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Full Length Research Paper

Evaluation of the hepatoprotective activity of some plants belonging to the tribe Cynareae growing in Egypt

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In an attempt to confront the growing problem of liver diseases in Egypt and worldwide, some plants belonging to the tribe Cynareae were evaluated for their hepatoprotective activities. The aerial parts of *Carduus getulus, Centaurea alexandrina, Centaurea calcitrapa, Cynara cornigera* and *Onopordum alexandrinum*, the leaves of *Cynara scolymus* and the seeds of *C. cornigera* and *O. alexandrinum* were selected for the present study. The ethanol extract of the stated parts of the six plants were subjected to hepatoprotective assays using Wistar albino rats at doses of 500 and 250 mg/kg, depending on the case. The liver injury was induced in rats using carbon tetrachloride. The biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin were estimated as reflections of the liver condition. The histopathological studies where carried out with silymarin as reference hepatoprotective drug. Best results were obtained after treatment of animals with *C. cornigera* and *O. alexandrinum*.

Key words: Hepatoprotection, Cynareae, *Carduus*, *Centaurea*, *Cynara*, *Onopordum,* albino rats.

INTRODUCTION

Although liver diseases are worldwide problem, it is much more exaggerated in Egypt (Strickland, 2006). The liver is one of the largest organs in the human body and the chief site for intense metabolism and detoxification (Ram, 2001). It has a surprising role in the maintenance, performance and regulating homeostasis of the body. Moreover, it is involved with almost all the biochemical pathways responsible for growth, fight against diseases, nutrient supply, energy provision and reproduction (Ward and Daly, 1999).

In spite of the tremendous advances in modern medicine, no effective drugs are available to overcome some liver problems (Chatterjee, 2000). This fact puts a challenge for drug scientists to explore the potential of hepatoprotective activity of plants based on traditional use (Witte et al., 1983). A large number of medicinal preparations are recommended for the treatment of liver disorders (Chatterjee, 2000). Members of the family Asteraceae, especially the tribe Cynareae are known for their efficacy in relieving some liver disorders. In particular, *Silybum marianum* (Muriel and Mourelle, 1990; Muriel et al., 1992; Flora et al., 1998; Evans, 2002; Madani et al., 2008) and *Cynara scolymus* (Adzet et al., 1987 a, b; Gebhardt, 1997) are very active hepatoprotective agents. Silymarin, obtained from *S. marianum* is a widely used plant derived hepatoprotective agent (Morazzoni and Bombardelli, 1995).

The Cynareae tribe is represented in Egypt by many genera such as *Silybum, Cynara, Centaurea, Carduus, Onopordum* and others (Täckholm, 1974; Boulos, 2002). Some common Cynareae plants in Egyptian flora were selected for this study to evaluate their hepatoprotective effect as they are related chemo-taxonomically to the

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most active hepatoprotective plants.

MATERIALS AND METHODS

The aerial parts of *Carduus getulus* Pomel, *Centaurea alexandrina* Delile and *Centaurea calcitrapa* L. were collected from Borg El Arab during April 2008. The seeds of *Cynara cornigera* Lindl. and *Onopordum alexandrinum* Boiss. were collected from Borg El Arab during May 2008. *Cynara scolymus* L. leaves were collected from El Behira Governorate, Egypt, during March 2008. All the plants were identified in the Department of Botany, Faculty of Science, Alexandria University, and the voucher specimens are deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Egypt.

Extraction

The air dried powdered aerial parts of *C. getulus, C. alexandrina, C. calcitrapa, C. cornigera* and *Onopordum alexandrinum* and the leaves of *C. scolymus* (200 g each) were extracted with 70% ethanol by percolation at room temperature. The seeds of *C. cornigera* and *O. alexandrinum* (200 g each) were defatted using petroleum ether before extraction with 70% ethanol. The ethanol extracts of each plant was separately concentrated under vacuum using rotary vacuum evaporator to give 15.4, 15.9, 21.6, 16, 20.1, 21.0, 14.0 and 10.0 g of total alcohol extracts, respectively.

Experimental animals and chemicals

Wistar albino rats (150 - 200 g) of both sexes obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, were used. The animals were housed under constant temperature (22 ± 2°C), humidity (55%) and light/dark conditions (12/12 h). They were provided with Purina chow and access to drinking water *ad libitum*. All solvents used were of analytical grade. Silymarin was obtained from Sigma Aldrich (St. Louis, USA).

Hepatoprotective activity

Male Wistar rats were divided into 4 groups, of six animals each. Group I was used as control group, while Groups II, III and IV received 1.25 ml of chloromethane (CCl_4) in liquid paraffin (1:1) per kg body weight intraperitoneally. Group II received only CCl_4; Group III was administered silymarin at a dose of 10 mg/kg p.o; Group IV was divided into eight subgroups (n = 6); the subgroups 1 to 6 were treated with the prepared extracts at 500 mg/kg, while subgroups 7 and 8 were treated with the aerial parts of *O. alexandrinum* and *C. cornigera* at 250 mg/kg (Table 1). Drug treatment was started 5 days prior to CCl_4 administration and continued till the end of the experiment. After 48 h of CCl_4 administration, the animals were sacrificed under ether anesthesia. Blood samples were collected by cardiac puncture and the serum was separated for determining the different bio-chemical parameters. The liver was then immediately removed; a small piece was fixed in 10% formalin and kept for histopathological assessment.

Determination of biochemical parameters

Four biochemical parameters; aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin were estimated as reported by Edwards and Bouchier (1991). The enzyme activities were measured using diagnostic strips (Reflotron®, ROCHE) and were read on a Reflotron® Plus instrument (ROCHE).

Histopathological study

For this study, the liver was immediately removed; fixed in 10% formalin, dehydrated with ethanol xylene mixtures and fixed with paraffin wax. Thin sections (3 µM) were stained in Mayer's hematoxylin solution followed by eosin-phloxine solution. Details of the experimental procedures were described by Alqasoumi et al. (2009).

Statistical analysis

An analysis of variance (ANOVA) was used to determine the mean differences. Differences between the control and CCl_4 treated group were compared for significance using student's t-test for non-paired samples (Woolson and Clarke, 2002). Values are presented as mean ± S.E.

RESULTS AND DISCUSSION

Hepatoprotective activity

Carbon tetrachloride is metabolically activated by the cytochrome P-450 dependent mixed function oxidase in the endoplasmic reticulum to form trichloromethyl free radical ($CCl_3^{\cdot}$) that is rapidly converted to trichloromethyl peroxide ($Cl_3COO^{\cdot}$), which combines with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation (Snyder and Andrews, 1996). Some of the lipid peroxidation products are reactive aldehydes, such as 4-hydroxynonenal, which can form adducts with proteins (Weber et al., 2003). These interactions lead to changes in the structures of the endoplasmic reticulum and cell membranes, and hence to increase in plasma membrane permeability to Ca^{2+} and consequently necrotic cell death (Weber et al., 2003). The loss of metabolic enzyme activity, reduction of protein synthesis and loss of glucose-6-phosphatase activity, exacerbation liver damage (Recknagel and Glende, 1973; Azri et al., 1992).

Treatment of the animals with the hepatotoxic agent carbon tetrachloride resulted in significant ($p<0.001$) increase in the liver aminotransferases (AST and ALT) levels which are indications of hepatocytes damage (Zafar and Ali, 1998). Severe jaundice was reflected by the elevated level of serum bilirubin (Lin et al., 1997) (Table 1). On the other hand, treatment of the animals with silymarin resulted in significant decrease ($p<0.001$) in the elevated levels of AST, ALT, ALP and bilirubin ($p<0.001$) (Table 1). Silymarin acts as an antioxidant by scavenging peroxidant free radicals and by increasing the intracellular concentration of GSH (Valenzuela et al., 1989). It also exhibits a regulatory action on the cellular membrane permeability and increases its stability against xenobiotics injury. This is done by increasing the synthesis of ribosomal RNA (by stimulating DNA

Table 1. Effect of different extracts on the serum biochemical parameters (n = 6).

Treatment	Dose mg/kg (orally)	Bio-chemical parameters							
		AST (U/L)		ALT (U/L)		ALP (U/L)		Bilirubin (mg/dl)	
		Mean ± S.E	% Decrease	Mean ± S.E.	% Decrease	Mean ± S.E	% Decrease	Mean ± S.E	% Decrease
Normal	saline	81.63 ± 8.37	-	26.8 ± 5.16	-	335.66 ± 23.07	-	0.52 ± 0.04	-
CCl_4	1.25 ml/Kg	308.66 ± 16.28***	-	278.66 ± 29.67***	-	859.33 ± 43.06***	-	2.94 ± 0.32***	-
Sily.+CCl_4	10	135.33 ± 8.50***	56.15	72.73 ± 10.26**	73.90	396.66 ± 23.45***	53.84	0.89 ± 0.11**	69.72
1	500	290.00 ± 25.35*	6.04	203.3 ± 19.85*	27.03	844.00 ± 17.52*	1.78	1.86 ± 0.34*	36.73
2	500	180.00 ± 20.07**	41.68	122.33 ± 20.03**	56.10	686.33 ± 19.13*	20.13	1.16 ± 0.13**	60.54
3	500	218.66 ± 26.50*	29.15	213.33 ± 26.50*	23.44	790. 66 ± 41.47*	7.99	1.69 ± 0.19*	42.51
Normal	saline	106.00 ± 10.92	-	27.73 ± 5.51	-	374.00 ± 21.27	-	0.55 ± 0.06	-
CCl_4	1.25 ml/Kg	367.66 ± 30.89***	-	307.33 ± 30.43***	-	832.00 ± 53.56***	-	3.18 ± 0.24***	-
Sily.+CCl_4	10	157.33 ± 38.99**	57.20	86.30 ± 19.89**	71.91	456.66 ± 39.32**	45.11	0.82 ± 0.12***	74.21
4	500	280.33 ± 26.83*	23.75	190.33 ± 22.74*	38.06	782.33 ± 20.13-	5.96	2.19 ± 0.25*	31.13
5	500	386.66 ± 42.25 *	-	322.00 ± 67.44*	-	856.33 ± 39.50*	-	2.84 ± 0.27*	10.69
6	500	283.66 ± 15.82*	22.84	168.33 ± 29.14*	45.22	613.00 ± 14.10*	26.32	1.86 ± 0.24*	41.50
Normal	saline	108..23 ± 9.20	-	35.21 ± 2.74	-	482.66 ± 19.78	-	0.41 ± 0.02	-
CCl_4	1.25 ml/Kg	417.83 ± 22.56***	-	361.16 ± 16.79***	-	886.50 ± 20.53***	-	3.15 ± 0.04***	-
Sily.+CCl_4	10	196.17 ± 20.79***	53.05	165.31 ± 12.69***	54.22	642.33 ± 22.22***	27.54	1.19 ± 0.04***	62.22
7	500	213.83 ± 8.12***	48.82	172.5 ± 11.56***	52.17	827.33 ± 22.79	6.65	2.64 ± 0.1*	16.19
8	500	370.33 ± 9.74	11.36	324.33 ± 12.99	10.19	895.16 ± 11.20	-	2.84± 0.1	9.84
Normal	saline	105.86 ± 15.31	-	34.53 ± 6.55	-	366.66 ± 41.52	-	0.54 ± 0.06	-
CCl_4	1.25 ml/Kg	368.33 ± 21.22***	-	305.33 ± 27.61***	-	826.33 ± 35.57***	-	3.19 ± 0.22***	-
Sily.+CCl_4	10	182.66 ± 19.60**	50.45	108.83 ± 17.25**	64.35	428.33 ± 33.00***	48.16	0.94 ± 0.12***	70.58
2	250	280.66 ± 18.44*	23.80	183.33 ± 14.84*	39.95	706.00 ± 27.22*	14.56	2.38 ± 0.25*	25.53
6	250	310.33 ± 13.61*	15.74	229.00 ± 11.78*	24.99	734.33 ± 37.23-	11.13	2.49 ± 0.31*	22.09

1: *Cynara cornigera* seed; 2: *Onopordum alexandrinum* aerial parts; 3: *Centaurea calcitrapa* aerial parts; 4: *Centaurea alexandrina* aerial parts; 5: *Carduus getulus* aerial parts; 6: *Cynara cornigera* aerial parts; 7: *Cynara scolymus* leaves;, 8*: Onopordum alexandrinum* seeds. * p<0.05, **p<0.01, ***p<0.001 CCl_4 group compared with normal, silymarin and drug compared with CCl_4 group.

polymerase-I), exerting a steroid like regulatory action on DNA transcription thereby stimulating protein synthesis and faster regeneration of liver cells (Gakova et al., 1992; Dehmlow et al., 1996a, b; Saller et al., 2007). Silymarin efficacy is not limited to the treatment of toxic and metabolic liver damage but also effective in acute and chronic hepatitis and in inhibiting fibrotic activity (Hikino and Kiso, 1988; Mayer et al., 2005).

Two *Cynara* species were tested for their hepatoprotective effect. *C. scolymus* leaves, *C. cornigera* aerial parts and seed extracts at doses of 500 mg kg^{-1} were administered intraperitoneally to rats prior to CCl_4 administration. The best results were obtained with *C. cornigera* aerial

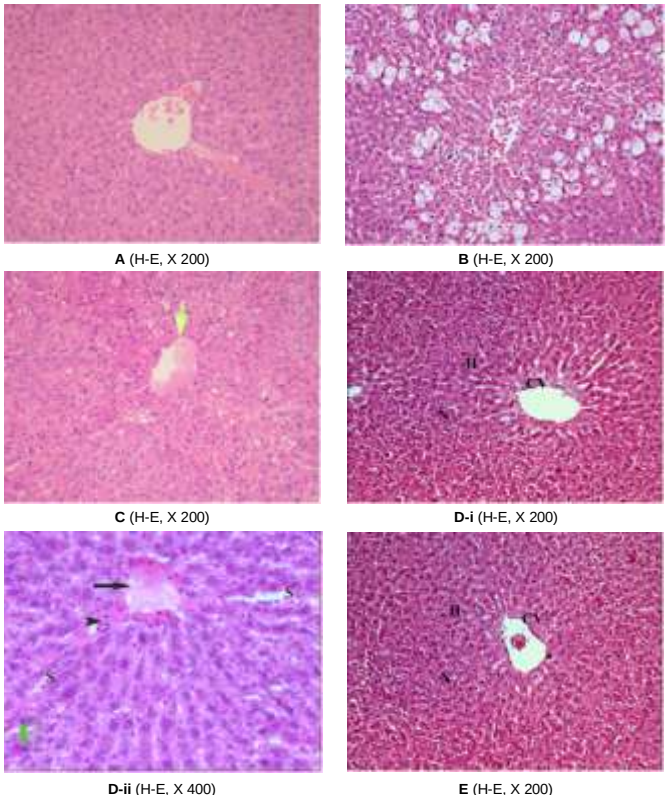

Figure 1. Histopathological appearance of liver cells; (A) normal cells; (B) liver cells of rats treated with CCl_4 showed centrilobular necrosis and extensive fatty change, which was observed on the mid-zonal or entire lobe at 24 h after treatment; (C) liver cells of rats treated with CCl_4 and silymarin showed no necrosis or fatty deposition but had only minimal portal inflammation; (D) liver cells of rats treated with 500 mg kg^{-1} *Cynara cornigera* aerial parts and CCl_4 showed marked protection with dilation of sinusoids and congestion in central vein**;** (E) liver cells of rats treated with 500 mg kg^{-1} *Onopordum alexandrinum* and CCl_4showed marked protection with mild congestion in central vein and dilated in the sinusoids**.**

parts where significant decrease in the levels of AST (22.84%), ALT (45.22%), ALP (26.32%) and bilirubin (41.50%) were observed (Table 1). *C. scolymus* leaves extract significantly decreased the levels of AST (48.82%) and ALT (52.17%). Meanwhile, the effect on bilirubin (16.19%) ($p<0.05$) was weak, while the effect on ALP (6.65%) was weak and statistically insignificant (Table 1). The administration of 500 mg kg^{-1} of the seeds of *C. cornigera* showed weak activity on elevated AST (6.04%) and ALT (27.03%). There was also a very weak effect on the level of ALP (1.78%), while the level of bilirubin was decreased by 36.73%. Treatment with 250 mg kg^{-1} of *C. cornigera* aerial parts resulted in 15.74, 24.99, 11.13 and 22.09 % decrease in the levels of AST, ALT, ALP and bilirubin, respectively (Table 1).

Treatment of animals with 500 mg kg^{-1} *O. alexandrinum* aerial parts significantly decreased the levels of AST (41.68%), ALT (56.10%), ALP (20.13%) and bilirubin (60.54%). No significant results were observed after the treatment with 500 mg kg^{-1} of the seeds of *O. alexandrinum*. Significant decrease in the elevated levels of AST (23.80%), ALT (39.95%) ALP (14.56%) and bilirubin (25.53%) was obtained after treatment with 250 mg kg^{-1} of *O. alexandrinum* aerial part (Table 1). Moreover, treatment of animals with 500 mg kg^{-1} of extracts of *C. calcitrapa* and *C. alexandrina* aerial parts showed weak hepato-protective effect as follows: AST (29.15 and 23.75%), ALT (23.44 and 38.06%), ALP (7.99 and 5.96%) and bilirubin (42.51 and 31.14%), respectively. Meanwhile the administration of 500 mg kg^{-1} of *C. getulus* aerial parts have weak effect on the level of bilirubin (10.69%), while other biochemical parameters were not improved.

Histopathological study

The histological appearance of the hepatocytes reflects their condition (Prophet et al., 1994). The improvement in biochemical parameters after treatment with *C. cornigera* and *O. alexandrinum* aerial parts were confirmed with the histopathological study of the corresponding liver tissues (Figure 1). Exposure of the hepatocytes to toxic agents such as CCl_4 led to histopathological changes from the normal cell appearance (Figure 1A). The hepatocytes of the rat livers treated with a single dose of 1.25 ml kg^{-1} CCl_4 in liquid paraffin showed centrilobular hepatocyte necrosis, microvesicular fatty changes, and extensive fatty changes were observed on the mid-zonal or entire lobe at 48 h (Figure 1B). Liver tissue of rats treated with CCl_4 and silymarin (10 mg kg^{-1}) showed protective effect with the absence of necrosis, and fatty depositions with minimal portal inflammation (Figure 1C). The liver of the rats treated with 500 mg kg^{-1} extract of *C. cornigera* aerial parts and CCl_4 showed marked protection with the appearance of hepatocytes similar to the normal cells. The dilation of sinusoids and congestion in central vein was observed (Figure 1D). The liver of the rats treated with *O. alexandrinum* 500 mg kg^{-1} and CCl_4 also showed marked hepato-protection with mild congestion in central vein and dilation in the sinusoids. The dilation of sinusoids is evident in the centrilobular areas (Figure 1E).

Conclusion

Different parts of six Cynareae plants were evaluated for their hepatoprotective effect against CCl_4-induced toxicity. The results obtained indicated that the aerial parts of *C. cornigera* and *O. alexandrinum* were the most effective in improving liver biochemical parameters and histopathological appearance of hepatocytes. *C. scolymus* was effective in reducing the levels of AST and ALT, however much less improvement was observed with ALP and bilirubin. Further evaluation of the hepato-protective effect of the tribe Cynareae members and the isolation of their corresponding active principles are

therefore recommended based on the obtained results.

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