62(2):371-81.
15. Farombi EO. *African Indigenous Plants with Chemotherapeutic potentials and biotechnological plants with production of bioactive prophylactic agents*. Afr. J. Biotech., 2003; 2: 662 – 671.
16. Erasto P, Grierson DS, Afolayan AJ. *Bioactive Sesquiterpene lactones from the leaves of Vernonia amygdalina.J. Ethnopharmacol*., 2006; 106: 117-120.
17. Iwu MM. *Empirical investigation of dietary plants used in Igbo-Ethnomedicine. In: Iwu MM. Plants in indigenous medicine and diet. Nina Etkined Redgrove Publishers Co, New York*, 1986; pp. 131-50.
18. Akah P, Njoku O, Nwanguwa A, Akunyili D. *Effect of aqueous leaf extract of Vernonia amygdalina on blood glucose and triglyceride levels in alloxan-induced diabetic rats (Rattus rattus). Ani Resnt'l*.,

2004; 1(2): 90-94.
19. Akah PA and Okafor CI. *Hypoglycaemic effect of Vernonia amygdalina (Del) in experimental rabbits. Plant Medicinal Researech*. 1992; 1: 6-10.
20. Trease GE and Evans MC. *Textbook of Pharmacognosy. 12th ed.* Bailliere, Tindall, London, 1983; pp. 343-383.
21. Miller LC and Tainter ML. *Estimation of EC50 and its error by means of log-probit graph paper*. *Proc. Soc. Exp. Biol. Med*, 1944; 57: 261-269.
22. Conn HJ. *Biological Stains: A handbook on the nature and uses of the dyes employed in the biological laboratory*. 5th ed. Geneva: N.Y Biotech publication, 1946 pp. 98.
23. Szkudelsiki T. *The mechanism of Alloxan and Streptozotocin action in beta-cells of the rat Pancreas*. Physiol. Res., 2001; 50: 536 – 546.
24. Tiwari AK and Rao JM. *Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Current Science*, 2002; 83(1): 30-37.