Hepatoprotective activity of *Ceriops decandra* (Griff.) Ding Hou mangrove plant against CCl₄ induced liver damage

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Abstract

In folklore medicine, *Ceriops decandra* is used to treat hepatitis. However, a scientific evaluation has not been performed. Thus, the present study was carried out to identify the hepatoprotective activity of plant parts (leaf, bark, collar, flower and hypocotyls) of *C. decandra*. In vitro antioxidant studies were carried out with DPPH, HRSA, NO, FRAP and LPO assays. The LD₅₀ was calculated and *in vivo* hepatoprotective activity was carried out with the leaf extract, which was found to be the most potent. The *in vivo* hepatoprotective activity was performed as follows: Group 1, control animals; Group 2, carbon tetrachloride (CCl₄)-treated animals; Group 3, silymarin (100 mg kg⁻¹ bw p.o.) treated animals; Groups 4, 5 and 6, *C. decandra* treatment groups (100, 200 and 400 mg kg⁻¹ bw). Histopathological scores were calculated with standard protocols. Of the selected different plant parts, the leaf extract showed maximum antioxidant scavenging properties. A study of the oral acute toxicity found *C. decandra* extract to be non-toxic up to 2000 mg kg⁻¹ bw. The *in vivo* hepatoprotective nature of the leaf extract was identified as dose dependent and the levels of SGOT, SGPT, ALP, bilirubin, CHL and LDH were found to be significantly decreased (*p* < 0.05) compared with hepatotoxic groups. Histopathological scores did not show any significant variations between control and high dose (400 mg kg⁻¹ bw) of leaf extract-treated animals. Preliminary phytochemical analysis of the leaf extract revealed the presence of phenolic groups, alkaloids, triterpenoids, flavonoids, catechin and anthraquinone. In conclusion, the hepatoprotective nature of the *C. decandra* leaf extract might be due to the occurrence of unique secondary metabolites and their antioxidant scavenging properties.

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Keywords: Antioxidant; *Ceriops decandra*; Hepatoprotective; Mangrove

1. Introduction

The liver is a vital organ that functions as a centre for metabolism of nutrients (carbohydrates, proteins and lipids) and excretion of xenobiotics from the body, thereby providing protection against foreign substances through detoxification and elimination processes [1,2]. Due to increasing incidences of xenobiotic/chemically
induced hepatotoxicity, there is a need for safe protective agents, in lieu of synthetic chemical compounds [3]. Therefore, there is particular interest in developing new drugs from plant sources, to treat liver diseases. According to the World Health Organization, herbal drugs from medicinal plants constitute a major part of the traditional medicine system [4]. Recently, in developing countries the utilization of medicinal plants has gained prominence and popularity because of their safety, efficacy and cost effectiveness [5]. In this regard, the marine environment is an excellent reservoir for biologically active products. It is one of the richest sources of floral wealth and diversity [6]. Marine plants remain the greatest unexploited source of potential pharmaceutical products. Traditionally, marine halophytes such as mangroves and mangrove associates have been used to treat several human and animal ailments including liver diseases [7]. Scientifically, marine halophytes have been shown to have antibacterial [8], antifungal, antiplasmodial and anti-viral properties [9,10]. For instance, the hepatoprotective nature of terrestrial plants (Murraya koenigii [1], Feronia limonia [2,3,5], Tecomella undulata [11], Clerodendron glandulosum [6,12], etc.) has been studied extensively. However, few studies have reported the treatment of liver diseases with mangrove plants (Lumnitzera racemosa (stilt root) [13] and Rhizophora mucronata (leaf) [14]). In view of this, we set out to scientifically validate the use of one such traditionally used mangrove plant, Ceriops decandra (Griff.) Ding Hou. In folklore medicine, the bark and leaf parts of C. decandra are used as cure for ulcer and hepatitis [15]. Moreover, several chemical constituents such as carotenoids, flavonoids, chlorophyll a and b, lipids and waxes, polyphenols, proteins, steroids, tannins and triterpenes have also been reported to have hepatoprotective properties [15]. The present study focused on identifying the in vivo hepatoprotective properties against carbon tetrachloride induced hepatotoxicity in Wistar albino rats.

2. Experimental

2.1. Collection and extraction

Different plant parts such as leaf (Herbarium id: AUOCAS000109), bark (AUOCAS00110), collar (AUOCA00111), hypocotyls (AUOCAS00112) and flower (AUOCAS00113) of C. decandra were collected from the Pichavaram mangrove forest (Lat. 11°20’N; Long. 79°47’E) in Tamilnadu, India. The sample specimen was preserved at School of Marine Sciences, Alagappa University, Thondi Campus, Thondi, India in ICMR sponsored cabinet facility. Samples were washed twice in tap water to remove the adhering contaminants and were shade dried. 500 g of the dried materials were extracted with petroleum ether at 60°C and then extracted (3:1, v/v) with ethanol:water mixture by percolation method and concentrated under vacuum. The extracts of mangroves were screened for the presence of phytochemical constituents by standard protocols [13].

2.2. In vitro antioxidant assay

2.2.1. Determination of DPPH radical scavenging activity [14]

Altogether, 10 μl of different concentrations (between 1.9 and 500 μg ml⁻¹) of plant extracts and vitamin C were mixed with 190 μl of 150 μM DPPH solution. The aliquot mixture was incubated at 37°C for 20 min. The solvent alone was used as control. The samples were measured at 517 nm with a Perkin Elmer UV-visible spectrophotometer.

2.2.2. Determination of hydroxyl radical scavenging activity (HRSA) [16]

Next, 100 μl of different concentrations (1.9–500 μg ml⁻¹) of extracts and vitamin C were mixed with the following chemical combinations: 1000 μl of iron-EDTA solution, 500 μl of 0.018% EDTA solution, 1000 μl of 0.85% (w/v) DMSO (in 0.1 M phosphate buffer, pH 7.4), and 500 μl of 0.22% (w/v) vitamin C. The aliquots were incubated at 85°C for 15 min in water bath. After that, 17.5% (w/v) of the ice-cold trichloroacetic acid and 300 μl of Nash reagent were mixed and incubated for 15 min at room temperature. The samples were measured at 412 nm with a Perkin Elmer UV-visible spectrophotometer Bio D99647 Made in Germany.

2.2.3. Determination of nitric oxide radical scavenging activity (NO) [17]

In this phase, 3 ml of different concentrations (1.9–500 μg ml⁻¹) of extracts and vitamin C were mixed with methanol and incubated for 150 min at 25°C. The incubated samples were mixed with Greiss reagent and read at 546 nm with Perkin Elmer UV-visible spectrophotometer.

2.2.4. Determination of lipid peroxidation inhibition assay (LPO) [18]

Liver sample homogenate from male Wistar albino rats was prepared with 0.15 M ice-cold KCl in a Teflon tissue homogenizer and the content of the protein was adjusted to 500 μg ml⁻¹. 1 ml of tissue homogenate was mixed with 25 μM FeSO₄, 100 μM ascorbate,
10 mM KH$_2$PO$_4$ and the volume was brought to 3 ml with distilled water and incubated at 37 °C for 30 min. Subsequently, the homogenate mixture was incubated with different concentrations of extracts and vitamin C (1.9–500 μg ml$^{-1}$). The mixture was read at 532 nm in Perkin Elmer UV-visible spectrophotometer.

2.2.5. Determination of ferric reducing antioxidant power assay (FRAP) [19]

Different concentrations (1.9–500 μg ml$^{-1}$) of extracts and vitamin C were mixed with 3 ml of FRAP reagent [2.5 ml of 10 mM TPTZ (300 mM acetate buffer, pH 3.6, 10 mM TPTZ, 2,4,6-tripyridyl-S-triazine) solution in 40 mM HCl and 20 mM FeCl$_3$·6H$_2$O, 40 mM HCl, 2.5 ml of 20 mM FeCl$_3$ and 25 ml of 0.3 M acetic buffer (pH 3.6)] and incubated for 5 min at 25 °C. The absorbance was read at 593 nm with Perkin Elmer UV-visible spectrophotometer.

2.2.6. Determination of superoxide radical scavenging assay (SOD) [20]

To determine the SOD, 200 μl of different concentrations (1.9–500 μg ml$^{-1}$) of extracts and vitamin C were mixed with 0.05 M sodium carbonate (480 μl; pH 10.5), 3 mM xanthan gum (20 μl), 3 mM EDTA (20 μl), 0.15% BSA (20 μl) and 0.75 mM of NBT (20 μl). The aliquots were incubated for 20 min at 25 °C. After that, 20 μl of 6 mM CuCl$_2$ was added to terminate the reaction. The absorbance was read at 560 nm in Perkin Elmer UV-visible spectrophotometer.

2.3. Oral acute toxicity analysis (determination of LD$_{50}$)

Wistar albino rats weighing 180–230 g were housed in large cages, kept in proper temperature, humidity and light/dark (12 h) cycles. Animals were fed with standard pellet diet and water ad libitum. All the animal studies experimental protocol was approved by Internal Research and Review Board (IRB), Ethical Clearance (EC), Biosafety and Animal Welfare Committee, Madurai Kamaraj University, Madurai, Tamilnadu, India. Female albino rats were selected for the toxicity analysis. Ten animals were kept fasting overnight, and subsequently the extracts were given orally at the dose of 250 mg kg$^{-1}$ bw and observed for 24 h. If six or more animals died, the administered dose was designated as a toxic dose. If mortality was observed in three animals, the same dose was administered to confirm the toxic dose. If mortality was not observed, the protocol was repeated at the next higher dose, i.e., 300 mg kg$^{-1}$ bw. Then, 1/10th of the LD$_{50}$ concentration was used for the in vivo hepatoprotective activity experiments.

2.4. In vivo hepatoprotective activity

Male Wistar albino rats (120–150 g) were divided into nine groups consisting of six animals per group.

Group 1: Animals received three doses of 5% acacia gum (1 ml kg$^{-1}$ bw p.o.) at 12 h intervals (0 h, 12 h and 24 h).

Group 2: Hepatotoxin group received three doses of vehicle [5% acacia gum (1 ml kg$^{-1}$ bw p.o.)] at 12 h intervals and a single dose of carbon tetrachloride [2 ml kg$^{-1}$ bw i.p. diluted in liquid paraffin (1:1)] administered after 30 min of second dose of vehicle.

Group 3: Positive control received doses of silymarin (100 mg kg$^{-1}$ bw p.o.) at 0 h, 12 h and 24 h intervals and a single dose of carbon tetrachloride [2 ml kg$^{-1}$ bw i.p. diluted in liquid paraffin (1:1)] administered after 30 min of second dose of vehicle.

Groups 4, 5 and 6 received low, medium and high doses of C. decandra leaf extracts (100, 200 and 400 mg kg$^{-1}$ bw p.o., respectively) at 0 h, 12 h and 24 h intervals and a single dose of carbon tetrachloride [2 ml kg$^{-1}$ bw i.p. diluted in liquid paraffin (1:1)] administered after 30 min of second dose of vehicle.

2.5. Liver function tests (LFT) analysis

First, 36 h after administration of CCl$_4$ intoxication, all the animals were anesthetized with mild ether and blood samples were collected by eye bleeding method and serum was separated using centrifugation method. The serum was used to estimate the following liver function tests (LFT) with the standard method [21]: serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP). Total protein (TPN) [22], albumin (ALB) [23], sugar (SUG) [24], cholesterol (CHL) [25] and bilirubin (BIL) [26] was assayed using Randox laboratory kits.

2.6. Microscopic evaluations of liver – histopathological analysis

Animals from each group were sacrificed and the abdomen was cut open to take out the liver. The liver samples were fixed in Bouin solution for 12 h, embedded into liquid paraffin, sectioned using micrometer (5 μm thickness) and stained with haematoxylin–eosin dye by conventional methods [27]. The sections were viewed under the light microscope for histopathological
examination and the liver pathology was described by Jamshidzadeh scores [28]. Histological damage was scored as follows: 0 = no visible cell damage; 1 = <25% of tissue show damaged hepatocytes; 2 = <25–50% of the tissue show damaged hepatocytes; 3 = extensive hepatic lesions; 4 = global hepatic necrosis.

2.7. Statistical analysis

The data were represented as the mean ± standard mean (SM) for six parallel measurements using Office 2014 package for Windows. Statistical analysis was conducted by Student’s t-test, and p < 0.05 was considered significant. IC₅₀ was calculated with the 50% inhibitory concentration (using SDAS/XP add ins program) from the dose response curve retrieved by plotting percentage inhibition versus concentration.

3. Results

3.1. In vitro antioxidant assay

Of the selected plant parts of *C. decandra*, the leaf extract showed minimum IC₅₀ values of 68.83 ± 4.83, 74.78 ± 1.32, 60.32 ± 2.98, 129.72 ± 4.87 and 142.38 ± 7.03 μg ml⁻¹, in DPPH, HRSA, NO, FRAP and LPO assays, respectively. However, the minimum IC₅₀ value identified with hypocotyl extract in the SOD assay was 169.92 ± 2.02 μg ml⁻¹ (Table 1).

3.2. Oral acute toxicity analysis (determination of LD₅₀)

In oral acute toxicity studies, mice treated with *C. decandra* leaf extract did not exhibit mortality up to 14 days afterwards, and the extract was found to be nontoxic up to 2000 mg kg⁻¹ bw. Ultimately, 400 mg kg⁻¹ bw concentration of the leaf extract was selected as the highest dose for the in vivo hepatoprotective studies.

3.3. In vivo hepatoprotective activity – liver function tests (LFT)

The in vivo hepatoprotective studies demonstrated that the level of SGOT, SGPT, ALP, bilirubin, CHL, sugar and LDH was significantly increased (p < 0.05) in carbon tetrachloride intoxicated rats, when compared with Group 1 animals. However, the rats treated with *C. decandra* leaf extracts all showed significant decrease (p < 0.05) in all the raised enzyme parameters. The level of SGOT (182.83 ± 19.93 IU L⁻¹), SGPT (169.06 ± 8.83 IU L⁻¹), ALP (217.83 ± 17.83 IU L⁻¹), BIL (1.92 ± 0.49 IU L⁻¹), CHL (117.29 ± 8.73 mg dl⁻¹), SUG (118.73 ± 14.83 mg dl⁻¹) and LDH (1720.93 ± 287.65 UL⁻¹) was at a maximum in the 400 mg kg⁻¹ bw of the leaf extract-treated animals. However, the level of TPN (7.25 ± 1.63 g dl⁻¹) and ALB (3.38 ± 2.73 g dl⁻¹) was found to be increased with the high dose (400 mg kg⁻¹ bw) of *C. decandra* leaf extract-treated animals (Table 2).

3.4. Microscopic evaluations of liver – histopathological analysis

Histopathological score revealed that the animals treated with low (100 mg kg⁻¹ bw) and medium (200 mg kg⁻¹ bw) doses of extract showed decreases in ballooning degeneration, necrosis, central vein congestion and sinusoidal space congestion when compared with the hepatotoxin treated groups, but no histopathological changes (except for mild ballooning degeneration) observed in high dose (400 mg kg⁻¹ bw) treated animals (Fig. 1 and Table 3).

3.5. Preliminary phytochemical analysis

The preliminary phytochemical analysis of the leaf extract shows the presence of sugars, protein, phenolic groups, alkaloids, triterpenoids, flavonoids, catechin, tannins and anthraquinones.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Leaf</th>
<th>Bark</th>
<th>Collar</th>
<th>Hypocotyl</th>
<th>Flower</th>
<th>Vitamin C</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH</td>
<td>68.83 ± 4.83</td>
<td>&gt;500</td>
<td>256.92 ± 7.63</td>
<td>186.93 ± 15.03</td>
<td>&gt;500</td>
<td>2.87 ± 1.26</td>
</tr>
<tr>
<td>HRSA</td>
<td>74.78 ± 1.32</td>
<td>442.52 ± 10.93</td>
<td>178.92 ± 10.73</td>
<td>381.92 ± 17.83</td>
<td>478.54 ± 15.93</td>
<td>44.24 ± 1.50</td>
</tr>
<tr>
<td>NO</td>
<td>60.32 ± 2.98</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>166.29 ± 14.83</td>
<td>&gt;500</td>
<td>4.98 ± 1.28</td>
</tr>
<tr>
<td>LPO</td>
<td>142.38 ± 7.03</td>
<td>&gt;500</td>
<td>158.52 ± 18.93</td>
<td>265.98 ± 76.84</td>
<td>&gt;500</td>
<td>31.79 ± 1.21</td>
</tr>
<tr>
<td>SOD</td>
<td>198.05 ± 7.94</td>
<td>&gt;500</td>
<td>410.03 ± 26.02</td>
<td>169.92 ± 2.02</td>
<td>&gt;500</td>
<td>24.31 ± 0.71</td>
</tr>
<tr>
<td>FRAP-reducing power</td>
<td>129.72 ± 4.87</td>
<td>148.72 ± 7.03</td>
<td>372.82 ± 18.94</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>56.69 ± 1.11</td>
</tr>
</tbody>
</table>

Values are presented as ±SM values of six replicates.
Table 2
Effect of dose dependant *C. decandra* leaf extract on the serum analysis of the CCL4-induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Hepatotoxic group</th>
<th>Silymarin positive control 100 (mg kg⁻¹ bw)</th>
<th><em>C. decandra</em> leaf extract (mg kg⁻¹ bw)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGOT (IU L⁻¹)</td>
<td>163.41 ± 29.72</td>
<td>353.63 ± 190.74</td>
<td>193.98 ± 9.74</td>
<td>328.82 ± 16.92</td>
</tr>
<tr>
<td>SGPT (IU L⁻¹)</td>
<td>156.73 ± 24.03</td>
<td>276.75 ± 21.87</td>
<td>92.63 ± 8.69</td>
<td>196.73 ± 8.19</td>
</tr>
<tr>
<td>ALP (mg dl⁻¹)</td>
<td>1.62 ± 0.37</td>
<td>3.42 ± 0.57</td>
<td>2.28 ± 0.94</td>
<td>3.02 ± 0.87</td>
</tr>
<tr>
<td>BIL (mg dl⁻¹)</td>
<td>98.87 ± 8.58</td>
<td>236.52 ± 15.29</td>
<td>124.63 ± 13.36</td>
<td>232.72 ± 72.93</td>
</tr>
<tr>
<td>CHL (mg dl⁻¹)</td>
<td>83.52 ± 13.39</td>
<td>217.52 ± 23.93</td>
<td>128.37 ± 13.61</td>
<td>196.83 ± 29.83</td>
</tr>
<tr>
<td>LDH (UL⁻¹)</td>
<td>938.37 ± 168.68</td>
<td>2984.93 ± 239.93</td>
<td>1884.70 ± 348.47</td>
<td>2437.82 ± 162.62</td>
</tr>
<tr>
<td>TPN (g dl⁻¹)</td>
<td>8.68 ± 1.03</td>
<td>2.93 ± 0.72</td>
<td>5.97 ± 0.81</td>
<td>3.43 ± 0.96</td>
</tr>
<tr>
<td>ALB (g dl⁻¹)</td>
<td>4.38 ± 0.84</td>
<td>0.91 ± 0.83</td>
<td>3.01 ± 0.38</td>
<td>0.83 ± 0.93</td>
</tr>
</tbody>
</table>

Data represents the mean ± SM of six animals. SGOT: serum glutamic oxaloacetic transaminase; SGPT: serum glutamic pyruvic transaminase; ALP: alkaline phosphatase; BIL: bilirubin; CHL: cholesterol; SUG: sugar; LDH: lactate dehydrogenase; TPN: total protein; ALB: albumin; ns: non-significant.

* p < 0.05, significant from control or CCL4-treated rats.

Table 3
Dose dependant histopathological scores of *C. decandra* leaf extract in CCL4-induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ballooning degeneration</th>
<th>Necrosis</th>
<th>Congestion in central vein</th>
<th>Congestion in sinusoidal spaces</th>
<th>Hepatocytes deformation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hepatotoxic group</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Silymarin positive control 100 (mg kg⁻¹ bw)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><em>C. decandra</em> leaf extract (mg kg⁻¹ bw)</td>
<td>100</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

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

4. Discussion

*C. decandra* is reported to have antiviral [15], antioxidant, anti-inflammatory [29,30], antidiabetic [31] and larvicidal properties [32]. However, no studies address the hepatoprotective activity of *C. decandra*. In the present study, we aimed to identify the antioxidant and hepatoprotective activities of *C. decandra*. In biological systems, the abnormal production of free radicals leads to cell necrosis and tissue damage [33]. With that in mind, we aimed to characterize the antioxidant properties of plant extracts, and found that *C. decandra* leaf extract generally has good oxygen scavenging properties. As indicated from DPPH scavenging assays, *C. decandra* leaf extract directly prevents free radicals from damaging biomolecules such as proteins, lipids, amino acids and unsaturated fatty acids in biological systems [10]. In living cells, the formation of hydroxyl and lipid radicals directly damages DNA and leads to cytotoxicity [20]. We found that the quenching properties of *C. decandra* leaf extract, as measured by LPO and HRSA assays, reduce cytotoxicity. Vidyalakshmi et al. [34] reported up to 80% reduction of hydroxyl radicals in rats treated with *Mussaenda glabra* leaf extract. In biological systems, nitric oxide radicals and superoxide anions cause renal injury [35]. We directly attribute the in vitro nitric oxide and superoxide radical scavenging properties to the *C. decandra* leaf extract, and posit that reduction in the level of nitric oxide could reduce tissue damage [20].

The FRAP method directly describes the ferrous complex reducing ability of the leaf extract [36]. Shajiselvin and Muthu reported that the methanolic extract of *Beneicasa hispida* showed the FRAP reduction at an IC₅₀ value of 65.00 µg ml⁻¹ [37]. The acute toxicity values of the *C. decandra* leaf extract was found to be greater than 2000 mg kg⁻¹ bw, with no indication of toxicity [38]. Other studies have reported similar properties in the *Coccinia indica* leaf extract [39]. In the in vivo hepatoprotective studies we conducted, animals were
dosed with CCl\textsubscript{4}, which produces \*CCl\textsubscript{3} and \*OOCCl\textsubscript{3} free radicals. These radicals attack membrane lipids in the endoplasmic reticulum, thereby raising the level of SGOT, SGPT, AST, ALP and LDH and decreasing metabolic functions (synthesis of proteins and storage of cholesterol and sugars) in blood serum [40–42]. The C. decandra leaf extract-treated animals show a decreased level of enzymatic properties, which might be due to stabilization of the hepatic cellular membrane, inhibition of lipid peroxidation, and radical scavenging properties [43]. The protective nature of the C. decandra leaf extract might be due to enhanced scavenging antioxidant properties in the presence of unique secondary metabolites such as flavonoids, polyphenols and alkaloids. These compounds are known to have antioxidant properties and have applications in treatment of liver diseases [10,14,20,44,45]. In addition, the level of total protein and albumin was found to be increased in C. decandra leaf extract-treated animals when compared with the CCl\textsubscript{4}-treated rats, which might be due to the stabilization of endoplasmic reticulum [46]. In histopathological analysis, the normal cellular membrane architecture was altered in CCl\textsubscript{4}-treated rats, but the C. decandra leaf extract-treated animals showed a more stable cellular membrane architecture, which further corroborates the protective qualities of the leaf extract [47,48].
observations have been observed in *L. racemosa* [10,14] and *R. mucronata* mangrove plants [20].

5. Conclusions

We conclude that the *Ceriops decandra* leaf collected from Pichavaram mangrove forest, Tamilnadu, India, has protective activity against carbon tetrachloride-induced hepatotoxicity in Wistar albino rats. Further studies are highly recommended to identify the active components of the extract and molecular mechanisms responsible for this hepatoprotection.

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