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Hypolipidemic efficacy of *hyphaene thebaica* (doum) in experimental nephrotic syndrome

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ABSTRACT

The water extract of the fruits of *Hyphaene thebaica* were given orally to male albino rats for 30 days to evaluate its effect on serum cholesterol, triglycerides, lipoproteins: HDL - LDL & VLDL (high - low and very low density lipoprotein) and apolipoproteins (A-1 & B). Our findings exhibited a highly significant decrease ($p < 0.05$) in all parameters except for HDL which showed insignificant decrease when compared to control group. Thus the water extract can reduce hyperlipidemia in nephrotic syndrome and leads to decrease the risk of glomerulosclerosis atherosclerosis and consequently the natural, safe and non-toxic *Hyphaene thebaica* fruit could be of great merit for use as hypolipidemic drug. Further, the phytochemical analysis of the potent water extract indicated the distribution of 14 polyphenolic compounds to which its activity maybe attributed. Among the isolated compounds tricin 5-*O*-rutinoside, kaempferol 3-*O*-rutinoside and rhamnazin 3-*O*-rutinoside were isolated, purified and identified from the fruit for the first time. The structure elucidation was based on ^{1}H and ^{13}C NMR.

Keywords: *Hyphaene thebaica* fruits, flavonoids, hypolipidemic efficacy.

INTRODUCTION

Nephrotic syndrome is a well recognized renal disorder of varied etiology; and is characterized by a special plasma lipids and lipoproteins profile whatever the cause is. This lipids and lipoproteins profile shows hypercholesterolemia, raised LDL and VLDL pattern, and decreased HDL concentration. The latter is especially manifest in human nephrotic syndrome [1]. Nephrotic syndrome patients frequently have hyperlipidemia and heypercoagulability and the incidence of myocardial infarction (MI) is eight times higher than normal in patients with nephortic syndrome [2].

The association between hyperlipidemia and the nephrotic syndrome is well established although; the underlying pathophysiological mechanisms have yet to be fully defined. Recently, it has even been suggested that secondary hyperlipidemia resulting from nephrotic syndrome can exacerbate the primary renal disorder [3, 4, 5]. Despite lack of knowledge of the causes and consequences of nephrotic dyslipidemia, many nephrologists understandably believe that efforts should be made to correct these abnormalities, based on proven adverse effects of similar lipid patterns in other populations [6, 7]. As well as **s**tudies in nephortic animals showed that progressive renal disease could be accelerated by diet induced hyperlipidemia and, vise versa that the development of glomerulosclerosis could be retarded by antihyperlipidemic drugs[8,9]. Doum (*Hyphaene thebaica*) is an African palm tree, common in Upper Egypt, originally native to the Nile valley, bearing an edible fruit which is glubose-quandrangular, about 6 x 5 cm with a shinny orange-brown to deep chestnut skin (epicarp). It was considered sacred by the Ancient Egyptians and its seeds were found in many pharaohs' tombs e.g. Tutankhamun's tomb [10].

Using of the fruit which is rich in polyphenols, flavonoids, saponins and tannins [11] in folk medicine is not surprising due to its nutritional and pharmacological properties. Doum extracts are being

used in the treatment of haematuria, bleeding especially after child birth [12], and also as hypo-lipidemic and haematinic suspension [13]. The tea of doum is popular in Egypt and believed to be good for diabetes [14]. Roots of doum were used in treatment of Bilharziasis, while the resin of the tree has demonstrated, diuretic, diaphoretic properties and also recommended for tap worm as well as against animal bites [15]. Meanwhile, Hetta and Yassin[16] reported that constituents of the doum exhibited a significant decrease in serum total cholesterol and Non-HDL cholesterol in rats; this can reduce the risk of atherosclerosis and subsequent cardiovascular diseases.

Therefore, attempts were done to clarify the role of water extract of doum fruit on some biochemical and hematological parameters in rats to establish safety or otherwise, as well as haematinic potentials. Moreover, the water extract was phytochemically investigated and afforded 14 flavonoid and phenolic compound namely; vitexin (**1**), isovitexin (**2**), tricin-5-*O*-glucopyranoside (**3**), luteolin-7-*O*-glucopyranoside (**4**), kaempferol-3-*O*-rutinoside (**5**), quercetin-3-*O*-glucopyranoside (**6**), querectin-7-*O*-glucopyranoside (**7**), rhamnazin-3-*O*-rutinoside (**8**), gallic acid (**9**), methyl gallate (**10**), luteolin (**11**), tricin (**12**), quercetin (**13**) and kaempferol (**14**). Among which tricin-5-*O*-glucopyranoside, kaempferol-3-*O*-rutinoside, rhamnazin-3-*O*-rutinoside were isolated for the first time from the fruit of doum.

MATERIALS AND METHODS

Plant material: The fruit of the plant was collected from Gohayna Wednesday market. Sohag, Egypt. The plants were authenticated by Dr. Mahmoud El-Dabaa**,** Department of Botany, National Research Center, Cairo, Egypt. A voucher specimen (no: D 13) has been deposited in the National Research Centre Herbarium.

General: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR: Joel spectrometer (Kyoto, Japan) in DMSO-d_6; UV: Shimadzu spectrophotometer model UV-240 (Kyoto, Japan); ESI-MS: on a Finnigan MAT 4600 spectrometer; CC: Sephadex LH-20 (Fluka); PC was carried out on Whatman No.1 and 3MM paper using the following solvent systems: (1) BAW (*n*-BuOH/AcOH/H_2O, 4:1:5(upper layer)); (2) H_2O; and (3) AcOH/H_2O (15:85).

Preparation of plant extract: The fruit was cleaned, debris removed and then separated into pulp and seed. The pulp was dried and ground into powder. The fine powder was sieved through a 0.25mm sieve (Endicott= s Test Sieves ltd, London, UK). 1 kg powdered dry fruit was successfully extracted with water at 60°C for 10 hr. The extract was concentrated under reduced pressure and evaporated to dryness.

Phytochemical screening of the plant extract: The extract (150g) was applied on Sephadex LH-20 column, using H_2O and H_2O/MeOH mixtures of decreasing polarities as solvent system. Five fractions (I-IV) were eluted which were further applied on preparative paper chromatography and/or Sephadex LH-20 columns, using different solvent systems. This resulted in the isolation of 14 flavonoid compounds.

Experimental animals: Thirty male albino rats weighing between 160-190 gm were obtained for the present study from the animal house of the National Research Centre. Rats were fed on a stock diet composed of whole wheat meal, skimmed milk powder, fish flour, dry yeast, stabilized vitamin A, D and NDP energy of 12 %. Diet was given in plenty to the rats throughout the experimentation period (for 3 months). Animals were provided with water *ad-libitum*. The rats were divided into three equal groups (each group consisting of 10 rats);

Group I: saline-treated group served as healthy controls.

Group II,III: had their kidneys made nephrotic by injecting them intraperitoneally with 0.2 cc low speed supernatant (3000-4000 rpm for 1-1.5 hours) of 1:2 blood free own kidney in saline obtained by previous unilateral nephrectomy with 0.3 Freund's complete adjuvant (is an antigen emulsified in mineral oil, used to stimulate production of tumor necrosis factor) . Each rat was injected whole six injections one each 14 days) according to the technique of Heymann et al [17]. Rats of group III were treated orally with water extract of *Hyphaene thebaica*. The rats were kept under normal standard breeding conditions of temperature and humidity for the period of the study. They were then sacrificed by decapitation twenty four hours after the last treatment.

Unilateral Nephrectomy: General anesthesia of rats was done through inhalation of diethyl ether; the hair at the site of operation was clipped and shaved then washed by soap and water, and disinfected by alcohol 70%, followed by Tincture iodine 4%. A longitudinal incision in the skin and muscles was made, and by blunt forceps the wound was widened till appearance of the kidney. Strong ligation to the renal artery and renal vein were made separately followed by excision of kidney. After nephrectomy, cooptation of the wound occurred by lamberts' sutures. Intraperitoneal injections of operated rats had been done after complete recovery for about four weeks.

Biochemical analysis: Blood samples were obtained from the eye under ether anesthesia. Blood was collected by using syringe into dry clean test tubes, then allowed to clot and centrifuged at 4000 rpm for 10 minutes to separate the serum. Serum was collected into dry clean test tubes and glucose and low density lipoprotein (LDL-Cholesterol) were determined immediately. The rest of serum was frozen at -20^{o}C for the subsequent estimation of the other parameter.

Determination of serum total cholesterol: Total cholesterol was determined using the enzymatic method of Allain et al [18]. The total cholesterol kit of Bio-Merieux laboratory reagents and products is used. Bio-Merieux 69280 Marcy, l'etoile, France.

Determination of serum Triglycerides: Serum triglycerides were determined by the quantitative enzymatic calorimetric determination of triglycerides according to Wahlefeld [19] by using Stanbio Enzymatic 2930 East Houston Street, San Antonio Texas 78202 USA.

Determination of Serum Phospholipid: Serum phospholipid was determined by enzymatic method according to Trinder [20], using phospholipids kit of Quimica Clinica Aplicada 43870 Amposta, Tarragona, Spain.

Determination of serum HDL-Cholesterol: Was done according to Glatter equation [21] where; HDL= total cholesterol - LDL – TG/5.

Determination of serum LDL-Cholesterol: Serum LDL cholesterol was determined according to Steinberg [22], by using LDL cholesterol/ phospholipids kit of Bio Merieux laboratory reagents and products Bio Merieux 69280 Marcy, l'etoile, France.

Determination of serum VLDL- Cholesterol: was done according to Glatter equation [21].

Determination of serum apolipoprotein A-1 and B: Apolipoprotein A-1 and B were estimated by imrnunoprecipitin analysis method according to Finley et al [23]. Apo A-1 kits obtained from Atlantic Antibodies, An INCSTAR Company INCSTAR corporation-Stillwater, Minnesota, U.S.A.

Statistical analysis of data: Data are expressed as mean ± S.E. Statistical significance of the difference was analyzed using one way-ANOVA and post-hoc Duncan test for multiple group comparison. P values of <0.05 were considered statistically significant. The correlation coefficient ® which is a measure of the degree of closeness of the linear relationship between two variables (X and Y) was determined, r always lies between -1 and +1

RESULTS

There was a highly significant decrease in all parameters of lipids, lipoprotein level, a highly significant increase in apolipoproteins A, an insignificant decrease in HDL in group III compared with its level in group II as shown in Tables 1 and 2, while a highly significant increase ($p < 0.05$) in all parameters of lipids, lipoprotein, apolipoproteins (A&B) except for HDL which showed insignificant change in group II compared with its level in group I. (Tables 1 and 2)

According to the significant biological activity of the water extract of *Hyphaene thebaica* fruits, phytochemical investigations were applied using column and preparative paper chromatography which resulted in 14 compounds: vitexin (**1**), isovitexin (**2**), tricin-5-*O*-rutinoside (**3**), luteolin-7-*O*-glucopyranoside (**4**), kaempferol-3-*O*-rutinoside (**5**), quercetin-3-*O*-glucopyranoside (**6**), querectin-7-*O*-glucopyranoside (**7**), rhamnezin-3-O-rutinoside (**8**), gallic acid (**9**), methyl gallate (**10**), luteolin (**11**), tricin (**12**), quercetin (**13**) and kaempferol (**14**). The structure of these compounds was determined by their chromatographic behaviors as well as spectroscopic analysis whereby compounds (**3**), (**5**) and (**8**) were isolated from the fruits for the first time "Figure 1". Thus, their spectral data is represented as follow:

*Tricin-5-O-rutinoside (**3**)*; yellowish powder, $C_{29}H_{34}O_{16}$, ESI-MS (negative, *m/z*): 654.56; UV (λ_{max}, nm) MeOH: 244, 269; +NaOMe: 253, 272 sh, 395 + NaOAc: 262, 275 sh, 320, 412; +NaOAc/H_3BO_3: 270, 302 sh, 348, 420; + $AlCl_3$: 245, 270 sh, 300, 355; ^{1}H-NMR (500 MHz, DMSO) δ: 6.89 (s, H-2', H-6'), 6.83 (br. s, H-3), 6.39 (d, *J*= 2.1 Hz, H-8), 6.13 (d, *J*= 2.1 Hz, H-6), 5.16 (d, *J*=7.8 Hz, H-1"), 4.69 (d, *J*=1.8 Hz, H-1'"), 3.74 (dt, *J*=10.9, 1,0 Hz, Hβ-6"), 3.73 (s, 3' & 5'-OMe), 3.52 (dd, *J*=3.5, 2.0 Hz, Hα-6"), 3.45-3.24 (m, H-2", H-3", H-4", H-5", H-2'", H-3'", H-4'", H-5'"), 1.14 (3H, d, *J*=6.3 Hz, C-6'"). ^{13}C-NMR (DMSO-d_6): aglycone moiety: (ppm) 162.4 (C–2); 106.3 (C-3); 177.0 (C-4); 158.3 (C-5); 104.3 (C-6); 161.0 (C-7); 98.5 (C-8); 158.5 (C-9); 108.1 (C-10); 120.4 (C-1'); 104.4 (C-2'); 148.1 (C-3'); 139.4 (C-4'); 148.1 (C-5'); 104.4 (C-6'); Sugar moieties: (ppm) 104.0 (C-1"); 73.59 (C-2"); 76.04 (C-3"); 70.8 (C-4"); 76.75 (C-5"); 68.84 (C-6"); 100.38 (C-1'"); 71.22 (C-2'"); 70.8 (C-3'"); 72.54 (C-4'"); 68.84 (C-5'"); 18.3 (C-6'"), 56.3 (3' &5'-OMe).

*Kaempferol-3-O-rutinoside; nicotiflorine (**5**)*, yellow powder, $C_{27}H_{30}O_{15}$, ESI-MS (negative, *m/z*): 593.51; UV (λ_{max}, nm) MeOH: 265, 350; +NaOMe: 275, 310;

+ NaOAc: 273, 355; +NaOAc/H_3BO_3: 271, 355; + $AlCl_3$: 272, 408; ^{1}H-NMR (500 MHz, DMSO) δ: 8.07 (d, *J*=8.5 Hz, H-2', H-6'), 6.80 (d, *J*=8.5 Hz, H-3', H-5'), 6.42 (d, *J*=2.0 Hz, H-8), 6.22 (d, *J*=2.0 Hz, H-6), 5.13 (d, *J*=7.8 Hz, H-1"), 4.52 (d, *J*=1.8 Hz, H-1'"), 3.81 (dt, *J*=10.9 & 1,0 Hz, Hβ-6"), 3.63 (dd, *J*=3.5 & 2.0 Hz, H-2'"), 3.52 (dd, *J*=9.5 & 3.5 Hz, H-3'"), 3.48 (m, H-5'"), 3.26-3.48 (m, H-2", H-3", H-4", H-5"), 3.37 (m, Hα-6"), 3.27 (m, H-4'"), 1.12 (d, *J*=6.3 Hz, C-6'"). ^{13}C NMR (DMSO, 125 MHz) δ: 158.74 (C-2), 135.68 (C-3), 178.63 (C-4), 164.74 (C-5), 100.13 (C-6), 166.20 (C-7), 95.05 (C-8), 159.59 (C-9), 105.39 (C-10), 122.94 (C-1'), 132.53 (C-2'), 116.30 (C-3'), 161.67 (C-4'), 116.30 (C-5'), 132.53 (C-6'), 104.72 (C-1"), 75.93 (C-2"), 77.40 (C-3"), 71.62 (C-4"), 78.32 (C-5"), 68.73 (C-6"), 102.60 (C-1'"), 72.46 (C-2'"), 72.46 (C-3'"), 74.06 (C-4'"), 69.89 (C-5'"), 18.06 (C-6'").

*Rhamnazin -3-O-rutinoside (**8**)*; yellow amorphous powder, $C_{29}H_{34}O_{16}$, ESI-MS (negative, *m/z*): 637.57; UV (λ_{max}, nm) MeOH: 257, 358; +NaOMe: 262, 413; +NaOAc: 259, 365; +NaOAc/H_3BO_3: 256, 364; + $AlCl_3$: 292, 370; ^{1}H-NMR (500 MHz, DMSO) δ: 8.42 (d, *J*=1.8 Hz, H-2'), 7.96 (dd, *J*=8.5 & 1.8 Hz, H-6'),7.43 (d, *J*=8.5 Hz, H-5'), 6.64 (d, *J*=1.8 Hz, H-8), 6.57 (d, *J*=1.8 Hz, H-6), 6.31 (d, *J*=7.3 Hz, H-1"), 5.35 (br s, H-1'"), 4.53 (dd, *J*= 11.6 & 1.2, Hα-6"), 4.39 - 4.10 (m, H-2", H-3", H-4", H-5", H-2'", H-3'", H-4'", H-5'"), 3.99 (dt, *J*=10.9 & 1,0 Hz, Hβ-6"), 3.93 (s, 3'-OMe), 3.73 (s, 7-OMe), 1.48 (d, *J*= 6.1, H-6'"); ^{13}C NMR (DMSO, 125 MHz) δ: 158.1 (C-2), 135.1 (C-3), 178.8 (C-4), 162.3 (C-5), 98.6 (C-6), 165.9 (C-7), 92.6 (C-8), 157.4 (C-9), 106.3 (C-10), 121.94 (C-1'), 114.3 (C-2'), 149.5 (C-3'), 150.3 (C-4'), 116.4 (C-5'), 123.7(C-6'), 103.8 (C-1"), 76.0 (C-2"), 77.5 (C-3"), 71.5 (C-4"), 78.5 (C-5"), 68.3 (C-6"), 102.6 (C-1'"), 72.5 (C-2'"), 72.6 (C-3'"), 73.9 (C-4'"), 69.7 (C-5'"), 18.5 (C-6'"), 56.1 (3'-OMe), 55.9 (7-OMe).

DISCUSSION

In the human body, high levels of cholesterol, triglycerides, low density lipoprotein (LDL) and oxidized LDL particles in the bloodstream are strongly associated with atheroma formation in the walls of arteries (atherosclerosis), which is the principal cause of cardiovascular diseases and stroke. In contrast, high density lipoprotein (HDL) particles have been identified as a mechanism by which cholesterol can be removed from atheroma. HDL particles transport cholesterol back to the liver for excretion. Increased concentrations of HDL correlate with lower rates of atheroma progressions and even regression. There is a strong inverse relationship between triglyceride level and HDL level. However, the negative impact of raised levels of triglycerides is lower than the ratio between LDL and HDL [24]. Our results showed a significant decrease in total cholesterol, triglycerides and LDL. Meanwhile, we found a significant increase in HDL, that in agreement with [14]. These results could reduce atheroma formation in the walls of arteries (atherosclerosis), thus reducing the risk of cardiovascular diseases and stroke. Moreover, lowering the blood concentration of triglycerides helps to lower the amount of LDL. Hayek et al. [25] and Nigdikar et al. [26] reinforce the physiological relevance of these observations because they found that consumption of polyphenols or catechin is associated with reduced susceptibility of LDL to oxidation and aggregation.

Since polyphenols have been shown to reduce the development of atherosclerosis in animal models [27, 28] therefore the improvement of the lipoprotein profile in response to doum supplementation was expected, parallel to reductions in the concentrations of both LDL and apo B, and increases in HDL and apo A-I as the range of flavonoid glycosides isolated from *Hyphaene thebaica* in this study is extensive and includes a flavone O-glycoside (tricin-5-*O*-rutinoside; **3** & luteolin-7-*O*-glucopyranoside; **4**), C-glycosides (vitexin; **1** & isovitexin; **2**), flavonol-3-O-glycosides (kaempferol-3-O-rutinoside; **5**, quercetin-3-O-glucopyranoside; **6** & querectin-7-O-glucopyranoside; **7**) and a number of methoxyflavonol O-glycosides (rhamnezin-3-O-rutinoside; **8**). This effect of Doum could be attributed to the flavonoids content and must be considered as beneficial in the reduction of cardiovascular risk in these patients because they are consistent with the report of National Cholesterol Education Program (2002), which suggested that the normal total blood cholesterol level should be < 200 mg/dl, and the desirable LDL level is considered to be less than 100 mg/dL, although a newer target of < 70 mg/dL can be considered in higher risk individuals.

Also, in men without cardiovascular disease there is a benefit from lowering abnormally high cholesterol levels [29] and it has been extensively demonstrated in clinical trials that treatment of dyslipidemic patients with drugs that decrease LDL cholesterol levels significantly reduces the risk for coronary heart disease [30, 31]. It is postulated that ingesting antioxidants and minimizing free radicals may reduce the contribution of LDL to atherosclerosis [32]. Doum contains tannins, flavonoids, and saponins which are antioxidants and play an important role in scavenging free radicals [33, 34] so they are helpful to prevent oxidation of LDL decreasing its harmful effects.

The data from animal experiments suggest that treatment that corrects hyperlipidemia may have an ameliorative effect on renal function. Thus, there are strong indications that lipoproteins may play a critical role in mediating the development of glomerulosclerosis [35, 7].

CONCLUSION

The results suggested a positive relationship between total phenolic and flavonoid content of *Hyphaene thebaica* with reducing hyperlipidemia in nephrotic syndrome and lead to decrease the risk of glomerulosclerosis and atherosclerosis. Thus, the natural, safe and non-toxic *Hyphaene thebaica* plant could be of great merit for use as hypolipidemic drug.

Table 1: Serum lipid fractions in different experimental groups

Group	VLDL(mg/dl)	HDL(mg/dl)	Ph.L(mg/dl)	TG(mg/dl)	T.Ch(mg/dl)
GroupI (N=10), Mean ±S.D	13.27±0.59	41.38±0.86	127.99±3.13	66.38±1.96	84.69±3.59
GroupII(N=10), Mean ±S.D	32.45*±0.99	54.81*±0.90	210.00*±5.80	162.24*±3.95	142.41*±5.01
GroupIII(N=10), Mean ±S.D	22.43$^{**\#}$±1.18	46.47** ±1.36	157.1$^{**\#}$±36.40	112.15$^{**\#}$±2.90	100.95$^{**\#}$±4.23

- *Group I= control rats, Group II= Nephrotic rats, Group III= Nephrotic rats treated with Doum*
- *Statistical differences were observed between:*

**p < 0.05: control & nephrotic rats*
***p< 0.05: nephrotic & nephrotic treated with doum*
#p<0.05: control & nephrotic treated with doum

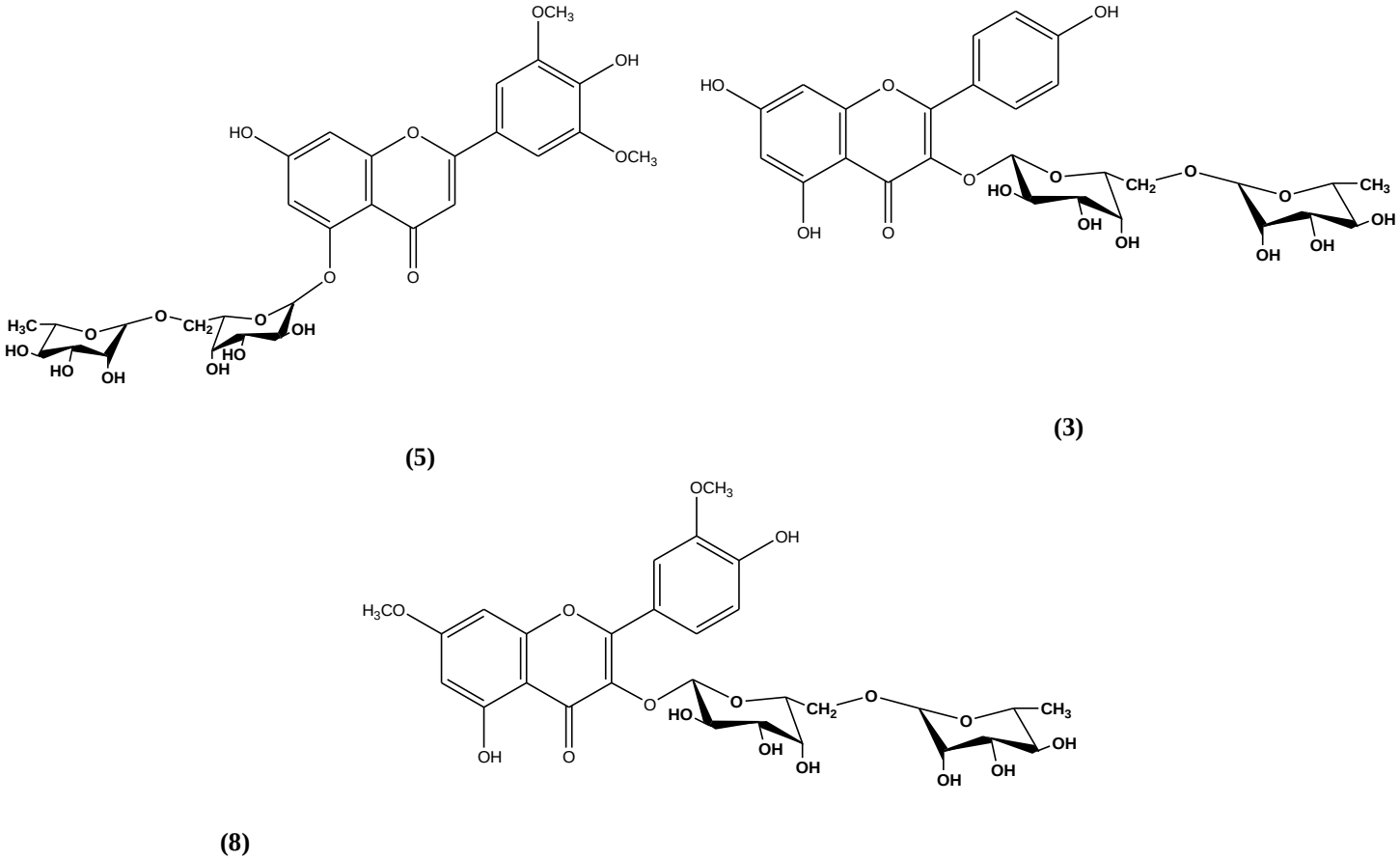
- *Total cholesterol (T.Ch), Triglycerides (TG), Phospholipids (Ph.L), High-Density Lipoprotein (HDL) and Very Low-Density Lipoprotein (VLDL).*

Table 2: Serum Apolipoprotein A-1 & Apolipoprotein B levels in in different experimental groups

Group	Apolipoprotein A-1 (mg/dl)	ApolipoproteinB (mg/dl)
GroupI (N=10), Mean ±S.D	45.21±2.49	31.65±1.99
GroupII(N=10), Mean ±S.D	102.98*±3.49	81.22*±2.96
GroupIII(N=10), Mean ±S.D	120.95$^{**\#}$±4.23	61.60$^{**\#}$±2.22

- *Group I= control rats, Group II= Nephrotic rats, Group III= Nephrotic rats treated with Doum*
- *Statistical differences were observed between:*

**p < 0.05: control & nephrotic rats*
***p< 0.05: nephrotic & nephrotic treated with doum*
#p<0.05: control & nephrotic treated with doum



(5) **(3)**

(8)

Fig. 1: Structure of isolated compounds for the first time of *Hyphaene thebaica* fruits

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