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Hepatoprotective activity of *Schouwia thebica* webb

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Abstract—Oral administration of alcoholic extracts of *Schouwia thebica* Webb showed that extracts are safe for human use. The studied extracts are considered safe, since they failed to induce death of mice in doses up to 4000 mg/kg body weight. Hepatoprotective activity was studied for the total alcoholic extracts. The total extract was fractionated in turn with diethyl ether, chloroform, ethyl acetate, and *n*-butanol, respectively. These extracts were tested for possible hepatoprotective activity. It was found that the ethyl acetate and *n*-butanol extracts of *S. thebica* Webb showed hepatoprotective activity. These extracts significantly reduced the increase in activities of ALT, AST, and GGT, and levels of glucose, triglycerides, and cholesterol in serum of CCl_4-treated rats. The extracts showing activity were found to contain flavonoids; one new compound, chrysoeriol-7-*O*-xylosoide- (1,2)-arabinofuranoside (**2**), in addition to another known four compound chrysoeriol (**1**), quercetin (**3**), quercetin-7-*O*-rhamnoside (**4**), and kaempferol-3-*O*-β-D-glucoside (**5**). The isolated new compound was mainly found to be responsible for this activity when tested on animals in the laboratory. The structures were established by melting point, UV spectroscopy, EI-Mass, Fab-Mass, and 1D and 2D NMR spectroscopic techniques on a 600 MHz instrument.

Plants and plant extracts have been used for thousands of years for the treatment of disease. More recently[1] there has been renewed interest in the use of natural products because of their reduced side effects on the human body in comparison to synthetic medicinal drugs[2]. Plants from the Cruciferae family have been widely used in folk medicine. Many of the plants belonging to this family are of economic importance, being either a very common food produce,[3] or constitute important articles in oil[4] production. *Schouwia thebica* Webb, a member of this family, has been used by Bedouins for the treatment of liver disorders. Our study has been carried out to validate this activity and to isolate the compounds responsible for such activity.

Plant material. The aerial parts of *S. thebica* Webb were collected from the Sinai in 2003. The identity of the plant was verified by the Late Prof. N. El-Hadidi, Professor of Botany, Botany Department, Faculty of Science, Cairo University, and by comparison with plant description Flora of Egypt.[5,6] A voucher specimen of the titled plant is kept in the herbarium of the Desert Research Center. Plant material was air-dried in shade, reduced to fine powder, packed in tightly closed containers, and stored for phytochemical and biological studies.

Extraction. The air dried powder of *S. thebica* (aerial parts) (1 kg) was extracted by percolation in 90% ethanol (4 l) at room temperature for two days and filtered. The residue was re-percolated again. This process was repeated four times. The combined ethanol extracts were concentrated under reduced pressure at a temperature not exceeding 35 °C to yield a dry extract (290 g). Water (400 ml) was added and the resultant mixture successively extracted with diethyl ether, chloroform, ethyl acetate, and *n*-butanol, respectively. Each extract was dried over anhydrous sodium sulfate, and concentrated under reduced pressure at a temperature not exceeding 35 °C to yield dry extracts (5.5, 6, 18.5, and 60 g) respectively. All extracts were tested for their toxicity and hepatoprotective activity.

Toxicological study. The LD_{50} of each of the extracts was determined using the method of Finney.[7] For this purpose, albino mice (25–28 g) were divided into groups each of 5 animals. Preliminary experiments were done for each extract to determine the minimal dose that kills

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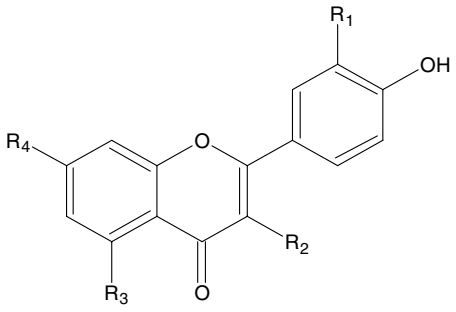
all mice and the maximal dose that fails to kill any animal. Several oral doses at equal intervals were chosen in between these doses. Animals were kept under observation for 24 h during which symptoms of toxicity and rate of mortality in each group were recorded.

Hepatoprotective activity. Forty mature male Sprague–Dawley rats of 180-200 g body weight were obtained from the Laboratory Animal Colonies, Ministry of Public Health, Helwan, Egypt. The animals were kept under good hygienic conditions and fed on standard diet and watered ad libitum. These rats were randomly divided into 8 groups of 5 animals, each. The 1st group was given corn oil in an oral dose of 2.5 ml/kg of body weight (b.wt) for 5 successive days and kept as a negative control. Animals of the 2nd group were given a single oral dose of 50% CCl_4 in corn oil (2.5 ml/kg b.wt) and kept as a positive control. The 3rd group was treated with silymarine, a standard drug, in a daily oral dose of 25 mg/kg b.wt for 5 successive days. The 4th, 5th, and 6th groups were orally administered total alcohol extract, ethyl acetate extract plus *n*-butanol extract, with a dose of 400 mg/kg b.wt for 5 successive days. The 7th and 8th groups were treated with compounds **1** and **2** (Fig. 1), respectively, with a dose of 100 mg/kg for 5 days. Twenty-four hours following the last dose, rats of the 3rd −8th groups were given a single oral dose of 50% CCl_4 in corn oil in a dose of 2.5 ml/kg b.wt. Two days later, blood samples were obtained from the orbital plexus of each rat and clear sera were separated by centrifugation at 3000 rpm for 15 min. The sera were assayed for determination of the activity of alanine aminotransferase (ALT),[8] aspartate aminotransferase (AST)[8] and γ-glutamyl transferase (GGT).[9] The levels of glucose,[10] total bilirubin,[11] triglycerides,[12] cholesterol,[13] total proteins[14] and albumin[15] in serum were determined. The results were statistically analyzed using Student's '*t*' test.[16]

Isolation. The ethyl acetate and *n*-butanol extracts were the only ones to show activity. TLC showed the same pattern of spots when each extract was chromatographed on silica gel G using the solvent system (a), [ethyl acetate–methanol–water (30:5:4)], and visualized by UV and/or an aluminum chloride spray reagent.[17] Therefore, both extracts were combined (78 g) and a portion applied to a silica gel column which was eluted gradually with ethyl acetate and methanol. Five flavonoid containing fractions were isolated. These were each subjected to preparative thin-layer chromatography using solvent system (a). Bands corresponding to each flavonoid were separately extracted with methanol, concentrated and for final purification each one was submitted to a column of Sephadex LH-20 eluted with methanol–water 1:1, giving compounds **1**–**5** (Fig. 1).

Apparatus. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected; mass spectra (Electrospray negative ion) sample dissolved in acetonitrile on a Micromass Quattro spectrometer. ^1H and ^{13}C NMR spectra, using external electronic referencing through the deuterium resonance frequency of the solvent, were determined at 600.17 or 150.91 MHz, respectively, with a JEOL ECA600 NMR spectrometer fitted with an auto-tune 5 mm X/H probe. Carbon-atom types were established in the ^{13}CNMR spectrum by employing a combination of broad-band proton-decoupled and DEPT (90 and 135) experiments. [$^1J_{C-H}$ and $^2J_{C-H}$ and $^3J_{C-H}$] ^1H–^{13}C correlations were established by using HMQC and HMBC pulse sequences, respectively. ^1H–^1H correlations were determined by double quantum filtered COSY. Pye Unicam pu 8800 spectrophotometer for UV spectral analysis.

Chrysoeriol ***1***. (130 mg) Yellow crystals, $R_f = 0.86$ (system a), mp 336 °C, UV, λ_{max} in MeOH: nm 345, 270 (sh), 351; ($AlCl_3$): 272, 303, 371, 391; ($AlCl_3$/HCl):



Compound	R1	R2	R3	R4
1	OCH_3	H	OH	OH
2	OCH_3	H	OH	O-xylose-arabinose
3	OH	OH	OH	OH
4	OH	OH	OH	O-rhamnose
5	H	glucose	OH	OH

Figure 1. Structures of the compounds isolated from *Schouwia thebica* Webb.

272, 303, 355, 391; (NaOAc): 276, 356, 402; (NaOAc/H_3BO_3): 275, 350; (NaOMe): 266, 333, 402. ^{1}H NMR (DMSO-d_6): δ 7. 64 (dd, J = 2.8 Hz, H6′), 7.57 (d, J = 2 Hz, H2′), 6.97 (d, J = 8 Hz, H5′), 6.95 (s, H3), 6.87 (d, J = 2.1 Hz, H8), 6.15 (d, J = 2.1 Hz, H6), and 3.86 (s, OCH_3).^{13}C NMR (DMSO-d_6): δ 155.4 (C-2), 103.1 (C-3), 177.3 (C-4), 162.3 (C-5), 99.1 (C-6), 168.6 (C-7), 94.2 (C-8), 156.7 (C-9), 103.9 (C-10), 131.4 (C-1′), 111.5 (C-2′), 147.1 (C-3′), 149.8 (C-4′), 116.7 (C-5′), 121.6 (C-6′), 55.8 (OCH_3). EI-MS *m/z* (% rel int): 300 (M^+) (100), 285 (50), 272 (16), 269 (8), 152 (22), 152 (12), 148 (20).

Chrysoeriol-7-O-xylosoide (1–2)-arabinofuranoside **2**. (130 mg) yellow crystals, R_f = 0.46 (system a), mp 365 °C, UV, λ_{max} in MeOH: nm 347, 270 (sh), 354; ($AlCl_3$): 272, 305, 373, 393; ($AlCl_3$/HCl): 272, 305, 357, 393; (NaOAc): 278, 357, 404; (NaOAc/H_3BO_3): 276, 352; (NaOMe): 267, 335, 404. ^{1}H NMR (DMSO-d_6): δ 7. 68 (dd, J = 2.8 Hz, H6′), 7.59 (d, J = 2 Hz, H2′), 6.98 (d, J = 8 Hz, H5′), 6.97 (s, H3), 6.89 (d, J = 2.1 Hz, H8), 6.17 (d, J = 2.1 Hz, H6) and 3.87 (s, OCH_3). ^{13}C NMR (DMSO-d_6): δ 154.6 (C-2), 103.4 (C-3), 176.9 (C-4), 162.4 (C-5), 98.9 (C-6), 168.9 (C-7), 94.5 (C-8), 156.6 (C-9), 104.1 (C-10), 131.1 (C-1′), 111.6 (C-2′), 146.8 (C-3′), 149.6 (C-4′), 116.5 (C-5′), 121.9 (C-6′), [Xylose; 106.2 (C-1″), 73.8 (C-2″), 76.8 (C-3″), 67.8 (C-4″), 66.7 (C-5″), arabinose; 108.8 (C-1‴), 76.1 (C-2‴), 79.3 (C-3‴), 73.9 (C-4‴), 64.4 (C-5‴)], 55.5(OCH_3). CI-MS *m/z* (% rel int): 564 (M^+) (100), 438(60), 306(70), 285 (53), 272 (26).

Quercetin 3. (55 mg) Yellow crystals, mp 312–314 °C, R_f = 80 (system a).UV: λ_{max} (MeOH): (nm) 255, 301, and 371, (NaOMe) 245, 330, ($AlCl_3$) 272, 301, 454, ($AlCl_3$/HCl) 270,357, 426, (NaOAc) 275, 324387, (NaOAc/H_3BO_3) 262,385. ^{1}H NMR (DMSO-d_6) δ 7.64 (1 H, d, J = 8.5 H-2′), 7.49 (1H, q, J = 8.5, H-6′), 6.85 (1H, d, J = 8.5,H-5′), 6.37 (1H, d, J = 2.5, H-6) and 6.14 (1H, d, J = 2.5, H-8). ^{13}C NMR (DMSO-d_6): δ 156.6 (C-2), 136.2 (C-3), 176.3 (C-4), 161.2 (C-5), 98.8 (C-6), 164.5 (C-7), 93.7 (C-8), 148.2 (C-9), 103.4 (C- 10), 120.4 (C-1′), 116.1(C-2′), 145.5 (C-3′), 147.2 (C-4′), 115.5 (C-5′) and 122.4 (C-6′).

Quercetin-7-O-rhamnoside **4**. (65 mg) Yellow amorphous powder. R_f = 0.34 (system a). UV λ_{max}, (MeOH): nm 254, 266(sh), 344; ($AlCl_3$): 270, 301(sh), 370, 408; ($AlCl_3$/HCl): 269, 297(sh), 255, 402; (NaOAc): 254, 266 (sh), 346; (NaOAc/H_3BO_3): 254, 266(sh), 346; (NaOMe): 271, 331(sh), 402. ^{1}H NMR (DMSO-d_6): δ 7.4 (1H, d, J = 8.5 Hz, H-2′), 7.3 (1H, dd, $J_{6',5'}$ = 8.5, $J_{6',2'}$ = 2.1 Hz, H-6′), 6.8 (1H, d, J = 8.5 Hz, H-5′), 6.4 (1H, d, J = 2.5 Hz, H-8), 6.2 (1H, d, J = 2.5 Hz, H-6), 5.2 (1H, d, J = 2.5 Hz, H-1 rhamnose), 1.2 (3H, d, J = 6 Hz, CH_3); sugar protons at δ 3.2–3.9. ^{13}C NMR (DMSO-d_6) δ 149.0 (C-2), 134.80 (C-3), 163.9 (C-4), 159.0 (C-5), 99.80 (C-6), 163.20 (C-7), 92.50 (C-8), 155.20 (C-9), 103.20 (C-10), 121.30 (C-1′), 114.80 (C-2′), 144.80 (C-3′), 146.70 (C-4′), 115.60 (C-5′), 119.70 (C-6′), 100.70 (C-1″ sugar); 68.10(C-2″), 70.30(C-3″), 70.60(C-4″), 75.20 (C-5″) and 17.60 (–CH_3).

Kaempferol-3-O-β-D-glucoside **5**. (50 mg), Yellow crystals. R_f = 0.56(system a) UV λ_{max} in MeOH : (nm) 265, 325 (sh), 350; ($AlCl_3$): 265, 290(sh), 420; ($AlCl_3$/HCl) 265, 300, 420 (NaOAc) 215, 300, 365 (NaOAc/H_3BO_3) 270, 360 (NaOMe) 275, 325, 400. ^{1}H NMR (DMSO-d_6): δ 7.9 (2H, d, J = 9 Hz, H2′ and H6′), 6.7 (2H, d, J = 9 Hz, H3′ and H5′), 5.6 (1H, d, J = 2.5 Hz, H8), 5.5 (1H, d, J = 2.5 Hz, H6), 5.1 (d, J = 7 Hz, H 1″ glucose) and δ 3.1–3.8 (m, remaining sugar protons). EI-MS *m/z* (% rel int): 447 (M^+) (100), 285(95), 315(18), 448(68). ^{13}C NMR (DMSO-d_6) δ: 153.3 (C-2), 132.6 (C-3), 174.3 (C-4), 159.6 (C-5), 98.6 (C-6), 160.4 (C-7), 95.5 (C-8), 153.2(C-9), 103.4 (C-10), 121.1 (C-1′), 130.3 (C-2′), 114.8 (C-3′), 157.4 (C-4′), 114.8 (C-5′), 130.3 (C-6′), 102.2 (C-1″), 75.4 (C-2″), 79.0 (C-3″), 71.2 (C-4″), 79.0 (C-5″), and 59.8 (C-6″).

The studied extracts are considered safe, since they failed to induce death of mice in doses up to 4000 mg/kg body weight.

Hepatoprotective activity. Oral administration of CCl_4 to male rats showed significant elevations of serum activities of ALT, AST, and GGT when compared with negative control rats. Moreover, the serum levels of glucose, total bilirubin, triglycerides, and cholesterol were significantly increased, while total proteins and albumin were significantly reduced (Table 1). In comparison with the CCl_4-treated group, significant improvements were seen in the serum activity of ALT, AST, and GGT, and in the serum level of glucose, triglycerides, and cholesterol in rats that previously had been medicated with silymarine, ethyl acetate extract or butanol extract (Table 1). These improvements are attributed to the accumulation of triglycerides in the hepatocytes, after blocking of the secretion of hepatic triglycerides into plasma.[16] The biochemical mechanism of CCl_4 toxicity is based on mitochondrial damage that leads to an accumulation of fat within 60 min, damage of endoplasmic reticulum within 30 min[16] and damage of lysosomes,[18] which result in death of the hepatocytes. Liver microsomal oxidizing systems connected with cytochrome P-450 produce reactive metabolites of CCl_4; for example, a trichloromethyl radical (CCl_3) or a trichloroperoxy radical (CCl_3O_3). This radical induces lipid peroxidation, disturbance in calcium (Ca^{2+}) homeostasis and finally cell death occurred.[19] The treatment with *S. thebica* extracts proved more effective than silymarine. This hepatoprotective effect of *S. thebica* against CCl_4—hepatotoxicity is attributed to the presence of flavonoids, which are known to be hepatoprotective and antioxidants,[20,21] that act as free radical scavengers for the lipoperoxidants. The major active compounds of *S. thebica* tested in this study showed both anti-oxidant and hepatoprotective activity. Therefore, the mechanism of *S. thebica* as a hepatoprotective agent against CCl_4-induced alteration in the liver may be due to an anti-oxidant effect of the isolated compounds; chrysoeriol and chrysoeriol-7-*O*-xylosoide (1-2)-arabinoside. There was an insignificant improvement in the level of total bilirubin, total proteins and albumin in serum of rats medicated with any of the tested extracts and compounds. The livers of the rats treated with CCl_4 were found to be en-

Table 1. Effect of CCl_4, silymarine, and alcoholic extracts of *Schouwia thebica* on hepatic functions of male rats ($n = 5$)

Parameters	Control negative	Control positive (CCl_4)	Silymarine + CCl_4	Total alcohol extract + CCl_4	Ethyl acetate extract + CCl_4	Butanol extract + CCl_4	Chrysoeriol + CCl_4	Chrysoeriol-7-*O*-xylosoid-arabinofuranosid + CCl_4
ALT (U/L)	43.60 ± 1.15	87.27 ± 2.10 (b)	65.19 ± 1.88***	75.87 ± 2.43**	70.83 ± 2.40***	69.43 ± 2.39***	69.42 ± 2.37***	68.43 ± 2.39***
AST (U/L)	35.63 ± 1.28	74.00 ± 2.78 (b)	56.60 ± 1.77***	65.73 ± 2.27*	62.63 ± 2.17**	60.64 ± 1.27***	62.65 ± 2.47***	61.64 ± 2.27***
GGT (U/L)	7.79 ± . 0.40	15.00 ± 0.61 (b)	10.50 ± 0.42***	12.60 ± 0.62*	11.10 ± 0.61***	11.00 ± 0.64***	12.05 ± 0.67**	12.03 ± 0.65**
Glucose (mg/dl)	128.39 ± 3.25	193.80 ± 4.62 (b)	140.96 ± 3.01*	171.28 ± 3.55**	159.38 ± 3.52***	158.34 ± 3.50***	165.36 ± 3.50***	163.36 ± 3.70***
Total bilirubin (mg/dl)	0.44 ± 0.03	0.30 ± 0.02 (a)	0.38 ± 0.02*	0.32 ± 0.03	0.34 ± 0.02*	0.35 ± 0.03	0.33 ± 0.02	0.33 ± 0.03
Triglycerides (mg/dl)	162.86 ± 4.67	197.39 ± 5.25 (b)	171.24 ± 4.74**	182.16 ± 4.01*	179.12 ± 4.01*	177.22 ± 4.01**	176.21 ± 5.01**	177.22 ± 6.01*
Cholesterol (mg/dl)	72.21 ± 3.45	98.84 ± 4.14 (b)	80.93 ± 3.36**	89.38 ± 3.54	85.28 ± 3.24*	86.38 ± 3.25*	88.37 ± 3.22	88.34 ± 3.15
Total proteins (g/dl)	6.39 ± 0.44	5.46 ± 0.30 (a)	6.10 ± 0.30	5.63 ± 0.38	5.70 ± 0.38	5.81 ± 0.23	5.61 ± 0.26	5.67 ± 0.23
Albumin (g/dl)	3.90 ± 0.17	2.62 ± 0.19 (b)	3.10 ± 0.23	3.10 ± 0.20	3.18 ± 0.20	3.05 ± 0.20	3.05 ± 0.20	3.07 ± 0.20

CCl_4, Carbon tetrachloride. Groups of silymarine and plant extracts are compared with control positive group. Control positive group was compared with control negative group. Significant at (a) $P \leqslant 0.01$, (b) $P \leqslant 0.001$.
* Significant at $P \leqslant 0.05$.
** Significant at $P \leqslant 0.01$.
*** Significant at $P \leqslant 0.001$.

larged, pale in color, and friable. Slight enlargement was seen in the livers of rats treated with silymarine, plant extracts or isolated compounds **1** and **2**.

A new compound named chrysoeriol-7-*O*-xylosoide (1-2)-arabinofuranoside **2** and four known compounds found to be chrysoeriol **1**, quercetin **3**, quercetin-7-*O*-rhamnoside **4**, and kaempferol-3-*O*-β-D-glycoside **5** were identified by comparing with published data their EI-MS, ^{1}H NMR, ^{13}C NMR, HMQC, HMBC spectra, and UV spectra in methanol and with different shift reagents. The new compound **2**, which in positive CI mass spectra exhibited a molecular ion at 564, upon acid hydrolysis yielded an aglycon and two sugars. The latter were identified as xylose and arabinose, by HPLC and TLC using system ([ethyl acetate–methanol–acetic acid–water (65:15:10:15)] and visualized by naphthoresorcenolsulforic acid.[17] The aglycon was found to be identical with compound **1**, by TLC and the use of UV shift reagents. Substitution at position 7 was indicated by the UV spectra upon addition of diagnostic shift reagents and from correlations in the HMQC and HMBC NMR spectra, which exhibited cross-peaks between the methoxy protons and C-3′ and the anomeric proton at C-7. The furanose form was indicated by the presence of two methylene signals in the DEPT135 spectrum. Thus, by comparison of the spectral data with published data[22,23] compound **2** was identified as chrysoeriol-7-*O*-xylosoide (1-2)-arabinofuranoside. Compound **3** migrated with quercetin and its spectral data was in agreement with those published for this compound.[22] Acid hydrolysis[23] of compound **4** revealed the sugar rhamnose, which was identified by TLC as before, and a aglycon which was found to be identical with compound **3** by comparative TLC and UV with shift reagents. Substitution at position 7 was indicated by UV spectra upon addition of diagnostic shift reagents.[22] Thus by comparison with published[23] data compound **4** was identified as quercetin 7-*O*-rhamnoside. Compound **5** was identified from its spectral data and after hydrolysis it gave glucose as it is free glycon moiety, whilst the aglycone was found to be identical to kaempferol.[22,23]

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