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Hepatoprotective activity of *Artocarpus lakoocha* leaf extract against paracetamol-induced hepatotoxicity

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ABSTRACT

Introduction: The search for effective and safe medications to combat liver problems remains an ongoing quest. This study aimed to evaluate any hepatoprotective potential of an aqueous methanolic leaf extract from *Artocarpus lakoocha* against paracetamol (PCM)-induced liver toxicity in Wister rats.

Methods: In vitro testing of the plant extract entailed phytochemical screening. An in vivo study was also performed involving a single exposure to PCM either alone or in combination with the standard hepatoprotective agent, silymarin, or *A. lakoocha* leaf extract administered orally over 8 days. Animal weight, acute toxicity, hematological parameters (red blood cell count [RBC], white blood cells [WBCs], thrombocytes, hemoglobin [Hb], erythrocyte sedimentation rate [ESR]), and serum biochemical markers of hepatic damage (total bilirubin, aspartate aminotransferase [AST], C-reactive protein [CRP], alanine transaminase [ALT]) were measured and post mortem liver tissues were also examined histopathologically using eosin-hematoxylin staining and microscopy.

Results: Phytochemical analysis of *A. lakoocha* leaf extract exposed a predominant content of flavonoids, tannins, alkaloids, saponins, phenols, and steroids. In addition, *A. lakoocha* extract prevented PCM-induced weight loss ($P<0.05$), decreased Hb plus RBC, and elevated WBC ($P<0.05$) during the protocol. Both the plant extract and silymarin reversed PCM-elevated serum concentrations of hepatic biomarkers (+35.6% to +840%) and the histopathological injury. Additionally, the plant extract had a dose-related hemato-biochemical hepatoprotective effect, and the findings were analogous to those with the hepatoprotective standard, silymarin.

Conclusion: These study outcomes substantiate a protective effect of *A. lakoocha* leaf extract against PCM-induced hepatotoxicity in the animal model.

Implication for health policy/practice/research/medical education:
Methanolic extract of *Artocarpus lakoocha* showed significant hepatoprotective activity and might be used as adjunctive therapy to prevent liver injury caused by drugs, especially paracetamol.

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Introduction

The liver is the primary body organ responsible for the metabolism of macronutrients and exogenous xenobiotic compounds among other functions (1). Impairment of liver function is harmful to health, and is responsible for a large number of deaths worldwide. Moreover, hepatic damage is a prominent cause of morbidity arising from the development of oxidative stress and lipid peroxidation after continuous exposure to liver toxins, from infection, excessive alcohol, consumption and medications (2). One of the most common causes of liver disease is drug-induced liver damage, which presents a considerable clinical and regulatory challenge. Drug-induced hepatotoxicity manifests a wide range of symptoms, from asymptomatic

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elevation of liver enzymes to fulminant hepatic failure (3). Paracetamol (PCM) or acetaminophen is a clinically important over-the-counter drug, commonly used as an analgesic and antipyretic. Although, PCM is a safe drug at therapeutic doses, it may cause hepatic necrosis, nephrotoxicity, hepatic lesions and ultimately death in overdose (4). PCM is activated and catalytically transformed by cytochrome P450 enzymes to the potentially dangerous metabolite N-acetyl-p-benzoquinoneimine (NAPQI/NABQI), which initiates oxidative stress and glutathione depletion (5). Despite developments in modern medicine, there is a deficiency of reliable medications that protect the liver against harm and/or facilitate regeneration of hepatic cells. In this respect, active plant extracts have been employed to treat a variety of clinical conditions, including liver disease. As a consequence, the search for effective and safe medications to combat liver problems remains an ongoing quest (3).

Artocarpus lakoocha, also called "Monkey Jack" belongs to the family Moraceae. It is native to the sub-Himalayan areas of India and Pakistan, South China and South-East Asia. It is cultivated in Uttar Pradesh, Bengal, Khasi Hills and the Western Ghats. Its bark consists of 8.5% tannins and it is used to treat skin disorders when it is chewed. Additionally, when the bark is applied externally, it not only draws pus, but aids the healing of broken skin and pimples (6).

Traditionally, different parts of the *A. lakoocha* plant are used in different countries to treat an assortment of ailments. Hence, the fruit pulp which is edible, is employed as a tonic for the liver. The leaves are used for curing liver cirrhosis, tuberculosis, ulcers, diabetes, hypertension and also for wound healing. The seeds present efficacy in diarrhea and the roots are used for inflammation, female contraception, anemia, asthma, dermatitis and cough, while the sap is utilized as an ointment (7). Overall, the plant has been reported to possess antioxidant, anthelmintic, insecticidal (8), antiviral (9), anti-tyrosine, skin whitening (10), antibacterial, antitubercular, antifungal, antiplatelet, and antiarthritic properties (11).

Different parts of the *A. lakoocha* plant display antioxidant activity, and there are several reports in the literature substantiating this assertion (Figure 1) (8,12-17).

In view of its antioxidant activity (12-14), and after a review of the scientific literature regarding *A. lakoocha*, it was established that its fruit has been used as a liver tonic, however, the leaves are actually employed in managing cirrhosis (18). Consequently, this study was designed to evaluate any propensity of an *A. lakoocha* leaf extract against PCM-induced liver injury in rats, versus the clinically effective hepatoprotective agent silymarin, as a standard reference comparator.

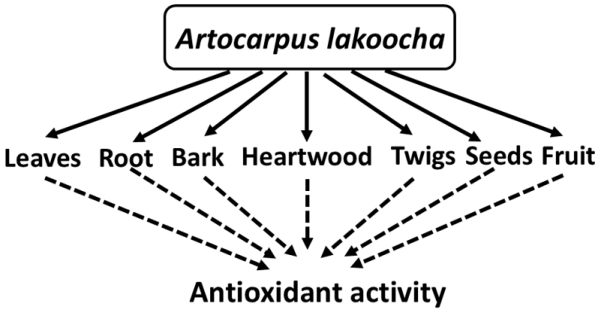

Figure 1. Antioxidant activity that has been demonstrated in extracts from various plant parts of *Artocarpus lakoocha* (8,12-17).

Materials and Methods

Preparation of plant extract

The leaves of *A. lakoocha* were collected from the district of Lahore, Pakistan. After identification, its specimen was preserved in the herbarium of the University of Health Sciences, Lahore, Pakistan, with a voucher number (278-03-13) for future reference. The leaves were dried and ground into a coarse powder for extraction at room temperature. The powdered material was macerated in methanol for 72 hours. Following this procedure, the obtained extract was filtered through double muslin and refiltered through Whatman filter paper 1. The filtrate was further concentrated to a semi-solid form by evaporation of the extraction solvent at 40 °C in a water bath. The resultant extract was dried, weighed and stored at -20 °C for further use.

Drugs and chemicals

Chemicals were all of analytical grade, including PCM (Sigma Aldrich, Germany), silymarin (Abbott Laboratories), sodium chloride (Pakistan Chemical), methanol and chloroform, (Sigma Aldrich, German. The followings were obtained from the sources in brackets: xylazine (Prix Pharmaceuticals, Pakistan), ketamine (Global Pharmaceuticals), and test kits for aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin and alkaline phosphatase (ALP) were obtained from Human Diagnostics.

In vitro studies

Phytochemical evaluation with qualitative tests

The majority of pharmacological activities are derived from phytochemical elements present in the plant. Therefore, a qualitative phytochemical analysis was performed to identify several possible constituents (18).

Flavonoids: 0.2 g of plant extract was placed in a test tube, 5 mL of diluted NaOH solution and 5.0 mL of 1M HCl were added. A yellow tint converted into a colorless solution indicated the presence of flavonoids.

Tannins: 0.5 g of plant extract was dissolved in 10 mL of distilled water, and the solution was boiled then filtered. Ferric chloride solution (0.1 %) was subsequently added to the filtrate and the appearance of a dark brown, green, or blue-black color showed the presence of tannins.

Alkaloids: A few drops of Hager's reagent were added to

1.0-2.0 mL of filtrate. The presence of a yellowish colored precipitate at the bottom of test tube detected the presence of alkaloids.

Saponins: The plant extract (0.2 g) was dissolved in distilled water (5.0 mL) and heated until boiling on a water bath. The formation of a foam indicated the presence of saponins.

Phenols by the ferric chloride test: A small quantity of distilled water and 2.0 mL of 5% ferric chloride solution were added to the plant extract and mixed. A green, blue, or violet colored precipitate showed the presence of polyphenols.

Phenols by the dilute iodine test: To the plant extract (1.0 mL) was added a few drops of dilute iodine solution. A transient red color gave an indication of a polyphenolic presence.

Phenols by the lead acetate test: The plant extract in water was mixed with a few drops of lead acetate solution. The appearance of a white precipitate manifested a polyphenol content.

Steroids: A small quantity of plant extract was mixed with 2.0 mL of chloroform in a test tube. Then, 2.0 mL of concentrated sulfuric acid was added to the mixture, followed by gentle shaking. The appearance of a reddish-brown color showed the existence of steroidal ring structures.

Carbohydrates: Benedict's solution (2.0 mL) was added to a small quantity of plant extract in a test tube, which was shaken and boiled. Formation of a reddish-brown precipitate revealed the presence of carbohydrates.

Protein by the ninhydrin test: Ninhydrin solution (2.0 mL; 0.2%) was mixed with a small quantity of plant extract and boiled. A resultant violet color of the mixture signified the presence of proteins.

Protein by the biuret test: To 1.0 mL of plant extract was added 1.0 mL of sodium hydroxide solution (40%) and 2 drops of $CuSO_4$ solution (1%), producing an initial blue color. The subsequent formation of a pinkish or purple-violet color indicated the presence of proteins.

Anthraquinones: To 5 g of dried plant extract was added benzene (10 mL), which was then filtered and 5 ml of ammonia solution (10%) was admixed. The development of a violet color in the ammonia phase showed the presence of free hydroxy anthraquinones) (Table 1).

In vivo studies

Experimental animals

Normal healthy Sprague-Dawley rats weighing 245.0 ± 15.0 g were kept in the vivarium, University of Health Science, Lahore, Pakistan. After acclimatization, the animals were divided into six groups (n=8). The animals were provided with standard rodent diet and free access to water, and the laboratory conditions were maintained at a temperature of 25.0 ± 3.0 °C (humidity > 65%), on an alternating 12 h/12 h light/dark cycle.

Dose preparation

Distilled water was used to dissolve both the plant extract and silymarin. PCM suspension was made with gum tragacanth (0.5%) in normal saline and was given orally (p.o.) to induce hepatotoxicity (19).

Acute toxicity studies

Sprague-Dawley rats were randomized into 5 groups (n = 8), the first group being classed as the control. *A. lakoocha* plant extract of was given via the oral route to the other four remaining groups at doses of 250, 500, 1000, 2000 mg/kg, respectively. All animals were subsequently observed for toxic symptoms for a period of 72 hours (20).

Paracetamol-induced hepatotoxicity

In order to induce hepatotoxicity, the experimental animals (other than controls), were administered PCM (500 mg/kg, p.o.). This individual dose of PCM was chosen because previous studies have shown that a single administration at this dose level, either intraperitonially or orally, produces consistent hepatic injury constituting a benchmark model of hepatotoxicity (21,22)

Seventy-two hours after PCM dosing, the animal groups were treated according to the following protocol:

- Group 1 received distilled water orally (p.o.) daily for 8 days (Control).
- Group 2 animals were administered a single dose of PCM (500 mg/kg, p.o.) and 72 hours later distilled water (p.o.) daily for 8 days.
- Group 3 animals were administered PCM (500 mg/kg, p.o.) and 72 hours later silymarin (100 mg/kg, p.o.) daily for 8 days, as the standard reference comparator drug.

Table 1. The phytoconstituents present in *Artocarpus lakoocha* extract and the type of test used to perform the phytochemical screening

Group	Phytoconstituent	Identification test name	Results
1	Flavonoids	Alkaline reagent test	Positive
2	Tannins	Gelatin test	Positive
3	Alkaloids	Hager's test	Positive
4	Saponins	Foam test	Positive
5	Phenols	Ferric chloride test, Dilute iodine test and Lead acetate test	Positive
6	Steroids	Salkowski test	Positive
7	Carbohydrate	Benedict test	Negative
8	Proteins	Ninhydrin test and Biuret test	Negative
9	Anthraquinones	Bontrager test	Negative

- Group 4 animals were administered PCM (500 mg/kg, p.o.) and 72 hours later *A. lakoocha* extract (250 mg/kg, p.o.) daily for 8 days.
- Group 5 animals were administered PCM (500 mg/kg, p.o.) and 72 hours later *A. lakoocha* extract (500 mg/kg, p.o.) daily for 8 days.
- Group 6 animals were administered PCM (500 mg/kg, p.o.) and 72 hours later *A. lakoocha* extract (750 mg/kg, p.o.) daily for 8 days.

Assessment of liver biochemical and histopathological parameters

The weight of all experimental animals was measured one day before the study and one day before the end of the protocol. The animals were fasted overnight, weighed and killed using ketamine and xylazine anesthesia (10:1 mixture, 2.0 mL/kg), and post mortem blood samples were collected by cardiac puncture in EDTA tubes. Following centrifugation at 3000 rpm for 15 minutes, the resultant serum was stored at -20 °C for biochemical analysis. Post-mortem, livers were immediately dissected, washed with normal saline and stored in 10% formalin for histopathological analysis.

Hematological and biochemical analysis

Hematological parameters including erythrocytes (RBC), white blood cells (WBCs), thrombocytes, hemoglobin (Hb), erythrocyte sedimentation rate (ESR), and liver biomarkers including total bilirubin, aspartate transaminase (AST), C-reactive protein (CRP), and alanine transaminase (ALT) were assayed during the protocol by commercially available kits.

Histopathological analysis

Liver tissue samples were subjected to standard histopathological techniques using graded methanol, fixation with xylene followed by embedding in paraffin wax. Staining of slides was performed with eosin and hematoxylin stain, and the images were viewed on an optical microscope at 10-40X resolution (23).

Statistical analysis

Data were presented as mean ± SEM (n = 8). Statistical significance between different experimental groups was evaluated by two-way ANOVA with post hoc Bonferroni's test. Values of $P < 0.05$ were considered statistically significant.

Results

Phytochemical analysis of *Artocarpus lakoocha* leaf extract

Phytochemical analysis of *A. lakoocha* extract indicated that it contained a variety of phytochemicals (Table 1).

Acute toxicity study on *Artocarpus lakoocha* leaf extract

An acute toxicity assessment of *A. lakoocha* extract in rats revealed that there were no signs or symptoms of toxicity up to a dose of 2000 mg/kg. It was therefore verified that the plant leaf extract at this dose level was below the maximum tolerated dose (MTD) (24).

Effect of *Artocarpus Lakoocha* leaf extract treatment on rat body weight following exposure to PCM

PCM induced a substantial weight loss in the animals by the end of the protocol, and this effect was reversed by the combined treatment with silymarin (100 mg/kg). Co-treatment of PCM and *A. lakoocha* extract (250 mg/kg) resulted in an animal weight outcome that was significantly different from both the PCM administered group and the control animals, such that it was in favor of the PCM alone treated group. By way of contrast, co-treatment of PCM with either of the two highest doses of *A. lakoocha* extract (500 or 750 mg/kg) yielded a complete reversal of PCM weight loss to levels which were statistically comparable to controls (Table 2).

Effect of *Artocarpus lakoocha* leaf extract on hematological parameters to PCM induced toxicity

PCM treatment decreased the erythrocyte count and blood Hb level, and these actions were subsequently reversed by co-treatment with either silymarin or the two highest doses of *A. lakoocha* extract (Table 3). In addition, PCM elevated the WBC count and ESR, while silymarin reduced these two parameters. The *A. lakoocha* extract, at all doses on the other hand, normalized the PCM elevated WBC count and reduced the ESR to a degree which was marginally above the value of the controls (Table 3).

Table 2. Effects of *Artocarpus lakoocha* extract on rat body weight following treatment with paracetamol (PCM) or combinations with either *Artocarpus lakoocha* or silymarin

Treatment groups	Animal weight (g)	
	Initial weight (g)	Final weight (g)
Control	260 ± 28.32	230 ± 29.18
PCM (500 mg/kg)	262.25 ± 22.23	166.75 ± 18.64*
PCM (500 mg/kg) + silymarin (100 mg/kg) standard	249.75 ± 26.02	223.5 ± 16.91$^{\#}$
PCM (500 mg/kg) + *A. lakoocha* extract (250 mg/kg)	252 ± 25.90	184.25 ± 17.74$^{*@}$
PCM (500 mg/kg) + *A. lakoocha* extract (500 mg/kg)	254.75 ± 23	216 ± 18.61$^{\#\times}$
PCM (500 mg/kg) + *A. lakoocha* extract (750 mg/kg)	248.5 ± 25.78	219 ± 14.19$^{\#\times}$

Values are shown as mean ± SEM. The Bonferroni post hoc test was used in conjunction with one-way ANOVA for statistical analysis. $^{*}P < 0.05$ versus control; $^{\#}P < 0.05$ versus PCM; $^{@}P < 0.05$ versus PCM + silymarin; $^{\times}P < 0.05$ versus PCM + *A. lakoocha* (250mg/kg).

Table 3. Effects of *Artocarpus lakoocha* extract on hematological parameters in rats treated with paracetamol (PCM) or combination with either *Artocarpus Lakoocha* or silymarin

Treatment groups	RBCs ($\times 10^6$ /µL)	WBCs (10^9/L)	Thrombocytes ($\times 10^5$ /µL)	Hemoglobin (g/dL)	ESR
Control	7.02 ± 0.18	11.27 ± 0.34	4.13 ± 0.54	13.70 ± 0.31	3.3 ± 0.17
PCM (500 mg/kg)	4.12 ± 0.64*	19.40 ± 0.75*	5.52 ± 0.37*	10.46 ± 0.71*	8.00 ± 0.63*
PCM (500 mg/kg) + silymarin (100 mg/kg) standard	6.76 ± 2.14$^{\#}$	10.56 ± 0.39$^{\#}$	4.56 ± 0.76$^{\#}$	12.73 ± 0.33	4.38 ± 0.17$^{*\#}$
PCM (500 mg/kg) + *A. lakoocha* extract (250 mg/kg)	5.0 ± 0.75$^{*@}$	12.22 ± 0.74$^{\#@}$	5.03 ± 0.10*	10.63 ± 2.30*	4.91 ± 0.11$^{*\#@}$
PCM (500 mg/kg) + *A. lakoocha* extract (500 mg/kg)	5.73 ± 1.18	11.52 ± 0.69$^{\#\times}$	4.63 ± 0.25$^{\#}$	11.89 ± 2.26	4.36 ± 0.25$^{*\#@\times}$
PCM (500 mg/kg) + *A. lakoocha* extract (750 mg/kg)	6.69 ± 0.54$^{\#}$	10.88 ± 0.83$^{\#}$	4.35 ± 0.19$^{\#}$	12.23 ± 1.90	3.63 ± 0.23$^{\#@\times\&}$

WBC, White blood cells; RBCs, Erythrocytes; Hb, Hemoglobin; ESR, Erythrocyte sedimentation rate.
Values are shown as mean ± SEM. The Bonferroni post hoc test was used in conjunction with one-way ANOVA for statistical analysis. *p < 0.05 versus control; #*P* < 0.05 versus PCM; @*P* < 0.05 versus PCM + silymarin; ˣ*P* < 0.05 versus PCM + *A. lakoocha* (250 mg/kg), &*P* < 0.05 versus PCM + *A. lakoocha* (500 mg/kg).

Effect of *Artocarpus lakoocha* leaf extract on serum biochemical markers of liver cell injury by PCM induced toxicity

PCM treatment significantly elevated the serum albumin level (+ 47.8%), but silymarin and the two lower doses of *A. lakoocha* extract reversed the PCM boosted serum albumin concentration. However, the highest dose of the plant extract (750 mg/kg), did not modify the increase in serum albumin level induced by PCM (Figure 2A). PCM also raised the serum concentration of AST by 35.6%, the level of which was returned to concentrations comparable to control by silymarin and all three doses of *A. lakoocha* extract (Figure 2B).

The serum concentration of the acute phase reactant, CRP, was sharply increased by a factor >840%, while all three doses of *A. lakoocha* extract and silymarin decreased serum CRP to levels that were not significantly different from the controls (Figure 2C). Likewise, PCM administration raised the serum concentration of ALT by 88.5%, and this response was abolished to the control levels by cotreatment with either silymarin or *A. lakoocha* extract (250-750 mg/kg) (Figure 2D).

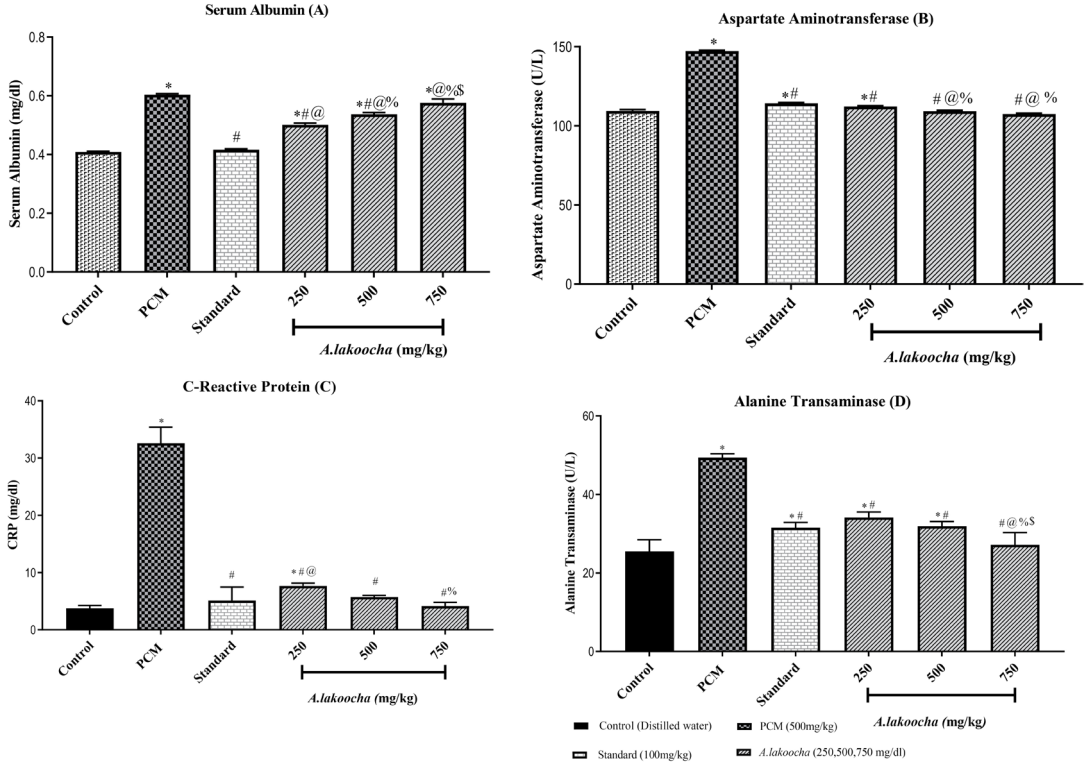

Figure 2. Effect of *Artocarpus lakoocha* leaf extract on serum biochemical parameters in paracetamol (PCM)-induced hepatotoxicity in rats (mean ± SD, n=8). (**A**) Albumin, (**B**) Aspartate aminotransaminase [AST], (**C**) C-reactive protein [CRP], and (**D**) Alanine transaminase [ALT] serum levels of control, PCM treated, and PCM co-treatment groups of rats: [PCM + silymarin [standard], PCM + *A. lakoocha* extract (250 mg/kg), PCM + *A. lakoocha* (500 mg/kg), PCM + *A. lakoocha* (750 mg/kg)]. **P* < 0.05 versus control; #*P* < 0.05 versus PCM; @*P* <0.05 versus PCM + silymarin; ˣ*P* < 0.05 versus PCM + *A. lakoocha* (250mg/kg), $*P* <0.05 versus PCM + *A. lakoocha* (500 mg/kg).

Effect of *Artocarpus lakoocha* extract and silymarin on PCM induced liver histopathology

Liver tissue samples from the animal groups were prepared for evaluation histopathologically, to establish any possible hepatoprotective activity of *A. lakoocha* extract. Samples from the control group showed normal histological architecture. Thus, hepatocytes were arranged in radial hepatic cords around the central vein. Sinusoidal spaces were clearly visible between the hepatic cords, with endothelial cells lining the sinusoids. The hepatocytes displayed normal cytoplasm with centrally located round nuclei. No signs of inflammation, degeneration, or necrosis were evident. Portal triads containing bile ducts, hepatic arteries, and portal veins were intact and well-organized (Figure 3A). The PCM alone treated group exhibited hepatocellular atrophy in the centri-lobular area and ballooning degeneration in the mid-zonal area. There was localized aggregation of lymphocytes and Kupffer cells (arrow) indicating a chronic immune response. It was noteworthy that fewer binucleated hepatocytes (star) were observed, indicating a deficiency of regeneration (Figure 3B). Samples from the PCM plus silymarin treated group presented normal histological structures with hepatocytes arranged in radial hepatic cords around the central vein. There was minimal infiltration of inflammatory cells observed within the sinusoidal spaces, suggesting a mild immune response. Overall, the liver architecture was well-preserved (Figure 3C). In addition, the PCM plus *A. lakoocha* (250 mg/kg) treated animal group revealed hepatocytes arranged in radial hepatic cords around the central vein while mild granular hydropic degeneration (arrow head) was evident in some hepatocytes. A few hepatocytes displayed signs of necrosis, including karyorrhexis (horizontal arrow) and pyknosis (vertical arrow). The endothelial cells and overall architecture of the hepatocytes were intact (Figure 3D). In the PCM plus 500 mg/kg plant extract treated group, there was evidence of cloudy degeneration in hepatocytes with a pronounced hemorrhage (star) in the parenchyma, indicating vascular damage. The presence of binucleated hepatocytes (arrow) suggested active mitosis and regenerative processes. Mild dilation of sinusoidal spaces was also noted although the overall liver architecture remained largely intact (Figure 3E). In the liver sections following PCM plus *A. lakoocha* extract (750 mg/kg), the photomicrographs exhibited normal histological architecture. The hepatocytes were arranged in radial hepatic cords around the central vein. Sinusoidal spaces were clearly visible between hepatic cords with endothelial cells lining the sinusoids. The hepatocytes displayed normal cytoplasm with centrally located round nuclei and fewer bi-nucleated hepatocytes were also evident. There were no signs of inflammation,

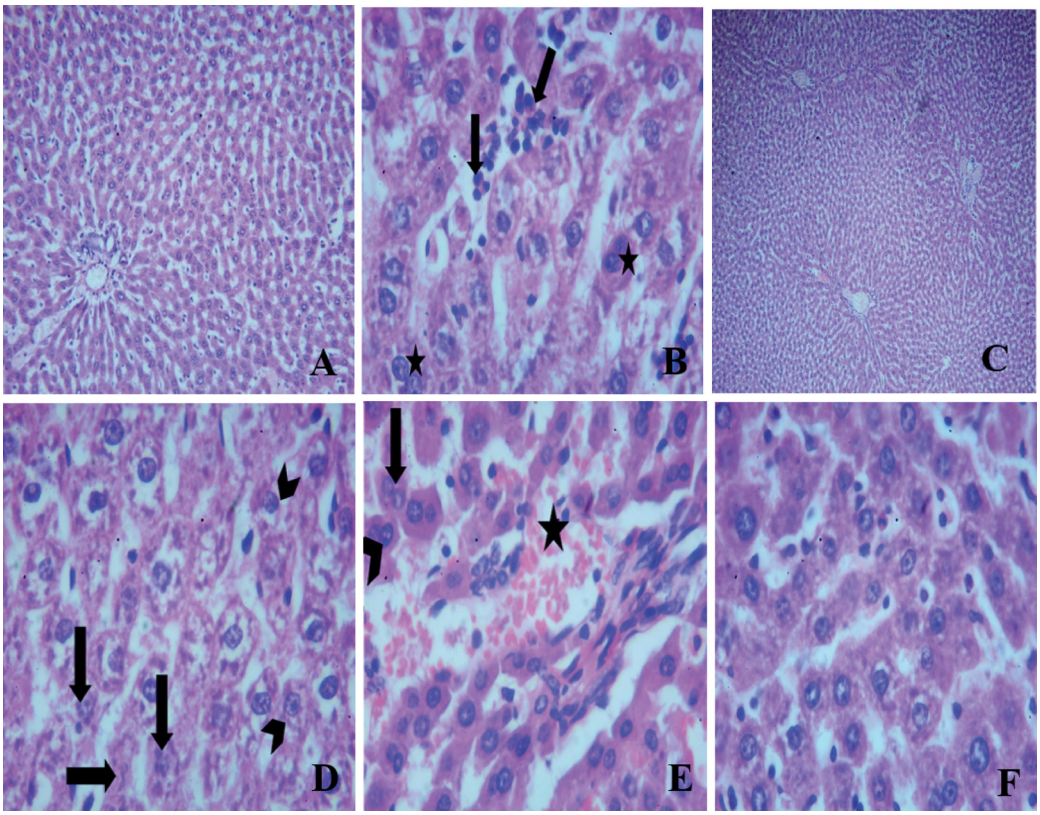

Figure 3. Liver histopathology sections taken from various treatment groups. (**A**) Control, 20X; (**B**) PCM, 40X, showing aggregation of lymphocytes and Kupffer cells (arrow), and binucleated hepatocytes (star); (**C**) PCM + silymarin, 10X; (**D**) PCM + *A. lakoocha* (250 mg/kg), 40X, showing karyorrhexis (horizontal arrow) and pyknosis (vertical arrow); (**E**) PCM+ *A. lakoocha* (500 mg/kg), 40X, showing a pronounced hemorrhage (star) and binucleated hepatocytes (arrow); (**F**) PCM + *A. lakoocha* (750 mg/kg) 40X.

degeneration, or necrosis. Portal triads containing bile ducts, hepatic arteries, and portal veins were intact and well-organized (Figure 3F), which was suggestive of an overall hepatoprotective action.

Discussion

It is well established that antioxidant activity is present ubiquitously in studied component parts of the *A. lakoocha* plant (Figure 1), and the leaves are no exception in this respect (13,16). Consequently, the initial study aim was to identify phytochemical components in our methanolic leaf extract of *A. lakoocha*, and subsequently, to evaluate any possible hepatoprotective activity against PCM hepatotoxicity in experimental animals. In the in-vitro studies, qualitative phytochemical analysis revealed that the methanolic extract of the plant leaves contained the secondary metabolites, including flavonoids, tannins, alkaloids, saponins, phenols, and steroids. Proteins, anthraquinones and carbohydrates were absent in the plant extract, and these overall outcomes were also in accord with a review on *A. lakoocha* phytochemistry and pharmacology (8). In respect to these findings, it is interesting that phenolic antioxidant plant constituents have been considered to provide a putative self-protective function in plants (16).

Arising from in-vivo studies, any hepatoprotective propensity of the plant extract was manifested in a combination profile. This consisted of hematological parameters (erythrocyte or RBC, WBC, platelets [thrombocytes], Hb level, ESR), liver biochemical markers (total bilirubin, AST, CRP, ALT), and histopathological studies.

Changes in hematological parameters provided symptomatic data on animal physiological status. The results demonstrated a significant reduction in RBC count and Hb level in all PCM-treated groups when compared to the controls. The reduction in RBC count and Hb may well have been attributable to destruction of mature RBCs, stemming from PCM hematotoxicity and leading to insufficiency of tissue blood flow and reduced oxygen levels (25). In this context, a similar reduction in RBC count and Hb level in rats, has been reported to PCM toxic effects (26). Correspondingly, we observed an elevation in WBC, platelets, and ESR in the animal group treated with PCM alone in comparison with other treatment groups. Co-administration of either *A. lakoocha* or silymarin with PCM reversed the hematological changes evoked by the PCM, and in the case of the plant extract the retrogression was graded. Thus, our findings support an amelioration of PCM induced hematotoxicity by *A. lakoocha* in the experimental animals.

Regarding the liver enzymes, PCM induced a marked escalation in the levels of serum biomarkers after treatment with PCM alone. Hence, our findings demonstrated that a single dose of PCM elevated the concentrations of serum albumin, AST, CRP, and ALT. In contrast, treatment with the standard agent, silymarin, or three doses of *A. lakoocha* leaf extract normalized liver enzyme levels, and this outcome was more pronounced with the highest dose of the plant extract (750 mg/kg) except in the case of serum albumin. PCM also evoked a precipitous increase in the serum concentration of CRP generated by the liver in response to inflammation and hepatic injury (27). Taken as a whole, the findings were also corroborated by the histopathological studies in liver tissues whereby animals treated with PCM displayed adverse tissue changes, while treatment with either *A. lakoocha* extract or silymarin tended to normalize and maintain tissue integrity.

The standard comparator agent, silymarin, has previously been shown to reduce liver-related deaths in clinical trials (28). Such an effect has been ascribed to antioxidant free radical scavenging, thereby reducing lipid peroxidation, in addition to modifying enzyme systems connected with hepatocellular injury (29).

Silymarin contains phenolic antioxidant and anti-inflammatory flavonolignans (30), while in this connection, *A. lakoocha* is also a source of the antioxidant polyphenol, resveratrol (31), which additionally happens to have anti-inflammatory activity (32). Intriguingly, not only does silymarin protect against PCM induced hepatotoxicity as we have shown here, but it has recently been reported that analogous PCM liver injury mitigating activity is shared by resveratrol (33). It is a distinct possibility therefore, that there is a hepatoprotective mechanistic commonality between resveratrol containing *A. lakoocha* and silymarin in terms of their antioxidant properties with respect to hepatotoxicity. Consequently, silymarin is an effective hepatoprotective agent and the current findings indicate that the leaf extract from *A. lakoocha* may well have a similar ability to induce a comparable intensity of hepatoprotection.

Conclusion

Arising from the current hemato-biochemical and histopathological studies, it was concluded that the methanolic extract of *A. lakoocha* leaves was comparable to the comparator standard agent, silymarin, in its intensity of ability to protect rats from acute liver damage caused by PCM. We are currently conducting further research with the constituent isolated compounds to better understand the precise mechanism underlying *A. lakoocha* hepatoprotective activity.

Limitation of the study

Since this is an initial study designed to establish if there is any hepatoprotective propensity of *A. lakoocha* leaf extract, no specific analysis of the constituents against liver damage has currently been performed. However, the investigation has now positively confirmed that the plant extract is hepatoprotective, so a logical step might involve an evaluation of a combination of the constituents ostensibly in the proportions occurring in the extract.

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Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical considerations

All experiments were approved by the Ethical Review Committee, University of Health Science, Lahore (PAEC/13/26).

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