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ORIGINAL ARTICLE

Hepatoprotective and Antioxidant Properties of *Rhizophora mucronata* Mangrove Plant in CCl_4 Intoxicated Rats

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Purpose: To identify the hepatoprotective effect of bark, collar, hypocotyl and stilt root extracts of *Rhizophora mucronata* mangrove plant.
Methods: Rats were divided into four groups of six animals. Group 1 was the control; Group 2 was treated with the CCl_4 hepatotoxin Group 3 was treated with silymarin (positive control) group; and Group 4 was the treatment group. The phytochemcial components of the extracts were analyzed. Moreover, *in vitro* antioxidant properties such as, DPPH, HR, NO, FRAP, LPO and SOD were measured for the stilt root extract, which was the most potent.
Results: Of the selected extracts, stilt root showed better hepatoprotective activity. The hepatoprotective activity of the *R. mucronata* stilt root extract was dose-dependent (75–300 mg/kg body weight) showed that the level of SGOT, SGPT, ALP, bilurubin, cholesterol, sugar and lactate dehydrogenase were significantly ($p < 0.05$) reduced by all the doses when compared with the levels in the hepatotoxin group rats. The maximum reduction of SGOT (191.36 ± 24.32 IU/L), SGPT (81.54 ± 5.73 IU/L), ALP (228.63 ± 18.56 IU/L), bilurubin (2.52 ± 0.83 mg/dL), cholesterol (129.87 ± 6.42 mg/dL), sugar (121.63 ± 7.38 mg/dL) and lactate dehydrogenase (1601.00 ± 305.65 IU/L) and no histopathological alteration other than mild fatty changes was observed with the high dose (300 mg/kg) of stilt root extract. Phytochemcial analysis of the extracts showed the presence of various chemical constituents, including flavonoids, alkaloids, coumarins and polyphenols. Further, the IC_{50} values were 58.33 ± 2.87 μg/mL, 64.78 ± 1.32 μg/mL, 72.14 ± 0.94 μg/mL, 25.79 ± 0.93 μg/mL, 163.38 ± 0.81 μg/mL and 22.80 ± 0.93 μg/mL for the DPPH, HR, NO, FRAP, LPO and SOD radical scavenging activity, respectively.
Conclusion: We conclude that the *R. mucronata* stilt root extract could be used as an alternative herbal medicine for the treatment of liver damage following the successful completion of clinical trials.

1. Introduction

Liver is understood to be a major organ in the metabolism, detoxification and excretion of various xenobiotics from the body. In general, toxins absorbed from the intestinal tract gain access to the liver resulting in a variety of liver diseases that, together, are a serious worldwide health problem.[1] Modern medicines have little to offer for alleviation of hepatic diseases and only limited numbers of drugs are available for the treatment of liver disorders. The synthetic drugs used for the treatment of liver diseases are inadequate and can have serious side-effects. Traditionally, many of the folk remedies of plant origin have long been used for the treatment of liver diseases.[2,3] Therefore, there is an urgent need to develop new and effective drugs for the treatment of liver diseases. Marine halophytes, such as mangroves and related species, are known to have many and various metabolites possessing antibacterial and antifungal,[4–6] antiviral,[7] antidiarrhoeal,[8] hepatoprotective,[9] antifeedant,[10] insecticidal,[11] cytotoxicity[12] and antiplasmodial[13–15] properties. There are few reports of the scientific evaluation of mangrove plant extracts against liver diseases and the present study was undertaken to investigate the hepatoprotective and antioxidant activity of parts of the *Rhizophora mucronata* against CCL_4-induced hepatotoxicity.

2. Materials and methods

2.1. Collection of plant materials

Fresh samples of different parts (bark, collar, hypocotyls and stilt roots) of the *R. mucronata* were collected from the Karangkadu mangrove forest (latitude 9° 38′ N and longitude 78° 57′E) in the Ramanathpuram district on the South East coast of India.

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Authentication of the plant species was done by Professor K. Kathiresan, Centre of Advanced Study in Marine Biology, Annamalai University, Porto Novo, Tamil Nadu, India. Voucher specimens of all the samples collected are maintained in the herbarium cabinet facility sponsored by the Indian Council of Medical Research, New Delhi in the Department of Oceanography and Coastal Area Studies, Alagappa University, Tamilnadu, India. All samples were washed three times with tap water and twice with distilled water to remove adhering salts and associated contaminants.

2.2. Extraction

The samples of parts of *R. mucronata* were shade dried and preferred for percolation by soaking in ethanol/water (3:1 v/v). After complete extraction, each filtrate was concentrated in a rotary vacuum evaporator (>45°C) and then freeze-dried (−80°C). Phytochemical components, including alkaloids, pheonolic compounds, coumarins, flavonoids, proteins and sugars, were analyzed by standard protocols.[16]

2.3. Experimental animals

Male Wistar albino rats (150−200 g body weight) were maintained under standard husbandry conditions (23 ± 2°C, relative humidity 55 ± 10%, 12 hours dark/12 hours light cycle). Animals were allowed access to standard laboratory feed (Sai Durga Feeds and Foods, Bangalore) and tap water *ad libitum*. The experimental protocol was approved by the Animal Ethical Committee, Alagappa University, Karaikudi, Tamilnadu, India.

2.4. Hepatoprotective activity

The 78 rats used for this experiment were divided into four groups of six animals.

Group 1 was the control group and received a dose of the 5% acacia mucilage vehicle (1 mL/kg body weight p.o. at time zero, 12 hours and 24 hours).

Group 2 (the hepatotoxin-treated group) received three doses of vehicle as described for Group 1 and a single dose of carbon tetrachloride (2 mL/kg body weight i.p.) diluted 1:1 (v/v) in liquid paraffin at 30 minutes after time zero.

Group 3 received three doses of silymarin (100 mg/kg body weight p.o.) at time zero, 12 hours and 24 hours. Carbon tetrachloride diluted in liquid paraffin (1:1, v/v) (2 mL/kg body weight i.p.) was administered 30minutes after time zero.

Group 4 [including does dependant hepatoprotective activity of stilt root extract (75, 150, 300 mg/kg body weight p.o.)] received three doses of each mangrove extract (300 mg/kg body weight p.o.) at time zero, 12 hours and 24 hours. Carbon tetrachloride (CCl_4) diluted 1:1 (v/v) in liquid paraffin (2 mL/kg body weight i.p.) was administered 30 minutes after time zero.

After 36 hours of CCL_4 treatment, all animals were anesthetized mildly with ether and blood samples were collected using sterile capillary tube by the eye bleeding method. The collected blood was allowed to clot at room temperature and serum was separated by centrifugation at 2500 rpm for 10 minutes. The serum was used for the estimation of biochemical parameters to determine the functional state of the liver. Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were assayed by standard protocols.[17] Total protein (TPN),[18] albumin (ALB),[19] sugar (SUG),[20] cholesterol (CHL)[21] and bilurubin (BIL)[22] were also assayed using Randox laboratory kits. Animals from each group were sacrificed, the abdomen was opened and the liver was removed, fixed in Bouins solution (75 mL of saturated picric acid, 25 mL of 40% (v/v) formaldehyde and 5 mL of glacial acetic acid) for 12 hours , then embedded in paraffin using conventional methods[23] and cut into 5 μm thick sections and stained using haematoxylin-eosin dye and finally mounted in diphenyl xylene. The section was observed under a light microscope for any histopathological change. The liver pathology was scored as described.[24] Histological damage was assessed using the following scoring system: 0, no visible cell damage; 1, focal hepatocytes damage on <25% of tissue; 2, focal hepatocytes damage on <25−50% of the tissue; 3, extensive, but focal hepatocytes lesion; 4, global hepatocytes necrosis.

2.5. Determination of DPPH radical scavenging activity[25]

10 μL of various concentrations (1.9−500 μg/ml) of *R. mucronata* stilt root extract and vitamin C were added separately to 190 μL of 150μM DPPH in ethanol, vortex mixed then incubated for 20 minutes at 37°C. Solvent alone was maintained as control. The decrease in absorbance at 517 nm of the test mixture due to quenching of DPPH free radicals was measured in a Perkin Elmer UV/Vis spectrophotometer. The IC_{50} values were determined as the concentration of the test mixture that gave 50% reduction of the absorbance compared to the control blank.

$$\text{DPPH scavenging activity } (\%) = \left\{ \left[A_{\text{control}} - A_{\text{sample}} \right] / A_{\text{control}} \right\} \times 100$$

2.6. Determination of hydroxyl radical (HR) scavenging activity[26]

100 μL of various concentrations (1.9−500 μg/mL) of *R. mucronata* stilt root extract and vitamin C were added separately with 1 mL of iron/EDTA solution (0.13% (w/v) ferrous ammonium sulfate and 0.26% (w/v) EDTA), 0.5 mL of 0.018% (w/v) EDTA and 1 mL of 0.85% (v/v) DMSO (in 0.1 M phosphate buffer, pH 7.4) followed by 0.5 mL of 0.22% (w/v) vitamin C. The tubes were capped tightly and incubated at 85°C for 15 minutes. After that, the tubes were uncapped and ice-cold trichloroacetic acid (17.5%, w/v) was added to each immediately, 3 mL of Nash reagent (7.5 g of ammonium acetate, 300 μL of glacial acetic acid and 200 μL of acetyl acetone made to 100 mL with distilled water) was added to each tube and incubated at room temperature for 15 minutes. The absorbance at 412 nm was measured. The IC_{50} values were determined as the concentration of the test mixture that gave 50% reduction of the absorbance compared to the control blank.

$$\text{HR scavenging activity } (\%) = \left\{ \left[A_{\text{control}} - A_{\text{sample}} \right] / A_{\text{control}} \right\} \times 100$$

2.7. Determination of nitric oxide (NO) radical scavenging activity[27]

Various concentrations (1.9-500 μg/mL) of *R. mucronata* stilt root extract (3 mL) and vitamin C were dissolved in methanol and incubated at 25°C for 150 minutes. The prepared sample was reacted with Greiss reagent (1% (w/v) sulphanilamide, 2% *O*-phosphoric acid and 0.1% *N*-(1-napthyl)ethylenediamine hydrochloride). The absorbance at 546 nm of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylene diamine was measured . The IC_{50} values were determined as the concentration of the test mixture that gave 50% reduction in the absorbance compared to the control blank.

Table 1 Phytochemical constituents of *R. mucronata* extracts

Constituent	Collar	Bark	Stilt root	Hypocotyls
Reducing sugar	+	+	+	+
Protein	+	+	+	+
Phenolic group	+	+	+	+
Alkaloid	+	+	+	+
Steroid	−	−	−	−
Triterpenes	+	+	+	+
Flavonoids	+	+	+	+
Catachin	+	+	+	+
Tannin	+	+	+	+
Anthroquinone	+	+	+	−

+ = presence; − = absence.

$$\text{NO scavenging activity } (\%) = \left\{\left[A_{\text{control}} - A_{\text{sample}}\right] / A_{\text{control}}\right\} \times 100$$

2.8. Determination of lipid peroxidation (LPO) inhibition assay[28]

Liver homogenate was prepared from male Wistar albino rats. The liver was excised after decapitation and exanguination, washed three times with ice-cold 0.15 M KCL in a Teflon tissue homogenizer and the protein content was adjusted to 500 μg/mL. In the control system, 1 mL of tissue homogenate, lipid peroxidation was initiated by the addition of 25μM $FeSO_4$, 100μM ascorbate, 10 mM KH_2PO_4, the volume was made to 3 mL with distilled water and incubated at 37°C for 30 minutes. The homogenate was incubated with different concentrations of *R. mucronata* stilt root extract and vitamin C (1.9–500 μg/mL). The extent of inhibition of lipid peroxidation was evaluated by estimation of thiobarbituric acid-reactive substances (TBARS) by measuring the absorbance at 532 nm. The IC_{50} values were determined as the concentration of the test mixture that gave 50% reduction in the absorbance compared to the control blank.

$$\text{LPO scavenging activity } (\%) = \left\{\left[A_{\text{control}} - A_{\text{sample}}\right] / A_{\text{control}}\right\} \times 100$$

2.9. Determination of ferric reducing antioxidant power assay (FRAP)[29]

Various concentrations (1.9–500 μg/mL) of stilt root extract (1ml) and vitamin C were mixed with 3 mL of FRAP reagent ((a) 300 mM acetate buffer, pH 3.6; (b) 10 mM (2,4,6-tripyridyl-*S*-triazine) in 40 mM HCl (TPTZ); (c) 20 mM $FeCl_3.6H_2O$. The working reagent was prepared by mixing (a) (b) and (c) at a ratio of 10:1:1 (by vol.) at the time of use) and incubated at 25°C for 5 minutes. The absorbance at 593 nm was measured. The IC_{50} values were determined as the concentration of the test mixture that gave 50% reduction in the absorbance compared to the control blank.

$$\text{FRAP reducing activity } (\%) = \left\{\left[A_{\text{control}} - A_{\text{sample}}\right] / A_{\text{control}}\right\} \times 100$$

2.10. Determination of superoxide (SOD) radical scavenging assay[30]

200 μL volume of *R. mucronata* stilt root extract at various concentrations (1.9–500 μg/mL) and vitamin C was mixed with 480 μL of 0.05 M sodium carbonate (pH 10.5), 20 μL of 3 mM xanthane, 20 μL of 3 mM EDTA, 20 μL of 0.15% (w/v) bovine serum albumin and 20 μL of 0.75 mM NBT. The mixture was incubated at 25°C for 20 minutes and the reaction was terminated by adding 20 μL of 6 mM CuCl. The absorbance at 560 nm was measured. The IC_{50} values were determined as the concentration of the test mixture that gave 50% reduction in the absorbance compared to the control blank.

$$\text{SOD scavenging activity } (\%) = \left\{\left[A_{\text{control}} - A_{\text{sample}}\right] / A_{\text{control}}\right\} \times 100$$

2.11. Statistical analysis

All statistical analysis was done with Microsoft Office 2007 with the XP/SDAS addin and the level of statistical significance was set at $p < 0.05$.

3. Results

Analysis of the plant extracts showed the presence of reducing sugars, protein, phenolic groups, alkaloids, triterpenoids, flavonoids, catachin, tannin and anthroquinone. However, steroids were not present in any plant part (Table 1). The present study found that the level of SGOT (313.5 ± 16.53 IU/L), SGPT (232.65 ± 17.38 IU/L), ALP (956.36 ± 64.72 IU/L), bilurubin (3.12 ± 0.48 mg/dL), cholesterol (243.45 ± 15.43 mg/dL), sugar (162.66 ± 7.65 mg/dl) and LDH (2785.00 ± 236.50 IU/L) were increased significantly ($p < 0.05$) in the hepatotoxin treatment group (Group 2) compared to the control group. However, the content of total protein (3.48 ± 0.47 g/dL) and albumin (1.27 ± 0.46 g/dL) were significantly ($p < 0.05$) decreased compared to the control group. Of the selected plant parts, the stilt root extract-treated rats showed a significant level

Table 2 Effect of *R. mucronata* plant parts on the biochemical parameters of CCl_4-induced hepatotoxicity in rats

	Control	Hepatotoxin group (CCl_4)	Silymarin Positive control	Plant part			
				Bark	Collar	Hypocotyl	Stilt root
SGOT (IU/L)	167.33 ± 13.27	313.5 ± 16.53*	181.85 ± 8.630*	310.60 ± 20.35*	195.35 ± 15.63*	215.85 ± 27.3*	185.36 ± 26.35*
SGPT (IU/L)	60.67 ± 5.39	232.65 ± 17.38*	84.13 ± 6.370*	215.68 ± 20.36*	210.37 ± 23.65*	228.63 ± 26.3*	76.54 ± 6.37*
ALP (IU/L)	135.27 ± 9.67	956.36 ± 64.72*	192.85 ± 10.260*	863.37 ± 74.36*	573.37 ± 38.73*	1083.93 ± 65.7*	234.63 ± 23.86*
BIL (mg/dl)	1.28 ± 0.13	3.12 ± 0.48*	1.46 ± 0.190*	3.85 ± 0.37^{ns}	2.96 ± 0.87*	3.10 ± 0.3*	2.43 ± 0.63*
CHL (mg/dl)	90.34 ± 7.64	243.45 ± 15.43*	110.35 ± 6.880*	218.65 ± 16.85*	215.87 ± 27.65*	223 ± 17.63*	137 ± 6.38*
SUG (mg/dl)	85.66 ± 10.34	162.66 ± 7.65*	101.36 ± 7.630*	147.35 ± 18.65*	157.83 ± 17.85*	168.37 ± 12.53^{ns}	115.63 ± 8.96*
LDH (U/L)	886 ± 137.65	2785 ± 236.50*	1842 ± 23*	2680 ± 376*	2075 ± 364.25*	2237 ± 265.23*	1631.00 ± 335.65*
TPN (g/dl)	7.80 ± 0.36	3.48 ± 0.47*	6.33 ± 0.46*	3.13 ± 0.73*	3.56 ± 0.87*	3.26 ± 0.96^{ns}	3.83 ± 0.73*
ALB (g/dl)	4.27 ± 0.98	1.27 ± 0.46*	2.98 ± 0.43*	1.35 ± 0.39*	1.63 ± 0.83*	1.86 ± 0.30*	1.95 ± 0.16*

Data are given as mean ± SD of six animals. *Significant difference ($p < 0.05$) from control or CCl_4-treated rats.
ALP = alkaline phosphatase; BIL = bilurubin; CHL = cholesterol; LDH = lactate dehydrogenase; ns = not significant ($p > 0.05$); SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase; SUG = sugar.

of maximum reduction in SGOT (185.36 ± 26.35 IU/L), SGPT (76.54 ± 6.37 IU/L), ALP (234.63 ± 23.86 IU/L), cholesterol (137 ± 6.38 mg/dL), sugar (115.63 ± 8.96 mg/dL) and LDH (1631.00 ± 335.65 IU/L) compared to the hepatotoxin-treated rats but the level of total protein (3.83 ± 0.73 g/dL) and albumin (1.95 ± 0.16 g/dL) were increased significantly ($p < 0.05$) in stilt root extract-treated rats when compared to hepatotoxin-treated rats (Table 2). Histopathological scores of different plant parts showed that the maximum level of fatty changes (3), focal necrosis (3), congestion in central vein (3) and congestion in sinusoidal spaces (2) were found in hepatotoxin-treated rats. However, maximum reduction of histopathological scores was observed in the

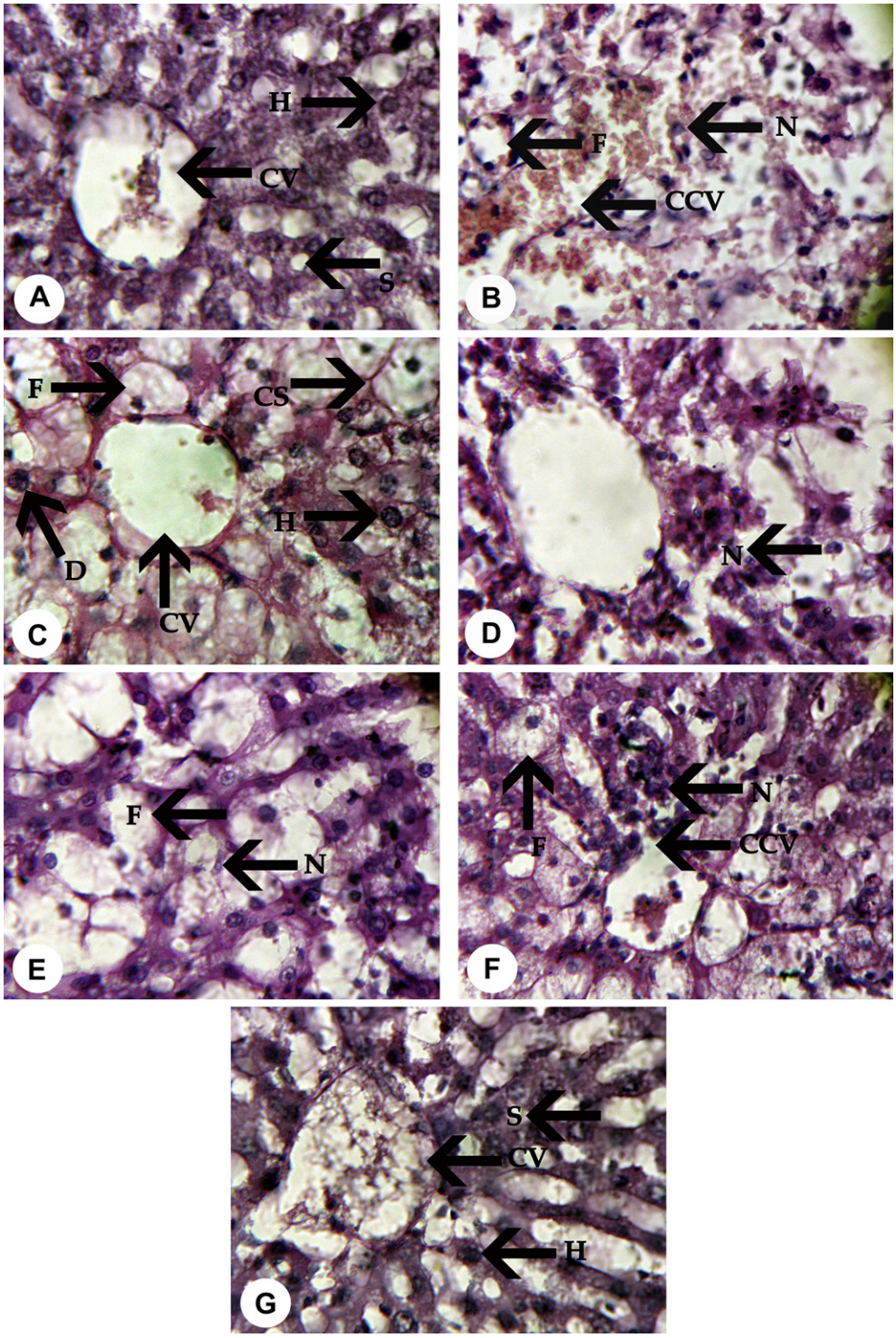

Figure 1 Effect of *R. mucronata* plant parts on CCl_4-induced hepatotoxicity in rats (A) Control group; (B) CCl_4-treated rats; (C) silymarin-treated rats (100 mg.kg^{-1} bw); (D) bark-treated rats, (E) collar-treated rats (F) hypocotyl-treated rats; (G): stilt root-treated rats. Liver sections were stained with haematoxylin and eosin (40× 10×). CCV = congestion in central vein; CS = congestion in sinusoidal spaces; CV = central vein; D = hepatic deformities; F = fatty changes; H = hepatocytes; N = necrosis, PV = portal vein; S = sinusoids; V = vacuoles.

Table 3 Hepatoprotective effect of *R. mucronata* on the histopathological scores of treated rats

	Fatty changes	Hydrophobic changes	Focal necrosis	Congestion in central vein	Congestion in sinusoidal spaces	Hepatocytes deformation	Total
Control	0	0	0	0	0	0	0
Hepatotoxin group(CCl_4)	3	0	3	3	2	0	11
Silymarin (100 mg/kg body weight)	1	2	0	0	0	1	4
Bark	3	0	1	1	2	0	7
Collar	1	0	1	2	0	0	4
Hypocotyl	2	0	1	2	1	0	6
Stilt root	1	0	0	0	0	0	1

0 = no visible cell damage; 1 = focal hepatocytes damage on <25% of tissue; 2 = focal hepatocytes damage on <25–50% of the tissue; 3 = extensive, but focal hepatocytes lesion; 4 = global hepatocytes necrosis.

R. mucronata stilt root extract-treated rats (1) followed by collar extract-treated rats (4) (Figure 1 and Table 3).

The dose-dependent (75, 150 and 300 mg/kg body weight) hepatoprotective activity of *R. mucronata* stilt root extract showed that the levels of SGOT, SGPT, ALP, bilurubin, cholesterol, sugar and lactate dehydrogenase were significantly ($p < 0.05$) reduced in all doses when compared to the hepatotoxin-treated rats but the maximum reduction of SGOT (191.36 ± 24.32 IU/L), SGPT (81.54 ± 5.73 IU/L), ALP (228.63 ± 18.56 IU/L), bilurubin (2.52 ± 0.83 mg/dL), cholesterol (129.87 ± 6.42 mg/dL), sugar (121.63 ± 7.38 mg/dL) and lactate dehydrogenase (1601.00 ± 305.65 U/L) were observed with the high dose (300 mg/kg) of stilt root extract. Moreover, the level of total protein (4.01 ± 0.73 g/dL) and albumin (2.27 ± 0.16 g/dL) were significantly ($p < 0.05\%$) increased with the maximum dose (300 mg/kg) of stilt root extract (Table 4). Histopathological scores revealed that the low (75 mg/kg) and medium (150 mg/kg) doses showed reduction (5 and 2, respectively) in fatty changes, focal necrosis, hydrophic changes, congestion in central vein and congestion in sinusoidal spaces when compared to the hepatotoxin groups (Table 5) and no histopathological alteration was observed with the high dose (300 mg/kg) of stilt root extract with mild fatty changes (Figure 2 and Table 5).

The *in vitro* antioxidant assay of stilt root extract revealed that the minimum IC_{50} value was 22.80 ± 0.93 µg/mL for the superoxide radical scavenging assay followed by the FRAP reduction assay (25.79 ± 0.93 µg/mL). Moreover, the results are comparable with the positive control of vitamin C (Table 6).

4. Discussion

The extent of hepatic damage is based on the formation of trichloro free radicals from carbon tetrachloride molecules, which directly affects the polyunsaturated fatty acids of the endoplasmic reticulum and thus the liver microsomal membrane, increasing the enzymatic levels (SGOT, SGPT, AST, ALP and LDH) and decreasing metabolic functions (synthesis of protein, albumins and inhibits the storage of cholesterol and sugars) in liver cells.[3] The present findings revealed that extracts of *R. mucronata* parts, such as bark, collar, hypocotyls and stilt root, had a range of hepatoprotective activity but the maximum percentage of hepatoprotective activity was observed in stilt root extract. This might be owing to the presence of chemical classes such as flavonoids,[31,32] polyphenols,[33] alkaloids[34] and coumarins.[35] The possible hepatoprotective mechanism of the action might be due to the inhibition of the cytochrome P450-dependent oxygenase activity and preventing the lipid peroxidation[31] and stabilization of hepatocyte membrane.[32] In biological system, oxidative stress is the state of imbalance between the level of the antioxidants defense system and re production of oxygen-derived species such as superoxide radicals; hydroxyl radicals etc., which cause the cell damage. The *in vitro* DPPH free radical scavenging property of the *R. mucronata* stilt root extract suggests the prevention of the free radical species from damaging biomolecules such as lipoproteins, polyunsaturated fatty acids (PUFA), DNA, amino acids, proteins and sugars in a biological system.[36,37] Moreover, hydroxyl radicals and lipid peroxides cause the cell damage by inducing DNA strand breakage[38] and the *in vitro* hydroxy radicals and lipid peroxide scavenging property of the stilt root extract might prevent the hepatotoxicity effect in liver cells.[39,40] Similar results are reported with the leaf extract of *Mussaenda glabra*.[41] Additionally, nitric oxide (NO*) and superoxide (O_2^{*-}) anion free radicals cause ischemic renal injury effects in liver cells.[42] The *in vitro* reduction of the nitric oxide free radical scavenging property of the *R. mucronata* stilt root extract enhances the capacity to prevent liver damage. The free radical scavenging property of the *R. mucronata* stilt root extract is able to donate hydrogen atoms to the free radicals and to convert them into more stable products strongly inhibiting liver damage.[43,44] The FRAP method describes the reducing ability of antioxidant compounds of the stilt root extract from Fe^{III}-TPTZ to Fe^{II} complex.[29,45,46] Histopathological examination of liver sections revealed that the normal liver architecture was disturbed by CCL_4 intoxication. In liver sections

Table 4 Effect of dose-dependent *R. mucronata* stilt root extract on the biochemical parameters of CCl_4-induced hepatotoxicity in rats

	Control	Hepatotoxin group (CCl_4)	Silymarin Positive control	*R. mucronata* stilt root extract (mg/kg body weight)		
				75	150	300
SGOT (IU/L)	154.84 ± 24.36	348.98 ± 20.89*	193.98 ± 9.74*	337.34 ± 18.58*	217.87 ± 19.38*	191.36 ± 24.32*
SGPT (IU/L)	54.83 ± 13.23	217.75 ± 21.87*	92.63 ± 8.69*	167.63 ± 15.34*	101.83 ± 14.73*	81.54 ± 5.73*
ALP (IU/L)	167.82 ± 13.82	1023.34 ± 117.38*	324.39 ± 84.87*	972.69 ± 138.96*	398.67 ± 87.97*	228.63 ± 18.56*
BIL (mg/dl)	1.47 ± 0.43	3.86 ± 0.76*	2.28 ± 0.94*	2.89 ± 0.63*	2.68 ± 0.95*	2.52 ± 0.83*
CHL (mg/dl)	98.87 ± 8.58	265.63 ± 21.43*	124.63 ± 13.36*	267.38 ± 23.68^{ns}	168.63 ± 11.64*	129.87 ± 6.42*
SUG (mg/dl)	78.95 ± 14.84	183.34 ± 13.84*	128.37 ± 13.61*	154.65 ± 14.54*	142.64 ± 17.38*	121.63 ± 7.38*
LDH (U/L)	938.37 ± 168.68	3010.00 ± 198.54*	1884.70 ± 348.47*	2063.74 ± 187.38*	1938.21 ± 217.36*	1601.00 ± 305.65*
TPN (g/dl)	8.68 ± 1.03	3.08 ± 0.87*	5.97 ± 0.81*	3.43 ± 0.96*	3.69 ± 0.39*	4.01 ± 0.73*
ALB (g/dl)	4.38 ± 0.84	1.87 ± 0.64*	3.01 ± 0.38*	1.36 ± 0.49^{ns}	1.95 ± 0.93*	2.27 ± 0.16*

Data are given as mean ± SD of six animals. *Significant difference ($p < 0.05$) from control or CCl_4 –treated rats.
ALP = alkaline phosphatase; BIL = bilurubin; CHL = cholesterol; LDH = lactate dehydrogenase; ns = not significant ($p > 0.05$); SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase; SUG = sugar.

Table 5 Dose-dependent histopathological scores of *R. mucronata* stilt root extract in CCl_4-induced hepatotoxicity in rats

	Fatty changes	Hydrophobic changes	Focal necrosis	Congestion in central vein	Congestion in sinusoidal spaces	Hepatocytes deformation	Total
Control	0	0	0	0	0	0	0
Hepatotoxin group (CCl_4)	3	0	3	3	2	0	11
Silymarin							
(100 mg/kg body weight)	1	2	0	0	1	0	4
Low dose (75 mg/kg body weight)	2	0	1	1	1	0	5
Medium dose (150 mg/kg body weight)	1	0	1	0	0	0	2
High dose (300 mg/kg body weight)	1	0	0	0	0	0	1

0 = no visible cell damage; 1 = focal hepatocytes damage on <25% of tissue; 2 = focal hepatocytes damage on <25–50% of the tissue; 3 = extensive, but focal hepatocytes lesion; 4 = global hepatocytes necrosis.

of rats treated with different doses (75, 150 and 300 mg/kg) of the *R. mucronata* stilt root extract and intoxicated with CCL_4-treated rats are proved to have improved cellular membrane architecture when compared with the CCL_4-treated rats, which further corroborate the hepatoprotective activity of the stilt root extract. It is concluded from the present study that the extract of *Rhizophora mucronata* stilt roots collected from the Karangadu mangrove forest, Ramanathapuram District, Tamilnadu, India has potential hepatoprotective activity

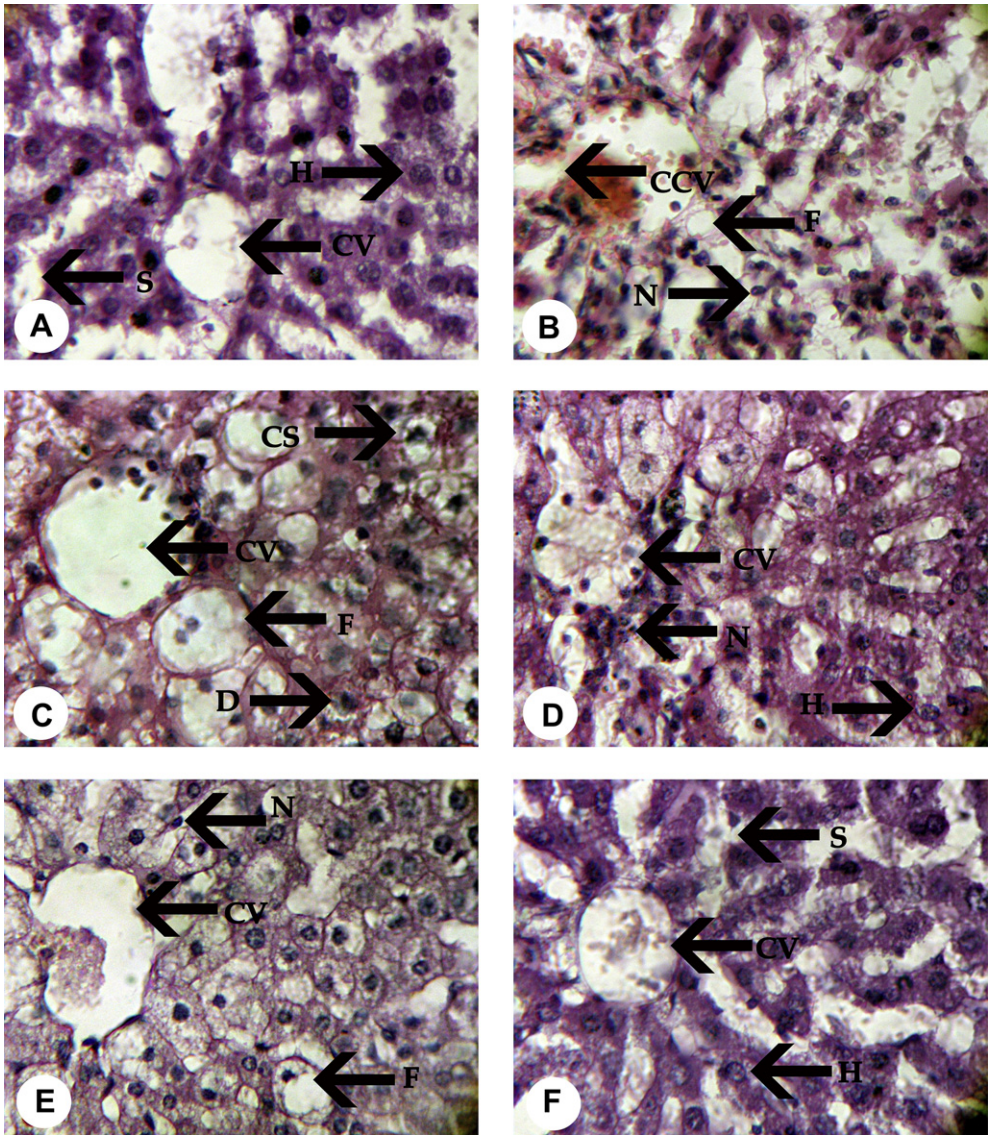

Figure 2 Dose-dependent effect of *R. mucronata* stilt root extract on CCl_4-induced hepatotoxicity in rats (A) Control group; (B) CCl_4-treated rats; (C) silymarin-treated rats (100 mg/kg); (D) 75 mg/kg body weight (low dose) of *R. mucronata* stilt root extract-treated rats; (E) 150 mg/kg body weight (medium dose) of *R. mucronata* stilt root extract-treated rats; (F) 300 mg/kg body weight (high dose) of *R. mucronata* stilt root extract-treated rats. Liver sections stained with haematoxylin and eosin (40× 10×). CCV = congestion in central vein; CS = congestion in sinusoidal spaces; CV body weight = central vein; D = hepatic deformities; F = fatty changes; H = hepatocytes; N = necrosis; PV = portal vein; S = sinusoids; V = vacuoles.

Table 6 IC_{50} values of *R. mucronata* stilt root extract and vitamin C with various antioxidant properties

Scavenging assay	*R. mucronata* stilt root IC_{50} (μg/ml)	Vitamin C IC_{50} (μg/ml)
DPPH	58.33 ± 2.87	2.87 ± 1.26
HR	64.78 ± 1.32	44.24 ± 1.50
NO	72.14 ± 0.94	4.98 ± 1.28
FRAP	25.79 ± 0.93	56.69 ± 1.11
LPO	163.38 ± 0.81	31.79 ± 1.21
SOD	22.80 ± 0.93	24.31 ± 0.71

Data are given as ±SD values of six replicates.

against carbon tetrachloride-induced hepatotoxicity in Wistar albino rats providing a new way to develop potential hepatoprotective drugs.

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