PROTECTIVE EFFECTS OF *FUMARIA VAILLANTII* EXTRACT ON CARBON TETRACHLORIDE-INDUCED HEAPTOTOXICITY IN RATS

Seyed Reza Mortazavi[1], Marjan Nassiri-Asl[1]*, Zahra Farahani-Nick[2], Shahram Savad[2], Seyed Kamal Farzam[3]

[1]Department of Pharmacology, Qazvin University of Medical Sciences, Iran

[2]School of Medicine, Qazvin University of Medical Sciences, Iran

[3]Department of Pathology, Qazvin University of Medical Sciences, Iran

Summary

Fumitory is widely used in Iran as a traditional medicine for treatment of several diseases like stimulation of liver function and gall bladder. In the present study the hepatoprotectiv effects of methanolic extract of one of the species, *Fumaria vaillantii* were investigated against carbon tetrachloride (CCl_4) induced hepatocellular injury in rat. Three and six doses of methanol extract of fumitory (50 mg/kg, p.o.) at 12 h intervals were administered 48 h after a single oral dose of CCl_4 (1.25 ml/kg). Blood and liver tissue were collected for the assessment of serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) 12 h after the last dose of extract. The liver tissue was used for histopathological assessment of liver damage. Post-treatment with the administration of six methanol extract (50 mg/kg, p.o.) decreased CCl_4 induced alterations in AST, ALT and ALP by 94.98, 97.64 and 54.56 percent respectively. We also observed that hepatic necrosis and fatty changes caused by CCl_4 were treated by *F. vaillantii* extract. Overall results indicate that the methanol extract of *F. vaillantii* possesses hepatoprotective effects on CCl_4 induced hepatotoxicity in rats.

Key Words: *Fumaria vaillantii*, Carbon tetrachloride, Hepatoprotective effects

***Corresponding author:** Dr. Marjan Nassiri-Asl
Address: Department of Pharmacology, Qazvin University of Medical Sciences, Qazvin, Iran, P.O. Box: 341197-5981, Tel: +982813336001, Fax: +982813324940
E-Mail: marjannassiriaslm@gmail.com

Introduction

Fumitory (Fumariaceae) grows in wide variety parts of Iran (1). Seven species of the annual plants of genus fumaria grow in Iran. However, *Fumaria officinlis* is the medicinal species of this genus which is not found in Iran (2). Fumitory has been used in Iranian folk medicine in skin diseases, for stimulation of liver function and gall bladder and as antiscabies, antiscorbite, antibronchite, diuretic, expectorant, antipyretic, diaphoretic, appetizer and antineoplastic (3). *Fumaria officinalis* has antispasmodic effect on the bile ducts and the gastrointestinal tract and has been used for spastic discomfort. It is also amphicholeretic. In folk medicine, it also has been used similar to Iranian species (4, 5).

One of the species which has been reported in Iran is *Fumaria vaillantii* loisei. There is lack of any pharmacological studies about hepatoprotective effects of it. However, the hepatoprotective activity of other species, *Fumaria parviflora* was shown against paracetamol induced hepatic damage (6).

Liver is well known to be the major organ responsible for the metabolism of drugs and toxic chemicals, and therefore is the primary target organ for nearly all the toxic chemicals (7, 8). Different chemical substances are known to cause hepatic injuries, such as acetaminophen, carbon tetrachloride ($CC1_4$) and D-galactosamine (9). CCl_4 is a model of hepatotoxicant. Single administration of it could lead acute liver injuries such as centrilobular necrosis and steatosis in rats (10, 11).

Thus, the main purpose of the present study was to investigate the potential effects of methanol extract of *Fumaria vaillantii* in reducing serum enzymes and ameliorating histopathological abnormality in the liver of rats which caused by CCl_4, which could provide helpful information for the therapy or prevention of such liver disease.

Materials and Methods

Animals

Male Sprague Dawley rats (200-250 g) were obtained from the Razi Institute (Karaj, Iran). The animals were housed in colony rooms with 12/12 h light/dark cycle at 21 ±

2°C and had free access to food and water. All animal experiments were carried out in accordance with Qazvin University of Medical Sciences, Ethical Committee Acts.

Preparation of extracts

Fumaria vaillantii was collected from Damavand Mountain in the spring (a region in Tehran provinces, Iran) and authenticated by Qazvin Agriculture and National Resources Research Center, Iran. Aerial parts of it were dried in shade and followed by grinding. Then, the powder was extracted using maceration with the methanol. The extract was then concentrated under reduced pressure to the desired volume. In the maceration method, 100 g of the powder was macerated in 1 liter methanol for 3 days and, subsequently, the solution was filtered and concentrated in a rotary evaporator at 50°C. The yield of the extract was 3% (w/w). The extract was diluted by saline.

Experimental groups and dose selection

In this study we used the dose of CCl_4 (1.25 ml/kg, p.o.) which has hepatotoxic effects as previous studies (6, 12). The rats were divided into ten groups of animals. Group 1 served as control and received normal saline (1.25 ml/kg, p.o.). Group 2 was administrated CCl_4 (1.25 ml/kg, p.o.). Group 3, three doses of methanol extract of *F. vaillantii* (50 mg/kg, p.o.) at 12 h intervals were administered 48 h after a single oral dose of CCl_4 (1.25 ml/kg). The dose of *F. vaillantii* used in this study was selected on the basis of the preliminary studies. Group 4, three doses of methanol extract of *F. vaillantii* (50 mg/kg, p.o.) at 12 h intervals were administered to animals without giving them CCl_4. Group 5, six doses of methanol extract of *F. vaillantii* (50 mg/kg, p.o.) at 12 h intervals were administered 48 h after a single oral dose of CCl_4 (1.25 ml/kg). Group 6, six doses of methanol extract of *F. vaillantii* (50 mg/kg, p.o.) at 12 h intervals were administered to animals without giving them CCl_4.

Serum biochemistry

At 12 h after the last dose, all treated animals were anesthetized by ether inhalation for blood sample collection. Blood samples were drawn by cardiac puncture. Serum was separated after coagulating at 37°C for 30 min and centrifuging at 2500 rpm. Serum was

analyzed for the biochemical parameters aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) (12).

Histopathological examinations

In group 1, 48 h after administration of CCl_4 and in other groups at 12 h after the last dose, liver tissues were dissected and fixed in 10% neutral buffered formalin solution for 24 h. The fixed tissues were processed routinely, and were then embedded in paraffin; sectioned to 5μm thickness (13). The extent of $CC1_4$-induced necrosis and steatosis was evaluated by assessing morphological changes in liver sections stained with hematoxylin and eosin (H&E), and examined under light microscopy by a pathologist.

Statistical analysis

The data were expressed as mean values ± SEM. Differences between group means were calculated by a one-way analysis of variance (ANOVA). Differences with a $p < 0.05$ were considered significant.

Results

Serum levels of AST, ALT and ALP

The changes of serum AST, ALT and ALP levels are shown in Table 1. Normal rats receiving only three or six methanol extract (50 mg/kg, p.o.) resulted in no significant changes in serum AST, ALT and ALP levels compared to control. Also, there were no significant difference between the serum AST, ALT levels in rats receiving three or six methanol extract (50 mg/kg, p.o.) 48 h after CCl_4 compared to control. Although in three administration of methanol extract the ALP level was still higher than in the control rats.

Post-treatment with the administration of three methanol extract of *F. vaillantii* (50 mg/kg, p.o.) decreased CCl_4 induced alterations in AST, ALT and ALP levels by 91.3, 93.05 and 37.02 percent respectively ($p<0.001$, $p<0.001$, $p<0.01$) while post-treatment with the administration of six methanol extract (50 mg/kg, p.o.) decreased CCl_4 induced alterations in AST, ALT and ALP levels by 94.98, 97.64 and 54.56 percent respectively ($p<0.001$) (Table 1).

Table 1. Effect of *F. vaillantii* extract on the activity of serum enzymes in rats

Group	AST (U/I)	ALT (U/I)	ALP (U/I)
1	136.9±12.5	67.4±5.6	218.1±6.8
2	3208±288.9***	5517±462.1***	1030.5±116.48***
3	275.3±26.9###	384.2±47.1###	647.5±59.5***##
4	148.2±7.9###	87.6±6.1###	430±62.2###
5	161.2±19.6###	130.3±8.6###	468.2±38.4###
6	255.8±126.1###	103.3±26.6###	370.3±28.3###

Group 1: Control; Group 2: CCl_4 (1.25 ml/ kg, p.o.); Group 3: Three doses of methanol extract of *F. vaillantii* (50 mg/ kg, p.o.) at 12 h intervals were administered 48 h after CCl_4; Group 4: Three doses of methanol extract of *F. vaillantii* (50 mg/ kg, p.o.) at 12 h intervals were administered to animals without giving them CCl_4.; Group 5: Six doses of methanol extract of *F. vaillantii* (50 mg/ kg, p.o.) at 12 h intervals were administered 48 h after CCl_4; Group 6: Six doses of methanol extract of *F. vaillantii* (50 mg/ kg, p.o.) at 12 h intervals were administered to animals without giving them CCl_4. Each value represents the mean ± S.E.M. of ten rats.

****P* < 0.001 vs. control group; ##*P* < 0.01, ###*P* < 0.001 vs. CCl_4 alone group; ALT, alanine aminotransferase; AST, aspartate aminotransferase; and alkaline phosphatase (ALP).

Histological assessment

A liver tissue section from normal rats is shown in Figure 1A. In rats receiving CCl_4 alone, the liver histology showed extensive necrosis of hepatocytes in centrilobular regions of the liver, atrophy and fatty change (Figure 1B). In contrast, the liver tissues were normal in rats receiving three and six doses of methanol extract of *F. vaillantii* (50 mg/kg, p.o.), 48 h after a single oral dose of CCl_4 treatment (Figure 1C). Also, there were no histological difference between the liver of rats receiving three or six methanol extract (50 mg/kg, p.o.) 48 h after CCl_4 compared to control.

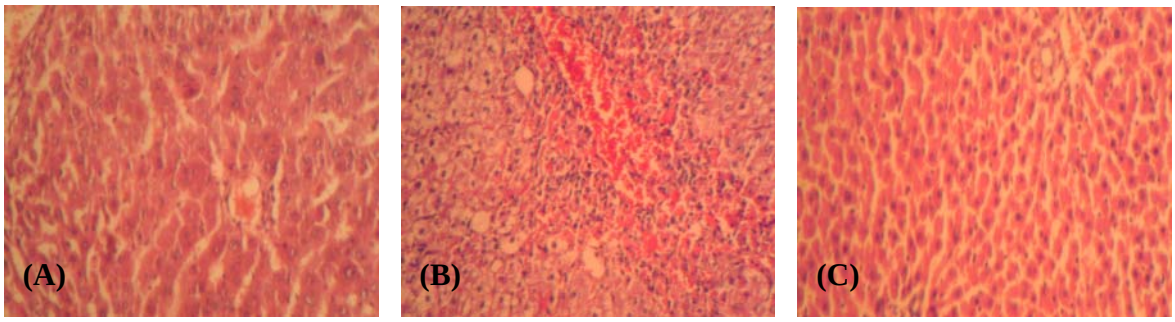

Figure 1. Light microphotographs of HE-stained sections of the formalin fixed livers. (A) Control group (B) CCl_4 group represent fatty change and necrosis (C) Post-treated group with six doses of methanol extract of *F. vaillantii* after CCl_4.

Discussion

The aim of the present study was to investigate the hepatoprotective effects of methanol extract of *F. vaillantii* on liver damage caused by $CC1_4$ in rats. Our results showed that CCl_4 administration caused severe acute liver damage in rats, demonstrated by significant elevation of serum AST, ALT levels and classic histopathological changes. It seems that post-treatment of methanol extract of it significantly reduces the ALT, AST and ALP levels in comparison with CCl_4 group. Histopathological studies also provided supportive evidence for the biochemical analysis.

Chronic administration of $CC1_4$ to rats induces severe disturbances of hepatic function together with histological observable liver fibrosis (9). Hepatoprotective effects of other species of fumaria and active constituents of them have been reported in previous studies (6, 12, 14). The hepatoprotective activity of an aqueous-methanol extract of *F. parviflora* against paracetamol has been due to inhibitory effect on microsomal drug metabolizing enzymes (MDME) (6). Monomethyl fumarate, active component of methanol extract of *F. indica*, has hepatoprotective effects against thioacetamide *in vitro* and against hepatotoxicities induced by CCl_4, paracetamol and rifampicin *in vivo*. Also, in this study, four possible mechanisms have been discussed for its effects: inhibitory effects on microsomal enzymes or on lipid peroxidation; stimulatory effects on hepatic regeneration, free radical scavenging effects (12).

Moreover, oral administration of protopine, an active constituent of fumaria, before administration of CCl_4, acetaminophen or thioacetamide significantly impeded the elevation of ALT and liver damage in mice. In addition, it exhibited biphasic effects on the hepatic cytochrome P450 in mice (14). Similarly, in other study, inhibition of MDME was established for hepatoprotective effects (15). On the other hands, the cytoprotective effect of the *F. densiflora* and *F. officinalis* extracts on primary cultures of rat hepatocytes which intoxicated with CCl_4 was associated with their alkaloids (16).

Fumaria vaillantii contains protopine, fumaridine, fumaramine, adlumidine, d-bicuculline, vaillantine (2, 3-didemethylmuramine) and rutin (17, 18). Antioxidant and antilipoperoxidant activities of alkaloid and phenolic extracts from *F. vaillantii* and other species of fumaria have been established (19). Recently, hydroalcoholic extract of *F. vaillantii* inhibited the development of atherosclerosis in rabbit and this effect has been related to antioxidant effects of its flavonoids like rutin (20). Thus, it seems that hepatoprotective effects of *F. vaillanii* may be due to inhibitory effects on microsomal drug metabolizing enzymes like *F. parviflora* or antioxidant activity of it. However, further studies need to clear the exact hepatorpotective effects of it.

In conclusion, the results of the present study indicated that methanol extract of *Fumaria vaillantii* has hepatoprotective effects on acute liver injuries induced by CCl_4 in rat.

Acknowledgment

The authors are thankful to the Vice Chancellor of Research, Qazvin and Mashhad University of Medical Sciences for financial support.

References

1. Zargari A. Medicinal Plants. Tehran University Press, 1989: 170-171.

2. Khalilighi-Sigaroodi F, Yazdani D, Taghizadeh M, Rezazadeh S. Quantities determination of an effective component of *Fumaria parviflora* Lam. J Med Herb 2005; 4: 62-71.

3. Amin GH. Popular Medical Plants of Iran. Farhang Press: Tehran, 1991: 51-52.

4. Gruenwald J, Brendler BA, Jaenicke C. PDR for Herbal Medicines. 3rd ed. Thomson Healthcare Inc: Montvale, 2004: 400.

5. Hentschel C, Dressler S, Hahn EG. *Fumaria officinalis* (fumitory) clinical applications. Fortschr Med 1995; 113: 291-292.

6. Gilani AH, Janbaz KH, Akhtar MS. Selective protective effect of an extract from *Fumaria parviflora* on paracetamol-induced hepatotoxicity. Gen Pharmacol 1996; 27: 979-983.

7. Bissel DM, Gores GJ, Laskin DL, Hoorhagle JH. Drug induced liver injury:mechanisms and test systems. Hepatology 2001; 33: 1009-1013.

8. Larrey D. Drug-induced liver diseases. J Hepatol 2000; 32: 77-88.

9. Vogel HG, Vogel WH. Drug Discovery and Evaluation, Pharmacological Assay Berlin: Springer; 1997: 942-943.

10. Weber LWD, Bull M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Crit Rev Toxicol 2003; 33:105-136.

11. Janakat S, Al-Merie H. Optimization of the dose and route of injection, and characterisation of the time course of carbon tetrachloride-induced hepatotoxicity in the rat. J Pharmacol Toxicol Methods 2002; 48; 41-44.

12. Rao KS, Mishra SH. Antihepatotoxic activity of monomethyl fumarate isolated from *Fumaria indica*. J Ethnopharmacol 1998; 60: 207-213.

13. Wang CY, Ma FL, Liu JT, Tian JW, Fu FH. Protective effect of salvianic acid A on acute liver injury induced by carbon tetrachloride in rats. Biol Pharm Bull 2007; 30: 44-47.

14. Wei HL, Liu GT. Protective action of corynoline, acetylcorynoline and protopine against experimental liver injury in mice. Yao Xue Xue Bao 1997; 32: 331-336.

15. Janbaz KH, Saeed SA, Gilani U. An assessment of the potential of protopine to inhibit microsomal drug metabolizing enzymes and prevent chemical-induced hepatotoxicity in rodents. Pharmacol Res 1998; 38: 215-219.

16. Taborska E, Bochorakova H, Sousek J, Sedmera P, Vavreckova C, Simanek V. *Fumaria densiflora* DC. alkaloids. Collection Czechoslovak Chem Commun 1996; 61: 1064-1072.

17. Ibragimova MU, Israilov IA, Yunusov MS, SYu Yunusov. Alkaloids of *Fumaria vaillantii* structure of vaillantine. Chem Nat Comp 1976: 10: 480-481.

18. Sousek J, Guedon D, Adam T, Bochorakova H, Taborska E, Valka I, Simanek V. Alkaloids and organic acids content of eight fumaria species. Phytochem Anal 1998: 10: 6-11.

19. Sousek J, Vavreckova C, Psotova J, Ulrichova J, Simanek V. Antioxidant and Antilipidperoxidant activities of alkaloid and phenolic extracts of eight fumaria species. Acta Hort 1999 (ISHS); 501:239-244.

20. Madani H, Talebolhosseiny M, Asgari S, Mahzooni P, Razban E. Preventive Effect of hydroalcoholic extract of *Silybum marianum* and *Fumaria vaillantii* in atherosclerosis. Pharm Sci 2007; 29-34.