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Histopathological study of the liver of Alloxan induced diabetic rats and macerated *Allium sativum* (garlic) Ameliorative Effect

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Abstract

Histopathological assessment of the macerated *Allium sativum* (garlic) on cytoarchitectural alterations in alloxan (150mg/kg) induced diabetic rat liver was studied. Twenty five (25) weaning rats weighing between 105-205g were divided into five (5) groups of 5 rats each. Group I and group II served as the normal control (NC) and diabetic control group (DC) respectively. The diabetic experimental groups III,IV and V were administered macerated preparation of *Allium sativum* (garlic) at a dose of 6.6g/kg and standard pellets orally for 7 ,14 and 21 days respectively. Thereafter, the animals were sacrificed, liver were weighed and fixed immediately in 10 % formal saline, transported to the laboratory, processed to paraffin wax, cut at 5 microns, stained using Heamatoxylin and Eosin technique and observed histopathologically under light microscope. The result revealed preserving cellular architecture, re-appearance of hyperplastic hepatocytes and cellular restoration, vascular dilatation and pyknotic nuclei in group III as mild, group IV as moderate restoration and group V as complete regeneration when compared to non-diabetic and diabetic control group that showed focal area of necrosis, vascular congestion, hyperplasia, vacuolation, inflammation and cellular degeneration. Statistical value in the weight of the body and liver were not significant at the value ($p > 0.05$) compared to control.

These findings are suggestive of a possible hepato-protective role played by the macerated preparation of *Allium sativum* (garlic) in single administration.

Keywords: *Allium sativum*, *Alloxan*, Diabetes mellitus, Histopathology, liver and weaning rats.

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INTRODUCTION

Following our previous studies of restorative effect of *Allium sativum* on diabetic mellitus rat's kidney, the effect on the live cannot be over-emphasized. Diabetes mellitus known as a disorders characterized by chronic hyperglycemia, polyuria, polydipsia, polyphagia, emaciation, and weakness due to disturbance in carbohydrate, fat, and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action. Present number of diabetics worldwide is 150 million and this is likely to increase to 300 million or more by the year 2025 [14]. Reasons for this rise include increase in sedentary lifestyle, consumption of energy rich diet, obesity, higher life span etc [28]. Regions with greatest potential are Asia and Africa, where diabetes mellitus (DM) rates could rise to two-third folds than the present rate [4].

Unfortunately, DM in the younger age group has been on the rise and there is an urgent need to combat this disease. DM patients are prone to some long-term complications like nephropathy, retinopathy and neuropathy [21]. These long-term complications resulted in diabetic patients' life expectancy accounting to only two-thirds of the general population [1].

Many herbal medicines have been recommended for the treatment of diabetes [18; 3]. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones due to the plethora of active ingredients present in a single herb [26]. On the basis of the above, mono-herbal therapy is considered the preferred therapeutic approach to the effective management of diabetes mellitus given its multifactorial pathogenicity [26; 8].

Allium sativum L. commonly known as garlic, is a specie in the onion family *Alliaceae*. It has a characteristic pungent spicy flavour that mellows and sweetens considerably with cooking. It could either be eaten raw or cooked, or has been used throughout recorded history because of its potential medical properties [25]and [12]. Garlic is rich in antioxidants, which help destroy free radicals – particles that can damage cell membranes, interact with genetic material, and possibly contribute to the aging process as well as the development of a number of diseased conditions, including cardiovascular diseases and cancer.

.Allicin is the main constituent of *Allium sativum*, It is very reactive in lowering serum cholesterol level. The transformation of allicin into the biological active allicin molecule upon crushing of a garlic clove is extremely rapid [13] and [23].In addition to allicin, Diallyldisulfide (DADS), an active principle of garlic is known for its anti-hyperlipidemic properties. However, a study reported that garlic powder preparation did not significantly affect plasma lipids levels [7].

No further studies have been conducted to elucidate possible Histopathological alterations induced by alloxan and possible restorative effect of *Allium sativum* (garlic) on the liver. The liver is intimately associated with the storage of glycogen, the maintenance of the normal blood sugar level and the formation of ketone bodies. The important rôle of the liver in carbohydrate metabolism makes the investigation of liver function in diabetes mellitus pertinent. This study therefore explores the ameliorative effect of macerated effect of *Allium sativum* (garlic) in alloxan induced diabetic groups, keeping in view histopathological alterations in diabetic treated and untreated groups by highlighting the hepato-protective role of the plant.

MATERIALS AND METHODS

Drugs and Chemicals

Alloxan, Sodium chloride, formaldehyde, sodium trioxocarbonade V, sodium bicarbornate, xylene, 70% alcohol, 90% alcohol, absolute alcohol, haematoxylin, eosin, egg albumin, distilled water, paraffin wax were all procured from BDH Chemicals, England. All other chemicals were of analytical grade.

Animals

25 weanling rats (105-205g) were obtained from the University of Uyo animal house. They were maintained on standard pellets (guinea feed) and water *ad libitum.* Permission and approval for animal studies were obtained from the college of health sciences animal ethnics committee, University of Uyo.

Sourcing of Plant material

Freshly harvested bulbs of *Allium sativum* were obtained in October, 2012 from Itam market, Uyo, Akwa Ibom State, Nigeria. The plant was identified and authenticated by the Department of Botany, University of Uyo, Uyo, Nigeria.

Preparation of Allium sativum (Garlic)

The fresh bulbs of *Allium sativum* (Garlic) which weighed (350g) were washed and air dried for 10 minutes. The bulb plants were macerated mechanically with a piston and mortar. The preparation was stored in a refrigerator at 10^{o}C until used for the experiments reported in this study.

Induction of Diabetics

The animals were fasted overnight and diabetes was induced by a single intra-peritoneal injection of a freshly prepared solution of alloxan (150mg/kg body

weight) in 0.9% NaCl saline solution into all the animals in group I, III, IV and V.while group I containing Normal control rats were not given anything except their standard pellet (Guinea feed) and water *ad libitum*. After 72 hours for the development of diabetes, the rats with moderate diabetes having glucosuria and hyperglycemia (blood glucose level range above 250mg/dl) were considered as diabetic and used for plant (herbal) treatment. The macerated plant bulbs and standard pellet (guinea feed) were administered at a concentration of 6.6g/kg (6600mg/kg) body weight/rats/day for 7, 14 and 21 days.

Experimental animal /Study design

The animals were divided into five groups of five (5) rats each and treated as follows:

Group I (NC): Normal control rats were administered standard pellets and water *ad libitum* for 21 days.

Group II (DC): Diabetic control rats were administered with 150mg/kg of alloxan solution, standard pellets and water *ad libitum* for 21 days.

Group III: Diabetic rats were given macerated preparation of *Allium sativum* (garlic) at a dose of 6.6g/kg and standard pellets for 7 days.

Group VI: Diabetic rats were given macerated preparation of *Allium sativum*_(garlic) at a dose of 6.6g/kg and standard pellets for 14 days.

Group IV: Diabetic rats were administered macerated preparation of *Allium sativum*_(garlic) at a dose of 6.6g/kg and standard pellets for 21 days.

The fasting blood glucose levels (BGL) of all rats were recorded at regular intervals during the experimental period. For acute study, the BGL was monitored after 72 hours of administration of a single dose of the macerated preparation of *Allium sativum* and standard pellet (Guinea feed) and the end of 7, 14 and 21days for prolonged treatments.

The BGL was monitored in the blood of the diabetic rats by tail tipping method. The blood was dropped it in the dextrostix reagent pad, which was inserted into microprocessor digital blood glucometer and the readings were noted.

Sample collection for Histopathological analysis.

At the end of the stipulated 21 days feeds were withdrawn, the rats were subjected to a 12 hours fast but had access to water. Sacrificed using chloroform vapour. Whole blood was collected by cardiac puncture (under light anaesthesia). The blood was transferred to plain sample bottles and allowed to clot for about 3 (three) hours. The clotted blood was then centrifuged at 3000 revolution per minute for 30 minutes to recover serum from clotted cells. Serum was separated using sterile syringes and stored under refrigerated condition before biochemical analysis were carried out.

The harvested liver was carefully dissected out, trimmed of all fat and connective tissue blotted dry to remove any blood. The tissues were fixed in 10% formal saline, and then transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicularly to the long axis of the kidney, liver and pancreas. The sections were designated "vertical sections". Serial sections of 5 µm thick were obtained from a solid block of tissue, fixed on clean albuminized slides to prevent sections coming off the slides and later stained with Haematoxylin and Eosin staining techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mount in DPX mountant, allowed to dry at room temperature and observed Histopathologically under digital light microscope.

Gross morphometrical analysis

The weights of body of the rats were measured daily using the weighing balance. The values of all the morphometric analysis were compared statistically using SPSS 17 Software

Photomicrography

Records of the Histological results were obtained by photomicrography using digital photomicrographic microscope at the Gross Anatomy Research Laboratory, Department of Human Anatomy, College of Health sciences, University of Uyo,Uyo,Akwa- Ibom, Nigeria as illustrated in Plate.1 to 5.

RESULTS

PLATE 1 Normal control of Liver tissue showed normal cellular architecture with portal triad, central vein, numerous hepatocytes and sinusoidal lining.

PLATE 2 Diabetic control of Liver tissue induced with 150mg/kg of Alloxan showed cellular abnormalities with area of vascular degeneration, necrosis, vascular congestion and cellular degeneration as compared with normal control group.

PLATE 3 Liver tissue treated with macerated preparation of Allium sativum (garlic) at a dose of 6.6g/kg and standard pellets for 7 days showed slight area of cellular restoration with marked vascular congestion, and cellular degeneration with pyknotic nuclei as compared with normal and diabetic control group.

PLATE 4 Liver tissue treated with macerated preparation of Allium sativum (garlic) at a dose of 6.6g/kg and standard pellets for 14 days showed moderate area of cellular restoration vascular

congestion and pyknotic nuclei as compared with normal and diabetic control groups.

PLATE 5 Liver tissues treated with macerated preparation of Allium sativum (garlic) at a dose of 6.6g/kg and standard pellets for 21 days showed complete restoration as compared with normal and diabetic control groups.

Finally, histological profile from the group treated with macerated Allium sativum (garlic) at a dose of 6.6g/kg at various days 7, 14 and 21 displayed tremendous recovering and restorative effect of the cellular components thereby signifying hepato-protective role of Allium sativum (garlic) on the liver tissues, however the histopathological alteration effect were completely restored.

Histopathological findings

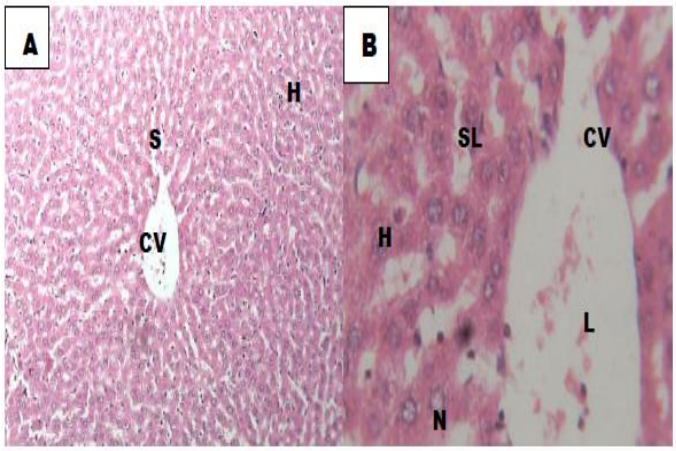

Plate 1 : Normal control of Liver tissue at magnification A(x100) and B(x400) stained with H & E technique.

Note: **CV**-central vein, **L**- lumen, **H**-hepatocytes, **N**- nucleus and SL-sinusoidal layer.

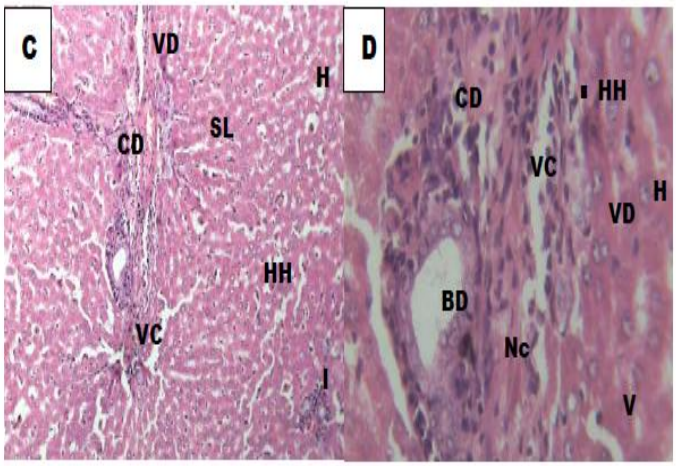

Plate 2: Diabetic control of Liver tissue induced with 150mg/kg of Alloxan at magnification C(x100) and D(x400) stained with H & E technique

Note: CV-central vein, PT- portal triad, HH-hyperplastic hepatocytes, Nc- necrosis, SL-sinusoidal layer and V- vacuolation

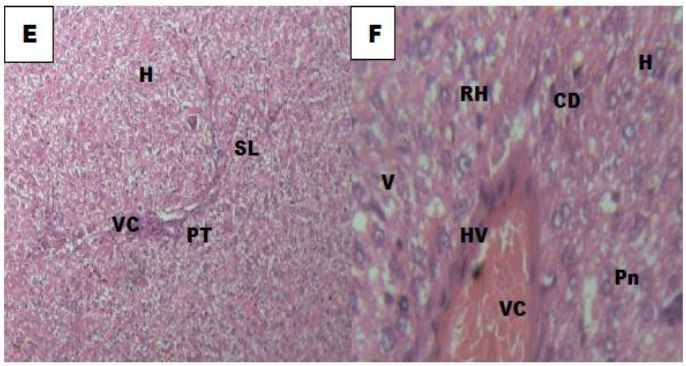

Plate 3: Diabetic Liver tissues treated with 6.6g/kg of Allium sativum for 7 days at magnification E(x100) and F(x400) stained with H & E technique.

Note: CV-central vein, PT- portal triad, RH-restorative hepatocytes, SL-sinusoidal layer, V- vacuolization Vc- vascular congestion, Pn-pyknotic nucleus and Hv- hepatic vein.

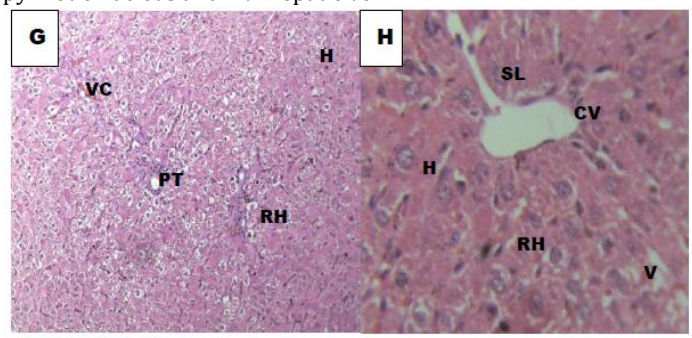

Plate 4: Diabetic Liver tissues treated with 6.6g/kg of Allium sativum for 14 days at magnification G(x100) and H(x400) stained with H & E technique.

Note: CV-central vein, PT- portal triad, RH-restorative hepatocytes, SL-sinusoidal layer, V- vacuolization, Vc- vascular congestion, Pn-pyknotic nucleus and Hv- hepatic vein

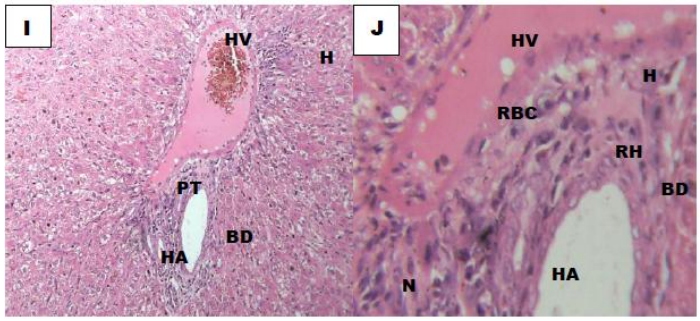

Plate 5: Diabetic Liver tissue treated with 6.6g/kg of Allium sativum for 21 days at magnification I(x100) and J(x400) stained with H & E technique.

Note: CV-central vein, PT- portal triad, RH-restorative hepatocytes, SL-sinusoidal layer, V- vacuolization Vc- vascular congestion, Pn-pyknotic nucleus and Hv- hepatic vein.

DISCUSSION

This study was undertaken to study the anti-diabetic and hepato-protective activity of macerated garlic in alloxan- induced diabetic rats on cyto-architectural alterations. Administration of alloxan (150mg/kg) which was maintained over a given period of time. 3-weeks of daily treatment with macerated garlic, standard pellet (Guinea feed), and water *ad libitum* caused a significant histopathological and reversible

effect ranging from mild to complete restoration in the Liver treated with the garlic after the establishment of diabetics in the rats. Normal control (NC) animals group were found to be stable while diabetic control group showed high level of cellular abnormalities including necrosis, cellular and vascular degeneration, vascular congestion, hyperplasia of the hepatocytes and vacuolation .It is well established that alloxan administration to experimental rats selectively causes pancreatic β-cell membrane disruption and cyto-toxicity after its intracellular accumulation [19]. The anti-hyperglycaemic activity caused by macerated garlic preparation is due to the presence of flavonoids and sulphur containing compounds in garlic [24, 11] proposed that garlic can act as an anti-diabetic agent by increasing either the pancreatic secretion of insulin from the β-cells or its release from bound insulin

The increase in the activities of serum AST and ALT indicated that diabetes may have induced hepatic dysfunction. Supporting the finding by [17], that liver cells were necrotized in diabetic patient.

An abnormality in glucose metabolism influences lipid metabolism as reported by (Oberley, 1988). Clinical knowledge of the level of serum lipids in an important biochemical tool in the toxicity or beneficial effects of foreign compounds. Serum lipids and lipid peroxidation are predominantly resident in body tissue. The physiological and pathological state of body tissues is highly associated with metabolism, level of serum lipids and lipid peroxidation.In situation where there is high activity of these lipids in body tissues due to oxidative damage, associated with lipid metabolism, the administration of an antioxidant such as allicin may ameliorate tissue dysfunction since antioxidant are known to improve tissue integrity [2]. From this study, it was observed that administration of *Allium sativum* (garlic) extract to alloxan induced diabetic rats revealed preserving cellular architecture, reappearance and cellular restoration, vascular congestion, re-appearance of hepatocytes with pyknotic nuclei migrating from the sinusoidal lining layer in group III as mild, group IV as moderate restoration and group V as complete regeneration in the liver tissues when compared to non-diabetic and diabetic control groups.

Findings indicate possible hepato-protective and anti-diabetic role played by the macerated preparation of *Allium sativum* (garlic) in single administration.

CONCLUSION

Diabetes mellitus and its complications is associated with free radical mediated cellular injury and lipid metabolism. Most probable causes for cyto-architectural alteration such as cellular vascular degeneration, necrosis, vacuolation and hepatocytic hyperplasia. This study has shown the protective effect of macerated *Allium sativum* (garlic) on liver as a major organ in carbohydrate metabolism, thus, justifying the possibility of using the macerated extract in management of diabetes mellitus and its complications.

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