ORIGINAL ARTICLE

Elevation of serum pancreatic amylase and distortion of pancreatic cyto-architecture in type 1 diabetes mellitus rats treated with Ocimum gratissimum

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ABSTRACT

Background: Diabetes mellitus has been shown to cause severe impairment in exocrine pancreatic function and cyto-architecture. *Ocimum grattissimum* has been reported to lower blood glucose levels in experimental diabetic animals. This study, therefore, aims to investigate if treatment with *O. grattissimum* can alleviate these pancreatic complications of diabetes mellitus. The phytoconstituents and median lethal dose of the plant extract were determined. **Materials and Methods:** Eighteen rats were divided into three groups of six rats each. Diabetes mellitus was induced by single intraperitoneal injection of 65 mg/kg streptozotocin. Group 1 was the control and were given normal feed only; Group 2 was of diabetic untreated rats, while Group 3 was *O. grattissimum*-treated diabetic rats at a dose of 1,500 mg/kg. After 28 days, blood was collected by cardiac puncture of the anaesthetised animals and the serum was obtained for analysis of serum pancreatic amylase. Permanent preparations using routine biopsy method were employed for histological preparations. **Results:** Results showed that the level of pancreatic serum amylase in the test groups (diabetic and diabetic-treated) were significantly higher ($P < 0.05$) than the control group, while the diabetic-treated group was significantly lower than the diabetic group. Atrophic acinar tissue without β-cells was noted in the diabetic and diabetic-treated groups. Patchy areas of necrosis, oedematous interstitium, haemorrhagic and necrotic acinar cells were present in diabetic-treated groups. **Conclusion:** Direct association exists between the hyperglycaemic state caused by diabetes mellitus and the elevation of the serum pancreatic amylase and distortion of pancreatic cyto-achitecture. *O. grattissimum*-treatment reduced serum pancreatic amylase level to near normal and limit the extent of structural damage.

Key words: *Ocimum gratissimum,* pancreatic histology, serum pancreatic amylase, Type 1 Diabetes mellitus

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INTRODUCTION

Previous studies have assessed exocrine pancreatic function in Diabetes Mellitus (DM). It has been clearly demonstrated that a significant number of Type I Diabetes Mellitus (TIDM) have impaired exocrine pancreatic function, as shown by a reduced duodenal output of enzymes in response to endogenous (meal, nutrients) and exogenous stimuli (Secretin + cholecystokinin infusion).

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Chey *et al,*[1] reported that endocrine function was impaired in 77% of TIDM patients. Blood glucose levels were not taken into account in these studies. Several theories have been postulated to explain these findings: Atrophy of exocrine tissue may be due to lack of trophic insulin action; pancreatic fibrosis could be the result of angiopathy and neuropathy and may lead to impaired exocrine function. Apart from direct pancreatic function test, indirect tests, such as urinary para-amino-papuric-acid (PAPA) recovery, or faecal tests such as fat excretion, chymotrypsin and elastase — concentrations, are abnormal in a substantial proportion of DM.[2,3]

Despite the high frequency of reduced exocrine function in DM, few patients develop overt exocrine pancreas insufficiency. Pancreatic exocrine insufficiency becomes clinically manifest only when less than 10% of the secretory capacity is preserved. In contrast to TIDM,

abnormalities of exocrine function are observed less frequently in Type 2 Diabetes Mellitus (T2DM).[3] These observations are consistent with the concept that insulin plays a rule in the maintenance of normal exocrine pancreatic function; this is supported by the presence of insulin receptors on acinar cells.[4] The physiological rule of insulin on stimulated pancreatic exocrine secretion has been demonstrated by Lee *et al.,*[5] using insulin anti-serum.

Secretion factors have been implicated in the reduced pancreatic enzyme output observed in DM; these include reduction in post-prandial Cholecystokinnin (CCK) secretion, delayed gastric emptying and nutrients delivery to the duodenum (giving rise to impaired post-prandial CCK Secretion) and autonomic neuropathy. Neural mechanisms are more relevant than hormone in the regulation of pancreatic secretion. Recent studies with CCK receptor antagonists indicate that over 70% of meal-stimulated exocrine secretion is dependent on neural mechanisms.[6] In contrast to animal studies, no association between exocrine function with the duration of DM, presence or absence of neuropathy, micro-angiopathy or metabolic control was found in humans.[7,8]

A hormonal imbalance may also contribute to impairment of exocrine function. Alteration in insulin, amylin and glucagon or somatostatin secretion in DM may directly influence the exocrine pancreas. In T1DM, pancreatic enzyme output is reduced. Pancreatic polypeptide (PP) and somatostatin may contribute to the regulation of acinar cell function. Infusion of exogenous PP to plasma levels comparable to those observed after meal inhibits pancreatic secretion.[9] This is indicative of a possible role of this peptide in the physiological control of exocrine pancreatic secretion.

In a prospective study of patients with DM in which pancreatic morphology was evaluated in detail by endoscopic retrograde cholangiopancreatography (ERCP), it was found that abnormal pancreatic ductograms were present not only in a high percentage of T1DM but also in T2DM with islet cell antibodies.[10] Pancreatic enzyme output to the duodenum, especially that of amylase is frequently impaired in DM, though, this does not automatically result in mal-digestion of fat and carbohydrates.[1] Steatorrhoea occurs when lipase secretary capacity of the pancreas is reduced to below 10%. Most patients with DM do not have symptoms of overt exocrine pancreatic insufficiency, i.e. steatorrhoea or weight loss.[1] This can be explained by the fact that digestive enzymes are secreted in excess by the pancreas.

There are clear evidences that the pancreatic cyto-architecture and pancreatic activities are very frequently and severely altered in diabetic conditions. Also, *Ocimum gratissimum* (OG) is reported to lower blood glucose levels in experimental diabetic animals. This study, therefore, aims to investigate if OG treatment in diabetic state can alleviate these pancreatic complications of DM.

MATERIALS AND METHODS

The leaves of OG were obtained from the University of Calabar Botanical Garden and identified by the Chief Herbarium Officer of Botany Department of University of Calabar. The fresh leaves were rinsed with water to remove sand and debris and then allowed to air dry. The leaves were then dried under shade for 2 days and then transferred into Astell Hearson Oven and dried at a temperature range of 40–45 ^{o}C. The dried leaves were then ground in an electric blender into fine powder to give a gram weight of 527 g. This 527 g weight was soaked in 2.65 liters of water (distilled water) overnight for about 15 hours and stirred at regular intervals. The mixture was filtered using a satin mesh material and the final filtrate was obtained by using Whatman's filter paper size 1. The final filtrate was dried in the Astell Hearson Oven at 45 ^{o}C to obtain a brown gummy paste. A mettler P163 electronic weighing balance was used to weigh the gummy paste before stock solution was prepared. The stock solution of the extract was prepared by dissolving 15 g of extract in 10 ml of water to give a concentration of 1,500 mg/ml and refrigerated at 4 ^{o}C. The median lethal dose (LD_{50}) of the plant extract was determined by the method of Lorke (1983).[11]

The phytoconstituents of the extract was determined and screened for the presence of carbohydrates, tannins, alkaloids, saponins, phenolics, anthraquinones and cardiac glycosides as described by Trease and Evans (1984)[12] and Sofowora (1984).[13]

Eighteen male albino wistar rats were used for the study, the animals were divided into three groups and were assigned randomly into each group which was made up of six rats each and housed in cages assigned to them. The first group was made up of the control animals, which were fed with normal rat chow (feed). The second group was streptozotocin-induced diabetic rats, which were left untreated. The third group was streptozotocin-induced diabetic rats treated with aqueous leaf extract of OG. The experimental procedures involving the animals and their care were in line with the approved guidelines by the local (University of Uyo, Akwa Ibom State) research and ethical committee established and guided by the Helsinki Declaration on Animal research.

TIDM was induced in twelve male albino wistar rats by a single injection of 65 mg/kg streptozotocin. The injection was given intraperitoneally. The state of diabetes was observed after 48 hours by the symptoms of polyuria and glucosuria and this state was confirmed using uristic test strip (Bayer Health Care LLC, USA). Also, the blood glucose

level was tested 1 week after induction using a Glucometer (ACCU-CHECK Advantage II, Roche Diagnostics (GmbH, Germany) and ACCU-CHECK Advantage II test strips.

One week after induction of diabetes mellitus, the extract was administered per oral to the diabetes mellitus-treated (DMT) group at a dose of 1,500 mg/kg body weight daily for 28 days. Administration was facilitated by the use of a syringe and orogastic tube.

Measurement of serum pancreatic amylase was carried out by the method of Landt *et al*.[14] The assay utilised an immunoabsorbent prepared by coating latex beads with a monoclonal antibody specific for pancreatic amylase. Treatment of specimen serum with immunoabsorbent removed pancreatic amylase, and measurement of residual amylase activity with standard total amylase methodology allowed estimation of the pancreatic amylase content. Extraction efficiency of pancreatic amylase was consistent at amylase concentrations up to 1,000 U/L (y = 0.97 x + 16.7 U/L; r = 0.9995).

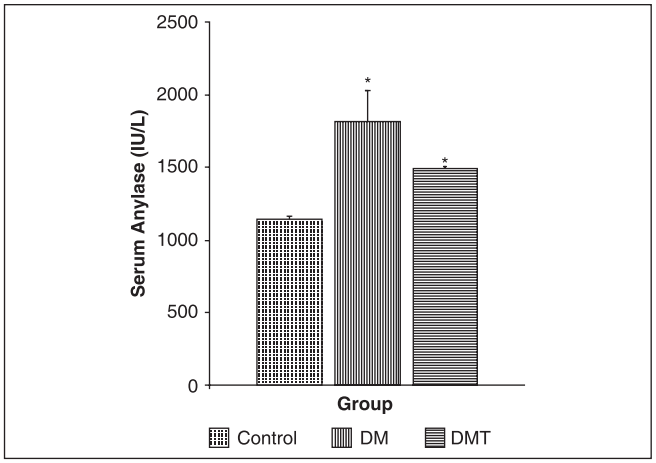

Figure 1: Comparison of serum amylase levels in the different experimental groups *P < 0.05 vs. control. A = P < 0.05, DM vs. DMT

RESULTS

The mean values in the control, DM and DMT experimental groups were: 1,136 ± 22.6, 1,806 ± 214.6 and 1,484 ± 118.1, mmol/L, respectively. The test groups (DM and DMT) were significantly higher (P < 0.05) than the control group. The DMT was significantly lower than the DM group [Figure 1].

Histology of the pancreas shows normal features in the control. Atrophic acinar tissue without β-cells was noted in the DM and DMT groups. More pathologic changes were seen in the DMT group, patchy areas of necrosis, oedematous interstitium and haemorrhagic and necrotic acinar cells [Figures 2-4].

DISCUSSION

There were raised levels of serum pancreatic amylase in the DMT and DM test groups compared to the control. This finding is of unique interest considering the hypothesis that insulin contributes to the regulation of acinar cell function, supported by the presence of insulin receptors on acinar

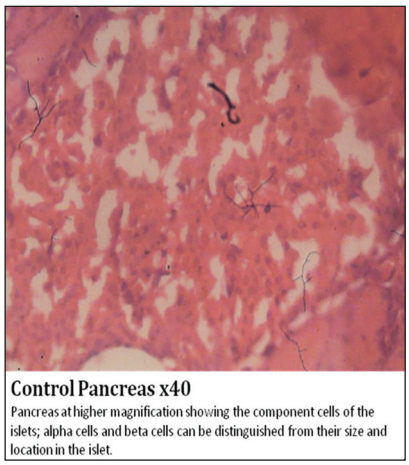

Figure 2: Histology of the pancreas in the control group

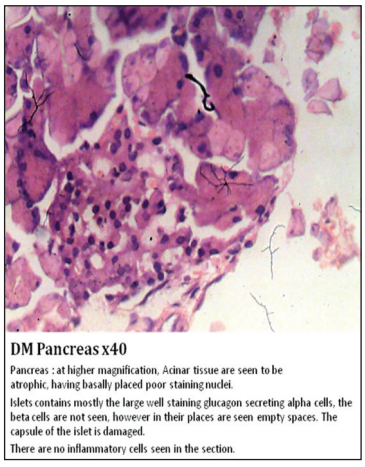

Figure 3: Histology of the pancreas in the DM group

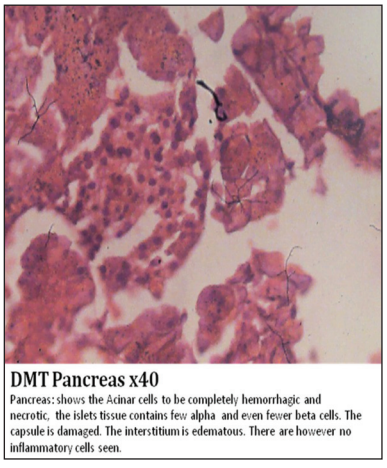

Figure 4: Histology of the pancreas in the DMT group

cells.[4] Exocrine pancreatic insufficiency has been observed in diabetic patients.[15] However, the underlying mechanism is not well known. Reduced cystolic Ca^{2+} signals in pancreatic cells may contribute to lower digestive enzyme secretion. It is well known that adenosine triphosphate (ATP) regulates cytosolic Ca^{2+} signals in acinar cells. Little is known as to whether DM impairs glucose metabolism that produces ATP in acinar cells. In the experiment reported by Hans *et al*.,[15] streptozotocin-induced diabetic C57BL/6 mouse model were used. Four weeks after being diabetic, pancreatic acinar cells were isolated, and amylase secretion and contents, glucose utilisation and oxidation, the activities of several key enzymes for glucose metabolism and ATP and nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) contents were determined. Compared with controls, diabetic mice had lower body weight, cholecystokinin-8 and acetylcoline-stimulated amylase secretion was significantly impaired, total amylase activity in acinar cells of streptozotocin-diabetic mice was markedly reduced, glucose utilization and oxidation were suppressed, measured enzyme activities for glucose metabolism, ATP and NADPH contents were significantly reduced. This data indicate that in glucose metabolism, ATP and NADPH production are very important for acinar cells normal function. It is very obvious that the above reduction could have contributed to the development of exocrine pancreatic insufficiency.

Pancreatic acinar cells secrete enzymes including pancreatic amylase. Snook,[16] reported that in rats rendered diabetic by streptozotocin, pancreatic amylase output was markedly reduced. On the other hand, when pancreatic exocrine secretion was stimulated by exogenous CCK, exocrine output was not reduced but, on the contrary, even significantly increased.[17] Also, Quiros *et al.*,[18] had reported elevation of serum pancreatic amylase and lipase in paediatric diabetic ketoacidosis (DKA). In multivariate analysis, an elevated blood urea nitrogen (BUN) concentration was associated with elevated serum amylase in the above study. It is logical that because of the close anatomical and functional relations between the endocrine and the exocrine pancreas, a disturbance or disease in one system will inevitably affect the other. However, other pathophysiological dynamics could alter this relationship as reported above. Therefore, from the result in this study, we could deduce that the animals had developed DKA, with associated rise in BUN. It is, therefore, possible that increase secretion of pancreatic amylase could be a compensatory response. T1DM is known to be commonly associated with DKA. Our result, therefore, proposes that DKA with elevated BUN in DM trigger increase exocrine pancreatic secretion. However, the inability to measure the acid base indices during the experiments was the major limitation of this study.

The control group showed normal pancreatic tissue, with normal acinar tissue and islet cells on histology. Absence of β-cells were observed in the DM and DMT groups thus confirming its effective destruction by streptozotocin administration. Diffuse pancreatic tissue atrophy in both the DM and DMT groups noted did not appear to affect their exocrine secretion. The presence of haemorrhagic and necrotic acinar cells in the DMT group could, however, have accounted for the slight differences in amylase levels in the DM and DMT groups.

It is evident from this result that the hypoglycaemic action of OG consequentially mitigated the degree of DKA in the diabetic treated group. This explains the observation that serum concentration of amylase in the diabetic treated group was significantly lower than the diabetic untreated group. We can, therefore, infer that the ability of OG to raise pancreatic amylase levels is secondary to its hypoglycaemic property.

CONCLUSION

Hyperglycaemia, as the hallmark of DM, has been directly or indirectly implicated in most systemic DM complications. This assertion is clearly evident from the results of this study, pointing to a direct association between the hyperglycaemic state and the elevation of the serum pancreatic amylase and distortion of pancreatic cyto-achitecture. OG treatment was observed to reduce serum pancreatic amylase level to near normal and limit the extent of structural damage. These findings lay strong credence to the efficacy of OG as an anti-diabetic agent.

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