Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(13)60025-8 © 2013 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

Preliminary phytochemical, acute oral toxicity and antihepatotoxic study of roots of *Paeonia officinalis* Linn.

Feroz Ahmad*, Nahida Tabassum

Department of Pharmaceutical Sciences, University of Kashmir, Hazratbal, Srinagar, J&K, India

PEER REVIEW

Peer reviewer

Dr. Majid Ahmad, Assistant Professor (Pharmacology), King Saud University, Saudi Arabia.
E-mail: Majidsays@yahoo.com

Comments

Looking at the biochemical and histopathological results, the work has proven the claim of the traditional system of medicines. The authors have conducted toxicity and pharmacognostic activity, which is very nice and important. I recommend this article to be published.

(Details on Page 68)

ABSTRACT

Objective: To carry out a preliminary phytochemical, acute oral toxicity and antihepatotoxic study of the roots of *Paeonia officinalis* (*P. officinalis*) L. **Methods:** Preliminary phytochemical investigation was done as per standard procedures. Acute oral toxicity study was conducted as per OECD 425 guidelines. The antihepatotoxic activity of aqueous extract of root of *P. officinalis* was evaluated against carbon tetrachloride (CCl₄) induced hepatic damage in rats. Aqueous extract of *P. officinalis* at the dose levels of 100 and 200 mg/kg body weight was administered daily for 14 d in experimental animals. Liver injury was induced chemically, by CCl₄ administration (1 mL/kg *i.p.*). The hepatoprotective activity was assessed using various biochemical parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum alkaline phosphatase (SALP), total bilirubin and total protein (TP) along with histopathological studies. **Result:** Phytochemical screening revealed that the roots of *P. officinalis* contain alkaloids, tannins, saponins, glycosides, carbohydrates, flavonoids, terpenes, steroids and proteins. The aqueous extract did not cause any mortality up to 2000 mg/kg. In rats that had received the root extract at the dose of 100 and 200 mg/kg, the substantially elevated AST, ALT, SALP, total bilirubin levels were significantly lowered, respectively, in a dose dependent manner, along with CCl₄ while TP levels were elevated in these groups. Histopathology revealed regeneration of the livers in extract treated groups while Silymarin treated rats were almost normal. **Conclusions:** The aqueous extract of *P. officinalis* is safe and possesses antihepatotoxic potential.

KEYWORDS

Paeonia officinalis, Antihepatotoxic, Liver, Peony

1. Introduction

The liver is a vital organ of paramount importance involved in the maintenance of metabolic function and detoxification from the exogenous and endogenous challenges, like xenobiotics, drugs, viral infection and

chronic alcoholism. If during all such exposures to these challenges, the natural protective mechanisms of the liver are overpowered, the result is a hepatic injury^[1]. Damage to liver is always associated with cellular necrosis and increase in serum levels of many biochemical markers like SGOT, SGPT, ALP and

*Corresponding author: Feroz Ahmad, Department of Pharmaceutical Sciences, University of Kashmir, Hazratbal, Srinagar, J&K, India.
Tel: +91-9697359523
E-mail: ferozahmad85@gmail.com

Article history:
Received 7 Oct 2012
Received in revised form 15 Oct, 2nd revised form 20 Oct, 3rd revised form 22 Oct 2012
Accepted 20 Nov 2012
Available online 28 Jan 2013

bilirubin[2].

Unavailability of rational therapy in modern medicine and no or very less positive influence of synthetic drugs in liver damage have urged researchers in this field to look for herbal drugs with better hepatoprotective action. Numerous medicinal plants and their formulations are used for liver disorders in ethno medical practices and in traditional system of medicine in India[3]. About 160 phytoconstituents from 101 plants have been reported to possess hepatoprotective activity[4]. In India, about 40 polyherbal commercial formulations are available and prescribed by physicians to treat hepatic disorders[5].

The root of *Paeonia officinalis* (Ood Saleeb) (*P. officinalis*) has been used in Unani, Ayurvedic and Homoeopathic systems of medicine for years[6,7]. However, no phytochemical investigation, toxicity study or anti-hepatoprotective study has been carried out on this plant[7].

Carbontetrachloride (CCl_4) is a widely used industrial chemical and a potent hepatotoxin. It induces hepatotoxicity by producing free radicals, putting oxidative stress hence causing lipid per oxidation in liver tissues leading to necrotic liver damage[8].

So the present study was undertaken to study the phytochemical, toxicity and antihepatotoxic profile of the roots of *P. officinalis* against CCl_4 induced hepatotoxicity in albino rats.

2. Materials and methods

2.1. Plant material

Dried roots of *P. officinalis* were obtained from a local Unani Hospital in Kashmir. The plant material was identified and authenticated at 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

Aqueous extract of root of *P. officinalis* (APO) was prepared by the method given by Alkofahi[9]. Dried *P. officinalis* roots were pulverized the powdered material (1 kg) was macerated in distilled water for 24 h with occasional shaking and then it was allowed to stand for 18 h. The contents were kept for elution and then filtered. The extract was evaporated to dryness under reduced pressure and controlled temperature (40–50 °C) (yield 100.0 g/kg).

2.3. Preliminary phytochemical screening

The powdered plant material was subjected to preliminary

phytochemical screening. The presence of saponins, tannins, alkaloids, flavonoids, anthraquinones, glycosides and reducing sugars were determined by the standard qualitative and quantitative methods[10].

2.4. Animals

Albino rats of Wistar strain, both sexes, weighing 125–150 g, were procured from the animal house of Indian Institute of Integrative Medicine (IIIM) canal road, Jammu. The animals were kept in polypropylene cages (6 in each cage) under standard laboratory conditions (12 h light and 12 h dark: day and night cycle) and had a free access to commercial pelleted diet (Ashirwad Industries) and tap water *ad libitum*. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. IAEC/PHARM.S/CL/KU/2012). All the chemicals used were of the analytical grade from standard companies and the water used was always the double distilled water.

2.5. Acute oral toxicity study

Acute toxicity study was carried out *in vivo* in the albino rats. Solutions of the dried extracts were prepared using 2% gum acacia in distilled water. The study was conducted as per Organization of Economic Cooperation and Development (OECD/OCDE) Test guidelines on Acute Oral Toxicity under a computer-guided Statistical Programme–AOT425statPgm, version 1.0. Up and Down Procedure was conducted, using the dose progression of 175 mg/kg *p.o.*, 550 mg/kg *p.o.* and 2000 mg/kg *p.o.* of the aqueous extract[11].

2.6. CCl_4 induced hepatotoxicity

The animals were divided into five groups, each group had six animals. Group 1 served as control animals received a single daily dose of gum acacia (*p.o.*, 1 mL of 20 g/L, body weight)[12]. Group 2 received carbon tetrachloride (*i.p.*, 1 mL/kg body weight, 1:1 v/v mixture of CCl_4 and olive oil) alone, group 3 received Silymarin suspension (100 mg/kg body weight *p.o.*) along with CCl_4 as in group 2 rats, while group 4 and 5 received orally 100 and 200 mg/kg body weight of aqueous extracts of *P. officinalis* in 20 g/L gum acacia, respectively along with carbon tetrachloride as in group second. The aqueous extract was given daily while carbon tetrachloride was given every 72 h for 14 d[13].

2.7. Assessment of hepatoprotective activity

After 14 d of drug treatment, the rats were fasted overnight and on the 15th day the rats were anaesthetized with diethyl ether and blood sample from each animal collected by

retro-orbital plexus puncture in sterilized centrifuge tubes. The blood samples were allowed to coagulate at 30 °C for 45 min. Serum was separated by centrifugation at 2 500 r/min at 30 °C for 15 min and subjected to biochemical investigations using standard test kits to assess liver function. The following biochemical investigations were carried out in serum: serum alanine transaminase (ALT)^[14], serum aspartate transaminase (AST)^[15], serum alkaline phosphatase (ALP)^[16], total bilirubin^[17], and total serum protein^[18].

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

2.8. Histopathological studies

Thin sections (5 µmol/L) were cut and stained with routine hematoxylin and eosin stain for photo microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue^[19].

2.9. Statistical analysis

All the results were expressed as mean±SEM. One-way analysis of variance (ANOVA) was used for the statistical analysis of data. Student's test was used for determining the significance. A probability value of $P<0.05$ was considered as significant and $P<0.01$ was considered highly significant.

3. Results

3.1. Phytochemical investigation

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

and proteins.

3.2. Acute oral toxicity study

The aqueous extract did not cause any mortality up to 2000 mg/kg and was considered as safe.

3.3. Biochemical tests

The effect of APO on serum marker enzymes is presented in Table 1. In the present study, CCl₄ given in the dose range of 1 mL/kg bodyweight (along with olive oil 1:1) produced a significant rise in AST, ALT, ALP and serum bilirubin levels and a significant fall in the total protein levels on exposure to CCl₄, indicating considerable hepatocellular injury. Administration of aqueous extract of *P. officinalis* at the doses of 100 and 200 mg/kg produced a highly significant fall in the AST, ALT, ALP, total bilirubin levels and a significant rise in the total protein levels in a dose dependent manner.

3.4. Histopathological observations

Histopathological examination of the liver slides of rats of normal control showed normal parenchyma and normal portal tract. Livers of the rats administered only CCl₄ (toxic control) showed inflammation of the portal triad; fatty change and necrosis of the periportal zone and they also showed necrosis, sinusoidal dilatation, inflammation, hemorrhage and vascular congestion of the centrilobular area. Rat livers treated with CCl₄ along with 100 mg/(kg·d) of Silymarin showed almost normal appearance of liver parenchyma (Figure 1).

However, a necrotic focus was seen in the periportal area. Animals that had received CCl₄ along with aqueous extract of 100 mg/(kg·d) of *P. officinalis* showed periportal inflammation, mild sinusoidal dilatation in the central zone and moderate degree of inflammatory cell infiltration in the portal triad. Liver from animals administered CCl₄ along with 200 mg/(kg·d) of aqueous extract of *P. officinalis* showed showing scattered fatty vacuolation.

Table 1. Effect of aqueous extract of the roots of *P. officinalis* on biochemical parameters against CCl₄ induced hepatotoxicity in rats.

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TB (mg/dL)	TP (g/dL)
Normal control (2% gum acacia, 1 mL/kg <i>p.o.</i>)	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>i.p.</i>)	379.20±26.13 ^b	768.60±42.15 ^b	345.49±22.02 ^b	7.85±0.64 ^b	5.21±0.56 ^b
CCl ₄ + Silymarin (100 mg/kg <i>p.o.</i>)	97.88±5.83 ^c	284.33±5.40 ^c	141.39±13.08 ^c	1.83±0.22 ^c	15.27±1.01 ^{ac}
CCl ₄ + 100 mg/kg <i>p.o.</i> of aqueous extract	120.76±7.91 ^{ad}	320.17±6.38 ^{bd}	184.04±11.67 ^{bc}	4.48±0.41 ^{bd}	14.78±1.02 ^{ad}
CCl ₄ + 200 mg/kg <i>p.o.</i> of aqueous extract	101.53±5.47 ^d	295.36±2.88 ^d	153.06±7.62 ^d	2.91±0.13 ^d	19.85±0.86 ^{ad}

Data expressed in mean±SEM, $n=6$. ^a: $P<0.05$, ^b: $P<0.01$, significant differences from the normal control group; ^c: $P<0.05$, ^d: $P<0.01$, significant differences from the CCl₄ group.

Figure 1. Histopathology of liver tissues.

(A) Liver from control group rat (CV=central vein, PT=portal triad). (B) Liver from animal treated with CCl_4 only. (C) Liver from animal treated with CCl_4 and 100 mg/(kg·d) of Silymarin. (D) Liver from animal treated with CCl_4 and 100 mg/(kg·d) of aqueous extract of *P. officinalis*. (E) Liver from animal treated with CCl_4 and 200 mg/(kg·d) of aqueous extract of *P. officinalis*.

4. Discussion

From the results of acute oral toxicity study of the aqueous extract it can be concluded that LD_{50} of the drug is greater than 2000 mg/kg bodyweight, which means that even a dose of 2000 mg/kg bodyweight of aqueous extract of the root powder of *P. officinalis* is safe for administration.

CCl_4 given intraperitoneally, at the dose of 1 mL/kg body weight every 72 h for 14 d, has been reported to produce hepatotoxicity. So, in the present study CCl_4 was administered at the dose of 1 mL/kg bodyweight (along with olive oil 1:1) on day one of the study and then every 72 h during 14 d study.

In the present study, CCl_4 given in dose of 1 mL/kg bodyweight (along with olive oil 1:1) produced a significant rise in AST, ALT, ALP and serum bilirubin levels and a significant fall in the total protein levels on exposure to CCl_4 , indicating considerable hepatocellular injury. The aqueous extract of *P. officinalis* was administered at two dose levels of 100 and 200 mg/(kg·d), for 14 d along with CCl_4 . The results of this experiment reveal that the extract of the root of *P. officinalis* has a definitive antihepatotoxic effect ($P < 0.01$) against the deleterious effect of CCl_4 upon the structure and function of liver as estimated by various parameters. Both doses (100 and 200 mg/kg) of the aqueous extract effectively attenuated the increased levels of AST, ALT, ALP and total bilirubin; effectively increased the total protein levels produced by CCl_4 and caused subsequent recovery towards normalization comparable to the control and the standard group animals. The hepatoprotective effect was pronounced more at the dose of 200 mg/(kg·d) than 100 mg/(kg·d).

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

The roots of *P. officinalis* contain alkaloids, tannins, saponins, glycosides, terpenes, flavonoids, carbohydrates, steroids and proteins as revealed by phytochemical screening. The LD_{50} of aqueous extract of the roots of *P. officinalis* was found to be greater than 2000 mg/kg. Aqueous extract of the roots of *P. officinalis* when administered for 14 d showed hepatoprotective/antihepatotoxic effect against CCl_4 induced hepatocellular damage in rats. The dose of 200 mg/(kg·d) of *P. officinalis* was found to be much more hepatoprotective than 100 mg/(kg·d) dose as evidenced by biochemical parameters and marked regenerative activity observed in rat that received 200 mg/(kg·d) dose. However, Silymarin was found to be more effective as a hepatoprotective than the aqueous extracts of the roots of *P. officinalis*.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

The research work was supported by the Department of Pharmaceutical sciences, University of Kashmir.

Comments

Background

The root of *P. officinalis* has been used in Unani, Ayurvedic and Homoeopathic systems of medicine for years. However, no phytochemical investigation, toxicity study or anti-hepatoprotective carried out on this plant.

Research frontiers

This drug is being used as a hepatoprotective in the traditional Indian system of medicines and this is the first scientific study as far as toxicity, pharmacognostic and antihepatotoxic activity is concerned.

Related reports

Phytochemical screening revealed that the roots of *P. officinalis* contain alkaloids, tannins, saponins, glycosides, carbohydrates, flavonoids, terpenes, steroids and proteins. The aqueous extract did not cause any mortality up to 2000 mg/kg and was considered as safe. The substantially elevated AST, ALT, SALP, total bilirubin levels were significantly lowered in a dose dependent manner, in rats that had received the root extract at the dose of 100 and 200 mg/kg, respectively, along with CCl₄ while TP levels were elevated in these regeneration of the livers in extract treated groups while Silymarin treated rats were almost

Innovations and breakthroughs

This is the first scientific study on this herbal drug and proves the traditional claim of the roots being hepatoprotective.

Applications

This drug has been used for years in the traditional system of medicines, however there was no scientific study conducted on this plant so far. This study has checked the toxicity profile, pharmacognostic constituents, and antihepatotoxic activity.

Peer review

Looking at the biochemical and histopathological results, the work has proven the claim of the traditional system of medicines. The authors have conducted toxicity and pharmacognostic activity, which is very nice and important. I recommend this article to be published.

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] Halder PK, Biswas M, Bhattacharya S, Karan T, Ghosh AK. Hepatoprotective activity of *Dregea volubilis* fruit against paracetamol-induced liver damage in rats. *Indian J Pharm Educ Res* 2012; **46**(1): 20–25.
- [4] Sreedhar V, Nath LKR, Gopal NM, Raju D, Venugopal K, Sekhar KKR. Hepatoprotective activity of *Vitex vestita* roots against paracetamol-induced hepatic injury in rats. *J Pharm Res* 2011; **4**(1): 288–290.
- [5] Bairwa NK, Sethiya NK, Mishra SH. Protective effect of stem bark of *Ceiba pentandra* linn. against paracetamol-induced hepatotoxicity in rats. *Pharmacogn Res* 2010; **2**(1): 26–30.
- [6] Lev E, Amar Z. *Practical materia medica of the medieval Eastern Mediterranean according to the Cairo Genizah*. Israel: Brill Academic Publishers; 2007, p. 235.
- [7] Ahmad F, Tabassum N, Rasool S. Medicinal uses and phytoconstituents of *Paeonia officinalis*. *IRJP* 2012; **3**(4): 85–87.
- [8] Dawkins MJR. Carbon tetrachloride poisoning in the liver of the new-born rat. *J Path Bact* 1963; **85**: 189.
- [9] 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.
- [10] Trease GE, Evans WC. *Pharmacognosy*. 16th ed. London: Bailliere Tindall Ltd; 2009, p. 60–75.
- [11] Acute Oral Toxicity (AOT) (OECD Test Guideline 425) Statistical Programme (AOT425StatPgm). 2001; Version: 1.0 (<http://www.oecd.org/oecd/pages/home/displaygeneral/0,3380,EN-document-524-nodirectorat-no-24-6775-8,FF.html>).
- [12] Eesha BR, Mohanbabu AV, Meena KK, Sarath B, Vijay M, Lalit M, et al. Hepatoprotective activity of Terminalia paniculata against paracetamol induced hepatocellular damage in Wistar albino rats. *Asian Pac J Trop Med* 2011; **4**(6): 466–469.
- [13] Verma N, Singh AP, Amresh G, Sahu PK, Rao ChV. Protective effect of ethyl acetate fraction of *Rhododendron arboreum* flowers against carbon tetrachloride-induced hepatotoxicity in experimental models. *Indian J Pharmacol* 2011; **43**(3): 291–295.
- [14] Bergmeyer HV, Horder M. IFCC methods for measurement of catalytic concentrations of enzymes. *Clin Chim Acta* 1980; **105**: 147–172.
- [15] Bergmeyer HV, Bowers GN, Horder M, Mas AW. Optimization of methods for aspartate aminotransferase and alanine aminotransferase. *Clin Chem Acta* 1978; **24**: 58–73.
- [16] Rick W. *Klinische chemie und miroskopie*. 6th ed. Berlin: Springer Verlag; 1990, p. 294.
- [17] Jendrassik L, Grof P. Modified Jendrassik and Grof's method. *Biochem* 1938; **2**(297): 81.
- [18] Doumas BT. Standards for total serum protein assays—a collaborative study. *Clin Chem* 1975; **21**: 1159–1166.
- [19] Sabarense CM, Rocha KS, Rosa DD, Martins JH, Pereira MM, Silva FF, et al. A new computational method for hepatic fat microvesicles counting in histological study in rats. *Biochem Biophys Res Commun* 2012; **418**(2): 284–289.

[1] Latha TB, Srikanth A, Eswar KK, Mastan K, Srinivasa