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RESEARCH ARTICLE

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Hypoglycemic and modifying effect of aqueous cocoa powder extract on diabetic-induced histologic changes in the pancreas of alloxan diabetic rats

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ABSTRACT

Diabetes mellitus is a chronic disorder of carbohydrate metabolism with its characteristic hyperglycemia induced clinical features. Forty eight female Albino rats weighing between 200-250g were used for the study. The animals were randomly divided into six groups (200 mg/kg, 300 mg/kg, 500 mg/kg, 1000 mg/kg, glibenclamide, and diabetic untreated) each group consisting of eight rats. Diabetes mellitus was induced using a single intraperitoneal administration of 120mg/kg bodyweight alloxan after an overnight fast into all the rats. Establishment of diabetes was confirmed after 48 hrs of alloxan administration. All the animals were given normal rat chow and water freely for 40 days. Blood was obtained from the tail vein of each rat on daily basis for glucose determination using one torch glucometer. The animals were anaesthetized using diethyl ether on day 40 of the experiment to harvest the pancreas which was fixed, using 10% formalin, for histological studies. A significant ($p<0.05$) reduction in plasma glucose was observed and noted to start at about 30 mins of administration of the cocoa powder. The reduction in plasma glucose became more pronounced at 120mins of administration in all the different doses of aqueous cocoa powder extract. Some loss (atrophic) of β-cells and compensatory hyperplastic changes were also noted to occur in the pancreas of diabetic untreated and extract treated rats respectively. Conclusively, administration of aqueous cocoa powder extract lowers plasma glucose level and modifies diabetic-induced histologic changes in the pancreas of alloxan diabetic rats. The observed reduction in plasma glucose in the glibenclamide (a sulfonylurea) group indicates that alloxan at a dose of 120mg/kg bodyweight could give both type 1 and 11 diabetes mellitus features.

Key words: Diabetes mellitus, alloxan, hypoglycemia, atrophy, hyperplasia.

INTRODUCTION

Diabetes mellitus is a metabolic syndrome characterized by chronic hyperglycemia. The peculiar clinical features of polyuria, polydipsia, weight loss and secondary complications observed in diabetes mellitus are primarily a reflection of the hyperglycemic state. The pathognomonic hyperglycemia results from either total or partial deficient insulin secretion or cellular resistance to secreted insulin. Diabetes is characterized by hyperglycemia

which results in high supply of metabolic substrate to the mitochondria, increase glucose oxidation, increase free radical production, reduced total antioxidant capacity and finally oxidative stress. This resultant oxidative stress causes damage to a wide range of structural molecules including carbohydrate, lipids, proteins and nucleic acids that forms the basis of vascular and multiorgan complications in diabetes mellitus. In the pancreas, oxidative stress, through superoxide production, is the common pathogenic factor known to be responsible for β-cell dysfunction which eventually result in abnormal insulin secretion and impaired glucose tolerance (IGT) [2]. Thus, hyperglycemia could be described as a connector between diabetes mellitus and the various complications that are associated with the disease condition [13].

The effects of the hyperglycemia-induced increased free radicals are naturally annulled by the body's antioxidant defense system involving both enzymatic and nonenzymatic system that usually work synergistically with each other to neutralize free radicals in the body. Complications from diabetes mellitus therefore arises when the total antioxidant capacity of the body is exceeded or overwhelmed such that the free radical scavenging ability of the antioxidant system is reduced or loss.

The pancreas is an important organ that plays a major role in the availability of biofuels (from carbohydrates, protein and lipids) for normal physiological cellular activities. The regulatory function of the pancreas in carbohydrate metabolism lies in the secretion of Insulin and glucagon from the β-cells and α-cells respectively as determined by plasma glucose concentration. The role of genetic and environmental factors such as viral infection, obesity and lack of exercise in a genetically predisposed individual had been stressed [9]. Globally, the incidence of diabetes is increasing and the diabetic population had been projected to likely increase to 300 million or more by the year 2025 [17].

The current method for the management of diabetes mellitus entails dietary and lifestyle modifications; moderate exercise; and drug. Drug therapies include parentheral insulin and orally administered sulfonylureas, biguanides, α-glucosidase inhibitors, and α-amylase inhibitors, which are used singly or in combination to achieve a better glycemic control. The challenges with diabetes management are many ranging from financial constraints, drug unavailability, non-drug compliancy of diabetic patients, to the various side effects from these hypoglycemic agents all of which needed to proffer solution to [15] in order to prevent usually associated psychosocial problems.

Generally, cost effective and scientifically proven plants (herbs, shrubs or trees in part or in whole) with anti-hyperglycemic properties are increasingly being sought for in the management of diabetes mellitus in developing countries and the whole world. Africa, like India, offers a reservoir for plants with high medicinal importances that are yet to be researched upon. The aim of this research is therefore to join other researchers in the search for a cost effective antihyperglycemic agent with little or no adverse effect for the management of diabetes mellitus and prevention of its associated complications.

MATERIALS AND METHODS

Commercially available Nigerian pure cocoa powder was purchased from appetizing food company Ibadan, and used for the study, which was done at the department of Chemical Pathology and Immunology, Olabisi Onabanjo University.

Experimental design

A total of forty eight apparently normal female Albino rats weighing between 200-250g were purchased from anatomy department, University of Ibadan and used for the study. The animals were randomly divided into six groups each consisting of eight rats. Diabetes was induced using 120mg/kg alloxan which was administered intraperitoneally into all the rats except the normoglycemic controls. Establishment of diabetes was confirmed after 48 hrs of alloxan administration and treatment with the aqueous extract and glibenclamide was commenced immediately except in the diabetic untreated control group. The room temperature of 25°C-28°C and the natural cycle of daylight and night darkness were used. Glibenclamide (a sulphonylurea) was administered to a group of rats to ascertain the presence of proportions of the β-cells that were not destroyed by alloxan. Glibenclamide was intended to enhance or augment insulin secretion by residual pancreatic β-cells.

The various doses of aqueous cocoa powder (200mg/kg, 300mg/kg, 500mg/kg and 1000mg/kg) and glibenclamide (5mg/kg) were freshly prepared shortly prior to administration. The rats were fed with normal rat chow (obtained

from FA feeds, Ijebu-Ode) and water was given ad libitum for the whole research period. The test substance was administered orally once daily for 40 days in a constant volume over the range of the chosen doses by varying the concentration of the dosing preparation in water at room temperature. Each dose was administered in a single dose using oral cannula after overnight fasting.

Preparation of animals

The animals were randomly selected and arranged into six groups using the different doses of aqueous cocoa powder, glibenclamide and diabetic untreated control. The animals were tail-marked for individual identification, and kept acclimatized to the laboratory conditions for seven days before induction. The care of the animals was in accordance with the U.S. Public Health Service Guidelines [10].

Specimen collection, storage and processing

Fasting blood sample was collected from the tail vein daily for forty days to determine the blood glucose concentration using acu-check glucometer. The collected blood was applied directly onto the glucometer strip as specified by the manufacturer At the expiration of forty days experimental period, the rats were sacrificed (using diethyl ether as anesthetic agent), and then dissected. The pancreas in diabetic untreated control group rats and treated (both aqueous cocoa powder and glibenclamide) diabetic group rats was harvested and kept in 10% (v/v) formalin solution till fixed on plate for histologic studies.

Histopathology

From the pancreas obtained from both the treated (cocoa extract and glibenclamide) and diabetic untreated control groups, a section of 5µg thickness each were cut and stained by heamatoxylin and eosin (H&E) for histological examination.

Data analysis

Data analysis was done using SPSS version 17 statistical package. Mean and standard error of mean (Mean ± SEM) were used to describe variables which were expressed graphically and also using bar chart. Statistical significance level was set at $p < 0.05$.

RESULTS

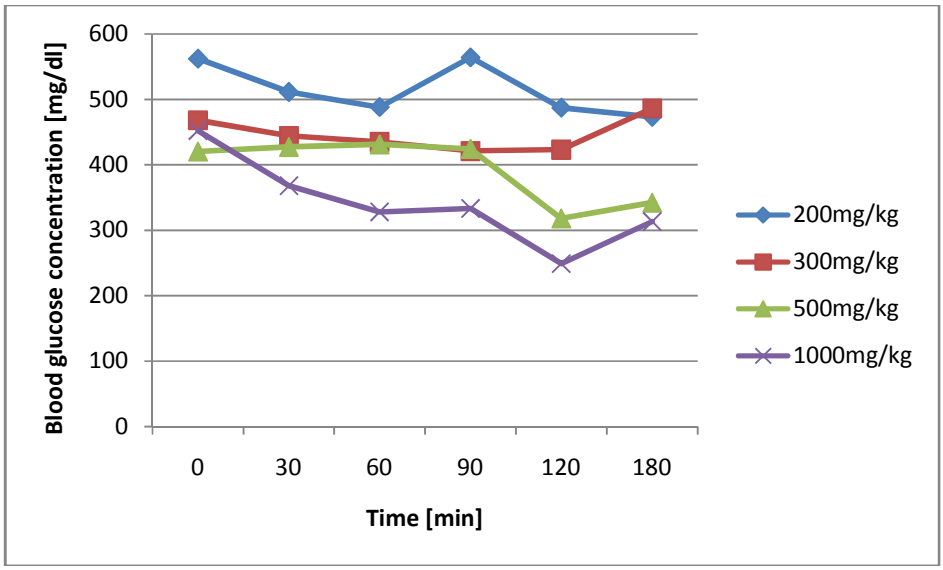

Figure 1: Blood glucose response to oral aqueous cocoa powder extract intake

Figure 1 above shows the establishment of hypoglycemic effect of aqueous cocoa powder extracts at 30 mins of the administration of different doses of the extract.

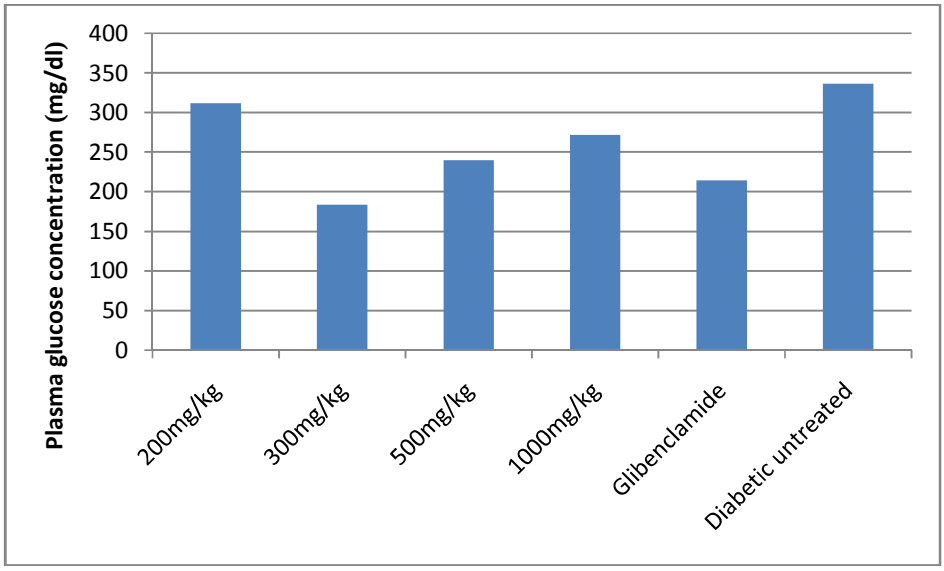

Figure 2: Changes in plasma glucose concentrations amongst the treated and control groups

Figure 2 above shows the glucose level amongst the experimental and control groups. A dose of 300mg/kg shows a significant hypoglycemic effect compare to other doses and diabetic untreated group.

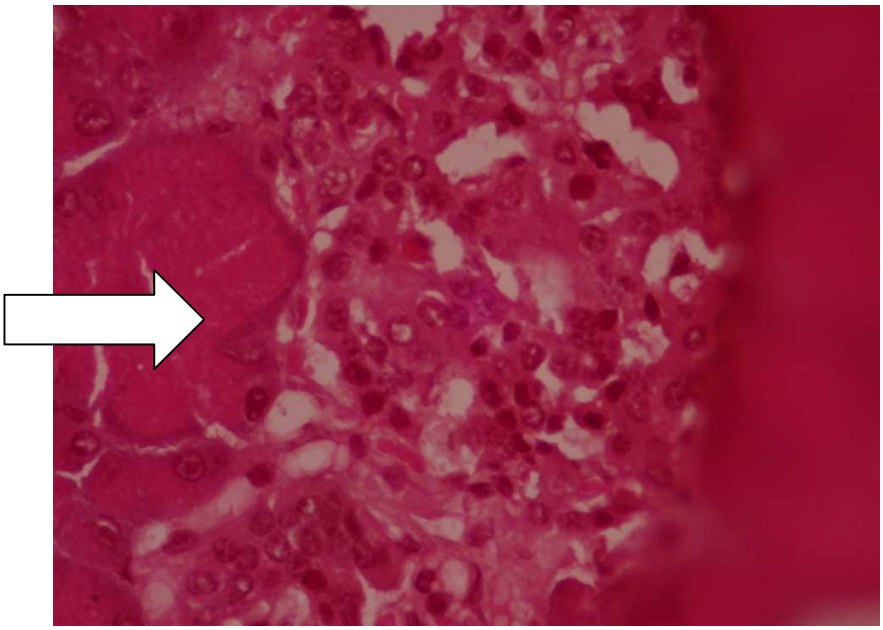

Figure 3: Changes in Islet with 200mg/kg cocoa powder dose

Photomicrograph sample of pancreas of rats administered with 120 mg/kg bodyweight of alloxan and treated with 200mg/kg bodyweight cocoa powder extract. Solid arrow shows the pancreatic Islets of beta cells which appears enlarged or hyperplastic

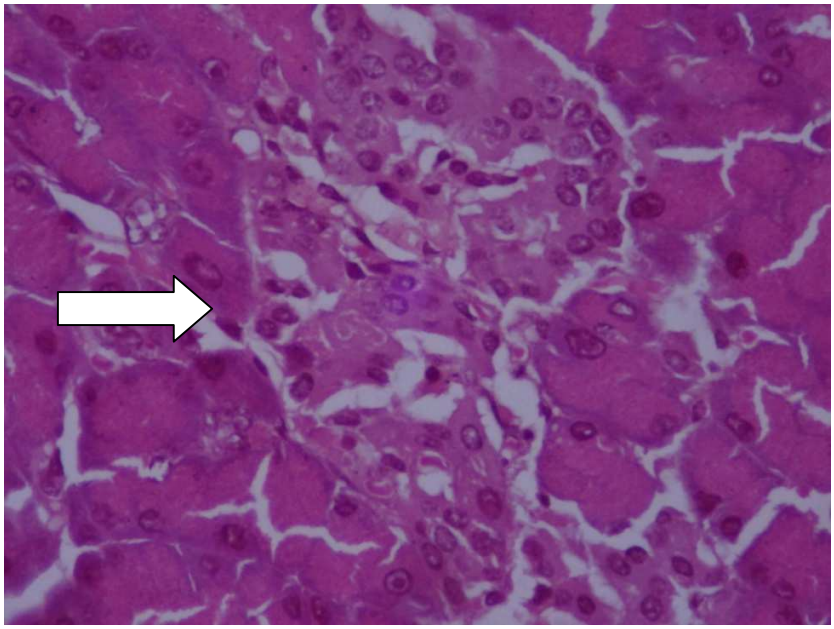

Figure 4: Changes in Islet with 300mg/kg cocoa powder dose

Photomicrograph sample of pancreas of rats administered with 120 mg/kg bodyweight of alloxan and treated with 300mg/kg bodyweight cocoa powder extract. Solid arrow shows the pancreatic Islets of beta cells which appears reduced in size in comparison to that seen in the control group.

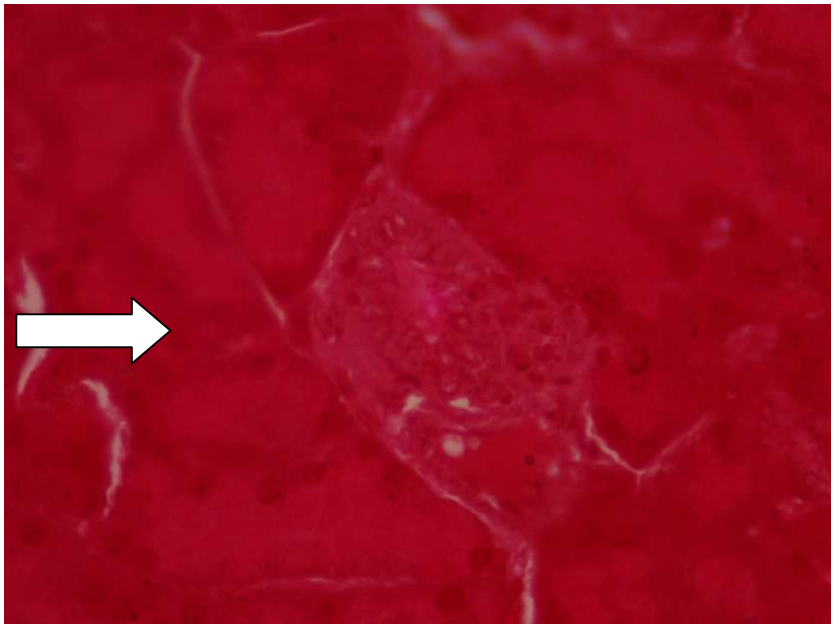

Figure 5: Changes in Islet with 500mg/kg cocoa powder dose

Photomicrograph sample of pancreas of rats administered with 120 mg/kg bodyweight of alloxan and treated with 500mg/kg bodyweight cocoa powder extract. Solid arrow shows the pancreatic Islets of beta cells which appears reduced in size (similar to figure) in comparison to that seen in the control group.

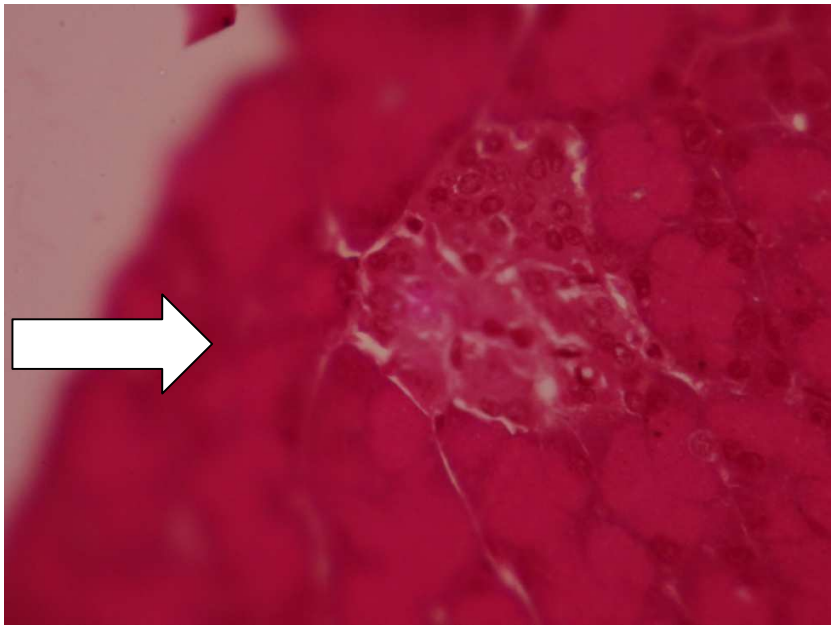

Figure 6: Changes in Islet with 1000mg/kg cocoa powder dose

Photomicrograph sample of pancreas of rats administered with 120 mg/kg bodyweight of alloxan and treated with 1000mg/kg bodyweight cocoa powder extract. Solid arrow shows the pancreatic Islets of beta cells which appears reduced in size in comparison to that seen in the control group (similar to plate 2 and 3).

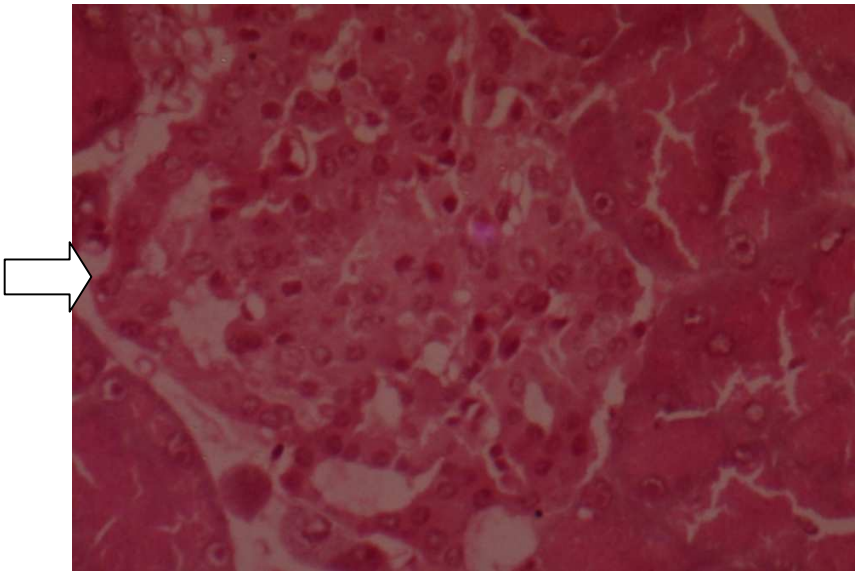

Figure 7: Changes in Islet with Glibenclamide at 5mg/kg dose

Photomicrograph sample of pancreas of rats administered with 120 mg/kg bodyweight of alloxan and treated with 5mg/kg bodyweight glibenclamide. Solid arrow shows the pancreatic Islets of beta cells which appears atrophic or reduced in size in comparison to that seen in the control group.

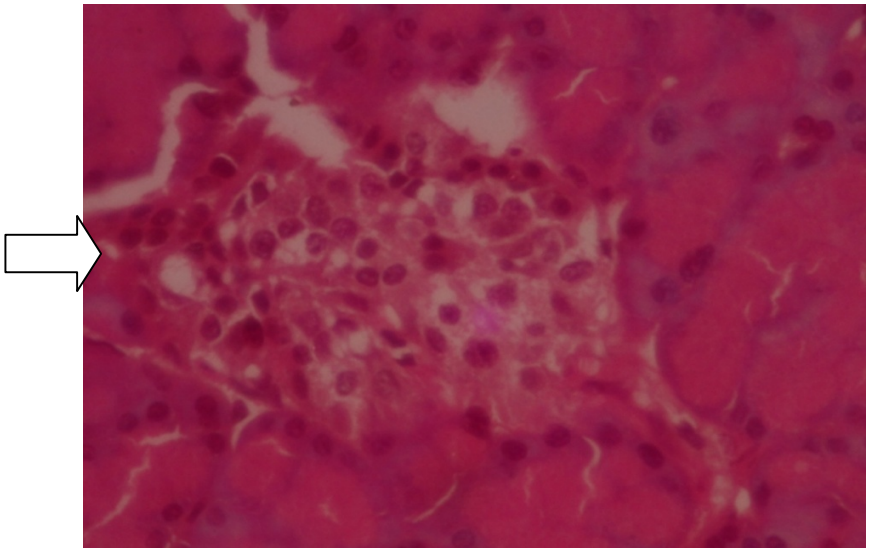

Figure 8: Changes in Islet in diabetic untreated group

Photomicrograph sample of pancreas of rats administered with 120 mg/kg bodyweight of alloxan and not treated. Solid arrow shows the pancreatic Islets of beta cells which appear shrunk.

DISCUSSION

From this study, a reduction in plasma glucose was observed following oral administration of different doses of aqueous cocoa powder extract in the experimental (aqueous cocoa powder extract treated) as compared to diabetic untreated groups. The antihyperglycemic effect of aqueous cocoa powder extract was noted to start at 30mins of administration of the extract and became more pronounced at 120mins in all the different doses of aqueous cocoa powder extract administered (figure 1). The initially observed reduction in plasma glucose could either be from the action of the extract or the administered alloxan which had been discovered to cause reduction in plasma glucose concentration within 30mins of administration [8]. This effect of alloxan is short-lived and is consequent to a transient stimulation of insulin secretion from the β-cells [8]. The observed hypoglycemia in alloxan-induced diabetic rats could be due to antioxidant activity of polyphenol compounds therein present. Similar finding was observed in streptozotocin-induced diabetic rats [14, 1].

Glibenclamide was observed to show some hypoglycemic activity which ordinarily is not expected in type 1 diabetes mellitus. This observation reflects non-total damage of alloxan to the pancreatic β-cells at the induction dose of 120mg/kg body weight used in this study. It probably indicates that alloxan could be used to induce both type 1 and type 2 diabetes mellitus in a dose dependent manner in laboratory animals. In this regards, at higher doses (≥ 150mg/kg) alloxan will result in type 1 diabetes mellitus while at low dose (120mg/kg) it gives mixed features of both type 1 and type 2 diabetes mellitus due to the presence of some proportions of insulin producing β-cells in the islet.

The β-cells are responsible for insulin production and exhaustion of β-cells will therefore result in insulin deficiency which will result in disorder of carbohydrate, protein and fat metabolism

From this study, some loss of β-cells was observed in the pancreas as evidenced by pressure islet atrophy in diabetic untreated control group (figure 8). Similar observation was reported in the pancreas of human with chronic diabetes mellitus [6]. The pressure could be from large deposits of a homogenous eosinophilic material which largely occupies the islet in the diabetic untreated control group.

The islet cells in 300mg/kg, 500mg/kg and 1000mg/kg experimental groups appeared similar (figures 4, 5 and 6). However, the islet in 200mg/kg group appears enlarged/hyperplastic (figure 3). This islet cell hyperplasia may be of physiologic or compensatory importance in early stage of diabetes mellitus. In glibenclamide treated rats the islet

cells appeared decreased in size (atrophic) while shrinkage in islet cell mass was observed in diabetic untreated group (figures 7 and 9). All these histological appearances are indicative of the observed biochemical parameter changes reported in this study.

A dose dependent increase in plasma flavanol concentration and the total antioxidant capacity after oral intake of cocoa had been reported [11]. This may act to mop up the circulating reactive oxygen species (ROS) generated by the hyperglycemia and the administered alloxan and also the flavonols may probably have arrested further destruction of the remaining β-cells in the islet thereby allowing other phytochemicals present in the powder to induce regenerative activities. This may be used to explain the reduction in plasma glucose observed in the different doses of cocoa powder extract administered to the rats. Also, flavomols have been reported to cause increase in β-cells [3] and this is probably due to the presence of some stable cells in the islets with regenerating ability [4].
The hypoglycemic effect of cocoa powder, as observed in this study, may also be probably due to the type or class of flavonol present. Cocoa had been reported to be rich in flavonol of the class catechins which reduces dietary carbohydrate bioavailability either by inhibiting intestinal glucose transporters [14] or inhibiting pancreatic α-amylase thereby reducing glucose uptake with consequent reduction in blood glucose level.

In addition to polyphenol content, cocoa is also rich in methylxanthine, namely caffeine and theobromine [16]. Caffeine had been reported to decrease insulin-mediated glucose uptake and glucose catabolism [7]. The caffeine and antioxidant content of the administered cocoa powder is expected to increase with increasing quantity of the cocoa powder (i.e. 1000mg/kg > 500mg/kg > 300mg/kg > 200mg/kg). Thus, the relatively observed hyperglycemia in a dose of 1000mg/kg, and non-significant ($p > 0.05$) hypoglycemia in 200mg/kg, as compared to 300mg/kg and 500mg/kg (figure 2), are probably due to high caffeine and low antioxidant levels respectively in those concentrations.

The presence of high methylxanthines (theobromine and caffeine) content in cocoa powder could inhibit the antioxidant capacity of flavonoids present in the powder and this explains the insignificant ($p > 0.05$) hypoglycemia observed in 1000mg/kg cocoa powder concentration (figure 2).

Alloxan is noted to cause injury to the pancreas β-cells and pancreatic regeneration after injury has been reported to occur in animal models [18]. However, plant therapy was reported not to regenerate β-cells of the endocrine pancreas [18]. The mode of action of antihyperglycaemic plants may be affectation of circulating insulin levels [5]. Due to the observed reduction in plasma glucose by glibenclamide in this study, the used alloxan dosage did not probably destroy all the pancreatic β-cells and the remaining cells can secrete insulin which to some extent lowers the plasma glucose.

Conclusively, administration of aqueous extract of cocoa powder lowers plasma glucose level and could be of managerial importance in the management of diabetes mellitus. In addition to lifestyle modifications through increased physical activities to adequately metabolize ingested glucose and reduced calorie intake or bioavailability, aqueous cocoa powder ingestion either solely or as adjuvant to drugs with established antihyperglycemic properties will improve the quality of life of diabetics.

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