Background: The endocrinial abnormalities in diabetes mellitus as one of the numerous metabolic disorders is associated with derangement in exocrine functions of the pancreas and ultimately influences blood glucose regulation.
Aim: The study was aimed at assessing the role of alpha-amylase and glycogen synthase in anti-diabetic potential of *Terminalia catappa* in diabetic rats.
Materials and Methods: Thirty five (35) Wistar rats were assigned to 5 groups of 7 animals each. Group 1 served as the control administered distilled water at 5ml/kg bodyweight and group 2 was a non diabetic group given orally, 130/kg body weight of aqueous leaf extract of *Terminalia catappa*. Groups 3, 4 and 5 received a single dose of 150mg/kg body weight of alloxan solution.
intraperitoneally to induce diabetes and rats with blood glucose levels ≥200mg/dl after 72 hours were considered diabetic. This was followed by oral administration of 5ml/kg bodyweight of *Terminalia catappa* leaf extract orally and subcutaneous administration of insulin, 0.75U/kg body weight to groups 3 (diabetic), 4(diabetic + extract) and 5 (diabetic + insulin) respectively.

**Results:** The results showed significant (P<.05) increase in serum level of alpha-amylase and glycogen synthase in both non-diabetic extract treated and diabetic groups when compared to control. But these enzymes significantly (P<.05) reduced in diabetic extract and insulin treated groups when compared to the diabetic group.

**Conclusion:** Therefore the hypoglycaemic potential of *Terminalia catappa* leaf extract could be attributed to its ability to reduce alpha-amylase level while lowered glycogen synthase might be secondary to reduction in blood glucose.

**Keywords:** Alpha-amylase; glycogen synthase; diabetes mellitus; *Terminalia catappa*.

### 1. INTRODUCTION

Diabetes mellitus is a growing health problem and it is the most common non infective disease [1] especially in developed countries. Diabetes mellitus is a chronic cluster of metabolic disorder characterized by hyperglycaemia that results from defect in insulin secretion, insulin inaction or both and by increased hepatic glucose production [2,3]. Hyperglycemia is reported to be linked to numerous injurious effects of diabetes mellitus with manifesting clinical complications. The prevalence of this disorder is on the increase globally and it has been projected that 500 million adults will be affected with diabetes mellitus by 2030 [4]. This becomes a huge concern in the health domain globally culminating to increasing quest into prevention and management regimes.

Treatment regimes ranging from the use of insulin to oral hypoglycaemic drugs as well as lifestyle modifications are employed to tackle diabetes. For some years now, new therapeutic approaches are being investigated to regulate postprandial level of blood glucose due to the several side-effects of commercially available anti-diabetic drugs [1]. In view of the apparent side effect of the orthodox drugs, several herbal medicinal plants have been reported to be effective in treatment of diabetes mellitus [5]. Digestion of carbohydrate involves complex steps of enzymatic actions. Apart from the well known approaches which enhances glucose uptake, the use of drugs with target on the function of the digestive enzymes to reduce blood glucose have been employed in the management of diabetes. Among the most important digestive enzymes in humans is the pancreatic alpha amylase that catalyses the hydrolysis of alpha-1,4 glycosidic linkages of the starch, amyllopectin, amylose, glycogen and numerous maltodextrins [1,6]. The final step of the digestion process of carbohydrate especially starch is by the action of alpha-glucosidase or maltase which act upon 1,4-alpha bonds and produces glucose as the final product of the digestion [7]. If there be excess conversion of starch to sugars, it will increase the sugar level in blood, and then the role of insulin will come into action by ordering cells to metabolize the excess sugar moieties and store as energy sources i. e. glycogen [1]. Synthesis of glycogen as a major form of energy storage molecule in the liver and skeletal muscle involves processes which require some enzymatic activities [8]. The process is initiated by the autocatalytic protein glycogenin, but the rate by which glycogen synthesis occurs is controlled by a rate limiting enzyme [9,10], uridine diphosphate glycosyltransferase glycogen synthase [11,12]. But in some cases, due to excess activity of amylase enzyme and insulin deficiency or resistance to insulin, the level of blood glucose will rise which might results in hyperglycaemia. To control hyperglycaemia several studies on inhibition of amylase enzyme activity have been carried out. This led to development of anti-diabetic drugs such as acarbose and voglibose which is of microbial origin [13,14,15,16,17] and miglitol being a 1-deoxynojirimycin derivative [18] to reduce serum level of amylase. Due to severe side-effects of commercially available anti-diabetic medications, different plant extracts with alpha-amylase inhibitory activity are being investigated that might decrease postprandial blood glucose levels. Research findings revealed that the inhibitory effect of some natural products is more potent and safer than some commercial drugs [19,20]. Therefore this research seeks to know the role of alpha amylase and glycogen synthase in the anti-diabetic function of...
Terminalia catappa leaf extract in alloxan induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Procurement of Plant

Fresh leaves of Terminalia catappa were collected at the premises of the University of Calabar and the area was free of pesticides and other contaminants. The leaves were authenticated by a botanist (Mrs E. G. Udona) at the Department of Botany and Ecological studies, University of Calabar, Nigeria. The plant was kept in the herbarium with a voucher number UUPH 22(a).

2.2 Preparation and Extraction of Plant Extract

The leaves were then washed with clean water to remove debris. The water was blotted out and kept overnight at room temperature to dry up. The clean leaves were pulverized and 5000g of the pulverized leaves were soaked in 5 litres of deionised water for 18 hours. The mixture was filtered using muslin cloth and evaporated to dryness using thermostatic water bath at 45°C until a semi solid paste of 204.18g of the extract was obtained after evaporation representing a percentage yield of 4.08%. The extract was stored in refrigerator for later use.

2.3 Animals/Experimental Design

Thirty five (35) adult male albino Wistar rats weighing between 150-200g were procured from the animal house, Department of Physiology, Faculty of Basic Medical Sciences, University of Calabar and were used for the study. In the test study, the experimental animals were randomly distributed into five (5) groups of seven (n=7) rats per group as follows (see Table 1).

2.4 Induction of Diabetes

Diabetes was induced by intraperitoneal injection of alloxan monohydrate at a dose of 150mg/Kg body weight [21,22,23]. The animals were assessed for development of diabetes after 72 hours [24] by obtaining blood sample from the tip of the tail. The blood sample was dropped into glucose strip to measure the glucose level using a glucometer (One Touch Ultra, Life Scan Inc, U.S.A). Blood glucose of ≥200mg/dl was considered diabetic (normal range of blood glucose in rat is 80 – 120mg/dl) and were used for the experiments [22,24].

2.5 Determination of Fasting Blood Glucose (FBG)

The animals were fasted overnight (about 14 hours) and the blood glucose was determined between 8:00am to 10:00 am to avoid the effect of homeostatic changes in blood glucose metabolism. Blood sample was obtained by pricking the tip of the tail. Measurement of glucose was done using glucometer (one Touch, Life Scan USA) on days 1, 4, 7, 10 and 14 [25].

2.6 Determination of Serum-Alpha Amylase Level

Serum alpha-amylase level was analysed with rat alpha-amylase analysis kit using Sandwich-ELISA (Enzyme Linked Immunosorbent Assay) method. Standards or samples were added to the appropriate microelisastripplate wells and combined to the specific antibody. The absorbance or optical density (OD) was measured spectrophotometrically at a wavelength of 450nm and readings are obtained using Microtiter Plate Reader, the Optical Density value is proportional to the concentration of alpha-amylase.

Table 1. Animal grouping

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment regime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Control)</td>
<td>Non diabetic rats administered with only distilled water orally at a dose of 5ml/kg body weight.</td>
</tr>
<tr>
<td>Group 2</td>
<td>Non diabetic rats administered orally with aqueous leaf extract of Terminalia catappa at a dose of 130mg/kg body weight.</td>
</tr>
<tr>
<td>Group 3 (Diabetic control)</td>
<td>Administered with only distilled water orally at a dose of 5ml/Kg body weight.</td>
</tr>
<tr>
<td>Group 4</td>
<td>Diabetic rats treated with aqueous leaf extract of Terminalia catappa at a dose of 130mg/Kg body weight by oral administration.</td>
</tr>
<tr>
<td>Group 5</td>
<td>Diabetic rats treated with exogenous Insulin at a dose of 0.75U/Kg body weight by subcutaneous administration.</td>
</tr>
</tbody>
</table>
2.7 Determination of Glycogen Synthase Level

Serum glycogen synthase level was analysed with rat glycogen synthase analysis kit using Sandwich-ELISA (Enzyme Linked Immunosorbent Assay) method. Standards or samples are added to the appropriate microelisastripplate wells and combined to the specific antibody. The absorbance or optical density (OD) was measured spectrophotometrically at a wavelength of 450nm and readings are obtained using Microtiter Plate Reader, the Optical Density value is proportional to the concentration of glycogen synthase.

2.8 Statistical Analysis

The results were presented as Mean ± Standard error of mean (SEM). Differences between means were compared using ANOVA and post Hoc analysis and P<.05 was considered significant.

The data obtained from the result was subjected to statistical testing using Microsoft Excel and SPSS 20.0 software.

3. RESULTS

3.1 Effect of Terminalia Catappa Leaf Extract on Alpha-Amylase Level

The effect of Terminalia catappa leaf extract on amylase level in rat is shown in (Fig. 1). There was no significant change in amylase level in non-diabetic rats administered with extract on day 10 (70.50 ± 0.62) but a significant increase was observed at day 4 and 14 (77.33 ±0.14 and 73.50 ± 0.23) when compared to corresponding control (73.50 ± 0.23 and 69.83 ± 0.75) at P<.05. There was a significant increase on day 4, 10 and 14 (159.0 ± 0.81; 165.8 ± 0.75; 200.83 ± 1.33) respectively in amylase level of diabetic control group compared to corresponding control group (11.13 ± 0.60; 12.55 ± 0.39; 9.58 ± 0.19) at P<.05.

A significant decrease was observed on day 4, 10 and 14 (106.2 ± 0.75; 104.0 ± 0.62; 122.83 ± 0.89) respectively in amylase level of diabetic rats treated with Terminalia catappa leaf extract compared to corresponding diabetic control group (159.0 ± 0.81; 165.8 ± 0.75; 200.83 ± 1.33) at P<.05. A significant decrease was observed on day 4, 10 and 14 (81.83 ± 0.49; 91.00 ± 0.47; 78.17 ± 0.36) respectively in amylase level of diabetic rats treated with insulin compared to corresponding diabetic control group (159.0 ± 0.81; 165.8 ± 0.75; 200.83 ± 1.33) at P<.05.

3.2 Effect of Terminalia Catappa Leaf Extract on Glycogen Synthase Level

The effect of Terminalia catappa leaf extract on glycogen level in rats is shown in (Fig. 2). There was a significant increase on day 4, 10 and 14 (2.24 ± 0.03; 1.72 ± 0.03; 1.89 ± 0.05) respectively in glycogen level in non-diabetic rats administered extract compared to corresponding control group (1.34 ± 0.04; 1.59 ± 0.01; 1.63 ± 0.00) at P<.05. Glycogen level in diabetic control group significantly increased on day 4, 10 and 14 (11.13 ± 0.60; 12.55 ± 0.39; 9.58 ± 0.19) respectively compared to corresponding control group (1.34 ± 0.04; 1.59 ± 0.01; 1.63 ± 0.00) at P<.05.

In diabetic group treated with Terminalia catappa leaf extract, the glycogen level significantly decreased on day 4, 10 and 14 (6.51 ± 0.17; 8.37 ± 0.01; 8.41 ± 0.04) respectively compared to corresponding diabetic control group (11.13 ± 0.60; 12.55 ± 0.39; 9.58 ± 0.19) at P<.05.

3.3 Percentage Relative Change in Terminalia Catappa Aqueous Leaf Extract Effect on Alpha Amylase and Glycogen Synthase Level

The percentage relative changes in the effect of Terminalia catappa aqueous leaf extract on alpha-amylase as well as glycogen synthase is shown in (Table 2). A positive change in amylase and glycogen synthase level was observed in the diabetic control rats when compared to the control. A negative change in alpha amylase and glycogen synthase level was observed in diabetic rat treated with the leaf extract when compared to the diabetic control.

3.4 Fasting Blood Glucose (FBG) Level in Alloxan Induced Diabetic Rats and Extract Treated Diabetic Rats

The result showing changes in fasting blood glucose level is represented in (Table 3). Blood glucose level was compared within group between day 1 and other days in control and non-diabetic groups but comparison in diabetic groups were between days 1 and 4 with other days. The glucose level in the control group showed no significant difference between day 1
and other days. In non-diabetic extract treated group, blood glucose reduced in day 4, 7 and 10 with significant ($P < 0.05$) changes in days 4 and 7. The glucose level in the diabetic group was significantly ($P < 0.05$) higher in day 4, 7, 10 and 14 compared to day 1. But in extract and insulin treated groups, there were significant ($P < 0.05$) reduction in days 7, 10 and 14 compared with day 4.

Fig. 1. *Terminalia catappa* leaf extract effect on alpha-amylase level

$N=7$; $a$ Significant change compared to control of same day ($P<.05$)

$b$ Significant change compared to diabetic control of same day ($P<.05$)

Table 2. Percentage relative changes in *Terminalia catappa* aqueous leaf extract effect on alpha-amylase and glycogen synthase

<table>
<thead>
<tr>
<th>Groups</th>
<th>Alpha-amylase (u/l) % Relative Change from Day 4-10</th>
<th>Glycogen synthase (ng/ml) % Relative Change from Day 4-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-4.31</td>
<td>-4.31</td>
</tr>
<tr>
<td>Non-diabetic + extract</td>
<td>-8.83</td>
<td>-8.83</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>4.27</td>
<td>4.27</td>
</tr>
<tr>
<td>Diabetic + Extract</td>
<td>-2.07</td>
<td>-2.07</td>
</tr>
<tr>
<td>Diabetic + Insulin</td>
<td>11.21</td>
<td>11.21</td>
</tr>
</tbody>
</table>

Table 3. Fasting blood glucose level in alloxan induced diabetic rats and *Terminalia catappa* leaf extract treated rats in mg/dl

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fasting Blood Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>59.25±8.6</td>
</tr>
<tr>
<td>Non-diabetic + extract</td>
<td>61.0±2.0</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>56.2±6.5</td>
</tr>
<tr>
<td>Diabetic + extract</td>
<td>42.8±3.7</td>
</tr>
<tr>
<td>Diabetic + Insulin</td>
<td>44.5±1.5</td>
</tr>
</tbody>
</table>

Result is presented as mean ±SEM.; *= significant change compared to control,

$a$= significant change compared to day 4.; Significant level is $P< 0.05$
4. DISCUSSION

The role of alpha-amylase and glycogen synthase in relation to the hypoglycaemic and anti-diabetic effect of *Terminalia catappa* aqueous leaf extract was studied. The fasting blood glucose level in non-diabetic group treated with extract reduced in days 4, 7 and 10. Similarly, significant reduction in glucose level was observed in diabetic group treated with the extract. This result agrees with the report of Ahmed and colleagues [26] that *Terminalia catappa* leaf extract reduces blood glucose in diabetes. Glucose release from carbohydrate digestion involves enzymatic action of alpha amylase for the initial hydrolysis of alpha-1,4 glycosidic bond [1]). The result showed that serum alpha-amylase level in the diabetic group was significantly increased when compared to the control group across day 4, 10 and 14. The level of increase in amylase was not dependent on the duration as this is reflected in the percentage relative changes observed between different days during the experiment. This result is contrary to the popularly reported low level concentration of serum amylase which have been empirically observed in clinical settings in patients with type 1 diabetes, type 2 diabetes with insulin dependence, or advanced overt pancreatitis [27,28,29]. It is upheld that the action of insulin is critical for the production of pancreatic amylase [30,31] as such the common etiology in these conditions may be depleted secretion of insulin from the pancreas [32]. Although some studies reported low amylase level in type II diabetes mellitus, attributing it to the decline in the trophic effect of insulin on the pancreatic acinar cells [33,34,35], others claimed existence of transient and acute increases (from several to ten times beyond the upper normal level) in serum amylase concentration as a result of destruction of acinar cells in the pancreas [36]. However, repeated acute pancreatitis may eventually results in exhausted acinar cells and restricted flow of enzymes from pancreas parenchyma into the circulation [37,38,39] leading to low serum amylase.

Fig. 2. *Terminalia catappa* leaf extract effect on glycogen synthase level

N= 7;  a Significant change compared to control of same day (P<.05)
   b Significant change compared to diabetic control of same day (P<.05)
diabetes could be a compensatory response triggered in diabetes ketoacidosis. This view was in consonance with previous work in paediatrics by Quiros [45]. Although Yokoyama [27] and Nakajima [46] reported of low serum amylase frequently observed in diabetic ketoacidosis before treatment with insulin in clinical settings, many in previous studies [41,42,43] explained that the unexpectedly high serum amylase may result from the fact that diabetic ketoacidosis involves numerous etiologies that can contribute to high serum amylase. These includes acute pancreatitis (mild to moderate grades) [42] renal dysfunction, and dehydration, which all result in increase serum amylase.

Estimation of serum amylase has been useful in determining several endocrine and metabolic disease pathogenesis [47,46]. Traditionally, serum amylase level measurement has been commonly used to determine the presence of acute pancreatitis and biliary tract disease [48]. And these conditions are reported to predispose to higher serum amylase. But in chronic pancreatitis, low serum amylase was reported and this was attributed to total destruction of pancreatic acinar cells [37,38,39]. It might interest to note that overt diabetes reflects an end stage destruction of the pancreas which might be equivalent to chronic or repeated pancreatitis associated with low alpha amylase expression [49], while the experimental diabetes in animal study may mimic the state of acute pancreatitis characterized with high serum amylase. However, this assertion requires appropriate experimental protocol to unravel the underlying mechanism as considered by other researchers [27,46].

The view that low serum amylase is experienced in diabetes must have been the reason for developing some pharmaco-therapies particularly against diabetes, such as dipeptidyl peptidase-4 inhibitors and glucagon-like peptide 1 receptor agonists capable of raising amylase. But this is contradictory to the current position of which postprandial hyperglycaemia is attributed to the action of amylase and anti-diabetic drugs with ability to inhibit the enzyme are useful in treatment of diabetes. Considering that excessive inhibition of pancreatic alpha-amyrase might cause abnormal bacterial fermentation of undigested carbohydrates in the colon resulting in flatulence and diarrhoea [50], advocating for reduction of alpha amylase as a new therapeutic target in treatment of diabetes as it is currently may pose a serious risk of indigestion if low level of amylase is a well established disturbance in diabetes. But in line with the current search for the new therapeutic target, serum level of alpha amyrase in the diabetic rats treated with the aqueous leaf extract of Terminalia catappa in this study significantly decreased when compared to the diabetic group across day 4, 10 and 14 and this was not dependent on the duration of the treatment. This observed diminution in amylase could be attributed to the role of some phyto-constituents present in the leaf extract. Previous in-vitro study by Mccue and Shetty [51] has indicated that phenols are capable of inhibiting α-amylase and reducing amylase level via reduction in the diabetic ketoacidosis and associated blood urea nitrogen thereby hindering glucose release from carbohydrate in diet and subsequently reduces diabetic hyperglycaemia. This hypothesis is in line with the recent claim that pharmacologically active components of plants such as vitamins, carotenoids, flavonoids, anthocynins and other phenolic compounds can inhibit alpha amylase and reduce blood sugar in diabetic patients [52]. Therefore phytochemical analysis of Terminalia catappa and investigation on the effect of some extract fractions is needed to elucidate the possible constituent(s) involved in the observed reduction in alpha amylase. It could be noted that alpha amylase catalysis the first step of carbohydrate digestion which normally precedes the action of insulin, thus new therapeutic approaches being investigated to regulate post-prandial glucose levels considers inhibition of amylase as an interesting and novel target for treatment of diabetes mellitus. From this finding, Terminalia catappa can be recommended as a potent natural agent that can reduce blood glucose level in diabetes through the reduction of serum alpha amylase enzyme.

Glucose as the end product of carbohydrate digestion is metabolised via oxidative and non-oxidative glucose pathways. The non-oxidative glucose metabolism leads to synthesis of glycogen and this involves enzymatic function of glycogen synthase. In this study serum glycogen synthase level was significantly higher in diabetic group when compared to the control group on day 4, day10 and day14. Again the observed increase was not dependent on the duration of the diabetes as there was no much difference in the level of glycogen on these days comparatively. Regulation of glycogen synthase is by insulin dependent and insulin independent mechanisms. In the muscle, insulin stimulates the non-oxidative glucose disposal [19] but in the liver glycogen synthase is regulated mainly by
high energy substrate while insulin has just a potentiating effect [8]. It is known that glycogen synthase is regulated both allosterically and by covalent modification via phosphorylation [8]. High energy substrate like glucose (which is of high concentration in the blood in diabetes mellitus) promotes liver glycogen storage [53]. Knowing well that glucose entry into the liver is via a facilitative non insulin dependent GLUT 2 transporter [54,55], insulinopenia present in diabetes does not impede the entry of glucose into the liver. In hyperglycaemic condition, glucose influx into the liver promotes glycogen synthesis which is brought about by a glucose stimulated dephosphorylation of phosphorylase a and glycogen synthase b leading to inactivation of glycogen breakdown and activation of glycogen synthesis [8]. Therefore, the significantly raised glycogen synthase observed in this study may be attributed to hyperglycaemia associated with diabetes which is a non hormonal hyperglycaemic induced activation of key glycogenesis enzyme; glycogen synthase b. Stimulation of glycogen synthase by insulin is by inhibition of glycogen synthase kinase 3 (GSK3). But possible involvement of an insulin-independent pathway leading to GSK3 inactivation [56] has been reported. There is evidence in rat muscle [57] and recent study in humans that has suggested a GSK3-independent mechanism [58]. Experimental report has shown a dramatic and sustained increase in glycogen synthase activity following re-addition of glucose which is independent of GSK3 inactivation and the mechanism leading to GS activation was independent of that utilized by insulin [12]. In addition, post-prandial hyperglycaemia is considered to further augment non oxidative glucose metabolism compared to constant glucose level [59]. Therefore the observed increase in the glycogen synthase in diabetic rat may be as a result of hyperglycemia or activation of insulin independent mechanisms.

The decrease in glycogen synthase level observed in the *Terminalia catappa* leaf extract treated group might suggest that the reduction of blood glucose diminished the primary stimulus of the non hormonal action of glucose on glycogen synthase which occur in the liver. The putative mechanism for the reduction in glycogen synthase level could be the reduction in non-oxidative glucose metabolism due to the reduction in glucose level or possible activation of the oxidative glucose metabolism in the muscle by increasing insulin release. It could be suggestive from this study that the anti-hyperglycaemic effect of the extract is via the reduction of postprandial blood glucose level or increase in insulin secretion in diabetic rats which lowers the initially elevated blood glucose level being the main factor in the control of liver glycogen synthesis.

5. CONCLUSION

The results shows that aqueous leaf extract of *Terminalia catappa* reduces serum alpha amylase in alloxan induced diabetic Wistar rats but the reduction in glycogen synthase may however be secondary to the reduced blood glucose. Therefore reduction in alpha amylase level may be the possible mechanism in which *Terminalia catappa* leaf extract expresses its anti-diabetic potential in rats.

ETHICAL APPROVAL

As per international standard or University standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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