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Ameliorating Potentials of 3 Medicinal Plants on Relative Pancreatic Weights in Streptozotocin Induced Diabetic Rats

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Abstract

The ameliorating potentials of cocoyam (*Colocasia esculenta* L.), ginger (*Z.officinale* Roscoe) and unripe plantain (*Musa paradisiacae* L.) incorporated feeds on the relative pancreatic weights of streptozotocin induced diabetic rats was investigated. The blood glucose level of all the rats was measured with a glucometer, the assay of the phytochemical and crude fibre compositions of the test feeds were carried out using standard techniques. The administration of the test feeds for 21 days to the diabetic rats of groups 5 to 7, resulted in 58.75%, 29.81% and 38.13% decreases in their hyperglycemia. Administration of the cocoyam incorporated feeds to the diabetic rats of group 5, resulted in 2.71% and 19.52% increases in their body weights and growth rates respectively, administration of the ginger incorporated feeds to the diabetic rats of group 6, resulted in 9.88% and 60.24% increases in their body weights and growth rates, unlike the diabetic rats of group 7, administered unripe plantain feed that had 5.12% decrease in weight with a corresponding 29.52% decrease in growth rate but higher than the diabetic control rats that recorded 28.69, 28.62% and 29.46% decreases in body weights with corresponding 248.9%, 239.9% and 250.14% decreases in growth rates. Chemical analysis of the test feeds indicate that they contained considerable amounts of phytochemicals and crude fibre. Results indicate the ability of cocoyam, ginger and unripe plantain to ameliorate the relative pancreatic weight of diabetics.

Keywords: Diabetes; Streptozotocin; Rats; Incorporated feeds

Introduction

Medicinal plants are being used traditionally in many parts of the world in the treatment of diabetes mellitus where access to formal healthcare is limited [1] and these medicinal plants could play important roles in the lives of rural people, particularly those in remote parts of developing countries.

The ginger (*Zingiber officinale* Roscoe) rhizome is commonly found in most Nigerian homes where a small percentage of its flour (not more than 10%) is used mainly as a spice for flavoring a variety of dishes and drinks. Ginger also has a lot of medicinal relevance such as: suppression of prostaglandin synthesis through inhibition of cyclooxygenase- 1 and cyclooxygenase- 2 [2].

Cocoyam *(Colocasia esculenta L.)* is a herbaceous perennial plant belonging to the "Araceae" family. In most African countries, cocoyam is mainly cultivated by small-scale farmers [3]. Like many plants of the Araceae family, cocoyam grows from the fleshy corm (tuber) that can be boiled, baked or mashed into a meal and used as a staple food or snack. The corms supply easily digestible starch and are known to contain substantial amounts of protein, vitamin C, thiamine, riboflavin, niacin and dietary fiber [4]. Cocoyam has also been reported in folklore medicine in the management of diabetes mellitus although there is paucity of such information in literature.

Plantain (*M. paradisiaca*) belongs to the "Musacae" family and its cultivated in many tropical and subtropical countries of the world. Scientifically, unripe plantain has been documented as a hypoglycemic plant [5] although its mechanism of hypoglycemic action remains unclear.

In our previous studies [5,6], we reported the ameliorating potentials of ginger, cocoyam and unripe plantain flours on relative tissue weights and renal growths in streptozotocin induced diabetic rats but we did not report their ameliorating actions on relative pancreatic weight.

Since intraperitoneal injections of streptozotocin (STZ), has been reported to cause degeneration of the pancreatic β-cells and pancreatic tumors in experimental animals [7], we decided to investigate the effect of cocoyam, ginger and unripe plantain incorporated feeds on the relative pancreatic weight of diabetic rats induced with graded concentrations of streptozotocin (55, 65 and 70mg/kg body weight).

Materials and Methods

Plant materials

The cocoyam variety (*Colocasia esculenta* L.) known by its local name in Nigeria as *Edeofe* and the ginger (*Zingiber officinale* Roscoe) variety (UGI) were freshly obtained at harvest from the National Root Crops Research Institute, Umudike, Nigeria while the false horn unripe plantain variety (*M. paradisiacae*) was bought from Umuahia Main Market, Abia State, Nigeria. They were authenticated in the Department of Botany, Michael Okpara University of Agriculture, Umudike, Nigeria.

Processing of the plant materials

The samples were properly washed, peeled and oven dried at 50°C for 48 hours until constant weight was obtained before being pelletized and incorporated into the rat feeds.

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Chemical analysis

The AOAC methods [8] was used in the analysis of the crude fibre, flavonoid, saponin and tannin composition of the flours of the incorporated feeds while the gravimetric method of Harbone [9] was used in the determination of the total alkaloid contents of the flours of the test feeds.

Selection of animals

Forty male albino rats of the Wistar strain (146.76-228.74 g) obtained from the animal house of the Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria, were used for the study. The rats were kept in metabolic cages in the animal house of the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Nigeria. The rats were acclimatized for two weeks to their diets and water prior to the commencement of the experiment and were maintained under a constant 12-h light and dark cycle and at room temperature. The experimental procedures were approved by the Ethical committee of Michael Okpara University of Agriculture, Umudike, Nigeria. The National Institutes of Health Principles of Laboratory Animal Care [10] were observed.

Induction of diabetes

Freshly prepared solution of streptozotocin (0.1g dissolved in 5 ml of freshly prepared sodium citrate buffer 0.1M, pH 4.5) was injected intraperitoneally to the rats at a dosage of 65mg/kg body weight at fasting state [11]. Blood was collected from the tail vein and the blood glucose concentration was analyzed prior to the commencement of the dietary feeding using a blood glucose meter (Double G Glucometer, USA) and subsequently, twice in a week, throughout the duration of the experiment. The STZ-treated rats with fasting blood glucose levels > 200mg/dl after seven (7) days of induction of STZ, were considered to be diabetic.

Experimental procedure

The experimental rats with stable diabetic condition were then divided into 6 sub-groups (groups 2 to 7) with six rats per group while the non-diabetic group formed the first group as follows:

Group 1: Normal rats administered standard rat pellets (Non-diabetic control)

Group 2: Diabetic control rats administered 55mg/kg body weight STZ

Group 3: Diabetic control rats administered 65mg/kg body weight of STZ

Group 4: Diabetic control rats administered 70mg/kg body weight STZ

Group 5: Diabetic rats administered cocoyam incorporated feed

Group 6: Diabetic rats administered ginger incorporated feed

Group 7: Diabetic rats administered unripe plantain incorporated feed

Their diets and water were both administered *ad libitum* for 21days, after which the rats were anesthetized with chloroform and their pancreas collected and weighed. The body weights of the rats were recorded on a daily basis, using an electronic weighing balance (Model Scout Pro, Ohaus Corporation, USA) and were calculated as:

$$\text{Percentage change in weight} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

While the percentage growth rate of the animals was calculated as:

$$\text{Percentage growth rate} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Experimental duration}} \times 100$$

Similarly, the percentage change in fasting blood sugar of the animals was calculated as:

$$\text{Percentage change in fasting blood glucose (FBG)} = \frac{\text{Initial FBG} - \text{Final FBG}}{\text{Initial FBG}} \times 100$$

Finally, the relative pancreatic weight of the animals was calculated as:

$$\text{Relative pancreatic weight } (g/100g) = \frac{\text{Total weight of pancreas}}{\text{Final body weight}} \times 100$$

Statistical analysis

Data was subjected to analysis using the Statistical Package for Social Sciences (SPSS), version 15.0. Results were presented as the means ± standard deviations of triplicate experiments. One way analysis of variance (ANOVA) was used for comparison of the means. Differences between means were considered to be significant at $P < 0.05$ using the Duncan Multiple Range Test.

Results

The administration of STZ at a dosage of 55, 65 and 70 mg/kg body weight to the rats of groups 2 to 4, produced stable diabetic condition within 7 days in most of the experimental rats. Administration of the cocoyam incorporated feed to the diabetic rats of group 5, resulted in 58.75% decrease in the resulting hyperglycemia, administration of the ginger incorporated feed to the diabetic rats of group 6 resulted in 29.81% decrease in the resulting hyperglycemia while the administration of the unripe plantain incorporated feed to the diabetic rats of group 7, resulted in 38.13% decrease in their hyperglycemia compared with the diabetic controls and non-diabetic rats (Table 1).

The diabetic control rats of groups 2 to 4, had 28.69%, 28.62% and 29.46% decreases in weight at the end of the experimentation. The diabetic rats administered cocoyam and ginger incorporated feeds recorded 2.71% and 9.88% gain in weights while the diabetic rats administered unripe plantain incorporated feeds recorded 5.12% reduction in weight compared with the non-diabetic rats that recorded 6.21% gain in weight at the end of the experiment (Table 2).

The diabetic control rats of groups 2 and 4, and the diabetic rats administered unripe plantain incorporated feeds, recorded 248.9%, 239.9%, 250.14% and 29.52% decreases in growth rates respectively while the diabetic rats administered cocoyam and ginger incorporated feeds, recorded 19.52% and 60.24% increases in growth rates compared with the non-diabetic rats that recorded 60.14% increase in growth rates (Table 2).

Groups	Initial	Final	PC (%)
Group 1	87.00 ± 7.55	93.67 ± 8.50	-7.67 (increase)
Group 2	232.67 ± 12.36	280.00 ± 5.29	-20.34 (increase)
Group 3	352.67 ± 150.88	308.00 ± 54.88	-12.67 (decrease)
Group 4	242.00 ± 20.88	269.50 ± 13.87	-11.36 (increase)
Group 5	373.00 ± 126.06	153.88 ± 30.09	58.75 (decrease)
Group 6	312.00 ± 48.08	219.50 ± 111.01	29.81 (decrease)
Group 7	210.00 ± 9.80	129.92 ± 52.80	38.13 (decrease)

Values are reported as means ± standard deviations. PC-Percentage change in fasting blood glucose; n=6 animals per group

Table 1: Fasting blood glucose of diabetic and non-diabetic rats (mg/dl).

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The pancreatic weight of all the experimental and normal control rats were not significantly different (P>0.05) from each other at the end of the experimentation (Table 3).

However, the relative pancreatic weights of the diabetic control rats of groups 2 to 4 were significantly different (P<0.05) from that of the non diabetic rats at the end of the experimentation. In addition, the relative pancreatic weights of the diabetic rats administered the test diets were not significantly different from that of the non diabetic rats at the end of the experimentation (Table 3).

The composition of the cocoyam incorporated feed comprised of 77% cocoyam flour, 9% soy bean flour, 4% vitamin mixture, 2% salt, 4% banana flavour and 4% groundnut oil; the composition of the ginger incorporated feed comprised of 10% ginger flour, 35.22% soybean flour, 15.65% vitamin mixture, 7.8% salt, 15.67% banana flavour and 15.67% groundnut oil while the composition of the unripe plantain incorporated feed comprised of 77% unripe plantain flour, 9% soybean flour, 4% vitamin mixture, 2% salt, 4% banana flavour and 4% groundnut oil.

The chemical composition of the cocoyam incorporated feed indicated that it contained on the average, 1.51% crude fibre, 2.65% flavonoid, 1.01% alkaloid, 0.70% saponin and 1.06% tannin; that of the unripe plantain incorporated feed contained on the average, 1.45% crude fibre, 2.09% flavonoid, 1.84% alkaloid, 0.57% saponin and 0.89% tannin while the ginger incorporated feed contained on the average, 1.52% crude fibre, 3.61% flavonoid, 2.15% alkaloid, 0.79% saponin and 1.23% tannin (Table 4).

Discussion

Streptozotocin selectively destroys the insulin secreting β-cells, leaving less active cells which results in a diabetic state [12] and this explains the diabetic conditions of the animals administered varying doses of STZ. However, the 58.75%, 29.81% and 38.13% decreases in hyperglycemia in the diabetic rats administered cocoyam, ginger and unripe plantain respectively, confirm the ability of cocoyam, ginger and unripe plantain to ameliorate hyperglycemia.

The reduction in the body weight of the diabetic animals could be explained on the basis of loss of structural proteins as these structural proteins contribute to body weight [5].

However, the increase in the body weight of the diabetic rats treated with cocoyam, ginger and unripe plantain incorporated feeds is suggestive of better glycemic control by the test plants.

STZ administration causes β-cell destruction within the pancreas, leading to type 1 diabetes mellitus, and consequently insulin production deficiency. Insufficiency in energy production from glucose metabolism affects growth and development [13]. This explains the reduction in the growth rates as well as the significant increase in the relative pancreatic weight of the diabetic control rats. The lack of significant difference in the relative pancreatic weights of the diabetic rats treated with cocoyam, ginger and unripe plantain incorporated feeds, compared with the non-diabetic rats, is suggestive of the regeneration of the β-cells since the pancreas has been reported to contain stable (quiescent) cells that have the capacity of regeneration to replace the lost cells [14].

Analysis of the phytochemical and crude fibre constituents of the incorporated feeds indicated that they contained considerable amounts of alkaloids, flavonoids, saponins, tannins and crude fibre.

Flavonoids, alkaloids, tannins and flavonoids, as polyphenolic compounds, have been associated with hypoglycemic activity [15,5].

Groups	Initial	Final	PC(%)	PG (%)
Group 1	203.47 ± 19.15	216.10 ± 21.86*	-6.21(increase)	60.14 (increase)
Group 2	182.20 ± 5.57^{bc}	129.93 ± 5.38^{ns}	28.69 (decrease)	-248.90 (decrease)
Group 3	175.97 ± 5.35^{bc}	125.60 ± 20.69^{ns}	28.62(decrease)	-239.9 (decrease)
Group 4	178.33 ± 18.60^{bc}	125.80 ± 4.12^{ns}	29.46 (decrease)	-250.14 (decrease)
Group 5	151.45 ± 16.33^{ab}	155.55 ± 14.78^{ns}	-2.71 (increase)	19.52 (increase)
Group 6	128.10 ± 7.35^{a}	140.75 ± 6.58^{ns}	-9.88(increase)	60.24(increase)
Group 7	121.10 ± 2.97^{a}	114.90 ± 2.69^{ns}	5.12 (decrease)	-29.52 (decrease)

Values are reported mean ± SD. PC-Percentage change in weight; PG-Percentage Growth Rate; *P<0.05 within the groups (column). ^{a-b}P<0.05 versus normal control. ^{a}P<0.05 versus diabetic control

^{bc}P>0.05 versus normal control within the groups (column). NS: Not Significantly different from each other within the groups. *P<0.05 within the group

Table 2: Body weights of non-diabetic and diabetic rats (g).

Groups	Pancreatic weight (g)	Relative Pancreatic weight (g/100 g)
Group 1	0.35 ± 0.07^{ns}	0.16 ± 0.01
Group 2	0.35 ± 0.07^{ns}	0.27 ± 0.07^{b}
Group 3	0.34 ± 0.02^{ns}	0.28 ± 0.05^{b}
Group 4	0.34 ± 0.06^{ns}	0.27 ± 0.03^{b}
Group 5	0.31 ± 0.01^{ns}	0.20 ± 0.02^{ab}
Group 6	0.33 ± 0.02^{ns}	0.23 ± 0.00^{ab}
Group 7	0.28 ± 0.02^{ns}	0.24 ± 0.01^{ab}

^{b}P<0.05 vs normal control. ^{ab}P>0.05 versus diabetic control within the groups. Values in the table are the means ± SD of triplicate experiments. NS: Not-Significantly different from each other within the groups (column)

Table 3: Pancreatic and relative pancreatic weights of diabetic and non-diabetic rats.

	Flavonoid	Alkaloid	Saponin	Tannin	Crude fibre
Cocoyam	2.65 ± 0.35^{a}	1.01 ± 0.03^{a}	0.70 ± 0.00^{b}	1.06 ± 0.00^{b}	1.51 ± 0.22^{b}
Plantain	2.09 ± 0.13^{a}	1.84 ± 0.06^{ab}	0.57 ± 0.05^{a}	0.89 ± 0.07^{a}	1.45 ± 0.10^{a}
Ginger	3.61 ± 0.01^{b}	2.15 ± 0.21^{b}	0.79 ± 0.01^{b}	1.23 ± 0.04^{c}	1.52 ± 0.20^{b}

Means with different superscripts along each column are significantly different (P < 0.05)

Table 4: Phytochemical composition of compounded feeds (%).

Dietary fibre on the other hand, decreases the digestion and conversion of starch to simple sugars, an important factor in the management of diabetes. Despite the possession of higher quantities of these bioactive constituents by the ginger incorporated feed than the cocoyam and unripe plantain incorporated feeds, it was observed to exhibit lower hypoglycemic action in this study than the cocoyam and unripe plantain incorporated feeds which is a significant finding in this study. Although the reason behind this cannot be explained presently, it worth's being noted.

Conclusion

This study shows that the ability of cocoyam, ginger and unripe plantain to ameliorate the relative pancreatic weight of diabetics.

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