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## Evaluation of the Hepatoprotective Effect of *Fumaria parviflora* and *Momordica balsamina* from Saudi Folk Medicine Against Experimentally Induced Liver Injury in Rats

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**Abstract:** In a project to evaluate the efficacy of traditional Saudi plants used for liver problems the two plants *Fumaria parviflora* Lam. (Fumariaceae) and *Momordica balsamina* Linn. (Cucurbitaceae) were studied. The ethanol extract of the aerial part of *Fumaria parviflora* and the leaves of *Momordica balsamina* 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 results of the biochemical parameters measurements, histopathological study was performed on the liver of rats treated with two extracts. The normal appearance of hepatocytes indicated a good protection of the extracts from carbon tetrachloride hepatotoxicity. All the results were compared with silymarin, the reference hepatoprotective drug.

**Key words:** *Fumaria parviflora*, *Momordica balsamina*, carbon tetrachloride, hepatoprotection, silymarin, rats

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### INTRODUCTION

Natural products still represents a vital source for biologically active drugs with unique mechanisms of actions. Many traditional plants are used for the treatment of liver problems. Study of these led to the discovery of active compounds yet developed to successful drugs. Silymarin (Saller *et al.*, 2007), schisandrin B (Zhu *et al.*, 1999; Cyong *et al.*, 2000), phyllanthin, hypophyllanthin, picroside I and kutkoside (Ram, 2001) are examples of natural antihepatotoxic compounds derived from traditional herbs. In Saudi Arabia, many plants are claimed to treat liver problems (Mossa *et al.*, 1987), however, most of these claims lack the scientific prove. Here was an interest in screening of such plants for their hepatoprotective effect and in advanced steps identify their active constituents. The aerial parts of *Fumaria parviflora* are used in Saudi folk medicine for bile secretion, spleen disorders and jaundice. Other used includes diaphoretic, diuretic, anthelmintic and laxative. It is also claimed to have antimicrobial activity so used for treatment of bacterial and skin diseases (Mossa *et al.*, 1987; Ghazanfar, 1994; El-Shanawany, 1996). Extract of the aerial parts of *Fumaria parviflora* showed significant anticholinesterase activity (Orhan *et al.*, 2004). It also proved to be active against larvae of *Haemonchus contortus* (Hördegen *et al.*, 2006). Pharmacological studies

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indicated that *Fumaria parvi* posses anti-inflammatory, anti-nociceptive and antidiabetic activities (Akhtar *et al.*, 1984; Heidari *et al.*, 2004). Different parts of *Momordica balsamina* are used as galactagogue, anthelmintic, laxative and tonic. It is also used for disorders of the liver (Mossa *et al.*, 1987; El-Shanawany, 1996). Recent study indicated that the fruit pulp extract of *Momordica balsamina* has promising anti-HIV, antimicrobial and antiplasmodial effects (Bot *et al.*, 2007; Benoit-Vical *et al.*, 2006; Jigam *et al.*, 2004).

## MATERIALS AND METHODS

### Plant Materials

The aerial parts of *Fumaria parviflora* Lam. (Asteraceae) and the leaves of *Momordica balsamina* Linn. (Cucurbitaceae) were purchased from the local market in Riyadh, in April 2003. The plants 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.

### Extraction

The dried ground aerial parts of *Fumaria parviflora* and leaves of *Momordica balsamina* (50 g from each plant) were extracted to exhaustion by percolation at room temperature with 90% ethanol and the extract was evaporated *in vacuo* to leave 7.4 and 6.3 g of residue.

### 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* (Abdel-Kader and Alqasoumi, 2008). 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 1 was kept as a control group. Groups 2, 3, 4 and 5 received 0.125 mL of CCl<sub>4</sub> in liquid paraffin (1:1) per 100 g body weight intraperitoneally. Group 2 received only CCl<sub>4</sub> treatment. Group 3 was administered silymarin at a dose of 10 mg kg<sup>-1</sup> p.o. Groups 4 and 5 were treated with 250 and 500 mg kg<sup>-1</sup> of extracts respectively. Drug treatment was started 5 days prior to CCl<sub>4</sub> administration and continued till the end of the experiment. After 48 h, following CCl<sub>4</sub> 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 (Boon *et al.*, 2006). The enzyme activities were measured using diagnostic strips (Reflotron<sup>®</sup>, ROCHE) and were read on a Reflotron<sup>®</sup> Plus instrument (ROCHE).

### Statistical Analysis

For each set of experiments where two or more than two groups were compared, an analysis of variance (ANOVA) test was used to determine the significance of the differences. Differences between the control and CCl<sub>4</sub>-treated group were compared for significance using student's t-test for non paired samples (Woolson and Clarke, 2002). All the values shown are the mean±SE mean.

### 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 infiltration by immersion in different conc of ethanol (70-100%), xylene (3 times, 1 h each) and finally paraffin wax (4 times, 1 h 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 (Prophet *et al.*, 1994). The slides were then deparaffinized, hydrated and to stained in Mayer's hematoxylin solution for 15 min. The slides were then washed in lukewarm running tap water for 15 min, placed in distilled water, 80% ethyl alcohol for 1 to 2 min then counterstained in eosin-phloxine solution for 2 min. The slides were then dehydrated and cleared through 2 changes each of 95% ethyl alcohol, absolute ethyl alcohol and xylene, 2 min each and finally mounting with resinous medium.

## RESULTS AND DISCUSSION

Male Wistar rats were used to estimate the serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin serum enzymes as indicator for liver protection against carbon tetrachloride hepatotoxicity. Treatment of animals with the hepatotoxic agent carbon tetrachloride resulted in significant increase of transaminases (SGOT and SGPT) and alkaline phosphate levels (ALP) due to hepatocytes damage (Zafar and Ali, 1998). Severe jaundice was reflected by increase level of serum bilirubin (Lin *et al.*, 1997) (Table 1).

Treatment of animals with the known hepatoprotective agents silymarin as a reference standard resulted in significant decrease in the elevated levels of SGOT, SGPT, ALP and bilirubin ( $p < 0.001$ ) (Table 1). Silymarin act as an antioxidant by scavenging prooxidant free radicals and by increasing the intracellular concentration of GSH. It also exhibits a regulatory action of cellular membrane permeability and increase in its stability against xenobiotics injury; increasing the synthesis of ribosomal RNA by stimulating DNA polymerase-I, exerting a steroid like regulatory action on DNA transcription and stimulation of protein synthesis and regeneration of liver cells (Saller *et al.*, 2007; Dehmlow *et al.*, 1996a, b; Gakova *et al.*, 1992). Silymarin efficacy is not limited to the treatment of toxic and metabolic liver damage; it is also effective in acute, chronic hepatitis and in inhibiting fibrotic activity (Hikino and Kiso, 1988; Mayer *et al.*, 2005).

Treatment of the animals with doses of 500 mg kg<sup>-1</sup> body weight of *Fumaria parviflora* aerial parts ethanol extract prior to CCl<sub>4</sub> administration resulted in decrease in the levels of SGOT (24.1%),

Table 1: Effects of ethanolic extracts of *Fumaria parviflora* and *Momordica balsamina* on serum biochemical parameters

Treatments (n = 6)	Dose mg kg <sup>-1</sup> (Orally)	Biochemical parameters			
		SGOT (units L <sup>-1</sup> )		SGPT (units L <sup>-1</sup> )	
		Mean±SE	Decrease (%)	Mean±SE	Decrease (%)
Normal (control)	Normal saline	115.10±20.61		45.01±12.78	
CCl <sub>4</sub> only (toxicity control)	1.25 mL kg <sup>-1</sup>	500.16±27.37 <sup>a****</sup>		403.66±25.09 <sup>a****</sup>	
Silymarin (standard drug)+CCl <sub>4</sub>	10	174.33±30.68 <sup>b****</sup>	65.1	112.05±17.91 <sup>b****</sup>	72.2
<i>F. parviflora</i> +CCl <sub>4</sub>	250	456.50±30.36 <sup>b</sup>	8.7	351.66±30.56 <sup>b</sup>	12.8
<i>F. parviflora</i> +CCl <sub>4</sub>	500	378.33±28.21 <sup>b*</sup>	24.3	316.33±27.06 <sup>b*</sup>	21.6
Normal (control)	Normal saline	98.93±14.58		47.30±10.73	
CCl <sub>4</sub> only (toxicity control)	1.25 mL kg <sup>-1</sup>	482.33±25.28 <sup>a****</sup>		400.16±19.04 <sup>a****</sup>	
Silymarin+CCl <sub>4</sub>	10	204.16±28.25 <sup>b****</sup>	57.6	171.50±25.24 <sup>b****</sup>	57.1
<i>M. balsamina</i> +CCl <sub>4</sub>	250	367.16±27.88 <sup>b*</sup>	23.8	286.00±14.19 <sup>b****</sup>	28.5
<i>M. balsamina</i> +CCl <sub>4</sub>	500	301.00±28.85 <sup>b****</sup>	37.5	243.66±33.20 <sup>b****</sup>	39.1

Treatments (n = 6)	Dose mg kg <sup>-1</sup> (Orally)	Biochemical parameters			
		ALP (units L <sup>-1</sup> )		Bilirubin (mg dL <sup>-1</sup> )	
		Mean±SE	Decrease (%)	Mean±SE	Decrease (%)
Normal (control)	Normal saline	496.66±34.43		0.59±0.05	
CCl <sub>4</sub> only (toxicity control)	1.25 mL kg <sup>-1</sup>	940.33±30.32 <sup>a****</sup>		2.99±0.20 <sup>a****</sup>	
Silymarin (standard drug)+CCl <sub>4</sub>	10	553.33±27.20 <sup>b****</sup>	41.1	1.13±0.13 <sup>b****</sup>	62.2
<i>F. parviflora</i> +CCl <sub>4</sub>	250	796.83±26.61 <sup>b****</sup>	15.2	3.02±0.26 <sup>b</sup>	-
<i>F. parviflora</i> +CCl <sub>4</sub>	500	746.83±43.17 <sup>b****</sup>	20.5	2.65±0.29 <sup>b</sup>	11.3
Normal (control)	Normal saline	490.33±28.22		0.60±0.07	
CCl <sub>4</sub> only (toxicity control)	1.25 mL kg <sup>-1</sup>	984.83±27.98 <sup>a****</sup>		3.11±0.28 <sup>a****</sup>	
Silymarin+CCl <sub>4</sub>	10	614.83±23.97 <sup>b****</sup>	37.5	1.18±0.13 <sup>b****</sup>	62.0
<i>M. balsamina</i> +CCl <sub>4</sub>	250	809.33±37.62 <sup>b****</sup>	17.8	1.92±0.22 <sup>b****</sup>	38.2
<i>M. balsamina</i> +CCl <sub>4</sub>	500	755.83±27.14 <sup>b****</sup>	23.2	1.47±0.12 <sup>b****</sup>	52.7

\*p<0.05; \*\*p<0.01; \*\*\*p<0.001, <sup>a</sup>as compared with the normal saline (control) group; <sup>b</sup>as compared with the CCl<sub>4</sub> only group

SGPT (21.6%), ALP (20.5%) and bilirubin (11.3%) levels. The lower dose of the extract (250 mg kg<sup>-1</sup>, body weight) show only significant decrease of the levels of APL (15.2%) (Table 1).

*Momordica balsamina* (Leaves) crude extract when given to rats in the two used doses (250 and 500 mg kg<sup>-1</sup>, body weight) before CCl<sub>4</sub> treatment resulted in highly significant (p<0.001) decrease in the levels of SGOT, SGPT, ALP and bilirubin (Table 1). The obtained results indicated a high degree of protection against the hepatotoxic effect of CCl<sub>4</sub>.

The histological appearance of the hepatocyte reflects their conditions (Fig. 1A). Exposure of hepatocytes to toxic agents such as CCl<sub>4</sub> leads to histopathological changes from the normal cell appearance. The hepatocytes of rat livers treated with a single dose of 1.25 mL CCl<sub>4</sub> kg<sup>-1</sup>, showed centrilobular hepatocyte necrosis, microvesicular fatty change and extensive fatty change were observed on the midzonal or entire lobe at 24 h (Fig. 1B). Liver tissue of rats treated with CCl<sub>4</sub> and silymarin showed good recovery with absence of necrosis, fatty depositions and recovery to normal histological appearance of hepatocytes (Fig. 1C). The central vein has minimal portal inflammation. Histological appearance of rat liver treated with 500 mg kg<sup>-1</sup> *Fumaria parviflora* aerial parts total extract showed minimal portal inflammation and more or less normal appearance (Fig. 1D). Sections of the rat liver treated with CCl<sub>4</sub> and *Momordica balsamina* (500 mg kg<sup>-1</sup>) showed significant recovery with disappearance of fatty deposition and necrosis. The portal tracts appeared almost clear indicating a potent hepatoprotective activity (Fig. 1E).

The current study aimed to evaluate the effect of *Fumaria parviflora* and *Momordica balsamina* as protective for hepatocytes. The higher dose of *Fumaria parviflora* showed significant decrease in

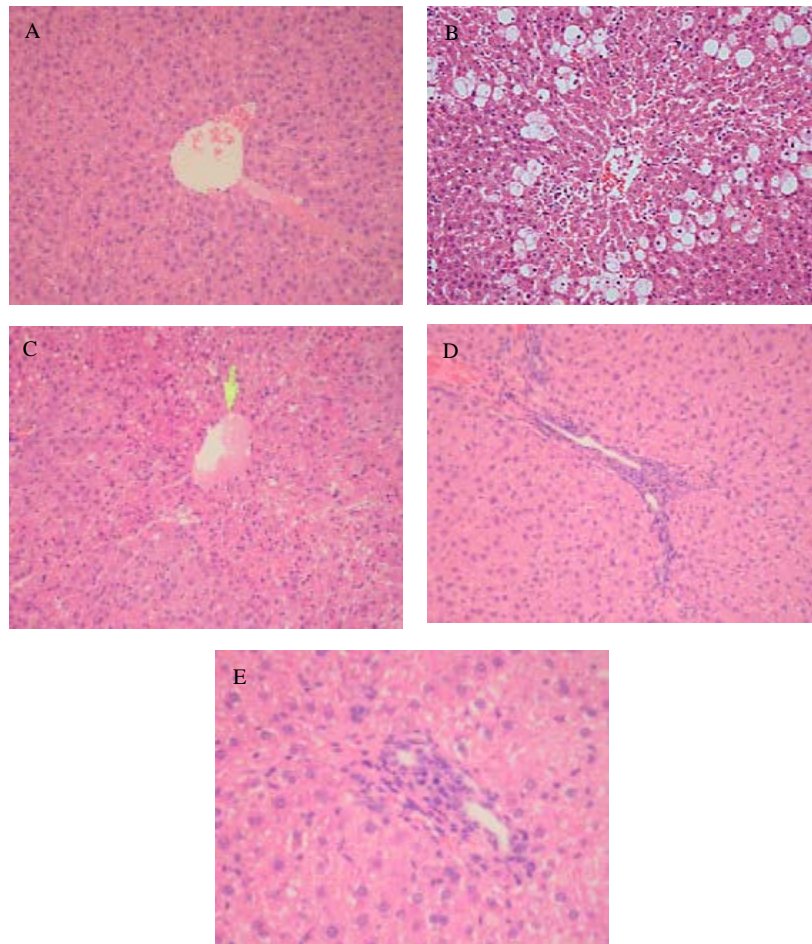


Fig. 1: Histopathological appearance of liver cells, (A) normal cells, (B) liver cells of rats treated with  $\text{CCl}_4$ , (C) liver cells of rats treated with  $\text{CCl}_4$  and silymarin, (D) liver cells of rats treated with  $\text{CCl}_4$  and *Fumaria parviflora*, (E) liver cells of rats treated with  $\text{CCl}_4$  and *Momordica balsamina*

the level of biochemical markers as well as notable recovery of the histological characters of the hepatocytes. *Momordica balsamina* showed superior protective effect in both biochemical markers assessment and histopathological appearance of hepatocytes.

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