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Original Research

Effect of 70% ethanolic extract of roots of *Paeonia officinalis* Linn. on hepatotoxicity

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Abstract

Background: Paeonia officinalis, commonly known as European peony (family: Paeoniaceae) is native to south-eastern Europe and has been introduced widely elsewhere (including Kashmir) as a garden plant. Roots of P. officinalis have been reported to possess abortifacient, antihypertensive, and antiulcer activity. It has been used in traditional Unani, homeopathic, and Chinese systems of medicine for curing liver disorders such as bladder stones and jaundice, besides being used in treating stomach ache, diarrhea, labor pains, nightmares, epilepsy, and lunacy.

Purpose: The present study was undertaken to investigate the hepatoprotective potential of the 70% ethanolic extract of the roots of P. officinalis.

Methods: In the present study, the efficacy of a 70% ethanolic extract of the roots of P. officinalis was evaluated against carbon tetrachloride (CCl_4)-induced hepatic damage in rats. P. officinalis, at doses of 100 mg/kg and 200 mg/kg, was administered orally once daily for 14 days.

Results: CCl_4 produced a significant increase in the serum levels of aspartate transaminase, alanine transaminase, alkaline phosphatase, and total bilirubin in rats, while a decrease in total protein levels was observed. In rats that had received extracts of P. officinalis along with CCl4, substantially elevated levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin were lowered significantly in a dose-dependent manner, while total protein levels were elevated. Histopathology revealed regeneration of the liver in extract-treated groups while silymarin-treated rats were almost normal.

Conclusion: The results of this study indicate that roots of P. officinalis possess hepatoprotective activity.

Keywords: Carbon tetrachloride; Hepatoprotective; *Paeonia officinalis*; Peony

1. Introduction

The liver is a vital organ of paramount importance because it is involved in the maintenance of metabolic functions and detoxification of exogenous and endogenous substances such as xenobiotics, drugs, viral infection, and chronic alcoholism. Liver diseases (also called hepatic diseases) have become a global problem, which account for about 20,000 deaths every year.[1] Damage to liver is always associated with cellular necrosis and an increase in the serum levels of many biochemical markers such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and bilirubin.[2]

Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and traditional systems of medicine in India.[3] About 160 phytoconstituents from 101 plants have been reported to possess hepatoprotective activity.[4,5]

The roots of *P. officinalis* (Ood Saleeb) have been used in Unani, Ayurvedic, and homeopathic systems of medicine for years.[6] In Unani medicine, its roots are used as an ingredient in many antioxidant preparations.[6,7] In Ayurvedic medicine, root is a part of medicinal preparations used for treating disease states such as jaundice, dropsy, hepatitis, hepatomegaly, liver dysfunction, cirrhosis, and sluggish liver.[7] Roots are also used in homeopathy for the treatment of liver problems.[8,9] An aqueous

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extract of this root has been reported to possess hepatoprotective activity; however, no scientific study has been conducted to confirm the antihepatotoxic potential of its ethanolic extract.[10] With this background, the present study was undertaken to investigate the hepatoprotective potential of the 70% ethanolic extract of the roots of *P. officinalis* against carbon tetrachloride (CCl_4)-induced hepatotoxicity in albino rats.

CCl_4 is a widely used industrial chemical and a potent hepatotoxin. It induces hepatotoxicity by producing free radicals, which in turn induce oxidative stress that causes lipid peroxidation in liver tissues, leading to necrotic liver damage.[11]

2. Materials and methods

2.1. Plant material

Dried roots of *P. officinalis* were obtained from a local Unani hospital in Kashmir, after proper identification and authentication of plant material at the Centre for Biodiversity & Taxonomy, University of Kashmir, Srinagar. A sample of the plant material was deposited in the herbarium of the Department of Taxonomy, University of Kashmir, under voucher specimen number 1051/KASH for future reference.

2.2. Preparation of extract

An alcoholic extract (70% v/v ethanol) of the roots of *P. officinalis* was prepared following the standard method.[12] The roots were pulverized and subjected to extraction. The powdered material (1 kg) was exhaustively extracted (3 cycles/hour) with 70% ethanol in a Soxhlet apparatus (Borosil 3740-extraction apparatus Borosil, Connaught Place, New Delhi, India) by continuous hot extraction. The extract was evaporated to dryness under reduced pressure and controlled temperature conditions (40–50°C) (yield 10%, w/w).

2.3. Preliminary phytochemical screening

The powdered plant material was subjected to preliminary phytochemical screening. The standard qualitative and quantitative methods were used to test the presence of saponins, tannins, alkaloids, flavonoids, anthraquinones, glycosides, and reducing sugars.[13]

2.4. Animals

Albino rats of Wistar strain, both sexes, weighing 125–250 g, were procured from the animal house of the Indian Institute of Integrative Medicine (IIIM), Jammu. The animals were kept in polypropylene cages (6 in each cage) under standard laboratory conditions (12-hour light and 12-hour dark: day and night cycle), and had free access to a commercial pelleted diet (Ashirwad Industries, Mohali, Punjab, India) and tap water *ad libitum*. All studies were performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Institutional Animal Ethical Committee, Department of Pharmaceutical Sciences, India (Reg. No. IAEC/PHARM.S/CL/KU/2012). All the chemicals used were of analytical grade from standard companies, and double-distilled water was used always.

2.5. Acute oral toxicity study

The acute toxicity study was carried out *in vivo* in albino rats. Solutions of the dried extracts were prepared using 2% gum acacia in distilled water. The study was conducted as per the Organization of Economic Cooperation and Development (OECD/OCDE) test guidelines on acute oral toxicity using a computer-guided statistical program (AOT425statPgm, version 1.0, Developed by Westat (Rockville, United States) for the U.S. Environmental Protection Agency).[14]

P. officinalis roots have been reported to be toxic at higher doses[15]; the main test of the up-and-down procedure was conducted as per the guidelines, using the dose progressions of 175 mg/kg, 550 mg/kg, and 2000 mg/kg of the oral ethanolic extract.

2.6. CCl_4-induced hepatotoxicity

Animals were divided into five groups, with six rats in each group. Rats of the first group served as control animals and received a single daily dose of gum acacia [1 mL of 2%, w/v, per oral (p.o.) body weight (b.w.)].[16] The second group received only CCl_4 [1 mL/kg b.w., intraperitoneal (i.p.), 1:1 v/v mixture of CCl_4 and olive oil].[17] Rats of the third group received a silymarin suspension (100 mg/kg b.w., p.o.) along with CCl_4, while rats in the fourth and fifth groups received orally 100 mg/kg b.w. and 200 mg/kg b.w. of ethanolic extracts of *P. officinalis* in 2% gum acacia (w/v), respectively, along with CCl_4. The ethanolic extract was given daily to the respective groups, while CCl_4 was administered every 72 hours for 14 days.[17]

2.7. Assessment of hepatoprotective activity

After 14 days of drug treatment, the rats were fasted overnight. On the 15th day, they were anesthetized with diethyl ether, and blood from each animal was collected, by retro-orbital plexus puncture, in sterilized centrifuge tubes. Blood samples were then allowed to coagulate at 30°C for 45 minutes. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 minutes and subjected to biochemical investigations using standard test kits to assess liver function. The following biochemical investigations were carried out in serum: serum ALT,[18] serum AST,[19] serum ALP,[20] total bilirubin,[21] and total serum protein.[22]

After collecting the blood samples, animals from all groups were sacrificed by cervical dislocation. Abdomen of each animal was cut open to excise the liver. These livers were then washed with normal saline and fixed in 10% neutral formalin solution to be processed separately for histological observation.

2.8. Histopathological studies

Thin sections (5 μM) of the livers were cut and stained with routine hematoxylin and eosin stain for photomicroscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue.[23]

3. Statistical analysis

All the results were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used for the statistical analysis of data. Student *t* test was used for determining the significance. A p value <0.05 was considered significant and $p < 0.01$ was taken to be highly significant.

4. Results

4.1. Preliminary phytochemical screening

Phytochemical screening revealed that the roots of *P. officinalis* contain alkaloids, tannins, saponins, glycosides, carbohydrates, flavonoids, terpenes, steroids, and proteins.

4.2. Acute oral toxicity

The 70% ethanolic extract did not cause any mortality up to a dose of 2000 mg/kg and was considered safe.

4.3. Biochemical estimations

The effect of *P. officinalis* on serum marker enzymes is presented in Table 1. In the present study, CCl_4 given at a dose of 1 mL/kg b.w. (along with olive oil 1:1) produced a significant rise in serum AST, ALT, ALP, and bilirubin levels and a significant fall in the total protein levels, indicating considerable hepatocellular injury. Administration of the 70% ethanolic extract of *P. officinalis* at doses of 100 mg/kg and 200 mg/kg produced a significant reduction in the AST, ALT, ALP, and total bilirubin levels and a significant rise in the total protein levels in a dose-dependent manner.

4.4. Histopathological observations

A histopathological examination of the liver slides of normal control rats showed normal parenchyma and normal portal tract (Fig. 1A). Liver of the rats administered with only CCl_4 (toxic control) showed inflammation of the portal triad, fatty change and necrosis of the periportal zone, necrosis, sinusoidal dilatation, inflammation, hemorrhage, and vascular congestion of the centrizonal area (Fig. 1B). The livers of rats treated with CCl_4 along with silymarin (100 mg/kg/day) showed almost normal-appearing liver parenchyma, with occasional necrotic foci in the periportal area (Fig. 1C). Animals that had received CCl_4 along with an ethanolic extract of *P. officinalis* (100 mg/kg/day) showed mild sinusoidal dilatation, inflammatory cell infiltration, and hemorrhage around the bile duct across the entire liver tissue region (Fig. 1D). The livers from animals administered with CCl_4 along with an ethanolic extract of *P. officinalis* (200 mg/kg/day) showed mild inflammatory cell infiltration across the entire liver tissue (Fig. 1E).

5. Discussion

From the results of the acute oral toxicity study of the ethanolic extract, it can be concluded that the LD_{50} of the drug is greater than 2000 mg/kg b.w, that is, an ethanolic extract of the root powder of *P. officinalis* is safe for administration up to a dose of 2000 mg/kg b.w.

CCl_4 at a dose of 1 mL/kg b.w. (i.p.) every 72 hours for 14 days has been reported to produce hepatotoxicity. Therefore, in the present study, CCl_4 was administered at a dose of 1 mL/kg b.w. (along with olive oil 1:1) on Day 1 of the study and then every 72 hours during the 14-day study period.

The results of this experiment reveal that the 70% ethanolic extract of the roots of *P. officinalis* has a definitive antihepatotoxic effect ($p < 0.01$) against the deleterious effects of

Table 1
Effect of the 70% ethanolic extract of the *Paeonia officinalis* roots on biochemical parameters against CCl_4-induced hepatotoxicity in rats.

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TB	TP
Normal control (2% gum acacia, 1 mL/kg p.o.)	88.75 ± 11.14	269.14 ± 12.46	122.64 ± 9.78	1.48 ± 0.11	9.97 ± 1.61
Toxic control (CCl_4; 1 mL/kg i.p.)	379.2 ± 26.13**	768.60 ± 42.15**	345.49 ± 22.02**	7.85 ± 0.64**	5.21 ± 0.56**
CCl_4 + silymarin (100 mg/kg p.o.)	97.88 ± 5.83***	284.33 ± 5.40***	141.39 ± 13.08***	1.83 ± 0.22***	15.27 ± 1.01*, ***
CCl_4 + 100 mg/kg p.o. of ethanolic extract	216.61 ± 31.96**, ****	345.29 ± 14.32**, ****	233.41 ± 13.18**, ***	4.66 ± 0.18**, ****	12.08 ± 0.87*, ****
CCl_4 + 200 mg/kg p.o. of ethanolic extract	173.37 ± 15.39**, ****	307.72 ± 10.87**, ****	167.24 ± 9.23**, ****	2.52 ± 0.24**, ****	14.14 ± 0.76*, ****

Data are expressed as the mean ± SEM; $n = 6$.
*$p < 0.05$, significant differences from the normal control group.
**$p < 0.01$, significant differences from the normal control group.
***$p < 0.05$, significant differences from the CCl_4 group.
****$p < 0.01$, significant differences from the CCl_4 group.
ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate transaminase; CCl_4 = carbon tetrachloride; i.p. = intraperitoneal; IU = international units; p.o. = per oral; SEM = standard error of the mean; TB = total bilirubin; TP = total protein.

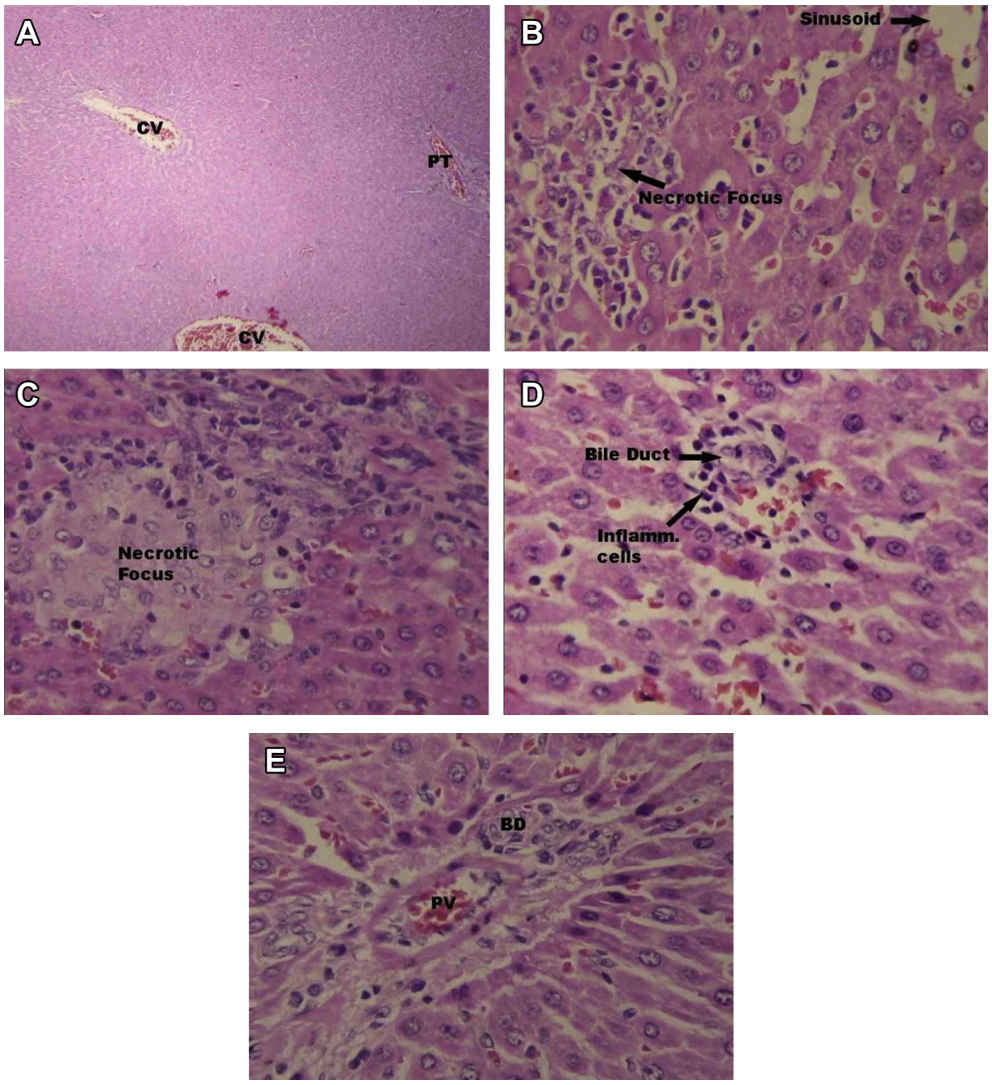

Fig. 1. Histopathology of liver tissues. (A) Liver from a control group rat: showing a normal portal tract and a large portal vein. (B) Liver from an animal treated with CCl_4 only: showing sinusoidal dilatation and a focus of necrosis with inflammatory cell infiltration and hemorrhage. (C) Liver from an animal treated with CCl_4 and silymarin (100 mg/kg/day): showing normal liver parenchyma and occasional necrotic foci in the periportal area. (D) Liver from a rat treated with CCl_4 and an ethanolic extract of *Paeonia officinalis* (100 mg/kg/day): showing inflammatory cell infiltration and hemorrhage around a bile duct across the entire liver. (E) Liver from rats treated with CCl_4 and an ethanolic extract of *P. officinalis* (200 mg/kg/day): showing inflammatory cell infiltration across the entire liver tissue. BD = bile duct; CCl_4 = carbon tetrachloride; CV = central vein, PT = portal triad; PV = portal vein.

CCl_4 on the structure and function of liver. Both doses (100 mg/kg and 200 mg/kg) of the 70% ethanolic extract attenuated the increase in the levels of AST, ALT, ALP, and total bilirubin effectively; increased the total protein levels produced by CCl_4 significantly; and caused subsequent recovery toward normalization, comparable to the levels in control and the standard group animals. The hepatoprotective effect was more pronounced at the dose of 200 mg/kg/day than at 100 mg/kg/day.

Histopathological studies also confirm the hepatoprotective role of the 70% ethanolic extract of the *P. officinalis* roots in antagonizing the deleterious effects of CCl_4 on the histology of liver. While CCl_4-treated rats showed extensive histological changes, the animals treated concurrently with CCl_4 and the 70% ethanolic extract of *P. officinalis* at doses of 100 mg/kg/day and 200 mg/kg/day showed only moderate to mild changes. The dose of 200 mg/kg/day of the ethanolic extract was found to be more effective than the dose of 100 mg/kg/day in protecting the liver against the hepatocellular injury caused by CCl_4.

Various other *Paeonia* species, such as *Paeonia lactiflora*[24] and *Paeonia radix*,[25] have been observed to possess antihepatotoxic activity against CCl_4-induced liver injury. It has been established that the hepatoprotective activity of these *Paeonia* species is due to the presence of glycosides in them. Total glucosides of peony have been found to protect hepatocytes from the oxidative damage induced by CCl_4. Since we have already reported that roots of *P. officinalis* also contain glycosides, it can be assumed that the hepatoprotective activity of *P. officinalis* roots is due to the antioxidant activity of its

glycoside content; however, this needs to be verified experimentally in future studies.

In the future, studies should be conducted on this drug to verify whether its hepatoprotective activity is due to the total glycoside content; we also need to study the effect of higher doses of this medicinal herb on liver protection.

6. Conclusion

The results of this study strongly indicate the protective effect of *P. officinalis* roots against acute liver injury in rats, which may be attributed to its glycoside content.

References

1. Latha TB, Srikanth A, Eswar KK, Mastan K, Srinivasa RY, Bhavani B. Comparative hepatoprotective efficacy of Kumaryasava and Livfit against carbon tetrachloride induced hepatic damage in rats. *Pharmacologyonline*. 2009;1:1127−1134.
2. Wolf PL. Biochemical diagnosis of liver diseases. *Ind J Clin Biochem.* 1999;14:59−90.
3. Usha P, Benjamin S, Raghu K. An efficient micropropagation system for *Vitex negando* L., an important woody aromatic medicinal plant, through shoot tip culture. *Res J Bot*. 2007;2:102−107.
4. Stickel F, Schuppan D. Herbal medicine in the treatment of liver diseases. *Digest Liver Dis*. 2007;39:293−304.
5. Tabassum N, Qazi MA, Shah A. Curative activity of ethanol extract of *Taraxacum officinale* Weber. against CCl_4 induced hepatocellular damage in albino rats. *J Pharm Res*. 2011;4:687−689.
6. Sifiuddin H. *Unani Adviya Mafarruda*. 8th ed. New Delhi: Tariki Urdu Bureau; 1999.
7. Ahmad F, Tabassum N, Rasool S. Medicinal uses and phytoconstituents of *Paeonia officinalis*. *IRJP*. 2012;3:85−87.
8. Lev E, Amar Z. *Practical Materia Medica of the Medieval Eastern Mediterranean According to the Cairo Genizah*. Israel: Brill Academic Publishers; 2007:235.
9. Manolova L. *Natural Pharmacy.* 1st ed. Pittsburgh: Dorrance Pub; 2003:25−59.
10. Ahmad F, Tabassum N. Preliminary phytochemical, acute oral toxicity and antihepatotoxic study of roots of *Paeonia officinalis* Linn. *Asian Pac J Trop Biomed*. 2013;3:64−68.
11. Ahmad F, Tabassum N. Experimental models used for the study of antihepatotoxic agents. *J Acute Dis*. 2012;1:1−5.
12. Alkofahi A, Masaadeh H, Al-Khalil S. Antimicrobial evaluation of some plant extracts of traditional medicine of Jordan. *Alex J Pharm Sci*. 1996;10:123−126.
13. Trease GE, Evans WC. *Pharmacognosy.* 11th ed. London: Bailliere Tindall Ltd.; 1989:60−75.
14. *Acute Oral Toxicity (AOT) (OECD Test Guideline 425) Statistical Programme (AOT425StatPgm). Version 1.0*; 2001. http://www.oecd.org/oecd/pages/home/displaygeneral/0,3380,EN-document-524-nodirectorate-no-24-6775-8,FF.html.
15. Singh MP, Panda H. *Medicinal Herbs with Their Formulations*. 1st ed. Delhi: Daya Publishing House; 2005:622.
16. Eesha BR, Mohanbabu AV, Meena KK, et al. Hepatoprotective activity of *Terminalia paniculata* against paracetamol induced hepatocellular damage in Wistar albino rats. *Asian Pac J Trop Med*. 2011;4:466−469.
17. Rao GM, Rao CV, Pushpangadan P, Shirwaikar A. Hepatoprotective effects of rubiadin, a major constituent of *Rubia cordifolia* Linn. *J Ethnopharmacol*. 2006;103:484−490.
18. Bergmeyer HV, Horder M. IFCC methods for measurement of catalytic concentrations of enzymes. *Clin Chim Acta*. 1980;105:147F−172F.
19. Bergmeyer HV, Bowers GN, Horder M, Mas AW. Optimization of methods for aspartate aminotransferase and alanine amino transferase. *Clin Chem Acta*. 1978;24:58−73.
20. Rick W. *Klinische Chemie und Miroskopie*. 6th ed. Berlin: Springer Verlag; 294.
21. Jendrassik L, Grof P. Modified Jendrassik & Grof's method. *Biochem Z.* 1938;2:81−89.
22. Doumas BT. Standards for total serum protein assays-a collaborative study. *Clin Chem.* 1975;21:1159−1166.
23. Amresh G, Rao CV, Singh PN. Antioxidant activity of *Cissampelos pareira* on benzo (a) pyrene induced mucosal injury in mice. *Nutr Res*. 2007;27:625−632.
24. Kim ID, Ha BJ. The effects of paeoniflorin on LPS-induced liver inflammatory reactions. *Arch Pharm Res*. 2010;33:959−966.
25. Li R, Guo W, Fu Z, Ding G, Zou Y, Wang Z. Hepatoprotective action of Radix Paeoniae Rubra aqueous extract against CCl4-induced hepatic damage. *Molecules*. 2011;16:8684−8694.