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ORIGINAL ARTICLE

Ameliorative Potential of a Traditionally Used Plant *Fraxinus micrantha* against Oxidative Stress and Paracetamol-Induced Hepatotoxicity

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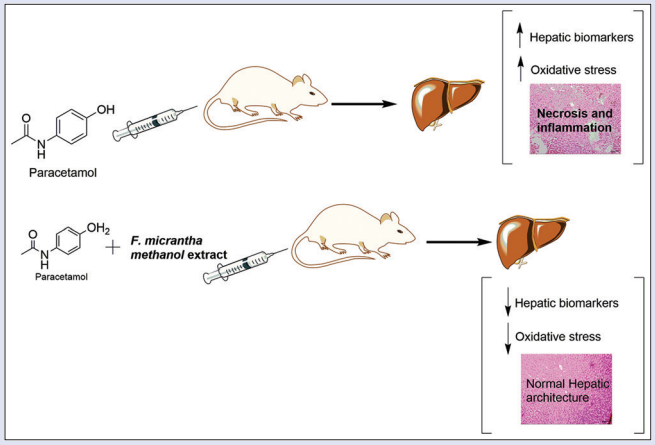
ABSTRACT

Background: Liver disorders are one of the serious health issues. The treatment of liver disorders and their associated complications must be done with care due to the adverse effects of present-day medications. Therefore, there is an urgent need to develop effective and non-toxic herbal drugs for hepatoprotection. **Objective:** The objective of the present study is to investigate the hepatoprotective potential of traditionally used plant *Fraxinus micrantha* against paracetamol (PCM)-induced hepatotoxicity in rats. **Methods:** Hepatotoxicity was induced by a standardized single oral dose of paracetamol (PCM) at 3 g/kg body weight. Standard drug (silymarin 50 mg/kg) and test drugs (chloroform and methanol extracts) were administered orally to rats for 7 days. All the animals were sacrificed on the 8th day, and blood was withdrawn by retro-orbital vein puncture, collected in fresh centrifugation tubes, and centrifuged at 10,000 rpm for 10 min to separate serum for various estimations. Livers were isolated immediately for various biochemical and histopathological studies. **Results:** Treatment with chloroform extract (200, 400, and 800 mg/kg) and methanol extract (200 and 400 mg/kg) ameliorated the elevated levels of hepatic markers, but a significant reduction in these levels was observed by treatment with methanol extract at 800 mg/kg. In addition, treatment with chloroform and methanol extracts resulted in the amelioration of oxidative stress along with the histopathological changes. High-performance liquid chromatographic analysis of methanol extract of *F. micrantha* revealed the presence of various polyphenols such as rutin, naringenin, kaempferol, physicion, quercetin, and gallic acid. **Conclusion:** The results of the present study revealed that the observed hepatoprotective effect of methanol extract of *F. micrantha* may be due to the presence of various polyphenols and also provides pharmacological evidence for the use of *F. micrantha* bark in folk medicine for the treatment of liver diseases.

Key words: *Fraxinus micrantha*, hepatoprotection, histopathology, polyphenols

SUMMARY

- *Fraxinus micrantha*, a traditionally used medicinal plant exhibited potential hepatoprotective and antioxidant activity against PCM induced hepatotoxicity The bioactive methanol extract of *F. micrantha* showed the presence of various polyphenols (gallic acid, rutin, naringenin, quercetin, kaempferol and physocion) in HPLC analysis suggesting that these compounds may be responsible for the hepatoprotective activity of plant.



Abbreviations used: ALP: Alkaline phosphatase; AOPPs: Advanced oxidative protein products; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; GSH: Reduced glutathione; HPLC: High-performance liquid chromatography; IAEC: Institutional Animal Ethics Committee; SGOT: Serum glutamic-oxaloacetic transaminase; SGPT: Serum glutamic-pyruvic transaminase; TBARS: Thiobarbituric acid reactive substances; TrxR: Thioredoxin reductase.

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INTRODUCTION

The liver is a prime and most significant organ of the body associated with detoxification and helps in maintaining the physiological and metabolic homeostasis of an organism.[1,2] The liver plays an imperative role in the regulation of various physiological processes, secretion, and metabolism.[2] Damage to hepatocytes or hepatotoxicity occurs mainly due to the oxidative stress, multidimensional functions, and various xenobiotics which lead to the distortion of liver functions.[3] Several metabolic disorders are associated with significant hepatotoxicity and can even lead to death.[4] Major risk factors pertinent to cirrhosis, hepatitis, and hepatotoxicity include xenobiotics, pollutants, free radicals, food additives, and alcohol. Liver diseases remain one of the most serious health problems, and their management is still a challenge to present-day medications.[5] The treatment of liver diseases and their

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associated disorders is necessary and therefore must be done with extensive care. Although the currently available therapies offer liver protection and stimulate liver functions or help in the regeneration of hepatic cells, yet their usage is associated with many adverse effects and can be toxic at particular dose.[6] Therefore, there is an urgent need for the development of effective, non-toxic, indigenous, and inexpensive botanical sources for hepatoprotective crude or purified hepatoprotective drugs.

Fraxinus micrantha is one of the ashes of olive family, Oleaceae, found in Asia mainly in India and Nepal. It is usually found in Himachal Pradesh and Uttar Pradesh region in India.[7] *F. micrantha* have been studied since time immemorial for its therapeutic and economical value globally. The inner bark of the plant has been used traditionally by the local inhabitants of Dharchula, Himalayas, as an infusion for the treatment of liver enlargement, jaundice, and other liver diseases.[8] Previous studies have reported that ethanol extract of *Fraxinus rhynchophylla* ameliorated the CCl_4-induced liver fibrosis in rats through its antioxidant potential.[9] Furthermore, the leaves and stem bark extract of *Fraxinus angustifolia* also attenuated the paracetamol- and streptozotocin-induced liver injuries and diabetes. These results suggest that hepatoprotective and antidiabetic activities pertain to the presence of various antioxidant compounds in the plant.[10] Younis *et al.* have also reported that methanol extract of leaves of *Fraxinus xanthoxyloides* showed protection against CCl_4-induced liver damage due to its antioxidant constituents.[11] *In vitro* antioxidant activities of extracts of *F. floribunda* and *F. angustifolia* have also been reported.[12,13] The methanol extract of *F. micrantha* aerial parts also showed marked anti-inflammatory activity in carrageenan-induced inflammation model in mice.[14] It was further shown that oxidative stress and various inflammatory processes might be the causes of various liver disorders.[15,16] Handa *et al.* 1986 have shown the effect of some coumarin and flavonoid components of *Fraxinus* spp. in liver protection. These components cause an increase in the bile secretion and showed low toxicity and marked choleretic effects in various animal models.[17] Based on these earlier findings for the use of *Fraxinus* species in liver disorders, it was envisaged to explore *F. micrantha* bark for its hepatoprotective ability in PCM-induced hepatotoxicity.

METHODS

Plant material and extraction

The dried bark of *F. micrantha* was procured from Sri Venkateswara University, Tripura, and the identity of the plant was confirmed by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Andhra Pradesh. A voucher specimen number 711 has been deposited in the same department's herbarium. The plant material was coarsely powdered and subjected to successive Soxhlet extraction using different solvents in increasing order of their polarity, viz. petroleum ether, chloroform, methanol, and finally, the marc was digested with water to obtain the aqueous extract. Each extract was concentrated using rotary evaporator (IKA Works Inc., North America) and stored at low temperature, which was further used for pharmacological studies.

Drugs and chemicals

Paracetamol, 1,1,3,3-tetramethoxypropane (Sigma-Aldrich, Bangalore, India), reduced glutathione (GSH) (Loba Chemie, Mumbai, India), thiobarbituric acid (Loba Chemie, Mumbai, India), tris buffer (Merck Specialities, Mumbai, India), glacial acetic acid, sodium dihydrogen phosphate, and disodium hydrogen phosphate (Thermo Fisher Scientific, Mumbai, India) were used in the present study.

Experimental animals

Male Wistar rats weighing 200–250 g procured from National Institute of Pharmaceutical Education and Research with no prior drug treatment were employed in the present study. Animals were housed in the animal house of Guru Nanak Dev University with free access to water and laboratory pellet chow diet. The rats were exposed to normal cycle of light and dark. The experimental protocol number 226/CPCSEA-2015-35 was duly approved by the Institutional Animal Ethics Committee of Guru Nanak Dev University, and animals were taken care of as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

Preliminary phytochemical screening of extracts

The extracts prepared from the powdered bark of *F. micrantha* were subjected to preliminary phytochemical screening using standard methods for different classes of phytoconstituents.[18]

Acute toxicity studies

The acute toxicity studies were carried out according to OECD guidelines. The extracts were found to be safe up to 2000 mg/kg. No behavioral, biochemical, and histopathological changes were observed with no mortality. The animals were observed for 14 days, and there was no change in the weight and feed on animals.

Plasmid DNA nicking assay

DNA protective activity was measured using plasmid nicking assay given by Lee *et al.* 2002.[19] The methanol extract prepared from *F. micrantha* bark was evaluated for its antioxidant effect by its ability to protect plasmid DNA from the devastating effect of hydroxyl radicals generated by Fenton's reagent.

Induction of hepatotoxicity

Hepatotoxicity was induced by a standardized single oral dose of PCM at 3 g/kg body weight. PCM was freshly prepared and administered to overnight-fasted rats.

Experimental protocol

Nine groups, each comprising six male Wistar albino rats (200–250 g), were used in the present study. Standard drug (silymarin) and test drugs (chloroform and methanol extracts) were administered orally to rats for 7 days. Tween 80 (0.5% aqueous) was used as a vehicle to prepare the suspension of various test doses.

Group I (normal): Hepatotoxicity was not induced to any animal of this group. Group II (PCM intoxicated): Hepatotoxicity was induced by a single oral dose of paracetamol (PCM) at 3 g/kg. Group III (positive control): Rats with hepatotoxicity were treated with silymarin (50 mg/kg) for 7 days orally. Group IV–VI (chloroform extract – 200, 400, and 800 mg/kg): After induction of hepatotoxicity, chloroform extract of *F. micrantha* (200, 400, and 800 mg/kg p. o.) was administered for 7 consecutive days. Group VII–IX (methanol extract – 200, 400, and 800 mg/kg): The methanol extract of *F. micrantha* (200, 400, and 800 mg/kg p. o.) was administered to PCM-intoxicated rats for 7 consecutive days.

Biochemical estimations

Blood was withdrawn under anesthesia (phenobarbital sodium, 40 mg/kg *i.p.*) by retro-orbital vein puncture, collected in fresh centrifugation tubes, and centrifuged at 10,000 rpm for 10 min to separate serum for various estimations. All the animals under study were sacrificed by cervical dislocation on the 8th day after overnight fasting. Livers were isolated immediately for various biochemical and histopathological studies.

Estimation of liver marker enzymes

Serum parameters such as serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase, albumin, total cholesterol, and triglycerides were estimated using commercially available kits (Erba Lachema). The concentrations were expressed in milligrams per deciliter.

Estimation of oxidative stress in liver tissues

The quantitative measurement of thiobarbituric acid reactive substances (TBARS) was performed as per previously described method.[20] The results were expressed as nanomoles per milligram of protein. GSH level in tissue was determined using a previously standardized method,[21] and the results were expressed as micrograms of GSH per milligram of protein. The liver advanced oxidative protein products (AOPPs) were determined as standardized by Witko-Sarsat *et al.*[22] The results of the assay were expressed as micromole per milligram of protein.

Estimation of liver thioredoxin reductase activity

Liver thioredoxin reductase (TrxR) activity was performed according to the method of Holmgren and Bjornstedt.[23] One unit of activity was defined as 1 μM 2-nitro-5-thiobenzoate (TNB) formed per minute per milligram of protein.

Histopathological studies

The liver tissues were preserved in 10% formalin in a stoppered container. The prepared slides were then examined with the help of a binocular microscope, and pictures were taken with the help of camera fixed on microscope.

High-performance liquid chromatography of methanol extract of *Fraxinus micrantha*

The methanol extract was subjected to high-performance liquid chromatography (HPLC) at a concentration of 10 mg/ml using HPLC grade acetonitrile and 0.1% formic acid. HPLC apparatus was fitted with RP-18e Chromolith column (5 μm Merck 100–4.6), an efficient degasser, and a photodiode array detector (Shimadzu). Analysis of different compounds was carried out using an isocratic system. Injection volume was 5 μl, flow rate was 0.8 ml/min, and column temperature was 35 degrees.

Statistical analysis

All the results were expressed as mean ± standard error mean. Data were statistically analyzed by one-way ANOVA followed by Tukey's test using GraphPad Prism Version 7 software. GraphPad Software, 2365 Northside Dr. Suite 560, San Diego, CA. $P < 0.05$ was considered to be statistically significant.

RESULTS

Preliminary phytochemical screening of extracts

A preliminary phytochemical screening study showed the presence of lipids and steroids in hexane extract and alkaloids in chloroform extract. Methanol extract gave positive tests for alkaloids, glycosides, and flavonoids. Aqueous extract contained carbohydrates and proteins.

Plasmid DNA nicking assay

The methanol extract of *F. micrantha* showed no protection at the lowest concentration. However, a dose-dependent protection was observed at higher concentrations which showed a significant hydroxyl radical scavenging activity in plasmid DNA nicking assay [Figure 1].

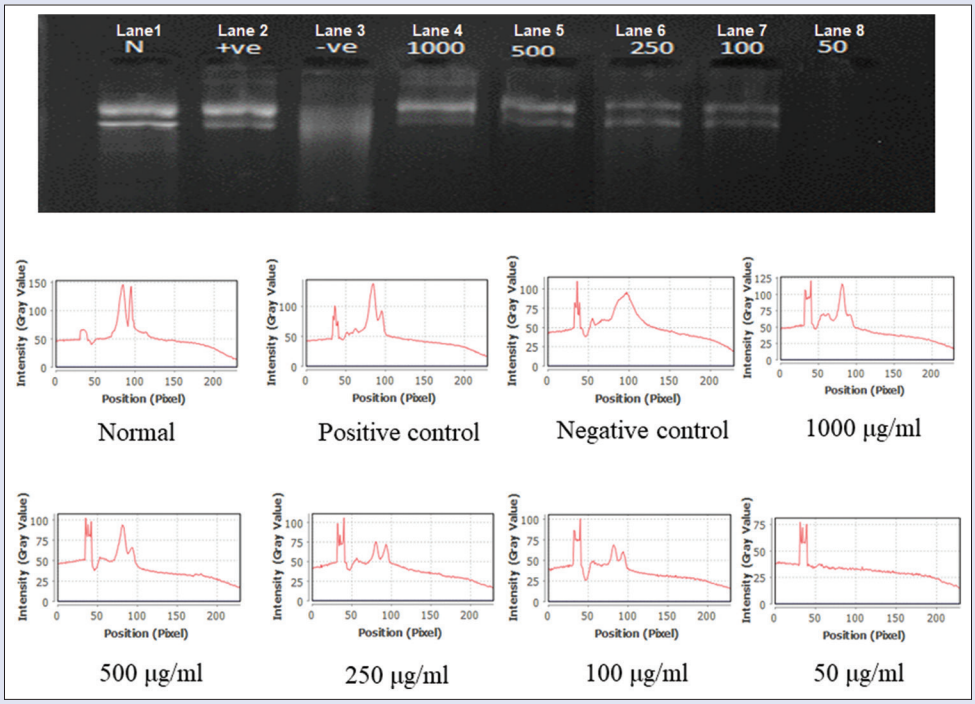

Figure 1: Inhibition of DNA scission by methanol extract of *Fraxinus micrantha* at different concentrations showing percentage amount of supercoiled DNA conserved against OH radicals created by Fentons's reagent (FR). Lane 1: Normal control; Lane 2: Positive control (rutin); Lane 3: Negative control (Fenton's reagent); Lane 4-8: Fenton's reagent + concentrations of extract (50, 100, 250, 500, and 1000 μg/ml)

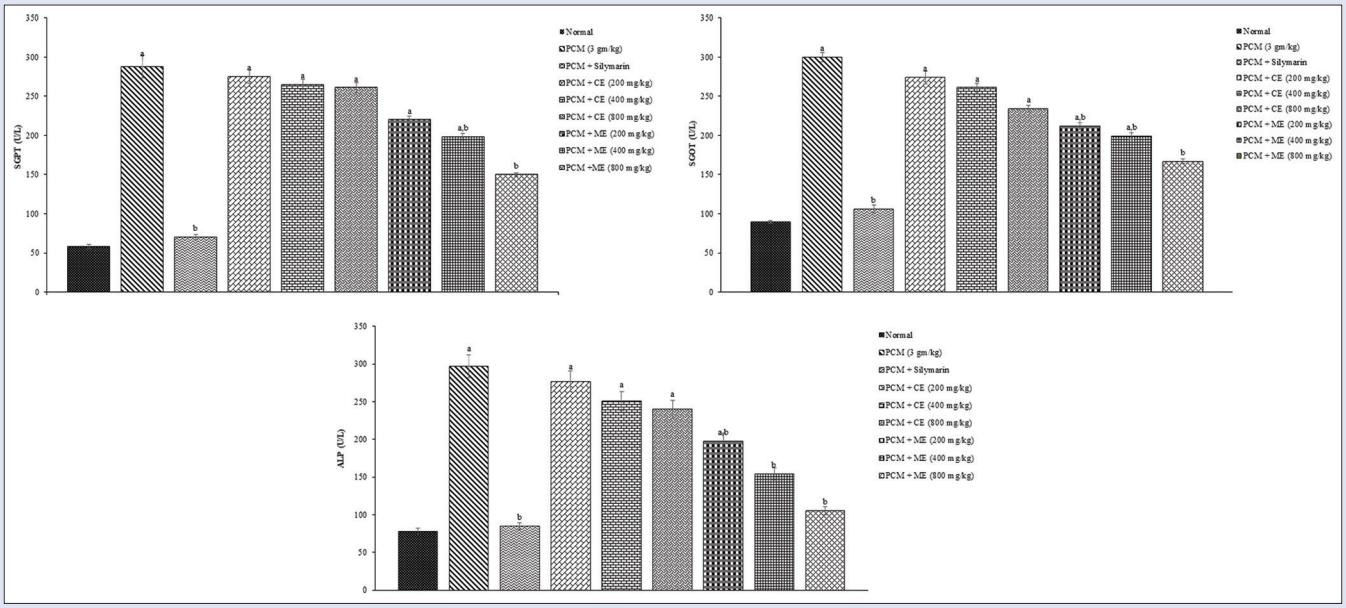

Figure 2: The effects of chloroform and methanol extract on serum glutamic-pyruvic transaminase, serum glutamic-oxaloacetic transaminase, and alkaline phosphate levels. Values are expressed as mean ± standard error mean (n = 6). [a]P < 0.05 versus normal control; [b]P < 0.05 versus PCM (hepatotoxic)

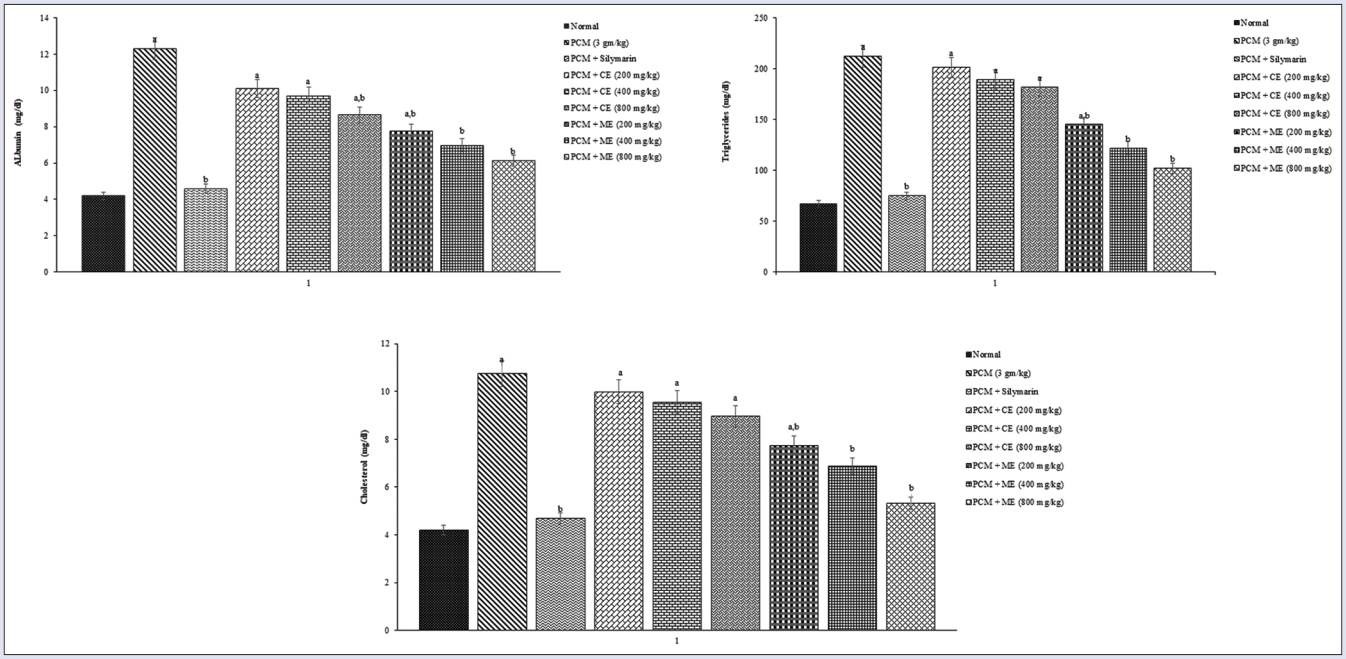

Figure 3: The effects of chloroform and methanol extract on albumin, triglycerides, and cholesterol levels. Values are expressed as mean ± standard error mean (n = 6). [a]P < 0.05 versus normal control; [b]P < 0.05 versus PCM (hepatotoxic)

Effect of various interventions on hepatocellular enzyme levels in PCM-induced hepatotoxicity

PCM-induced hepatotoxicity resulted in a significant increase in serum SGPT, SGOT, ALP, Albumin (ALB), triglycerides, and cholesterol levels as compared to the control group. Administration of chloroform and methanol extracts (200, 400, and 800 mg/kg) attenuated the PCM-induced hepatocellular enzyme alterations [Figures 2 and 3]. However, methanol extract (800 mg/kg) exhibited better protection than chloroform extract in PCM-induced hepatotoxicity.

Effect of various interventions on oxidative stress markers in PCM-induced hepatotoxicity

Liver damage by PCM resulted in a significant increase in oxidative stress (decrease in the levels of GSH and increase in the levels of TBARS and AOPPs) when compared to the control group. Oral administration of chloroform and methanol extracts (200, 400, and 800 mg/kg) showed protection against PCM-induced hepatotoxicity, but a significant reduction in oxidative stress was observed in methanol extract at 800 mg/kg [Table 1].

Table 1: Effect of various interventions of *F. micrantha* on tissue biomarker changes

Groups	GSH (µg/mg of protein)	TBARS (nmol/mg of protein)	Hepatic TrxR (U/min/mg protein)	Hepatic AOPPs (µmol/mg protein)
Normal	11.54±0.14	17.34±0.56	0.63±0.13	76.43±0.12
PCM (3 g/kg)	4.67±0.21[a]	38.54±0.45[a]	0.072±0.024[a]	98.54±0.64[a]
PCM + silymarin	10.73±0.11[b]	18.34±0.76[b]	0.60±0.12[b]	78.34±0.45[b]
PCM + CE (200 mg/kg)	4.87±0.54[a]	37.22±0.23[a]	0.087±0.013[a]	95.62±0.94[a]
PCM + CE (400 mg/kg)	5.32±0.32[a]	35.67±0.92[a]	0.096±0.016[a]	92.23±1.07[a]
PCM + CE (800 mg/kg)	6.87±0.17[a]	32.45±0.37[a]	0.17±0.09[a,b]	91.87±0.34[a]
PCM + ME (200 mg/kg)	6.12±0.22[a]	31.77±0.45[a]	0.27±0.11[a,b]	92.34±0.54[a]
PCM + ME (400 mg/kg)	8.39±0.23[b]	26.23±0.63[a,b]	0.42±0.10[b]	86.23±0.67[b]
PCM + ME (800 mg/kg)	10.13±0.22[b]	19.64±0.33[a,b]	0.57±0.14[b]	80.23±0.87[b]

The effects of chloroform and methanol extract on GSH, TBARS, hepatic TrxR, and hepatic AOPPs levels. Values are expressed as mean±SEM (n=6). [a]P<0.05 versus normal control; [b]P<0.05 versus PCM (hepatotoxic). GSH: Reduced glutathione; TBARS: Thiobarbituric acid reactive substances; TrxR: Thioredoxin reductase; AOPPs: Advanced oxidative protein products; SEM: Standard error of mean; PCM: Paracetamol; ME: Methanol extract of *F. micrantha*; CE: Chloroform extract of *F. micrantha*

Effect of various interventions on liver thioredoxin reductase in PCM-induced hepatotoxicity

The levels of TrxR were significantly decreased in PCM-intoxicated group, which were reversed by the treatment with chloroform and methanol extracts (200, 400, and 800 mg/kg) for 7 days. Moreover, methanol extract at 800 mg/kg showed better protection [Table 1].

Histopathological studies

Histopathological studies were carried out for liver tissues and witnessed all the altered biochemical changes. Normal heptic architecture was observed in normal group [Figure 4A]. As was expected, the histopathology examination of the liver revealed that PCM destroyed the hepatic architecture with necrosis and multiple portal-central bridging [Figure 4B]. Photomicrographs of the liver of silymarin (50 mg/kg)-treated animals showed near-normal architecture with mild inflammation [Figure 4C]. Group IV to VI animals treated with chloroform extract (200, 400, and 800 mg/kg) showed focal and portal inflammation with spotty necrosis [Figure 4D-F]. Photomicrographs show confluent necrosis and inflammation in methanol extract at doses of 200 and 400 mg/kg groups [Figure 4G and H)]. However, mild inflammation and normal hepatic architecture were observed in 800 mg/kg methanol extract-treated group [Figure 4I].

High-performance liquid chromatography of bioactive methanol extract of *Fraxinus micrantha*

HPLC results of the bioactive methanol extract revealed the presence of rutin, naringenin, kaempferol, Physicion, quercetin, and gallic acid [Table 2].

Table 2: Polyphenolic compounds detected by high-performance liquid chromatography in methanol extract of *Fraxinus micrantha*

Ret. time	Area	Height	Area (%)	Name
2.414	35,751,193	1,759,900	100.00	Gallic acid
13.944	26,452,127	1,890,244	100.000	Rutin
15.840	15,548,797	1,628,143	99.995	Naringenin
16.509	32,630,280	1,971,484	99.999	Quercetin
16.793	39,794,685	1,097,255	100.000	Physicion
17.755	35,623,574	1,937,922	100.000	Kaempferol

DISCUSSION

Acetaminophen or paracetamol (PCM) is a derivative of para-aminophenol which is commonly used as an analgesic and antipyretic drug.[24] PCM is a well-known nonsteroidal anti-inflammatory drug which is toxic at larger dose and causes acute liver damage and hepatic necrosis.[25] The mechanism by which PCM causes hepatotoxicity has been attributed to the formation of highly toxic metabolite N-acetyl-p-benzoquinone imine which further causes oxidative stress and depletion of glutathione.[26,27]

In the present study, a single oral dose of PCM (3 g/kg) resulted in significant hepatic damage as demonstrated by pertinent biochemical and histopathological changes. PCM treatment significantly increased the levels of SGPT, SGOT, ALP, ALB, triglycerides, and total cholesterol pertinent to liver damage as they leak into the blood due to hepatotoxicity.[28]

Treatment with chloroform extract (200, 400, and 800 mg/kg) and methanol extract (200 and 400 mg/kg) moderately ameliorated the elevated levels of SGPT, SGOT, ALP, ALB, triglycerides, and total cholesterol, but a significant reduction in these levels was observed by treatment with the highest dose of methanol extract (800 mg/kg). This reversal of hepatic biomarkers by *F. micrantha* treatment may be due to the membrane stabilization, hepatocyte regeneration, and thereby preventing their escape which is in line with previous studies.[29]

It is further supposed that the main culprit owing to the hepatic damage is lipid peroxidation due to the administration of PCM. The increase in the level of TBARS and decreased GSH levels in liver pertains to enhanced lipid peroxidation due to the failure of antioxidative defense mechanism. GSH is the abundant intracellular antioxidant which is involved in the protection against oxidative stress damage and also in diverse detoxification mechanisms.[30] This further leads to the degradation of cellular macromolecules and causes tissue damage.[31] Furthermore, PCM treatment demonstrated the increased levels of AOPPs (products of oxidative modification of proteins) due to oxidative stress modification of proteins, thereby affecting various cellular metabolisms.[32,33] Moreover, a decline in TrxR levels observed here with PCM-induced toxicity are in line with previous findings of Erkekoglu *et al.* who documented that inhibition of TrxR in di(2-ethylhexyl) phthalate-induced hepatotoxicity leads to reduction in the activity of enzymes that are dependent on thioredoxin, thereby decrease the free radical scavenging ability, and lead to oxidative stress, apoptosis, and necrosis.[34]

Treatment with chloroform (200, 400, and 800 mg/kg) and methanol extract (200 and 400 mg/kg) resulted in the amelioration of oxidative stress. However, a marked protection against oxidative stress was observed in animals treated with methanol extract 800 mg/kg. These observed improvements in the above oxidative stress markers indicate the protective role of *F. micrantha* against hepatic damage.

The induction of toxicity by PCM and hepatoprotective effect of *F. micrantha* extracts as evident from serum and tissue biochemical analysis is further supported by the histopathological observations.

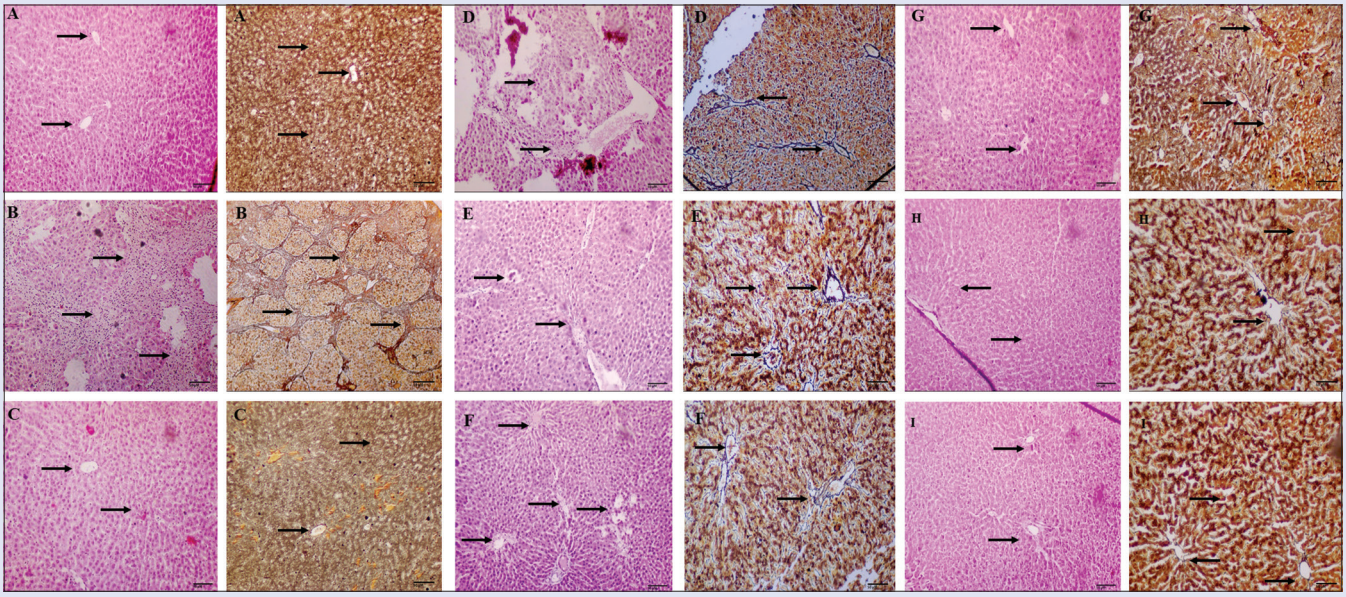

Figure 4: (A) Photomicrographs of liver showing normal hepatic architecture. (B) Necrosis and multiple portal-central bridging by PCM hepatotoxicity. (C) Near-normal architecture with mild inflammation in silymarin-treated group. (D-F) Focal and portal inflammation in animals treated with chloroform extract (200, 400, and 800 mg/kg). (G and H) Photomicrographs show confluent necrosis and inflammation in methanol extract at doses of 200 and 400 mg/kg groups,. (I) Mild inflammation and normal hepatic architecture in 800 mg/kg methanol extract-treated group (H and E, ×100 and Reticulin, ×100)

PCM intoxication significantly leads to the destruction of hepatic architecture along with necrosis, inflammation, and portal-to-central bridging. Treatment with *F. micrantha* continuously for 7 days not only reduced the inflammation and necrosis in liver tissues as compared to PCM-intoxicated group but also significantly decreased the morphological changes in liver tissues leading to the normal hepatic architecture.

Phytochemical screening of methanol extract of *F. micrantha* showed the presence of various polyphenols. The HPLC analysis of methanol extract of *F. micrantha* revealed the presence of various polyphenols such as rutin, naringenin, kaempferol, physocion, quercetin, and gallic acid [Table 2]. The methanol extract of *F. micrantha* also showed a significant antioxidant activity in plasmid DNA nicking assay.

Many research groups have demonstrated that free radical scavenging activity is an important mechanism for hepatoprotective activity.[35] Various polyphenols from plants such as *Cichorium glandulosum*, apple pomace, *Folium microcos*, and *Cleome viscosa* are reported to exert protective effects in liver toxicity due to their antioxidative potential.[36-39] Gowri *et al*. 2008 have also reported that polyphenols from *Commiphora berryi* are responsible for hepatoprotection and decrease the oxidative stress.[40] Our results also suggest that the observed hepatoprotective effect of methanol extract of *F. micrantha* pertains to the presence of various polyphenols. The present study provides a pharmacological evidence for the appropriate use of *F. micrantha* bark in folk medicine for the treatment of liver diseases.

CONCLUSION

The results of the present investigation suggest that the various polyphenols present in the methanol extract of *F. micrantha* bark might be responsible for the hepatoprotective activity due to their potent antioxidant effect. Further studies are required to ascertain the underlying mechanism of *F. micrantha* in hepatoprotection.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Samuel AJ, Mohan S, Chellappan DK, Kalusalingam A, Ariamuthu S. *Hibiscus vitifolius* (Linn.) root extracts shows potent protective action against anti-tubercular drug induced hepatotoxicity. J Ethnopharmacol 2012;141:396-402.
2. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44-84.
3. Pal RK, Manoj J. Hepatoprotective activity of alcoholic and aqueous extracts of fruits of *Luffa cylindrical* Linn in rats. Ann Biol Res 2011;2:132-41.
4. Patel RK, Patel MM, Patel MP, Kanzaria NR, Vaghela KR, Patel NJ. Hepatoprotective activity of *Moringa oleifera* Lam. fruit on isolated rat hepatocytes. Pharmacogn Mag 2008;4:118-23.
5. Wolf PL. Biochemical diagnosis of liver disease. Indian J Clin Biochem 1999;14:59-90.
6. Gagliano N, Grizzi F, Annoni G. Mechanisms of aging and liver functions. Dig Dis 2007;25:118-23.
7. Kumar S, Kashyap P. Antiproliferative activity and nitric oxide production of a methanolic extract of *Fraxinus micrantha* on Michigan cancer foundation-7 mammalian breast carcinoma cell line. J Intercult Ethnopharmacol 2015;4:109-13.
8. Garbyal SS, Aggarwal KK, Babu C. Traditional phytomedicinal knowledge of Bhotias of Dharchula in Pithoragarh. Indian J Tradit Knowl 2005;4:199-207.
9. Peng WH, Tien YC, Huang CY, Huang TH, Liao JC, Kuo CL, *et al*. Fraxinus rhynchophylla ethanol extract attenuates carbon tetrachloride-induced liver fibrosis in rats via down-regulating the expressions of uPA, MMP-2, MMP-9 and TIMP-1. J Ethnopharmacol 2010;127:606-13.
10. Medjahed Z, Atmani-Kilani D, Fauconnier ML, Richard G, Atmani D. Hepatoprotective and antidiabetic activities of *Fraxinus angustifolia* Vahl extracts in animal models: Characterization by high performance liquid chromatography analysis. Turk J Med Sci 2016;46:910-20.
11. Younis T, Khan MR, Sajid M. Protective effects of *Fraxinus xanthoxyloides* (Wall.) leaves against CC_4 induced hepatic toxicity in rat. BMC Complement Altern Med 2016;16:407.
12. Palash SA. Antioxidant potential of *Fraxinus floribunda* bark extracted through various aqueous processing. Free Rad Antioxid 2015;5:6-12.

13. Ayouni K, Berboucha-Rahmani M, Kim HK, Atmani D, Rob Verpoorte R, Choi YH. Metabolomic tool to identify antioxidant compounds of *Fraxinus angustifolia* leaf and stem bark extracts. Ind Crop Prod 2016;88:65-77
14. Kumar S, Kashyap P. *In-vivo* anti-inflammatory activity of methanol extract of *Fraxinus micrantha*. ARC J Pharm Sci 2015;1:1-4.
15. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci 2004;74:2157-84.
16. Shah NA, Khan MR, Naz K, Khan MA. Antioxidant potential, DNA protection, and HPLC-DAD analysis of neglected medicinal Jurinea dolomiaea roots. Biomed Res Int 2014;2014:726241.
17. Handa SS, Chakraborty KK, Sharma A. Antihepatotoxic activity of some Indian herbal formulations as compared to silymarin. Fitoterapia 1986;57:307.
18. Khandelwal KR. Preliminary phytochemical Screening in Practical Pharmacognosy. Pune: Nirali Parkashan; 2004. p. 1449-156.
19. Lee JC, Kim HR, Kim J, Jang YS. Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica* var. Saboten. J Agric Food Chem 2002;50:6490-6.
20. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
21. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963;61:882-8.
22. Witko-Sarsat V, Friedlander M, Khoa TN, Capeillère-Blandin C, Nguyen AT, Canteloup S, *et al.* Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. J Immunol 1998;161:2524-32.
23. Holmgren A, Björnstedt M. Thioredoxin and thioredoxin reductase. Methods Enzymol 1995;252:199-208.
24. Aghababian RV. Essentials of Emergency Medicine. Burlington: Jones and Bartlett Publishers; 2010. p. 814.
25. Hurkadale PJ, Shelar PA, Palled SG, Mandavkar YD, Khedkar AS. Hepatoprotective activity of *Amorphophallus paeoniifolius* tubers against paracetamol-induced liver damage in rats. Asian Pac J Trop Biomed 2012;2:S238-42.
26. Shah VN, Deval K. Hepatoprotective activity of leaves of *Parkinsonia aculeata* Linn against paracetamol induced hepatotoxicity in rats. Int J Pharm 2011;1:59-66.
27. Lee WM, Squires RH Jr., Nyberg SL, Doo E, Hoofnagle JH. Acute liver failure: Summary of a workshop. Hepatology 2008;47:1401-15.
28. Gutierrezl RM, Solı's RV. Hepatoprotective and inhibition of oxidative stress in liver of *Prostechea michuacana*. Rec Nat Prod 2009;3:46-51.
29. Vadivu R, Krithika A, Biplab C, Dedeepya P, Shoeb N, Lakshmi KS. Evaluation of hepatoprotective activity of the fruits of *Coccinia grandis* Linn. Int J Health Res 2008;1:163-8.
30. Shi J, Sun B, Shi W, Zuo H, Cui D, Ni L, *et al.* Decreasing GSH and increasing ROS in chemosensitivity gliomas with IDH1 mutation. Tumour Biol 2015;36:655-62.
31. Messarah M, Klibet F, Boumendjel A, Abdennour C, Bouzerna N, Boulakoud MS, *et al.* Hepatoprotective role and antioxidant capacity of selenium on arsenic-induced liver injury in rats. Exp Toxicol Pathol 2012;64:167-74.
32. Sefi M, Ben Amara I, Troudi A, Soudani N, Hakim A, Zeghal KM, *et al.* Effect of selenium on methimazole-induced liver damage and oxidative stress in adult rats and their offspring. Toxicol Ind Health 2014;30:653-69.
33. Kocic G, Sokolovic D, Jevtovic T, Cvetkovic T, Veljkovic A, Kocic H, *et al.* Short communication: Effect of commercial or depurinized milk diet on plasma advanced oxidation protein products, cardiovascular markers, and bone marrow CD34+stem cell potential in rat experimental hyperuricemia. J Dairy Sci 2014;97:6823-7.
34. Erkekoglu P, Zeybek ND, Giray BK, Rachidi W, Kızılgün M, Hininger-Favier I, *et al.* The effects of di(2-ethylhexyl) phthalate on rat liver in relation to selenium status. Int J Exp Pathol 2014;95:64-77.
35. Boll M, Weber LW, Becker E, Stampfl A. Mechanism of carbon tetrachloride-induced hepatotoxicity. Hepatocellular damage by reactive carbon tetrachloride metabolites. Z Naturforsch C 2001;56:649-59.
36. Sharma S, Rana S, Patial V, Gupta M, Bhushan S, Padwad YS. Antioxidant and hepatoprotective effect of polyphenols from apple pomace extract via apoptosis inhibition and nrf2 activation in mice. Hum Exp Toxicol 2016;35:1264-75.
37. Wu H, Zhang G, Huang L, Pang H, Zhang N, Chen Y, *et al.* Hepatoprotective effect of polyphenol-enriched fraction from *Folium microcos* on oxidative stress and apoptosis in acetaminophen-induced liver injury in mice. Oxid Med Cell Longev 2017;2017:3631565.
38. Nguyen TP, Tran CL, Vuong CH, Do TH, Le TD, Mai DT, *et al.* Flavonoids with hepatoprotective activity from the leaves of *Cleome viscosa* L. Nat Prod Res 2017;31:2587-92.
39. Tong J, Yao X, Zeng H, Zhou G, Chen Y, Ma B, *et al.* Hepatoprotective activity of flavonoids from *Cichorium glandulosum* seeds *in vitro* and *in vivo* carbon tetrachloride-induced hepatotoxicity. J Ethnopharmacol 2015;174:355-63.
40. Gowri SNL, Manavalan R, Venkappayya D, David RC. Hepatoprotective and antioxidant effects of Commiphora berryi (Arn) Engl bark extract against CCl4- induced oxidative damage in rats. Food Chem Toxicol. 2008;46:3182–5.