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Phenolic compounds and Hepatoprotective activity of *Centaurea aegyptiaca* L. on Carbon Tetrachloride-induced Hepatotoxicity in Rats

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ABSTRACT

Objectives: This study aimed to isolate phenolic compounds from the 70% aqueous methanol extract of the aerial parts (leaves, flowers and stems) of *Centaurea aegyptiaca* , evaluate *in-vivo* hepatoprotective activity and determine total phenolic and total flavonoid contents. **Methods:** 70% aqueous methanol extract of the aerial parts subjected to different chromatographic separation techniques. The identities of the isolated compounds were established on the basis of their spectral data and comparing with previously reported data. The crude extract was evaluated for *in-vivo* hepatoprotective activity on CCl_4-induced hepatotoxicity in rats. Total phenolic and total flavonoid contents of the aqueous methanol extract were determined using Folin-Ciocalteu and aluminum chloride colorimetric methods, respectively. **Results:** The aqueous methanol extract of the aerial parts of *Centaurea aegyptiaca* afforded seven compounds. A phenolic acid ester; protochatechuic acid methyl ester (**1**) along with six known flavonoids; apigenin-6-C-*β*-D-glucopyranoside (isovitexin) (**2**), apigenin-8-C-*β*-D-glucopyranoside (vitexin) (**3**), quercetin-3-*O*-*β*-D-glucopyranoside (isoquercetin) (**4**), apigenin (**5**), 3-*O*-methylquercetin (**6**) and quercetin (**7**). Administration of the extract at doses of 100 and 200 mg/kg b.wt showed a hepatoprotective activity similar to that of the standard drug; silymarin at a dose of 25 mg/kg b.wt. Comparative histopathological study of liver exhibited moderate changes in liver histoarchitecture when compared to the CCl_4 group. The methanolic extract showed high concentration of phenolic and flavonoid contents. **Conclusion:** The aqueous methanol extract of the aerial parts of *C. aegyptiaca* afforded seven phenolic compounds for the first time from this species, with promising *in-vivo* hepatoprotective activity.

INTRODUCTION

Genus *Centaurea* L. (Asteraceae), encompassing about 500 species, is distributed around the Mediterranean area and West Asia[1]. *Centaurea* species have been the object of various phytochemical studies, with a diversity of bioactive phytochemicals and prevalence of flavonoids [2], flavonoid aglycones[3], lignans[4], sesquiterpene lactones[5], essential oils[6], alkaloids [7] and other biologically active constituents. Some species of the genus *Centaurea* have been used as medicinal plants in folk medicine for their uses in gastrointestinal[8] and inflammatory disorders[9], cytotoxic activity[4,10], in addition to the anti-microbial[11,12] and antioxidant activities[13]. There are 17 different species of *Centaurea* growing wild in Egypt[14]. *C. aegyptiaca* L., known locally as Murrar Masry, is a biennial or short-lived perennial herb grows widely in the Egyptian desert, Red Sea coastal strip, Sinai, Gebel Elba[15] and it has no reported folkloric use but the closely related species *C. alexandrina,* is reported to be used as a hypoglycemic agent in folk medicine in Egypt[16].

Reviewing the current literature of *C. aegyptiaca* very few phytochemical and biological

studies were reported, only sesquiterpene lactones[1,17,18,19] were isolated from this species while nothing dealt with the isolation of phenolic compounds except only one report dealt with the characterization of the phenolic profile utilizing LC-HRESI-MS-MS[20]. Thus, the present study deals with the isolation and identification of secondary metabolites from the aqueous methanol extract of aerial parts (leaves, flowers and stems) of *C. aegyptiaca*, determination of the total phenolic and flavonoid contents of the extract, as well as studying its hepatoprotective activity.

MATERIALS AND METHODS

General

^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker AMX- 400, Avance 400, and Avance 300 spectrometers with standard pulse sequences operating at 400 MHz in ^{1}H NMR and 100 MHz in ^{13}C NMR. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard. ESI-MS spectrum was recorded in the negative mode, using a XEVO TQD triple quadruple instrument. Column chromatography (CC): was carried out on Polyamide 6S, (Riedel-DeHaen, Hannover, Germany), Sephadex LH-20 (Pharmacia Fine Chemicals) and silica gel (Fluka, Sigma-Aldrich Chemicals, Germany); PC was carried out on Whatman No. 1MM paper (46×57 cm). Precoated TLC plates, silica gel 60 F $_{254}$ (E-Merck, Darmstadt, Germany), (20x20 cm). Carbon tetrachloride (BDH Chemicals, England), Silymarin, gallic acid, rutin and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Folin-Ciocalteu solution and 95% ethanol were purchased from Merck Co. (Santa Ana, CA, USA). The following solvent systems were used: S_1 BAW (*n*-butanol: acetic acid: water 4:1:5 upper phase), S_2 acetic acid/water (15:85v/v). All chemicals and solvents used were of analytical grade.

Plant material

Aerial parts (leaves, flowers and stems) of *C. aegyptiaca* used in this study were collected from wild plants growing in Wadi Hagool, Suez city: Egypt, during March 2014. The plant material was authenticated by Prof. Dr. Ibrahim El-Garf, Department of Botany, Faculty of Science, Cairo University. A voucher specimen was kept in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, MUST University, Egypt.

Extraction and isolation

The dried aerial parts of *C. aegyptiaca* (2.8 kg) were finely ground and macerated in 70% methanol at room temperature and the crude extract was evaporated to dryness under reduced pressure (200 g). A sample (100 g) of the dry extract was fractionated on polyamide 6S column. The column was eluted with water then water-methanol step gradient. The obtained fractions (500 ml of each fraction) were subjected to paper chromatography using S_1 and S_2 as developing solvents, and the similar fractions were collected together to give five major fractions (I-V). Fractions (II and III) were concentrated and the concentrates were taken up with *n*.butanol (*n*-BuOH). The *n*-BuOH extracts were evaporated and the residues were weighed. Fractionation of fraction II (residue) (10 g) on Sephadex LH-20 CC using *n*-butanol saturated with water (upper layer) gave five sub-fractions. The sub-fraction (5-7) (1g) was further fractionated on silica gel column eluted with CH_2Cl_2/ethylacetate step gradient as solvent system. The sub-fractions eluted with CH_2Cl_2: ethylacetate (90:10) were collected to give a pure compound **1** (80 mg). Fraction III (residue) (5 g) was subjected to Sephadex LH-20 CC starting elution with MeOH: H_2O (20:80) to give three sub-fractions. The sub-fraction (12-20) was subjected to silica gel column eluted with *n*-hexane: ethylacetate and ethylacetate: methanol step gradient. Two compounds were isolated; **2** (20 mg) from *n*-hexane-ethylacetate fraction (10:90) and **3** (35 mg) from ethyl acetate–methanol fraction (97:3). Fraction IV (6 g) has been applied on Sephadex LH-20 CC eluted isocratically with *n*-butanol saturated with water (upper layer) gave five sub-fractions. Sub-fraction (2-12) subjected to Sephadex LH-20 CC starting elution using MeOH: H_2O (20:80) resulted in isolation of compound **4** which was further purified on Sephadex LH-20 CC using MeOH: H_2O to afford the purified compound **4** (25 mg). Fraction V (2 g) has been applied on Sephadex LH-20 CC starting elution using MeOH: H_2O (30:70) as a solvent system and gradually decrease polarity to 100% MeOH to give three collective sub-fractions. Each was further purified on Sephadex LH-20 CC using MeOH: H_2O mixtures to afford the purified compounds of **5** (28mg), **6** (50mg) and **7** (55mg) **Figure 1**.

Total phenolic content

The concentration of total phenolics of the plant extract was determined according to the method described by **Kumar *et al*., 2008** [21] using gallic acid as a standard. Briefly, a mixture of 100 µl of plant extract (100 µg/ml), 500 µl of Folin- Ciocalteu reagent and 1.5 ml of Na_2CO_3 (20 %) was shaken and diluted up to 10 ml with water. After 2 h, the absorbance was measured at 765 nm (using a spectrophotometer). All determinations were carried out in triplicate. The total phenolic concentration was expressed as gallic acid equivalents (GAE).
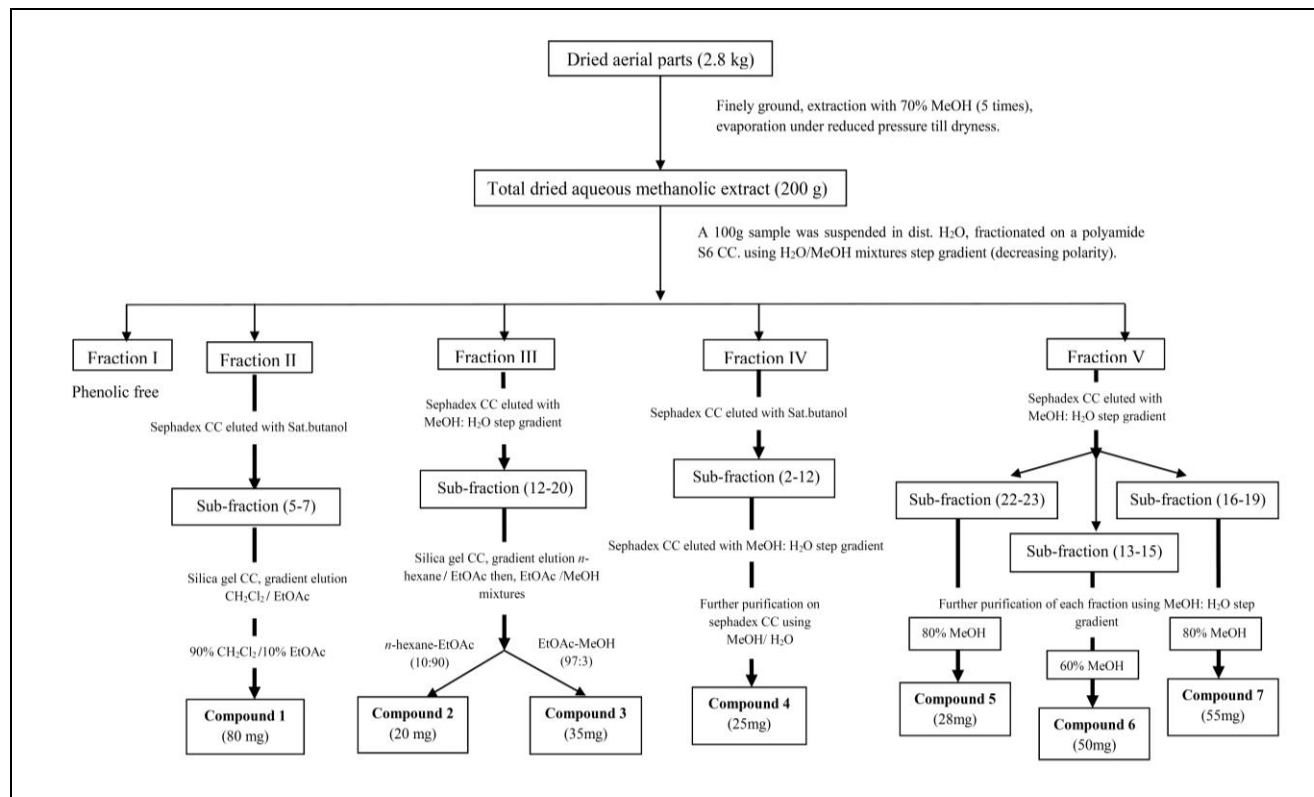
Figure 1. Flow chart of extraction, fractionation and isolation of compounds from aerial parts of *C. aegyptiaca*

Total flavonoid content

Total flavonoid concentration of plant extract was determined according to the reported procedure by **Kumaran & Karunakaran, 2007** [22]. 100 µl of plant extract (10 mg/ml) in methanol was mixed with 100 µl of 20 % AlCl$_3$ in methanol and a drop of acetic acid and then diluted to 5 ml with methanol. The absorbance was measured at 415 nm after 40 min against the blank.The blank consisted of all reagents and solvent without AlCl$_3$. All determinations were carried out in triplicate. The total flavonoid concentration was expressed as rutin equivalents (RE).

Determination of median lethal dose (LD$_{50}$)

The LD$_{50}$ of the methanolic extract of *Centaurea aegyptiaca* L. was estimated according to **Kärber's procedure (1931)**. Male albino mice (25-30 g) were divided into groups, each of six animals. Preliminary experiments were carried out to determine the minimal dose that kills all animals (LD$_{100}$) and the maximal dose that fails to kill any animal. Several doses at equal logarithmic intervals were chosen in between these two doses; each dose was injected in a group of six animals. The number of dead animals in each group, 24 hours after injection was determined and the median lethal dose (LD$_{50}$) was calculated.

Determination of in-vivo hepatoprotective activity
Animals

Adult male rats Sprague-Dawley strain (120-180 g) were purchased from the animal house colony, Science Park, European countryside, Giza, Egypt. They were fed a standard diet of commercial rat chow, tap water ad *libitum*. The animals were allowed one week under these conditions to acclimatize before the commencement of the experiment. Handling procedures were conducted in accordance with the Institutional Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication no. 85-23, revised 1985).

Experimental Design

Forty two animals (42) were divided into 7 groups, 6 rats in each as follows: **Group I:** Rats served as negative control and were orally administered normal saline for 21 days. **Group II:** Rats were orally administered 70% dried methanol extract of *C.aegyptiaca* L. solubilized in distilled water (100 mg/kg b.wt.) for 21 days. **Group III:** Rats were orally administered 70% dried methanol extract of *C. aegyptiaca* L. solubilized in distilled water (200 mg/kg b.wt.) for 21 days. **Group IV:** Rats served as positive control and were orally administered normal saline for
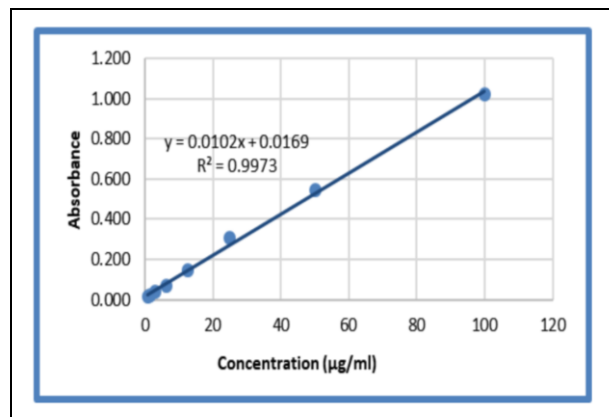
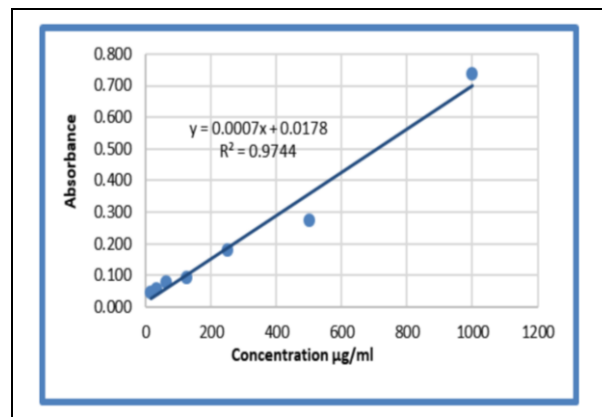**Figure 2. Standard calibration curve of gallic acid for colorimetric determination of total phenolic content.**

Figure 3. Standard calibration curve of rutin for colorimetric determination of total flavonoid content.

15 days followed by administration of CCl_4 (1.5 mg/kg b.wt., interpritonial) twice weekly until day 21. **Group V:** Rats were orally administered silymarin (25 mg/kg b.wt.) as a reference hepatoprotective drug for 15 days followed by administration of CCl_4 (1.5 mg/kg b.wt., interpritonial) twice weekly until day 21. **Group VI:** Rats were orally administered 70% dried methanol extract of *C. aegyptiaca* L. solubilized in distilled water (100 mg/kg b.wt.) for 15 days followed by administration of CCl_4 (1.5 mg/kg b.wt., interpritonial) twice weekly until day 21. **Group VII:** Rats were orally administered 70% dried methanol extract of *C. aegyptiaca* L. solubilized in distilled water (200 mg/kg b.wt.) for 15 days followed by administration of CCl_4 (1.5 mg/kg b.wt., interpritonial) twice weekly until day 21.

Biochemical assessment

At the end of the experiment, blood samples were obtained from the retro-orbital vein plexuses, under ether anesthesia. ALT and AST activities in serum were determined according to **Reitman & Frankel, 1957** [23]. All animals were sacrificed, then livers were removed and a portion of the liver was homogenized and used for determination of the lipid peroxidation (LPO) which was determined by the estimation of biochemical paramteres; malondialdehyde (MDA) content according to **Mihara & Uchiyama, 1978** [24], the reduced glutathione (GSH) according to the method of **Beutler et al., 1963** [25] and nitric oxide (NO) according to **Miranda et al., 2001** [26].

Histopathological studies

Autopsy samples taken from the livers of rats in different groups were fixed in 10% formal saline for twenty four hours and washed with tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin-eosin stain for examination through the light electric microscope [27].

Statistical analysis

The results were expressed as mean ± S.E. Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer's Multiple Comparison Test. $P \leq 0.05$ was considered statistically significant and denoted by asterisk "*".

RESULTS AND DISCUSSION

Phytochemical analysis

From the aqueous methanol extract of the aerial parts of *C. aegyptiaca* seven compounds were isolated for the first time from this species. One phenolic acid ester was isolated and identified together with six known flavonoids.

The compounds were identified as protocaechuic acid methyl ester (**1**) apigenin-6-C-*β*-D-glucopyranoside (isovitexin)(**2**), apigenin-8-C-*β*-D-glucopyranoside (vitexin)(**3**) and their aglycone; apigenin (**5**), quercetin-3-*O*-*β*-D-glucopyranoside (isoquercetin) (**4**), 3-*O*-methyl quercetin (**6**) and their aglycone; quercetin (**7**), by using different spectroscopic analyses and comp-aring with previously reported data[28,29,30,31] **Figure 4.**

Identification of isolated compounds

Protocaechuic acid methyl ester (**1**): white amorphous powder. **ESI-MS**: m/z 167 [M-H]⁻ . **¹H-NMR** (CDCl₃, 400 MHz) δ_H 7.66 (1H, brs, H-2), 7.58 (1H, dd, *J* =1.8, 8.4, Hz, H-6), 6.93 (1H, d, *J* = 8.4 Hz, H-5), 3.90 (3H, s, Me-ester-7). **¹³C-NMR** (CDCl₃,
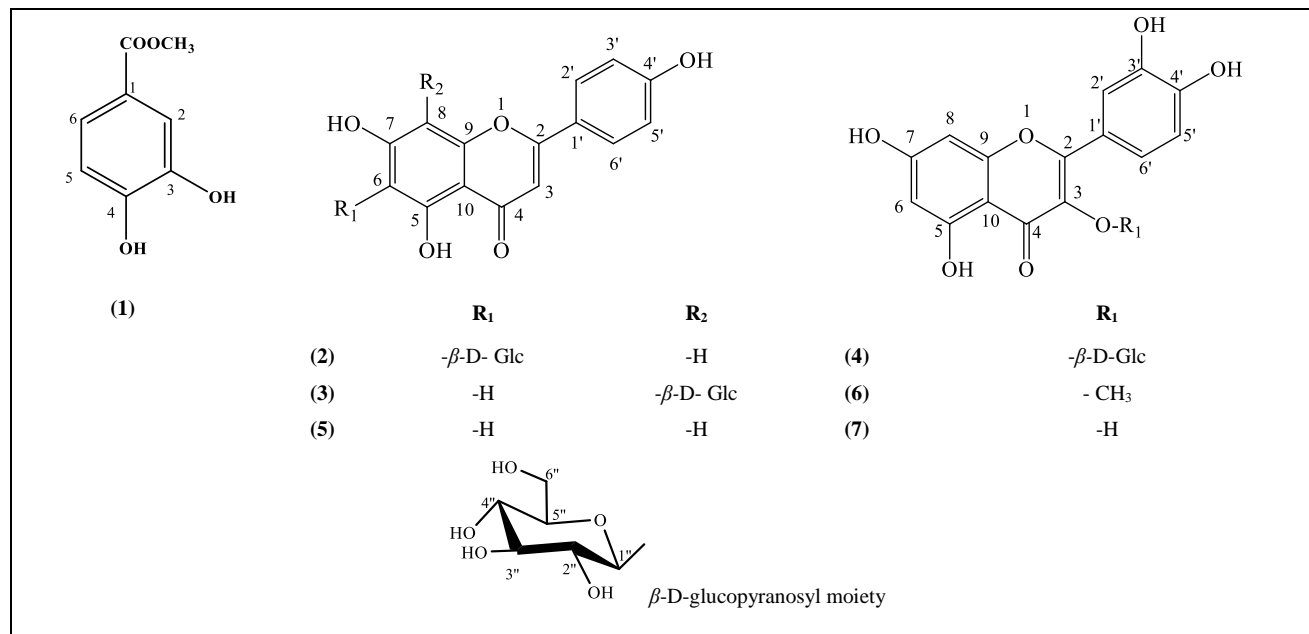
Figure 4. Structure of the isolated compounds

100 MHz) δ_C 167.6 (C-7), 148.9 (C-4), 143.1 (C-3), 123.7 (C-1), 123.6 (C-6), 116.6 (C-2), 114.8 (C-5), 52.1 (Me-ester).

Apigenin-6-C-β-D-glucopyranoside (isovitexin) (**2**): yellow amorphous powder **¹H-NMR** (DMSO-d_6, 400 MHz) δ_H 7.94 (2H, d, J = 8 Hz, H-2`,6`), 6.94 (2H, d, J = 8 Hz, H-3`,5`), 6.79 (1H, s, H-3), 6.52 (1H, s, H-8), 4.59 (1H, d, J =9.8 Hz, H-1``), 3.17-4.07 (6 H, overlapped, H-2``–H-6``). **¹³C- NMR** (DMSO-$d_6$, 100 MHz) $\delta_C$ 183.2 (C-4), 164.8 (C-2), 164.5(C-7),162.4 (C-5), 160.9 (C-4`), 157.5 (C-9), 129.7 (C-2`,6`), 122.4 (C-1`), 117.3 (C-3`,5`),110.1 (C-6), 104.7 (C-10), 104.1 (C-3), 94.9 (C-8), 82.8 (C-5``), 80.2 (C-3``), 72.6 (C-1``), 71.9 (C-2``), 71.5 (C-4``), 62.7 (C-6``).

Apigenin-8-C-β-D-glucopyranoside (vitexin) (**3**): yellow amorphous powder **¹H-NMR** (DMSO-d_6, 400 MHz) δ_H 8.02 (2H, d, J = 8.0 Hz, H-2`,6`), 6.89 (2H, d, J = 8.0 Hz, H-3`,5`), 6.78 (1H, s, H-3), 6.28 (1H, s, H-6), 4.69 (1H, d, J =9.8 Hz, H-1``), 3.17-3.86 (6 H, overlapped, H-2``–H-6``). **¹³C- NMR** (DMSO-$d_6$, 100 MHz) $\delta_C$ 182.5 (C-4), 164.4 (C-2), 163.3 (C-7), 161.6 (C-5), 160.9 (C-4`), 156.5 (C-9), 129.4 (C-2`,6`), 122.1 (C-1`), 116.3 (C-3`,5`), 105.1 (C-8), 104.4 (C-10), 102.9 (C-3), 98.7 (C-6), 82.2 (C-5``), 79.1 (C-3``), 73.8 (C-1``), 71.3 (C-2``), 71.0 (C-4``), 61.8 (C-6``).

Quercetin-3-O-β-D-glucopyranoside (isoquercetin) (**4**): yellow amorphous powder **¹H-NMR** (DMSO-d_6, 400 MHz) δ_H 7.67 (dd, J = 2.1, 8.6 Hz, H-6'), 7.53 (d, J = 2.1 Hz, H-2'), 6.82 (d, J = 8.6 Hz, H-5'), 6.40 (d, J = 1.8 Hz, H-8), 6.20 (d, J = 1.8 Hz, H-6) 5.37

(d, J = 7.63 Hz, H-1``), 3.28-3.65(6 H, overlapped, H-2``–H-6``). **¹³C- NMR** (DMSO-$d_6$, 100 MHz) $\delta_C$ 177.5 (C-4), 164.6 (C-7), 161.6 (C-5), 156.8 (C-2), 156.6 (C-9), 148.8 (C-4`), 145.8 (C-3`), 133.6 (C-3), 122.0 (C-6`),121.6 (C-1'),116.2 (C-5`), 115.8 (C- 2`),104.0 (C-10), 101.2 (C-1``), 98.9 (C-6), 93.8 (C-8), 74.9 (C-5``), 74.7 (C-3``), 71.6 (C-2``), 70.0 (C-4``), 61.5 (C-6``).

Apigenin (**5**): yellow amorphous powder **¹H-NMR** (CD₃OD, 400 MHz) δ_H 7.93 (2H, d, J = 8.5 Hz, H-2`,6`), 6.92 (2H, d, J = 8.5 Hz, H-3`,5`), 6.74 (1H, s, H-3), 6.41 (1H, d, J = 2 Hz, H-8), 6.22 (1H, d, J = 2 Hz, H-6). **¹³C-NMR** (CD₃OD, 400 MHZ) δ_C 181.5 (C-4),163.8 (C-2), 163.6 (C-7), 161.4 (C-4`), 161.3 (C-5), 157.2 (C-9), 128.3 (C-2`/6`), 121.1 (C-1`), 116.8 (C-3`,5`), 103.6 (C-10), 102.8 (C-3), 98.7 (C-6), 93.9 (C-8).

3-O-methyl quercetin (**6**): pale yellow needle crystals **¹H-NMR** (CD₃OD, 400 MHZ) δ_H 7.62 (1H,brs, H-2'), 7.52 (dd, J = 2.1, 8.8 Hz, H-6'), 6.91 (d, J = 8.4 Hz, H-5'), 6.19 (1H,s, H-8), 6.38 (1H,s, H-6), 3.78 (3H,s, O-CH₃). **¹³C-NMR** (CD₃OD, 400 MHZ) δ_C 178.6 (C-4), 164.5 (C-7) , 161.2 (C-5) , 156.9 (C-2), 156.6 (C-9), 148.5 (C-4'), 145.0 (C-3'), 138.1 (C-3), 121.5 (C-1'), 120.9 (C-6'), 115.1 (C-2'), 115.1 (C-5'), 104.4 (C-10), 98.5(C-6), 93.4 (C-8), 59.2 (O-CH₃).

Quercetin (**7**): yellow amorphous powder **¹H-NMR** (CD₃OD, 400 MHz) δ_H 7.68 (1H, brs, H-2'), 7.55 (dd, J =1.9, 8.0 Hz, H-6'), 6.90 (d, J = 8.0 Hz, H-5'), 6.45 (1H, s, H-8), 6.21 (1H, s, H-6). **¹³C-NMR** (CD₃OD, 400 MHZ) δ_C 178.30(C-4), 164.43 (C-7), 161.14 (C-5), 156.58 (C-9), 148.17 (C-4'), 147.2 (C-2)

Table 1. Effect of treatment with the 70% methanol extract of the aerial parts of *C. aegyptiaca* on serum ALT and AST in CCl4-induced hepatotoxicity in rats.

Group	Dose (mg/kg b.wt.)	ALT (U/L)	AST (U/L)
GpI (Negative control)		76.67 ± 2.305	216.7 ± 21.65
GpII (Methanolic extract I)	100	61.17 ± 7.208	123.8 ± 6.478
GpIII (Methanolic extract II)	200	58.17 ± 3.683	133.0 ± 11.33
GpIV (Positive control CCl4)	1.5	355.3 ± 34.64 [a]	442.3 ± 18.78 [a]
GpV (Silymarin + CCl4)	25	285.5 ± 44.35[a]	337.5 ± 21.55
GpVI (Methanolic extract I + CCl4)	100	287.3 ± 34.04[a]	293.0 ± 38.82[b]
GpVII (Methanolic extract II + CCl4)	200	297.5 ± 65.31[a]	317.3 ± 75.20

Table 2. Effect of treatment with the 70% methanol extract of the aerial parts of *C. aegyptiaca* on hepatic MDA, GSH and NO in CCl4-induced hepatotoxicity in rats.

Group	Dose (mg/kg b.wt.)	MDA ng/g.tissue	GSH pg/g.tissue	NO umol/g.tissue
GpI (Negative control)		8.400±0.6245	49.47±2.892	7.300±0.6245
GpII (Methanolic extract I)	100	26.93±1.302 [a]	15.30±0.5508 [a]	22.93±0.8413[a]
GpIII (Methanolic extract II)	200	14.97±0.7535	23.77±0.7265[a]	13.20±0.7000[a]
GpIV (Positive control CCl4)	1.5	65.27±3.169 [a]	5.367±0.4333[a]	48.30±2.650[a]
GpV (Silymarin + CCl4)	25	9.800±0.2646 [b]	40.60±0.7638[ab]	8.800±0.2646[b]
GpVI (Methanolic extract I + CCl4)	100	19.10±0.7638[abc]	18.90±0.6658[abc]	17.20±0.9452[abc]
GpVII (Methanolic extract II + CCl4)	200	11.50 ±0.4163[bd]	28.30±1.069[abcd]	10.53±0.4177[bd]

Data were expressed as mean ± SE (n=6).
[a] Significantly different from normal control at P<0.05,
[b] Significantly different from CCl4 group at P<0.05.
[c] Significantly different from Silymarin group at P<0.05.
[d] Significantly different from methanolic extract I (100mg/Kg) + CCl4 group at P<0.05.

145.5 (C-3′), 136.2 (C-3), 122.4 (C-1′), 120.5 (C-6′), 116.1 (C-2′), 115.5 (C-5′), 103.4 (C-10), 98.7 (C-6), 93.8 (C-8).

Phenol and flavonoid content

Results of the analysis of polyphenols constituents of the methanolic extract showed high concentration of the phenols (51.186±2.2845mg GAE/gram dry extract) and flavonoids (109.81±6.5mg RE/ gram dry extract) contents.

The hepatoprotective activity

CCl4 administration produced significant elevations of serum ALT and AST compared to the normal control group. The elevated activities of these enzymes are indicative of cellular leakage and liver injury. However, pretreatment of rats with 100 and 200 mg/kg b.wt. of *C. aegyptiaca* extract significantly decreased these serum biochemical indices (**Table 1**, **Figures 5-6**) as compared with the CCl4 group which revealed the hepatoprotectivity of *C. aegyptiaca* extract. Also treatment with CCl4 decrease the hepatic GSH level and increase MDA and NO levels (**Table 2, Figures 7-9**) compared to the normal control group. Treatment with the extracts prior to CCl4 administration, significantly decrease CCl4 -induced elevated levels of malondialdehyde (MDA) and nitric oxide (NO), while increase the level of reduced
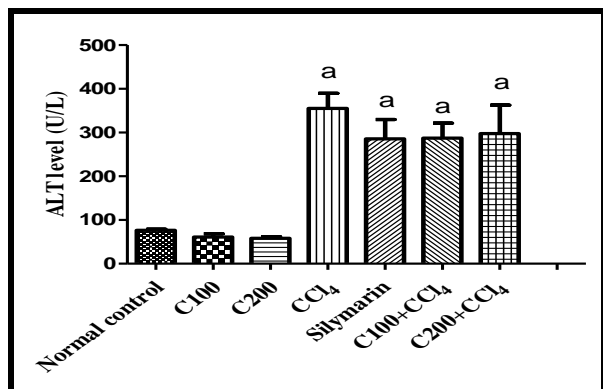
Figure 5. Effect of treatment with the methanolic extracts of the aerial parts of *Centaurea aegyptiaca* L. on serum ALT in CCl₄ -induced hepatotoxicity in rats.
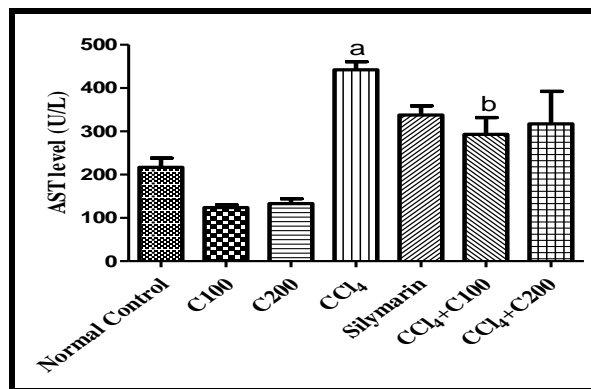

Figure 6. Effect of treatment with the methanolic extracts of the aerial parts of *Centaurea aegyptiaca* L. on serum AST in CCl₄ -induced hepatotoxicity in rats.
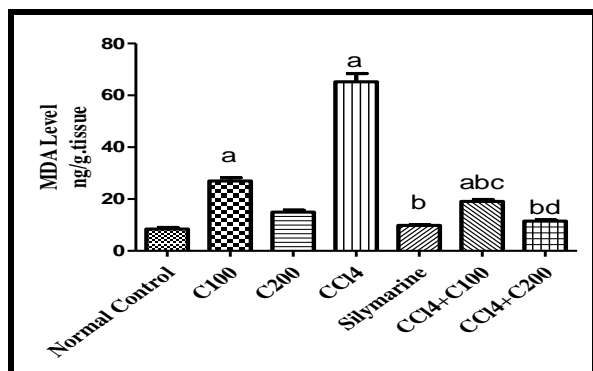

Figure. 7. Effect of treatment with the methanolic extracts of the aerial parts of *Centaurea aegyptiaca* L. MDA in CCl₄ -induced hepatotoxicity in rats.
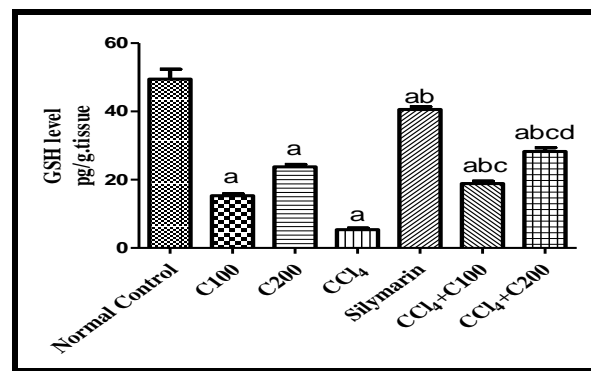

Figure. 8. Effect of treatment with the methanolic extracts of the aerial parts of *Centaurea aegyptiaca* L. GSH in CCl₄ -induced hepatotoxicity in rats.
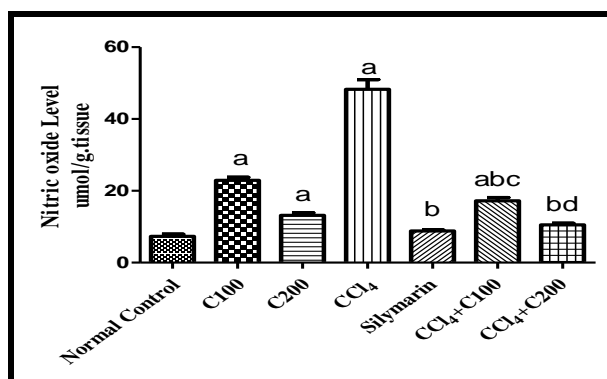

Figure 9. Effect of treatment with the methanolic extracts of the aerial parts of *Centaurea aegyptiaca* L. NO in CCl₄ -induced hepatotoxicity in rats.

glutathione (GSH). The hepatotoxicity induced by CCl₄ is due to its metabolite $CCl_3^\bullet$, a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage[32]. The increased value of GSH level in liver tissues of the rats treated with two doses of *C. aegyptiaca* extract and Silymarin may be due to *de novo* GSH synthesis or GSH regeneration[33]. The increase in MDA levels in liver
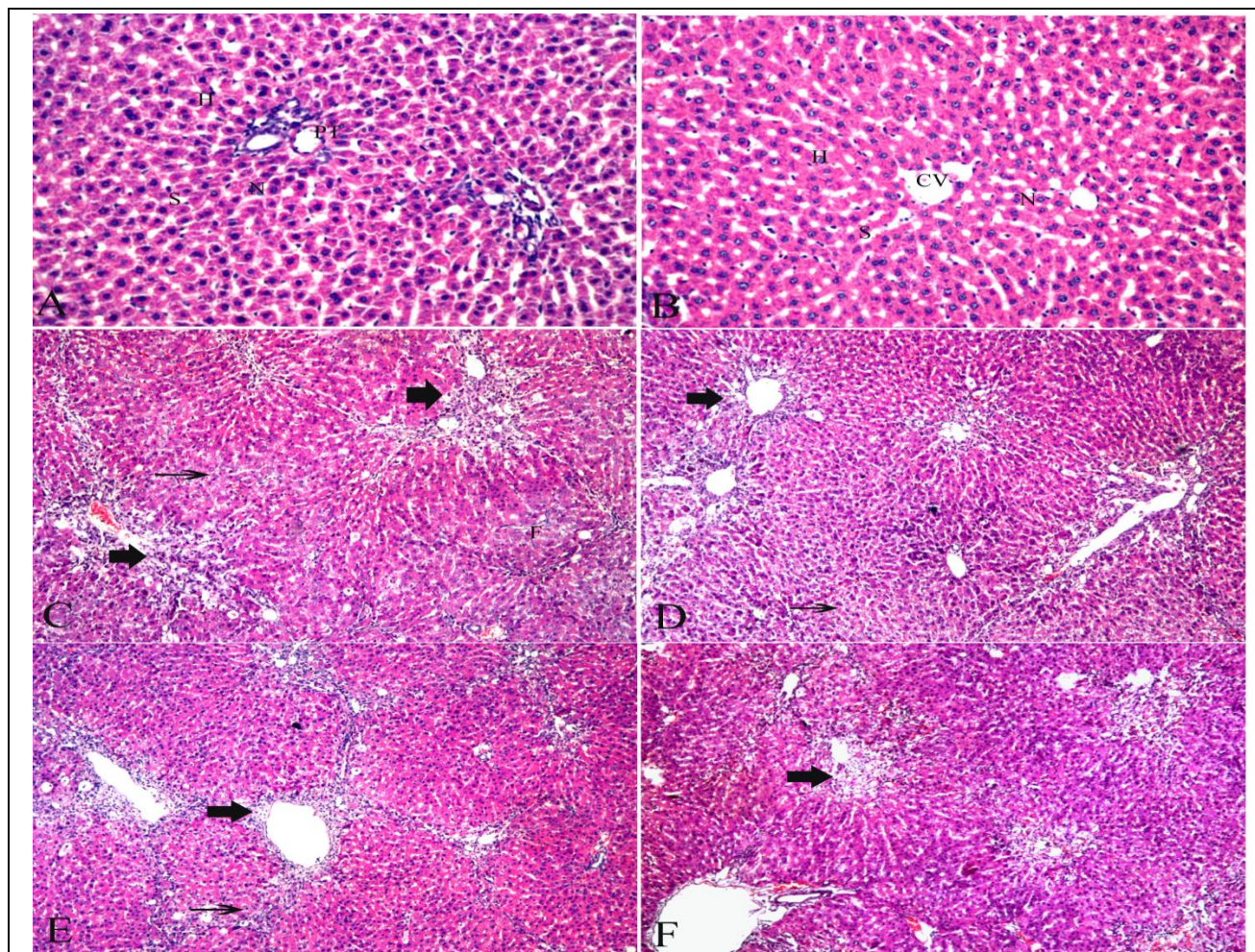
Figure. 10. Photomicrographs of hepatic histopathology. A. Normal liver section from control group showing normal hepatic lobule has central vein (CV), hepatocyte (H) with hepatic sinusoids(S) and prominent nucleus (N). **B**. Liver section from group treated with the plant extract at dose 100 mg/kg showing normal hepatic architecture central vein (CV), with hepatic sinusoids (S) and prominent nucleus (N). **C**. Histopathological examination of liver of the group treated with CCl_4 revealed degenerative changes in the hepatic tissues including inflammatory cells infiltration (thick arrow), hepatocytes degeneration (thin arrow), fatty changes, massive necrosis .Also, in the CCl_4 group dilatation with congestion of the central vein and pyknotic nuclei we also observed (Fig.10 C). **D**. However, in the group treatment with CCl_4 and silymarin and showed moderate improvement in histological structure of liver tissues, inflammatory cells infiltration (thick arrow), hepatocytes degeneration (thin arrow) were also noticed (Fig.10 D). **E**. Treatment with CCl_4 and low dose of plant showed mild improvement. Hepatic cords were slightly distorted, necrosis some hepatocytes (thin arrow) with inflammatory cells infiltration (thick arrow), congestion and dilation of portal tract (Fig.10E). **F**. Histopathological examination of liver sections treated with CCl_4 + high dose of plant (200 mg/kg) showed nearly normal hepatic architecture with minimally necrotic cells and inflammatory cells infiltration (thick arrow) (Fig.10F) (H & E. stain, x400).

suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals[34]. Several studies have reported the inhibitory effects of flavonoids on lipid peroxidation[35]. Sulfhydryl compounds such as glutathione (GSH) are well known to be an antioxidant substance in organisms, playing a critical role against CCl_4-induced injury by covalently binding to CCl_4. This is considered as the initial reactant in the chain reaction of oxidation, and then result in the lipid peroxidation and the cell membrane disruption[36]. Treatment with *C. aegyptiaca* extract (100 and 200 mg/kg) resulted in elevating the content of liver GSH compared with the control group. Several diseases have been associated with the changes in GSH levels, and reduced the resistance to the oxidation stress. The level of GSH was used to monitor the balance of oxidative stress and chemopreventive ability[37]. In our study, the *C. aegyptiaca* extract exhibited protective effect against liver damage from CCl_3. Furthermore the

GSH- related antioxidant system has been improved. In conclusion, the treatment with *C. aegyptiaca* extract could reduce damage induced by CCl₄. The mechanism of protection including the inhibition of lipid peroxidation, increasing the content of GSH, elevating the expression of antioxidant enzymes, all of which result in recuperation of biological parameters. Furthermore, the relationship of their antioxidant effects to the hepatoprotective mechanism, such as changes in antioxidant enzyme activity and the effects and mechanisms of the pure compounds from this plant requires further study.

Histopathological findings

Hepatoprotective effect of the *C. aegyptiaca* extract was further confirmed by the histopathological study of the liver. Histology of the liver section of control animals (**Figure 10A**) showed normal hepatic cells. Photomicrograph of section from liver treated with extracts of *C. aegyptiaca* (100& 200 mg/kg) showed normal structure of liver tissues (**Figure 10B**). Liver sections of the CCl₄ treated group showed multiple focal necrotic areas with or without inflammatory cells infiltration surrounding the portal area as well as the central vein in association with ballooning degeneration and fatty changes in the other hepatocytes all over the parenchyma (**Figure 10C**). Liver sections of the group protected with silymarin showed few areas of degeneration and necrosis with inflammatory cell infiltration as well as fatty change in some of the hepatocytes were detected in the parenchyma(**Figure 10D**). Liver sections of the group treated with extracts then experimentally inducted showed moderate focal necrosis and degeneration in the hepatocytes surrounding the central vein and the portal area with fatty change in others hepatocytes all over the parenchyma (**Figure 10E**& **10F**) .

In conclusion, the activity of the methanolic extract of the aerial parts of *Centaurea aegyptiaca* L. either at a dose of (100 or 200 mg/kg b.wt.) showed a similar hepatoprotective activity and a comparable potency as the standard drug, silymarin (25mg/kg b.wt.), depending on the biochemical results showed in this study. However, the protective, curative and antioxidant qualities of *C. aegyptiaca* may be attributed to the presence of active principles in the plant extract especially flavonoids and other polyphenolic compounds.

CONCLUSION

The aqueous methanol extract of the aerial parts (leaves, flowers and stems) of *Centaurea aegyptiaca* afforded seven phenolic compounds (**1-7**) which are isolated for the first time from this species. Also it is the first record for estimation of total phenolic, total flavonoid contents and the *in-vivo* hepatoprotective activity of *Centaurea aegyptiaca*.

Conflict of Interest

The authors declare that they don't have any conflict of interest.

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