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Evaluation of the Hepatoprotective effect of *Ephedra foliate*, *Alhagi maurorum*, *Capsella bursa-pastoris* and *Hibiscus sabdariffa* Against Experimentally Induced Liver Injury in Rats

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Abstract – In a project to study the hepatroprotective effect of some plant extracts four plants *Ephedra foliate* Boiss, *Alhagi maurorum* Medikus, *Capsella bursa-pastoris* (L.) Medik. and *Hibiscus sabdariffa* L. were studied. The ethanol extract of the aerial part of the first three plants and the flowers of *H. sabdariffa* were subjected to hepatoprotective assays using Wistar albino rats. Liver injury induced in rats using carbon tetrachloride. The biochemical parameters; serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin were estimated as reflection of the liver condition. Based on the good results of the biochemical parameters measurements, histopathological study was performed on the liver of rats treated with *E. foliate*. The normal appearance of hepatocytes indicated a good protection of the extract from carbon tetrachloride hepatotoxicity. All the results were compared with silymarin, the reference hepatoprotective drug.
Keywords – *Ephedra foliate*, *Alhagi maurorum*, *Capsella bursa-pastoris*, *Hibiscus sabdariffa*, carbon tetrachloride, hepatoprotection, silymarin, rats

Introduction

The liver is an organ of prime importance and plays a significant role not only in metabolism and detoxification of exogenous toxins and therapeutic agents, but also in the bioregulation of fats, carbohydrates, amino acids and proteins. A number of pharmacological and chemical agents act as hepatotoxins and produce a variety of liver ailments (Ram, 2001). Numerous herbal extracts are used for liver problems, however, considerable number of them lack the scientific prove for these claims. Silymarin (Morazzoni and Bombardelli, 1995; Mourelle, *et al*., 1988; Chander, 1989), schisandrin B (Zhu, *et al*., 1999; Maeda, *et al*., 1981; Cyong, *et al*., 2000), phyllanthin, hypophyllanthin (Ramachandra Row, *et al*., 1966), picroside I and kutkoside (Ram, 2001; Ansari, *et al*., 1988) are examples of natural antihepatotoxic drugs derived from traditional herbs. We are interested in screening of some plant extracts for possible hepato-protective effects. In the present study four plant extracts; *E. foliate*, *A. maurorum*, *C. bursa-pastoris* and *H. sabdariffa* were tested against experimentally induced liver injury in rats. *A. maurorum* known locally as "Aqul" or "Camel's Thorn" is used in Saudi folk medicine for the treatment of liver problems, migraine, and cataract, as tonic, digestive, antipyretic, laxative, diuretic, aphrodisiac and anti-inflammatory (Al-Yahya, *et al*., 1990; Ghazanfar, 1994; Mossa, *et al*., 1987). The plant is reported to contain alkaloids, flavonoids, tannins, sterols and ascorbic acid (Al-Yahya, *et al*., 1990; Abbas, *et al*., 1992; Ghazanfar, 1994). *C. bursa-pastoris*, known locally as "Shepherd's Purse", is used locally as remedy for liver, hemorrhages, respiratory problem and diuretic (Al-Yahya, *et al*., 1990; Mossa, *et al*., 1987; El-Shanawany, 1994). Phytochemical study revealed the presence of alkaloids, flavonoids saponins and ascorbic acid (Al-Yahya, *et al*., 1990; El-Shanawany, 1994). The choice of *H. sabdariffa* was based on the fact that it is rich in phenolic compounds and has antioxidant effect (Ross, 2003). Our phytochemical screening revealed that *E. foliate* is rich in phenolic compounds.

Experimental

Plant materials – The aerial parts of *Ephedra foliate*

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Boiss (Ephedraceae), *Alhagi maurorum* Medikus (Fabaceae), *Capsella bursa-pastoris* (L.) Medik. (Brassicaceae) and *Hibiscus sabdariffa* L. (Malvaceae) were collected from different parts of Saudi Arabia. All the plant materials were identified by Dr. Mohammad Atiqur Rahman, taxonomist of the Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Voucher specimens (# 11046, 10978, 12552 and 7881 for the four plants respectively) were deposited at the herbarium of this center.

Preparation of the extracts – From each plant material 50 g were extracted to exhaustion by percolation at room temperature with 90% ethanol, and the extracts were evaporated *in vacuo* to leave 5.75, 7.69, 6.68 and 8.3 g of *E. foliate* Boiss, *A. maurorum* Medikus, *C. bursa-pastoris* (L.) Medik. and *H. sabdariffa* L., respectively.

Test animals – Wistar albino rats (150 - 200 g) of either sex roughly the same age (8 - 10 weeks), 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 free access to drinking water *ad libitum* (Ahmed, *et al*., 2003). The experiments and procedures used in this study were approved by the Ethical Committee of the College of Pharmacy, King Saud University.

Chemicals – Silymarin (Sigma Chemical Company, USA).

Hepatoprotective activity – Male Wistar rats were divided into five groups' six animals each. *Group I* was kept as a control group. *Groups II, III, IV* and *V* received 0.125 mL of CCl_4 in liquid paraffin (1 : 1) per 100 g body weight intraperitoneally. *Group II* received only CCl_4 treatment. *Group III* was administered silymarin at a dose of 10 mg/kg p.o. Groups *IV* and *V* were treated with 250 and 500 mg/kg of extracts or fractions respectively. Drug treatment was started 5 days prior to CCl_4 administration and continued till the end of the experiment. After 48 h, following $CC1_4$ administration the animals were sacrificed using ether anesthesia. Blood samples were collected by heart puncture and the serum was separated for evaluating the biochemical parameters. The liver was immediately removed and a small piece was fixed in 10% formalin for histopathological assessment.

Determination of the enzyme levels – The biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin were estimated by reported methods (Edwards and Bouchier, 1991). The enzyme activities were measured using diagnostic strips (Reflotron®, ROCHE) and were read on a Reflotron® Plus instrument (ROCHE).

Statistical analyses – For each set of experiments where two or more than two groups were compared, an analysis of variance (ANOVA) test were used to determine the significance of the differences. Differences between the control and CCl_4-treated group were compared for significance using student's *t*-test for non paired samples (Woolson, 1987). All the values shown are the mean ± S.E.

Histopathology – The livers of treated animals were immediately removed and a small piece was fixed in 10% formalin for histopathological assessment. All specimens were placed in cassettes and loaded into tissue baskets. The specimens were subjected to dehydration, clearing and inflatration by immersion in different conc of ethanol (70 - 100%), xylene (3 times, 1 hr each) and finally paraffin wax (4 times, 1hr each). The tissues were then transferred into moulds filled with paraffin wax. After orienting the tissues by hot forceps the moulds were chilled on cold plates and excess wax were trimmed off using a knife. The rotary microtome (*Leitz* 1512) was used for making thin sections (3 μm). The sections were placed onto clean slides that were drained vertically for several minutes before placing them onto a warming table at 37 - 40 °C (Edna, *et al*., 1994). The slides were then deparaffinized, hydrated and stained in Mayer's hematoxylin solution for 15 minutes. The slides were then washed in lukewarm running tap water for 15 minutes, placed in distilled water, 80% ethyl alcohol for 1 to 2 minutes then counterstained in eosin-phloxine solution for 2 minutes. The slides were then dehydrated and cleared through 2 changes each of 95% ethyl alcohol, absolute ethyl alcohol, and xylene, 2 minutes each and finally mounting with resinous medium.

Results and Discussion

Hepatic toxicity is reflected by increase in the biochemical parameter levels such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin. Treatment of rats with carbon tetrachloride resulted in sever damage of hepatocytes, biliary obstruction and transport inability across the liver as indicated by high levels of SGOT, SGPT, ALP and bilirubin (Table 1) (Malloy and Erelyn, 1937; Edwards and Bouchier, 1991; Reitman and Frankel, 1957; Kind

Table 1. Effects of Ethanolic Extracts of *E. foliate*, *A. maurorum*, *C. bursa-pastoris* and *H. sabdariffa* on Serum Biochemical Parameters

Treatment (n = 6)	Dose mg/kg (Orally)	Biochemical Parameters							
		SGOT (units/l)		SGPT (units/l)		ALP (units/l)		Bilirubin (mg/dl)	
		Mean ± S.E.	% Decrease	Mean ± S.E.	% Decrease	Mean ± S.E.	% Decrease	Mean ± S.E.	% Decrease
Normal (control)	Normal saline	105.41 ± 21.73		35.68 ± 11.10		400.83 ± 26.75		0.58 ± 0.05	
CCl_4 only (toxicity control)	1.25 ml/kg	411.50[a] ± 25.05***		309.83[a] ± 23.97***		996.33[a] ± 25.35***		2.88[a] ± 0.19***	
Silymarin + CCl_4	10	184.83[b] ± 26.43***	55.1	104.88[b] ± 15.01***	66.1	601.50[b] ± 28.45***	39.6	1.05[b] ± 0.16***	63.5
Ephedra foliate + CCl_4	250	320.00[b] ± 27.12*	22.2	266.33[b] ± 22.42	14.0	911.33[b] ± 21.96*	8.5	2.24[b] ± 0.21*	22.2
Ephedra foliate + CCl_4	500	236.00[b] ± 26.66***	42.6	187.50[b] ± 17.64**	39.5	784.50[b] ± 29.06***	21.2	1.55[b] ± 0.14***	46.2
Normal (control)	Normal saline	96.90 ± 19.87		35.96 ± 8.62		371.16 ± 39.24		0.61 ± 0.08	
CCl_4 only (toxicity control)	1.25 ml/kg	371.00[a] ± 35.63***		320.33[a] ± 46.61***		907.00[a] ± 32.26***		3.85[a] ± 0.38***	
Silymarin + CCl_4	10	124.11[b] ± 19.11***	66.5	112.05[b] ± 20.77**	65.02	448.33[b] ± 40.97***	50.6	1.17[b] ± 0.24***	69.6
Alhagi maurorum + CCl_4	250	378.00[b] ± 29.30	–	316.33[b] ± 27.44	–	964.33[b] ± 25.31	–	3.72[b] ± 0.38	–
Alhagi maurorum + CCl_4	500	333.33[b] ± 28.60	10.1	289.33[b] ± 15.40	9.7	929.33[b] ± 36.44	–	3.20[b] ± 0.23	16.8
Capsella bursa-pastoris + CCl_4	250	356.16[b] ± 32.60	4.0	308.66[b] ± 20.60	3.6	944.16[b] ± 39.15	–	3.02[b] ± 0.30	21.5
Capsella bursa-pastoris + CCl_4	500	271.00[b] ± 26.38*	26.9	248.16[b] ± 31.84	22.4	787.50[b] ± 54.64	13.2	2.63[b] ± 0.26*	31.7
Hibiscus sabdariffa + CCl_4	250	275.16[b] ± 27.67	25.8	227.83[b] ± 22.42	28.8	792.00[b] ± 30.21*	12.7	2.68[b] ± 0.28*	30.4
Hibiscus sabdariffa + CCl_4	500	205.66[b] ± 31.08**	44.5	201.33[b] ± 15.71*	37.1	716.00[b] ± 29.07**	21.0	2.50[b] ± 0.32*	35.0

*p < 0.05; **p < 0.01; ***p < 0.001, [a] as compared with the normal saline (control) group; [b] as compared with the CCl_4 only group.

and King 1954). Pretreatment of rats with silymarin, significantly decreased the raised levels of SGOT, SGPT, ALP and bilirubin induced by CCl_4 (55.1, 66.1, 39.6 and 63.5% respectively, $p < 0.001$) (Table 1) indicating a good recovery from the hepatotoxic agent.

The hepatoprotective effect offered by *E. foliata* (whole plant) crude extract at 500 mg/kg doses, was found to be significant in all parameters studied with 42.6, 39.5, 21.2 and 46.2% reduction in SGOT, SGPT, ALP and bilirubin, respectively. At the lower doses (250 mg/kg) the extract resulted in a significant reduction in SGOT, ALP and bilirubin ($p < 0.05$) (Table 1). Although the lower doses resulted in a 14% reduction in the SGPT level. However, the results were not statistically significant.

A. maurorum (aerial parts) crude extract at the doses of (250 and 500 mg/kg) failed to reduce the raised level of the biomarkers indicating that the plant extract is free from any hepatoprotective effect.

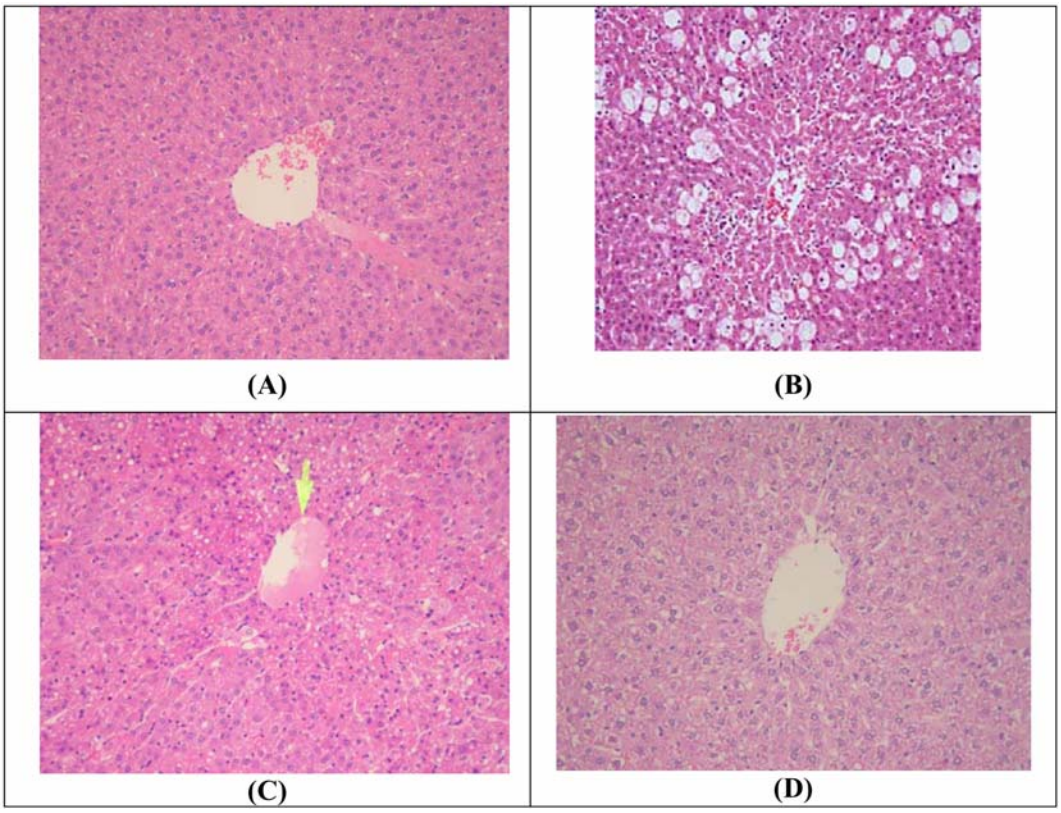

Fig. 1. Histopathological appearance of liver cells; (A) normal cells; (B) liver cells of rats treated with CCl_4; (C) liver cells of rats treated with CCl_4 and silymarin; (D) liver cells of rats treated with CCl_4 and *Ephedra foliata*.

The serum levels of SGOT and bilirubin in the group of *C. bursa-pastoris* (aerial parts) crude extract treated animals showed significant decreases by (26.9 and 31.7%) respectively, at the dose of 500 mg/kg body weight ($p < 0.05$) (Table 1). The smaller dose of the extract, although it lowered the levels of all parameters, however the results were not statistically significant.

The increased serum levels of SGOT, SGPT, ALP and bilirubin in the group of *H. sabdariffa* (flowers) crude extract treated animals showed significant decreases (44.5, 37.1, 21.0 and 35.0% respectively) at the dose of 500 mg/kg body weight ($p < 0.05$) (Table 1). The smaller dose of the extract lowered significantly only the levels of ALP and bilirubin (12.7 and 30.4% respectively) ($p < 0.05$).

According to the obtained results from the hepato-protective study using the liver enzyme levels as an indication for hepatoprotection, *E. foliate* gave the best results followed by *H. sabdariffa*. The extract of *C. bursa-pastoris* showed weak effect while that of *A. maurorum* was totally inactive.

Based on the above results the livers of rats pretreated with *E. foliata* were subjected histological study. The histological appearance of the hepatocyte reflects their conditions (Edna, *et al*., 1994). Exposure of hepatocytes to toxic agents such as CCl_4 leads to histopathological changes from the normal histological appearance (Fig. 1). The hepatocytes of rat livers treated with a single dose of 1.25 mL CCl_4/kg, showed centrilobular hepatocyte necrosis and extensive fatty change were observed on the midzonal or entire lobe at 24 h (Fig 1B). Liver tissue of rats treated with CCl_4 and silymarin showed good recovery with absence of necrosis and fatty depositions (Fig. 1C), only minimal portal inflammation has been noticed. *E. foliata* at 500 mg/kg dose revealed a tremendous progress with disappearance of fatty deposition and necrosis. It showed only mild portal inflammation indicating a good recovery of hepatocytes.

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