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Hepatoprotective and antioxidant properties of marine halophyte *Luminetzera racemosa* bark extract in CCL_4 induced hepatotoxicity

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ABSTRACT

Objective: To identify the hepatoprotective and antioxidant activity of *Luminetzera racemosa (L. racemosa)*bark extract. **Methods:** Wistar albino rats were divided into 6 groups: Group 1 served as control; Group 2 served as hepatotoxin (CCL_4 treated) group; Group 3 served as positive control (Silymarin) treated groups; Group 4, 5 and 6 served as (100, 200 and 300 mg/kg bw p.o.) *L. racemosa* bark extract treated groups. Moreover, *in vitro* antioxidant indexes, including DPPH, hydroxyl radical scavenging activity (HRSA), NO, ferric reducing antioxidant power (FRAP), lipid hydroperoxide (LPO) and super oxide dismutase (SOD) were also analyzed in the bark extract. **Results:** The results suggested that, the level of serum glutamate oxyloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatise (ALP), bilurubin, cholesterol, sugar and lactate dehydrogenase (LDH) were significantly ($P<0.05$) increased in hepatotoxin treated rats when compared with the control group. But, the maximum reduction of SGOT [(225.36 ±13.65) IU/L], SGPT [(96.85±17.36) IU/L], ALP [(315.37±17.16) IU/L], bilirubin [(2.97±0.46) mg/dL], cholesterol [(163.73±17.54) mg/dL], sugar [(127.35±27.35) mg/dL] and LDH [(1 784.00±268.36) IU/L] were observed with 300 mg/kg bw of bark extract treated rats. Histopathological scores showed that, no visible changes were observed with high dose (300 mg/kg bw) of bark extract treated rats except mild fatty changes. The in vitro antioxidant assays showed that, the IC_{50} values were observed as (44.17±6.87) μg/mL, (42.45±2.81) μg/mL, (62.37±3.98) μg/mL, (54.24±3.09) μg/mL, (87.25±5.90) μg/mL and (71.54±5.42) μg/mL for DPPH, HRSA, NO, FRAP, LPO and SOD radical scavenging activities, respectively. **Conclusions:** The hepatoprotective and antioxidant activities of the bark extract might be to the presence of unique chemical classes such as flavonoids, alkaloids and polyphenols.

1. Introduction

Reactive oxygen species (ROS), plays important role in the production of tissue injury through covalent binding and lipid peroxidation and can augment fibrosis as seen from increased collagen synthesis[1]. Scavenging of free radicals by antioxidants could reduce the fibrosis process in the tissue[2]. Recently, clinical importance of herbal drugs has received considerable attention. Free radicals may also contributory factor in the function of the immune system[3]. Several medicinal plants have been screened based on the integrative approaches on drug development against liver diseases[4]. Even though, studies related with the scientific evaluation of the mangrove plants against liver diseases are too restricted. In this regard, the present study was made an attempt to investigate the hepatoprotective and antioxidant property of the *Luminetzera racemosa(L. racemosa)* bark extract against carbon tetrachloride induced hepatotoxicity in Wistar albino rats.

2. Materials and methods

2.1.Collection and preparation of extracts

The bark samples of *L. racemosa* were collected during the spring season from Pichavaram mangrove forest (Lat. 11°

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20′ N; Long. 79° 47′ E), Tamil Nadu, India. The specimen sample was authenticated by Prof. Kathiresan K, Centre of Advanced Study in Marine Biology, Annamalai University, Porto Novo, Tamil Nadu, India. The voucher specimen (AUOCAS033 and AUOCAS034) was also maintained in the herbarium cabinet facility, sponsored by Indian Council of Medical Research, New Delhi at School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi, India. The collected parts were washed thrice with distilled water to remove the contaminants and air dried in shade. Coarsely powdered sample (500 g) was defatted with petroleum ether (60-80 ℃) and then extracted with 1 L of 95% (V/V) ethanol and water mixture by percolation method. The extract was concentrated under vacuum to the solvent free residues. Preliminary phytochemical analysis such as phenolic group, alkaloids, flavonoids, catachin, triterpenoids, tannins, and anthroquinones were calculated by the standard protocols[5].

2.2. Carbon- tetrachloride induced hepatotoxicity

Male Wistar albino rats (150-200 g) were divided into nine groups consisting of 6 animals per group. Group 1 (Control) received three doses of 5% acacia mucilage (1 mL/kg bw po.) with 12 hrs intervals (0 hr, 12 hrs and 24 hrs). Group 2 (Hepatotoxin group) received three doses of vehicle [5% acacia mucilage (1 mL/kg bw po.)] at 12 hrs intervals and a single dose of carbon tetrachloride (2 mL/kg bw ip.) diluted in liquid paraffin (1:1) 30 min after the administration of first dose of vehicle. Group 3 (Positive control) received three doses of silymarin (100 mg/kg bw po.) at 0 hr, 12hrs and 24 hrs intervals. Carbon tetrachloride diluted in liquid paraffin (1:1) (2 mL/kg bw ip.) was administrated 30 min after the administration of first dose of silymarin. Group 4, 5 and 6 (Treatment groups) received with different doses of (100, 200 and 300 mg/kg bw po.) *L. racemosa* bark extract at 0 hr, 12 hrs and 24 hrs intervals. Carbon tetrachloride diluted in liquid paraffin (1:1) (2 mL/kg bw ip.) was administrated 30 min after the administration of first dose of bark extract. All the animal model studies and experimental protocols have been approved by Institutional Animal Ethics Committee, Alagappa University, Tamil Nadu, India.

2.3. Liver function test

After 36 h of administration of CCl_4, all the animals were anesthetized with mild ether and blood samples were collected by eye bleeding method. The collected blood was allowed to clot at room temperature and serum was separated by centrifugation at 2 500 rpm for 10 min. The serum was used for the estimation of biochemical parameters to determine the functional state of liver. Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were assayed by following the method of Vidya *et al*(2007)[6]. Total protein and albumin[7], sugar[8], cholesterol[9] and bilirubin[10] were assayed using randox laboratory kits.

2.4. Analysis of histopathological scores

Animal from each group were sacrificed and the abdomen was cut open to remove the liver. Then the liver was fixed in bouins solution (mixture of 75 mL of saturated picric acid, 25 mL of 40% formaldehyde and 5 ml of glacial acetic acid) for 12 hrs, then embedded in paraffin using conventional methods and cut into 5 μm thick sections and stained using haematoxylin-eosin dye and finally mounted in di-phenyl xylene[11]. The section was observed under microscope for any histopathological changes. The liver pathology was scored as described by Jamshidzadeh[12]. Histological damage was expressed using the following score system: 0= no visible cell damage; 1= focal hepatocytes damage on <25%-50% of the tissue; 3=extensive, but focal hepatocytes lesion; 4= global hepatocytes necrosis.

2.5. In vitro antioxidant assay

In vitro antioxidant assays such as DPPH scavenging and nitric oxide radical scavenging[13], hydroxyl radical scavenging[14], lipid peroxide radical scavenging, ferric reducing antioxidant(FRAP) and superoxide radical scavenging[15] were calculated with different concentrations (1.9 μg/mL to 500 μg/mL) of *L. racemosa* bark extract and vitamin C (Positive control). Statistical calculations such as IC_{50} values and SD values calculated with office XP/SDAS add-ins program.

3. Results

The results of the present study suggested 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 [(2 785.00±236.50) IU/L] were significantly (P<0.05) increased in hepatotoxin treatment (Group 2), but the content of total protein [(3.48±0.47) g/dL] and albumin [(1.27 ±0.46) g/dL] were significantly (P<0.05) found less when compared with the control group (Group 1). But, different concentration of (75, 150 and 300 mg/kg bw) bark extract treated rats showed significant (P<0.05) reduction in the elevated level but, the maximum reduction of SGOT [(225.36 ±13.65) IU/L], SGPT [(96.85±17.36) IU/L], ALP [(315.37±17.16) IU/L], bilirubin [(2.97±0.46) mg/dL], cholesterol [(163.73± 17.54) mg/dL], sugar [(127.35±27.35) mg/dL] and LDH [(1 784.00±±268.36) IU/L] were observed with the high dose extract treated rats (Table 1). Histopathological scores revealed 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, *L. racemosa* bark extract (75 mg/kg bw and 150 mg/kg bw) treated rats showed reduction in fatty changes, focal necrosis, hydrophic changes, and no visible changes were observed with high dose (300 mg/kg bw) of bark extract treated rats except mild fatty changes when compared with the hepatotoxin treated groups (Figure 1). The *in vitro* antioxidant assays showed that, the IC_{50} values were observed as (44.17±6.87) μg/mL, (42.45±2.81) μg/mL, (62.37±3.98) μg/mL, (54.24±3.09) μg/mL, (87.25±5.90) μg/mL and (71.54±5.42) μg/mL for DPPH, hydroxyl radical scavenging activity (HRSA), NO, FRAP, lipid hydroperoxide (LPO) and super oxide dismutase (SOD) radical scavenging activities, respectively, and the results are comparable with the positive control of the vitamin C (Table 2). The preliminary phytochemical analysis of the bark extract showed the presence of reducing sugars, protein, phenolic groups, alkaloids, triterpenoids and tannins. However, the

Table 1

Effect of *L. racemosa* bark extract on the biochemical parameters in CCl_4 induced hepatotoxicity in rats

Parameters	Control	Hepatotoxin group(CCl_4)	Silymarin positive control	*L. racemosa* bark extract (mg/kg bw)		
				100	200	300
SGOT(IU/L)	167.33±13.27	313.5±16.53*	181.85±8.63#	304.4±18.28#	261.43±32.29#	225.36±13.65#
SGPT(IU/L)	60.67±5.39	232.65±17.38*	84.13±6.37#	204.02±21.12#	194.54±17.31#	96.85±17.36#
ALP(IU/L)	135.27±9.67	956.36±64.72*	192.85±10.26#	671.26±118.12#	490.23±64.14#	315.37±17.16#
BIL(mg/dL)	1.28±0.13	3.12±0.48*	1.46±0.19#	2.97±0.87#	2.26±0.44#	2.97±0.46#
CHL(mg/dL)	90.39±7.69	243.45±15.43*	110.55±6.88#	217.64±21.36#	181.32±6.24#	163.73±17.54#
SUG(mg/dL)	85.66±10.34	162.66±7.65*	101.36±7.63#	172.32±8.39	148.00±4.54#	127.35±27.35#
LDH(U/L)	886.00±137.65	2 785.00±236.50*	1 842.00±31.00#	2 621.46±175.47#	2 041.01±158.06#	1 784.00±268.36#
TPN (g/dL)	8.68±1.03	3.08±0.87*	5.97±0.81#	4.43±1.96#	5.72±0.25#	6.95±0.26#
ALB (g/dL)	4.38±0.84	1.87±0.64*	3.01±0.38#	2.36±0.49#	3.02±0.93#	4.00±0.84#

BIL: bilurubin, CHL: cholesterol, SUG: sugar; * $P<0.05$ *vs.* control, #$P<0.05$ *vs.* CCl_4 –treated rats.

Table 2

IC_{50} values of *L. racemosa* bark extract and vitamin C with various antioxidant activities (μ g/mL).

Parameter	*L. racemosa* bark extract	Vitamin C
DPPH	44.17±6.87	2.87±1.26
HRSA	42.45±2.81	44.24±1.50
NO	62.37±3.98	4.98±1.28
FRAP	54.24±3.09	56.69±1.11
LPO	87.25±5.90	31.79±1.21
SOD	71.54±5.42	24.31±0.71

steroids and anthroquinone were not observed with the bark extract.

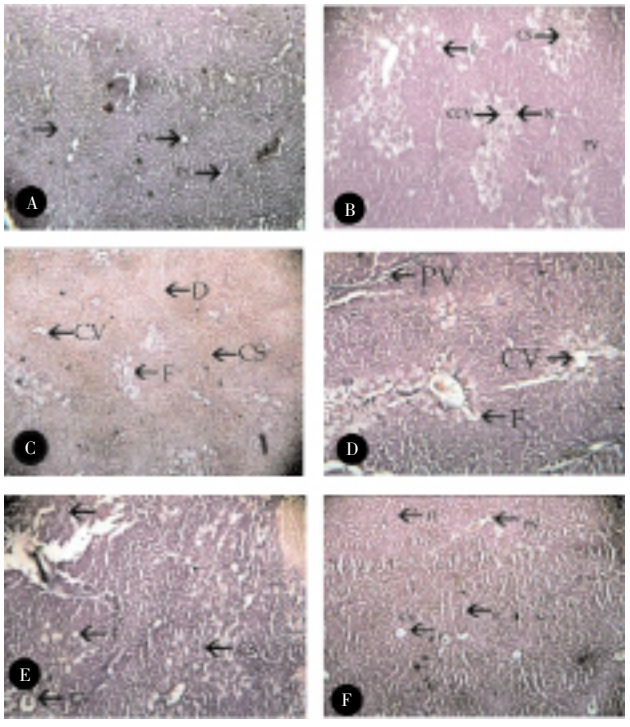

Figure 1. Liver sections stained with haematoxylin and eosin (40×). (A) Control group; (B) CCl_4 treated rats; (C) Silymarin treated rats (100 $mg\cdot kg^{-1}\cdot bw$); (D) 100 $mg\cdot kg^{-1}\cdot bw$ of *L. racemosa* bark extract treated rats (E) 200 $mg\cdot kg^{-1}\cdot bw$ *L. racemosa* bark extract treated rats (F) 300 $mg\cdot kg^{-1}\cdot bw$ of *L. racemosa* bark extract treated rats. CV: Central vein; S: Sinusoids; H: Hepatocytes; N: Necrosis, F: Fatty changes; V: Vacuoles; CS: Congestion in sinusoidal spaces; CCV: Congestion in central vein; PV: Portal vein D: hepatic deformities.

4. Discussion

World health organization survey indicates that, a total of about 70%–80% of the world's population rely on, non commercial medicine mainly the herbal sources, in the primary health care units[16]. Although, plants and plant materials play several important roles in biological and pharmacological activities in humans as well as in animals[17]. In this connection, one such marine plant part of the *L. racemosa* bark extract was selected for *in vivo* hepatoprotective activity against CCL_4 induced hepatotoxicity in Wistar albino rats. The result of the present studies suggest that, the hepatotoxic effect of CCL_4 is due to the conversion by the cytochrome– p450 mixed function enzyme in the smooth endoplasmic reticulum of the liver and other organs to the highly reactive CCL_3 molecules[18]. Highly reactive tricoloro free radicals formation directly attacks the poly unsaturated fatty acids of the endoplasmic reticulum and thus causes over production of SGOT, SGPT, ALP, bilirubin and LDH enzymes[19]. Pre–treatment of *L. racemosa* bark extract showed the antagonizes activity against the elevated enzyme parameters. Reduction in the level of SGOT, LDH and SGOT enzymes towards the normal value is an indication of the hepatic cell regeneration process[18]. Reduction in the level of ALP with the concurrent depletion with the raised bilirubin level suggest the stability of the biliary function. In CCL_4 intoxicated rats the level of total protein and albumin were significantly reduced this might be due to the functional failure of the cytochrome p450 complexes[18]. But, *L. racemosa* bark extract treated rats showed raised level of total protein and albumin concentration, this might be due to the stabilization of endoplasmic reticulum[20]. Suppression of the cholesterol level by the extracts suggests the bile acids synthesis inhibition was reversed[21]. The protective

effect exhibited by the high dose (300 mg/kg bw) of *L. racemosa* bark extract was comparable with the standard drug silymarin (100 mg/kg bw). The histopathological examination of the liver sections confirmed that, normal liver cellular architecture was damaged with CCL_4 treated rats. However, the liver sections from bark extract treated rats showed that, the reduction in histopathological scores as well as cellular damage and thus further, confirming the hepatoprotective effect of the *L. racemosa* bark extract[22]. Moreover, in biological systems lipid bi-layers of the cellular membrane are potential target site for the free radicals and this oxidants plays several pathological conditions including aging, hepatotoxicity, cancer and inflammation. The *in vitro* antioxidant studies revealed such as DPPH, superoxide radical scavenging, hydroxyl radical scavenging and lipid peroxide radical scavenging activity of the *L. racemosa* bark extract and thus suggest the ability of the good antioxidant activities[14,23,24]. The hepatoprotective and antioxidant activity of the bark extract might be due to the presence of unique phytochemical constituents such as flavonoids, alkaloids[25] and polyphenols[26] and its free radical scavenging activities. In conclusion, the present studies demonstrated that, pre administration of the *L. racemosa* bark extract could provide good hepatoprotective activity against CCL_4 intoxication in Wistar albino rats and could be used as an herbal medicine for the treatment of liver damage after completing the successful clinical trials.

Conflict of interest statement

We declare that we have no conflict of interest.

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