



Research Article

Glyceamic Modulatory Effects of a Recipe from Some
Commonly Consumed Fruit Pulp in Normal and
Streptozotocin-induced Diabetic Rats

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ARTICLE INFO:

Keywords:

Diabetes,
Glyceamic,
Rats,
Recipe,
Streptozotocin

ABSTRACT

Plants continue to play an important role in the treatment and management of many diseases, including diabetes mellitus, in many developing economies. Most often, a cocktail of plant mixtures is normally used in traditional practice rather than single plant, which are believed to offer more benefits. *Adansonia digitata*, *Hyphaene thebaica*, *Ziziphus spina-christi*, and *Ziziphus mauritiana* are some of those plants that are reported to have medicinal benefits and, most importantly, anti-diabetic activity. This study evaluated the hypoglycemic and antidiabetic activity of the aqueous extracts of the fruit pulps and a recipe combining all the fruits in experimental rats. Aqueous extracts of the different fruits and the recipe were orally administered to normoglycemic and Streptozotocin (STZ)-induced diabetic rats, and their blood glucose was monitored over a period of 3 hours in the screening study. Two different doses of the recipe were also administered daily for four (4) weeks to STZ-diabetic rats to study the antidiabetic potential of the recipe. All the extracts did not significantly reduce the fasting blood glucose (FBG) of the normoglycemic rats in the hypoglycemic screening study. In the antidiabetic screening study, all the extracts lowered the FBG of their respective groups with percent reductions of 32%, 21%, 17%, and 46%, respectively, for *Z. spina-christi*, *Z. mauritiana*, *A. digitata* and *H. thebaica* extractives. The maximum percent reduction observed for the recipe was 25.5%. The daily administration of the recipe for four weeks also produced significant reductions that were not dose dependent. The 200 mg/kg bd. wt. dose showed a maximum reduction of 59.9%. The percentage reductions observed in the recipe-treated group were, however, lower than the percentage reductions seen in the insulin and glibenclamide-treated groups. The study confirms the antidiabetic properties of the individual fruits and the recipe, and thus confirms the traditional use of the fruits in the management of diabetes mellitus.

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INTRODUCTION

Glycemic homeostasis is key to healthy life; deviation from normoglycemia is a cause of some major diseases such as diabetes mellitus (DM). It is characterised by hyperglycaemia owing to the poor metabolic control of the blood sugar glucose. Though other classifications do exist, DM is broadly classified into two as Type 1 diabetes mellitus (T1DM) more common in children and Type 2 diabetes mellitus (T2DM) that is more common in adults. The pathogenesis of these diseases is insulin deficiency arising from the progressive destruction of pancreatic beta cells through an autoimmune response and insulin resistance which is majorly attributed to obesity, caused by poor dietary and lifestyle habits respectively (Tan, 2019). Complications arising from poor blood glucose regulation include microvascular degeneration such as nephropathy, retinopathy, and neuropathy while macrovascular complications include heart attack, stroke, and peripheral vascular disease (Patel *et al.*, 2011). Diabetes mellitus is no doubt one of the leading non communicable diseases in the world and perhaps one of the oldest known to Man (Tan *et al.*; 2019). DM is categorized as among the first five leading causes of death worldwide with global prevalence rate among adults reported at 8.3% (387 million) and predicted to rise to 8.5% (592 million) by the year 2035 (IDF, 2014). Prevalence rate in Nigeria is estimated at 14 million (7%) of the population (WHO, 2016). Other studies in Nigeria have also shown prevalence in the range of 3.3-7.7% (Nyenwe *et al.*, 2003; Ejike *et al.*, 2015). Therapeutic approach to treating this disease includes biotechnological, pharmacological, and non-pharmacological approaches. Gene therapy, stem cell therapy (Tan *et al.*, 2019) is some of the latest which are very expensive with some procedures still under trial. Phrenologically, the best treatment remained the use of insulin, however, mono or combined therapy with oral hypoglycaemics such as Biguanides, Sulphonyl urea, thiazolidinediones, and other forms are also in

use (Sola *et al.*, 2015; Rena *et al.*, 2017; Tan *et al.*, 2019);, all of these have their shortcomings.

Plants play an alternate role in the treatment of diabetes in many developing economies where such drugs or interventions are either expensive or not available in addition to their other side effects. These facts are attested to by several studies conducted on herbs under a multitude of ethno-botanical grounds (Jain and Jain, 2011). Most often, a cocktail of plant mixture is normally used in traditional practice rather than single plant, which are believed to offer more benefits. *Adansonia digitata*, *Hyphaene thebaica*, *Z. spina-christi* and *Z. mauritiana* are some of these plants which offer different medicinal benefits and more importantly have anti diabetic activity (Muhammad *et al.*, 2016; Abdel-Zaher *et al.*, 2005; Kamis *et al.*, 2003; Shehu *et al.*, 2014). This study investigated the synergistic effect of fruits of these plants combined as a recipe in respect of glycaemic modulation towards preventing hyperglycaemia.

MATERIALS AND METHODS

Fruits Samples Collection

The fruit pulps of *A. digitata*, *Z. mauritiana* and *Z. spina-christi* used in this study were collected dried from Gamboru market, Maiduguri, Borno State, Nigeria, and *H. thebaica* was collected from Geidam town, Yobe State, Nigeria. The plants were authenticated by a taxonomist from the Department of Biological Sciences, University of Maiduguri. Voucher specimens were deposited at the Research Laboratory, Department of Chemistry, University of Maiduguri, Nigeria. The voucher specimen numbers allocated for the samples are CHM/12/032 (*H. thebaica*), CHM/12/006 (*A. digitata*), CHM/12/023 (*Z. mauritiana*) and CHM/12/028 (*Z. spina-christi*).

Aqueous Extracts Preparation

The air-dried fruit pulps of all the samples were pulverized using wooden mortar and pestle. Aqueous decoction extracts of *A. digitata*, *H. thebaica*, *Z. mauritiana* and *Z. spina-christi* were prepared by boiling 200 g

powder of each in 1 L of water for 10 minutes. For the recipe, 50 g of each fruit powder were combined in a container and shaken to form a uniform mixture. The powdered plant mixture (200 g) was then extracted as was done with the individual fruits. The resultant extracts were then independently filtered using Whatman filter paper (No. 1) and the filtrate was concentrated to dryness *in-vacuo* at 40 °C using rotary evaporator {R201D PEC Medicals U.S.A.} (Usman *et al.*, 2009).

Experimental Animals

Albino rats with body weights ranging between 180-200 g of both sexes were purchased from the Animal House, Department of Biochemistry, University of Maiduguri. They were kept in plastic cages at the Biochemistry Research Laboratory, University of Maiduguri for a minimum period of one week before the commencement of the experiments. They were fed with rat pellets (Vital Feeds Nig Ltd Jos, Nigeria) and water *ad libitum*. The experiments were conducted in compliance with the international guiding principles for biochemical research involving animals. Ethical clearance was obtained from the Research and Ethics Committee, Faculty of Pharmacy, University of Maiduguri with approval number FP/022018/TETFP03.

Experimental Design

The extracts of the individual fruits and the recipe were first screened for hypoglycaemic and antidiabetic activities respectively in normoglycaemic and streptozotocin-induced diabetic rats. Different doses of the extracts were also orally administered to another set of rats daily for four weeks to assess the long term hypoglycaemic and antidiabetic effects of the extracts. The rats were fasted for 18 hours before each experiment and blood was collected from tip of the rats' tails for glucose assay.

Normoglycaemic Study

The experimental rats were grouped into 6 of 5 rats each and were fasted overnight. Groups II to VI were administered 400 mg/kg body weight of the extracts from the recipe, *Z. spina-christi*, *Z. mauritiana*, *A. digitata*, and *H.*

thebaica respectively. Group I served as normal control. Blood glucose concentration was determined over a period of three hours (0 min., 30 min., 60 min., 120 min. and 180 min.).

Screening of Extracts on Streptozotocin-Induced Diabetic rats

Diabetes was induced by a single intraperitoneal injection of freshly prepared citrate buffered (0.1 M pH 4.5) solution of streptozotocin (STZ) at a dose of 60 mg/kg body weight. Rats with fasting blood glucose of more than 200 mg/dL after 72 hours of induction were considered diabetic and included in the study.

The rats were divided into 9 groups of 5 rats each. Groups 1 to 4 served as normal control, diabetic control, and positive controls (insulin and glibenclamide), while 5 to 9 were diabetic rats administered with 400 mg/kg doses of the extracts from the recipe, *Z. spina-christi*, *Z. mauritiana*, *A. digitata*, and *H. thebaica* respectively. Blood glucose levels was determined over a period of three hours (0 min., 30 min., 60 min., 120 min. and 180 min.).

Anti-Diabetic Study (Sub-chronic)

A total of thirty (30) rats divided into 6 groups of 5 each were used. Groups 1, 2, 3 and 4 served as normal control, diabetic control, and positive controls and were administered normal saline (first and second groups), insulin (0.4 unit), glibenclamide (5 mg/kg bd. wt.) respectively, while 5 and 6 served as diabetic groups that were administered 200 and 400 mg/kg body weights of the extract from the recipe. Fasting blood glucose levels was determined weekly for the 4 weeks duration of the study.

Blood Glucose Determination

Blood was collected from the tails of the rats and analysed for glucose concentration using glucometer and test strips (Accu Chek Advantage II, Roche, Switzerland).

Statistical Analysis

The results are presented as Mean \pm SEM of five replicate determinations. Students T-test was used to statistically analyse the

differences between two means, and differences were considered significant at confidence level of $P < 0.05$.

RESULTS AND DISCUSSION

Results of the screening of the aqueous extracts of the different fruits and the recipe for hypoglycemic and antidiabetic activities by oral administration of the different extracts to normoglycemic and STZ-induced diabetic is presented in Tables 1 and 2, respectively. In the normoglycemic study, the different extracts did not significantly reduce the fasting blood glucose in any of the test groups. Administration of the extracts to the test groups resulted to an initial increase in blood glucose levels during the first hour which returned to normal values by the end of the second hour.

Extracts of the individual fruits and the recipe significantly ($P < 0.05$) reduced the fasting blood glucose of the diabetic rats. The maximum percentage reduction of 32.3% in fasting blood glucose of the STZ-induced diabetic rats was seen in the group orally administered with the aqueous extract of *Z. spina-christi* at 120 mins. post extract administration (Table 2). The recipe showed a maximum of 25.5% reduction at also 120 mins. post extract administration. The reductions were, however, lower than the maximum reductions observed for insulin (91.6%) and glibenclamide (60.4%) treated groups.

The effect of the different doses of the recipe extract on STZ-induced diabetic rats administered the extract daily for four weeks is presented in table 3. Administration of the extract at the dose of 200 mg/kg significantly ($P < 0.05$) reduced the initial fasting blood glucose of the group from 374.00 ± 21.89 to 165.00 ± 16.48 representing almost 60% reduction. The blood glucose lowering effect was, however, not dose dependent as the group administered with the 400 mg/kg body weight dose produced 35% reductions. The reductions observed with the different doses of the recipe were also lower than the reductions observed

with insulin (75.2%) and glibenclamide (70.4%) treatments.

Medicinal plants are believed to have active principles that can cure/manage diseases and are relatively safe when used in treatment; this is especially true when edible fruits are considered for such purposes. The present study showed that *Z. spina-christi* and *H. thebaica* extracts produced greater antidiabetic activities than the recipe at the screening level. This could be possible especially if there is no synergism in the antidiabetic activities among the constituents of the recipe. Of the four (4) fruit extracts, the extracts of *Z. mauritiana* and *A. digitata* fruits may have only added up to the quantity but not the antidiabetic activities of the recipe resulting into decreased activity lower than that of the extract of *Z. spina-christi* and *H. thebaica*. This can be explained because the antidiabetic activities of these individual extracts were comparably lower than that of *Z. spina-christi* and *H. thebaica*. In fact, *H. thebaica* extract reduced antidiabetic activities by 46.2% at the 180th min. but its contributory effect in the recipe may not tally as seen in the result of the recipe, this may happen because of the method of extraction adopted where equal amount of the individual samples was mixed before extraction and their respective percentage yields cannot be the same. Hence their contribution in the recipe is not the same despite using equal amount of sample for each.

The four plants have been reported to exhibit some levels of hypoglycemic activity on individual basis at different occasions (Muhammad *et al.*, 2016; Abdel-Zaher *et al.*, 2005; Shehu *et al.*, 2014); this confirmed our findings that the extracts from *Z. spina-christi* and *H. thebaica* were able to at the screening level reduce the blood glucose levels significantly 2 and 3 hours post-prandially respectively. However, to the best of our knowledge the recipe (a combination) of the fruits' pulps from the four (4) plants have never been tested for such type of study neither at the screening nor at the sub-chronic levels.

Administration of the recipe over a period of 28 days resulted in significant decreases in the blood sugar levels, however, the decreases were lower than the reductions observed with the two standards, insulin and glibenclamide used in this study. Nevertheless, a significant reduction of 55.9% by the recipe at 200 mg/kg body weight is a good indicator of the potentials of the recipe in the management of diabetes mellitus as the locals claim to have been doing. Overall, the extracts from the recipe and/or the individual fruits if properly explored can exhibit a tremendous potential in managing diabetes mellitus. Therefore, it is imperative to say that these findings could be related with the phytochemicals found in the extractives as we reported earlier (Geidam *et al.*, 2019; 2020).

CONCLUSION

The study concludes that aqueous extracts from *A. digitata* (L.), *H. thebaica* (L.), *Z. mauritiana* (L), *Z. spina-christi* (L.) and a recipe formed from these fruit pulps possesses antidiabetic properties that warrants further investigation for the possible development of a safe and affordable drug. The study therefore, confirms the antidiabetic properties of the individual fruit and the recipe; and thus, provide scientific evidence for the traditional use of the fruits in the management of diabetes mellitus.

CONFLICT OF INTEREST

The authors hereby declare no conflict of interest.

FUNDING

This research is funded by TETFund (Institution-Based Research) fund vide award letter with Ref. No.: *TETFUND/DESS/UNI/MAIDUGURI/IBR/2016/VOL. I*).

ACKNOWLEDGEMENTS

The authors wish to acknowledge the technical assistance rendered by the Technicians in Research Laboratories of the Departments of Pure and Applied Chemistry and that of Biochemistry.

Table 1: Effect of Oral Administration of Aqueous Extracts of *A. digitata*, *H. thebaica*, *Z. mauritiana* and *Z. spina-christi* and a Recipe Comprising the different Fruits on Fasting Blood Glucose Concentration (mg/dL) in *Normoglycemic* Rats

Groups	Blood Glucose Concentrations (mg/dL)				
	0 min.	30 min.	60 min.	120 min.	180 min
Normal Control	108.4± 1.72	108.4± 2.73	109.6± 3.52	102.4± 4.30	107.0±4.30
Recipe (400 mg/kg bd. wt.)	81.6± 6.46	80.6± 2.33	96.0± 3.31	82.6± 1.98	82.8± 2.39
<i>Z. spina-christi</i> (400 mg/kg bd. wt.)	78.0±7.23	115.8±4.06	98.4±4.47	92.8± 5.60	88.4± 5.44
<i>Z. mauritiana</i> (400 mg/kg bd. wt.)	88.6± 6.96	117.8± 3.83	106.6± 2.99	100.8± 7.10	90.6± 0.81
<i>A. digitata</i> (400 mg/kg bd. wt.)	78.0± 7.23	105.6± 3.52	93.6± 1.60	88.4± 4.34	83.8± 2.78
<i>H. thebaica</i> (400 mg/kg bd. wt.)	81.8± 4.46	101.6± 2.13	102.0± 1.48	93.2± 1.28	93.8± 4.18

Values are presented as mean ± SEM (n=5)

Table 2: Effect of Oral Administration of Aqueous Extracts of *A. digitata*, *H. thebaica*, *Z. mauritiana* and *Z. spina-christi* and a Recipe Comprising the different Fruits on Fasting Blood Glucose Concentration (mg/dL) in STZ-Induced Diabetic Rats

Groups	Blood Glucose Concentration(mg/dL)				
	0 min.	30 min.	60 min.	120 min.	180 min.
Normal Control	108.4± 1.72	108.4± 2.73	109.6± 3.52	102.4± 4.30	107.0±4.30
Diabetic control	402.2± 31.59	401.8± 33.6	419.0± 27.37	350.4± 16.61 (12.9%)	382.5± 16.53 (4.89%)
Glabenclamide (5 mg/kg bd. wt.)	447.6± 24.07	415.2± 21.49* (7.2%)	364.8± 20.40* (18.5%)	263.2± 27.37* (41.2%)	177.2± 25.52* (60.4%)
Insulin (0.4 unit)	485.4 ±22.79	154.2 ± 29.79* (68.2%)	73.2 ±7.71* (84.9%)	51.8± 3.91* (89.3%)	40.8± 6.71* (91.6%)
Recipe (400 mg/kg bd. wt.)	520.2± 28.92	571.2± 11.90	480.67± 96.72* (7.6%)	387.33±20.40* (25.5%)	427.33±30.71* (17.9%)
<i>Z. spina-christi</i> (400 mg/kg bd. wt.)	555.0± 26.14	509.8±35.04* (8.1%)	421.8± 13.21* (24.0%)	375.4± 14.27* (32.3%)	395.2± 12.15* (28.8%)
<i>Z. mauritiana</i> (400 mg/kg bd. wt.)	436.4± 15.50	415.6± 16.05* (4.8%)	384.0± 16.41* (12.0%)	352.6± 15.00* (19.2%)	344.6± 3.89* (21.0%)
<i>A. digitata</i> (400 mg/kg bd. wt.)	430.6±11.40	456.0± 15.26	413,4± 9.74 (4.0%)	388.0± 13.92* (9.9%)	359.2± 17.85* (16.5%)
<i>H. thebaica</i> (400 mg/kg bd. wt.)	417.4± 19.23	389.6± 11.96	373.8± 9.7 (10.4%)	305.0± 21.53* (26.9%)	224.4± 13.15* (46.2%)

Values are presented as mean ± SEM

* Significantly lower (P<0.05) compared with 0 hour in the same group.

Table 3: Effect of Four Weeks Oral Administration of Aqueous Extract of different Doses of the Recipe Comprising the different Fruits on Fasting Blood Glucose Concentration (mg/dL) in STZ-Induced Diabetic Rats

GROUP(S)	Blood Glucose (mg/dL)		% Reduction
	Initial	Final	
Normal Control	108.60± 2.84	107.40± 3.40	-
Negative Control	515.00+ 10.35	527.00±28.60	-
Positive Control Insulin (0.4 unit)	557.60± 18.24	138.20± 19.05*	75.2
Positive Control Glabenclamide (5 mg/kg bd. wt.)	501.20± 32.48	148.20± 10.05*	70.4
Recipe (200 mg/kg bd. wt.)	374.00± 21.89	165.00±16.48*	55.9
Recipe (400 mg/kg bd. wt.)	423.40± 30.85	275.40 ±40.96*	35.0

Values are presented as mean ± SEM

* Significantly lower (P<0.05) compared with initial fasting blood glucose in the same group.

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