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Hypoglycaemic Effect of Extracts of *Hyphaene thebaica* (L) Mart Fruit Pulp in Normal and Alloxan-induced Diabetic Rats

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ABSTRACT

Hyphaene thebaica, *Boscia senegalensis*, *Balanite aegyptica* and *Anisopus mannii* are plants claimed to posses anti-diabetic properties. In this study extracts of parts of these plants were first screened for anti hyperglycaemic activity using alloxan induced diabetic rats. In the second part of the study, aqueous and methanolic fractions of the fruit pulp of *H. thebaica* were prepared and different doses administered to different batches of rats (both normal and alloxan diabetic rats) after an 18 hours fast. Blood glucose levels were measured at 0,1,2,4 and 6 hours after treatment. A maximum reduction in fasting blood glucose of 28.4% was observed in diabetic rats administered 400 mg/dl dose of *H. thebaica* fruit methanol extract during the screening study. In the second part of the study, the aqueous extract at 800 mg/kg dose lowered the fasting blood glucose of both normal and alloxan-induced diabetic rats by 21.1% and 27.6% respectively. The methanolic extract, on the other hand, had maximum reduction of 33.1% for normoglycaemic rats and 33.7% for diabetic rats at the same 800 mg/kg dose. The anti hyperglycaemic effect of the methanolic fraction is comparable or even better than the maximum reduction observed with the diabetic rats administered with standard (glibenclamide) drug. The results of the study therefore indicate that fruits of *H. thebaica* pulp passing hypoglycaemic activity.

Keywords: *Hyphaene thebaica*, Alloxan-induced Diabetes, Anti Hyperglycaemic Activity.

INTRODUCTION

Diabetes mellitus, characterised by abnormally high blood sugar, is a common metabolic disorder affecting people all over the world. Complications of the disease include

retinopathy, neuropathy and nephropathy among others. The International Diabetes Foundation (IDF) (2006) reported that 6% of the world population are said to be diabetics while Diabetes Association of Nigeria (DAN) puts diabetes population in the country at about 10 millions (Globade, 2007). These figures are alarmingly increasing daily.

The characteristic hyperglycaemic in diabetes mellitus can be handled with insulin or oral glycaemic agents. The oral glycaemic agents manage the disease through increasing insulin secretion, decrease hepatic glucose production and/or increase sensitivity to insulin (Kelly and Mandarino 2000). Management of the disease with insulin and/or oral hypoglycaemic agents is not without some short comings. These include short shelf life, requirement for constant refrigeration and in the event of excess dosage fatal hypoglycaemic for the insulin. In the case of the oral hypoglycaemic agents, the side effects include hypersensitivity reactions, weight gain and GIT disturbance (Richard *et al,* 2009)

Medicinal plants might provide useful sources of new oral hypoglycaemic compounds devoid of the side effects associated with insulin and the other diabetic drugs. In the last few decades over 400 medicinal plants have been reported to possess hypoglycaemic activity (Neelesh *et al,* 2010). This is due partly to the recommendation of the World Health Organisation, to search for safer and more effective hypoglycaemic agents from the plant kingdom (WHO, 1980).

Hypaene thebaica (L) Mart is a plant use for its fruits, leaves and roots for medicinal purposes in many parts of the world. The plant is a member of the palmae (Arecaceae) family, with the fruits being use in the treatment of bilharzias, bleeding, especially after child birth and also as haematinic (Adaya *et al*., 1977; Von May del, 1986). Other medicinal uses of the fruits include improvement of spermatic count of male rats (Halta, *et al,* 2005), and some antimicrobial activities (Irobi and Adebayo, 1999). The fruit is also claimed to possess anti diabetic property (Kamis, *et al* 2003).

In this study the *H. thebaica* and other similar plants claimed to possess anti diabetic activity were first screened for antihyperglycaemic activity, subsequent studies centered on the fruits of *H. thebaica* in relation to dose and extracts effect in normal and alloxan-induced diabetic rats.

MATERIALS AND METHODS

Sample Collection and Identification

Fresh fruit and leaves of *Hyphaene thebaica,* leaves of *Boscia senegalensis Anisopus mannii* and *Balanite aegyptica* fruit were collected from Konduga Local Government Area of Borno state, Nigeria. The different parts were authenticated by a plant taxonomist with the Department of Biological Science, University of Maiduguri, Nigeria. They were cleaned, debris removed, shade dried and ground into powder using mortar and pestle.

Extracts Preparation

Aqueous and 70% methanolic extracts were prepared by soaking 500g of the powder in 2.5L of distilled water or 70% methanol in a glass jar. For aqueous extract it was left at room temperature for two hours, while for the methanolic extract the extract was left for two days. The extracts were filtered and the process repeated three times. The extracts were concentrated to dryness at temperature (at 40°C).

Experimental Animals

White Wister strain albino rats weighing between 120 and 200g were used for the study. The rats were bought from the animal house of the Veterinary Pharmacology Department, University of Maiduguri. They were maintained under standard condition of light, temperature and humidity (12 hour light /dark 25°C ± 1). They were fed standard rats diet (growers mesh, ECWA feed Nigeria Ltd) and water ad libitum.

Induction of Diabetes

Diabetes was induced by a single intramuscular injection of 120mg/kg alloxan monohydrate dissolved in cold normal saline after an overnight fast (Prince and Menon, 2003). After two weeks, surviving rats with blood glucose of more than 200 mg/dl were considered diabetic and used for the study.

Screening of Extracts for Anti-diabetic Property

Aqueous or methanolic extract of *H.thebaica* (fruit and leaves), *B. senegalensis* (leaves) *B.aegiptica* (fruits) and *A. mannii* (leaves) were first screened for possible anti hyperglycaemic activity. Dose of 400 mg/kg of the extracts was orally administered to groups of alloxan-induced diabetic rats (n=5) and their blood glucose levels monitored for 4 hrs.

Anti-hyperglycaemic Property of Extracts of *H. thebaica* Fruit

In this study nine groups of rats were used. Groups 1, 2 and 9 were

normal, diabetic and positive controls respectively. Groups 3, 4 and 5 were normal rats respectively administered 200,400 and 800 mg/dl doses of either aqueous or methanolic extracts, and groups 6, 7 and 8 were alloxan-induced diabetic rats treated similarly as with groups 3, 4, and 5. Normal and diabetic controls were administered water, while the positive control group received glibenclamide (1 mg/kg).

The rats were fasted for 18 hours prior to extract, water or glibenclamide administration. The extracts and the glibenclimide were suspended in water and administered as a single dose using a BMI feeding tube (size 8). Blood samples collected through the tail were used to assay for glucose at 0,1,2,4 and 6 hours after extract, water or Glibenclamide administration.

Blood Glucose Determination

Blood glucose concentration was determined by the glucose oxidase enzymatic method using commercial glucometer and test strips (Accu Chek Advantage11, Roche, W.S.H) (Rhenry and Kirk, 2000).

Statistical Analysis

The results obtained are presented as mean±SEM. Differences between means were analysed using students t-test and $p<0.05$ was considered significant.

RESULTS

Screening of Extracts for Anti Diabetic Property

Results of the effect of aqueous or methanolic extracts of the different plants screened are presented in table 1. A maximum percent reduction in fasting blood glucose of 28.4% was seen in diabetic rats administered 400 mg/dl methanolic extract of *H. thebaica* fruit. The leaves methanolic extract showed a 17.7% reduction which is similar with that of *B. aegyptica* (17.6%) but slightly higher than that of *B. senegalensis* (15.8%), and *A. mannii* (14.3%). The percentage reduction seen in the *H. thebaica* fruit was almost 2 folds higher than that observed in the glibenclamide treated group. The extracts of the remaining groups were however comparable to that of glibenclamide group.

Effect of Different Doses of Aqueous Extract of *H. thebaica* Fruit on FBG

Effect of different doses of aqueous extract of *H. thebaica* on FBG of normal and alloxan induced diabetic rats is presented in table 2. At the sixth hour post administration of the extract reductions of 8.9%, 17.3% and 21.1% FBG were respectively seen in the normal rats administered 200, 400 and 800mg/kg doses. In the diabetic groups the extracts also dose dependently reduced the fasting

blood glucose of the experimental rats. A maximum reduction of 27.6% was observed in the rats administered the highest dose of 800 mg/kg and was similar to that of the positive control group (27.7%).

Effect of Different Doses of Methanolic Extract of *H. thebaica* Fruit on FBG

The effect of different doses of methanolic extract of *H. thebaica* fruit on FBG of normal and alloxan induced diabetic rats is presented in table 3. The methanolic extract dose dependently affected the FBG of both normal and the diabetic rats. The percent reductions in FBG at the sixth hour for the 3 doses were 14.9%, 29.2%, and 33.1% respectively in normal rats. In the diabetic rats the reductions were 13.5%, 26.9% and 33.5% respectively for the 3 different doses. The 33.5% reduction seen in the diabetic rats administered 800 mg/kg methanolic extract was 21% higher than that observed in the positive control group.

DISCUSSION

Screening the different plants for hypoglycaemic property, showed *Hyphaene thebaica* (L) mart fruit pulp methanolic extract at 400 mg/kg dose, to significantly ($p<0.05$) lower the fasting blood glucose of diabetic rats, with the percent reduction of 28.4% compared to 15.8% by the standard drug, Glibenclamides after 4 hour administration. The results is indicating that the capacity of the extract to lower FBG is almost double that of the standard drug, and therefore warranted further studies for its anti diabetic potential.

The aqueous extract of *H. thebaica* fruit at 800 mg/kg dose lowered the blood glucose of both normal and alloxan induced diabetic rats by 21.1% and 27.6% respectively. However, methanolic extract had maximum reduction of 33.1% for normoglycaemic rats and 37.7% for diabetic rats at the same dose (table 2), thus supporting the traditional claim of the anti diabetic ability of the fruit of *H. thebaica.* In the study both the aqueous and methanolic extracts of the fruit dose dependently lowered the fasting blood of the rats. The effect was however well expressed in the diabetic than the healthy rats and the methanolic extract showed the better anti diabetic activity compared to the aqueous extract. Alcoholic solvents have been reported to have the ability to penetrate cell membranes, permitting the extraction of high amounts of endocellular components (Ghisalberti, 1993) and

phytochemicals that may have hypoglycaemic activity.
Phytochemicals detected in the methanolic extract (results not presented) includes carbohydrates, glycosides, flavonoids and saponins. These and other secondary plant metabolites have been reported to posses hypoglycaemic effect in various studies (Rass, 2001; Ojowole, 2002). Presence of these detected phytochemicals may be responsible for the hypoglycaemic property observed with the extracts of the fruits of *H. thebaica*. Further studies are however required to pin down the phytochemical from the fruit that is responsible for the activity.

The treatment with the fruits extracts resulted in the significant reduction in the serum glucose levels of not only diabetic rats but on normoglycaemic rats as well, an effect similar to that of sulphonylureas, like glibenclamide. They produce hypoglycaemia by stimulating the pancreatic beta cells to release more insulin (Goth, 1985). The mechanism by which fruit of *H. thebaica* produces hypoglycaemia may thus be similar to that of glibenclamide.

In conclusion, fruit of *H. thebaica* was found to possess hypoglycaemic effect in both normal and alloxan diabetic models thereby supporting the traditional claim. Studies are underway to isolate the phytochemical responsibly for the hypoglycaemic effect and establish the possible mechanism of the activity.

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Table 1: Screening of Five Different Plant Extracts (400mg/kg) on Fasting Blood Glucose (mg/dl) Level of Alloxan Induced Diabetic Rats

Treatment	Period of Treatment [hr(s)]					% Reduction in Blood Glucose
	0	1	2	3	4	
Normal control	58.30±1.51	59.38±1.25	57.40±1.50	60.28±3.41	59.45±1.55	
Diabetic control	283.34±7.17	284.43±5.54	282.64±1.92	285.00±1.50	286.20±1.70	
Diabetic + extract A	274.50±13.20	263.00±11.05	247.62±12.61	218.00±9.98*	196.57±8.17*	28.38%
Diabetic + extract B	240.00±6.13	230.00±7.91	217.50±4.15	207.58±4.15	197.60±6.50*	17.7%
Diabetic + extract C	237.50±6.29	230.82±4.08	220.70±4.08	210.60±4.28*	200.00±5.77*	15.8%
Diabetic + extract D	242.50±8.54	231.70±6.08	218.8±5.91*	205.00±2.89	200.04±2.50*	17.6%
Diabetic + extract E	237.50±11.10	225.38±9.40	215.00±6.80	210.23±6.30	203.50±6.80	14.3%
Diabetic + Glibenclamide (1mg/kg)	237.60±6.3	220.00±4.10	211.03±2.90*	200.00±4.10*	205.00±5.80*	15.8%

Values in the table are Mean ± SEM of six determinations

* P<0.05 compared with initial blood glucose (0hr) in the respective group

Extract A = Methanolic extract of H. *thebaica* fruit pulp

Extract B = Methanolic extract of H. *thebaica* leaves

Extract C = Aqueous extract of Boscia *senegalensis* leaves

Extract D = aqueous extract of Balanite *aegytica* fruit

Extract E = Methanolic extract of *Anisopus mannii* leaves

Table 2: Effect of Different Doses of Aqueous Extract of the Fruit Pulp of *H. thebaica* (L) Mart on Fasting Blood Glucose (mg/dl) Levels of Normal and Alloxan-Induced Diabetic Rats (n=6)

	Period of Treatment [hr(s)]				
Treatment	**0**	**1**	**2**	**4**	**6**
Normal control	83.67±4.39	82.33±4.57	81.66±4.72	79.67±4.01	79.50±4.01
Diabetic control	302.02±9.49	305.60±7.17	305.64±9.62	296.50±7.17	294.70±12.32
Normal + 200 mg/kg	82.33±2.87	80.17±2.21 (2.6%)	76.83±2.57 (6.7%)	76.00±2.98 (6.7%)	75.00±2.16 (8.9%)
Normal + 400mg/kg	85.67±2.67	80.83±1.20 (4.8)	81.50±2.46 (4.9%)	70.00±2.27*** (18.3%)	70.83±1.682*** (17.3%)
Normal + 800mg/kg	81.00±2.88	74.00±2.78 (8.6%)	65.83±2.81*** (18.7%)	60.17±1.40*** (20.8%)	63.83±1.17*** (21.1%)
Diabetic + 200 mg/kg	233.33±2.62	227.83±2.71 (2.4%)	212.00±1.83*** (9.1%)	204±1.83*** (12.6%)	206.00±2.34*** (11.7%)
Diabetic + 400mg/kg	263.16±1.85	250.33±1.22 (4.9%)	225.83±1.25*** (4.2%)	207.83±2.18 (21%)	205.33±1.61*** (22.1%)
Diabetic + 800mg/kg	304.67±1.62	285.50±2.19*** (6.3%)	249.83±3.34*** (18.0%)	224.83±3.36*** (26.2%)	220.67±1.12*** (27.6%)
Diabetic + Glibenclamide (1mg/kg)	277.40±11.29	250.00±14.08 (9.9%)	220.00±14.08* (17.1%)	205.50±10.27** (26%)	200.52±10.75** (27.7%)

Values are expressed as mean ± SEM

Values in parenthesis are percentage decrease in blood glucose

* P<0.05 compared with initial blood glucose (0hr) in the respective group

** P<0.01 compared with initial blood glucose (0hr) in the respective group

*** P<0.001 compared with initial blood glucose (0hr) in the respective group

Table 3: Effect of Different Doses of Methanolic Extract of the Fruit Pulp of *H. thebaica* (L) Mart on Fasting Glucose (mg/dl) Levels of Normal and Alloxan – Induced Diabetic Rats (n=6)

Doses	Period of Treatment [hr(s)]				
	0	1	2	4	6
Normal control	83.67±4.39	82.38±4.57	81.66±4.72	79.67±4.01	79.50±4.01
Diabetic control	302.02±9.49	305.60±7.17	305.64±9.62	296.50±12.17	294.70±12.32
Normal + 200 mg/kg	70.50±1.34	65.33±1.12** (7.3%)	61.17±0.75*** (13.2%)	57.17±0.70*** (13.3%)	60.00±0.77 (14.9%)
Normal + 400mg/kg	66.67±2.99	60.17±2.46 (9.7%)	57.51±1.73** (22.8%)	47.14±3.85*** (29.3%)	47.17±2.57*** (29.2%)
Normal + 800mg/kg	69.83±2.46	63.00±2.11*** (9.8%)	52.83±1.68*** (24.3%)	47.17±1.45*** (32.50%)	46.67±1.26*** (33.1%)
Diabetic + 200 mg/kg	253.83±3.87	241±2.89* (4.8%)	222.67±2.42*** (12.3%)	214.17±2.04*** (15.6%)	219.33±2.95*** (13.5%)
Diabetic + 400mg/kg	272.50±3.59	258.33±3.69*** (5.2%)	231.33±4.70*** (15.1%)	222.33±5.38*** (18.4)	199.17±5.85*** (26.9%)
Diabetic + 800mg/kg	302.67±6.36	276.67±6.30* (8.6%)	245.67±5.89*** (18.8%)	200.00±4.44*** (33.9%)	201.00±2.52*** (33.5%)
Diabetic + Glibenclamide (1mg/kg)	277.40±11.29	250.00±14.08 (9.9%)	230.00±14.08* (17.1%)	205.50±10.27** (26%)	200.52±10.75** (27.7%)

Values are expressed as mean ± SEM

Values in parenthesis are percentage decrease in blood glucose

* P<0.05 compared with initial blood glucose (0hr) in the respective group

** P<0.01 compared with initial blood glucose (0hr) in the respective group

*** P<0.001 compared with initial blood glucose (0hr) in the respective group

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