Hepatoprotective activity of hydroalcoholic extract of *Cissampelos pareira* linn. leaves against CCl_4-induced acute and chronic hepatotoxicity

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Background and objective
Cissampelos pareira L. is a medicinal plant distributed across the tropics and used across the world traditionally for curing various pathological conditions. Hence, the present study has been carried out to evaluate the hepatoprotective effect of hydroalcoholic extract of *C. pareira* L.
Materials and methods
C. pareira L. leaves were extracted with a hydroalcoholic solvent. The resulting extract was subjected to an acute oral toxicity test on the basis of the OECD 423 guideline. Afterward, the selected dose of *C. pareira* hydroalcoholic extract (CPHE) was checked for hepatoprotective activity against CCl_4-induced acute and chronic hepatotoxicity. Measurements of serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, total bilirubin, and direct bilirubin were performed. At the end of the study, histopathological analysis of livers of the animals of various treatment groups was carried out.
Result and conclusion
Based on the acute oral toxicity study, three doses of CPHE were selected, namely, 100, 200, and 400 mg/kg. Administration of CPHE at 200 and 400 mg/kg prevented an increase in serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, and bilirubin levels against CCl_4-induced hepatotoxicity. The histopathological investigation of the portal triad structure of the liver clearly indicated that CPHE at 400 mg/kg showed significantly greater reduction in the necrotized area and normal appearance of the central vein, Kupffer cells and hepatocyte cells with no inflammatory cells. The results indicated that CPHE at 400 mg/kg protected the hepatic cells' membrane integrity against CCl_4-induced hepatotoxicity.

Keywords:
bilirubin, *Cissampelos pareira* L, histopathological analysis, serum glutamic oxaloacetic transaminase

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Introduction

The liver is the major organ of the body involved in carbohydrate, lipid, and protein metabolism [1], xenobiotic metabolism and detoxification, steroid hormone synthesis, and plasma protein synthesis and degradation [2]. Hence, it is exposed to a variety of chemicals that may be toxic to hepatocytes, making it prone to acute and chronic liver diseases. Acute and chronic liver diseases continue to be major international public health challenges despite considerable advancement in the field of hepatology [3]. There are various causes of liver diseases in India including obesity, high salt intake, overuse of medications, nutritional supplements, inappropriate use of alternative medicines, chemotherapy, infections, heavy metals, smoking, and excess alcohol consumption [4]. In recent years, researchers and health care professionals have been exploring complementary and alternative medicines for the treatment of liver disorders, specifically because of the view that natural products are associated with fewer side effects and are of great interest in the general population.

Cissampelos pareira L has been reported in folklore to be traditionally used as a hepatoprotective [5], anti-inflammatory, antitussive, antidiarrheal agent, and for the treatment of dyspepsia and dropsy [6–8]. *C. pareira* has been reported to be rich in phytoconstituents such as polyphenolic compounds like flavonoids (quercetin) [9], phytosterols (beta-sitosterol) [10], alkaloids (berberine, cissampeline) [11,12], and triterpenes [11], which could be responsible for the beneficial pharmacological effects.

In view of the reported traditional hepatoprotective effects of *C. pareira* L, the present study has been carried out to evaluate the hepatoprotective effect of the hydroalcoholic leaf extract of *C. pareira* against CCl_4-induced hepatotoxicity.

Materials and methods

Plant material

Fresh leaves of *C. pareira* were identified and collected from the creative farmer live plant site online. The plant species was identified and authenticated by BilwalMedchem and Research Laboratory Pvt Ltd (Reengus Rajasthan, India). A voucher specimen [voucher no. BSI/AZRC/I.12012/Tech./2019 (PI. ID.) 549] of the plant was deposited in the herbarium laboratory for future reference. Leaves of *C. pareira* were cleaned and reduced into small fragments and air-dried under shade at room temperature for 15 days. Afterward, the leaves were coarsely powdered in a mixer. The powdered material was stored in an air-tight container.

Drugs and chemical

All the solvents used for extraction were laboratory reagent grade and were purchased from LobaChemie Ltd (Mumbai, India). Silymarin (Silybon 140 mg tablet) was purchased from Himachal Pradesh, India, and Micro Labs Ltd (Bangalore, India). The suspensions of the above tablets were made in distilled water using Tween-80 as a suspending agent. Tween-80 used was pharma grade and procured from S.D. Fine Ltd (Mumbai, India). Different doses of the test drug were formulated as suspensions in distilled water using Tween-80 as a suspending agent.

Experimental animals

Wistar albino rats (160–200 g) of both sexes were procured and housed in the animal house of BilwalMedchem and Research Laboratory Pvt Ltd under standard environmental conditions of temperature (25±2 °C), relative humidity (45–55%) and 12 h dark/light cycles. All animals were provided a standard diet (standard pellets; Hafed, Rohtak, Haryana, India) and water *ad libitum*. All the animal experiments were in compliance with the Animal Ethical Committee, Committee for the Purpose of Control And Supervision of Experiments on Animals (CPCSEA), and were approved by the Institutional Animal Ethics Committee (IAEC) of BilwalMedchem and Research Laboratory Pvt Ltd, SKS Reengus Industrial Area (Ethical committee IAEC reg.no.2005/PO/RcBT/S/18/CPCSEA).

Extraction of leaves of *Cissampelos pareira*

Two hundred fifty grams of coarsely powdered leaves of *C. pareira* were defatted with 6000 ml of petroleum ether (60–80°C) using the soxhlet apparatus. Extraction was continued until a drop of solvent from the siphon tube, when evaporated on filter paper, did not leave a greasy spot. The extract was concentrated in a rotary evaporator to obtain marc. The obtained dried marc was 240 g and was further extracted with 1000 ml of hydroalcoholic solvent, ethanol : water (7 : 3). Extraction was continued (~10–12 cycles) until a drop of solvent from the siphon tube became clear. The greenish black extract thus obtained was filtered and collected. The filtered extract was evaporated under reduced pressure and dried in an oven to remove the remaining moisture, and finally weighed and kept in a sealed container until further use [8].

Acute oral toxicity study

An acute oral toxicity study was performed according to the Organization of Economic Co-operation and development (OECD) guideline 423 for testing of chemicals. Swiss male mice were used for the study. Animals were divided into four groups of three animals each (n=3) (the total number of animal used was 12). Extracts were prepared as a suspension by triturating with 2% Tween-80. Different doses of extract solution of 5, 50, 300, and 2000 mg/kg body weight, postoperatively, were administered. After the initial dosing, the animals were observed once during the first 30 min, followed by observation in the first 24 h (with special attention to the initial first 4 h), and then observed daily for a total of 14 days for any sign of acute toxicity [13].

Measurement of body weight

Body weights of rats were determined at 0, 7, and 14 days. The change in the percentage of body weight in the experimental rats was calculated. The results were compared with those of the normal control group of vehicle (NCGV) group.

$$\%\ \text{change in body weight} = \frac{(\text{Body weight before treatment} - \text{body weight after treatment})}{\text{Body weight before treatment}} \times 100.$$

CCl_4-induced acute and chronic hepatotoxicity in rats

Wistar albino rats weighing 160–200 mg/kg were divided into six groups and received their respective doses for 7 and 14 days: group I: NCGV served as normal controls and received vehicle; group II: negative control group of CCl_4 (NCGC) served as negative controls and received only CCl_4; group III: positive control group of silymarin (PCGS) served as the standard and received silymarin (25 mg/kg,

postoperatively); group IV: *C. pareira* hydroalcoholic extract (CPHE 100) served as test group I and received CPHE 100 mg/kg, postoperatively; group V: CPHE 200 served as test group II and received CPHE 200 mg/kg, postoperatively; and group VI: CPHE 400 served as test group III and received CPHE 400 mg/kg, postoperatively. Acute liver damage was induced by administration of 1 ml/kg dose of 30% CCl_4 in olive oil in a ratio of 1 : 1 on the seventh day of the study to all experimental rats of each group, except for NCGV. Afterward, on the eighth day of the acute study, rats were anesthetized and killed for estimation of biochemical parameters and histopathological investigation. Chronic liver damage was induced in rats by administration of 1 ml/kg dose of 30% CCl_4 in olive oil in a ratio of 1 : 1 on the seventh and 14th days of the study to all experimental rats of each group, except for NCGV. On 15th day of the chronic study, rats were anesthetized and killed for estimation of biochemical parameters and histopathological investigation [14]. To minimize the use of animals in experimentation, the normal control group of rats (NCGV) that received vehicle was the same for both acute and chronic models of hepatotoxicity.

Collection of blood samples

At the end of the experiment, overnight fasted rats were anesthetized with ether; while under anesthesia, they were painlessly killed between 9.00 am and 11.00 am to minimize the diurnal variations. Blood samples were harvested via an intracardiac needle from each rat into heparinized sample bottles. The heparin anticoagulated blood samples were centrifuged at 1000 rpm for 10 min, after which their plasma was collected and stored at–20°C for subsequent analysis.

Estimation of biochemical parameters (serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, total bilirubin, and direct bilirubin levels)

The collected plasma of the different groups of rats treated was stored in vacutainers and refrigerated at a temperature 15–24° C. The biochemical parameters of the different groups of rats treated like serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total bilirubin, and direct bilirubin were estimated both for the acute and for the chronic study using assay kits according to the methods described by the manufacturers (Avecon Health Care Private Limited, Ambala, Haryana, India).

Histopathology investigations of the liver

After collection of the blood samples (on the 8th day for the acute model and the 15th day for the chronic model), livers of the treated groups of rats different were excised from each group of experimental animals and washed with normal saline and then preserved in 10% formalin solution. As per the method described by Luna [15], the livers were collected in formaldehyde solution and 5 µg thick sections of livers of different groups of rats treated were cut and stained with hematoxylin and eosin, and glass slides were prepared for histopathological examination. The different sections of the liver were analyzed microscopically for the evaluation of histopathological changes and transverse sections of the liver were photographed with a photomicroscope.

Statistical analysis

All data were analyzed using Graph pad prism software (GraphPad Prism, San Diego, California, USA), version number 3.0, and values are expressed as mean±SEM and analyzed statistically by one-way analysis of variance, followed by Dunnett's test of multiple comparison. The mean values of treatment groups were compared with the mean of the NCGC. The criterion for statistical significance was P value less than 0.05 and P value less than 0.01.

Results

Acute oral toxicity

No sign of toxicity, moribund status or mortality was observed in mice treated with the test extract up to 14 days of observation. Hence, the extract was considered to be safe up to 2000 mg/kg. 1/20, 1/10, and 1/5 of 2000 mg/kg, that is 100, 200, and 400 mg/kg were selected and were designated as CPHE 100, CPHE 200, and CPHE 400.

Body weight measurements

The body weights of experimental rats were measured on days 0, 7, and 14 of the study. Normal control group rats showed normal body weights on 0, 7, and 14 days. The experimental rats of the negative control group (NCGC) of CCl_4 showed a decrease in body weight at 0, 7, and 14 days. The administration of silymarin significantly increased the body weight after 14 days of treatment, indicating that the rats were gaining weight in the normal growth process without the impact of drug treatment. CPHE 100, CPHE 200, and CPHE 400 also increased the body weight of rats after 14 days of treatment, indicating that the plant extracts did not have a hazardous impact on the normal metabolism or growth pattern of rats. The results are shown in Table 1.

Effect of various doses of *Cissampelos pareira* hydroalcoholic extract on biochemical parameters

In the acute model of hepatotoxicity in rats, administration of CPHE 200 and CPHE 400 for 7 days before CCl_4-induced hepatotoxicity restored the SGOT, SGPT, total bilirubin, and direct bilirubin levels ($P \leq 0.05$) close to normal in comparison with the NCGC group. Administration of the CPHE extract exerted an effect in a dose-dependent manner, leading to a decrease in SGOT, SGPT, total bilirubin, and direct bilirubin levels in CCl_4-induced hepatotoxic rats, and the results of CPHE 400 were found to be close to those of the reference drug silymarin.

In the chronic model of hepatotoxicity in rats, administration of CCl_4 on the seventh and 14th day of the experiment resulted in the development of chronic hepatotoxicity in rats. In the negative control group of rats (NCGC), the maximum serum levels of SGOT, SGPT, total bilirubin, and direct bilirubin were found after chronic intoxication with CCl_4 on the seventh and 14th day of study; this indicates the development of chronic hepatotoxicity in rats after the administration of CCl_4 two times. However, treatments with silymarin (25 mg/kg) and CPHE extract at different dose (200 and 400 mg/kg) levels in the PCGS, CPHE 200 and CPHE 400 groups of rats for 14 days of experiment significantly decreased the serum levels of SGOT, SGPT, total bilirubin, and direct bilirubin. The results are shown in Table 2.

Histopathological investigation of the liver in CCl_4-induced acute hepatotoxicity in various treatment groups

The histopathological investigation of the liver of the normal control group (NCGV) of rats for the acute and chronic study showed the normal architecture of hepatocytes in the portal triad structure of the liver. Furthermore, Kupffer cells appeared normal. However, very mild dilation of the central vein of the liver did not reveal any significant lesion of pathological importance.

Table 1 Effect of hydroalcoholic extract of leaves of *Cissampelos pareira* on body weight in rats

Groups	Treatment	Average body weight (g)±SEM		
		0 Day	7 Days	14 Days
I	NCGV	166.16±3.30	170.06±3.31	180.01±4.09
II	NCGC	164.50±4.34	159.08±4.21	150.09±6.35
III	PCGS Silymarin 25 mg/kg, postoperatively	167.58±3.60	173.12±4.63*	190.27±4.30**
IV	CPHE 100 mg/kg	165.15±4.22	169.40±5.26	172.64±4.95*
V	CPHE 200 mg/kg	169.14±4.90	174.13±6.90*	179.16±4.13*
VI	CPHE 400 mg/kg	163.82±3.18	168.43±4.93**	179.12±4.08**

Results are expressed as mean±SEM and analyzed statistically by one-way analysis of variance, followed by Dunnett's test of multiple comparison. The mean values of the treatment groups were compared with the mean value of the negative control group of CCl_4 (NCGC). CPHE, *C. pareira* hydroalcoholic extract; NCGC, negative control group of CCl4; NCGV, normal control group of vehicle; PCGS, positive control group of silymarin. The criteria for statistical significance were **P* value less than 0.05 and ***P* value less than 0.01.

Table 2 Effect of various doses of *Cissampelos pareira* hydroalcoholic extract on biochemical parameters in acute and chronic hepatotoxicity models

Serial number	Groups	SGOT (U/l)		SGPT (U/l)		Total bilirubin (mg/dl)		Direct bilirubin (mg/dl)	
		Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic
1	NCGV	845.4±44.72*	845.4 ±44.72*	805.3 ±42.63*	805.3 ±42.63*	0.54 ±0.06*	0.54 ±0.06*	0.15 ±0.01*	0.15 ±0.01*
2	NCGC	2898±43.82	3887±53.18	2888±52.82	3788±55.84	1.08±0.04	1.18±0.05	0.22±0.04	0.26±0.04
3	PCGS	978±43.41*	609±23.41*	911.6 ±43.63*	711.6 ±23.63*	0.62 ±0.04*	0.57 ±0.07*	0.16 ±0.04*	0.15 ±0.04*
4	CPHE 100	2381±44.08	2681±40.08	2560.6 ±44.61	2509±44.61	1.01±0.03	0.97±0.04	0.21±0.02	0.24±0.05
5	CPHE 200	1543.3 ±41.25*	1681 ±33.08*	2057 ±46.22*	1509 ±34.61*	0.78 ±0.02*	0.89 ±0.05*	0.20±0.01	0.23±0.07
6	CPHE 400	1098±42.68*	798±24.68*	930±42.08*	830±20.08*	0.68 ±0.02*	0.78 ±0.04*	0.17 ±0.07*	0.17 ±0.04*

Results are expressed as mean±SEM and analyzed statistically by one-way analysis of variance, followed by Dunnett's test of multiple comparison. The mean values of treatment groups were compared with the mean value of the negative control group of CCl_4 (NCGC). CPHE, *C. pareira* hydroalcoholic extract; NCGC, negative control group of CCl4; NCGV, normal control group of vehicle; PCGS, positive control group of silymarin; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase. The criteria for statistical significance were **P* value less than 0.05 and ***P* value less than 0.01.

In rats of the NCGC, histopathological investigation of the liver revealed fibrosis and hyperplasia of Kupffer cells, centrilobular sinusoidal dilatation/congestion and appearance of fatty vacuoles with loosened cell nucleus and increased glycogen, indicative of CCl_4-induced necrosis and injury of liver cells.

The rates in the PCGS, in histopathological investigation of liver in the acute study, showed no appearance of sinusoidal fibrosis and central vein fibrosis. Furthermore, Kupffer cells and hepatocytes were arranged in the normal architecture, indicative of protection conferred by silymarin against CCl_4-induced liver injury in this group.

The histopathological investigation of the liver of rats of the CPHE 100 group showed fatty vacuoles with loosened cell nucleus and increased glycogen, central lobular necrosis, and sinusoidal dilatation and mild hyperplasia and fibrosis of Kupffer cells, indicative of CCl_4-induced necrosis and injury of liver cells.

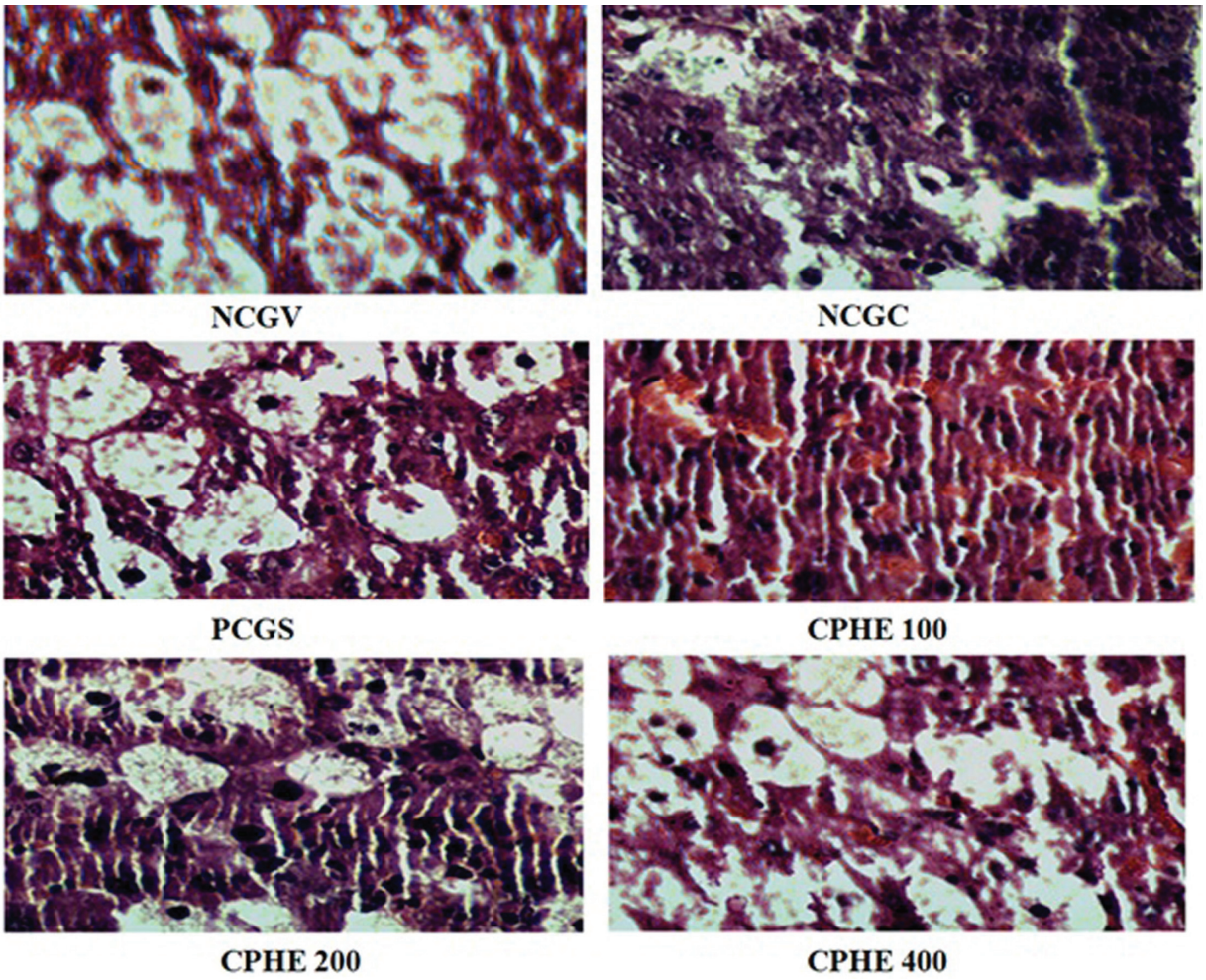
The histopathological investigation of the liver of rats of the CPHE 200 group of rats showed mild central vein dilatation, little sinusoidal congestion, decreased infiltration of inflammatory cell, and reduced necrosis of hepatocytes. However, prominent disarrangement of Kupffer cells in between the hepatocytes cells was also observed, which indicates mild protection against CCl_4-induced hepatic injury in this group.

The histopathological investigation of the portal triad structure of the liver of CPHE 400-treated groups of rats for the acute study showed the normal architecture of hepatocytes and Kupffer cells with very little central vein enlargement. This indicates the protection of CPHE 400-treated groups of rats against CCl_4-induced hepatic injury. The results are presented in Fig. 1.

Histopathological description of the liver in CCl_4-induced chronic hepatotoxicity in various treatment groups

The histopathological investigation of the portal triad structure of the liver of negative control groups

Figure 1



Histopathological sections of the liver of rats of various treatment groups against CCl_4-induced acute hepatotoxicity in various treated groups, magnification (hematoxylin and eosin, ×40).

(NCGC) of rats for chronic study showed congestion of sinusoids and dilatation of the central vein, Kupffer cell derangement, prominent necrosis of hepatocytes of the centrilobular zone and marked fibrosis of hepatocytes, indicative of CCl_4-induced necrosis and injury of liver cells.

The rats in the PCGS showed no appearance of sinusoidal fibrosis and central vein fibrosis in the histopathological investigation of the liver in the acute study. Furthermore, Kupffer cells and hepatocytes are arranged in the normal architecture, indicative of the protection conferred by silymarin against CCl_4-induced liver injury in this group.

The histopathological investigation of the liver of rats of the CPHE 100 group showed severe degenerative changes in liver cells, central vein fibrosis, sinusoidal dilatation, and derangement of Kupffer cells, indicative of CCl_4-induced necrosis and injury of liver cells.

The histopathological investigation of the liver of rats of the CPHE 200 group showed mild central vein dilatation, little sinusoidal congestion, absence of inflammatory cells near the central vein and a marked decrease in necrosis of hepatocytes and normal positioning of Kupffer cells, all indicative of the complete protection afforded by CPHE 200 against CCl_4-induced hepatic injury in this group.

The histopathological investigation of the liver of rats of the CPHE 200 group revealed marked depletion of the necrosed area and hepatocytes devoid of any inflammatory markers. Moreover, the positioning of Kupffer cells and hepatocyte cells with a healthy cytoplasm, nucleus and central vein indicates the normal architecture pattern of the portal triad structure of rat liver. No marked central vacuole formation and fibrosis were observed in this group, all indicative of the complete protection conferred by CPHE 400 against CCl_4-induced hepatic necrosis and injury.

The histopathological investigation of the portal triad structure of the liver both in the acute and chronic models of hepatotoxicity clearly shows that the CPHE 400 treatment displayed significantly greater reduction in the necrotized area and normal appearance of the central vein, Kupffer cells, and hepatocyte cells, with no inflammatory cells. No marked central vacuole formation and fibrosis were observed in the histopathological section of the liver of the CPHE 400-treated group. The results are shown in Fig. 2.

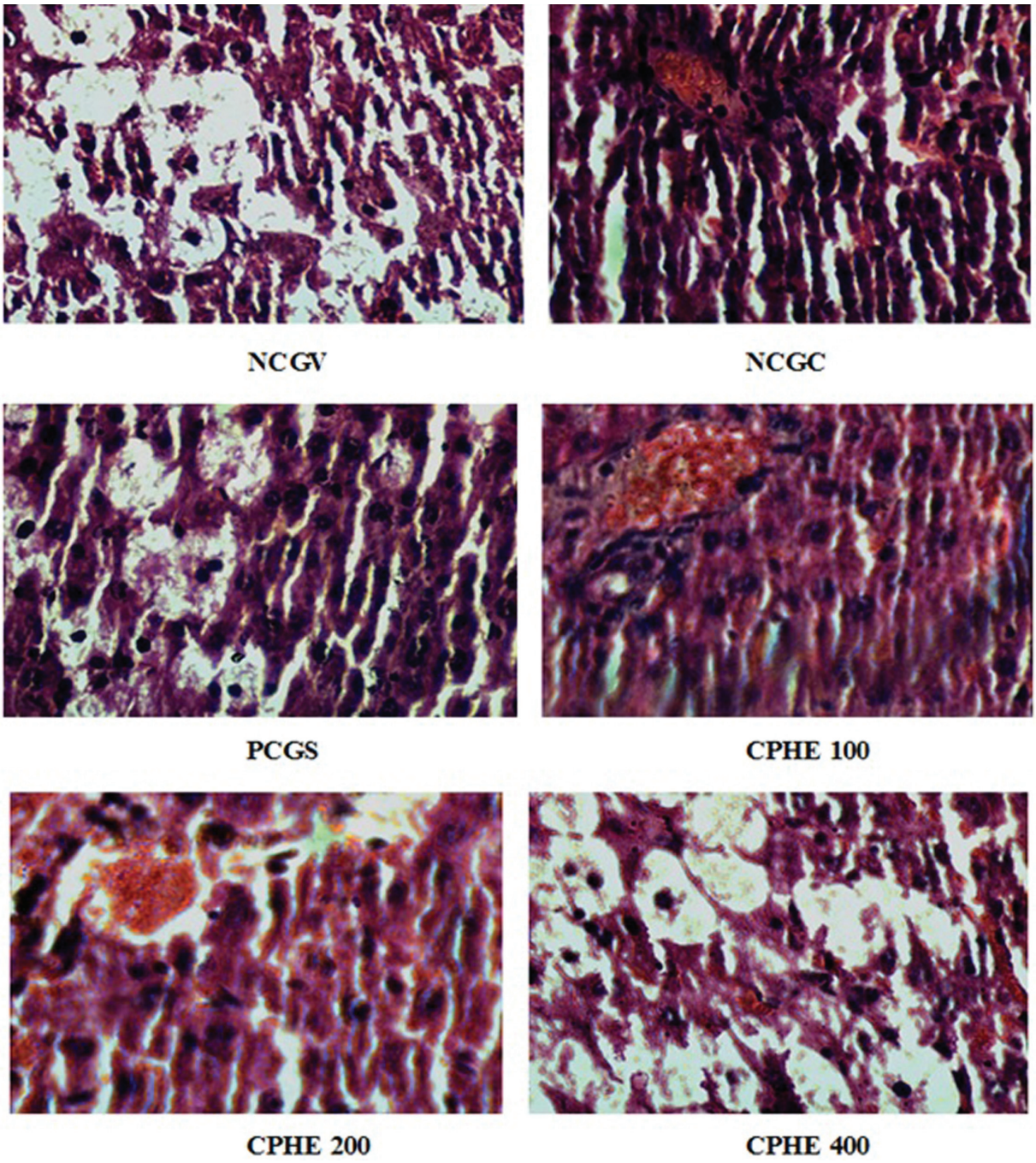
Discussion

The results of the biochemical parameters for the acute study for the rats in the NCGC group showed significantly higher levels of SGOT, SGPT, total bilirubin, and direct bilirubin levels, which clearly indicates CCl_4-induced hepatotoxicity and liver injuries in these rats. CCl_4 is metabolized by cytochrome pigment 450 in the endoplasmic reticulum and mitochondria of the liver and forms CCl_3O, which is a reactive oxidative free radical, which initiates lipid peroxidation and causes liver damage. Within 24 h of administration of a single dose of CCl_4, centrilobular necrosis and fatty changes occur in the liver. This injury in hepatocyte cells of the liver leads to the release of SGOT, SGPT, and bilirubin in plasma. The high levels of the above biochemical parameters are clearly signs of hepatotoxicity and liver injuries. Therefore, the results of the biochemical parameters of the acute study in rats clearly indicate that the acute model of CCl_4-induced hepatotoxicity is a successful model of hepatotoxicity and can be used for screening of hepatoprotective drugs.

Seven days pretreatment of silymarin (25 mg/kg) significantly restored SGOT, SGPT, total bilirubin, and direct bilirubin levels in CCl_4-induced hepatotoxic rats in comparison with the NCGC group of rats. Administration of the hydroalcoholic extract of CPHE (200 and 400 mg/kg) restored SGOT, SGPT, total bilirubin, and direct bilirubin levels in CCl_4-induced hepatotoxic rats and stabilized normal functions of the liver in the acute study. These results suggest that the extract protected the membrane integrity of the liver cells against CCl_4-induced leakage of serum enzymes into the circulation. Also, the CPHE 400 group of rats showed equivalent effects to silymarin. Therefore, in this study, the CPHE 400 extract of leaves of *C. pareira* was found to provide more effective protection against CCl_4-induced hepatotoxicity.

The histopathological examination clearly reveals that the hepatic cells, central vein, and sinusoids are almost normal in the CPHE 400-treated and silymarin-treated groups of rats in contrast to the NCGC group of rats. The study showed that CPHE 400 and silymarin showed a significant protective effect against CCl_4-induced hepatic injury in both acute and chronic models of hepatotoxicity, as evidenced from the results of biochemical parameters and histopathological examination. Thus, CPHE 400 can be considered an effective hepatoprotective agent that not only

Figure 2



Histopathological sections of the liver of rats of various treatment groups against CCl_4-induced chronic hepatotoxicity in various treated groups, magnification (hematoxylin and eosin, ×40).

ameliorates the damage caused by CCl_4-induced hepatotoxicity but also normalizes hepatic functions.

It can be hypothesized that CPHE treatment confers hepatoprotection by the following proposed mode of action: (a) stabilization of plasma membranes by maintaining the membrane integrity of the hepatic cells against CCl_4-induced leakage of serum enzymes, as observed from the decline of biochemical parameters (SGOT, SGPT, and bilirubin) in the CPHE 400-treated group of rats both in acute and chronic models of hepatotoxicity. (b) Repair of hepatic tissue against CCl_4-induced hepatotoxicity as observed in the histopathological section of the liver of the CPHE 400-treated group both in acute and chronic models of hepatotoxicity. (c) A previous scientific study has shown demonstrable beneficial effects of *C. pareira* root extract as an antioxidant; therefore, root extract of *C. pareira* exhibited free radical-scavenging activity that could lead to a decrease in the severity of oxidative damage in the liver [16].

Conclusion

Based on the results, it can be concluded that CPHE showed significant hepatoprotective potential in a dose-dependent manner, against CCl_4-induced acute and chronic hepatotoxicity in experimental rats. CPHE 400 mg/kg showed the most prominent hepatoprotective effect, equivalent to silymarin (25 mg/kg). Therefore, *C. pareira* may be used as a hepatoprotective agent against exogenously administered drugs. However, detailed investigation is required to isolate the active principle responsible for hepatoprotection.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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