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Hypoglycaemic, Hypolipidaemic and Possible Toxicity of the Methanolic Fruit Pulp Extract of *Hyphane thebaica* (L) Mart in Alloxan-induced Diabetic Rats

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ABSTRACT

The antidiabetic effect of methanolic extract of *Hyphaene thebaica* (L) mart was studied at sub-acute level. Three different doses (200, 400 and 800 mg/kg body weight) of the extract were administered to alloxan-induced diabetic rats daily for 45 days. Weekly fasting blood glucose and body weight were monitored and at the end of the experimental period the rats were killed, blood collected and effect of the extract on lipid profile, indices of liver and kidney function assessed from the serum collected after processing the blood. All the doses significantly ($P<0.05$) reduce the fasting blood glucose of the alloxan diabetic rats. The maximum percent reduction of 57.36% was observed within the group administered the 400mg/kg body weight extract. The effect was however not dose dependent, the extract also ameliorated the increases observed in the indices of liver and kidney function and also corrected imbalances observed in lipid profiles of diabetic control group. *Hyphaene thebaica* fruit has antidiabetic effect is further supported by the result of this study.

Keywords: Diabetes mellitus, *Hyphaene thebaica,* Hypoglycaemic, Alloxan-Induced Diabetic Rats.

INTRODUCTION

Diabetes mellitus (DM) is a syndrome characterized by disordered metabolism and abnormally high blood sugar resulting from low levels of the hormone insulin with or without abnormal resistances to insulin effects (Tierney *et al;* 2002), leading to risk of microvascular damage (Retinopathy, neuropathy and nephropathy) WHO, 2006. The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in the year 2000. The total number of people with diabetes has been projected to rise from 171 million in 2000 to 366 million by

2030 with the prevalence of the disease being higher in men than women (Sarah *et al,* 2004). The greatest increase in prevalence is however expected to occur in Asia and Africa, where most patients will likely be found by 2030 (WHO 2002).

Modification of life-style, such as diet and exercise and the use of insulin and or oral hypoglycaemic drugs are ways of managing *Diabetes mellitus* by orthodox treatment. These methods target glycaemic control, since studies have confirmed that for the type 2 diabetes, effective control of blood glucose substantially decrease the risk of developing diabetic complications (Ohkubo *et al.,* 1993). Management of *Diabetes mellitus* with insulin and or oral hypoglycaemic agents has certain setbacks necessitating the search for more effective and safer antidiabetic drugs. The scientific search for hypoglycaemic agents from medicinal plant as encouraged by the WHO has become even more important. In the last few decades over 500 herbal medicines have been reported to possess antidiabetic property (Jia *et al*., 2003).

Hyphaene is derived from Greek word 'hyphaino' (web), referring to the fibres from the leaves, which are used for weaving. *Hyphaene thebaica* belongs to the family Palmae (Arecaceae) and subfamily Barassoideae (Burdkit 1987). Each *H. thebaica* palm fruit is about 7.5 × 5cm in size and its mesocarp small, and taste like ginger, hence the name in English, 'ginger bread' in some places. It grows in the Sahel in the hot savannah between 12–18^{0}N from Senegal to Northern Nigeria, Chad, Zaria and North East Africa (Walter, 1971). Acute Hypoglycaemic studies of *H. thabaica* fruit pulp have been carried out, but the sub-acute antidiabetic activity was yet to be investigated.

MATERIALS AND METHODS

Sample Collection

Fresh fruit of *Hyphaene thebaica* was collected from Konduga local government area of Borno state, Nigeria. The plant was authenticated by plant taxonomist with the Department of Biological Science, University of Maiduguri. The fruit were cleaned, debris removed, shade dried and ground into powder using mortar and pestle.

Methanolic Extract Preparation

Hyphaene thebaica fruit pulp powder (500g) was macerated with 2.5 liters of 70% methanol in a glass jar for 2 days at room temperature, the extract was filtered and concentrated to dryness under reduced temperature and pressure on a rotary evaporator.

Experimental Animals

White wister strain albino rats weighing between 120 and 200g were used for the study. The rats were brought from the animal house of the Veterinary Pharmacology Department, University of Maiduguri. They were maintained under standard condition of light, temperature and humidity (12 hours light /dark 25°C ± 1). They were fed standard 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

Experimental Design

The rats were divided into six groups of five animals each. Groups 1, 2 and 6 were normal, diabetic and positive controls, while groups 3, 4 and 5 were diabetic rats orally administered 200, 400 and 800 mg/dl methanolic extract of the fruit of *H. thebaica* respectively. The groups 1 and 2 were administered distilled water and group 6 was administered with 1mg/kg glibenclamide.

The methanolic extract, glibenclamide and distilled water were administered to the rats daily for 45 days. The administration was by tube feeding (BMI feeding tube, size 8). Blood glucose was measured weekly throughout the experimental period. Twenty – four hours (24hrs) after the last treatment the rats were killed and blood collected. Serum harvested from the blood was used for the estimation of Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), urea, creatinine Na^+, K^+, cholesterol and triacylglycerols, LDL, HDL, Total protein and albumin.

Biochemical Analysis

Blood glucose concentration was estimated by the glucose oxidase enzymatic method using commercial glucometer and test strips (Accu-chek Advantage II Glucose, Roche U.S.) (Rhenry and Kirk, 2000). Serum Alanine (ALT) and Aspartate (AST) Amino transferases were assayed by the method of Reitman and Frankel (1957). Serum Alkaline Phosphatase (ALP) by the method of Mc Comb and Browser (1972). Serum total protein and albumin were assayed by the methods of Henry *et al,* (1974) as reported by

Kaplan *et al,* (1988). The diacytylmonoxine and Jaffe's reaction as described by Kaplan *et al,* (1998) were used in assaying serum urea and creatinine respectively. Serum sodium and potassium levels were estimated by Flame photometric method, (Kolthof, 1976). Total Cholesterol, HDL-Cholesterol and LDL were by Alan *et al* 1980 and Triacyglycerol by Tietz (1990).

RESULTS

Body Weight Changes

The percentage body weight changes due to different doses of methanolic extract orally administered to rats for 45 days (Table 1) showed significant ($P<0.05$) weight loss in Diabetic rats when compared with normal control. Extract administration to diabetic rats significantly ameliorated the body weight loss. The reduction in weight loss observed in the groups administered the 400 and 800 mg/kg doses were even higher when compared with the standard drug group.

Effect on Blood Glucose

The FBG of the diabetic control group was consistently high throughout the 45 days experimental period. Oral administration of the methanolic extract resulted in a significant reductions in the FBG of experimental groups (Table 2). Reductions of 51.6, 57.4 and 54.6% respectively were seen in groups administered 200, 400 and 800 mg/kg body weight doses. The standard drug showed a reduction of 68.2%

Serum Biochemistry

There was significant increases in the levels of indices of liver and kidney function under diabetic condition (Tables 3 and 4) when compared with normal control group. Treatment for 45 days significantly ($P<0.05$) decreased the elevated serum ALT, ALP, Creatinine and Urea concentration. The level of sodium further decreased ($P<0.05$) at higher dose when compared with diabetic control but potassium was unaffected.

Effect of Some Lipid Profiles

Diabetes was found to have caused significant increase ($P<0.05$) in the level of total cholesterol, triacylglycerol, and low density lipoproteins (LDL) and a decrease ($P<0.05$) in High Density Lipoprotein (HDL). However, administration of the extract at 800mg/kg lowered ($P<0.05$) the levels of total cholesterol, triacylglycerols and LDL and increased ($P<0.05$) High Density Liproprotein (HDL) at all the doses tested (Table 5).

DISCUSSION

Daily oral administration of different doses of the methanolic extract for the 45 days resulted in a significant decreases in the FBG of the diabetic rats. By the end of the 45 days the 200 mg/kg dose showed a FBG reduction of 51.6%.The percentage reductions for 400 and 800 mg/kg doses were 57.4% and 54.6% respectively. This reductions were comparable to the 68.2% seen in the group administered the standard oral hypoglycaemic drug-glibenclamide.

Ability of plant extracts to lower blood glucose after daily oral administration for many days have also been reported (Sakthi *et al.,* 2010; Gidado *et al.,* 2009). In both studied different doses of extract were administered daily for over a long period just as in this study. The antidiabetic effect of the *H. thebaica* fruit in this study was however not dose dependent but it further supported the ability of the extract to reduce fasting hyperglyceamia as we reported in an earlier study Shehu *et al.,* 2014.

The levels of serum and tissue lipids (Cholesterol, free fatty acids and phospholipids) are usually elevated in diabetes (Prince *et al;* 1999). Diabetes significantly increases the level of cholesterol, triacylglycerol and decrease high density lipoproteins. The sub-acute hypoglycaemic activity result suggested that methanolic extract of *H. thebaica* fruit pulp (800 mg/kg) decrease total cholesterol, triacyglycerol and lower density lipoprotein level but increased high density lipoprotein (Cardioprotective lipid) in diabetic rats. The hypolipidemic effect of *H. thebaica* methanolic extract may be attributed to a consequence of blood glucose reduction which have potential role in management of diabetes as well as prevent formation of atherosclerosis, coronary heart disease .Reduction in levels of creatinine, urea, ALT, and ALP in diabetic treated groups further confirms the utility of these extract in diabetes.

Doum palm contains tannins flavonoids and saponins which are antioxidant and play an important role in scavenging free radicals (Bhttacharya *et al;* 2000; Seartezzini *et al;* 2006). It is postulated that ingesting antioxidants and minimizing free radicals may reduce the contribution of LDL to antherosclerosis (El-genedy *et al,* 2009). Therefore antioxidant found in doum palm may have the ability to overcome these problems. In conclusion, that the extract reduce fasting

hyperglyceamia, corrected lipid profile imbalances and prevented liver and kidney damages, proofs the ability of *H. thebaica* to manage diabetes mellitus.

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Table 1: Body Weight Changes (Mean ± SEM) Following Oral Administration of Different Doses of Methanolic Extract of the Fruit Pulp of *Hyphaene thebaica* (L.) Mart in Alloxan-induced Diabetic Rats (n=5) for 45 Days

Treatment Doses mg/kg	Initial wt (g)	Final wt (g)	Percentage Change
Normal control	152.60±3.84	186.00±3.45	17.97±1.13
Diabetic control	167.20±3.94	134.00±3.91	-19.88±1.00[a]
Diabetic + 200	167.00±9.04	153.00±8.77	-8.44±0.46[b]
Diabetic + 400	181.20±8.22	176.40±7.97	-2.64±0.27[c]
Diabetic + 800	169.40±6.66	165.60±6.65	-2.25±0.05[d]
Diabetic + Glibenclamide (1mg/kg)	181.00±5.68	171.00±6.14	-5.44±0.51[e]

[a] P<0.05 Compared with Normal control

[bcde] = P<0.05 compared with diabetic control

Table 2: Effect of Different Doses of Methanolic Extract of *H. thebaica* (L.) Mart on Fasting Blood Glucose (Mean ± SEM) in (mg/dl) on Alloxan- induced Diabetic Rats (n=5) for 45 Days

Treatment Doses (mg/kg)	Initial Blood Glucose (mg/dl)	Final Blood Glucose (mg/dl)	Percentage Change in Blood Glucose
Normal control	82.17±2.22	83.00±2.25	1.02±2.18
Diabetic control	277.16±20.82	312.45±19.95[a]	12.52±15.42[a]
Diabetic + 200	288.08±17.32	138.60±16.76[b]	- 51.57±10.52[b]
Diabetic + 400	267.60±18.41	112.63±16.35[b]	- 57.36±13.56[b]
Diabetic + 800	262.04±7.40	118.72±8.34[b]	-54.58±5.08[b]
Diabetic + Glibenclamide (1mg/kg)	268.30±13.60	84.65±8.36[b]	-68.23±11.33[b]

Initial – values before administration of extract

Final – values at the end of 45 days experiment

[a] P<0.05 compared with normal control

[b] P<0.05 compared with diabetic control

Table 3: Effect of Oral Administration of Different Doses of *H. thebaica* Fruit (L.) Mart Methanolic Extracts on Some Indices (Mean ± SEM) of Liver Function in Alloxan – Induced Diabetic Rats (n=5) for 45 Days

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Protein (g/L)	Albumin (g/L)
Normal control	74.40±5.68	41.60±1.97	321.00±3.26	82.40±2.25	35.20±0.97
Diabetic control	100.80±2.87[a]	76.00±3.73[a]	376.20±2.40[a]	51.80±2.87[a]	23.60±1.69[a]
Diabetic + 200 mg/kg	88.00±7.89	50.40±2.58[b]	367.80±2.20	67.00±2.83[b]	28.40±1.17
Diabetic + 400 mg/kg	86.60±4.58	52.40±2.56[b]	343.80±7.17[b]	68.80±2.18[b]	24.60±1.33
Diabetic + 800 mg/kg	81.60±3.66	50.20±2.48[b]	334.40±3.49[b]	76.00±1.14[b]	28.80±0.58
Diabetic + Glibenclamide (mg/kg)	89.80±3.35	49.80±2.82[b]	346.60±4.77[b]	61.40±1.91[b]	29.00±1.14[b]

[a] = P<0.05 Significantly different from Normal control

[b] = P<0.05 Significantly different from Diabetic control

Table 4: Effect of Oral Administration of Different Doses of *H. thebaica* (L.) Mart Fruit Pulp Methanolic Extracts on some Indices of Kidney Function (Mean ± SEM) in Alloxan – induced Diabetic Rats (n=5) for 45 Days

Treatment	Urea (mmol/L)	Creatine (µmol/L)	Na^+ (mmol/L)	K^+ (mmol/L)
Normal control	5.08±0.12	61.00±1.30	147.60±2.06	5.66±0.13
Diabetic control	7.42±0.41[a]	81.40±4.23[a]	149.00±2.74	4.96±0.17[a]
Diabetic + 200 mg	6.46±0.39	66.60±3.19[b]	140.40±3.54	5.16±0.10
Diabetic + 400 mg	6.66±0.11[b]	64.00±3.52[b]	136.40±2.32[b]	5.28±0.21
Diabetic + 800 mg	5.02±0.07[b]	61.60±4.20[b]	137.40±2.71[b]	5.10±0.07
Diabetic + Glibenclamide (mg/kg)	6.08±0.22[b]	62.86±1.50	141.60±1.21	5.44±0.13

[a] = P<0.05 Significantly different from Normal control

[b] = P<0.05 Significantly different from Diabetic control

Table 5: Effect of Oral Administration of Different Doses of *H. thebaica* (L) Mart Fruit Pulp Methanolic Extract on some Lipid Profile (Mean ± SEM) in Alloxan – induced Diabetic Rats (n=5) for 45 Days

Treatment	Total Cholesterol (mmol/L)	Triacyglycerol (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Normal control	1.60±0.13	2.12±0.19	1.50±0.07	0.98±0.10
Diabetic control	2.24±0.08	3.10±0.10[a]	0.62±0.07[a]	3.08±0.16[a]
Diabetic + 200 mg/kg	1.88±0.09	1.26±0.18[b]	1.28±0.06[b]	1.22±0.20[b]
Diabetic + 400 mg/kg	1.84±0.08	0.98±0.10[b]	1.40±0.14[b]	1.34±0.07[b]
Diabetic + 800 mg/kg	1.60±0.13[b]	1.04±0.10[b]	1.44±0.05[b]	1.32±0.07[b]
Diabetic + Glibenclamide (1mg/kg)	1.96±0.05	1.52±0.12[b]	1.04±0.11[b]	1.36±0.05

[a]= P<0.05 Compared with normal control

[b] = P<0.01 Compared with diabetic control

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