Iranian Journal of Pharmaceutical Research (2007), 6 (4): 285-290
Received: March 2006
Accepted: September 2006

Original Article

Hepatoprotective Activity of *Capparis spinosa* Root Bark Against CCl_4 Induced Hepatic Damage in Mice

Nasrin Aghel[a*], Iran Rashidi[b] and Amir Mombeini[a]

[a]*Department of Pharmacognosy, School of Pharmacy, Ahwaz Joundishapour Medical Sciences University, Ahwaz, Iran.* [b]*Department of Pathology, School of Medicine, Ahwaz Joundishapour Medical Sciences University, Ahwaz, Iran.*

Abstract

Many hepatoprotective herbal preparations have been recommended in alternative systems of medicine for the treatment of hepatic disorders. No systematic study has been done on protective efficacy of *Capparis spinosa* (Capparidaceae) to treat hepatic diseases. Protective action of *C. spinosa* ethanolic root bark extract was evaluated in this study in an animal model of hepatotoxicity, which was induced by carbon tetrachloride.

Healthy male mice (30-35 g body weight, 6-8 week old) were divided into 7 groups. Group 1 was normal control group; Group 2, the hepatotoxic group was given CCl_4; Group 3 was administered olive oil (vehicle); Groups 4-6 received different doses of ethanolic root bark extract (100, 200 & 400 mg/kg) with CCl_4; Group 7 was administered overdose of the extract (800 mg/kg). The parameters studied were alanine transaminase and aspartate transaminase activities and duration of sleep. The hepatoprotective activity was also supported by histopathological studies of liver tissue.

Results of the biochemical studies of blood samples of CCl_4 treated animals showed significant increase in the levels of serum enzyme activities, reflecting the liver injury caused by CCl_4. Whereas blood samples from the animals treated with ethanolic root bark extracts showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells. The results revealed that ethanolic root bark extract of *C. spinosa* could afford significant dose-dependent protection against CCl_4 induced hepatocellular injury.

Keywords: Hepatotoxicity; CCl_4; *Capparis spinosa*; Liver enzymes; Histopathology.

Introduction

Capparidaceae are a medium-sized family of approximately 40-45 genera and 700-900 species, whose members present considerable diversity in habit, fruit, and floral features (1-3). Capparidaceae are pantropical in distribution, being most conspicuous in tropical seasonally dry habitants (4). *Capparis spinosa* (caper), a winter-deciduous perennial shrub, is a consistent floristic element of Mediterranean ecosystems, growing from May to October, i.e. entirely during the prolonged summer drought. Capers, a useful and beautiful plant in the Capparidaceae, can today be found growing wild all over Mediterranean, and are frequently cultivated (e.g., in France, Spain, Italy and Algeria; furthermore, Iran, Cyprus and Greece produce significant amounts of the plant); their origin is, though, supposed in the dry areas of Western or Central Asia.

* Corresponding author:
E-mail: aghelnas@yahoo.com

The caper plant is well known for the culinary properties of the caper, the immature flower buds which have been pickled in vinegar or preserved in granular salt. They have long been used in recipes of salads, pasta, meat, sauces and garnishes to add a pungent spicy flavor and aroma to food. The caper had other uses prior to its use in cooking. The first recorded use of the caper bush was for medicinal purposes in 2000 BC by the Sumerians. It has been suggested that Capers have been used or are still being used in reducing flatulence, in the treatment of rheumatism, anemia and gout. Further medical uses include ingesting for improving liver functions, as diuretics, kidney disinfectants (5). Infusions and decoctions from caper root bark have been traditionally used for dropsy, anemia, arthritis and gout (6, 7). The root-bark is analgesic, anthelmintic, antihaemorrhoidal, aperient, depurative, diuretic, emmenagogue, expectorant, hepatoprotective, tonic and vasoconstrictive (7-9). Externally, it is used to treat skin conditions, capillary weakness and easy bruising (10). The bark is harvested in the autumn and dried for later use (6).

Liver, an important organ actively involved in metabolic functions, is a frequent target of a number of toxicants (11). The principal cause of carbon tetrachloride CCl_4 induced hepatic damage is lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals (12, 13). The resulting hepatic injury was characterized by leakage of cellular enzymes into the blood stream and by centrilobular necrosis (14).

Presently, the use of herbal medicines for prevention and control of chronic liver diseases is in the focus of attention for the physicians, pharmaceutical manufacturers and patients; the reasons for such shift toward the use of herbals include the expensive cost of conventional drugs, adverse drug reactions, and their inefficacy.

Many hepatoprotective herbal preparations have been recommended in alternative systems of medicine for the treatment of hepatic disorders. No systematic study has been done on protective efficacy of *Capparis spinosa* to treat hepatic diseases. Therefore, the protective action of *Capparis spinosa* root bark extract was evaluated in an animal model of hepatotoxicity induced by carbon tetrachloride.

Experimental

Plant extract

The roots of *Capparis spinosa* were collected in July 2005 from Shoosh, province of Khuzestan, south-west of Iran. The plant was authenticated by Department of Botany, Faculty of Agriculture, Shahid Chamran University, Ahwaz, Iran and the voucher specimen has been deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ahwaz Joundishapour Medical Sciences University. Ahwaz, Iran. The collected roots were washed and the bark was peeled off and then air-dried at shade and room temperature.

Hydroalcoholic extract was prepared by using the soxhlet method. The coarsely powdered plant barks (200 g) were extracted with ethanol (80% v/v in water) for 5 h. After filtration using Whatman No.1 filter paper, the ethanolic extract was evaporated in vaccum below 50 °C. The yield of evaporation and solvent removal of hydroalcoholic extract of *Capparis spinosa* was 7.35% w/w, which was stored in refrigerator for further use.

Experimental animals

Healthy, 6-8 week old male mice (30-35g) were obtained from Razi Vaccine & Serum Research Institute, Karaj, Iran. Animals were maintained on a standard laboratory diet. Food and water were given *ad libitum*. They were housed in standard stainless-steel cages at a 12-h cycle of light and dark. Room temperature was kept at 24±2°C and humidity maintained at 50%. All the chemicals used were of the analytical grade from standard companies.

Treatment of animals

Mice were randomly divided into 7 groups with 7 animals in each group. Group 1 served as negative control and was administered a single daily dose of 0.2 ml of normal saline by oral gavage. Group 2, the positive control was given daily carbon tetrachloride (0.2 mg/kg/0.2 ml in olive oil) orally. Group 3 was administered a single daily dose of 0.2 ml olive oil (vehicle) orally. The drug control groups (4,

Table 1. Effects of *Capparis spinosa* root bark extracts on serum enzymes activitis in CCl_4-intoxicated mice.

Group	Treatment	ALT (U/L)	AST (U/L)
1	Normal saline	114.00±4.37	143.20±2.70
2	CCl_4 0.2 ml/kg	191.80±5.51*	239.40±4.27*
3	Olive oil 0.2 ml	117.14±2.99	145.28±2.95
4	Plant extract 100 mg/kg+ CCl_4	180.83±3.17*	230.16±3.23*
5	Plant extract 200 mg/kg+CCl_4	155.60±5.92**	168.20±4.69**
6	Plant extract 400 mg/kg+CCl_4	117.00±9.24**	147.20±3.54**
7	Plant extract 800 mg/kg	117.71±2.90	144.00±4.74

Mice were administered with *Capparis spinosa* extracts 100, 200 and 400 mg/kg orally once, one hour after the administration of carbon tetrachloride (orally), for 4 days. Data are expressed as mean±SEM (n=7).
* $p<0.05$ as compared with negative control (group 1).
** $p<0.05$ as compared with positive control (group 2).
One-way ANOVA and Dunnett test.

5 and 6) were given the plant extracts orally in doses of 100, 200 and 400 mg/kg/0.2 ml (in normal saline), respectively, one hour after the administration of carbon tetrachloride (0.2 mg/kg/0.2ml in olive oil), for four days (15, 16). To evaluate the effects of the root bark extract overdose on the studying factors, the 7th group was selected, which received 800 mg/kg daily of the extract. In the 5th day, all animals were given sodium thiopental (25 mg/kg/0.2 ml) intraperitoneally and the effects of extracts on CCl_4-induced prolongation of thiopental sleeping time were studied (17). All animals were sacrificed in the 6th day, blood samples were collected and serum was separated. The liver was excised, fixed in 10% buffered formalin for histopathological assessment of liver damage.

Assessment of liver damage

Liver damage was assessed by the estimation of serum activities of alanine aminotransferase (ALT) and asparate aminotransferase (AST) using commercially available test kits. Histopathological assessment of liver damage was done by studying Haematoxylin and Eosin (H&E) stained slides of liver tissue, including cell necrosis, fatty change, infiltration of kupffer cells and lymphocytes.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA), followed by the Dunnett test for multiple comparisons using SPSS software and $p<0.05$ was regarded as significant.

Results and Discussion

The results of hepatoprotective effects of *Capparis spinosa* extracts on CCl_4-intoxicated mice are shown in Table 1. In the CCl_4-treated control, serum ALT and AST were increased (191.80 and 239.40 U/ml, respectively), whereas these values showed 114.00 and 143.20 U/ml in normal saline group, respectively. In contrast, serum ALT and AST in the groups treated with 100, 200 and 400 mg/kg of root bark extracts decreased significantly ($p<0.05$) in a dose dependent manner toward normalization. Treatment with 400 mg/kg of root bark extract showed highly significant activity. There was no significant changes in the activities of serum ALT and AST in the overdose group, means the plant root bark extract is not hepatotoxic itself.

The results of *Capparis spinosa* root bark extract on the thiopental-induced sleeping time in mice are presented in Table 2. They indicated that CCl_4 produced significant increases in thiopental induced sleeping time compared to the control groups (1 and 3). The treatment with different doses of root bark extracts, especially with 400 mg/kg, resulted in decreased in thiopental induced sleeping time compared to the group received CCl_4 only.

Histophathological studies also provided supportive evidence for biochemical analysis. Histology of the liver section of normal control animals (group 1) showed normal hepatic cells

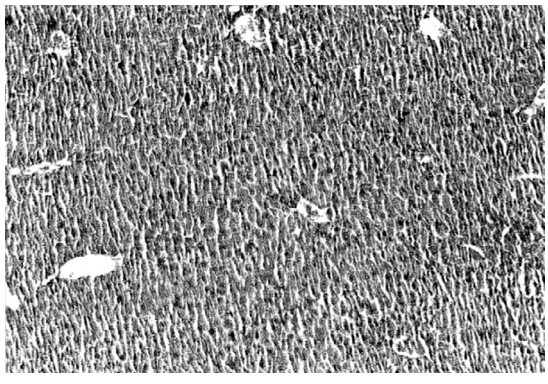

Figure 1. Light microphotograph of Haematoxylin & Eosin -stained section of formalin fixed liver of normal control group. Normal hepatic cell with well preserved cytoplasm, well brought out central vein (× 10).

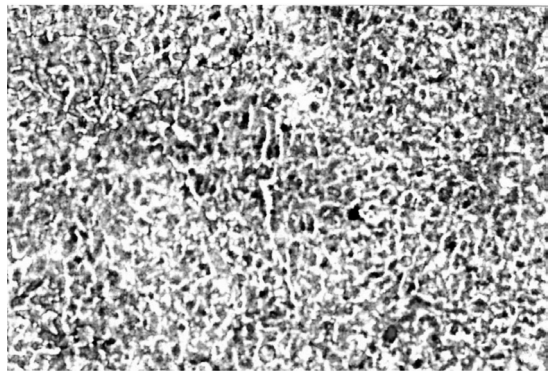

Figure 2. Light microphotograph of Haematoxylin & Eosin -stained section of formalin fixed liver of CCl_4 control group (0.2 ml/kg, orally). Massive fatty changes, necrosis, ballooning, degeneration and broad infiltration of the lymphocytes and Kupffer cells (× 10).

each with well preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein (Figure 1). The liver sections of CCl_4-intoxicated mice showed massive fatty changes, necrosis, ballooning degeneration and broad infiltration of the lymphocytes and Kupffer cells around the central vein and the loss of cellular boundaries (Figure 2). The extract toxicity control group (group 7), showed the normal parenchymal architecture with cords of hepatocytes, portal tracts and central veins without noticeable alterations compared to the normal saline control group. The toxin mediated changes in livers of mice pre-treated with *Capparis spinosa* root bark extracts in different doses one hour after the administration of CCl_4 were less intensity than those observed in the livers of carbon tetrachloride treated mice. The histological architecture of liver sections of mice treated with 400 mg/kg plant extract showed normal lobular pattern with a mild degree of fatty change, necrosis and lymphocyte infiltration almost comparable to the normal control (Figure 3).

The present study showed for the first time, *Capparis spinosa* root bark extract possess hepatoprotective activity as evidenced by the significant inhibition in the elevated levels of serum enzyme activities induced by CCl_4. *Capparis spinosa* root bark extract given orally (100, 200 & 400 mg/kg) once daily for 4 days showed dose dependent hepatoprotective activity. However, highly significant effect was seen with 400 mg/kg body weight against CCl_4 induced hepatic damage.

It is well established that CCl_4 induces

Table 2. Effects of *Capparis spinosa* root bark extracts on the thiopental-induced sleeping time.

Group	Treatment	Sleeping time (min)
1	Normal saline	43.6±2.37
2	CCl_4 0.2 ml/kg	145.00±4.84*
3	Olive oil 0.2 ml	45.14±2.46
4	Plant extract 100 mg/kg+CCl_4	137.16±2.15*
5	Plant extract 200 mg/kg+CCl_4	100.80±7.58**
6	Plant extract 400 mg/kg+CCl_4	47.40±3.55**
7	Plant extract 800 mg/kg	43.00±2.05

Mice were administered with *Capparis spinosa* extracts 100, 200 and 400 mg/kg orally once, one hour after the administration of carbon tetrachloride (orally), for 4 days. Data are expressed as mean±SEM (n=7).
* $p<0.05$ as compared with negative control (group 1).
** $p<0.05$ as compared with positive control (group 2).
One-way ANOVA and Dunnett test.

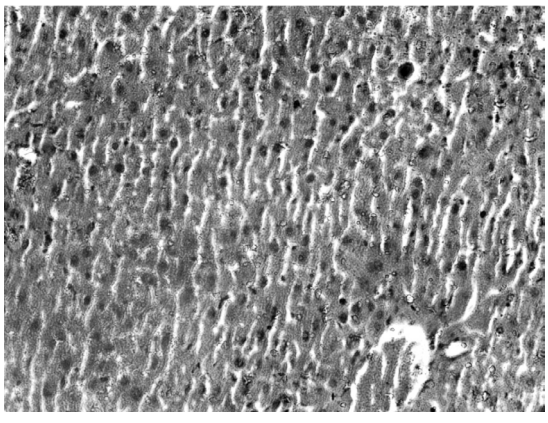

Figure 3. Light microphotograph of Haematoxylin & Eosin -stained section of formalin fixed liver of mice treated with single dose of CCl_4 (0.2 ml/kg, orally) + *Capparis spinosa* root bark extract (400 mg/kg, orally) one hour after $CCl_4 \times 4$ days. Well brought out central vein, normal lobular pattern with a mild degree of fatty change, necrosis and lymphocyte infiltration almost comparable to the normal control (× 10).

hepatotoxicity by metabolic activation, therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. CCl_4 is bio-transformed by the cytochrome P_{450} system in the endoplasmic reticulum to produce trichloromethyl free radical ($^{*}CCl_3$). This free radical then combined with cellular lipids and proteins in the presence of oxygen to form a trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethylperoxyl free radical leads to elicit lipid peroxidation, the destruction of Ca^{2+} homeostasis, and finally, results in cell death (18).

Many compounds known to be beneficial against carbon tetrachloride-mediated liver injury exert their protective action by toxin-mediated lipid peroxidation either via a decreased production of CCl_4 derived free radicals or through the antioxidant activity of the protective agents themselves (19). The mechanism by which *Capparis spinosa* exert its protective action against CCl_4 induced alternations in the liver may be (attributed to) due to the antioxidant effect of the plant extract; but the suggestion needs to be more expleted.

In conclusion, the results indicated that under the present experimental conditions, hydroalcoholic extract of *Capparis spinosa* root bark showed hepatoprotective effects against CCl_4 induced liver damage in mice.

Acknowledgement

The authors would like to thank Dr. H. Kalantari for his valuable comments and helps.

References

(1) Cronquist A. *An integrated system of classification of flowering plants*. Columbia University Press, New York (1981)

(2) Heywood VH. *Flowering plants of the world*. Oxford University Press, New York (1993)

(3) Mabberley DJ. *The plant-book: a portable dictionary of the higher plants*. Cambridge University Press, Cambridge (1997)

(4) Hall JC, Sytsma KJ and Iltis HH. Phylogeny of Capparaceae and Brassicaceae based on chloroplast sequence data, *American Journal of Botany.* (2002) 89: 1826-1842

(5) Ozcan M. Mineral composition of different parts of Capparis ovata Desf. Growing wild in Turkey, *J.Med. Food*. (2005) 8: 405-407

(6) Bown D**.** *Encyclopaedia of Herbs and their Uses.* Dorling Kindersley, London (1995)

(7) Chopra RN, Nayar SL and Chopra. IC**.** *Glossary of Indian Medicinal Plants (Including the Supplement)*. Council of Scientific and Industrial Research, New Delhi (1986)

(8) Chiej R**.** *Encyclopaedia of Medicinal Plants.* MacDonald and Co., London (1984)

(9) Huseini HF, Alavian SM, Heshmat R, Heydari MR and Abolmaali K. The efficacy of Liv-52 on liver cirrhotic patients: a randomized, double-blind, placebo controlled first approach. *Phytomedicine* (2005) 12: 619-24

(10) Chevallier A**.** *The Encyclopedia of Medicinal Plants.* Dorling Kindersley, London (1996)

(11) Meyer SA and Kulkarni AP. Hepatotoxicity. In: Hodgson E, Smart RC. (Eds.) *Introduction to biochemical toxicology*. 3rd ed. A. John Wiley & Sons, New York (2001) 487-90

(12) Castro JA, De Ferreyra EC, De Castro CR, Fenoes OM, Sasame H and Gillette JR. Prevention of carbon tetrachloride- induced necrosis by inhibition of drug metabolism-further studies on their mechanism of action. *Biochem. Pharmacol*. (1974) 23: 295-302

(13) Poli G. Liver damage due to free radicals. *Br. Med. Bull*. (1993) 49: 604-20.

(14) Muriel P, Alba N, Perez-Alvarez VM, Shibayama M and Tsutsumi VK. Kupffer cells inhibition prevents hepatic lipid peroxidation and damage induced by carbon tetrachloride. *Comp Biochem Physiol C Toxicol Pharmacol* (2001) 130: 219-26

(15) Shahjahan M, Sabitha KE, Jainu M and Shyamala Devi CS. Effect of *Solanum trilobatum* against carbon tetrachloride induced hepatic damage in albino rats.

Indian J Med Res (2004) 194-198

(16) Mohamed AF, Gafar Ali Hasan A, Hamamy MI and Abdel-Sattar E. Antioxidant and hepatoprotective effects of *Eucalyptus maculate*. *Med Sci Monit* (2005) 11: BR426-431

(17) Gilani AH, Janbaz KH, Shah BH. Esculetin prevents liver damage induced by paracetamol and CCL_4. *Pharmacol. Res*. (1998) 37: 31-35

(18) Mohona Rao GM, Rao CV, Pushpangadan P and Shirwaikar A. Hepatoprotective effects of rubiadin, a major constituent of *Rubia cordifolia J. Ethnopharmacol*. (2006) 103: 484-490

(19) Hewawasam RP, Jayatilaka KAPW, Pathirana C and Mudduwa LKB. Hepatoprotective effect of *Epaltes divaricata* extract on carbon tetrachloride induced hepatotoxicity in mice, *Indian J. Med. Res*. (2004) 120: 30-3430-34

This article is available online at http://www.ijpr-online.com