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Antagonistic Salubrious Effects of Macerated *Allium Sativum* (garlic) on Cytoarchitectural Alterations in the Pancreas of Alloxan Induced Diabetic Rats.

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Abstract

Histopathological and antagonistic salubrious effects of macerated *Allium sativum* (garlic) on cytoarchitectural alterations in pancreas of alloxan (150mg/kg) induced diabetic rats was studied. Twenty five (25) wistar rats weighing between 101- 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 test 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, pancreas were extracted, weighed and fixed immediately in 10% formal saline, transported to the laboratory, processed to paraffin wax, cut at 5 microns, stained using Heamtoxylin and Eosin technique and observed histopathologically under light microscope. The result revealed preserving cellular architecture, reappearance of islet cells of langherns, the serous gland, and centro-acinar cells in group III as mild restoration, in group IV as moderate restoration and in group V as complete regeneration and restoration as compared to the normal non-diabetic group revealed normal cellular architecture and diabetic control group II showing degenerated and disappearance of beta cell, distortion of islet cell of larghans, degeneration of centro-acinar cells and area of necrosis. These findings are suggestive of a possible anti-diabetic role played by the macerated preparation of *Allium sativum* (garlic) on pancreas in single administration.

Keywords*: Diabetes mellitus, Allium sativum, Histopathology, Alloxan, Pancreas and wistar rats.*

1.0 Introduction

Diabetes mellitus is a name given to a group of 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 (King et al., 1998). Reasons for this rise include increase in sedentary lifestyle, consumption of energy rich diet, obesity, higher life span etc (Yajmk, 2001). Regions with greatest potential are Asia and Africa, where diabetes mellitus (DM) rates could rise to two-third folds than the present rate (ADA, 1997).

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 (Natan, 1993).These long-term complications resulted in diabetic

patients' life expectancy accounting to only two-thirds of the general population (Ahmed, 2005).
Many herbal medicines have been recommended for the treatment of diabetes (Marles and Fransworth, 1995; Alarcon-Aguilara et al., 1998). Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones (Pari and Umamahas-Wari, 2000), due to the plethora of active ingredients present in a single herb (Twan and Rao, 2002). 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 (Tiwari an Rao, 2002; Ebong, 2008).

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 (Tattelman,2005) and (Katzer, 2005). 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(Kerst et al., 1999) and (Robinkov et al., 1994).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 (Christopher et al., 2001)
No further studies have been conducted to elucidate possible Histopathological alterations and possible restorative effect of *Allium sativum* (garlic) on pancreas, as a storage depot for digestive enzymes and hormones such as insulin which promote uptake of glucose for most cells, particularly liver, skeletal muscle, kidney and adipose tissues thus lowering the plasma glucose concentration(Etim et al., 2011). Injury to the to the pancreas leads to impaired functions and other morphological abnormalities.
This study therefore explores the antagonistic salubrious effect of macerated effect of *Allium sativum* (garlic), keeping in view histopathological alterations in diabetic treated and untreated groups by highlighting the protective role

2.0 Materials and Methods

2.1 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.

2.2 Animals
25 weanling rats (101-205g) 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.

2.3 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.

2.4 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.

2.5 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 II,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.

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

Group V: 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.

2.7 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 pancreas were 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.

2.8 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

2.9 Photomicrography

Records of the Histological and histochemical 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.

3.0 Results

PLATE-1 Normal control groups I (NC) showed normal cellular architecture of the columnar shaped serous acinar cells and scattered regions of large mass of cell column, islet cells of langherns with interspersed connective tissue within normal limit in the endocrine region.

PLATE-2 Diabetic control group (DC) showed abnormal cellular architecture, there is necrosis, degeneration of the serous gland, islet cells of largherns, and centro-acinar cells with loss of interspersed connective tissue as compared to non diabetic group I

PLATE-3 Diabetic experimental group III treated with macerated preparation of *Allium sativum* (garlic) at a dose of 6.6g/kg and standard pellets for 7 days showed cellular architecture, there is reappearance of islet cells, no visible vacuolization and the serous gland, islet cells of largherns and centro-acinar cells and interspersed connective tissue are well demonstrated as compared to non diabetic and diabetic control groups(I and II).

PLATE-4 Diabetic experimental group IV treated with macerated preparation of *Allium sativum* (garlic) at a dose of 6.6g/kg and standard pellets for 14 days showed preserving cellular architecture, there islet cells are visible, no visible vacuolization and the serous gland, islet cells of largherns and centro-acinar cells and interspersed connective tissue are well demonstrated as compared to non diabetic and diabetic control groups(I and II)

PLATE-5 Diabetic experimental group V treated with macerated preparation of *Allium sativum* (garlic) at a dose of 6.6g/kg and standard pellets for 21 days showed well preserved cellular architecture of islet cells that are visible, the serous gland, centro-acinar cells and interspersed connective tissue are well demonstrated as compared to non diabetic and diabetic control groups (I and II)

Finally, histopathological 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 protective and anti-diabetic role of *Allium sativum* (garlic) on the pancreas, however the cytoarchitectural alteration effect were completely restored.

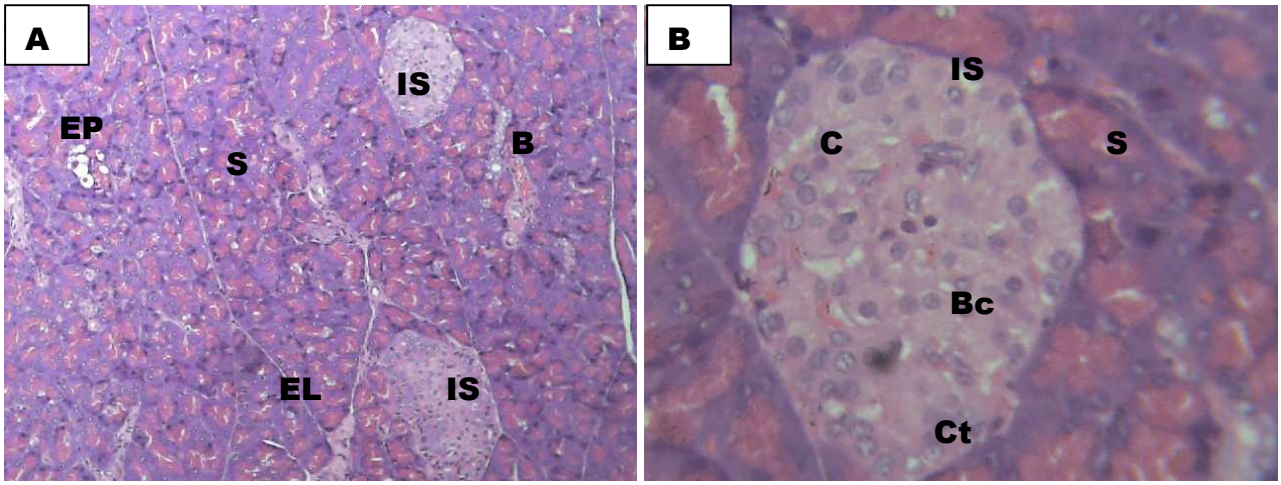

PLATE 1- Normal control of Pancreatic tissue at magnification A (x100) and B(x400) stained with H & E technique.
*Note: **El**-endocrine lobe, **IS**-Islet cells of langherns, **Ct**- connective tissue, **SG**- serous gland **EP**- exocrine portion, SA – serous acinar, **BV**-Blood vessel and **Bc**- Beta cells*

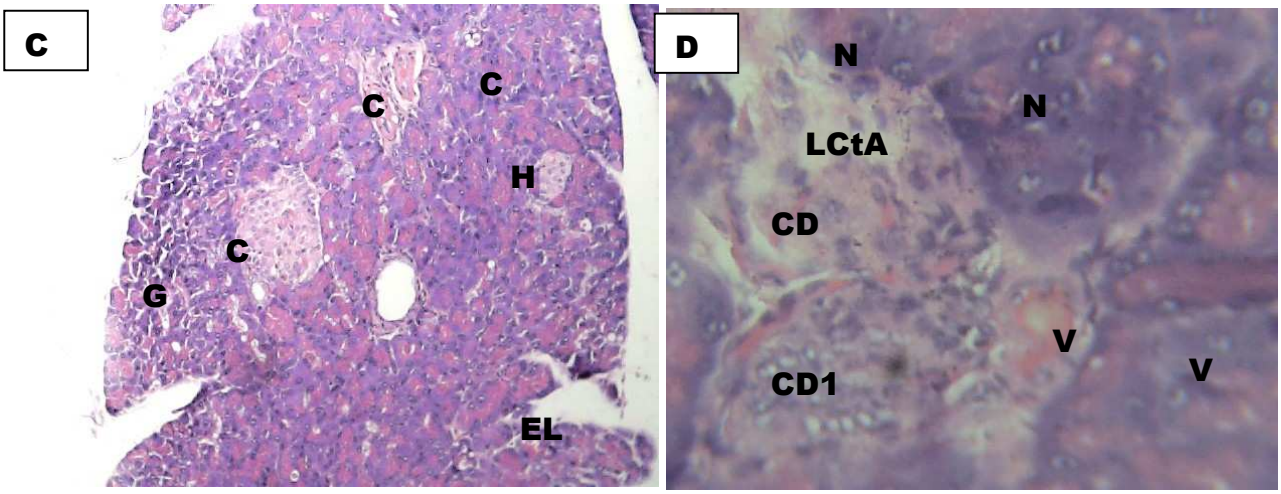

PLATE 2- Diabetic control of Pancreatic tissue induced with 150mg/kg of Alloxan at magnification C(x100) and D(x400) stained with H & E technique.
*Note: **El**-endocrine lobe, **IS**-Islet cells of langherns, **Ct**- connective tissue, **SG**- serous gland **EP**- exocrine portion, **SA** – serous acinar and **BV**-Blood vessel and **LCtA**- Loss of connective tissue area*

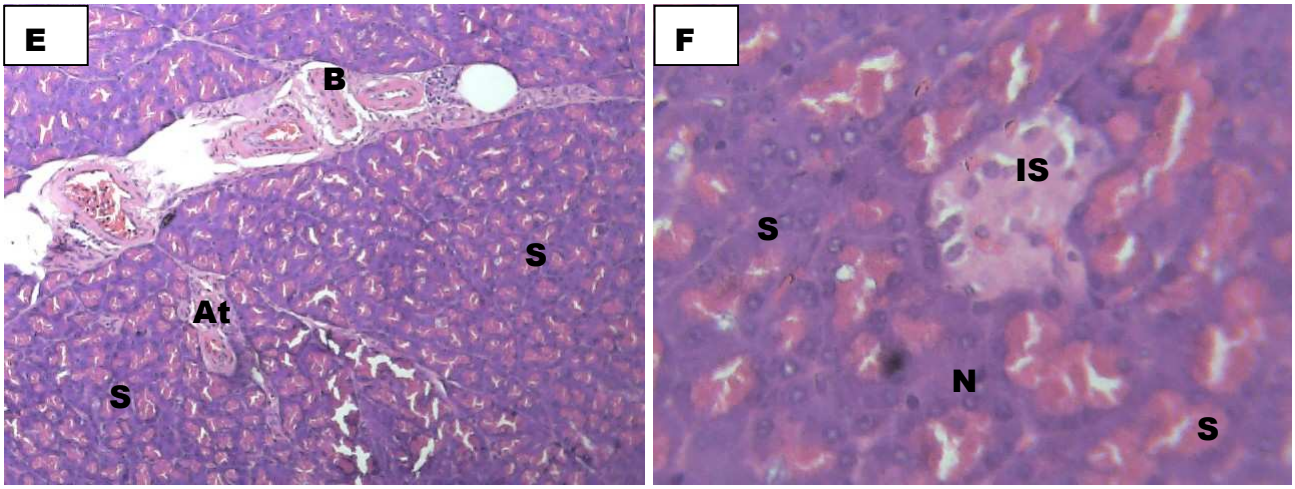

PLATE 3- Diabetic Pancreatic tissue treated with 6.6g/kg of Allium sativum for 7 days at magnification E(x100) and F(x400) stained with H & E technique.
*Note: **El**-endocrine lobe, **IS**-Islet cells of langherns, **Ct**- connective tissue, **SG**- serous gland **EP**- exocrine portion, **SA** – serous acinar and **BV**-Blood vessel.*

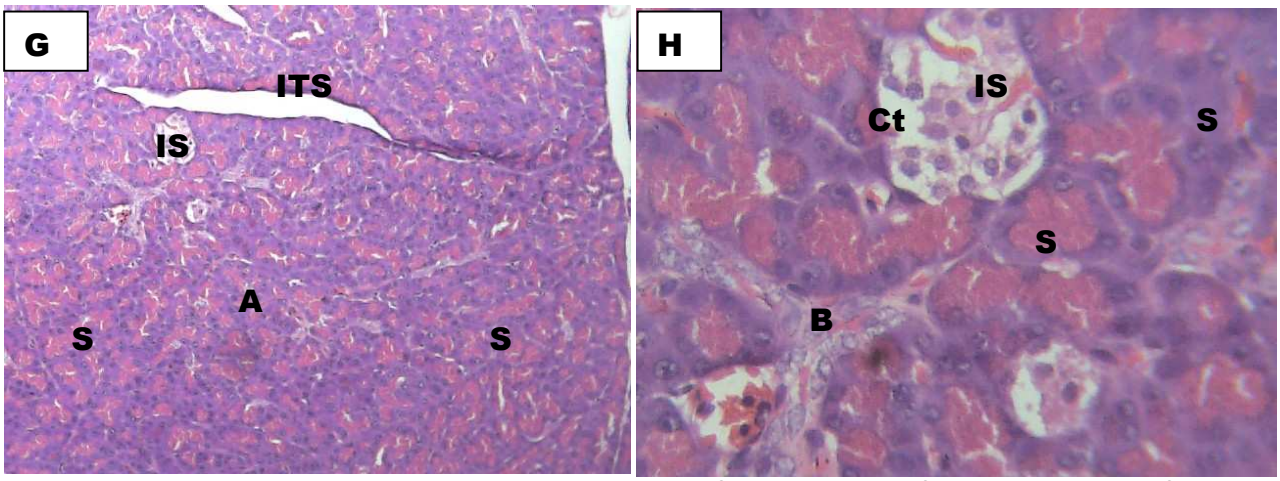

PLATE 4 Diabetic Pancreatic tissues treated with 6.6g/kg of Allium sativum for 14 days at magnification G(x100) and H(x400) stained with H & E technique.

Note: ***El****-endocrine lobe,* ***IS****-Islet cells of langherns,* ***Ct****- connective tissue,* ***SG****- serous gland* ***EP****- exocrine portion,* ***SA*** *– serous acinar and* ***BV****-Blood vessel.*

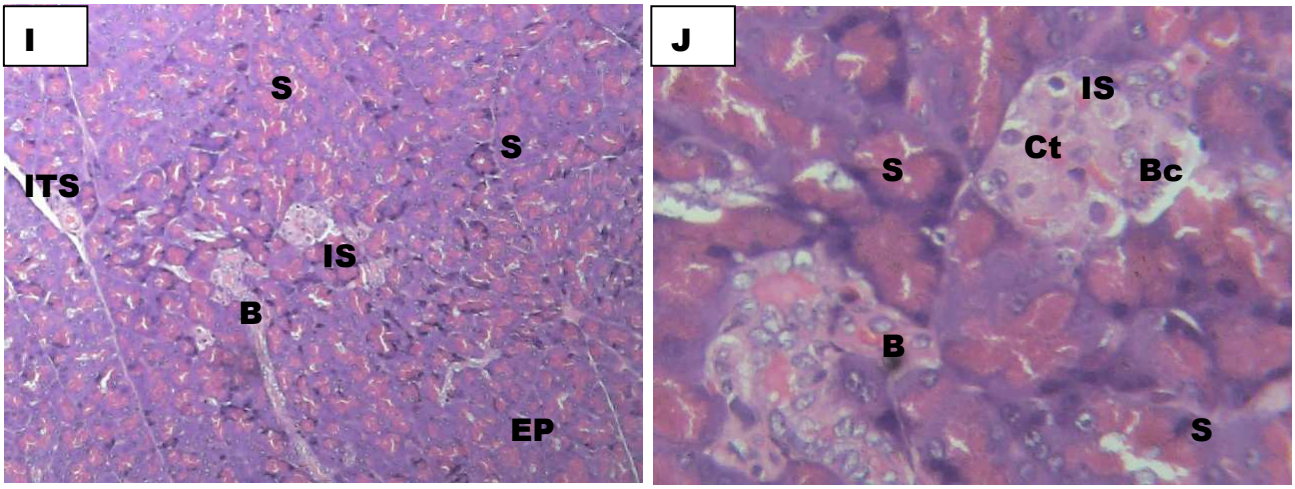

PLATE 5 Diabetic Pancreatic tissues treated with 6.6g/kg of Allium sativum for 21 days at magnification I(x100) and J(x400) stained with H & E technique.

Note: ***El****-endocrine lobe,* ***IS****-Islet cells of langherns,* ***Ct****- connective tissue,* ***SG****- serous gland EP- exocrine portion,* ***SA*** *– serous acinar,* ***BV****-Blood vessel and* ***Bc****- Beta cells*

4.0 Discussion

The present day study was undertaken to study the anti-diabetic and to evaluate reversible effect on cyto-architecture following 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 effect on the micro-morphological appearance of the constituents as well as reversible effect ranging from mild to complete restoration the pancreas treated with the garlic after the establishment of diabetics in the rats. Normal control (NC) animals were found to be stable while diabetic control group showed high level of cellular abnormalities including necrosis, decrease in beta cells, degeneration of islet cells, centro-acinar atrophy and disarrangement of cytoartitectural

component. It is also established that alloxan administration to experimental rats selectively causes pancreatic β-cell membrane disruption and cyto-toxicity after its intracellular accumulation (Mathew et al., 1973). The anti-hyperglycaemic activity caused by macerated garlic preparation is due to the presence of flavonoids and sulphur containing compounds in garlic (Swanston-Flatt et al., 1990). Jain and Vyas (1975) 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

Alloxan monohydrate, a beta cytotoxin induces diabetes in a wide variety of animal species by damaging the section insulin pancreatic ı-cells resulting in decrease in endogenous insulin release which lead to decrease glucose utilization by the tissues and a resultant diabetic (hyperglycemia) condition. 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.

From this study, it was observed that administrative effect of *Allium sativum* (garlic) extract on alloxan induced diabetic rats revealed preserving cellular architecture, reappearance of islet cells of langherns, the serous gland, and centro-acinar cells in group III as mild restoration, in group IV as moderate restoration and in group V as complete regeneration and restoration of the pancreas.

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

5.0 Conclusion

Diabetes mellitus and its complications are associated with free radical mediated cellular injury and lipid metabolism. Most probable causes for cyto-architectural degeneration in diabetes include abnormal lipid metabolism, vascular complications, increased glycation of protein, peroxidation of apolipoproteins, a deficiency of antioxidant activity of superoxide dismutase and glutathione peroxidase (Blomhoff et al., 2006). This study has shown effect of macerated *Allium sativum* (garlic) on the alloxan induced pancreatic cellular abnormalities justifying the possibility of using the extract in management of diabetes mellitus and its complications.

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References

Ahmed N, Advanced glycation endproducts: role in pathology of diabetic complications, Diab Res Clin Pract,2005, 67():3–21.

Ahren B and Corrigan CB (1994). Intermittent need for insulin in a subgroup of diabetic patients in Tanzania. Diabet Med, 2: 262-64.

Alarcon-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S, Aguilar-Contreras A, Contreras-Weber CC and Flores-Saenz JL (1998). Study of the anti-hyperglycemic effect of plants used as antidiabetics. *J. Ethnopharmacol*, **61**: 101-110.

American Diabetes Association (2007). Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 30Suppl. 1:542-7. (Med line).

BASTAKI S, Diabetes mellitus and its treatment, Int J Diabetes Metabolism, 2005, 13(3):111–134.

Blomhoff A, Olsson M, Johansson S, Akselsen HE, Pociot F (2006).Linkage diequilibrium and haplotype blocks in the MHC vary in an HLA haplotype specific manner assessed mainly by DRB1*04haplotypes. Genes Immun., 7: 130-140.

Christopher DG, Chattergee LM, Carlson JJ. Effect of garlic preparation on plasma lipid levels in moderator hypercholesterolemia adults. Atherosclerosis 2001;154(1):213−20.

Ebong, PE, Atangwho, I. J. Eyong, E. U, Egbung, G. E (2008). The antidiabetic efficacy of combined extracts from two continental plants. Azdirachta indica and Vernonia amygdalina. American Journal of Biochemistry and Biotechnology, 4(3): 239-244.

Ebomoyi, Maureen Isoken and Abuda, Onobu. Blood glucose and morphology of the liver and pancreas in garlic –fed Wistar rats .Journal of Medicinal Plants Research Vol. 4(18), pp. 1877-1882.

Etim O.E,Bassey E.I,Ita S.o,Udokang,I.C, Etim,I.E and Jackso,I.L .(2011). Modulatory effect of sweet almond extract on serum lipids,lipid peroxidation and histology of pancreas in alloxan –induced diabetic rats.International journal of pharmacy and pharmacology Vol 1.1(7)pp.155-161

Jain, R.C., Vyas, C.R., 1975. Garlic in alloxan-induced diabetic rabbits. American Journal of Clinical Nutrition 28, 684–685.

Katzer, G. (2005): Spice Pages: Garlic (Allium sativum, garlick). Retrieved 29/9/2010. http://www.uni-graz.at/~katzer/engl/Alli_sat.html

Kerst I, Keusgen M. Stabilization and pharmaceutical use of allinase. Pharmizie 1999;54(4):289−93.

King H, Rewersm M, Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. WHO Ad Hoc Diabetes Reporting Group, Diabetes Care,1993, 16(1):157–177.

King ML, Bidwell D. shikh A, Voller A, Banatvala JE (1998). Coxsackie – B- virus – specific 1gm responses in children with insulin-dependent (Juvenile-onset type 1) diabetes mellitus. Lancet. 1397-99.

King, MW and Marchersisni, S (2007). Cholesterol and Bile Metabolism Information Online Retrieved from http://med.unibs.it/smarchesi/cholesthtml.

Marles, J. R. and Farnsworth, N. R. (1995). Antidiabetic plants and their active constituents. Phytomedicine 2(2): 123-89.

Martha Thomson, Zainab M. Al-Amin, Khaled K. Al-Qattan, Lemia H. Shaban and Muslim Ali. Anti-diabetic and hypolipidaemic properties of garlic (Allium sativum) in streptozotocin-induced diabetic rats**.** Int ernational Journal Diabetes & Metabolism (2007) 15: 108-115

Mathew, P.T and K.T. Augusti, 1975. Hypoglycaemic effects of onion, Allium cepa linn on diabetes mellitus-a preliminary report. Indian J. Physiol. Pharmacol., 19: 213-217.PubMed |

Nathan DM, Long-term complications of diabetes mellitus,N Engl J Med, 1993, 328(23):1676–1685.

Oberley, L.W., Free radicals and diabetes, *J. Free Radicals Biol. Med*., 1998, **5**, 113–124.

Robinkov A. Zhuz X. Garfi G. Gaili G, Mirelma D. Alium lyses allinase from garlic: biochemical characterization and C DNA cloning, Appl Biochem Biotecnol 1994;48:149−71.

Swanston Flatt, S.K., Day, C., Bailey, C.J., Flatt, P.R., 1990.Traditional plant remedies for diabetes. Studies in thenormal and streptozotocin diabetic mice. Diabetologia 33,462–464.

Tattelman,E(2005): Health effects of garlic. *AmFamPhysician* 72:103-106.

Tiwari AK, and Rao. JM (2002). Diabetes Mellitus and multiple therapeutic approaches of photochemical present status and further projects. Current Science, 83(1): 30-27.

Teoh SL,Latiff AA and Dass S, The effect of topical extract of Momordica charantia (bitter gourd) on wound healing in nondiabetic rats and in rats with diabetes induced bystreptozotocin, Clin Exp Dermatol, 2009, 34(7):815–822.

Yajmk CS, Shelgikar KM, Naikss, Kanitkar SV, Orskor H, Alberti KG (2001). The Ketoacidosis resistance in fibro-calculous pancreatic-diabetes. Diabetes Res Clin Pract 15: 149-56.