



Preclinical Study on the Hepatoprotective Effect of Pollen Extract of *Pinus brutia* Ten. (Red Pine) in Mice and Phenolic Acid Analysis

Pinus brutia Ten. (Kızılcım) Polen Ekstresinin Karaciğer Koruyucu Etkisinin Preklinik Olarak Araştırılması ve Fenolik Asit Analizleri

İ Hasya Nazlı GÖK¹, İ Hina GÜL², İ Muhammad GÜLFRAZ², İ Muhammad Javaid ASAD², İ Nilgün ÖZTÜRK³, İ Fuat ŞANAL⁴, İ İlkey Erdoğan ORHAN^{1*}

¹Gazi University Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Turkey

²PMAS Arid Agriculture University, Institute of Biochemistry and Biotechnology, Rawalpindi, Pakistan

³Anadolu University Faculty of Pharmacy, Department of Pharmacognosy, Eskişehir, Turkey

⁴General Directorate of Forestry, Chairmanship of Inspection Committee, Ankara, Turkey

ABSTRACT

Objectives: Many agents, including those from herbal sources, have been sought as preventives or cures for hepatotoxicity. The pollen of *Pinus brutia* Ten., known as red pine (Pinaceae), is used against liver diseases in Anatolian folk medicine.

Materials and Methods: In the current study, pollen ethanol extract of *P. brutia* was investigated for its possible hepatoprotective activity using a mouse model of CCl₄-induced hepatotoxicity. Swiss albino mice were divided into five groups, and extract-treated groups were compared with a silymarin-treated group as the reference. The extract was tested at 100, 200, and 300 mg/kg (b.w.). Phenolic acids were analyzed using high-performance column chromatography (HPLC) in the extracts as pollens are usually known to be rich in phenolics.

Results: Our data revealed that the extract displayed the best hepatoprotection at a dose of 100 mg/kg when compared with silymarin (Legalon®), the reference drug. HPLC analysis indicated presence of protocatechuic acid (0.176 mg/g extract), p-hydroxybenzoic acid (0.001 mg/g extract), vanillic acid (VA) (0.537 mg/g extract), syringic acid (0.050 mg/g extract), and tr-cinnamic acid (0.310 mg/g extract), while the major phenolic acid was VA.

Conclusion: The outcomes of this study allow us to conclude that red pine pollen extract can serve as a promising hepatoprotective agent. Among the phenolic acids analyzed in the pollen extract, vanillic acid as the major one besides some other phenolic acids detected seems to be responsible for its remarkable hepatoprotective effect.

Key words: *Pinus brutia*, red pine, pollen, hepatoprotective activity, HPLC

ÖZ

Amaç: Hepatotoksisiteyi önlemek veya iyileştirmek için bitkisel kaynaklar dahil olmak üzere birçok bileşik araştırılmaktadır. Kızılcım olarak bilinen *Pinus brutia* Ten. bitkisinin polenleri Anadolu'da halk arasında karaciğer rahatsızlıklarında kullanılmaktadır.

Gereç ve Yöntemler: Bu çalışmada, *P. brutia* polen ekstresinin farelerde CCl₄ ile indüklenen hepatotoksisite modeli üzerindeki muhtemel hepatoprotektif etkisi araştırılmıştır. Swiss albino fareler 5 gruba ayrılmış ve ekstrakt uygulanan gruplar referans olarak silimarin (Legalon®) kullanılan grup ile karşılaştırılmıştır. Ekstreler 100, 200 ve 300 mg/kg (v.a.) konsantrasyonlarında çalışılmıştır. Polenlerin genellikle fenolik açıdan zengin olduğu bilindiğinden, ekstrede bulunan fenolik asitler yüksek performanslı sıvı kromatografisi (HPLC) kullanılarak analiz edilmiştir.

Bulgular: Sonuç olarak, ekstrenin referans ilaç olan silimarin (Legalon®) ile karşılaştırıldığında, en etkin hepatoprotektif etkiyi 100 mg/kg'lik dozda gösterdiği belirlenmiştir. HPLC analizi ekstrede protokatesik asit (0,176 mg/g ekstre), p-hidroksibenzoik asit (0,001 mg/g ekstre), vanilik asit (VA) (0,537 mg/g ekstre), siringik asit (0,050 mg/g ekstre), ve tr-sinnamik asit (0,310 mg/g ekstre) bulunduğunu, en yüksek miktardaki fenolik asidin ise VA olduğunu ortaya koymuştur.

Sonuç: Çalışmamız neticesinde, kılcım polen ekstresinin umut verici bir hepatoprotektif ajan olarak kullanılabilceği sonucuna varılmıştır. Polen ekstresinde analiz edilen fenolik asitler arasında, majör olan vanilik asidin yanı sıra bazı diğer fenolik asitlerin ekstrenin gösterdiği hepatoprotektif etkiden sorumlu olduğu düşünülmektedir.

Anahtar kelimeler: *Pinus brutia*, kılcım, polen, hepatoprotektif aktivite, HPLC

*Correspondence: iorhan@gazi.edu.tr, Phone: +90 312 202 30 11, ORCID-ID: orcid.org/0000-0002-7379-5436

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INTRODUCTION

The liver is one of the most important organs that regulate metabolic functions, hormones, and defense mechanisms in the body. On the other hand, the liver is exposed to many threats, such as alcohol, viruses, and xenobiotics; hence, protection of the liver is essential to the maintenance of liver function.^{1,2} The genus *Pinus* (Pinaceae) contains approximately 80 species with a worldwide distribution.³ In Turkey, *Pinus* contains seven species, *Pinus pinaster* Aiton, *P. brutia* Ten., *P. halepensis* Mill., *P. pinea* L., *P. sylvestris* L., *P. nigra* J.F. Arnold, and *P. radiata* D. Don.⁴ *P. brutia* Ten. (red pine) is spread out over Eastern Mediterranean countries such as Turkey, Greece, and Cyprus; Black Sea countries such as Ukraine and Georgia; and the Caucasus countries.⁵ Different parts of *P. brutia*, such as the bark, resin, tar, and cones, are used to treat asthma, bronchitis, cancer, diabetes, diarrhea, pneumonia, and tuberculosis in Turkish folk medicine.⁶⁻⁹

Pollens from many kinds of plants have been used as food traditionally for many years, even since pre-historic times.¹⁰⁻¹⁶ Pine pollen, which is the male spores of *Pinus*, have been used to protect the liver, combat senility and fatigue, treat gastrointestinal dysfunction, improve sexual function, and increase cerebral-cardiac blood vessel function for many years.^{17,18} The relevant literature survey shows that most previous studies on *P. brutia* were conducted on its bark. On the other hand, *P. brutia* bark contains some phenolic compounds, such as 4-hydroxybenzoic acid, resveratrol, gentisic acid, vanillin, vanillic acid (VA), catechin hydrate, p-coumaric acid (p-COU), ferulic acid (FA), protocatechuic acid (proCA), gallic acid (GA), myricetin, naringenin, caffeic acid (CA), luteolin, and kaempferol.¹⁹ On the other hand, there have been a few studies on the phytochemistry and biological activity on the pollens of *P. brutia*.²⁰⁻²⁶

There have been several studies describing the hepatoprotective effect of various pollens, such as bee pollen and that of chestnut, canola, *P. massoniana*, *Schisandra chinensis*, etc.²⁷⁻³² However, no reports on the hepatoprotective effect of pollen from *P. brutia* are available to date, although it is used against liver diseases around Muğla province in the Aegean region of Turkey (personal communication). Taking this information into account, we aimed to perform the present study in order to evaluate anecdotal claims of the hepatoprotective use of red pine pollen ethanol extract using carbon tetrachloride (CCl₄)-induced liver damage in mice and to identify major phenolic acids in the extract using high-performance column chromatography (HPLC).

MATERIALS AND METHODS

Experimental

Plant material

The pollen of *P. brutia* trees was collected from the forest area belonging to the General Directorate of Forestry, Ministry of Agriculture and Forestry, in the vicinity of Antalya province, Turkey in June 2015.

Preparation of the extract

Air-dried pollens (51.15 g) of *P. brutia* were macerated with 800 mL ethanol (80%) for 24 h twice at room temperature. The ethanol macerate was filtrated and evaporated to dryness *in vacuo*. The yield of the crude extract was 24.98% (w/w).

High-performance liquid chromatography analysis

Chemicals used for HPLC (methanol and formic acid) analysis were of chromatographic grade (Sigma-Aldrich, St. Louis, MO, USA). Phenolic acid standards, e.g., GA, proCA, p-OHBA (4-hydroxybenzoic acid), VA, CA, chlorogenic acid, syringic acid (SA), p-COU, FA, o-coumaric acid, rosmarinic acid, and trans-cinnamic acid (tr-CIN) used in HPLC analysis were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Merck (GmbH, Darmstadt, Germany). Analysis of phenolic acids in the extract was carried out with an Agilent 1100 series auto-sampler system from Agilent, GL Sciences Inc. (Waldbronn, Germany) equipped with a system controller, a DAD detector (G 1315B, 280 nm), and a quaternary LC pump (G1311A). The separation was carried out with a Zorbax Eclipse XDB-C18 column (150 mm, 4.6 mm i.d., and 5 μm particle size) (Agilent, Waldbronn, Germany) with the column temperature set at 25°C. Chromatographic separation was carried out using two solvent systems: A) methanol:water:formic acid (10:88:2, v/v/v) and B) methanol:water:formic acid (90:8:2, v/v/v), as reported elsewhere.³³ The analyses were performed by using a linear gradient program. The conditions were 100% A from 0 to 15 min, 100% A from 15 to 20 min, 85% A from 20 to 30 min, 50% A from 30 to 35 min 0% A from 36 to 42 min, and returned to 100% A. The flow rate was 1 mL/min, and the injection volume was 10 μL. Signals were detected at 280 nm. The extract was dissolved in a mixture of methanol and water (1:1, v/v) and injected into the HPLC.

Each compound was identified by its retention time and by spiking with the standards under the same conditions. The identities of phenolic acids were also confirmed with a photodiode array detector by comparison with the ultraviolet spectra of standards in the wavelength range of 220-320 nm. Each compound was quantified according to the peak area measurements, which were reported in calibration curves of the corresponding standards. Data are reported as means ± standard deviations of three independent analyses.

Animals

Swiss albino mice of either sex (50 to 70 g) were maintained under standard animal housing conditions fed with commercial mice chow and allowed water *ad libitum*. The experimental protocol was approved by an Institutional Ethic Committee constituted by PMAS Arid Agriculture University Rawalpindi for the animal study.

Hepatoprotective activity

Forty mice were divided into eight groups of five each (n=5). Group 1: The control group received 0.5 mL of saline (0.9%, v/v) in water. Group 2: Animals of this group received 0.5 mL of olive oil (0.5%). Group 3: Animals of this group received ethanol (0.2%, v/v). All of these animals received doses once per day for

the entire period (7 days) by *i.p.* injection, respectively. Animals of group 4 were administrated *i.p.* with CCl_4 dissolved in olive oil at a dose of 0.5 mL/kg/day body weight. Animals of groups 4 to 8 were administrated *i.p.* with CCl_4 dissolved in olive oil at a dose of 0.5 mL/kg/day body weight (b.w.).

Animals of group 5 were fed with silymarin dissolved in ethanol at a dose of 50 mg/kg/day. Animals of group 6 were fed with the pollen ethanol extract at dose of 100 mg/kg once per day by gavage, while animals of group 7 and 8 were fed with the extract at doses of 200 and 300 mg/kg, respectively, once *per* day by gavage. At the end of the experiments, all mice were sacrificed, serum was collected, the livers were removed, and washed with ice-cold physiological saline.

Acute oral toxicity study

An acute toxicity study was conducted for a selected suitable dose of plant extracts.³⁴ Approximately 100 to 300 mg of dried pollen extract was dissolved in 5 mL of ethanol, and 1 mL of each dose was given to animals by gavage.

Biochemical analysis

Organs were homogenized in 0.1 M Tris HCl buffer (pH 7.4) to give a 10% homogenate. This homogenate was used for the estimation of triglycerides, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol by using commercial kits (Randox Laboratory) and the enzymatic method of Bierman.³⁵ The enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP); total bilirubin; red blood cell count (RBC); white blood cell count (WBC); and platelet levels were estimated by using their respective diagnostic kits and an auto-analyzer (Merck). The body weights of animals were calculated by measuring the weight before and after treatment with the extract.

Statistical analysis

All values are expressed as mean \pm standard deviation. One-Way ANOVA was used to determine the consequences of different treatments by using the computer software GraphPad Prism 5.0.

RESULTS AND DISCUSSION

HPLC analysis

The pollen ethanol extract was analyzed by HPLC, which led to identification and quantification of the following phenolic acids: proCA (0.176 mg/g extract), p-hydroxybenzoic acid (0.001 mg/g extract), VA (0.537 mg/g extract), SA (0.050 mg/g extract), and tr-CIN acid (0.310 mg/g extract). The major phenolic acid was found to be VA (Figure 1).

Results of liver enzymes, lipid profiles, and blood cells

When the effects of the extract on mouse lipid profiles were examined, the pollen extract was found to reduce triglycerides and total cholesterol levels significantly at the 100 mg/kg dose. Nevertheless, the extract also reduced the HDL cholesterol level (Table 1). Although the extract applied at the 200 and 300 mg/kg doses decreased the triglyceride and cholesterol levels,

the activities were lower than that of the 100 mg/kg extract. The extract at a dose of 100 mg/kg exhibited a greater reducing effect than that of silymarin.

Our data indicated that plasma levels of AST and ALT enzymes were notably elevated in rats treated with CCl_4 . The ALT, AST, ALP, and bilirubin levels were diminished drastically with extract at the 100 mg/kg, 200 mg/kg, and 300 mg/kg doses (Table 2). The activity of the extract on these enzymes and proteins was higher than that of the reference drug, silymarin. Considering the pollen extract treatment on blood cells, the counts of reduced RBC and WBC was increased, so the extract presented a similar effect to that of silymarin (Table 3).

Histopathologic findings

Histopathologic data displayed that livers from the healthy mouse group showed normal hepatocyte structures. However, after administration of CCl_4 , complete loss of liver architecture was observed, whereas the damaging effects of CCl_4 were reversed by treatment with the pollen extract (Figure 2). The recovery of tissue was significant when it was treated with the 300 mg/kg dose of the pollen extract, which indicated that tissue regeneration was dose-dependent (Figure 2e), while similar results were obtained when the hepatoprotective agent was used, *i.e.*, silymarin (Figure 2b).

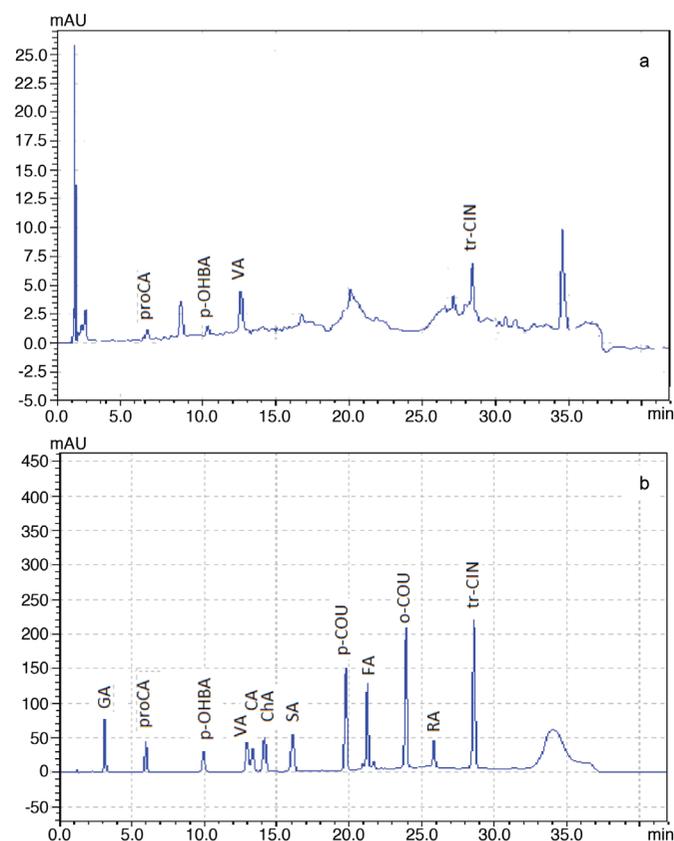


Figure 1. HPLC chromatograms of the pollen extract (a) and standard phenolic acids (b)

HPLC: High-performance column chromatography, GA: Gallic acid, proCA: Protocatechuic acid, VA: Vanillic acid, SA: Syringic acid, p-OHBA: 4-hydroxybenzoic acid, p-COU: P-coumaric acid, tr-CIN: Trans-cinnamic acid

Liver damage induced by CCl_4 in rats is one of the most preferable experimental models for the study of hepatoprotection. Several studies have been performed to determine the hepatoprotective or lipid-lowering effects of the various aforementioned pollen extracts as the liver is known to play the foremost role in lipid transformations.²⁷⁻³² Pollen grains are the tiny male particles released from trees, weeds, and grasses. The main function of

pollen grains is to fertilize other parts of plants. An early study on a flower pollen extract (0.4 mL/100 g b.w.), in which the name of the extract was mentioned as cernitins, was described to possess a hepatoprotective effect on livers damaged by alcohol by reducing serum AST and ALT levels.

Another pollen extract from a flower, whose scientific or local name was not indicated, was reported to exert a

Table 1. Effects of red pine pollen extract on the lipid profiles of mice

Group	Treatment	Triglycerides (mg/dL)	Cholesterol (mg/dL)	HDL (mg/dL)
1	Normal (vehicle)	81.15±1.22	65.38±2.34	59.24±2.36
2	Olive oil group	75.27±2.51	71.34±1.34	45.43±1.23
3	Ethanol group	82.35±2.37*	66.24±2.14*	54.26±1.45*
4	CCl_4 + olive oil	142.35±2.37	132.35±0.39	112.35±1.34
5	Silymarin + olive oil	89.31±1.32	74.12±1.25	62.17±0.38
6	Pollen extract at 100 mg/kg	82.31±1.27*	59.23±2.14*	47.26±1.45*
7	Pollen extract at 200 mg/kg	91.38±2.76	88.34±1.32	51.24±2.35
8	Pollen extract at 300 mg/kg	98.35±1.52	89.65±0.57	52.78±1.45

*Significant ($p < 0.05$) values vs. control/normal and expressed as mean ± SD, n=5
 CCl_4 : Carbon tetrachloride, HDL: High-density lipoprotein, SD: Standard deviation

Table 2. Effects of red pine pollen extract on liver enzyme and proteins in mice

Group	Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	Bilirubin (mg/dL)
1	Normal (vehicle)	81.15±1.21	65.38±2.34	59.24±2.36	0.146±0.028
2	Olive oil group	75.27±1.51	62.54±1.33	45.43±1.25	0.245±0.051
3	Ethanol group	62.35±2.37*	56.24±2.14*	44.26±1.45*	0.691±0.596
4	CCl_4 + olive oil	162.35±2.37	152.35±0.39	142.35±1.34	1.289±0.19
5	Silymarin + olive oil	89.31±1.32	84.12±1.25	72.17±0.38	0.571±0.22
6	Pollen extract at 100 mg/kg	68.31±1.27*	69.23±2.14*	67.26±1.45*	0.169±0.33
7	Pollen extract at 200 mg/kg	61.38±2.76	58.34±1.32	61.24±2.35	0.186±0.02
8	Pollen extract at 300 mg/kg	57.48±1.53	61.54±0.78	62.35±2.57	0.192±0.01

*Significant ($p < 0.05$) values vs. control/normal and expressed as mean ± SD, n=5
 ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, SD: Standard deviation, CCl_4 : Carbon tetrachloride

Table 3. Effects of red pine pollen extract on blood cell parameters in mice

Group	Treatment	RBCs ($10^6/\text{mm}^3$)	WBCs ($10^3/\text{mm}^3$)	Platelets ($10^3/\text{mm}^3$)
1	Normal (vehicle)	4.65±0.31	5.38±1.34	259.24±1.36
2	Olive oil group	4.87±0.51	6.24±0.33	245.23±1.26
3	Ethanol group	3.25±0.37	3.64±0.14	234.26±1.45
4	CCl_4 + olive oil	1.25±0.37	1.55±0.39	132.35±1.54
5	Silymarin + olive oil	3.91±0.32	4.42±0.25	242.15±1.38
6	Pollen extract at 100 mg/kg	4.81±0.27*	5.93±0.14	243.56±2.15
7	Pollen extract at 200 mg/kg	4.68±0.76	4.84±0.32	241.22±1.37
8	Pollen extract at 300 mg/kg	4.98±0.53	4.91±0.35	248.12±0.58

*Significant ($p < 0.05$) values vs. control/normal and expressed as mean ± SD, n=5
 RBC: Red blood cell, WBC: White blood cell, SD: Standard deviation, CCl_4 : Carbon tetrachloride

hepatoprotective effect via normalization of AST, ALT, and ALP levels as well as hypolipidemic and hypocholesterolemic activity in testosterone-androgenized rats.³⁶ The same extract was also shown to have a protective effect in a paracetamol-induced hepatotoxicity model in mice along with hypolipidemic effect.²⁷

Bee pollen from China was previously demonstrated effective in decreasing the amount of lipofuscin (fine yellow-brown pigment granules composed of lipid-containing residues of lysosomal digestion) in cardiac muscle, liver, and brain as well as adrenal gland cells in NIH mice.³⁷ Additionally, bee pollen extract with a rich polyphenol content from Poland was tested for its anti-atherogenic effect in apolipoprotein E-knockout mice at two doses of 0.1 and 1 g/kg body weight (b.w.) for 16 weeks.³² The extract led to a decrease in triglyceride and LDL cholesterol levels and displayed complete protection of the coronary arteries at 1 g/kg b.w. The effect was speculated to correlate with the polyphenol content of the pollen extract, which was supported by histopathological data on the cardiac vessels. In another study, a strong hepatoprotective effect of a pollen ethanol (70%) extract prepared from *Phoenix canariensis* hort. ex Chabaud as one of the palm species was shown in adult male Wistar albino rats.³⁸ The pollen extract was found to contain isorhamnetin-3-O-rutinoside and rutin as the phenolic compounds, which were concluded to contribute to its hepatoprotective effect. Yildiz et al.²⁹ studied the hepatoprotective effect of chestnut bee pollens collected from the western Black Sea region of Turkey at doses

of 200 and 400 mg/kg/day through CCl₄-induced liver damage in Sprague-Dawley rats. Particularly, bee pollen extract led to a significant decrease in AST and ALT levels at a dose of 400 mg/kg, whereas silybinin administered at a dose of 50 mg/kg in rats revealed a better hepatoprotective effect as compared with that of bee pollen extract at 200 mg/kg. Phytochemical analysis of chestnut pollen pointed to the presence of total phenolic compounds (28.87 mg GA equivalent/g), total flavonoids (8.07 mg quercetin equivalent/g), total anthocyanins (92.71 mg cyanidin-3-glucose equivalent/kg), and total carotenoids (29 mg β-carotene equivalent/100 g). Since the antioxidant activity of the extract in that study was also consistent with its hepatoprotective effect, the phenolic compounds analyzed in the extract were considered to contribute to its antioxidant and hepatoprotective effects. Similarly, the pollen extract of *Schisandra chinensis* of Chinese origin was reported to exert strong antioxidant and hepatoprotective effects against hepatotoxicity induced by CCl₄, which is consistent with our data.²⁸ Recently, Taishan *Pinus massoniana* pollen extract was shown to exert marked hepatoprotection in CCl₄-induced oxidative stress in the liver of rats tested at doses of 100, 200, and 400 mg/kg b.w., where AST, ALT, ALP, lactic dehydrogenase (LDH), malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase levels were significantly reduced.³¹ The strong hepatoprotective action of this pollen extract was concluded to be a result of its polysaccharide content, which was described as an acidic heteropolysaccharide with glucose

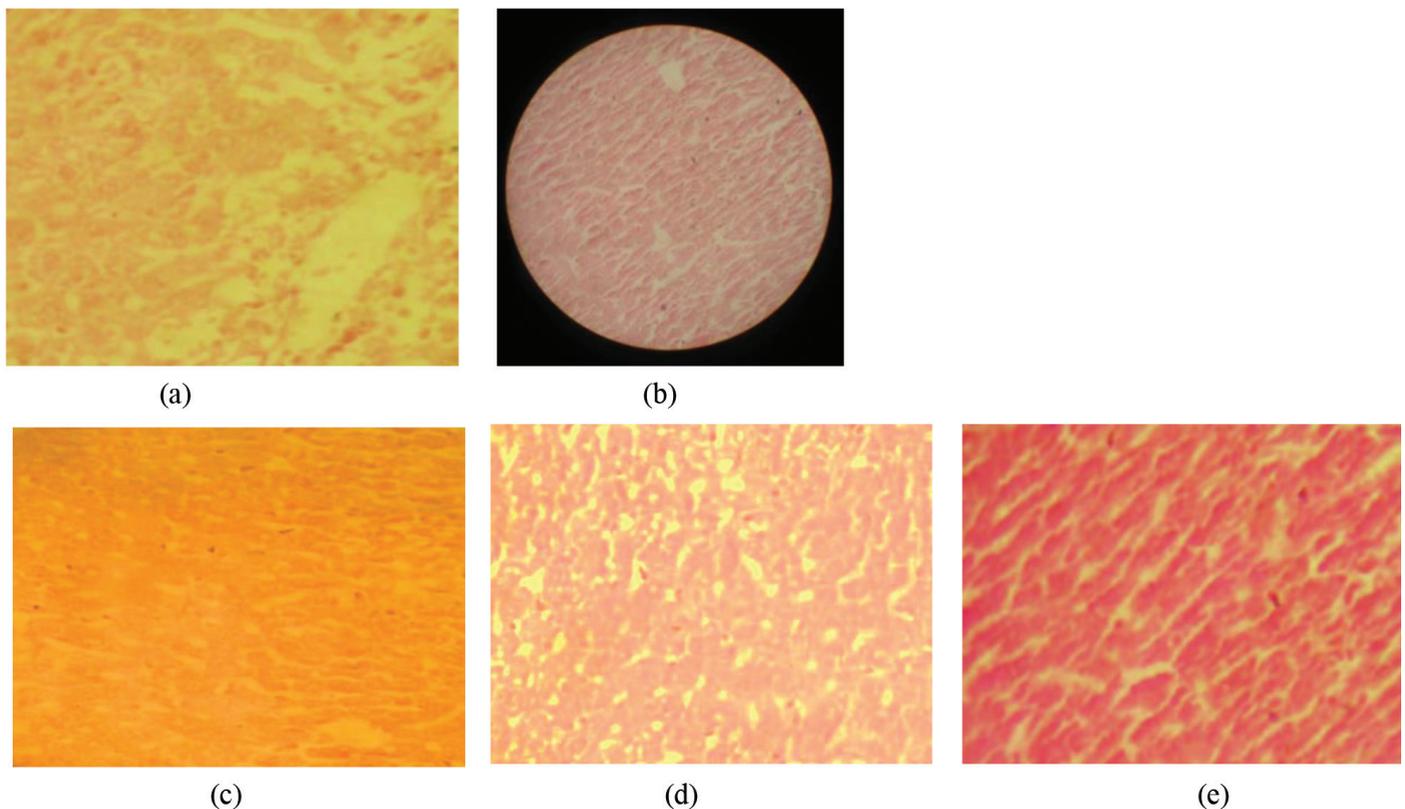


Figure 2. Histopathological results with cellular organization by red pine pollen extract (a) in CCl₄-induced liver damage; (b) by silymarin (c) pollen extract at 100 mg/kg; (d) pollen extract at 200 mg/kg; (e) pollen extract at 300 mg/kg

CCl₄: Carbon tetrachloride

and arabinose as the key constituent monosaccharides. On the other hand, Rzepecka-Stojko et al.³² mentioned a positive correlation between polyphenols present in bee pollen and its hepatoprotective and other biological activities.

In another study on honeybee products including chestnut honey, pollen, propolis, and royal jelly, their hepatoprotective activity was investigated using a CCl₄-induced model in rats.³⁹ Recovery of hepatotoxicity was observed by measuring AST and ALT levels as well as oxidative stress parameters such as MDA, SOD, and catalase (CAT). The use of bee pollen due to its discernible bioactivities was also suggested to be beneficial not only for human health but also for animal health (up to 20 g/kg diet) for production and health patterns of livestock.⁴⁰

On the other hand, VA was detected as the major phenolic compound in the extract along with some other phenolic acids. In fact, VA was reported to have a strong hepatoprotective activity in a number of plant or mushroom extracts.⁴¹ For instance, VA and SA were reported to be the active constituents in edible mushroom *Lentinula edodes* (shiitake) in concanavalin A-induced liver injury in mice.⁴² In another study, *L. edodes*, rich in VA and SA, was shown to exert strong hepatoprotection in mice with acute and chronic liver injury induced by CCl₄, which is in good agreement with our findings.⁴³ The phenolic composition of a Taiwanese mushroom species, *Xylaria nigripes*, with a high amount of epicatechin, catechin, and p-COU, was interpreted to be related to its activity against *in vivo* CCl₄-induced hepatotoxicity by Song et al.⁴⁴

Consistently, the leaf methanol extract of *Capparis spinosa* of Tunisian origin, found to contain rutin, resveratrol, coumarin, epicatechin, luteolin, catechin, kaempferol, VA, and GA, led to a notable decrease in serum ALT, AST, and LDH levels in CCl₄-induced acute liver damage, as well as in the amount of hepatic MDA formation, whereas it raised the activities of SOD, CAT, and GPx, and repaired injury that occurred in the liver.⁴⁵ In a similar study, a strong hepatoprotective effect was observed with the hot aqueous extract prepared from the leaves of *Asparagus albus* in male Wistar rats by Serairi-Beji et al.⁴⁶, where some phenolic acids, e.g., GA, VA, and 3,4-dimethoxybenzoic acid, along with several flavonoids, e.g., catechin, rutin, and quercetin, were identified through HPLC. The authors commented that the hepatoprotective effect of the extract was correlated with its polyphenolic content. A remarkable *in vivo* hepatoprotection was caused by *Artocarpus lakoocha* fruits which contain chromatotropic, gallic, vanillic, cinnamic, and FAs as well as quercetin and kaempferol, which is consistent with the findings of our study.⁴⁷ A *in vivo* study parallel to ours was conducted on the hepatoprotective effect induced by thioacetamide of the ethanol extract of *Prunus amygdalus* stem and leaves from Egypt.⁴⁸ Analysis of the extract using LC-DAD-ESI-MS in negative ion mode indicated the presence of a number of phenolics, including VA and homovanillic acid, which were correlated to hepatoprotection by the plant. Actually, all these previous studies have underlined a considerable contribution of VA to the hepatoprotective activity of a number of plants, which may also lead us to propose that VA might be the major

compound responsible for the hepatoprotective effect of red pine pollen extract.

CONCLUSION

Red pine pollen extract exhibited remarkable and dose-dependent hepatoprotection against CCl₄-induced liver damage in mice. Phenolic compounds, VA in particular, present in the pollen extract could be responsible for its notable hepatoprotective effect. We conclude that red pine pollen extract has the potential to serve as a promising hepatoprotective agent.

Conflicts of interest: No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of the paper.

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