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# Hepatoprotective and antioxidant activity of a mangrove plant *Lumnitzera racemosa*

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## ABSTRACT

**Objective:** To identify the hepatoprotective and *in vitro* antioxidant activity of *Lumnitzera racemosa* (*L. racemosa*) leaf extract. **Methods:** Animals in Group 1 served as vehicle control, Group 2 served as hepatotoxin (CCL<sub>4</sub> treated) group, Group 3 served as positive control (Silymarin) group, and Group 4, 5 and 6 served as (75, 150 and 300 mg/kg bw *p.o.*) *L. racemosa* leaf extract treated groups. Moreover, *in vitro* antioxidant DPPH, hydroxyl radical scavenging activity (HRSA), NO, ferric reducing antioxidant power (FRAP), lipid hydroperoxide (LPO) and super oxide dismutase (SOD) were also analyzed for the leaf extract. **Results:** The levels of the serum parameters such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), bilirubin, cholesterol (CHL), sugar and lactate dehydrogenase (LDH) were significantly increased in CCL<sub>4</sub> treated rats when compared with the control group ( $P < 0.05$ ). But the *L. racemosa* leaf extract treated rats showed maximum reduction of SGOT [(210.36 ± 19.63) IU/L], SGPT [(82.37 ± 13.87) IU/L], ALP [(197.63 ± 23.43) IU/L], bilirubin [(2.15 ± 0.84) mg/dL], cholesterol [(163.83 ± 15.63) mg/dL], sugar [(93.00 ± 7.65) mg/dL] and LDH [(1134.00 ± 285.00) IU/L] were observed with the high dose (300 mg/kg bw) of leaf extract treated rats. Histopathological scores showed that, no visible changes were observed with high dose (300 mg/kg bw) of leaf extract treated rats except few mild necrosis. The IC<sub>50</sub> values were observed as (56.37 ± 4.87) μg/mL, (57.68 ± 1.98) μg/mL, (64.15 ± 2.90) μg/mL, (61.94 ± 3.98) μg/mL, (94.53 ± 1.68) μg/mL and (69.7 ± 2.65) μg/mL for DPPH, HRSA, NO, FRAP, LPO and SOD radical scavenging activities, respectively. **Conclusions:** In conclusion, the hepatoprotective effect of the *L. racemosa* leaf extract might be due to the presence of phenolic groups, terpenoids and alkaloids and *in vitro* antioxidant properties.

## 1. Introduction

Oxidative stress plays an important role in many diseases including liver diseases[1]. The production of oxidative stress can be controlled by the antioxidant systems in living organisms. However, the over production of oxidative stress can lead to damage in DNA, cell membrane, protein and cellular membranes and consequently induces degeneration, destruction and toxicity of various molecules[2] and causes muscular dystrophy, cancer as well as liver diseases. Currently, many synthetic antioxidant drugs (BHT, TBHQ) have been used in drug composition. However, these synthetic drugs can cause many side effects and then lead to many potential health problems. In this connection, herbal drugs have gained importance and popularity in

recent years because of their safety, efficacy and cost effectiveness. Herbal based therapeutics for liver disorders have been practiced in India for a long time and popularized globally by leading pharmaceutical companies[3]. For instance, the hepatoprotective activity of terrestrial plants *viz* *Vitis vinifer*[4], *Phyllanthus maderaspatensis*[5], *Achillea millefolium*[6], *Cytisus scoparius*[7] and *Curcuma longa*[8] has been identified and popularized. But limited studies are available on the use of marine halophytes for the management of liver diseases. Hence, the present study was aimed to identify the hepatoprotective effect of the *Lumnitzera racemosa* (*L. racemosa*) leaf extract on the carbon tetrachloride induced hepatotoxicity in rat models.

## 2. Materials and methods

### 2.1. Extraction preparation

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The fresh matured leaves of *L. racemosa* was collected from Pichavaram mangrove forest (Lat. 11° 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 (AUOCAS031) was also maintained in the herbarium cabinet facilities sponsored by Indian Council of Medical Research, New Delhi. The leaves 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 °C) 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, catechin, triterpenoids, tannins, and anthroquinones were analyzed by the standard protocols<sup>[9]</sup>.

## 2.2. Animals

Male Wistar albino rats (150–200 g) were maintained under standard conditions [23±2 °C, relative humidity (55±10)% and 12 h L: 12 h D cycle] and allowed free accesses to food (Sai Durga Feeds and Foods, Bangalore, India) and water. Experimental protocols were approved by Institutional Animal Ethics Committee, Alagappa University, Tamil Nadu, India.

## 2.3. Hepatoprotective activity

The animals were divided into six groups, consisting of 6 animals each. Animals in Group 1 were treated with distilled water (5 mL/kg bw) for 9 days and served as control group. Group 2 was treated with distilled water (5 mL/kg bw) for 9 days and single dose of (2 mL/kg bw, ip) carbon tetrachloride on the 9th day with liquid paraffin (1:1) and hepatotoxin. Group 3 was treated with silymarin at the dose of 100 mg/kg bw, was administrated through oral gavage for 9 days with single dose of (2 mL/kg bw, ip) carbon tetrachloride on the 9th day with liquid paraffin (1:1) and served as positive control group. Animals of Group 4, 5 and 6 were treated with ethanolic extracts of 75, 150 and 300 mg/kg bw. They were administrated through oral gavage for 9 days with single dose of (2 mL/kg bw, ip) carbon tetrachloride on the 9th day with liquid paraffin (1:1) and served as treatment groups.

## 2.4. Analysis of liver function enzymes

On the 10th day, all 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 assessed by the method of Vidya *et al*<sup>[10]</sup>. Total protein, albumin, sugar, cholesterol and bilirubin were analyzed by

using standard randox laboratory kits.

## 2.5. Analysis of histopathological scores

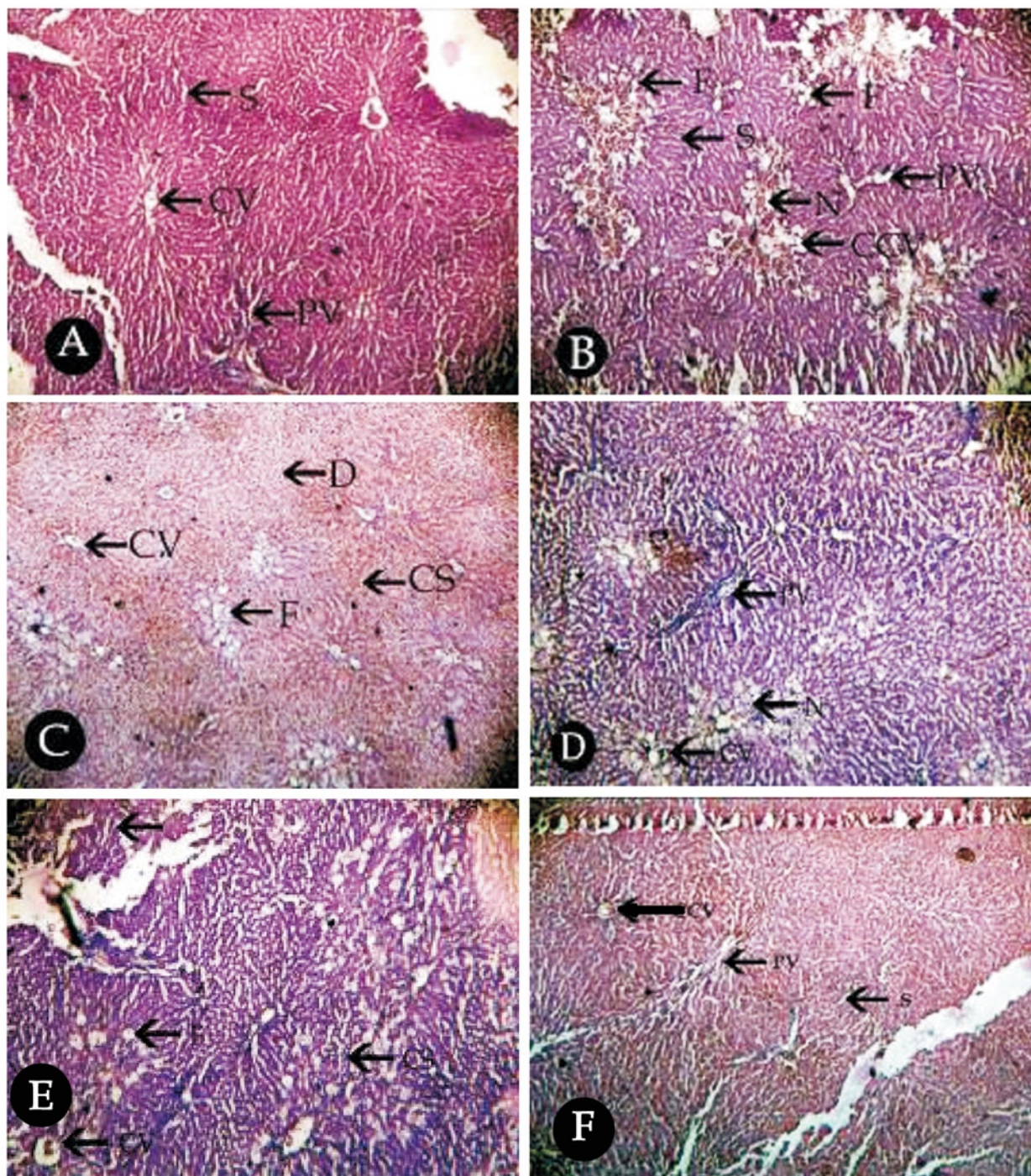
Animals were sacrificed and the abdomen was cut open to remove the liver. Then the liver was fixed in bouin's solution (mixture of 75 mL of saturated picric acid, 25 mL of 40% formaldehyde and 5 mL of glacial acetic acid) for 12 hrs, then embedded in paraffin using conventional methods<sup>[11]</sup>. They were cut into 5 µm thick sections and stained using haematoxylin–eosin dye and finally mounted in di-phenyl xylene. The section was observed under microscope for any histopathological changes. The liver pathology score was calculated as described by Jamshidzadeh *et al*<sup>[12]</sup>. 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.

## 2.6. Determination of in vitro antioxidant assay

Various concentrations (1.9 µg/mL to 500 µg/mL) of *L. racemosa* leaf extract was subjected for the determination of DPPH assay<sup>[13]</sup>, hydroxyl radical scavenging assay (HRSA)<sup>[14]</sup>, nitric oxide radical scavenging assay, lipid peroxide (LPO) radical scavenging assay, ferric reducing antioxidant power (FRAP) assay and superoxide radical scavenging assay<sup>[15]</sup> by using standard protocols with vitamin C (positive control). Statistical calculations such as IC<sub>50</sub> values and SD values were calculated with office XP/SDAS add-ins program.

## 3. Results

The results of the present study showed that, the levels of SGOT [(313.50±16.53) IU/L], SGPT [(232.65±17.38) IU/L], ALP [(956.36±64.72) IU/L], bilirubin [(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 significantly increased in hepatotoxin treated group ( $P<0.05$ ) (Group 2) when compared with control group. But the content of total protein [(3.48±0.47) g/dL] and albumin [(1.27±0.46) g/dL] were significantly decreased ( $P<0.05$ ). Administration (75, 150 and 300 mg/kg bw) of *L. racemosa* leaf extract treated rats showed significant reduction in the level of SGOT, SGPT, ALP, bilirubin, cholesterol, sugar and lactate dehydrogenase ( $P<0.05$ ) when compared with hepatotoxin treated rats. But, the maximum reduction of SGOT [(210.36±19.63) IU/L], SGPT [(82.37±13.87) IU/L], ALP [(197.63±23.43) IU/L], bilirubin [(2.15±0.84) mg/dL], cholesterol [(163.83±15.63) mg/dL], sugar [(93.00±7.65) mg/dL] and LDH [(1134.00±285.00) IU/L] were observed in the high dose Group (300 mg/kg bw). Moreover, the level of total protein [(6.01±0.57) g/dL] and albumin [(2.96±0.68) g/dL] were significantly increased in the high dose Group (300 mg/kg bw) ( $P<0.05$ ) (Table 1). Histopathological scores showed that, the maximum level of fatty changes, focal necrosis, congestion in central vein and congestion in sinusoidal spaces were found in hepatotoxin treated rats. However, *L. racemosa*



**Figure 1.** Dose dependant effect of *L. racemosa* leaf extract on  $\text{CCl}_4$  induced hepatotoxicity in Wistar rats.

A: Control group; B:  $\text{CCl}_4$  treated rats; C: Silymarin treated rats (100 mg/kg bw); D: 75 mg/kg bw (Low dose) of *L. racemosa* leaf extract treated rats; E: 150 mg/kg bw (Medium dose) of *L. racemosa* leaf extract treated rats; F: 300mg/kg bw of (High dose) *L. racemosa* leaf extract treated rats. Liver sections stained with haematoxylin and eosin (40 $\times$ ). 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.

leaf extract (75 mg/kg bw and 150 mg/kg bw) pre treated rats showed reduction in fatty changes, focal necrosis, hydrophic changes, and no visible changes except hydrophic changes were observed with high dose (300 mg/kg bw) (Table 2 and Figure 1). The *in vitro* antioxidant assays showed that the  $\text{IC}_{50}$  values were (56.37 $\pm$ 4.87)  $\mu\text{g/mL}$ , (57.68 $\pm$ 1.98)  $\mu\text{g/mL}$ , (64.15 $\pm$ 2.90)  $\mu\text{g/mL}$ , (61.94 $\pm$ 3.98)  $\mu\text{g/mL}$ , (94.53 $\pm$ 1.68)  $\mu\text{g/mL}$  and (69.7 $\pm$ 2.65)  $\mu\text{g/mL}$  for DPPH, HRSA, NO, FRAP, LPO and SOD

radical scavenging activities, respectively. Moreover, the results are also comparable with the positive control of the vitamin C. The preliminary phytochemical analysis of the plant part extracts showed the presence of reducing sugars, protein, phenolic groups, alkaloids, triterpenoids and tannins. However, the steroids, flavonoids, anthroquinone and catachins were not reported in the leaf extract.

**Table 1**  
Effect of *L. racemosa* leaf extract on the biochemical parameters in CCl<sub>4</sub> induced hepatotoxicity in rats (Mean ±SD).

Parameters	Control	Hepatotoxin group (CCl <sub>4</sub> )	Silymarin positive control	<i>L. racemosa</i> leaf extract (mg/kg bw)		
				75	150	300
SGOT (IU/L)	167.33±13.27	313.50±16.53*	181.85±8.63*	324.4±13.48**	250.43±23.42**	210.36±19.63**
SGPT (IU/L)	60.67±5.39	232.65±17.38*	84.13±6.37*	208.54±16.12**	176.54±14.34**	82.37±13.87**
ALP (IU/L)	135.27±9.67	956.36±64.72*	192.85±10.26*	539.26±78.12**	290.33±34.14**	197.63±23.43**
BIL (mg/dL)	1.28±0.13	3.12±0.48*	1.46±0.19*	2.93±0.68**	2.46±0.54**	2.15±0.84**
CHL (mg/dL)	90.39±7.69	243.45±15.43*	110.55±6.88*	212.64±12.36**	184.21±4.13**	163.83±15.63**
SUG (mg/dL)	85.66±10.34	162.66±7.65*	101.36±7.63*	146.44±6.39**	114.00±8.64**	93.00±7.65**
LDH (U/L)	886.00±137.65	2 785.00±236.50*	1 842.00±31.00*	2 589.46±250.47**	1 341.11±178.6**	1 134.00±285.00**
TPN (g/dL)	8.68±1.03	3.08±0.87*	5.97±0.81*	3.43±0.96**	4.69±0.39**	6.01±0.57**
ALB (g/dL)	4.38±0.84	1.87±0.64*	3.01±0.38*	1.36±0.49	1.95±0.93**	2.96±0.68**

BIL: bilirubin; CHL: cholesterol; SUG: sugar.

\**P*<0.05 vs control; \*\**P*<0.05 vs CCl<sub>4</sub>-treated group.**Table 2**  
Dose dependant histopathological scores of *L. racemosa* leaf extract in CCl<sub>4</sub> induced hepatotoxicity in rats.

Parameters	Fatty changes	Hydrophic changes	Focal necrosis	Congestion in central vein	Congestion in sinusoidal spaces	Hepatocytes Deformation	Total
Hepatotoxin group (CCl <sub>4</sub> )	3	0	3	3	2	0	11
Silymarin	1	2	0	0	1	0	4
<i>L. racemosa</i> treated group (75 mg/kg bw)	2	2	1	0	0	0	5
<i>L. racemosa</i> treated group (150 mg/kg bw)	1	1	1	0	0	0	3
<i>L. racemosa</i> treated group (300 mg/kg bw)	0	1	0	0	0	0	1

**Table 3**  
IC<sub>50</sub> values of *L. racemosa* leaf extract and vitamin C with various antioxidant activities (μg/mL).

Parameter	<i>L. racemosa</i> leaf extract IC <sub>50</sub>	Vitamin C IC <sub>50</sub>
DPPH radical scavenging	56.37±4.87	2.87±1.26
HRSA radical scavenging	57.68±1.98	44.24±1.50
NO radical scavenging	64.15±2.90	4.98±1.28
FRAP radical scavenging	61.94±3.98	56.69±1.11
LPO radical scavenging	94.53±1.68	31.79±1.21
SOD radical scavenging	69.70±2.65	24.31±0.71

#### 4. Discussion

In Indian system of medicine, many of the herbal materials are claimed to provide relief against many of the liver diseases. In this connection, the claimed herbal extracts should be verified in scientific manner. In the present study, one such extract from the mangrove plant was taken for the in vivo validation. The ethanolic leaf extract of *L. racemosa* posses significant hepatoprotective effect in the CCl<sub>4</sub> intoxicated models (*P*<0.05). The hepatoprotective effect of the plant extract might be attributed to the presence of unique chemical classes such as alkaloids[16] and polyphenols[17]. The hepatotoxicity effect of the CCl<sub>4</sub> might be due to the formation of the highly reactive free radicals, which directly affects the polyunsaturated fatty acids and directly alters the liver microsaomal membranes in the Wistar albino rats[18]. The pre administration of the *L. racemosa* leaf extract can reduce all the elevated biochemical parameters such as SGOT, SGPT, ALP, bilirubin and LDH levels. In hepatotoxin intoxicated rats, the reduction in the level of total protein and albumin might be due to the damage produced and localized in the endoplasmic reticulum which lead to its functional failure with decrease in protein synthesis and accumulation of triglycerides[19]. Intoxication of CCl<sub>4</sub> also results in inhibition of the synthesis of the bile acids from

the cholesterol and leads to elevated level of cholesterol[20]. Suppression of cholesterol level in the serum parameters suggests the inhibition of synthesis of the bile acids from cholesterol, and is reversed by the pre-administration of the *L. racemosa* leaf extract[21]. Reduction in the level of SGOT, SGPT towards the normal value is an indication of the stabilization of the plasma membrane as well as repair of hepatic tissue damage caused by CCl<sub>4</sub>[22]. Reduction in the level of ALP and bilirubin suggests the stabilization of the biliary function and the increased level of protein as well as albumin suggests the stabilization of the endoplasmic reticulum leading o the protein synthesis[20]. Oxidative stress is the state of imbalance between the level of antioxidant defence system and production of the oxygen derived species[23]. The increased level of oxygen and oxygen derived species such as superoxide radicals, hydroxyl radicals and peroxide radicals causes the oxidative stress[24]. The in vitro assays such as DPPH radical scavenging assay, superoxide radical scavenging assay, hydroxyl radical scavenging assay, lipid peroxide radical scavenging assay suggest the ability of the *L. racemosa* leaf extract to reduce the biological oxidative stress[25,26]. Hence, the hepatoprotective effect of the leaf extract may be achieved by the scavenging free radical activity of the oxidative stress[27,28]. Moreover, the histopathological analysis showed that, the normal liver

architecture was disturbed by the hepatotoxin treated rats. But, the liver sections obtained from the rats treated with ethanolic extract and intoxicated with hepatotoxin, the normal cellular architecture retained as compared with silymarin treated rats<sup>[20,29]</sup> thereby further confirming the protective effect of the leaf extract. It can be concluded from the present findings that, the *L. racemosa* could be efficiently used as a hepatoprotective agent after successful clinical trials.

### Conflict of interest statement

We declare that we have no conflict of interest.

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