National Research Centre[1], Pharmaceutical Industries Division, Chemistry of Natural Compounds Department; National Research Centre[2], Medical Research Division, Pharmacology Department, Dokki, Cairo, Egypt

# Comparative studies on hypocholesterolemic effect of different fractions of *Hyphaene thebaica* (Doum) in experimental animals

M. H. Hetta[1], N. Z. Yassin[2]

*Received December 29, 2004, accepted March 24, 2005*

*Dr. Mona H. Hetta, Department of Pharmacognosy, Beni-Sueif University, Beni-Sueif, Egypt. P.O. 32514 monahetta@yahoo.com*

*Pharmazie 61: 230–232 (2006)*

A comparison of different fractions of the fruit of *Hyphaene thebaica* (Doum) was performed in order to investigate their effects on serum cholesterol, triglycerides and lipoproteins: HDL (high density lipoprotein) cholesterol and Non-HDL cholesterol in normal rats. Female Sprague-Dawely rats were treated orally with different fractions of the Doum plant. We used atorvastatin and a natural extract of *Monascus purpureus* as references. The total cholesterol, HDL cholesterol, Non-HDL cholesterol and triglycerides were estimated. Three fractions of the Doum plant exhibited a highly significant decrease in serum cholesterol and Non-HDL cholesterol. One fraction exhibited a highly significant decrease in cholesterol level but with only a moderately significant effect in decreasing the Non-HDL level. Decreasing Non-HDL, especially LDL, cholesterol, can reduce the risk of atherosclerosis and subsequent cardiovascular diseases. The natural, safe and non-toxic Doum plant could be of great merit for use as a hypocholesterolemic drug.

## 1. Introduction

*Hyphaene thebaica* L. Mart (Doum palm) is an edible wild plant, with little or no toxic effect. It is native to Upper Egypt, Sudan, Kenya and Tanzania. It was considered sacred by the ancient Egyptians. Published literature mentions that the aqueous extract of the fruit has antihypertensive activity. It is also reported that the aqueous extract of the fruit contains alkaloids, reducing sugars and glycosides (Sharaf et al. 1972). The aqueous extract may have value in the treatment of fungal diseases since it has antifungal activity (Irobi and Adedayo 1999). Recently, aqueous and alcoholic extracts of the fruits (epicarp and mesocarp) has been found to induce significant anti-inflammatory, antipyretic, analgesic and antiepileptic activity (Amin and Mahmoud 1999). The thick roots of Doum are used in some cases for treatment of bilharziasis (Boulos 1983). The resin of the tree has diuretic and diaphoretic properties. As part of a series of experiments to obtain a natural product with cholesterol lowering capacity from plants, the aim of this work was to test the hypocholesterolemic effect of different fractions of *Hyphaene thebaica* in normal rats.

## 2. Investigations, results and discussion

Preliminary screening revealed the presence of steroids and triterpenoids in the chloroformic fraction (D), alkaloids in the chloroformic fraction (D) and the ethyl acetate fraction (E), saponins in the total water extract together with the ethanol/water extract (B) and the water after methanol fraction (G). It was also shown the presence of phenolic compounds in all the fractions except (D) and (G) and carbohydrates and glycosides in all the fractions except (D) and (E).

Oral treatment of mice with different doses (1.8, 3.5, 4, 2.5, 0.5, 7 and 3 g/kg b.w. for A, B, C, D, E, F and G respectively, which are the maximum concentrations soluble in 1 ml distilled water) of *Hyphaene thebaica* fruit extract were carried out to determine their lethal effect and any toxic symptoms. Results obtained showed no sign of toxicity for any of the fractions: total water extract (A); extract (B); extract (C); chloroform fraction(D); ethyl acetate fraction (E); methanol fraction (F); and water after methanol fraction (G).

The effect of the different extracts and the reference drugs on the total lipid level is summarized in the Table. All the fractions lowered TC and Non-HDL cholesterol levels significantly ($p < 0.001$) after 2 weeks of treatment. The methanol fraction (700 mg/kg b.w.) and extract (C) (400 mg/kg b.w.) did not induce any significant difference in HDL-C level from the control level. After 4 weeks, the most noteworthy fractions were extract D (chloroform) (250 mg/kg b.w.), extract G (water after methanol) (300 mg/kg b.w.) and extract A (total water) (180 mg/kg b.w.). They caused a highly significant decrease in TC and Non-HDL cholesterol ($p < 0.001$). The methanol fraction induced a highly significant effect in decreasing TC, ($p < 0.001$) and a significant effect on Non-HDL cholesterol ($p < 0.005$). HDL-C was slightly reduced by all the fractions except for the ethyl acetate fraction which had almost no effect on HDL-C. After the first 15 days TG levels had increased, but after 30 days, the TG levels were reduced, especially with extract (B) with high significance ($p < 0.001$). Extract (C) increased the TG level ($p < 0.05$). On comparing the effect of the fractions tested

**Table: Effect of different fractions and extracts of *Hyphaene thebaica* L. Mart on the lipid profile in normal albino rats after 2 and 4 weeks**

| Parameter | Dose p.o. (g/kg b.w.) | TC mg/dl | TG mg/dl | HDL mg/dl | Non HDL mg/dl |
|---|---|---|---|---|---|
| After 2 weeks | | | | | |
| negative control | – | 117.2 ± 10.60 | 113.80 ± 7.97 | 49.30 ± 4.31 | 70.80 ± 7.51 |
| A | 0.18 | 65.00 ± 4.92 | 201.75 ± 6.35 | 23.25 ± 0.80 | 41.75 ± 4.80 |
| B | 0.35 | 65.00 ± 3.98 | 167.16 ± 10.65 | 37.08 ± 4.37 | 27.91 ± 6.32 |
| C | 0.4 | 67.33 ± 1.74 | 209.00 ± 8.48 | 48.33 ± 1.94 | 19.00 ± 1.98 |
| D | 0.25 | 58.90 ± 2.80 | 158.33 ± 27.88 | 21.33 ± 1.45 | 37.56 ± 4.02 |
| E | 0.05 | 65.66 ± 4.17 | 196.66 ± 11.65 | 41.33 ± 5.00 | 24.33 ± 2.42 |
| F | 0.7 | 72.66 ± 8.51 | 204.50 ± 14.50 | 48.83 ± 6.37 | 23.83 ± 4.19 |
| G | 0.3 | 59.25 ± 2.73 | 165.50 ± 11.48 | 22.56 ± 0.77 | 36.68 ± 2.60 |
| Atorvastatin | 0.0009 | 83.00 ± 8.75 | 85.00 ± 10.50 | 39.50 ± 6.60 | 43.50 ± 4.71 |
| *Monascus purpureus* extract | 0.054 | 69.56 ± 5.69 | 95.16 ± 2.45 | 30.70 ± 3.58 | 38.85 ± 3.09 |
| After 4 weeks | | | | | |
| negative control | – | 91.05 ± 5.06 | 186.16 ± 4.92 | 39.15 ± 5.45 | 51.90 ± 5.19 |
| A | 0.18 | 57.08 ± 0.85*** | 177.33 ± 7.71 | 21.25 ± 0.35 | 35.83 ± 0.84** |
| B | 0.35 | 75.95 ± 3.37 | 112.80 ± 8.63*** | 27.50 ± 1.38 | 48.45 ± 3.97 |
| C | 0.4 | 69.95 ± 4.91 | 206.50 ± 8.30 | 21.33 ± 1.58 | 48.61 ± 5.73 |
| D | 0.25 | 53.80 ± 2.24*** | 176.33 ± 10.13 | 20.66 ± 1.97 | 33.16 ± 2.60 *** |
| E | 0.05 | 74.64 ± 6.11 | 173.20 ± 7.72 | 38.16 ± 8.33 | 44.64 ± 4.96 |
| F | 0.7 | 65.76 ± 3.38*** | 185.50 ± 6.00 | 29.00 ± 1.65 | 36.76 ± 2.77 |
| G | 0.3 | 51.33 ± 3.22*** | 164.00 ± 14.95 | 18.58 ± 0.86 | 32.75 ± 3.57 *** |
| Atorvastatin | 0.0009 | 86.24 ± 9.24 | 123.00 ± 14.04*** | 49.80 ± 6.80 | 36.44 ± 2.97 |
| *Monascus purpureus* extract | 0.054 | 83.16 ± 1.09 | 126.30 ± 9.42 *** | 44.66 ± 2.18 | 38.51 ±0.89 *** |

Means ± SEM
p.o per oral
Number of animals/group = 6 rats
*** highly significant $P < 0.001$
** significant $P < 0.005$
A: total water; B: extract B; C: extract C; D: chloroform fraction; E: ethyl acetate fraction; F: methanol fraction; G: water after methanol fraction
TC: total cholesterol; TG: triglycerides; HDL: high density lipoprotein; Non HDL: non high density lipoprotein

with the reference drugs, we noted that the mode of action of the fractions tested depends on lowering the TC and Non-HDL cholesterol levels in blood serum. This effect could be attributed to the presence of pectins. Pectins lower TC by decreasing the absorption of cholesterol from food. The faecal output of cholesterol is increased by pectin supplemented diets. Studies have shown that pectins only decrease the "bad" cholesterol fractions LDL and VLDL. They do not alter HDL cholesterol which protects the body from atherosclerosis (Sheehan-John et al. 1997; Alpana 1996).

The effect of the fractions may be attributed to the presence of flavonoids or phenolic compounds which act as antioxidants. The fatty acids in LDL and/or endothelial cell membrane attacked by free radicals, in a reaction called lipoperoxidation, are changed to lipoperoxides, which are atherogenesis, factors. Thus, preventing LDL and membrane fatty acids from lipoperoxidation by antioxidants like flavonoids or polyphenols could be an effective tool in the prevention of atherosclerosis (Remesy et al. 1996).

The effect of the fractions could also be attributed to the presence of sterols or fatty acids. As mentioned in the literature, plant sterols reduce cholesterol, LDL and the LDL/HDL ratio without affecting lipid-soluble provitamins, so the serum lipids become less atherogenic (Arnqvist et al. 2003; Jones et al. 1999). Unsaturated fatty acids (esters of linoleic, linolinolic and oleic acid with phospholipids) decrease cholesterol and triglyceride levels (Chilmonczyk et al. 2001).

The level of LDL in a person's blood is linked to atherosclerosis. Having a high level of LDL cholesterol puts a person at risk of having coronary heart disease. Recent studies have shown that plasma levels of Non-HDL cholesterol and HDL cholesterol modulate the risk of cardiovascular diseases. Increased plasma levels of Non-HDL cholesterol, especially LDL cholesterol, and decreasing levels of HDL cholesterol are associated with increasing risk of these diseases (Sehayek et al. 2003).

Further studies are in progress to compare and evaluate the main active groups responsible for the hypocholesterolemic action of the *Hyphaene thebaica* fruit, to make use of this natural source in the pharmaceutical industry.

## 3. Experimental

### *3.1. Phytochemical Study*

#### *3.1.1. Plant material*

Doum plants were collected from Upper Egypt and authenticated. Voucher specimens are deposited in the laboratory of the Chemistry of Natural Compounds Department, NRC, Dokki, Cairo, Egypt.

#### *3.1.2. Sample preparation*

Preparation of samples for the hypocholesterolemic study is illustrated in the Scheme.

**Scheme**

Weight of *Hyphaene thebaica* (L).Mart fruit

| | |
|---|---|
| Slices of the plant<br>↓ Extraction with dist $H_2O$<br>Total $H_2O$ extract (**A**)<br>↓ EtOH absolute: dist $H_2O$ (2 : 1)<br>Residue (extract **B**),<br>Supernatant (extract **C**), | Slices of the plant<br>↓ Extraction with $CHCl_3$<br>$CHCl_3$ extract (**D**)<br>↓ Extraction with EtAc<br>EtAc extract (**E**)<br>↓ Extraction with MeOH<br>MeOH extract (**F**)<br>↓ Extraction with dist $H_2O$<br>$H_2O$ extract (**G**) |

*3.1.3. Phytochemical screening*

Freshly prepared *Hyphaene thebaica* extracts were subjected to phytochemical screening tests for the detection of various constituents.

### *3.2. Pharmacological study*

*3.2.1. Animals*

Sixty four Sprague dawely female rats weighing between 150 and 200 g and albino mice (25–30 g) were obtained from a breeding colony of the National Research Centre (NRC), Dokki, Egypt. Animals were kept under the same hygienic conditions, and a well balanced diet and water were supplied *ad libitum*.

*3.2.2. Diet*

Normal diet consisted of vitamin mixture 1%, mineral mixture 4%, corn oil 10%, sucrose 20%, cellulose 0.2%, casein (95% pure) 10.5%, starch 54.3% (Banha Company).

*3.2.3. Drugs*

Atorvastatin, manufactured by Pfizer Egypt S.A.E. Cairo A.R.E. under authority of Pfizer Inc, USA. A natural extract of *Monascus purpureus* manufactured by Biopharm, Egypt.

*3.2.4. Hypocholesterolemic studies*

The animals were divided into eight groups: the first group serving as the negative control group, received a daily oral dose of 1 ml distilled water. The other seven groups were tested for the effects of the different extracts: $2^{nd}$ group was fed with 0.18 g/kg b.w. of total water extract, $3^{rd}$ group was fed with 0.35g/kg b.w. of extract B (residue after treatment of total water extract with ethanol: water, 2 : 1), $4^{th}$ group was fed with 0.4 g/kg b.w. of extract C (supernatant after treatment of total water extract with ethanol: water, 2 : 1), $5^{th}$ group was fed with 0.25g/kg b.w. of $CHCl_3$ fraction, $6^{th}$ group was fed with 0.05 g/kg b.w. of ethyl acetate fraction, $7^{th}$ group was fed with 0.7g/kg b.w.of MeOH fraction and $8^{th}$ group was fed with 0.30g/kg b.w. of $H_2O$ after MeOH fraction.
The reference drugs were used in doses of 0.9 mg/kg b.w. for atorvastatin and 54 mg/kg b.w. for the *Monascus purpureus* extract.
Blood samples from the retro-orbital plexus were drawn after overnight fasting (12–14 h) before treatment (0 week) then after 2 and 4 weeks of treatment.

TC, TG, and serum HDL-C levels were determined in the serum using an Olympus Automatic analyzer 400. HDL-C level was determined in the serum after treatment with phosphotungestic acid magnesium chloride. Non-HDL level was calculated by difference.

*3.2.5. Statistical analysis*

All the results tabulated was expressed as means ± SEM, and are compared using Student's *t* test. A p value $<0.001$ is considered highly significant, and $p < 0.005$ is considered significant.

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