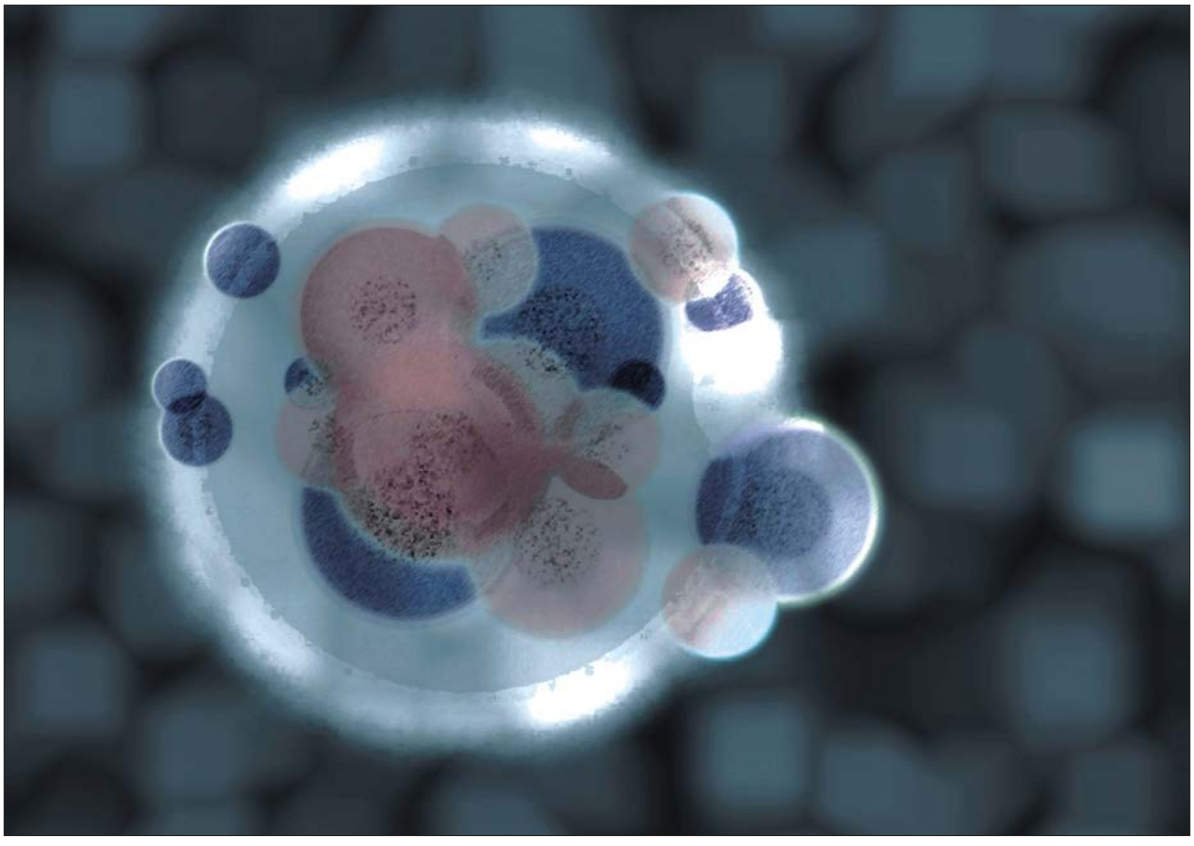

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Research Article
Effects of Methanol Extract of *Gongronema latifolium* Leaves on Glycaemic Responses to Carbohydrate Diets in Streptozotocin-induced Diabetic Rats

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Abstract

Background and Objective: 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 in streptozotocin-induced diabetic rats were investigated. **Materials and Methods:** Forty-five adult male Wistar albino rats divided into nine groups of 5 animals each were induced with diabetes by intraperitoneal injection of streptozotocin (STZ) (65 mg kg^{-1} b.wt.) and administered with carbohydrate diets GLE mixed with the diets for 21 days while estimating fasting and postprandial blood glucose (PBG) levels. **Results:** 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 administration of GLE mixed with carbohydrate diets. **Conclusion:** Herein, we deduce that methanolic extracts of *G. latifolium* leaves elicited hypoglycaemic responses in diabetic rats and hence, could be considered as a potential candidate to be explored in the industrial design of a potent antidiabetic drug.

Key words: Diabetes, *Gongronema latifolium*, carbohydrate, streptozotocin, hypoglycaemic, drug design, postprandial blood glucose

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes remains a major global metabolic health challenge, reaching epidemic proportions[1] and the continuous search for its cure has kept scientists busy over the years. Different kinds of carbohydrate 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[2]. The extent of postprandial hyperglycaemia and insulin secretion depends on the amount of food and carbohydrate consumed per meal.

Carbohydrates are the most abundant biomolecules found in living organisms on earth. They are a major source of metabolic energy, both for plants and for animals that depend on plants for food. Besides their energy-giving ability, carbohydrates in form of cellulose serve as a structural component in plants, sugar moiety of nucleic acids like DNA and RNA and recognition sites on cell surfaces. Nigeria was named the greatest producer and consumer of cassava in the world[3]. Cassava (*Manihot esculenta* Crantz) and yam (*Dioscorea* spp.) are tropical crops consumed by over two billion people and represent the main source of carbohydrate and energy source for approximately 700 million people living in the tropical and sub-tropical areas of Africa[4]. Plantain is cultivated in many tropical countries. It is rich in iron, vitamins, fibre, minerals and serotonin[5]. Traditionally, unripe plantain is usually recommended for managing diabetes and treating anaemia and liver disorders[6,7] while, *G. latifolium* leaves have 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 leaves with little attempts at investigating their potential glycaemic responses to 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[8], while the co-ingestion of large amounts of protein and fats results in a reduction of the difference between glycaemic indices of foods[9].

Gongronema latifolium (Amaranth globe) is a member of the family, Asclepiadaceae and the genus *Gongronema*[10]. It is a tropical rainforest plant which rampant in West Africa and is locally known as "Utasi" (by the Ibibios, Quas and Efiks), "Utazi" (by the Igbos in South East) and "Arokeke" (by the Yorubas in South Western part of Nigeria). It is an edible plant with a green leaf, yellow flower and stem. It has a uniquely sharp, slightly sweet and bitter taste[11,12]. *Gongronema latifolium* possesses anti-plasmodial activity[13] which informs its local use for malaria treatment. It is also used in treating sore gums, colic, dyspepsia and helminth[14]. *Gongronema latifolium* is highly medicinal harbouring numerous bioactive ingredients in its various parts including fruits, leaves, seeds, roots and stems. The technique for harnessing the medicinal value of the plant depends on the plant part where the active ingredient is residing. The vital constituents stored in the leaf can be extracted by blending, chewing or infusion. The fruits and seeds can be chewed. The roots can be prepared by decoction[15]. An infusion or decoction of the leaves, stems and roots is locally used in treating digestive complications such as anorexia, colic and stomach ache, dyspepsia, constipation, dysentery and intestinal worms[16-19]. Other medicinal properties of the plant include hypoglycemic, antihypertensive[20], hepatoprotective[21], hypolipidemic activity[22], nephroprotective activity[23], anticancer activity[24], antimicrobial activity[18,25,26], Immunomodulatory effect[27], antioxidant activity[28], antimalarial[29] and antiinflammatory activity[12].

The volume of information found in the literature on the potential food and drug uses of *G. latifolium* leaves is scanty[30], especially why it is served as post-delivery meals to women and nursing mothers. Also, the need to widen the 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 a further evaluation of the potential glycaemic properties of *G. latifolium* leaf extract, a widely available and highly consumed medicinal plant in Nigeria[20].

Therefore, study work aimed to investigate the effects of the methanol extracts of *Gongronema latifolium* leaves on glycaemic responses to carbohydrate diets [obtained from cassava (*Manihot esculenta*), yam (*Dioscorea rotundata*) and plantain (*Musa paradisiaca*)] in streptozotocin-induced diabetic rats in comparison with the standard drug, glibenclamide.

MATERIALS AND METHODS

Study area: This study was carried out at the Biochemistry Post-Graduate Laboratory of the University of Nigeria, Nsukka Enugu State, between June, 2019 to 2020.

Sample collection and preparation: Fresh mature leaves of *G. latifolium* were purchased from the 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 the Nsukka food market, around the University of Nigeria, Nsukka campus in July, 2020. The fresh leaves were then washed, air-dried at room temperature (25°C) for 7 days and pulverized into a coarse powder. Exactly 1 kg of the leaf powder was infused with 2.5 L of methanol by cold maceration with occasional stirring for 72 hrs and filtered[31]. The filtrate was evaporated to dryness in a rotary evaporator to obtain the methanol extract which was stored in a refrigerator at 4°C 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 a 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 a rotary evaporator and preserved in glass bottles at 4°C for use in each experimental diet preparation.

Preparation of experimental diets: The carbohydrate diets were prepared by accurately measuring quantities of the powdered food extracts equivalent to 200 mg kg^{-1} b.wt., as required for each rat in each group and dissolving the same in distilled water before feeding. The carbohydrate with *G. latifolium* leaf extract diets was prepared by measuring 200 mg kg^{-1} b.wt., of each carbohydrate food and adding 100 mg kg^{-1} b.wt., of the *G. latifolium* leaf extract for each rat in each group and dissolving both in distilled water before feeding.

Induction of diabetes in animals: Forty-five adult male Wistar albino rats were fasted overnight and injected with a single intra-peritoneal (i.p.) dose of streptozotocin (STZ) (Sigma, St. Louis, MO, USA) at 65 mg kg^{-1} b.wt. Just before the induction of diabetes, STZ was dissolved in a freshly prepared 0.1 M cold citrate buffer, pH 4.5[32]. Control animals have injected intraperitoneal citrate buffer alone at a single dose of 1.2 mL kg^{-1} b.wt. Because STZ is capable of inducing fatal hypoglycaemia as a result of massive pancreatic insulin release, STZ-treated rats were provided with a 10% glucose solution after the 6th hrs 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 hrs for hyperglycaemia to develop. After 3 days, the fasting blood glucose levels of the animals were measured with a One Touch Ultra Mini Glucometer. Animals with blood glucose concentrations $\geq$250 mg dL^{-1} were considered hyperglycaemic and selected for the study[33].

Experimental design: The 45 STZ-induced diabetic adult male Wistar albino rats were randomly divided into nine groups of five 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 2.50 mg kg^{-1} b.wt., of glibenclamide
Group 3: Diabetic, fed 200 mg kg^{-1} b.wt., of cassava diet only
Group 4: Diabetic, fed 200 mg kg^{-1} b.wt., of yam diet only
Group 5: Diabetic, fed 200 mg kg^{-1} b.wt., of plantain diet only
Group 6: Diabetic, fed 200 mg kg^{-1} b.wt., of cassava mixed with 100 mg kg^{-1} b.wt., of GLE
Group 7: Diabetic, fed 200 mg kg^{-1} b.wt., of yam mixed with 100 mg kg^{-1} b.wt., of GLE
Group 8: Diabetic, fed 200 mg kg^{-1} b.wt., of plantain mixed with 100 mg kg^{-1} b.wt., of GLE
Group 9: Diabetic, fed 100 mg kg^{-1} b.wt., 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 a standard one-touch Accu-Check Ultra Mini-glucometer (Roche Diabetes Care Inc., USA) and test strips. The rats in each group fasted overnight and tests were carried out before feeding the animals in the morning. The glucometer was powered on, the test strip was inserted and put at zero. A 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 a 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, 30, 60, 90 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 for up to 21 days.

RESULTS

Effects of GLE mixed with carbohydrate diets on body weight changes in streptozotocin (STZ)-induced diabetic rats: Table 1 displayed the body weights of the diabetic rats fed with carbohydrate diets (group 3, 4 and 5) showed a significant ($p<0.05$) decrease when compared to the positive control (group 1) that received water-only from day 7-21. However, treatment with GLE-carbohydrate diets caused a significant ($p<0.05$) increase in weights among the animals fed with the 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 groups 5, 6 and 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.

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 21 in groups 6, 7 and 8 with the GLE-plantain diet displaying the 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 the GLE-plantain diet-fed group, but significantly ($p<0.05$) different from group 2 that received glibenclamide (diabetic standard control).

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

Group No.	Feeding (kg^{-1} b.wt.)	Day 7 (mg)	Day 14 (mg)	Day 21 (mg)
1	Diabetic, untreated ($D.H_2O$)	-13.10±0.500^{e}	-22.23±2.523^{f}	-27.90±2.120^{f}
2	Diabetic, glibenclamide 2.5 mg	11.93±1.102^{ab}	12.80±2.120^{b}	16.06±0.290^{ab}
3	Diabetic, cassava 200 mg	-26.53±4.502^{c}	-31.66±5.166^{c}	-42.30±3.291^{d}
4	Diabetic, yam 200 mg	-26.60±1.997^{c}	-29.40±3.001^{c}	-38.70±4.091^{d}
5	Diabetic, plantain 200 mg	-18.86±0.949^{c}	-27.23±3.204^{c}	-35.56±2.740^{c}
6	Diabetic, cassava 200 mg with GLE 100 mg	03.06±0.088^{a}	01.50±0.030^{a}	07.70±0.500^{a}
7	Diabetic, yam 200 mg with GLE 100 mg	05.76±0.022^{a}	01.60±0.009^{a}	06.63±1.011^{a}
8	Diabetic, plantain 200 mg with GLE 100 mg	08.33±1.928^{a}	07.33±1.583^{a}	09.330±2.001^{a}
9	Diabetic, GLE extract 100 mg	14.00±3.000^{b}	16.20±2.177^{b}	23.86±3.202^{ab}

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

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

Group No.	Feeding (kg^{-1} b.wt.)	Day 7 (mg dL^{-1})	Day 14 (mg dL^{-1})	Day 21 (mg dL^{-1})
1	Diabetic, untreated ($D.H_2O$)	04.00±0.712^{b}	05.33±0.693^{b}	05.00±1.001^{b}
2	Diabetic, glibenclamide 2.5 mg	-17.33±3.321^{a}	-82.33±6.235^{a}	-51.66±6.193^{ab}
3	Diabetic, cassava 200 mg	34.00±2.742^{c}	39.33±4.120^{c}	48.66±3.190^{c}
4	Diabetic, yam 200 mg	32.66±0.993^{c}	36.00±3.013^{c}	41.33±2.847^{c}
5	Diabetic, plantain 200 mg	14.00±2.003^{bc}	15.66±1.864^{bc}	13.66±1.118^{bc}
6	Diabetic, cassava 200 mg with GLE 100 mg	-06.66±0.538^{a}	-07.33±1.059^{ab}	-10.66±2.973^{ab}
7	Diabetic, yam 200 mg with GLE 100 mg	-09.00±2.225^{a}	-28.33±3.111^{ab}	-14.00±1.008^{ab}
8	Diabetic, plantain 200 mg with GLE 100 mg	-06.66±0.619^{a}	-102.33±8.210^{a}	-157.66±11.149^{a}
9	Diabetic, GL extract 100 mg	-13.33±1.263^{a}	-143.00±9.209^{a}	-170.33±8.239^{a}

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

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

Group No.	Feeding (kg^{-1} b.wt.)	30 min ($mg\ dL^{-1}$)	60 min ($mg\ dL^{-1}$)	90 min ($mg\ dL^{-1}$)	120 min ($mg\ dL^{-1}$)
1	Diabetic, untreated ($D.H_2O$)	09.00 ± 1.204^{bc}	18.00 ± 2.004^{cd}	08.33 ± 0.759^{bc}	-02.33 ± 0.008^{a}
2	Diabetic, glibenclamide 2.5 mg	23.33 ± 2.530^{d}	19.00 ± 1.211^{cd}	13.67 ± 1.104^{c}	3.67 ± 0.041^{b}
3	Diabetic, cassava 200 mg	16.67 ± 0.988^{c}	29.33 ± 2.303^{e}	31.00 ± 2.502^{e}	24.67 ± 2.093^{d}
4	Diabetic, yam 200 mg	15.33 ± 2.006^{c}	25.00 ± 2.229^{d}	34.67 ± 2.604^{e}	41.67 ± 3.941^{e}
5	Diabetic, plantain 200 mg	09.00 ± 1.101^{bc}	14.33 ± 2.231^{c}	23.67 ± 2.029^{d}	17.33 ± 1.207^{cd}
6	Diabetic, cassava 200 mg with GLE 100 mg	05.67 ± 0.602^{b}	10.00 ± 0.707^{b}	11.33 ± 1.422^{c}	8.33 ± 0.957^{c}
7	Diabetic, yam 200 mg with GLE 100 mg	11.00 ± 0.094^{bc}	16.67 ± 1.112^{c}	13.33 ± 1.814^{c}	07.33 ± 0.693^{bc}
8	Diabetic, plantain 200 mg with GLE 100 mg	00.67 ± 0.003^{a}	02.33 ± 0.011^{a}	02.67 ± 0.029^{b}	-02.00 ± 0.158^{a}
9	Diabetic, GLE 100 mg	06.67 ± 1.53^{b}	00.67 ± 0.007^{a}	-07.33 ± 1.382^{a}	-07.00 ± 1.022^{a}

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

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

Group No.	Feeding (kg^{-1} b.wt.)	30 min ($mg\ dL^{-1}$)	60 min ($mg\ dL^{-1}$)	90 min ($mg\ dL^{-1}$)	120 min ($mg\ dL^{-1}$)
1	Diabetic, untreated ($D.H_2O$)	02.67 ± 0.706^{b}	01.33 ± 0.097^{b}	-09.67 ± 1.248^{a}	-37.67 ± 4.928^{a}
2	Diabetic, glibenclamide 2.5 mg	-03.67 ± 0.966^{a}	-29.00 ± 2.775^{f}	-23.00 ± 3.025^{f}	-34.33 ± 2.747^{f}
3	Diabetic, cassava 200 mg	13.33 ± 1.953^{d}	22.67 ± 2.852^{cde}	24.33 ± 1.947^{d}	22.33 ± 2.318^{d}
4	Diabetic, yam 200 mg	22.67 ± 2.007^{e}	37.67 ± 3.116^{e}	40.673 ± 3.063^{e}	33.33 ± 2.847^{e}
5	Diabetic, plantain 200 mg	12.33 ± 1.795^{cd}	16.33 ± 1.580^{cd}	12.33 ± 1.437^{c}	15.67 ± 2.071^{cd}
6	Diabetic, cassava 200 mg with GLE 100 mg	3.00 ± 0.950^{b}	6.67 ± 1.023^{bc}	4.33 ± 1.003^{b}	2.67 ± 0.7356^{bc}
7	Diabetic, yam 200 mg with GLE 100 mg	11.67 ± 1.863^{cd}	16.67 ± 1.936^{cd}	09.67 ± 1.689^{bc}	06.33 ± 0.981^{c}
8	Diabetic, plantain 200 mg with GLE 100 mg	13.67 ± 2.201^{d}	12.67 ± 1.547^{cd}	02.00 ± 0.618^{b}	00.33 ± 0.093^{b}
9	Diabetic, GLE 100 mg	10.33 ± 1.205^{c}	08.67 ± 0.860^{c}	01.33 ± 0.124^{a}	-4.67 ± 0.967^{a}

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

Effects of GLE mixed with carbohydrate diets on day 0 postprandial blood glucose (PBG) changes in diabetic rats: Table 3 presented that, there was a significant ($p<0.05$) increase in PBG level in groups 3, 4 and 5 fed with carbohydrate diets compared to the positive control group after 30, 60, 90 ad 120 min. However, a significant ($p<0.05$) reduction was observed in groups 6 (diabetic, GLE-cassava diet), 7 (diabetic, GLE-yam diet) and 8 (diabetic, GLE-plantain diet) after 30-120 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 and 120 min. In addition, compared to the diabetic, GLE-treated rats in group 9, glibenclamide had a lower PBG reduction efficacy.

Effects of GLE mixed with carbohydrate diets on day 7 PBG changes in 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 groups 3 (fed with cassava diet) and 4 (fed with yam diet) and group 5 (fed with plantain diet) when compared to group 1 (positive control). However, groups 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 groups 3, 4 and 5 fed with carbohydrate 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).

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 carbohydrate diets caused a significant ($p<0.05$) decrease in PBG level in groups 6 and 7 compared to their respective carbohydrate-fed groups with the 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).

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 plantain in groups 3, 4 and 5, respectively caused a significantly ($p<0.05$) increase in the level of PBG when compared to an untreated control group with cassava evoking the highest glycemic response. Nonetheless, treatment with GLE-carbohydrate diets caused a significant ($p<0.05$) reduction in PBG level in groups 6, 7 and 8 compared to their respective carbohydrate-fed groups 3, 4 and 5 with GLE-plantain diets stimulating the highest

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

Group No.	Feeding (kg^{-1} b.wt.)	30 min ($mg\ dL^{-1}$)	60 min ($mg\ dL^{-1}$)	90 min ($mg\ dL^{-1}$)	120 min ($mg\ dL^{-1}$)
1	Diabetic, untreated ($D.H_2O$)	21.00 ± 2.104^{c}	09.93 ± 1.118^{b}	07.33 ± 1.201^{b}	03.33 ± 0.058^{b}
2	Diabetic, glibenclamide 2.5 mg	10.00 ± 1.837^{b}	11.67 ± 2.209^{bc}	-05.33 ± 0.620^{a}	-17.00 ± 2.003^{a}
3	Diabetic, cassava 200 mg	10.00 ± 1.219^{b}	23.67 ± 3.001^{cd}	24.33 ± 1.293^{d}	22.00 ± 1.940^{c}
4	Diabetic, yam 200 mg	21.00 ± 2.104^{c}	28.33 ± 1.749^{e}	38.33 ± 2.827^{e}	34.67 ± 2.746^{d}
5	Diabetic, plantain 200 mg	13.67 ± 1.111^{bc}	30.00 ± 2.774^{d}	-08.33 ± 1.007^{a}	-10.67 ± 1.213^{a}
6	Diabetic, cassava 200 mg with GLE 100 mg	02.00 ± 0.054^{a}	14.67 ± 1.125^{c}	10.33 ± 0.981^{c}	01.00 ± 0.010^{b}
7	Diabetic, yam 200 mg with GLE 100 mg	02.33 ± 0.009^{a}	10.33 ± 2.013^{b}	06.33 ± 1.004^{d}	03.00 ± 0.085^{b}
8	Diabetic, plantain 200 mg with GLE 100 mg	04.33 ± 0.281^{a}	03.00 ± 0.013^{a}	-02.33 ± 0.009^{a}	08.33 ± 1.035^{a}
9	Diabetic, GLE 100 mg	02.33 ± 0.007^{a}	11.00 ± 1.107^{bc}	-02.00 ± 0.006^{a}	-07.33 ± 1.100^{a}

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

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

Group No.	Feeding (kg^{-1} b.wt.)	30 min ($mg\ dL^{-1}$)	60 min ($mg\ dL^{-1}$)	90 min ($mg\ dL^{-1}$)	120 min ($mg\ dL^{-1}$)
1	Diabetic, untreated ($D.H_2O$)	03.33 ± 0.978^{b}	-03.33 ± 0.809^{a}	-05.33 ± 0.638^{a}	-07.00 ± 1.003^{a}
2	Diabetic, glibenclamide 2.5 mg	06.33 ± 1.120^{b}	-30.67 ± 9.301^{a}	-35.00 ± 4.285^{a}	-39.33 ± 3.246^{a}
3	Diabetic, cassava 200 mg	21.33 ± 2.028^{c}	18.00 ± 2.151^{c}	25.00 ± 3.102^{c}	21.67 ± 4.339^{b}
4	Diabetic, yam 200 mg	13.67 ± 3.210^{cd}	25.00 ± 1.917^{d}	17.00 ± 2.164^{bc}	15.67 ± 1.947^{bc}
5	Diabetic, plantain 200 mg	17.00 ± 2.353^{d}	21.00 ± 2.453^{cd}	11.33 ± 1.947^{b}	10.33 ± 1.748^{b}
6	Diabetic, cassava 200 mg with GLE 100 mg	09.00 ± 0.937^{c}	05.67 ± 0.458^{b}	-01.00 ± 0.009^{a}	-12.67 ± 1.905^{a}
7	Diabetic, yam 200 mg with GLE 100 mg	-03.67 ± 0.101^{a}	-11.67 ± 1.850^{a}	-24.00 ± 3.310^{a}	-27.00 ± 3.108^{a}
8	Diabetic, plantain 200 mg with GLE 100 mg	-02.00 ± 0.009^{a}	-06.33 ± 0.794^{a}	-20.00 ± 3.002^{a}	-58.67 ± 4.028^{e}
9	Diabetic, GLE 100 mg	-22.67 ± 1.725^{a}	-38.67 ± 2.647^{a}	-27.67 ± 1.593^{a}	-43.00 ± 4.823^{e}

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

hypoglycemic effect after 30-120 min of feeding. Furthermore, the PBG lowering effect of the 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.

DISCUSSION

The results from this study demonstrate that methanolic extracts of *G. latifolium* leave elicited hypoglycaemic responses in diabetic rats. Diabetes mellitus is a life-threatening disease condition delineated by alterations in carbohydrate, lipid and protein metabolism[34]. The management of diabetes remains a global challenge as the search for an effective and lasting cure is yet to make headway. Most of the available anti-diabetic drugs only control blood sugar levels upon regular administration and are accompanied by side effects[35,36]. Jekayinfa *et al.*[34] 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[37]. Hence, a dire need for safer, better and more convenient treatment for diabetes mellitus 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. *Gongronema latifolium* is a famous medicinal plant that is widely used in West Africa consequent to its therapeutic and nutritional properties. Herein, we elucidated the effects of the methanol extracts of *G. latifolium* leaves on glycaemic responses to carbohydrate diets [obtained from cassava (*Manihot 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 previous studies by Cheng and Caughey[38], which got a 10.24% yield of the crude extract, the aqueous residue (45.80%) and the n-butanol fraction (25.14%), ethyl acetate fraction (10.70%) and n-hexane fraction (6.66%)[39]. 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%).

Table 1 displayed the body weights of the diabetic rats fed with carbohydrate diets (group 3, 4 and 5) showed a significant ($p<0.05$) decrease when compared to the positive control (group 1) that received water only from day 7-21. However, treatment with GLE-carbohydrate diets caused a significant ($p<0.05$) increase in weights among the animals fed with the 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 groups 5, 6 and 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 the excessive breakdown of tissue protein and fatty acids. Consumption of *G. latifolium* is not readily associated with an increase in weight, which agrees with the report of Okpala *et al.*[40]. 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[41].

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 groups 6, 7 and 8 with the GLE-plantain diet displaying the 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 the 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 a 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 the authors[20]. This superior effect on FBS may be consequent to the synergized effect of the bio-active compounds from the plantain and the medicinal plants[42]. The group that was treated with the anti-diabetic drug, glibenclamide, showed a reduction in fasting blood glucose level due to its insulin-stimulating actions on the beta cells of the pancreas[43]. The results support the claim by Tiwari and Rao[44], that there are beneficial effects of using combined diets in controlling hyperglycaemia. The results obtained are also in consonance with the report of Narasinga[45], who found that mixed feeding using hypoglycaemic agents shows promises ineffective 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[46]. 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 groups 3 (cassava diet), 4 (yam diet) and 5 (plantain diet) after 30-120 min of feeding compared to the positive control. However, a 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 and 120 min. In addition, compared to the diabetic, GLE-treated rats in group 9, glibenclamide had a lower PBG reduction efficacy. On day 7, the 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, groups 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 groups 3, 4 and 5 fed with carbohydrate 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 carbohydrate diets caused a significant ($p<0.05$) decrease in PBG level in groups 6 and 7 compared to their respective carbohydrate fed groups with the 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). Results obtained on the last day (day 21) showed that administration of cassava, yam and plantain in groups 3, 4 and 5, respectively caused a significantly ($p<0.05$) increase in the level of PBG when compared to an untreated control group with cassava evoking the highest glycemic response. Nonetheless, treatment with GLE-carbohydrate diets caused a significant ($p<0.05$) reduction in PBG level in groups 6, 7 and 8 compared to their respective carbohydrate-fed groups 3, 4 and 5 with GLE-plantain diets stimulating the highest hypoglycemic effect after 30-120 min of feeding. Furthermore, the PBG lowering effect of the 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 kinds 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[47]. These bioactive constituents can stimulate the activities of insulin on the pancreatic beta cells. In addition, the antidiabetic actions of phytochemicals such as polyphenol in *G. latifolium* have been reported[48]. Polyphenols attenuate hyperglycaemia and lipidaemia and reduce oxidative stress[49]. The phytochemical result of the methanolic fraction of *G. latifolium* showed that the plant is rich in terpenoids, flavonoids, glycosides, saponins and carbohydrates. It has been documented that saponins and flavonoids are good antidiabetic metabolites[50]. This is also in tandem with the work of Testa *et al.*[51], who demonstrated that ethanolic extracts from *G. latifolium* leaves possess hypoglycemic activity. It was suggested that the plant carries out its hypoglycemic action by activating hexokinase, phosphofructokinase, glucose-6-phosphate dehydrogenase and inhibiting glucokinase activity in the diabetic rats' livers[51]. Sherma *et al.*[52], also revealed that ethanolic extracts of *G. latifolium* seemed to exhibit substantial oxidative stress reducing, antilipid peroxidation potential and increased reduced glutathione/oxidized glutathione (GSH/GSSG) ratio, ascertaining its ethnopharmacological use in ameliorating the oxidative stress linked diabetes. Another report suggested that islet beta cell regeneration may be the underlying mechanism of the recovery of diabeticrats[53].

From the foregoing, the significant alleviation of blood glucose levels by *G. latifolium* observed herein is in agreement with the reports of scientists[51,54]. We observed that cassava diets induced higher glycaemic (glucose) responses as a sole diet compared to plantain diets that elicited lower responses. This result suggests that plantain diets are vital for human consumption as foods that induce low increments in glucose and insulin levels are considered to play very important roles in the prevention of diseases related to metabolic syndrome. Also, the 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[43].

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[2,55]. It is known that the more processed a food is, the higher the glycaemic response it will produce[56,57]. During the process of boiling 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 the case of pounded yam without further processing. Various studies have shown the importance of viscosity (a property of the fibre content of food) on postprandial blood glucose response to food[58]. In 1997, the FAO and WHO endorsed the use of the 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[32].

CONCLUSION

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

SIGNIFICANCE STATEMENT

This study discovers a robust hypoglycaemic action on glycaemic response in diabetic rats by methanolic extracts of *G. latifolium* leaves. This study will help researchers in the industrial design of a potent antidiabetic drug that will augment the already existing ones. Thus a new theory on glycaemic response may be arrived at.

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