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

Seyed Reza Mortazavi¹, Marjan Nassiri-Asl¹*, Zahra Farahani-Nick², Shahram Savad²,
Seyed Kamal Farzam³

¹Department of Pharmacology, Qazvin University of Medical Sciences, Iran
²School of Medicine, Qazvin University of Medical Sciences, Iran
³Department 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 hepatoprotective effects of methanolic extract of one of the species, Fumaria vaillantii were investigated against carbon tetrachloride (CCl₄) 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 CCl₄ (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 CCl₄ 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 CCl₄ were treated by F. vaillantii extract. Overall results indicate that the methanol extract of F. vaillantii possesses hepatoprotective effects on CCl₄ 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
Fumitory (Fumariaceae) grows in wide variety parts of Iran (1). Seven species of the annual plants of genus fumaria grow in Iran. However, *Fumaria officinalis* 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 (CC14) and D-galactosamine (9). CCl4 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 CCl4, 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 CCl₄ (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 CCl₄ (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 CCl₄ (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 CCl₄. 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 CCl₄ (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 CCl₄.

**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 CCl₄ 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 CCl₄-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 CCl₄ 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 CCl₄ 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 CCl₄ 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

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

Group 1: Control; Group 2: CCl₄ (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 CCl₄; 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 CCl₄.; Group 5: Six doses of methanol extract of *F. vaillantii* (50 mg/ kg, p.o.) at 12 h intervals were administered 48 h after CCl₄; 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 CCl₄. Each value represents the mean ± S.E.M. of ten rats.

***P < 0.001 vs. control group; ##P < 0.01, ###P < 0.001 vs. CCl₄ 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 CCl₄ 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 CCl₄ 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 CCl₄ compared to control.
Figure 1. Light microphotographs of HE-stained sections of the formalin fixed livers. (A) Control group (B) CCl₄ group represent fatty change and necrosis (C) Post-treated group with six doses of methanol extract of *F. vaillantii* after CCl₄.

**Discussion**

The aim of the present study was to investigate the hepatoprotective effects of methanol extract of *F. vaillantii* on liver damage caused by CCl₄ in rats. Our results showed that CCl₄ 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 CCl₄ group. Histopathological studies also provided supportive evidence for the biochemical analysis.

Chronic administration of CCl₄ 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 CCl₄, 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 CCl₄, 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 CCl₄ 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 antiliperoxidant 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 hepatoprotective 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 CCl₄ in rat.

**Acknowledgment**

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

**References**


