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Original article

Attenuation of oxidative damage-associated hepatotoxicity by piperine in CCl₄-induced liver fibrosis

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ABSTRACT

Objectives: The present research has demonstrated the impact of treatment with piperine on hepatic fibrosis induced by the administration of carbon tetrachloride.**Methods:** In order to study the outcome of piperine treatment on hepatic fibrosis caused by carbon tetrachloride administration in male Wistar rats, a total of 24 rats were collected and randomly classified into 4 separate groups each consisting of 6 animals. Group I was assigned as a control group and treated with olive oil for 4 weeks (1 ml/kg, i.p.) with two doses each week. Group II was designated as a toxic group and treated with carbon tetrachloride for 4 weeks (1 ml/kg, 1:1 combination with olive oil, i.p.) with two hepatic fibrosis induction doses each week. Group III was graded as a group I therapy and co-treated with carbon tetrachloride (1 ml/kg, 1:1 combination with olive oil, i.p.) for 4 weeks with two hepatic fibrosis induction doses a week and with piperine (25 mg/kg) for 4 weeks. Group IV was designated as group II therapy and co-treated with carbon tetrachloride (1 ml/kg, 1:1 combination of olive oil, i.p.) for 4 weeks with two hepatic fibrosis induction doses a week and piperine (50 mg/kg) for 4 weeks.**Results:** Piperine significantly reduced the carbon tetrachloride augmented activities of liver enzymes, lipid peroxidation, glutathione, hydroxyproline, nitrite, and carbonyl levels. Carbon tetrachloride enhanced Total cholesterol (TC), Triglyceride (TG), Low density lipoprotein (LDL), and Very low density lipoprotein (VLDL) levels, and decreased High density lipoprotein (HDL) level was restored to normal with piperine treatment. Moreover, piperine inhibited the over-expression of pro-inflammatory biomarkers and α -SMA.**Conclusion:** The data of the current study demonstrated piperine as a potent hepato-protective agent and recommends the use of piperine for preventing hepatic fibrosis.© 2021 The Authors. Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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1. Introduction

Liver fibrosis is a remedial reaction produced against liver damage caused by its exposure to drugs or toxic substances (Duval et al., 2015). The essential pathological characteristic depicted in hepatic fibrosis is the excess deposition of extracellular matrix (Tacke and Trautwein, 2015). Liver fibrosis can advance to produce more severe conditions such as cirrhosis, portal hypertension, and even hepato-cellular malignancy that resulted in higher rate of disease and death (Branchi et al., 2014).

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Carbon tetrachloride (CCl₄), a known toxic reagent that induces hepatic fibrosis and hepatic lesion, has been widely employed for hepatic studies (Huang et al., 1998). Liver damage is induced by the development of ROS and trichloromethyl free radicals triggered by liver cytochrome P450 2E1 (CYP2E1). Piperine inhibits NF-κB via activating Nrf2, blocking the down-stream inflammatory cytokines or mediators such as IL1β, IL6, TNFα, iNOS, Cox2, NO, PGE2, and MPO, stimulating anti-oxidant reaction machinery including CAT, GSH, GPx, SOD, GR, NQO1, and HO1, ROS scavenging, and reducing lipid per-oxidation (Rehman et al., 2020).

Piper nigrum contains around 2–8% of phytochemical piperine (Iahtisham-Ul et al., 2020). Piperine exhibits a broad array of biologic properties including anti-depressant, anti-anxiety, anti-thyroid, anti-tumor immune-stimulating, anti-inflammatory, and anti-metastatic (Pradeep and Kuttan, 2002). This alkaloid also stimulates the hepato-protection against the toxicity induced by CCl₄ and enhances the bioavailability of numerous drugs exhibiting structural and therapeutic diversity. In the current write-up, we will study the effectiveness of piperine for attenuating the oxidative damage associated with hepatotoxicity in CCl₄-induced liver fibrosis.

2. Materials and methods

2.1. Chemicals

CCl₄, bovine serum albumin (BSA), Piperine, reduced glutathione (GSH), 4-hydroxy-L-proline, hexadecyltrimethylammonium, 5,5-dithio-bis (2-nitrobenzoic acid) (Ellman's reagent), and tetramethylbenzidine. N-butanol, NADPH, EDTA, thiobarbituric acid (TBA), Tris Hcl, DNPH, CDNB, chloramine-T, trichloroacetic acid (TCA), NaOH, dL-Aspartate, FAD, PCA, Tween 20, DCPIP, α-ketoglutaric acid, NaCl, and sodium pyruvate were procured from Sigma-Aldrich (St. Louis, MO, USA). Piperine had a purity grade of 97%.

2.2. Animals

Male Wistar rats aged from 7 to 9 weeks with 180–220 g of body weight were employed in the current study. The rats were acquired from the Central Animal House Facility and housed at the standard temperature (25 ± 1 °C) and light/dark period (12/12 h) in the animal care facility. Normal food pellets and tap water were fed to these rats. Rat acclimatization was done for 7 days before treatment. All the tests have been carried out according to guidelines. The project has been accepted by the Institutional Board of Approval (No. RAKMHSU-REC-7-2017-F-P).

2.3. Treatment protocol and induction of hepatic fibrosis

In order to research the outcome of treatment with piperine (Sigma-Aldrich) for hepatic fibrosis caused by CCl₄ administration in male Wistar rats, a total of 24 Wistar rats were collected and randomly classified into 4 separate groups each containing 6 animals. Group I was assigned as a control group and given olive oil for 4 weeks (1 ml/kg, i.p.) with two doses each week. Group II was designated as a toxic group and treated with CCl₄ for 4 weeks (1 ml/kg, 1:1 combination with olive oil, i.p.) with two hepatic fibrosis induction doses each week. Group III was graded as the group I therapy and co-treated with CCl₄ (1 ml/kg, 1:1 combination with olive oil, i.p.) for 4 weeks with two hepatic fibrosis induction doses a week and with piperine (25 mg/kg) for 4 weeks. Group IV was designated as group II therapy and co-treated with CCl₄ (1 ml/kg, 1:1 combination with olive oil, i.p.) for 4 weeks with two doses each week for hepatic fibrosis induction, and piperine (50 mg/kg)

for 4 weeks with two doses per week for hepatic fibrosis induction. 12 h after the last CCl₄ injection, all the rats were euthanized under light ether anesthesia.

2.4. Determination of the liver enzymes functional tests

The hepatoprotective property of piperine was investigated by estimating total protein, albumin, bilirubin, ALP, ALT, and AST level in serum by diagnostics kits commercially available.

2.5. Assessment of catalase activity (CAT)

The enzymatic activity of the catalase was determined with the procedure described by Claiborne (1984).

2.6. Assessment of superoxide dismutase activity (SOD)

Super-oxide dismutase (SOD) activity was determined with the protocol described by Marklund and Marklund (1974).

2.7. Assessment of reduced glutathione (GSH)

The procedure described by Jollow et al. (1974) was used for estimating reduced glutathione levels.

2.8. Assessment of glutathione reductase activity (GR)

The activity of Glutathione reductase was determined with the protocol described by Carlberg and Mannervik (1975).

2.9. Assessment of glutathione peroxidase activity (GPx)

Glutathione peroxidase activity was measured by the modulus operandi of Mohandas et al., 1984.

2.10. Assessment of protein carbonyl

The assessment of protein carbonyl was done by following the modified version of the protocol of Levine et al. (1990) modified by Floor and Wetzel (1998).

2.11. Assessment of hepatic 4-hydroxyproline

The colorimetric quantification of hepatic 4-hydroxyproline was done by the chloramine-T technique of Lee et al. (2005) after minor modifications.

2.12. Assessment of malondialdehyde (MDA)

Lipid peroxidation (LPO) was performed with the protocol described by Wright et al. (1981).

2.13. Estimation of TNF-α, IL-6, TGF-β, and NFκ-B

TNF-α (Tumor necrosis factor α), IL-6 (Interleukin-6), TGF-β (Transforming growth factor β), and NFκ-B were determined by ELISA technique-based (enzyme-linked Immunosorbent assay) rat TNF-α, IL-6, TGF-β, and NFκ-B kits (Bioscience, Inc., San Diego, USA). Each of the pro-inflammatory cytokine was measured from colonic tissue samples with the help of ELISA. Sample preparation was done in phosphate-buffered saline (PBS) containing protease inhibitor cocktail and each sample was analyzed with the help of Elisa Plate Reader (Multiskan EX, Thermo) by following the manufacturer's instructions.

2.14. Assay for hepatic Immunohistochemistry

The assessment of α -smooth muscle actin was done by following the modified procedure of Hosoya et al. (2006).

2.15. Histopathological analysis of hepatic tissue

The wet hepatic tissues are fixed and dehydrated in a 10% neutral buffered solution. Following the prescribed histopathological investigation procedure, liver tissue samples were treated after fixation. For examination with hematoxylin and eosin (H&E) dye, a 5 μ m part of the liver was cut and dyed. In all groups, the pathological features of liver tissues were observed under the fluorescent microscope for histopathological improvement (Olympus Life Science, Europa GMBH, Wendenstrasse 14–18, 20,097 Hamburg, Germany).

2.16. Assessment of lipid profile

Serum samples were collected and analyzed for lipid profile. Triglyceride (TG), HDL-cholesterol, and Total cholesterol (TC) levels were estimated by kit (Genzyme Diagnostics). However, the estimation of LDL-cholesterol was done as the remaining difference of TC and HDL-cholesterol.

2.17. Statistical analysis

The individual group data were presented as mean \pm SEM. Mean \pm SEM was viewed as the individual community results. As statistical methods to evaluate disparities between groups, ANOVA (analysis of variance) and Tukey-Kramer multiple comparison tests were used. At $p < 0.05$, all the comparisons were considered to be statistically important.

3. Results

3.1. Effect of CCl₄ and piperine on the functions of liver enzymes

The CCl₄ treated Group II showed a remarkably elevated ($***p < 0.001$) level of ALT, AST, ALP, and Bilirubin whereas total protein and albumin declined in contrast with the control group. This depicts that CCl₄ can induce hepatotoxicity by enhancing the functions of liver enzymes. However, piperine treatment signif-

icantly decreases the functions of liver enzymes both at low-dose (25 mg/kg) in group III ($^{##}p < 0.01$) and at high-dose (50 mg/kg) in group IV ($^{###}p < 0.001$) as evident from Table 1.

3.2. Effect of piperine on glutathione and other antioxidant parameters

The effect of piperine treatment on CCl₄-induced reduction in anti-oxidant enzyme activities was investigated and the outcome is depicted in Table 2. The antioxidant enzymatic activities showed a considerable disparity ($***p < 0.001$) between groups I and II. With the piperine treatment, antioxidant enzymatic activities were re-established to normal.

3.3. Effect of CCl₄ and piperine on lipid profile

The lipid profile was significantly enhanced ($***p < 0.001$) except HDL that showed a reduction with CCl₄ treatment in comparison to control as illustrated in Table 3. The treatment with piperine at both doses (25 mg/kg & 50 mg/kg) decreases the lipid profile except HDL that showed an increasing trend.

3.4. Effect of piperine serum inflammatory cytokines levels

In CCl₄ treated group II the pro-inflammatory cytokine levels of TNF α , TGF β , NF- κ B, and IL6 depicted a considerable elevation ($***p < 0.001$) in contrast to the untreated group I. Nevertheless, piperine treatment in groups III and IV significantly reduced these cytokine levels near to normal at both dosages. A non-significant difference in these cytokines was observed between groups I and IV (Table 4).

3.5. Effect of piperine on CCl₄-induced MDA levels

The levels of oxidative stress biomarker MDA increased with the elevated oxidative stress. With the administration of CCl₄, MDA levels of group II depicted a sharp elevation than group I ($***p < 0.001$). The piperine treatment evidently decreased the aberrantly increased MDA levels of group III ($^{\#}p < 0.5$), and IV ($^{##}p < 0.01$) (Fig. 1).

Table 1
Effect of CCl₄ and Piperine on liver function enzymes in different experimental groups.

Treatment Groups	ALT (IU/L)	AST (IU/L)	Albumin (g/dl)	Bilirubin (mg/dl)	Total Protein (g/dl)	ALP (IU/L)
Group I	42.76 \pm 7.92	76.75 \pm 8.73	6.32 \pm 0.28	0.23 \pm 0.06	6.16 \pm 0.37	25.13 \pm 2.48
Group II	167.1 \pm 28.83 ^{***}	174.3 \pm 16.7 ^{***}	3.01 \pm 0.27 ^{**}	0.99 \pm 0.17 ^{***}	4.91 \pm 0.29 ^{ns}	44.32 \pm 4.87 ^{**}
Group III	123.1 \pm 14.03 [*]	110.9 \pm 9.14 [#]	4.93 \pm 0.19 [#]	0.69 \pm 0.10 [#]	5.32 \pm 0.32 ^{ns}	32.72 \pm 4.14 [#]
Group IV	70.93 \pm 7.86 ^{##}	95.1 \pm 9.31 ^{##}	5.92 \pm 0.61 ^{##}	0.32 \pm 0.10 ^{###}	6.26 \pm 0.33 [#]	29.12 \pm 2.32 ^{##}

Each value represents the mean of 6 rats \pm SE.

Table 2
Effect of Piperine on glutathione and other antioxidant parameters in CCl₄ induced liver fibrosis.

	Group I	Group II	Group III	Group IV
Reduced glutathione (GSH; nmol mg protein)	369.24 \pm 23.5	145.31 \pm 13.7 ^{***}	281.53 \pm 16.4 [#]	299.53 \pm 14.9 ^{##}
Oxidized glutathione (GSSG; nmol mg protein)	38.63 \pm 9.31	91.63 \pm 8.32 ^{***}	71.34 \pm 9.75 [#]	48.22 \pm 7.23 ^{###}
GSH/GSSG Ratio	9.551 \pm 1.52	1.580 \pm 0.19 ^{***}	3.946 \pm 0.73 [#]	6.211 \pm 0.86 ^{###}
GPx (nmol/min/mg protein)	219.57 \pm 24.5	97.32 \pm 9.15 ^{***}	146.73 \pm 11.2 [#]	165.12 \pm 14.7 ^{##}
GR (nmol min/min/mg protein)	233.75 \pm 21.7	101.43 \pm 12.3 ^{***}	174.72 \pm 18.1 ^{##}	214.22 \pm 21.8 ^{###}
SOD (units/min/mg protein)	14.531 \pm 1.63	6.533 \pm 0.91 ^{***}	8.559 \pm 0.79 [#]	11.158 \pm 1.77 ^{###}
Catalase (nmol H ₂ O ₂ consumed/min/mg protein)	8.54 \pm 0.97	4.11 \pm 0.19 ^{***}	6.21 \pm 0.54 [#]	7.95 \pm 0.65 ^{##}

Each value represents the mean of 6 rats \pm SE.

Table 3
Effect of CCl₄ and Piperine on lipid profile in experimental animals.

Treatment Groups	TC (IU/L)	HDL (IU/L)	LDL (g/dl)	TG (mg/dl)	VLDL (g/dl)
Group I	82.80 ± 14.7	29.43 ± 0.92	41.70 ± 3.45	89.10 ± 9.43	17.53 ± 3.32
Group II	162.10 ± 21.3***	17.23 ± 0.73***	98.32 ± 7.31***	163.3 ± 15.1***	39.22 ± 3.32***
Group III	123.14 ± 14.9##	21.28 ± 0.69#	67.33 ± 4.32##	101.4 ± 8.17##	26.04 ± 2.05##
Group IV	101.92 ± 9.41#	51.21 ± 0.41##	53.12 ± 4.03##	123.6 ± 9.31##	23.05 ± 2.19##

Each value represents the mean of 6 rats ± SE.

Table 4
Effect of Piperine on serum levels of inflammatory cytokines (TGF-β, NFκ-B, TNF-α and IL-6).

	Group-I	Group-II	Group-III	Group-IV
TGF-β (pg/ml)	876.64 ± 71.8	2121.63 ± 193.9***	1667.72 ± 107.8#	1004.32 ± 115.3***
TNF-α (pg/ml)	518.31 ± 78.63	1425.32 ± 153.5***	1123.73 ± 125.4#	732.64 ± 91.94##
NFκ-B (pg/ml)	872.65 ± 84.20	2002.7 ± 186.1***	1401.43 ± 132.7##	994.15 ± 88.135##
IL-6 (pg/ml)	761.83 ± 43.62	1537.62 ± 143.8***	1046.71 ± 92.47##	875.64 ± 78.70***

Each value represents the mean of 6 rats ± SE.

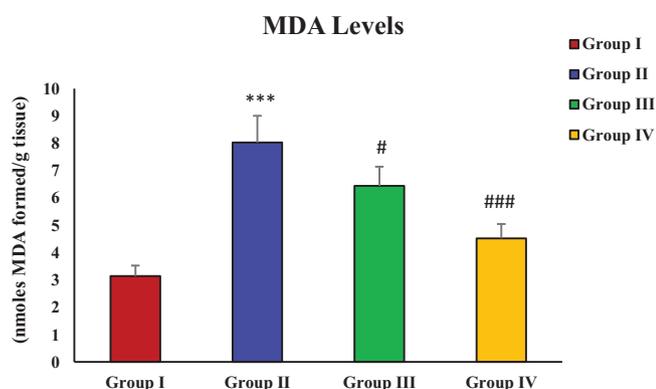


Fig. 1. Effect of piperine treatment on MDA levels in CCl₄-induced hepatic fibrosis.

3.6. Effect of piperine on CCl₄-induced hydroxyproline levels

With the administration of CCl₄ the concentration of hydroxyproline was remarkably raised in the toxic group (group II) in contrast with group I (control) (**p < 0.001). On the other hand, a considerable reduction in hydroxyproline levels was seen subsequent to piperine treatment in group III (#p < 0.05), and IV (##p < 0.01). Moreover, an immeasurable difference was observed in hydroxyproline levels between groups I and IV (Fig. 2).

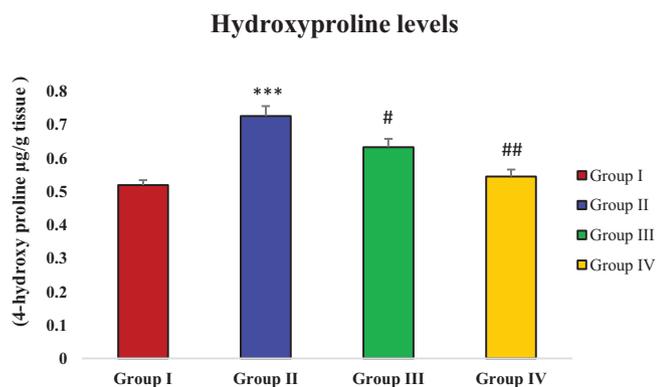


Fig. 2. Effect of piperine treatment on hydroxyproline levels in CCl₄-induced hepatic fibrosis.

3.7. Effect of piperine on CCl₄-induced nitrite level

With CCl₄ administration in the toxic group (group II), a considerable rise in nitrite level was observed in contrast with the control group (**p < 0.001). The piperine treatment with 25 mg/kg dosage depicted statistically no remarkable decrease in nitrite level of group III. However, the considerable decline in nitrite level was noticed in group IV (###p < 0.001) after treatment with piperine at the dosage of 50 mg/kg (Fig. 3).

3.8. Effect of piperine on CCl₄ induced protein carbonyl level

Protein Carbonyl concentration was found considerably elevated (**p < 0.001) in group II treated with CCl₄ in contrast with group I. However, a significant decline in protein Carbonyl level was observed with piperine administration in groups III and IV (#p < 0.05) (Fig. 4).

3.9. Effect of piperine on CCl₄ α-SMA expression

In the process of staining of α-smooth muscle actin (α-SMA) immunohistochemically, insignificant staining was observed in group I, taken as control while group II intoxicated with CCl₄ showed a marked α-SMA expression as evident in the form of the strongly brown-stained portion. The treatment with piperine at 25 mg/kg dose remarkably reduced the expression of α-SMA as evident in the form of reduced brown-stained portion when compared with group II. However, the piperine treatment at 50 mg/kg dose in group IV completely reversed the CCl₄ induced expression of α-SMA and returned the hepatic tissue to a normalized state similar to group I (Fig. 5).

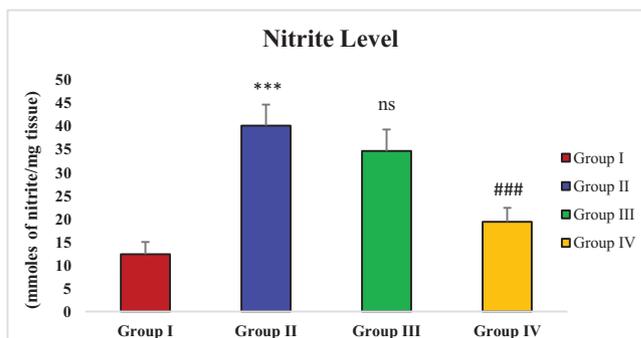


Fig. 3. Effect of piperine treatment on nitrite levels in CCl₄-induced hepatic fibrosis.

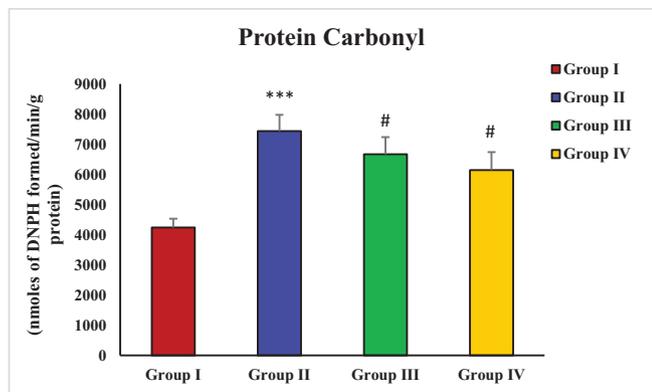


Fig. 4. Effect of piperine treatment on protein Carbonyl level in CCl₄ induced hepatic fibrosis.

3.10. Histopathological investigation

The histopathological investigation displayed supportive shreds of evidence for the liver enzymes and oxidative markers. Histopathological findings showed a normal structure with separate cells, precise central vein, and sinusoidal spaces for the animals' regular group. Group II, on the other hand, confirmed necroinflammation, ballooning degeneration, steatosis, and loss of cellular bourgeons. Piperine treatment at a dosage of 25 mg/kg (group III) and 50 mg/kg (group IV) displayed a relatively normal pattern with a moderate degree of necrosis and infiltration of inflammatory cells compared to group II (Fig. 6).

4. Discussion

Hepatic fibrosis develops as a reversible hepatopathy condition during the progressive course of chronic hepatic impairment to irreversible hepatic cirrhosis (Hafez et al., 2017). CCl₄ is the toxic reagent most frequently utilized to establish animal models with

hepatic fibrosis and hepatic cirrhosis (Scholten et al., 2015). The cytochrome P450 enzyme causes CCl₄ biotransformation into harmful metabolic product trichloromethyl free radicals leading to CCl₄ toxicity (Anusha et al., 2011).

In this study, the hepato-protective and anti-fibrotic potential of piperine was demonstrated in the Wistar rat model to ameliorate the hepatic fibrosis induced with CCl₄ by investigating different parameters. The increased levels of hepatic enzymes in serum have been accepted as non-invasive substitute markers to detect hepatocellular damage (Hann et al., 2012). Our findings showed the evident development of hepatic injury in CCl₄ treatment Wistar rats that was supported by significantly increased (**p < 0.001) levels of AST, ALP, ALT, and bilirubin, presenting CCl₄ as hepatonecrotic and inflammatory agent (Bahcecioglu et al., 2008). The treatment with piperine significantly retrieved the augmented activities of liver enzymes (Table 1), indicating the hepato-protective potential of piperine that perhaps averts the CCl₄-induced loss of functional integrity of membranes of hepatocytes and leaking of sensitive enzymes. Our finding concurs with the previous study that demonstrated the hepato-defensive action of piperine against CCl₄-induced hepatotoxicity (Desai et al., 2008).

The SOD activity serves as a sensitive indicator in hepato-renal injury since it reduces the toxic effects via scavenging the superoxide anions to produce hydrogen peroxide. In hepatic cells, glutathione and catalase form the critical systems for the detoxification of peroxides (Solanki and Jain, 2011). The cellular GSH, as well as the anti-oxidant enzymes including GPx and SOD are the critical representative elements of endogenous antioxidant systems that mainly participate in the scavenging of free radicals. In this study, GPx, GR, SOD, CAT, and GSH content was drastically depleted in rats treated with CCl₄. However, a remarkable restoration in GPx, SOD, GR, CAT, and GSH content was detected with the piperine treatment (Table 2), indicating the piperine's role in promoting the effective and quick consumption of ROS produced by CCl₄ induced P450 bio-activation. The present data concurs with the study of (Abdel-Daim et al., 2019), where piperine treatment in microcystin-induced neurotoxicity in mice has been found to

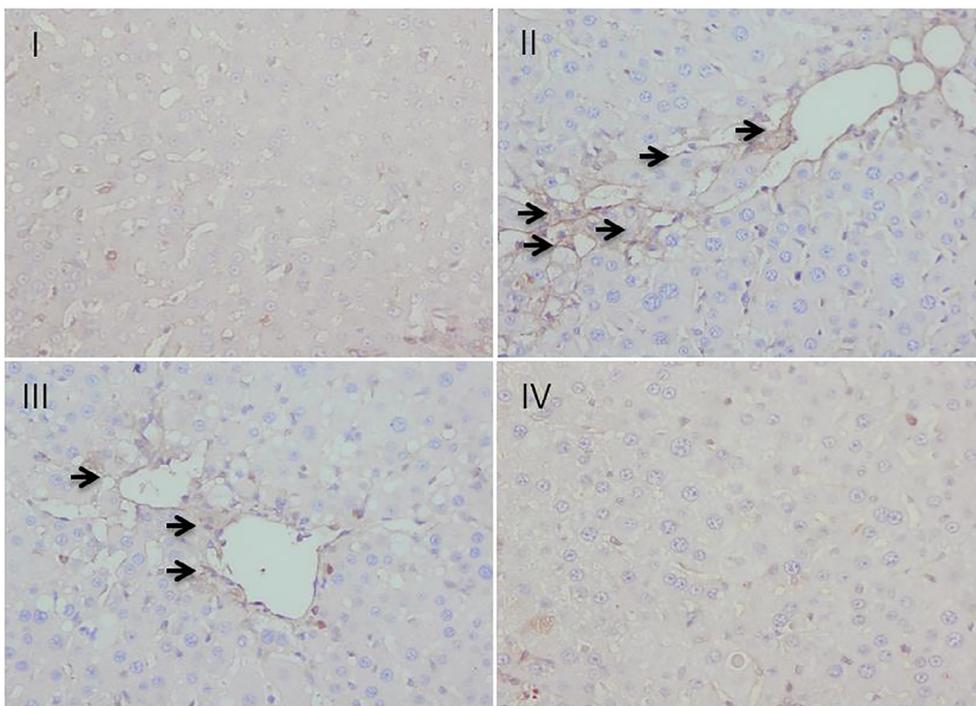


Fig. 5. Effect of piperine treatment on CCl₄-induced α -SMA expression.

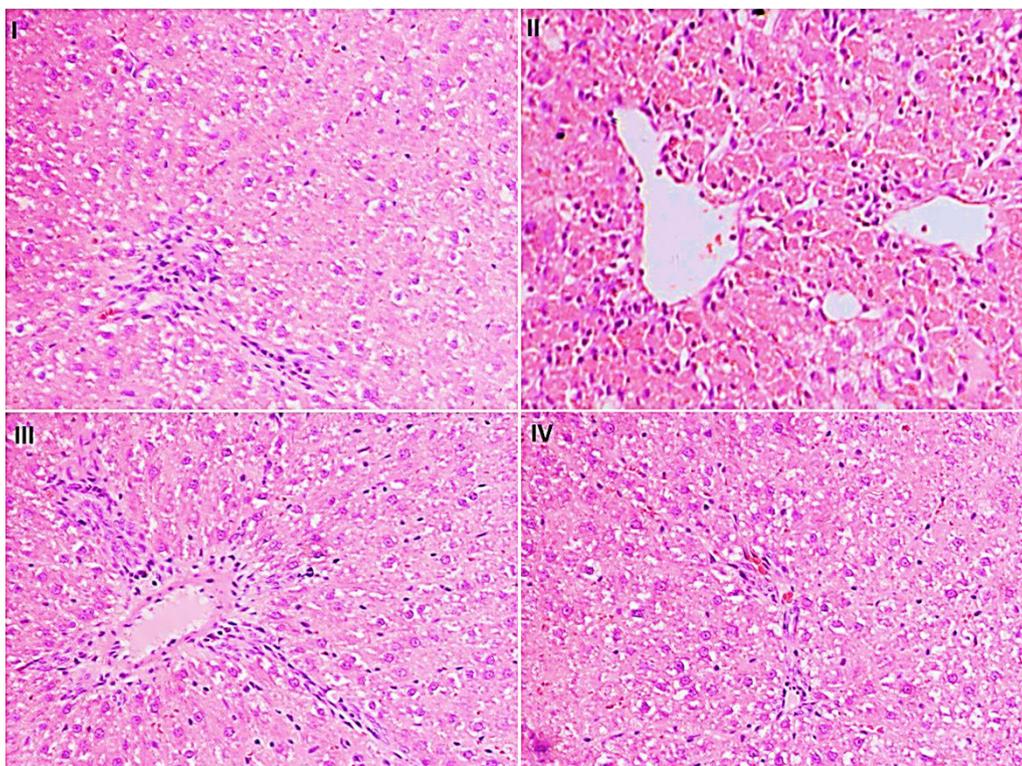


Fig. 6. Microphotograph of hematoxylin and eosin (H & E)-stained sections of liver.

increase GPx, CAT, SOD, and GSH content in the brain tissue. In this study, CCl₄ treated rats showed significantly enhanced lipid profile (TC, TG, LDL, and VLDL), but HDL level was decreased. With the piperine treatment, the lipid profile was retrieved (Table 3). A previous study conducted on cholesterol-rich diet-fed rats reported that methyl piperine remarkably prevented increasing serum TC and TC/HDL ratio (Tu et al., 2014).

The levels of proinflammatory cytokines including TNF α , TGF β , NF- κ B, and IL6 were drastically raised in group II treated with CCl₄ in contrast with the untreated group (group I). However, the piperine treatment considerably reduced these cytokines to almost average levels (Table 4), depicting its role as an anti-inflammatory agent. Our results concord with the established anti-inflammatory property of piperine (Aswar et al., 2015). Guo et al. demonstrated a considerable decrease in 2,4,6 trinitrobenzene sulfonic acid (TNBS) up-regulated proinflammatory cytokines TNF α , IL6, IL β 1, and NF- κ B after piperine treatment in TNBS induced colitis rats as compared to controls (Guo et al., 2020). Furthermore, piperine has been reported to alleviate microcystin-LR-induced hepato-toxicity and neurotoxicity via significantly reducing serum biochemical parameters, proinflammatory cytokines (TNF α , IL6, and IL β 1), and improving oxidative/anti-oxidative state (Abdel-Daim et al., 2019). Choi et al., 2019 also reported a decrease in TNF α , TGF β , IL1 β , and IL6 mRNA levels after piperine treatment during chronic pancreatitis.

MDA levels in CCl₄ treated group were sharply elevated. However, the groups (group III and IV) treated with piperine showed a decreasing trend in MDA levels (Fig. 1). A similar result has been observed with piperine treatment in the case of microcystin-induced elevation in MDA levels in nervous tissue (Mohammed et al., 2019). Hydroxyproline levels were drastically increased in the toxic group with CCl₄ administration in contrast with the control group. However, the hydroxyproline level was significantly reduced in piperine treated groups (Fig. 2). The results of the current study correspond to the survey conducted by Pradeep and

Kuttan (2002) who reported that piperine treatment considerably attenuated the elevated lung collagen hydroxyproline in mice with metastasized lungs. In CCl₄ toxic group, the nitrite level was remarkably increased in contrast with the control group. The piperine treatment only at a higher dose (50 mg/kg) significantly restored the nitrite level (Fig. 3). In a previous study, piperine treatment of lipopolysaccharide and concanavalin-A-treated balb/c mice significantly decreased the nitrite level (Pradeep and Kuttan, 2003). In this study, a significant rise in protein carbonyl level was reported in the group treated with CCl₄ in contrast with the control group. However, the piperine treatment considerably decreased the protein carbonyl level in treatment groups (Fig. 4). Our data is endorsed by the finding of Dhivya et al. (2017), they also reported a considerable decreased content of protein carbonyl in piperine pre-treated rats followed by isoproterenol treatment compared to isoproterenol intoxicated group in case of isoproterenol-induced myocardial ischemia. A markedly increased expression of α -SMA was observed in the group intoxicated with CCl₄. However, with the piperine treatment, α -SMA expression was remarkably reduced at 25 mg/kg and completely reversed at 50 mg/kg in treatment groups (Fig. 5). The current data is endorsed by the observations of Choi et al. (2019), who found that treatment with piperine during chronic pancreatitis decreased the expression of various fibrotic mediators, including α -SMA in pancreatic stellate cells and pancreas. Thus, in this study, we demonstrated piperine as a potential hepato-defensive agent against CCl₄-induced toxicity via regulating different biochemical parameters. These findings recommended that treatment with a higher piperine dosage (50 mg/kg) enhanced the histo-architecture and well-defined the standard arrangements for hepatic architecture.

5. Conclusions

This study demonstrated the hepatoprotective potential of piperine against CCl₄-induced hepatic fibrosis. Piperine exerts an

anti-fibrotic effect via inhibiting the excessive expression of hepatic markers, proinflammatory cytokines, α -SMA, preventing hepatic cell apoptosis, quenching free radicals, and enhancing antioxidant agents via modulating NF- κ B signaling. Thus, our study recommends the use of piperine as an effective treatment for preventing hepatic fibrosis.

Declaration of Competing Interest

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.

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