



Hepatoprotective and anti-inflammatory potential of chebulagic acid on carbon tetrachloride–induced hepatic fibrosis by antioxidative activities in rats

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Abstract

The aim of the present study was to evaluate the hepatoprotective, antioxidant, and anti-inflammatory potential of chebulagic acid on carbon tetrachloride–induced hepatic fibrosis in rats. The liver was damaged in the rats using CCL4 (2 ml/kg b.w dissolved in 20% corn oil) intragastrically twice/week for 8 weeks. After 8 weeks, activities of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and lactate dehydrogenase, lipid peroxidation markers, and enzymic and non-enzymic antioxidant enzymes, inflammatory and anti-inflammatory cytokines were analyzed and all these parameters were altered. Chebulagic acid administration reversed all the altered parameters to near-normal levels. Improved histological and immunohistochemical observations liver supported the biochemical investigated. Hence, chebulagic acid exhibits hepatoprotective effects against CCL4-induced liver damage.

Keywords CCL4 · Liver fibrosis · Chebulagic acid · Inflammation · Antioxidant

Introduction

The liver is one of the most important organs that play crucial roles in the physiological functions of our body. In the human body, the liver is the site of the regulation of glycogen storage, decomposition of RBCs, hormone and plasma protein production, and detoxification. Since the liver also plays a central role in detoxifying and transforming chemicals, it

is in a way exposed to their harmful effects increasing its susceptibility to diseases. Therefore, it may not be surprising that over 10% of the world's population suffers from liver diseases. The most common of these conditions are hepatitis, hepatic steatosis (fatty liver), fibrosis, cirrhosis, alcoholic, and drug-induced diseases (Latief and Ahmad 2018).

Hepatic fibrosis is a common pathological process resulting from various chronic hepatic injuries, which is characterized by an increase of extracellular matrix deposition in Disse's space and the imbalance between synthesis and degradation of the extracellular matrix. Accumulating evidence suggests that hepatic fibrosis is a reversible disease; therefore, an effective treatment would probably prevent or reverse the hepatic fibrotic process (Iredale 2008). In recent years, considerably clinical and experimental pieces of evidence show that oxidative stress caused by an imbalance between the oxidant and antioxidant systems of the body in favor of the oxidants should be a major apoptotic stimulus in different types of acute and chronic liver injury and hepatic fibrosis (Ghatak et al. 2011). Although early-phase liver fibrosis is considered, a reversible pathological process, late-stage liver fibrosis will deteriorate to cirrhosis, portal hypertension, or even hepatocellular carcinoma which leads to increased morbidity and mortality.

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CCl4 is a potent hepatotoxin producing centrilobular hepatic necrosis which is widely used for animal models of liver fibrosis. Hepatic fibrosis induced by CCl4 is associated with the exacerbation of lipid peroxidation and the depletion of antioxidant status (Fu et al. 2008). Accordingly, a number of herbal derivatives show protective effects of their natural antioxidants against hepatic fibrosis and hepatotoxicity either experimentally in cell culture (in vitro), in animals models (in vivo) or even in clinical trials (Frei and Higdon 2003).

Fruits of *Terminalia chebula* have been used in various Ayurvedic preparations for the treatment of various disorders and its fruit powder is one of the main constituents of Triphala, a well-known Ayurvedic medicine used to treat allergies and common health disorders. Chebulagic acid is one of the main bio-active constituents of *Terminalia chebula* fruit powder. Chebulagic acid has been shown to inhibit α -glucosidase activity (Gao et al. 2007). ROS generation from PMA (Phorbol 12-myristate 13-acetate)-stimulated leukocytes (Kinoshita et al. 2007) and CTL-mediated cytotoxicity (Hamada et al. 1997). In addition, it has been reported to suppress arthritis in mice (Lee et al. 2005) and LPS-induced nitric oxide (NO) generation in RAW 264.7 mouse macrophage cells (Murakami et al. 2005). However, the detailed molecular anti-inflammatory mechanism has not yet been studied. In the present study, we show for the first time that chebulagic acid inhibits NF- κ B activity and phosphorylation of MAP kinases in LPS-stimulated RAW 264.7 macrophages. Recently, chebulagic acid has been reported to have a gastroprotective effect on ethanol-induced gastric injury in rats (Liu et al. 2017). Scientific reports on the effect of chebulagic acid on CCl4-induced hepatic injury in rats are scarce. Therefore, the present study was designed to investigate the hepatoprotective and anti-inflammatory potential of chebulagic acid on carbon tetrachloride-induced hepatic fibrosis by antioxidative activities in rats.

Materials and methods

Chemicals

Carbon tetrachloride (CCL4) and silymarin were purchased from Sigma Chemical Company (St. Louis, MO, USA). Chebulagic acid was procured from Chem Faces, China. All other chemicals are analytical grade procured from SRL.

Experimental animals

Adult Male albino Wistar rats weighing about 200–220 g were obtained from Sri Muthukumaran Medical College Hospital & Research Institute, Mangadu, Chennai, Tamil

Nadu, India. Rats were housed in clean, sterile, and polypropylene cages under standard vivarium conditions 12-h light/12-h dark cycle and constant temperature (25 ± 2 °C) with free access to standard commercial rat chow (Pranav Agro Industries Ltd., Pune, Maharashtra, India) and water. The experimental protocol was approved by the Ministry of Social Justice and Empowerment, Government of India, and Institutional Animal Ethics Committee Guidelines (IAEC No: No. 12/09/2016).

Experimental design

A total of 42 rats (30 CCL4-induced hepatic injured rats and 12 normal rats) were used and experimental animals were divided into 7 groups, each group consists of a minimum of six rats ($n = 6$). Before the start of the experiment, randomly selected animals were tested for liver function. Animals with a normal range of liver function test (LFT) parameters were selected for experimentation and details are given below.

Group I	Normal control rats received 1 ml of double-distilled water
Group II	Drug control (normal healthy control rats received intragastrically chebulagic acid (80 mg/kg b.wt/day) dissolved in 1 ml of corn oil for 8 weeks
Group III	CCL4-induced liver injured control rats (rats received intragastrically 2 ml/kg b.wt of CCL4 dissolved in 20% corn oil twice/week for 8 weeks
Group IV	CCL4-induced liver injured rats received intragastrically chebulagic acid (20 mg/kg b.wt/day) dissolved in 1 ml of corn oil for 8 weeks
Group V	CCL4-induced liver injured rats received intragastrically chebulagic acid 40 mg/kg b.wt/day dissolved in 1 ml of corn oil for 8 weeks
Group VI	CCL4-induced liver injured rats received intragastrically chebulagic acid (80 mg/kg b.wt/day) dissolved in 1 ml of corn oil for 8 weeks
Group VII	CCL4-induced liver injured rats received intragastrically silymarin (200 mg/kg b.wt/day) dissolved in 1 ml of corn oil for 8 weeks

Sample collection

After experimental periods, the animals were deprived of food overnight and sacrificed by cervical decapitation. The blood was collected in two different tubes, i.e., one without ethylenediaminetetraacetic acid (EDTA) for estimation of serum liver function test and another with ethylenediaminetetraacetic acid (EDTA) for the estimation of interleukins. Liver tissues were excised immediately and rinsed in ice-chilled normal saline to remove the blood. The known weights of the tissues were minced and homogenized in 5 ml of 0.1 M Tris–HCl buffer (pH 7.4) in ice-cold condition. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

Biochemical analysis

The collected blood was allowed to clot and serum was separated by centrifugation at 3000 rpm for 15 min and the serum enzymes, namely aspartate aminotransferase (AST), serum glutamate pyruvate transaminase (ALT), serum alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), were assessed by commercial kit methods. Lipid peroxidation and hydroperoxides were estimated in liver tissues by the method of Niehius and Samuelsson (1968) and Jiang et al. (1992) respectively. Catalase (CAT) superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST), ascorbic acid (vitamin C), α -tocopherol (vitamin E), GSH, and protein were determined by the method of Sinha (1972). Stringer et al. (1989), Rotruck et al. (1973), Habig et al. (1974), Omaye et al. (1979), Baker et al. (1980), Ellman (1959), and Lowry et al. (1951) respectively.

Histopathological studies

A portion of liver tissue in each group of rats was selected and fixed in 10% formalin. The liver tissues were fixed in paraffin embedding. Thin Sects. (5 μ m) were cut and stained with hematoxylin and eosin and observed the changes in the liver architecture under a microscope.

Determination of inflammatory cytokines

Plasma concentrations of interleukin 1 β , interleukin 6 (IL6), interleukin 10 (IL10), tumor necrosis factor- α (TNF- α), and NF- κ B p65 were determined using commercial rat ELISA kits (Ray Biotech, Inc., Norcross, GA, USA), following the instructions in respective kit manuals. The concentration of proinflammatory cytokines was determined spectrophotometrically at 450 nm. Standard plots were constructed using standard cytokines and the concentrations for unknown samples were calculated from the standard plot.

Immunohistochemical observation of NF- κ B and TNF- α in hepatic tissue

Immunohistochemical analysis was carried out according to the method of Sambrook et al. (1989) with slight modification. The hepatic tissue sections were deparaffinized in xylene and rehydrated in ethanol series (100, 90, 70, 50, and 30%). After washing with phosphate-buffered saline (PBS: 50 mM mono- and diphosphate, 100 mM NaCl, pH 7.4), the slides were incubated with 3% H₂O₂ at room temperature for 15 min to quench endogenous peroxidase activity. The slides were incubated with citrate buffer at 60 °C for 15 min for antigen retrieval. Then, the slides were incubated with blocking buffer 3% BSA for 3 h at room temperature, and then, the slides were incubated overnight at 4 °C with monoclonal anti-NF- κ B and TNF- α (dilution 1:200 and 1:200, Santa Cruz, CA, USA). Subsequently, the sections were incubated with secondary antibodies (anti-rabbit and anti-mouse) for 2 h at 4 °C and then washed with PBS (phosphate-buffered saline). The slides were incubated with DAB solution (3,3-diaminobenzidine tetrahydrochloride 0.05%, 1X PBS-10 ml, and H₂O₂ 0.01%). Then, the sections were dehydrated with ethanol series (30, 50, 70, 90, and 100%). Finally, the sections were counter-stained with hematoxylin and mounted with DPX (distyrene, a plasticizer, and xylene) and photographs were taken using a Nikon microscope. Histological changes in the stained sections were viewed by a pathologist.

Statistical analysis

The results are expressed as mean \pm SD. Differences between groups were assessed by ANOVA using the SPSS software package for windows. Post hoc testing was performed for inter-group comparisons using the least significant difference (LSD). *P*-values < 0.05 were considered as significantly altered.

Results

Liver function and liver cell necrosis

The activities of liver function indices such as aspartate transaminase, alanine transaminase alkaline phosphatase, and cell necrosis indicator lactate dehydrogenase were significantly elevated in ccl4 induced rats compared to control rats. However, administration of different doses of (20, 40, and 80 mg/kg b.w) chebulagic acid to ccl4 induced rats significantly reduced the abovementioned parameters to near normal compared to untreated ccl4 induced rats. Among the three doses, a pronounced effect was found at

Table 1 Dose-dependent effect of chebulagic acid on liver marker enzymes in control and experimental animals

Group	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	LDH (IU/l)
Control	32.95 ± 2.65	36.05 ± 3.54	129.93 ± 8.74	82.20 ± 7.50
Normal + chebulagic acid (80 mg/kg b.wt)	34.11 ± 2.73	38.70 ± 3.14	134.36 ± 8.22	85.25 ± 7.35
CCL4 induced	288.60 ± 19.13c	331.67 ± 24.39c	458.13 ± 24.17c	179.59 ± 14.04
CCL4 induced + chebulagic acid (20 mg/kg b.wt)	229.59 ± 18.59d	272.98 ± 16.89d	318.34 ± 21.22d	135.20 ± 10.58
CCL4 induced + chebulagic acid (40 mg/kg b.wt)	149.32 ± 16.88e	170.45 ± 13.64e	204.27 ± 17.17e	105.79 ± 9.74
CCL4 induced + chebulagic acid (80 mg/kg b.wt)	90.26 ± 12.24	104.23 ± 10.09	165.82 ± 10.29	92.07 ± 5.27
CCL4 induced + silymarin (200 mg/kg b.wt)	87.42 ± 12.11	82.88 ± 8.67	160.41 ± 8.25	87.86 ± 4.79

Notes: Values are given as mean ± SD for six animals in each group. Values are considered significantly different at $P < 0.05$ with the post hoc LSD test * $P < 0.05$

^aControl vs. drug control (chebulagic acid alone treated control rats)

^bControl rats vs. CCL4 induced rats

^cCCL4 induced rats vs. chebulagic acid (20 mg/kg b.wt)

^dCCL4 induced rats vs. chebulagic acid (40 mg/kg b.wt)

^eCCL4 induced rats vs. chebulagic acid (800 mg/kg b.wt)

^fChebulagic acid–treated CCL4 rats vs. silymarin-treated diabetic rats

80 mg/kg b.w and that effect was comparable to that of silymarin—a hepatoprotective agent, used as a reference drug. Therefore, 80 mg/kg body weight was fixed as an effective dose and used for further analysis. Normal rats treated with chebulagic acid at a dose of 80 mg/kg b.w did not show any significant changes in the above-tested parameters (Table 1).

Measurement of lipid peroxidation markers

Table 2 shows the levels of TBARS and HP in the liver of control and experimental rats. CCL4 induced rats showed increased levels of TBARS and HP when compared to normal control rats. Oral administration of chebulagic acid and silymarin to ccl4 induced rats significantly decreased lipid and hydroperoxides in the liver. In addition, no significant

alternation in chebulagic acid–treated normal rats was found with respect to these lipid peroxidation markers.

Enzymic and non-enzymic antioxidant enzymes

Figure 1 and Table 3 illustrate the activities of enzymatic antioxidants (SOD, CAT, GPx, GST, and GR) and non-enzymatic antioxidants (vitamin C, vitamin E, and GSH) in the liver of control and ccl4 induced rats. The levels of enzymatic and non-enzymatic antioxidant were significantly decreased in ccl4 induced diabetic rats when compared to control rats. However, chebulagic acid and silymarin supplements restore the level of these enzymes to near-normal levels. These results indicate that chebulagic acid possesses antioxidant potential against ccl4-induced oxidative stress. No significant differences were observed in normal rats treated with chebulagic acid alone.

Table 2 Effect of chebulagic acid on the levels of TBARS and HP in the tissues of control and experimental rats

Group	TBARS (mM/100 g tissue)	Hydroperoxides (mM/100 g tissue)
Control	0.79 ± 0.07	65.08 ± 5.78
Normal + chebulagic acid (80 mg/kg b.wt)	0.87 ± 0.08	61.98 ± 5.30
CCL4 induced	2.36 ± 0.20 ^b	133.13 ± 8.34 ^b
CCL4 induced + chebulagic acid (80 mg/kg b.wt)	1.31 ± 0.12 ^c	73.72 ± 4.70 ^c
CCL4 induced + silymarin (200 mg/kg b.wt)	1.14 ± 0.13	70.50 ± 5.51

Notes: Values are given as mean ± SD for six animals in each group. Values are considered significantly different at $P < 0.05$ with the post hoc LSD test * $P < 0.05$

^aControl vs. drug control (chebulagic acid alone treated control rats)

^bControl rats vs. CCL4 induced rats

^cCCL4 induced rats vs. chebulagic acid

^dChebulagic acid–treated CCL4 rats vs. silymarin-treated diabetic rats

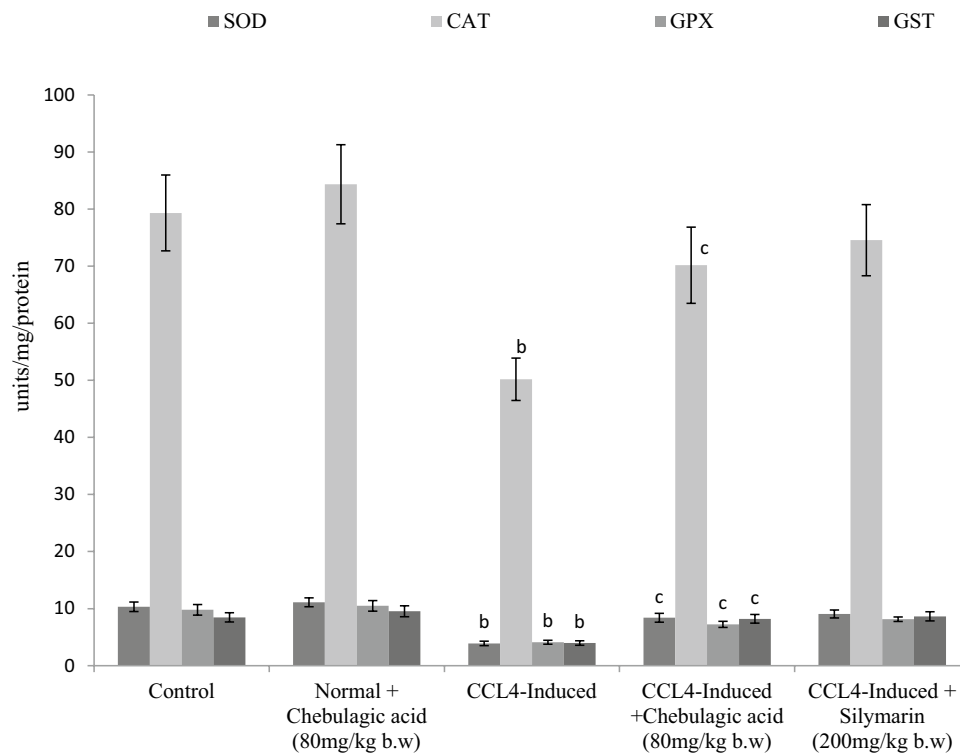


Fig. 1 Effect of chebulagic acid on the levels of hepatic antioxidant enzyme activities in control and experimental animals. Notes: Values are given as mean ± SD for six animals in each group. Values are considered significantly different at $P < 0.05$ with the post hoc LSD test * $P < 0.05$. (a) Control vs. drug control (chebulagic acid alone treated control rats). (b) Control rats vs. CCL4 induced rats. (c) CCL4 induced rats vs. chebulagic acid. (d) chebulagic acid–treated CCL4

rats vs. silymarin-treated diabetic rats. The activities of enzymes are expressed as follows: SOD, one unit of activity was taken as the enzyme quantity which gave 50% inhibition of nitroblue tetrazolium reduction in 1 min/mg protein; CAT, μ moles of H_2O_2 consumed/minute; GPx, μ g of glutathione consumed/minute/mg protein; GST, μ moles of 1-chloro 2,4-dinitrobenzene-GSH conjugate formed/minute/mg protein

The levels of inflammatory cytokines

The levels of inflammatory cytokines such as TNF- α , IL-1 β , IL-6, and NF- κ B p65 were significantly increased, whereas the levels of anti-inflammatory cytokine IL-10 were significantly decreased in ccl4 induced rats when compared with

control rats. However, oral administration of chebulagic acid and silymarin to ccl4 induced rats significantly declined these levels to near normalcy. No significant deviation was observed in the control rats treated with chebulagic acid alone (Fig. 2).

Table 3 Effect of chebulagic acid on the levels of non-enzymatic antioxidants in tissues of control and experimental rats

Group	Vitamin C (μ M/mg tissue)	Vitamin E (μ M/mg tissue)	Reduced glutathione (GSH) (μ M/mg tissue)
Control	4.00 ± 0.34	2.32 ± 0.19	60.09 ± 4.36
Normal + chebulagic acid (80 mg/kg b.wt)	4.43 ± 0.43	2.52 ± 0.22	61.17 ± 4.72
CCL4 induced	1.79 ± 0.10	0.91 ± 0.07	35.99 ± 3.09
CCL4 induced + chebulagic acid (80 mg/kg b.wt)	2.98 ± 0.14	1.88 ± 0.11	47.86 ± 4.34
CCL4 induced + silymarin (200 mg/kg b.wt)	3.27 ± 0.27	2.00 ± 0.15	50.71 ± 4.01

Notes: Values are given as mean ± SD for six animals in each group. Values are considered significantly different at $P < 0.05$ with the post hoc LSD test * $P < 0.05$

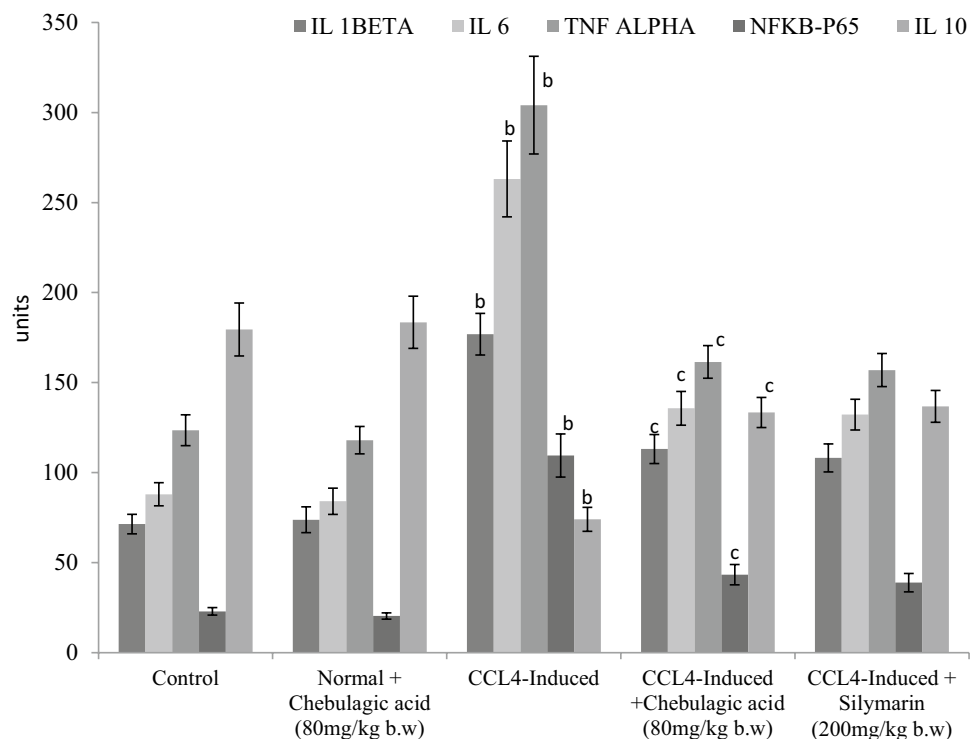
^aControl vs. drug control (chebulagic acid alone treated control rats)

^bControl rats vs. CCL4 induced rats

^cCCL4 induced rats vs. chebulagic acid

^dChebulagic acid–treated CCL4 rats vs. silymarin-treated diabetic rats

Fig. 2 Effect of chebulagic acid on the levels of IL-1 β , IL-6, TNF- α , p65 subunit of NF- κ B, and IL-10 in the plasma of control and experimental animals. Notes: Values are given as mean \pm SD for six animals in each group. Values are considered significantly different at $P < 0.05$ with the post hoc LSD test * $P < 0.05$. Units: TNF- α pg/ml, NF-KB p65-ng/ml, IL1 β IL-6, and IL10-pg/ml. (a) Control vs. drug control (chebulagic acid alone treated control rats). (b) Control rats vs. CCL4 induced rats. (c) CCL4 induced rats vs. chebulagic acid. (d) Chebulagic acid-treated CCL4 rats vs. silymarin-treated diabetic rats



Immunohistochemical expression of NF- κ B in the liver

The hepatic immunohistochemical expression of NF- κ B in control and experimental rats is shown in Figure 3. There was no nuclear signal or immune-reactivity for NF- κ B in the liver of control rats (Fig. 3A) and those treated with chebulagic acid alone (Fig. 3B), while the liver of rats administered with ccl4 showed higher immunostaining of NF- κ B, especially around the portal area, hepatocytes, and non-parenchymal cells (Fig. 3C). Treatment with chebulagic acid and silymarin showed no nuclear signal or no immunostaining of NF- κ B in the liver of ccl4 induced rats (Fig. 3D and E).

Immunohistochemical expression of TNF- α in the liver

The hepatic immunohistochemical expression of TNF- α in control and experimental rats showed in Fig. 4. The proinflammatory cytokine TNF- α expression was increased in the liver of ccl4 induced rats (Fig. 4C) when compared to control (Fig. 4A) and those treated with chebulagic acid alone (Fig. 4B). Chebulagic acid and silymarin-treated ccl4 induced rats showed diminished expression of TNF- α (Fig. 4D and E).

Liver histopathological examination

Changes in liver architecture revealed by H&E staining are illustrated in Fig. 1. The control and drug control rats

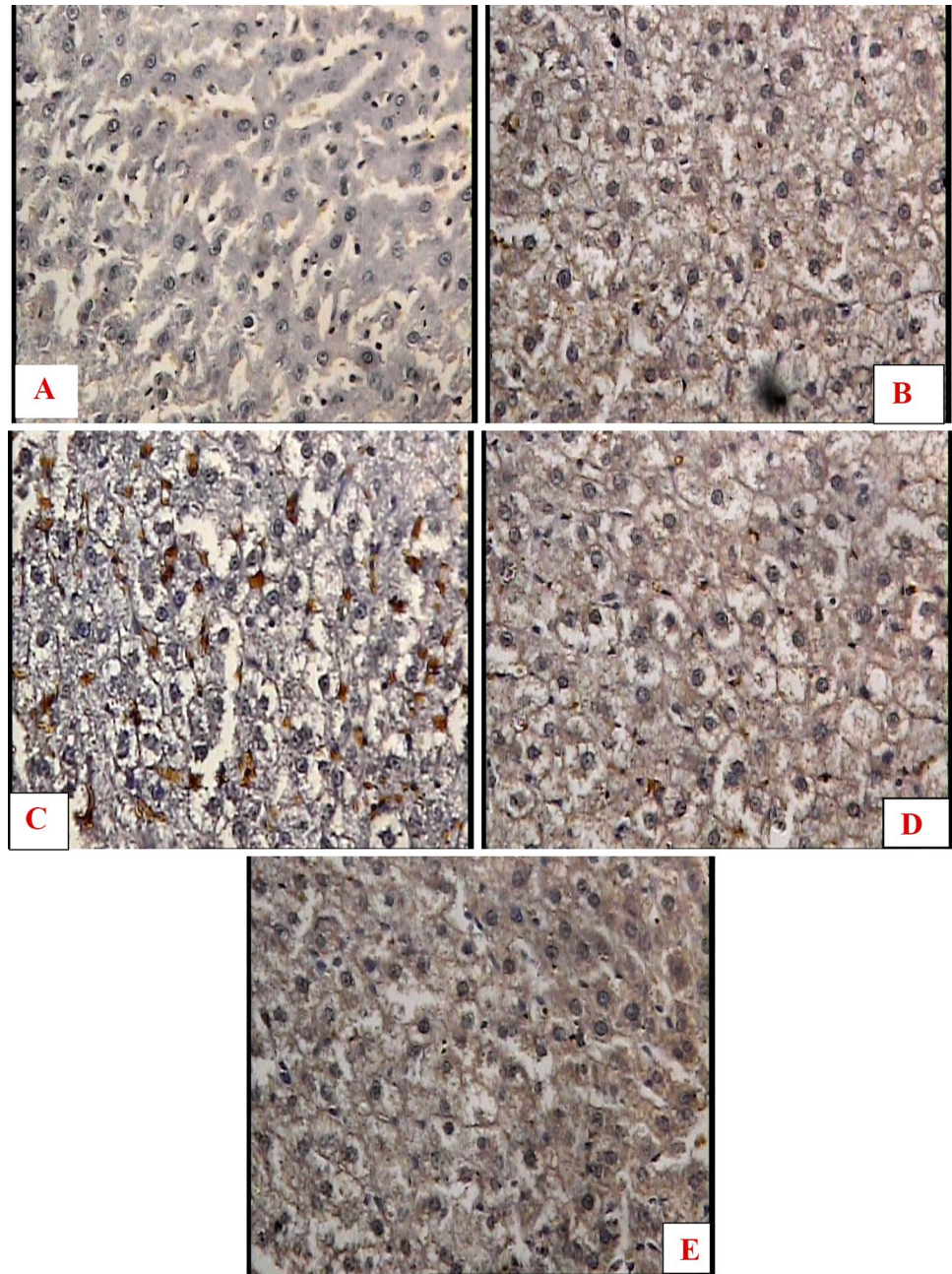
confirmed perfect lobular architecture with intact liver parenchyma and absence of inflammation or any damage (Fig. 5A and B). However, ccl4 induced rats showed hepatocellular necrosis, massive fatty infiltration, and inflammatory cellular infiltration along with disrupted hepatic cords (Fig. 5C). Histopathological disruptions observed in ccl4 induced rats were significantly reduced by the supplementation of chebulagic acid and silymarin (Fig. 5D and E).

Discussion

CCl4 is a widely used industrial solvent and it is the best-characterized animal model of xenobiotic-induced and oxidative stress-mediated hepatotoxicity (Ogeturk et al. 2005; Jaramillo-Juarez et al. 2008). The degree of hepatic damage is assessed by histological and biochemical parameters in circulation. In the present study, the levels of aspartate transaminase, alanine transaminase, alkaline phosphatase, and lactate dehydrogenase were significantly increased in ccl4 induced rats and these elevated enzymes levels confirm the hepatic damage/injury and hepatic cells necrosis caused by ccl4. However, those enzymes levels were found to be decreased to near normal in ccl4 induced rats upon treatment with chebulagic acid and silymarin.

Lipid peroxidation is an important parameter of oxidative stress. This process may cause peroxidative tissue damage in inflammation, cancer and toxicity of xenobiotics, and aging (Jalali Ghassam et al. 2014). Malondialdehyde is a

Fig. 3 Effect of chebulagic acid on the expression of NF- κ B in the liver of control and experimental rats (40 \times). Control (A), normal + chebulagic acid (B), CCL4 induced (C), CCL4 induced + chebulagic acid (D), CCL4 induced + silymarin (E)

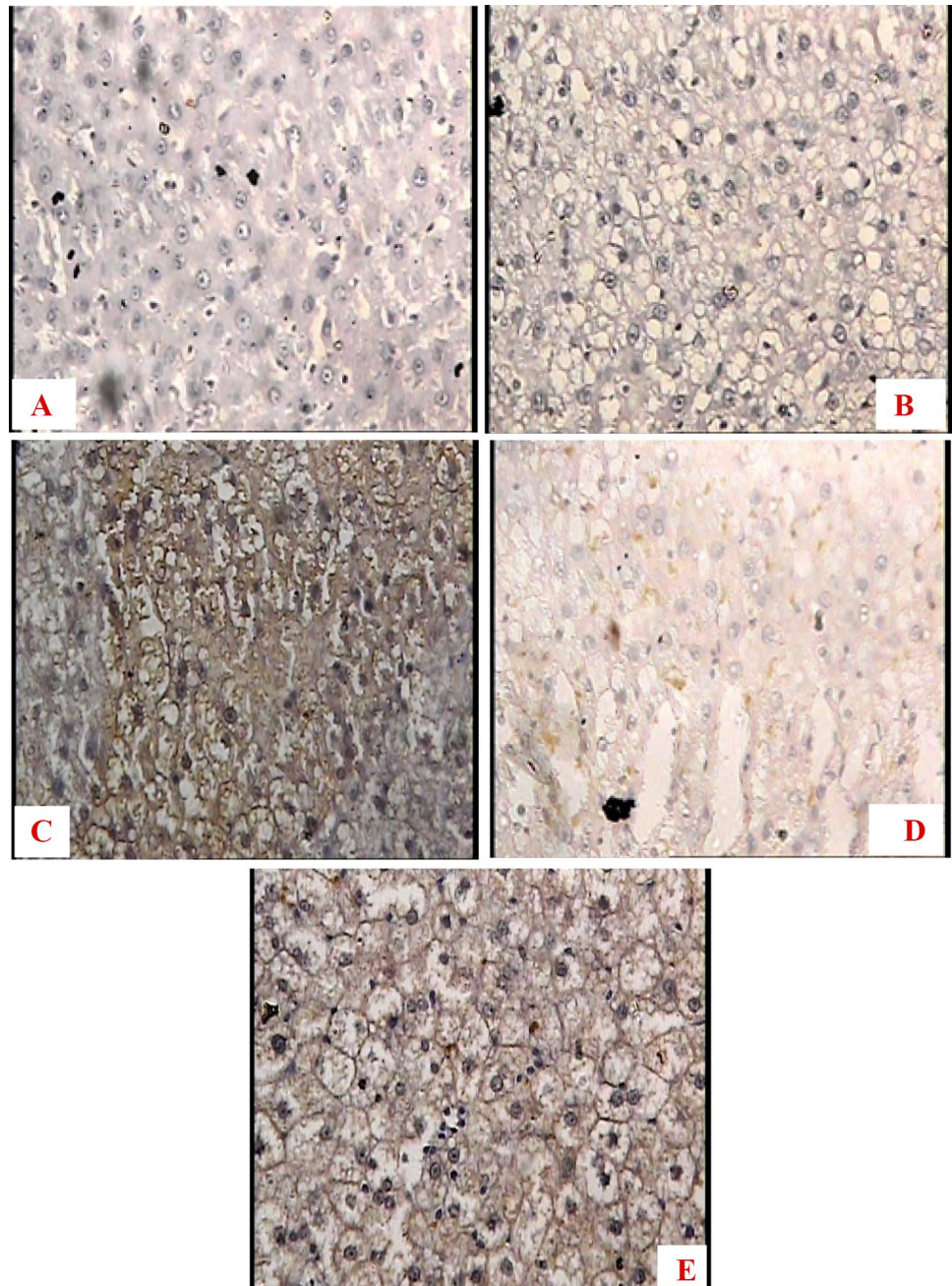


cytotoxic product that is a hallmark of lipid peroxidation. Elevated lipid peroxidation is responsible for the formation of lipid hydroperoxides. In our study, the levels of TBARS and HP are found to be higher in ccl4 induced rats which indicate membrane damage, alterations in structure and function of cellular membranes and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals. These results are in agreement with the previous study (Elgawish et al. 2015). Oral administration of chebulagic acid and silymarin to ccl4 induced rats significantly decreased levels of TBARS and HP to near normal. These

results indicate the antioxidant potential of chebulagic acid which protects the liver against lipid peroxidation.

Contrary to the levels of lipid peroxide markers, the levels of enzymic antioxidant (SOD, CAT, GPx, GST, and GR) and non-enzymic antioxidant (vitamin C, vitamin E, and GSH) were significantly declined. These results are consistent with a previous study that demonstrated that ccl4 treatment enhanced hepatic fibrosis which is associated with the exacerbation of lipid peroxidation and the depletion of antioxidant status (Chen et al. 2013). These changes were reversed upon treatment with chebulagic acid and silymarin.

Fig. 4 Effect of chebulagic acid on the expression of TNF- α in the liver of control and experimental rats (40 \times). Control (A), normal + chebulagic acid (B), CCL4 induced (C), CCL4 induced + chebulagic acid (D), CCL4 induced + silymarin (E)

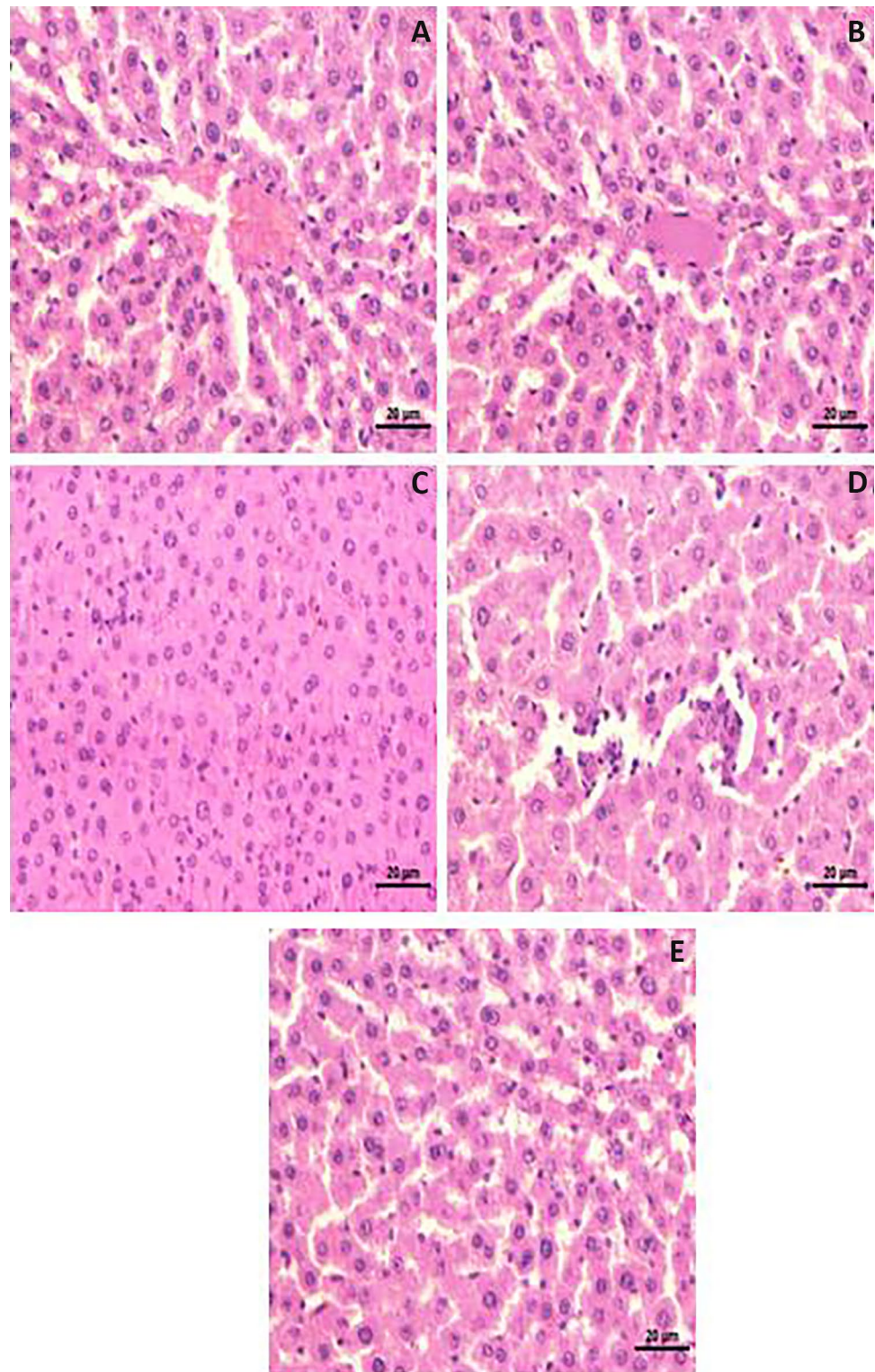


CCl4 induces the production of reactive oxygen species (ROS), thereby causing liver injury which is confirmed by elevated levels of lipid peroxidation markers (lipid peroxidation and hydroperoxides) in this study. The reactive oxygen species induce inflammation by upregulating the expression of inflammatory mediators. Among those, the inflammatory response is mediated by the action of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α , which are involved in the chemical-induced hepatotoxic process causes infiltration of neutrophils and monocytes into the damaged organ (Zhang et al. 2004; Novo and

Parola 2008; Weber et al. 2003). In our study, we observed that oral administration of chebulagic acid and silymarin to CCl4 induced rats prevented the increased plasma level of inflammatory cytokines (IL-1 β , IL-6, TNF- α , and NF- κ B) with an elevation of anti-inflammatory cytokine (IL-10). In addition, the immunohistochemical studies show that the elevated levels of TNF- α and NF- κ B were diminished in the liver of CCl4 induced rats after treatment with chebulagic acid and silymarin.

These results indicated that chebulagic acid was able to improve CCl4-induced hepatotoxicity by suppressing

Fig. 5 Effect of chebulagic acid on the changes of histological morphology of rat liver in control and experimental rats by hematoxylin and eosin (H&E) staining, magnification 40 \times . Control (A), normal + chebulagic acid (B), CCL4 induced (C), CCL4 induced + chebulagic acid (D), CCL4 induced + silymarin (E)



inflammatory response in rats. The anti-inflammatory properties of chebulagic acid are attributed to inhibition of NF- κ B, therefore, blocking the release of proinflammatory cytokines and amplification of inflammation via inhibition of downstream inflammatory mediators.

Similarly, a remarkable improvement was observed in the CCL4-induced histopathology of the liver after treatment with chebulagic acid and silymarin. These results are in agreement with the findings of previous studies (Elgawish et al. 2015; Liu et al. 2010). Altogether, our biochemical,

histological, and immunohistochemistry data clearly demonstrate that chebulagic acid exerts hepatoprotection against ccl4-induced hepatic damage in rats by its antioxidant and anti-inflammatory potential.

Conclusion

Results of the present study suggested that chebulagic acid has the hepatoprotective potential against CCl₄-induced hepatic injury through preventing oxidative stress and elevations of the inflammatory cytokines, keeping them at near control values.

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Declarations

Ethical approval The experimental protocol was approved by the Ministry of Social Justices and Empowerment, Government of India, and Institutional Animal Ethics Committee Guidelines (IAEC No: No. 12/09/2016).

Informed consent All authors are consented to publish this manuscript in this journal.

Conflict of interest The authors declare no competing interests.

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