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Effects of Methanol Extract of *Gongronema Latifolium* Leaves on Glycaemic Responses to Carbohydrate Diets in Streptozotocin-Induced Diabetic Rats

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Abstract

The search for natural plant extract as an alternative therapy to diabetes management has received enormous recognition globally. In this study, the effects of the methanol extracts of Gongronema latifolium leaves (GLE) on glycaemic responses to carbohydrate diets [obtained from cassava (Manihot esculenta), yam (Dioscorea rotundata) and plantain (Musa paradisiaca)] in streptozotocin-induced diabetic rats was investigated. Forty five adult male Wistar albino rats were induced diabetes by intraperitoneal (i.p.) injection of streptozotocin (STZ) (65 mg/kg b.w.) dissolved in citrate buffer, pH 4.5. The diabetic rats were divided into nine groups of 5 animals each. Group 1: the Positive control (diabetic, untreated) was fed with water only, group 2: the standard control (diabetic, treated) received 2.50mg/kg b.w of glibenclamide, a standard drug for diabetes, group 3, 4 ad 5 (carbohydrate diets treated only) were orally fed with 200 mg/kg b.w. of cassava diet, 200 mg/kg b.w. of yam

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diet and 200 mg/kg b.w. of plantain diet respectively, group 6, 7 ad 8 (GLE mixed with carbohydrate diets treated) were orally fed with 200 mg/kg b.w. of cassava mixed with 100 mg/kg b.w. of GLE, 200 mg/kg b.w. of yam mixed with 100 mg/kg b.w. of GLE and 200 mg/kg b.w. of plantain mixed with 100 mg/kg b.w. of GLE respectively, while group 9 (GLE treated only) was fed with 100 mg/kg b.w. of GLE only. The experiment lasted for 21 days with free access to feed and water. Animals' weights were measured on weekly basis as well as estimation of fasting blood sugar (FBG) and postprandial blood glucose (PBG). Results revealed that, administration of carbohydrate diets led to a significant ($p < 0.05$) decrease in body weights of the diabetic rats fed with carbohydrate diets. Conversely, administration of the GLE mixed with the diets caused a significant ($p > 0.05$) increase in body weights of the diabetic rats compared to the positive control. However, treatment with GLE alone showed a more effectuality which is comparable to that of the standard drug in restoring weight loss. In addition, the results of blood glucose estimation revealed a significant ($p < 0.05$) elevation in both fasting blood glucose (FBG) and Postprandial blood glucose (PBG) in animals fed with carbohydrate diets when compared to the positive control, but a significant ($p < 0.05$) decrease was observed in both parameters upon the admiistration of GLE mixed with carbohydrate diets. Similarly, administration of GLE alone caused an increase in both FBG and PBG which was significant ($p < 0.05$) compared to group fed with GLE mixed with carbohydrate diets. Howbeit, GLE elicited more potent and efficacious response when compared to the standard drug. Herein, we therefore deduce that methanolic extracts of G. latifolium leaves exhibited hypoglycaemic responses in the diabetic rats and the plant could be a worthwhile candidate in the design of a potent antidiabetic drug.

Keywords: Diabetes; *Gongronema latifolium*; Carbohydrate Diets; Fasting Blood Glucose; Postprandial Blood

INTRODUCTION

Diabetes remains a major global metabolic health problem, reaching epidemic proportions (American Diabetes Association, 2010) and the search for its cure continues. Different kinds of carbohydrates foods elicit different blood glucose and insulin concentrations, because of their chemical nature, especially the ratio of amylose to amylopectin forms of starch they contain, which may affect their rate and speed of digestion (Nguyen *et al.*, 1998). The extent of postprandial hyperglycaemia and insulin secretion depend on the amount of food and carbohydrate consumed per meal.

Nigeria was named the greatest producer and consumer of cassava in the world and yam remains a major source of carbohydrate meal in Africa. Unripe plantain has been a popular food for diabetic patients, while *G. latifolium* leaves has been part of various cultural delicacies in Nigeria and West Africa. However, the wide consumption and combination of these foods go on with little or no knowledge of their health implications and available reports focused mainly on the medicinal properties of the leaf with little attempts at investigating its potential glycaemic responses on carbohydrates. Also, salts, salty foods and condiments tend to speed up the rate of digestion of starch and increase the rate of absorption of glucose and as such, increase the glycaemic index (GI) of meals (Liljeberg and Bjorck, 1998) while the co-ingestion of large amounts of protein and fats results in a reduction of the difference between glycaemic indices of foods (Gulliford *et al*., 1989).

The volume of information found in literature on the potential food and drug uses of *G. latifolium* leaves is scanty (Afolabi and Eleyinmi, 2007), especially why it is served as post-delivery meals to women and to nursing mothers. Also, the need to widen raw material base of pharmaceutical industries and the current shift away from the use of synthetic chemicals in drug formulations to natural plant extracts, necessitate further evaluation of the potential glycaemic properties of *G. latifolium* leaf extract, a widely available and highly consumed medicinal plant in Nigeria. Therefore, this work aimed to investigate the effects of the methanol extracts of *Gongronema latifolium* leaves on glycaemic responses to carbohydrate diets [obtained from cassava (*Maninhot esculenta*), yam (*Dioscorea rotundata*) and plantain (*Musa paradisiaca*)] in streptozotocin-induced diabetic rats in comparison with the standard drug, glibenclamide.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh mature leaves of *G. latifolium* were purchased from Nsukka market in Enugu State, Nigeria and were botanically identified at Bioresources Development and Conservation Programme (BDCP), University of Nigeria Nsukka, Enugu State Nigeria. The three carbohydrate food items cassava (*Manihot esculenta*), yam (*Dioscorea rotundata*) and plantain (*Musa paradisiaca*) each of the same species, were bought from Nsukka food market, around the University of Nigeria, Nsukka campus in July 2012. The fresh leaves were then washed, air dried at room temperature (25^{o}C) for 7 days and pulverized into coarse powder. Exactly

1 kg of the leaf powder was infused with 2.5 litres of methanol by cold maceration with occasional stirring for 72 hours and filtered (Trease and Evans, 1998). The filtrate was evaporated to dryness in a rotary evaporator to obtain the methanol extract which was stored in a refrigerator at 4^0C before use.

Extraction of carbohydrate foods

The three carbohydrate foods (cassava, yam and plantain) were separately washed, peeled, cut into tiny pieces; blended in a warring blender with excess water and filtered through muslin cloth. The filtrates were allowed to rapidly sediment in shallow trays and washed several times with water to remove impurities from the surface. Sediments were evaporated to dryness using rotary evaporator and preserved in glass bottles at 4°C for use in each experimental diet preparations.

Preparation of Experimental Diets

The carbohydrate diets were prepared by accurately measuring quantities of the powdered food extracts equivalent to 200 mg per kilograms body weight (mg/kg b.w.) as required for each rat in each group and dissolving same in distilled water prior to feeding. The carbohydrate with *G. latifolium* leaf extract diets were prepared by measuring 200 mg per kilograms body weight (mg/kg b.w.) of each carbohydrate food and adding 100 mg per/kg b.w. of the *G. latifolium* leaf extract for each rat in each group and dissolving both in distilled water prior to feeding.

Induction of Diabetes in Animals

Forty-five adult male Wistar albino rats were fasted overnight and injected with a single intraperitoneal (i.p.) dose of streptozotocin (STZ) (Sigma, St. Louis, MO, USA) at 65 mg/kg b.w. Just prior to the induction of diabetes, STZ was dissolved in a freshly prepared 0.1M cold citrate buffer, pH 4.5 (Rakieten and Radkarni, 1963). Control animals were injected intra-peritoneal citrate buffer alone at a single dose of 1.2 ml/kg b.w. Because STZ is capable of inducing fatal hypoglycaemia as a result of massive pancreatic insulin release, STZ-treated rats were provided with 10% glucose solution after the 6^{th} hour to prevent severe hypoglycaemia. All animals were allowed free access to feed and water after STZ injection and left undisturbed for a minimum of 72 hours for hyperglycaemia to develop. After 3 days, fasting blood glucose levels of the animals were measured with One Touch Ultra Mini Glucometer. Animals with blood glucose concentrations ≥ 250 mg/dl were considered hyperglycaemic and selected for the study (Canepa *et al.*, 1990).

Experimental design

The 45 STZ-induced diabetic adult male Wistar albino rats were randomly divided into nine groups of 5 animals each. Three groups were orally fed with the carbohydrate diets alone; three other groups were fed with GLE mixed with carbohydrate diets daily while three control groups received only water, glibenclamide and GLE respectively as follows:

Group 1: Positive control (diabetic, untreated), fed water only

Group 2: Standard control (diabetic, treated), given 2.50mg/kg b.w of *glibenclamide*

Group 3: Diabetic, fed 200 mg/kg b.w. of cassava diet only

Group 4: Diabetic, fed 200 mg/kg b.w. of yam diet only

Group 5: Diabetic, fed 200 mg/kg b.w. of plantain diet only

Group 6: Diabetic, fed 200 mg/kg b.w. of cassava mixed with 100 mg/kg b.w. of GLE

Group 7: Diabetic, fed 200 mg/kg b.w. of yam mixed with 100 mg/kg b.w. of GLE

Group 8: Diabetic, fed 200 mg/kg b.w. of plantain mixed with 100 mg/kg b.w. of GLE

Group 9: Diabetic, fed 100 mg/kg b.w. of GLE only

All group treatments and administration lasted for 21 days with free access to feed and water. On weekly basis, animals' weights were measured and blood samples were collected from every rat in each group to estimate fasting blood sugar, postprandial blood glucose and serum protein concentrations.

Determination of Weight Changes

Each week, the body weights of each rat in each group before feeding were accurately measured using the laboratory weighing balance and recorded.

Blood Sample Collection

Blood samples (0.5 ml) were collected by cutting the rat tail vein with a new sterile surgical blade and used to determine the fasting blood sugar (FBS) and postprandial blood glucose (PBG) levels. Blood samples were also collected by ocular puncture into separate non-heparinized tubes and allowed to coagulate. The serum was collected and used to determine serum total protein.

Determination of Changes in Fasting Blood Sugar (FBS)

The fasting blood sugar (FBS) was carried out using standard one touch Accu-Check Ultra Mini-glucometer and test strips. The rats in each group were fasted overnight and test carried out before feeding the animals in the morning. Glucometer was powered on; the test strip inserted and put at zero. Tail-vein blood sample was taken; one drop was placed on the test strip and allowed to stand. The reading was taken as FBS concentration and then recorded.

Determination of Changes in Postprandial Blood Glucose (PBG)

The PBG tests were also done using standard one touch Accu-Check Ultra Mini-glucometer. The principle and reaction equations were the same as for the FBS tests. After the FBS tests on day 0, the rats in each group were given their respective group diets and blood samples were then collected at 0 min, 30 min, 60 min, 90 min and 120 min. By placing a drop of blood on a clean test strip of the glucometer, the PBG readings were taken. The test was later performed weekly for each rat in each group up to 21 days.

RESULTS

Effects of GLE mixed with Carbohydrate Diets on Body Weight Changes in Streptozotocin

(STZ)-induced Diabetic Rats

As displayed in table 1, body weights of the diabetic rats fed with carbohydrate diets (Group 3, 4 ad 5) showed significant ($p < 0.05$) decrease when compared to the positive control (Group 1) that received water only from day 7 to 21. However, treatment with GLE-carbohydrate diets caused a significant ($p < 0.05$) increase in weights among the animals fed with GLE-cassava diet (group 5), GLE-yam diet (group 6) and GLE-plantain diet (group 7) compared to their corresponding groups fed with carbohydrate diets alone (groups 2, 3 and 4). Meanwhile, GLE extract alone caused a significant ($p<0.05$) increase in the weights of group 9 when compared to group 5, 6 ad 7 and this increase is comparable to the one elicited in group 2 (diabetic standard control) by glibenclamide. This increase cut across days 14 and 21.

Table 1: Body weight changes in diabetic rats fed GLE mixed with carbohydrate diets

GROUP No	FEEDING (/Kg b.w.)	Δ DAY 7 (mg)	Δ DAY 14 (mg)	Δ DAY 21 (mg)
1	Diabetic, Untreated ($D.H_2O$)	-13.10^{e}	-22.23^{f}	-27.90^{f}
2	Diabetic, *Glibenclamide* 2.5 mg	11.93^{ab}	12.80^{b}	16.06^{ab}
3	Diabetic, Cassava 200 mg	-26.53^{c}	-31.66^{c}	-42.30^{d}
4	Diabetic, Yam 200 mg	-26.60^{c}	-29.40^{c}	-38.70^{d}
5	Diabetic, Plantain 200 mg	-18.86^{c}	-27.23^{c}	-35.56^{c}
6	Diabetic, $Cassava_{200mg}$ with GLE_{100mg}	03.06^{a}	$01.50a^{a}$	07.70^{a}
7	Diabetic, Yam_{200mg} with GLE_{100mg}	05.76^{a}	01.60^{a}	06.63^{a}
8	Diabetic, $Plantain_{200mg}$ with GLE_{100mg}	08.33^{a}	07.33^{a}	09.330^{a}
9	Diabetic, *GL* $Extract_{100mg}$	14.00^{b}	16.20^{b}	23.86^{ab}

Group values are mean ± SD, n = 5; values with different superscripts are statistically significant at $p < 0.05$

Effects of GLE mixed with Carbohydrate Diets on FBG changes in Diabetic Rats

Table 2 depicts the effect of GLE on blood glucose levels in STZ-induced diabetic rats. From the data, there was a significant ($p < 0.05$) elevation in FBG level in groups 3, 4 and 5 fed with carbohydrate diets alone compared to the positive control (group 1) with GLE-cassava diet eliciting the highest effect. Meanwhile, administration of *G. latifolium* extract (GLE) mixed with carbohydrate diets significantly ($p < 0.05$) reduced FBG from day 7 through day 21 in group 6, 7 and 8 with GLE-plantain diet displaying highest reduction. Also, groups fed with GLE-only showed a significant ($p<0.05$) and highest reduction in FBG and this reduction is comparable to GLE-plantain diet fed group, but significantly ($p<0.05$) different from group 2 that received glibenclamide (diabetic standard control).

Table 2: FBG changes in STZ-induced diabetic rats fed GLE mixed with carbohydrate diets

GROUP No	FEEDING (/Kg b.w.)	Δ DAY 7 (mg/dl)	Δ DAY 14 (mg/dl)	Δ DAY 21 (mg/dl)
1	Diabetic, Untreated ($D.H_2O$)	04.00^{b}	05.33^{b}	05.00^{b}
2	Diabetic, Glibenclamide 2.5 mg	-17.33^{a}	-82.33^{a}	-51.66^{ab}
3	Diabetic, Cassava 200 mg	34.00^{c}	39.33^{c}	48.66^{c}
4	Diabetic, Yam200 mg	32.66^{c}	36.00^{c}	41.33^{c}
5	Diabetic, Plantain 200 mg	14.00^{bc}	15.66^{bc}	13.66^{bc}
6	Diabetic, $Cassava_{200mg}$ with GLE_{100mg}	-06.66^{a}	-07.33^{ab}	-10.66^{ab}
7	Diabetic, Yam_{200mg} with GLE_{100mg}	-09.00^{a}	-28.33^{ab}	-14.00^{ab}
8	Diabetic, $Plantain_{200mg}$ with GLE_{100mg}	-06.66^{a}	-102.33^{a}	-157.66^{a}
9	Diabetic, GL Extract $_{100mg}$	-13.33^{a}	-143.00^{a}	-170.33^{a}

Group values are mean ± SD, n = 5; values with different superscripts are statistically significant at $p < 0.05$

Effects of GLE mixed with Carbohydrate Diets on Day 0 Postprandial BloodGlucose (PBG) Changesin diabetic Rats

As presented in Table 3, **there was** a significant ($p < 0.05$) increase in **P**BG level in groups 3, 4 and 5 fed with carbohydrate diets compared to the positive control group after 30, 60, 90 ad 120min . however, a significant ($p < 0.05$) reduction was observed in group 6 (diabetic, GLE-cassava diet), 7 (diabetic, GLE-yam diet) and 8 (diabetic, GLE-plantain diet) after 30 min to 120 min of feeding the diet compared to their corresponding carbohydrate fed groups 3, 4 and 5. When compared to the diabetic, glibenclamide-treated group 2, the PBG in groups 6, 7 and 8 were more significantly ($p < 0.05$) lower after 30 ad 60 min, while only group 7 (GLE-plantain diet) showed a significant ($p < 0.05$) PBG lowering effect compared to glibenclamide-treated group 2 after 90 ad 120 min. In addition, compared to the diabetic, GLE-treated rats in group 9, glibenclamide had a lower PBG reductions efficacy.

Table 3: Day 0 PBG changes in diabetic rats fed GLE mixed with carbohydrate diets

Group No	Feeding (/kg b.w)	Δ30min (mg/dl)	Δ60min (mg/dl)	Δ90min (mg/dl)	Δ120 min (mg/dl)
1	Diabetic, Untreated (D.H_2O)	09.00^{bc}	18.00^{cd}	08.33^{bc}	-02.33^{a}
2	Diabetic, $Glibenclamide_{2.5mg}$	23.33^{d}	19.00^{cd}	13.67^{c}	3.67^{b}
3	Diabetic, $Cassava_{200mg}$	16.67^{c}	29.33^{e}	31.00^{e}	24.67^{d}
4	Diabetic, Yam_{200mg}	15.33^{c}	25.00^{d}	34.67^{e}	41.67^{e}
5	Diabetic, $Plantain_{200mg}$	09.00^{bc}	14.33^{c}	23.67^{d}	17.33^{cd}
6	Diabetic, $Cassava_{200mg}$ with GLE_{100mg}	05.67^{b}	10.00^{b}	11.33^{c}	8.33^{c}
7	Diabetic, Yam_{200mg} with GLE_{100mg}	11.00^{bc}	16.67^{c}	13.33^{c}	07.33^{bc}
8	Diabetic, $Plantain_{200mg}$ with GLE_{100mg}	00.67^{a}	02.33^{a}	02.67^{b}	-02.00^{a}
9	Diabetic, $GLE_{100\ mg}$	06.67^{b}	00.67^{a}	-07.33^{a}	-07.00^{a}

Group values are mean ± SD, n = 5; values with different superscripts are statistically significant at $p < 0.05$

Effects of GLE mixed withCarbohydrate Diets on Day 7 PBG Changesin STZ-induced diabetic Rats

The result of PBG changes in the diabetic rats after day 7 (Table 4) revealed a significant ($p < 0.05$) elevation in PBG levels in group 3 (fed with cassava diet) and 4 (fed with yam diet) and 5 (fed with plantain diet) when compared to group 1 (positive control). However, group 6, 7 and 8 (fed with GLE-cassava, GLE-yam and GLE-plantain diets) displayed a significantly ($p < 0.05$) decrease in PBG level when compared to group 3,4 ad 5 fed with carbohydrates diets only. Group 9 fed with GLE only showed a significant ($p < 0.05$) reduction in PBG level when compared to group 2 (glibenclamide-treated group).

Table 4: Day 7 PBG changes in diabetic rats fed GLE mixed with carbohydrate diets

Group No	Feeding (/kg b.w.)	Δ 30min (mg/dl)	Δ 60min (mg/dl)	Δ 90min (mg/dl)	Δ 120min (mg/dl)
1	Diabetic, Untreated ($D.H_2O$)	02.67^{b}	01.33^{b}	-09.67^{a}	-37.67^{a}
2	Diabetic, $Glibenclamide_{2.5mg}$	-03.67^{a}	-29.00^{f}	-23.00^{f}	-34.33^{f}
3	Diabetic, $Cassava_{200mg}$	13.33^{d}	22.67^{cde}	24.33^{d}	22.33^{d}
4	Diabetic, Yam_{200mg}	22.67^{e}	37.67^{e}	40.67^{e}	33.33^{e}
5	Diabetic, $Plantain_{200mg}$	12.33^{cd}	16.33^{cd}	12.33^{c}	15.67^{cd}
6	Diabetic, $Cassava_{200mg}$ with GLE_{100mg}	3.00^{b}	6.67^{bc}	4.33^{b}	2.67^{bc}
7	Diabetic, Yam_{200mg} with GLE_{100mg}	11.67^{cd}	16.67^{cd}	09.67^{bc}	06.33^{c}
8	Diabetic, $Plantain_{200mg}$ with GLE_{100mg}	13.67^{d}	12.67^{cd}	02.00^{b}	00.33^{b}
9	Diabetic, $GLE_{100\ mg}$	10.33^{c}	08.67^{c}	01.33^{a}	-4.67^{a}

Group values are mean ± SD, n = 5; values with different superscripts are statistically significant at $p < 0.05$

Effects of GLE mixed with Carbohydrate Diets on Day 14 PBG Changes in diabetic Rats

The result of PBG changes in the diabetic rats on day 14 is displayed in Table 5. A significant ($p < 0.05$) elevation in the level of PBG was observed in group 3 (fed with cassava diet) and 4 (fed with yam diet) when compared to group 1 (untreated control group), while group 5 (fed with plantain diet) showed a significantly ($p < 0.05$) reduction in PBG level. Conversely, GLE mixed with carohydrate diets caused a significant ($p < 0.05$) decrease in PBG level in group 6 ad 7 compared to their respective carbohydrate fed groups with GLE-plantain diet evoking the highest PBG lowering effect. However, group 9 fed with GLE only showed no significant ($p < 0.05$) difference from group 2 (glibenclamide-treated group).

Table 5: Day 14 PBG changes in diabetic rats fed GLE mixed with carbohydrate diets

Group No	Feeding (/kg b.w.)	Δ 30min (mg/dl)	Δ 60min (mg/dl)	Δ 90min (mg/dl)	Δ 120min (mg/dl)
1	Diabetic, Untreated (D.H_2O)	21.00^{c}	09.93^{b}	07.33^{b}	03.33^{b}
2	Diabetic, $Glibenclamide_{2.5mg}$	10.00^{b}	11.67^{bc}	-05.33^{a}	-17.00^{a}
3	Diabetic, $Cassava_{200mg}$	10.00^{b}	23.67^{cd}	24.33^{d}	22.00^{c}
4	Diabetic, Yam_{200mg}	21.00^{c}	28.33^{e}	38.33^{e}	34.67^{d}
5	Diabetic, $Plantain_{200mg}$	13.67^{bc}	30.00^{d}	-8.33^{a}	-10.67^{a}
6	Diabetic, $Cassava_{200mg}$ with GLE_{100mg}	02.00^{a}	14.67^{c}	10.33^{c}	01.00^{b}
7	Diabetic, Yam_{200mg} with GLE_{100mg}	02.33^{a}	10.33^{b}	06.33^{d}	03.00^{b}
8	Diabetic, $Plantain_{200mg}$ with GLE_{100mg}	04.33^{a}	3.00^{a}	-02.33^{a}	-08.33^{a}
9	Diabetic, $GLE_{100\ mg}$	02.33^{a}	11.00^{bc}	-02.00^{a}	-07.33^{a}

Group values are mean ± SD, n = 5; values with different superscripts are statistically significant at $p < 0.05$

Effects of GLE mixed with Carbohydrate Diets on Day 21 PBG Changes in STZ-induced diabetic Rats

Table 6 showed that administration of cassava, yam and platain in group 3, 4 ad 5 respectively caused a significantly ($p < 0.05$) increase in the level of PBG when compared to untreated control group with cassava evoking the highest glycemic response. Nonetheless, treatment with GLE-carohydrate diets caused a significant ($p < 0.05$) reduction in PBG level in group 6, 7 and 8 compared to their respective carbohydrate fed groups 3, 4 and 5 with GLE-plantain diet stimulating the highest hypoglycemic effect after 30 min to 120 min of feeding. Furthermore, the PBG lowering effect of GLE-plantain diet and GLE only were not significant ($p < 0.05$) different from each other, but were significantly ($p < 0.05$) different when compared to glibenclamide-treated group 2 after 120 min.

Table 6: Day 21 PBG changes in diabetic rats fed GLE mixed with carbohydrate diets

Group No	Feeding (/kg b.w)	Δ30min (mg/dl)	Δ60min (mg/dl)	Δ90min (mg/dl)	Δ120 min (mg/dl)
1	Diabetic, Untreated ($D.H_2O$)	03.33^{b}	-03.33^{a}	-05.33^{a}	-07.00^{a}
2	Diabetic, $Glibenclamide_{2.5mg}$	06.33^{b}	-30.67^{a}	-35.00^{a}	-39.33^{a}
3	Diabetic, $Cassava_{200mg}$	21.33^{c}	18.00^{c}	25.00^{c}	21.67^{b}
4	Diabetic, Yam_{200mg}	13.67^{cd}	25.00^{d}	17.00^{bc}	15.67^{bc}
5	Diabetic, $Plantain_{200mg}$	17.00^{d}	21.00^{cd}	11.33^{b}	10.33^{b}
6	Diabetic, $Cassava_{200mg}$ with GLE_{100mg}	09.00^{c}	05.67^{b}	-01.00^{a}	-12.67^{a}
7	Diabetic, Yam_{200mg} with GLE_{100mg}	-03.67^{a}	-11.67^{a}	-24.00^{a}	-27.00^{a}
8	Diabetic, $Plantain_{200mg}$ with GLE_{100mg}	-02.00^{a}	-06.33^{a}	-20.00^{a}	-58.67^{e}
9	Diabetic, $GLE_{100\ mg}$	-22.67^{a}	-38.67^{a}	-27.67^{a}	-43.00^{e}

Group values are mean ± SD, n = 5; values with different superscripts are statistically significant at $p < 0.05$

DISCUSSION

Diabetes mellitus is a life threatening disease condition delineated by alterations in carbohydrate, lipid and protein metabolism (Das *et al.*, 1996). As a matter of fact, the management of diabetes remains a global challenge as the search for effective and lasting cure is yet to make a headway. Most of the available anti-diabetic drugs only control blood sugar levels upon regular administration and are accompanied by side effects (Upadhyay *et al.*, 1996; Cheng and Caughey, 2007). Das *et al.* (1996) documented that the diverse and numerous bioactive constituents of plants give medicinal plants an edge over synthetic drugs as better therapeutic agents in the treatment of different ailments including diabetes (Tiwari and Roa, 2002). Hence, the dire need for safer, better and convenient treatment for diabetes mellitus which can be achieved with medicinal plants.

Over the years, the use of Medicinal plants in folklore medicine for the management of diseases has been staggering and overwhelming. *G. latifolium* is a famous medicinal plant that is widely used in West Africa consequent to its therapeutic and nutritional properties. Herein, we elucidated the the effects of the methanol extracts of *G. latifolium* leaves on

glycaemic responses to carbohydrate diets [obtained from cassava (*Maninhot esculenta*), yam (*Dioscorea rotundata*) and plantain (*Musa paradisiaca*)] in streptozotocin-induced diabetic rats.

The pulverized leaf powder of *G. latifolium* extracted with methanol, gave a dry residual yield of 15.7%. This result compares with that of (Okpala *et al.*, 2014), who got 10.24% yield of the crude extract, the aqueous residue (45.80%), n-butanol fraction (25.14%), ethyl acetate fraction (10.70%) and n-hexane fraction (6.66%). Ezekwe *et al.* (2014) also obtained crude ethanol extract yield (8.36%), n-Hexane extract (32.7%), chloroform extract (25.6%), ethyl acetate extract (14.4%) and residual ethanol extract (29.1%).

As displayed in table 1, the body weights of the diabetic rats fed with carbohydrate diets (Group 3, 4 ad 5) showed significant ($p < 0.05$) decrease when compared to the positive control (Group 1) that received water only from day 7 to 21. However, treatment with GLE-carbohydrate diets caused a significant ($p < 0.05$) increase in weights among the animals fed with GLE-cassava diet (group 5), GLE-yam diet (group 6) and GLE-plantain diet (group 7) compared to their corresponding groups fed with carbohydrate diets alone (groups 2, 3 and 4). Meanwhile, treatment with GLE alone caused a significant ($p<0.05$) increase in the weights of group 9 when compared to group 5, 6 ad 7 and this increase is comparable to the one elicited in group 2 (diabetic standard control) by glibenclamide. This increase cut across days 14 and 21. These results reveal that significant ($p < 0.05$) weight losses were recorded among untreated diabetic rats compared to non-diabetic animals. This could be attributed to the losses in muscle and adipose tissues resulting from excessive breakdown of tissue protein and fatty acids. Consumption of *G. latifolium* is not readily associated with increase in weight, which is in consonance with the report of (Iweala and Obidoa, 2009). The physical form of any food is also a determinant of the rate at which the starch is hydrolyzed as conditions that are known to increase the digestibility of starches are those which produce obvious hydration of the granule, distinct changes in the chemical nature or disruption of the organized granule structure increasing the surface area for enzymatic action (Wong and O'Dea,1983).

Furthermore, the result of fasting blood glucose (FBG) (Table 2) revealed that there was a significant ($p <0.05$) elevation of FBG level in groups 3, 4 and 5 fed with carbohydrate diets compared to control groups with GLE-cassava diet eliciting the highest effect. Howbeit, administration of *G. latifolium* extract (GLE) mixed with carbohydrate diets significantly ($p < 0.05$) reduced FBG from day 7 through day 21 in group 6, 7 and 8 with

GLE-plantain diet displaying highest reduction. Also, groups fed with GLE-only showed a significant ($p<0.05$) and highest reduction in FBG and this reduction is comparable to GLE-plantain diet fed group, but significantly ($p<0.05$) different from group 2 that received glibenclamide (diabetic standard control). This result indicates that the mixing of *G. latifolium leaf extract* (GLE) in plantain diet may have superior blood glucose lowering effects compared to glibenclamide, the standard anti-diabetic drug which is in line with the finding of (Ugochukwu *et al*., 2003). This superior effect on FBS, may possibly be consequent to the synergized effect of the bioactive compounds from the plantain and the medicinal plants (Tiwari and Rao, 2002). The group that was treated with the anti-diabetic drug, *glibenclamid*e, showed a reduction in fasting blood glucose level due to its insulin-stimulating actions on the beta cells of the pancreas (Reaven, 1995). The results support the claim by (Aly and Mantaw, 2012) that there are beneficial effects of using combined diets in controlling hyperglycaemia. The results obtained is also in consonance with the report of (Atangwho *et al*., 2010) who found that mixed feeding using hypoglycaemic agents shows promises in effective amelioration of complications associated with diabetes which are linked to oxidative stress, liver dysfunction and lipid peroxidation. The results of reduced FBG by medicinal plants may encourage their use by diabetic patients (Egede *et al*., 2002). The combined dietary feeding of GLE and plantain diet had hypoglycaemic effects in both diabetic and non-diabetic rats.

Moreover, Postprandial blood glucose (PBG) responses in the diabetic animals at day 0, PBG concentrations showed significant ($p < 0.05$) increases in group 3 (cassava diet), 4 (yam diet) and 5 (plantain diet) after 30 min to 120 min of feeding compared to the positive control. However, significant ($p < 0.05$) reduction in PBG responses was observed in group 6 (diabetic, GLE-cassava diet), group 7 (diabetic, GLE-yam diet) and group 8 (diabetic, GLE-plantain diet) after feeding the diet compared to their corresponding carbohydrate fed groups 3, 4 and 5. When compared to the diabetic, glibenclamide-treated group 2, the PBG in groups 6, 7 and 8 were more significantly ($p < 0.05$) lower after 30 ad 60 min, while group 7 (GLE-plantain diet) alone showed a significant ($p < 0.05$) PBG lowering effect comparable to glibenclamide-treated group 2 after 90 ad 120 min. In addition, compared to the diabetic, GLE-treated rats in group 9, glibenclamide had a lower PBG reductions efficacy. On day 7, result revealed a significant ($p <0.05$) elevation in PBG levels in group 3 (fed with cassava diet) and 4 (fed with yam diet) and 5 (fed with plantain diet) when compared to group 1 (untreated control group). However, group 6, 7 and 8 (fed

with GLE-cassava, GLE-yam and GLE-plantain diets respectively) displayed a significantly ($p < 0.05$) decrease in PBG level when compared to group 3,4 ad 5 fed with carbohydrates diets only. Group 9 fed with GLE only showed more hypoglycaemic efficacy causing a significant ($p < 0.05$) reduction in PBG level when compared to group 2 (glibenclamide-treated group). On day 14, a significant ($p <0.05$) elevation in the level of PBG was observed in group 3 (fed with cassava diet) and 4 (fed with yam diet) when compared to group 1 (untreated control group), while group 5 (fed with plantain diet) showed a significantly ($p <0.05$) reduction in PBG level. Conversely, GLE mixed with carohydrate diets caused a significant ($p < 0.05$) decrease in PBG level in group 6 ad 7 compared to their respective carbohydrate fed groups with GLE-plantain diet evoking the highest PBG lowering effect. However, group 9 fed with GLE only showed a comparable hypoglycaemic action with no significant ($p < 0.05$) difference from group 2 (glibenclamide-treated group). Result obtained on the last day (day 21) showed that administration of cassava, yam and plantain in group 3, 4 ad 5 respectively caused a significantly ($p <0.05$) increase in the level of PBG when compared to untreated control group with cassava evoking the highest glycemic response. Nonetheless, treatment with GLE-carohydrate diets caused a significant ($p < 0.05$) reduction in PBG level in group 6, 7 and 8 compared to their respective carbohydrate fed groups 3, 4 and 5 with GLE-plantain diet stimulating the highest hypoglycemic effect after 30 min to 120 min of feeding. Furthermore, the PBG lowering effect of GLE-plantain diet and GLE only were not significantly ($p < 0.05$) different from each other, but were significantly ($p < 0.05$) different when compared to glibenclamide-treated group 2 after 120 min.

Intriguing research revealed that *G. latifolium* leaves contain numerous kind of phenolic compounds, alkaloids, flavonoids, lignan, hydroxycinnamic acids, terpenes, saponin, sterol, allicin, carotenoid, alkaloids, phytols and saponins, some of which are responsible for the reported antihyperglycaemic effect (Al-Hindi *et al.*, 2016). These bioactive constituents have the capacity to stimulate the activities of insulin on the pancreatic beta cells. In addition, the antidiabetic actions of phytochemicals such as polyphenol in *G. latifolium* has been reported (Khunti and Davies, 2013). Polyphenols attenuate hyperglycaemia, lipidaemia and reduce oxidative stress (Testa *et al.*, 2016). From the foregoing, the significant alleviation of blood glucose level by *G. latifolium* observed herein, is in agreement with the reports of (Ugochukwu and Babady, 2003; Okon *et al.*, 2019). We observed that cassava diets induced higher glycaemic (glucose) responses as a sole diet compared to plantain diets that effected

lower responses. This result suggests that plantain diets are vital for human consumption as foods that induce low increments in glucose and insulin levels and are considered to play very important roles in the prevention of diseases related to the metabolic syndrome. Also GLE-plantain diet elicited a comparable hypoglycemic effect to GLE-only. This superior effect is possible due to synergism between diet, bioactive compounds from medicinal plants and other agents (Tiwari and Rao, 2002).

. It has also been recommended that foods that produce low glycaemic responses, be fed to diabetic or obese subjects, because they prevent large fluctuations in plasma glucose concentration after meals, leading to their improved control and management (Frape *et al.*, 1998; Nguyen *et al.*, 1998). It is known that the more processed a food is, the higher the glycaemic response it will produce (Booher *et al.*, 1995; Thorne *et al.*, 2013). During the process of boiling of yam in water, gelatinization of the starch molecule occurs, thus increasing the availability of starch for digestion by digestive enzymes. This is what occurs when boiled yam is eaten directly as well as in case of pounded yam without further processing. Various studies have shown the importance of viscosity (a property of the fiber content of food) on postprandial blood glucose response to food (FAO/WHO, 1998). In 1997, the FAO and WHO endorsed the use of glycemic index (GI) method for classifying carbohydrate-rich foods and recommended that the GI values of foods be used in conjunction with information about food composition to guide food choices. With the increasing incidence of diabetes mellitus worldwide, dietary restriction and modification remain the cornerstones in the prevention and management of the disease (Rakieten and Radkarni, 1963).

CONCLUSION

This study establishes that methanolic extracts of *G. latifolium* leaves exhibited strong hypoglycaemic action on glycemic response in the diabetic rats challenged with carbohydrate diets and proved to be more potent compared to the standard drug. The plant should therefore be considered in the design of a potent antidiabetic drug.

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