



## ANTIOXIDANT, HYPOLIPIDEMIC AND ANGIOTENSIN CONVERTING ENZYME INHIBITORY EFFECTS OF ALKALOID FRACTION OF *Hyphaene thebaica* (DOUM PALM) ON HIGH FAT-FED OBESE WISTAR RATS

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### ABSTRACT

*This study investigated the in vivo effects of alkaloid fraction of H. thebaica on some antioxidant parameters, lipid profile and the activity of angiotensin converting enzyme of high fat-fed obese wistar rats. A total of twenty five (25) apparently healthy rats weighing between 150-240g were used for the experiment. Rats were divided into five experimental groups of five rats each, and treated with different concentrations of the doum extract. The rats were fed high fat diet for 30 days to induce obesity. The obese rats were treated with a standard drug (atorvastatin) and two different dose of H. thebaica extract for 14 days. The rats were sacrificed after two weeks of treatment and blood samples were collected for investigation of the antioxidant parameters, lipid profile and ACE activity. The results show a significant increase in the animals' body weight in all the groups. Fourteen (14) days treatments with both fractions of H. thebaica were found to significantly decrease the body weight. Administration H. thebaica extract significantly increases catalase activity, glutathione peroxidase and superoxide dismutase level while malondialdehyde decreased significantly ( $p < 0.05$ ). The level of total cholesterol, triglyceride, and LDL cholesterol was found to decrease in the group treated with alkaloid fraction of H. thebaica. On the other hand a significant ( $p < 0.05$ ) increase was observed in HDL cholesterol level. The ACE activity was also found to be inhibited after treatment with fractions of H. thebaica. The results show that Hyphaene thebaica contains some potent antioxidant, hypolipidemic and ACE inhibitory effects.*

**Keywords:** *H. thebaica*, antioxidant, angiotensin converting enzyme, obese, hypolipidemic

### INTRODUCTION

Obesity is a chronic disorder characterized by an abnormal or excessive accumulation of body fat. It is a medical problem that increases the risk of other diseases and health problems, such as heart disease, diabetes, high blood pressure and certain cancers (Leonhardt *et al.*, 2014). Activation of the renin-angiotensin system (RAS) has been shown to represent a link between obesity and hypertension (Segura and Ruilope 2007). Inhibition of RAS has been an important strategy for the treatment of hypertension and as a result, angiotensin converting enzyme (ACE) inhibitors have found a role as one class of antihypertensive drug (Segura-Campos *et al.*, 2010). Most obese individuals have elevated lipid levels, known as hyperlipidemia, a medical condition characterized by an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters, phospholipids and/or plasma lipoproteins such as very low-density lipoprotein and low-density lipoprotein,

and reduced high-density lipoprotein levels. This elevation of plasma lipids is among the leading risk factors associated with cardiovascular diseases (Shatat *et al.*, 2009).

Oxidative stress is defined as an imbalance between oxidants and antioxidants or disruption of redox signaling and control (Jones 2006). Plants extracts have been widely used for long as source of dietary antioxidants, and it is believed that two-thirds of the world plant species have medicinal uses (Krishnaiah *et al.*, 2011).

Doum palm fruit (*Hyphaene thebaica*) is a desert palm tree with edible oval fruit, originally native to the Nile valley (Hossam *et al.*, 2018). Various extracts of *H. thebaica* are used in the treatment of many diseases. Doum fruit drink brewed from hot water infusion of dried fruit pulp is widely consumed as a health tonic. Fruit and seed of *H. thebaica* have also been used in the treatment of hypertension, bilharzias and as hematinic suspension (Burkill 1997).

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Aqueous extract of doum fruits can reduce hyperlipidemia in nephrotic syndrome and leads to decrease the risk of glomerulosclerosis and atherosclerosis and consequently the natural, safe and nontoxic *H. thebaica* fruit could be of great merit for use as hypolipidemic drugs (Aboshora *et al.*, 2014).

## **MATERIALS AND METHODS**

### **Chemicals and Reagents**

Ethyl acetate, N Hydrochloric acid and Methanol. (Sigma, U.S.A). All other chemicals/reagents used were of analytical grade.

### **Collection, Identification and Preparation of Plant Material**

Doum palm fruit was collected from Rimi Market Kano municipal City, Kano State, Nigeria, in February, 2018. The fruit was identified and authenticated by a botanist at the herbarium in department of plant biology, faculty of Life Science, Bayero University, Kano. A voucher specimen was deposited in the Herbarium for future use. The doum fruit was cracked to separate epicarp from the seed and ground into powder. The obtained powder sieved using a mesh (2mm) and used for preparation of extract.

### **Experimental Animals**

Twenty five (25) apparently healthy albino wistar rats (150-240g) were obtained from the National Veterinary Research Institute, Vom in Jos, Plateau State. The animals were kept in well ventilated cages under standard conditions with 12 h light/dark cycle. They were allowed access to normal rat chow (Vital Feed, UAC) and water *ad libitum* throughout the period of the study. The animals were allowed to acclimatize for 2 weeks before commencement of experiment. Animals were used in accordance with the guideline of Bayero University ethics committee on the use of animals for research.

### **Experimental Design**

The rats were divided into 5 groups of 5 rats each:

Group 1: Control group (Standard diet, not treated), Group 2: Fat-fed obese control (High fat fed, not treated), Group 3: Standard control (High fat feed, treated with standard drugs Atorvastatin 70mg/kg), Group 4: 100mg/kg *H.thebaica* (High fat fed, treated with 100mg/kg alkaloid fraction of *H.thebaica*), Group 5: 250mg/kg *H. thebaica* (High fat fed, treated with 250mg/kg alkaloid fraction of *H. Thebaica*)

### **Preparation of High Fat Diet (HFD)**

High fat diet was prepared as described by Mickelsen *et al.*, (1955). The diet consists of 60g Crisco, 25g casein, 7g sucrose, 1g starch, 4g mineral salt, 3g palm oil, 2g of vitamin A and D, 1g of vitamin E was added to every 100g of normal fed (vital feeds).

### **Induction of Obesity**

Rats in all groups except group 1 (control) were given HFD, freshly prepared daily until they became obese. This lasted for three weeks. Obesity was defined as 0.35 kg/m<sup>2</sup>. The obese rats were treated orally with alkaloid fraction of doum palm and standard drug (atorvastatin) for a period of 2 weeks.

### **Preparation of Alkaloid Fraction of *H. thebaica***

Alkaloid extract was obtained as described by Osmium (2005). Briefly about 500g of the dried powdered doum fruits was measured and extracted with petroleum ether to remove the non-polar constituents. The residual extract was then subjected to extraction using methanol. The extract was evaporated to leave the crude alkaloid mixture.

### **Determination of Antioxidant Parameters**

The antioxidant parameters measured include Malondialdehyde (MDA) as described by Vashney and Kale (1990), Catalase activity according to the method of Brannan *et al.* (1981), reduced glutathione according to Ellman (1959), Superoxide Dismutase (SOD) activity as described by Misra and Fridovich (1972).

### **Serum Lipid Analysis**

Lipid profile was determined using enzymatic colorimetric methods. The serum levels of high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol (TC) and triglyceride (TG) were determined using commercially prepared kit. They were analyzed according to the manufacturer's instruction (K labkit CHEMELEX, S.A. pole industry).

### **Determination of ACE Activity**

The ACE activity was determined as described by Cushman and Cheung (1971).

### **Statistical Analysis**

All experiments were conducted in triplicate and results were expressed as Mean  $\pm$ SD. Data were analysed by one-way ANOVA using Graphical Instat3 software (2000) version 3.05 by Graphical Inc. Values of  $p < 0.05$  were considered significant.

**RESULTS AND DISCUSSION**

**Effects of fractions of *H. thebaica* on body weight**

The body weight of all the animals in experimental groups were found to increase significantly after induction of obesity as shown in table 1. High fat intake has been associated with increase in body weight, and can subsequently lead to obesity and other related

disorders. High fat-induced obesity in animal models is therefore considered to represent the most common route of obesity in humans (Buettner *et al.*, 2007). Treatment with two different doses of alkaloid fraction of *H. thebaica* was found to reduce the body weight of animals under the present experimental condition. However, the decrease was not statistically significant ( $P>0.05$ ).

**Table 1:** Effect of alkaloid fraction of *H. thebaica* (Doom palm) on body weight of fat fed obese wistar rats

Groups	Body Weight (g)		
	Before Induction	After Induction	After Treatment
Control	148.30±2.77 <sup>f</sup>	193.00±16.76 <sup>b</sup>	190.20±15.53 <sup>b</sup>
Obese control	214.20±3.56 <sup>c</sup>	348.80±21.68 <sup>g</sup>	349.00±22.87 <sup>g</sup>
Standard drug Atorvastatin (70mg/kg)	203.80±4.49 <sup>d</sup>	242.00±4.76 <sup>r</sup>	200.00±12.24 <sup>s</sup>
<i>H.thebaica</i> (100mg/kg)	146.40±6.12 <sup>a</sup>	212.00±43.82 <sup>j</sup>	208.00±41.72 <sup>j</sup>
<i>H.thebaica</i> (250mg/kg)	199.60±2.30 <sup>c</sup>	306.00± 27.02 <sup>n</sup>	297.40±28.51 <sup>n</sup>

Values are expressed as mean ± SD (n = 5)

Values with different superscript across the groups are significantly different at  $p<0.05$

**Effect of extracts on antioxidant parameters**

Results in Table 2 show concentration dependent antioxidant activity of alkaloid fraction of *H. thebaica*. Significant ( $p<0.05$ ) increase was observed in the level of catalase, glutathione peroxidase and superoxide dismutase in all treated group compared to the obese group. MDA was found to be significantly ( $p<0.05$ ) high in obese control group. Increased generation of free radicals is prominent in most diseases; the antioxidant system represents the main pathway to detoxify free radicals (Lobo *et*

*al.*, 2010). Induction of obesity has been reported to lessen the activity of antioxidant defense mechanism, due to the increased cellular accumulation of lipid peroxides and depletion of endogenous antioxidants due to the high-fat diet (Mashaal *et al.*, 2017). Alkaloid fraction of *H. thebaica* has shown effective antioxidant activity (table 2) in a concentration dependent manner. Lamiaa and Laith (2018) reported a similar finding of strong antioxidant activity of ethanol and ethyl acetate extracts of doom palm. Both extracts show an increase activity with increasing concentration.

**Table 2:** Effect of alkaloid fraction of *H. thebaica* on some serum oxidative stress and antioxidant enzyme markers in fat-feed obese Wistar rats

Groups	Catalase (U/ml)	Glutathione Peroxidase (U/ml)	Superoxide Dismutase (U/ml)	Malondialdehyde (µmol/ml)
Control	268.68±5.77 <sup>n</sup>	81.54±1.21 <sup>b</sup>	3.64±0.69 <sup>c</sup>	1.03±0.01 <sup>f</sup>
Obese Control	53.23±1.70 <sup>o</sup>	69.11±4.59 <sup>a</sup>	0.64±0.69 <sup>w</sup>	3.11±0.17 <sup>i</sup>
Standard drug (70mg/kg atorvastatin)	206.62±20.85 <sup>q</sup>	78.29±1.29 <sup>b</sup>	2.63±0.66 <sup>d</sup>	2.16±0.09 <sup>j</sup>
<i>H.thebaica</i> (100mg/kg)	169.97±14.11 <sup>m</sup>	78.29±1.29 <sup>b</sup>	1.59±0.63 <sup>s</sup>	2.25±0.48 <sup>j</sup>
<i>H.thebaica</i> (250mg/kg)	198±12.20 <sup>f</sup>	79.56±0.61 <sup>b</sup>	2.59±0.54 <sup>c</sup>	2.29±0.25 <sup>j</sup>

Values are mean±SD

Values with different superscripts in the same column are significantly different at  $P<0.05$

**Effects of alkaloid fraction of *H. thebaica* on lipid profile**

Table 3 shows the effect of alkaloid fractions of *H. thebaica* on the lipid profile of obese wistar rats. It was observed that high-fat induced

obesity resulted in a remarkable increase in lipid parameters including total cholesterol, triglycerides and LDL cholesterol and reduced level of HDL cholesterol.

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This is an indication of hyperlipidemia which is a major risk factor associated with obesity (Souravh *et al.* 2014). Treatment of high fat-induced obesity with alkaloid fraction of *H. thebaica* was found to significantly ( $p < 0.05$ ) reduced serum levels of total cholesterol, triglyceride and LDL cholesterol. A significant increase ( $p < 0.05$ ) was observed in the level of HDL cholesterol in all treated groups, Effects were observed in a dose-dependent manner. This is in consistent with the finding of Aida (2016) which reported the hypolipidemic effect of doum fruit. In a similar study, El Gendy *et al.*

(2008) observed that oral supplement of doum fruit significantly reduced serum levels of total cholesterol, triglyceride and LDL in human. Whereas HDL level was found to increase significantly. High levels of cholesterol, triglycerides and LDL cholesterol is directly related to arteriosclerosis which is the major cause of cardiovascular diseases (Ntchapda *et al.*, 2017). Thus, the result of this study proves the beneficial effect of doum palm in reducing the risk of cardiovascular diseases due to its hypolipidemic effect.

**Table 3:** Effect of alkaloid fraction of *H. thebaica* on serum lipid profile of fat-fed obese wistar rats.

Lipidprofile (mg/dl)	Control	Obese Control	Standard Drug atorvastatin (70 mg/kg)	<i>H. thebaica</i> (100 mg/kg)	<i>H. thebaica</i> (250 mg/kg)
Total Cholesterol	85.44 <sup>a</sup> ± 1.11	113.12 <sup>c</sup> ± 2.23	74.04 <sup>b</sup> ± 1.77	70.62 <sup>b</sup> ± 4.33	51.32 <sup>g</sup> ± 6.66
HDL Cholesterol	42.12 <sup>d</sup> ± 0.90	10.48 <sup>u</sup> ± 1.51	40.06 <sup>n</sup> ± 2.19	14.73 <sup>u</sup> ± 3.19	33.23 <sup>o</sup> ± 3.39
Triglycerides	95.76 <sup>t</sup> ± 4.85	113.8 <sup>y</sup> ± 1.60	80.34 <sup>i</sup> ± 3.51	70.22 <sup>f</sup> ± 5.23	50.14 <sup>r</sup> ± 2.65
LDL Cholesterol	24.14 <sup>z</sup> ± 1.20	79.66 <sup>c</sup> ± 1.20	17.92 <sup>p</sup> ± 1.03	41.65 <sup>k</sup> ± 2.08	10.76 <sup>e</sup> ± 0.74

Values are expressed as mean ± SD (n=5).

Values with different superscript across the group are significantly different  $P < 0.05$

**Effects of alkaloid fraction of *H. thebaica* on the activity of angiotensin converting enzyme (ACE)**

Hypertension is a global health problem associated with increased cardiovascular diseases. Obesity is an important factor that increases the risk of hypertension by altering endothelin and Renin Angiotensin Aldosterone System (RAAS). Angiotensin-converting enzyme, a decapeptidyl peptidase, is widely distributed not only in the cardiovascular system, but also in various non-cardiovascular tissues, such as the vascular endothelial cells of the lung, distal

tubular epithelial cells and human alveolar macrophage (Sharifi *et al.*, 2003). Table 4 shows the effect the alkaloid fraction of *H. thebaica* on ACE activity in serum, kidney and lungs of fat-fed obese wistar rats. It could be observed that induction of obesity increase ACE activity significantly. However, treatment with alkaloid fraction of *H. thebaica* was found to decrease ACE activity. 250mg/kg of alkaloid fraction show the highest activity between the groups. This indicates the antihypertensive effect of doum extracts by inhibition of ACE activity (Noha *et al.* 2018).

**Table 4:** Effect of the alkaloid fraction of *H. thebaica* on the activity of angiotensin converting enzyme in serum, lung and kidneys of fat-fed obese wistar rats.

Groups	ACE Activity (µmol/min/mg)		
	Serum	Kidney	Lungs
Control	0.452 ± 0.77 <sup>b</sup>	0.559 ± 0.054 <sup>a</sup>	0.514 ± 0.039 <sup>c</sup>
Obese control	0.718 ± 0.0908 <sup>**</sup>	0.942 ± 0.084 <sup>b,**</sup>	0.899 ± 0.042 <sup>d,**</sup>
Standard drug (Atorvastatin, 70 mg/kg)	0.521 ± 0.059 <sup>b</sup>	0.628 ± 0.048 <sup>a</sup>	0.053 ± 0.073 <sup>e</sup>
<i>H. thebaica</i> (100 mg/kg)	0.477 ± 0.054 <sup>b</sup>	0.552 ± 0.069 <sup>a</sup>	0.618 ± 0.032 <sup>c</sup>
<i>H. thebaica</i> (250 mg/kg)	0.483 ± 0.067 <sup>b</sup>	0.688 ± 0.049 <sup>a</sup>	0.620 ± 0.042 <sup>c</sup>

Values are expressed as Mean ± SD

Values with different superscript down the column are significantly different at ( $p < 0.05$ )

## CONCLUSION

Based on the findings in this study, it can be concluded that doum fruit (*H. thebaica*) some potent antioxidant, hypolipidemic and

antihypertensive effects, and can be used to protect the body against adverse effects of high fat diet-induced obesity, thereby reducing the risk of cardiovascular diseases.

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