Isolation, characterization, structural elucidation and in vivo hepatoprotective studies of phytoconstituents obtained from the fruits of *Cordia obliqua* Willd

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Abstract

**Purpose:** To isolate, characterize, and investigate the hepatoprotective effect of phyto-constituents from fruits of *Cordia obliqua* Willd in paracetamol-induced hepatotoxicity in Wistar rats.

**Methods:** Ethanol and aqueous extracts of *C. obliqua* fruits were screened for phytochemicals. The extracts were subjected to column chromatography and preparative thin-layer chromatography (TLC) to isolate four novel compounds. Compounds were characterized using infrared spectroscopy (IR), mass spectroscopy (MS), as well as ¹H and ¹³C nuclear magnetic resonance (NMR). The isolated compounds were assessed for acute toxicity, in vivo hepatoprotective and antioxidant potential (dose: 5 mg/kg) in paracetamol-induced (75 mg/kg) hepatotoxicity through oral route in Wistar rats.

**Results:** Phytochemical analysis of ethanol (COE) indicated the presence of fatty acids, anthraquinones, glycosides, saponins, alkaloids, tannins, flavonoids, coumarins, phenolics, triterpenes, and sterols. Compounds A, B, and C were identified from COE. Treatment at 50 mg/kg significantly reduced aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGTP), and total bilirubin (TB), as well as increased total protein and total alkalinity levels in serum compared to the positive control. Liver histo-architecture showed improvements compared to the positive control, indicating hepatic protection.

**Conclusion:** The isolated compounds (A, B and C) from COE exhibit hepatoprotective effects attributed to flavonoids and phenolics with free radical scavenging properties. Further research is needed to identify key mechanisms responsible for hepatoprotective effects.

**Keywords:** Cordia obliqua, Hepatoprotective effect,Antioxidant and isolated phyto-constituents
and necrosis, is a comprehensive term for liver damage [3]. Paracetamol is an over-the-counter antipyretic and analgesic drug with weak anti-inflammatory properties. Although it is regarded as safe at therapeutic doses, paracetamol induces fatal centrilobular hepatic necrosis at high doses. This hepatic necrosis is the result of an intricate series of events that includes metabolism of paracetamol to its highly reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), via the cytochrome P-450 enzyme (CYP). This CYP metabolism depletes glutathione levels, which in turn increases oxidative stress and alteration in mitochondrial permeability. As a result, mitochondrial ability to synthesize adenosine triphosphate (ATP) is lost, which then leads to necrosis [4].

Although several newly developed drugs are used for treatment of acute and chronic liver disorders, these drugs are associated with several adverse effects hence alternative and complementary therapies are becoming more and more popular [2]. Thus, continued study on plants and herbs that could potentially substitute chemical-based drugs is vital, as many medicinal plants have been proven to exhibit hepatoprotective characteristics. The synthesis of aspirin, an anti-inflammatory substance, from salicin, a natural substance obtained from bark of willow tree Salix alba L., is one of the most widely recognized instances to date. Despite these discoveries, only 10 % of world’s biodiversity has been assessed for possible biological activity [5]. More valuable natural lead compounds are yet to be discovered, indicating a large scope for identification of novel pharmacological entities derived from plants [5].

The Boraginaceae family of borage includes the genus Cordia, which includes trees and shrubs. The majority of Cordia (C) species plants, in particular, C. ulmilolia, C. latifolia, C. spinescens, C. salicifolia, C. martinecensis, C. verbenacea, C. obliqua, C. myxa, and C. dichotoma, have long been used in indigenous medicine as cough suppressant, appetite suppressants, febrifuges, diuretics, antimalarials, anthelmintics, astringents, anti-ulcer, anti-histaminic, antitumor, anti-inflammatory agents, cicatrizaants, and to treat leprosy, lung diseases, biliary obstruction, and urinary infections [6].

The genus is rich in secondary metabolites like dammarane-type hydrocarbons and triterpenes, arynaphthalene-type lignin, ursane and oleane-type triterpenes, flavonol glycosides, sesquiterpenes, fatty acids, polysaccharides, meroterpenoid naphthoquinone, prenylated hydroquinone, triterpenoids, and terpenoid hydroquinone. The isolated substances and extracts have been shown to possess antidiabetic, antimicrobial, analgesic, hepatoprotective, larvical, antioxidant, and anti-inflammatory activities [7]. In literature, the plant leaves have been reported to possess hepatoprotective activity [8], however, the fruits have not been investigated and reported for hepatoprotective activity. This study was designed to investigate the hepatoprotective and antioxidant effect of isolated phytoconstituents in ethanolic and aqueous extracts of Cordia obliqua fruit in paracetamol-induced liver damage in Wistar rats.

**EXPERIMENTAL**

**Collection of plant material**

The fruits of Cordia obliqua were collected from Chidambaram in September 2021, authenticated by Dr. P Shivakumar Singh, Department of Botany, Palamuru University, Mahabubnagar, Telangana, India and a voucher specimen (HPU:102/2019) was deposited in the herbarium.

**Chemicals and reagents**

All chemicals and solvents employed were of pharmaceutical grade.

**Extraction**

The fruits of C. obliqua were mechanically pulverized into powders after drying in shades and preserved in airtight containers. Using hot continuous percolation method in a Soxhlet apparatus, powdered fruit material was sequentially extracted with absolute ethanol (99 %) and distilled water. The extract was concentrated using a rotary evaporator, and freeze-dried in a lyophilizer to produce a dry powder.

**Phytochemical screening**

Preliminary phytochemical screening was carried out on ethanolic (COE) of Cordia obliqua fruits according to standard protocols [9].

**Isolation and characterization of phytoconstituents**

**Column chromatography**

A borosilicate column (7 x 53 cm²) packed with silica gel (# 100 - 200) as the stationary phase was used to isolate COE. Wet packing was employed to assemble the column. Silica gel was
mixed with hexane in a beaker with the stationary phase before being carefully transferred into the column while carefully excluding air bubbles. The cotton plug was kept on the stationary phase after wet packing while a filter paper was kept above it. The elution process utilized a solvent system composed of hexane and ethyl acetate. The eluting solvents were varied at percentages of 10, 20, and 25 % ethyl acetate. Fraction collection was carried out using 25 mL per fraction, and each fraction was evaluated on a thin layer chromatographic (TLC) plate. Fractions with identical retardation factor (Rf) values were combined and subsequently concentrated for further investigation. Silica gel GF254 (stationary phase), hexane and ethyl acetate (25:75 v/v), with a saturation time of 20 mins, were used to carry out TLC analysis, and the spots were visualized under ultraviolet (UV) at 254 nm and iodine vapour [10].

**FTIR spectroscopy**

Using a mortar and pestle potassium bromide (KBr) salt, COE, and COA were mixed and then pressed into thin pellets. Fourier transform infrared (FTIR) Spectrometer (Thermo Nicolet Nexus 670) with a scan range between 4,000 and 400 cm\(^{-1}\) was used to record spectroscopic results after the samples were put onto the FTIR spectroscope [11].

**Nuclear magnetic resonance (NMR) spectroscopy**

Tetramethylsilane was used as an internal standard in dimethyl sulphoxide (DMSO)-d\(_6\) to capture NMR spectra using a JEOL USA NMR Spectrometer (JNM-ECZ500R/S1). The coupling constants were expressed in Hz and the chemical shift values in ppm [12].

**Tandem liquid chromatography-mass spectrometric (LC-MS) analysis**

The Waters Xevo TQ MS System was used for LC/MS analysis of the phyto-constituents. Acetonitrile, 0.1 % formic acid solution (30:70 v/v) was employed for analysis in isocratic elution in positive ion scan mode [12].

**Animals**

Wistar rats weighing 150 – 200 g were procured from Sai Nath agency, Hyderabad, Telangana, India. The animals were housed in standard environmental conditions in metal cages. They were given free access to water and fed a standard rodent diet (Grain Based, Source: Sai Nath agency, Hyderabad). The protocol was approved by the Institute’s Animal Ethical Committee (approval no CPCSEA/IAEC/JLS/18/07/22/025), and followed international guidelines for animal studies.

**Acute toxicity study of the isolated compounds**

Acute toxicity studies of isolated compounds A, B, and C were evaluated following OECD Guidelines 423 in albino mice [13]. Compounds A, B, and C were carefully prepared, ensuring their stability, purity, and identity were rigorously maintained. These preparations were administered to test animals within a 1 – 2 h window following their formulation. In a randomized fashion, the animals were allocated to respective dose groups (5, 50, 300, and 2000 mg/kg) for each compound (A, B, and C). Control group received the vehicle (distilled water) devoid of test substance. To facilitate accurate assessment, the test substances were administered via oral gavage in a single, carefully controlled administration for each compound, ensuring precise and consistent exposure conditions [13].

**Hepatoprotective activity**

Rats were randomly divided into seven groups of six animals each and administered the following test solutions orally once daily for one week: Group I was normal control and received normal saline solution (10 mL/kg) orally for a week. Group II, which served as the toxic control, received 1 mL of vehicle (distilled water) daily and 2000 mg/kg of paracetamol on the 5\(^{th}\) day. Group-III (P1), received 2000 mg/kg of paracetamol on the 5\(^{th}\) day of treatment and 75 mg/kg of standard drug silymarin daily for one week. Group IV, V, VI, and VII are pre-treatment groups that received 2000 mg/kg of paracetamol on the 5\(^{th}\) day of treatment and 5 mg/kg of isolated compounds A, B, and C obtained from COE daily for 1 week [14].

**Biochemical analysis**

After hepatic injury had been induced using paracetamol, the animals were given light ether anesthesia 48 h later which was at the end of the one-week treatment with the compounds and silymarin. Blood was withdrawn via a cardiac puncture into sterilized centrifuge tubes, centrifuged at 3000 rpm for 10 mins to obtain serum for examination of biochemical parameters. The animals were sacrificed via cervical dislocation and the liver was quickly removed, weighed, and thoroughly cleaned of blood with ice-cold saline. A tiny portion was then...
homogenized in cold phosphate buffer (0.05 M, pH 7.4) and centrifuged for 10 mins at 3000 rpm at 4 °C. The supernatant that resulted was utilized to identify biological markers [14]. Serum was separated and used to determine biochemical parameters using commercially available kits obtained from Ranbaxy Diagnostic (New Delhi, India). Reitman and Frankel’s instructions were employed to estimate the serum alanine transaminase (ALT) and aspartate transaminase (AST), total protein (TP), total bilirubin (TB), total albumin (TA), and gamma-glutamyl transeptidase (GGTP) levels. Spectrophotometric technique described by Ohkawa et al [15] was employed to quantify the oxidative stress and antioxidant markers such as hepatic malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), respectively.

Histopathology studies

The liver was removed from the animals, and washed with normal saline. The tissue materials were fixed in 10 % buffered neutral formalin before being washed, dehydrated in descending grades of isopropanol, and finally rinsed with xylene. Thereafter, tissues were immersed in molten paraffin wax. Sections ranging in thickness from 5 to 6 µm were cut with a rotary microtome (Leica RM 2125 RTS, Singapore), processed in an alcohol-xylene series, stained with hematoxylin and eosin, and histologically examined. The liver sections were then scored and assessed based on the severity of the hepatic injury, as described by Zakaria et al [16], with slight modifications.

Statistical analysis

The data were presented as mean ± standard deviation (SD) (n = 6). Statistical analysis was carried out with GraphPad Prism software using one-way ANOVA, followed by Dunnet’s multiple comparison test. P < 0.01 was considered statistically significant.

RESULTS

Yield and physical properties

Powdered extracts were dark brown and had a percentage yield of 8.2 and 11.6 w/w for ethanol and aqueous extracts respectively.

Phytochemical screening

Phytochemical screening indicated presence of fatty acids, anthraquinones and glycosides, saponins, alkaloids, tannins, flavonoids, coumarins, phenolics, triterpenes, and sterols in both COE and COA.

Isolation of phytoconstituents

Column chromatography

Three compounds labeled A, B, and C, were effectively isolated and characterized from the ethanolic extract of Cordia obliqua via column chromatography and subjected to further analysis using IR, 1H-NMR, and 13C-NMR. These findings provide conclusive evidence of the compounds’ existence and elucidate their structural attributes.

Structural elucidation of isolated compounds A, B, and C

Isolated compound A

The infra-red (IR) spectral analysis of the isolated compound A showed a broad absorption peak at 3431 cm⁻¹ which indicated the presence of phenolic (OH) group. A sharp absorption peak at 1710 cm⁻¹, indicates presence of C=O group. The presence of aromatic C=C bond was indicated by absorption peaks at 1600 - 1561 cm⁻¹. Presence of C-O-C bond was confirmed by absorption peak at 1710 cm⁻¹ (Figure 1 A).

The 1H NMR spectra revealed presence of phenolic (OH) group at δ 8.03 - 8.015 ppm. The chemical shift values at δ: 7.68 - 7.66 (d), 7.59 - 7.58 (t), 7.36 - 7.30 (d), and 6.47 - 6.45 (s) ppm respectively, correspond to C7-H, C6-H, C5-H and C2-H. A singlet at δ 3.40 ppm is for C-OH group at δ 8.015 ppm. The chemical shift values at δ: 7.68 - 7.66 (d), 7.59 - 7.58 (t), 7.36 - 7.30 (d), and 6.47 - 6.45 (s) ppm respectively, correspond to C7-H, C6-H, C5-H and C2-H. A singlet at δ 3.40 ppm is for C-OH group (Figure 1 B). The 13C NMR spectra chemical shift values at δ 160 - 40 as a signal corresponding to carbon atoms as δ 160 (1C, s), 153 (1C, s), 144 (1C, s), 128 (1C, s), 124 (1C, s), 119 (1C, s), and 116 (1C, s) ppm (Figure 1 C). Based on the spectra data (IR, 1H-NMR and 13C-NMR) the isolated compound was found to be 8-hydroxy-3-methoxy-flavone (Figure 1 D).

Isolated compound B

The IR spectrum revealed presence of -OH, -C=O, -C=O-, and -C-O-C at 3353, 1657, 1562–1372, and 1096 cm⁻¹, respectively (Figure 2 A). Results of proton NMR revealed chemical shift values at δ 12.50, 10.78, and 9.38 as singlet and broad peaks, which correspond to presence of phenolic (OH) groups. The multiplet appeared at δ 7.68 - 7.53 corresponding to C2-H, C3-H, C5-H, and C6-H respectively. The singlet at δ 6.90-6.88 ppm appeared as a doublet for C5-H, whereas δ 6.41 was a doublet for C6-H. A singlet appeared at δ 3.85 ppm for C-OCH₃ (Figure 2 B).
The $^{13}$C NMR spectra of the sample showed 14 signals, which indicated the presence of 14 carbon atoms. Chemical shift values assigned to carbon atoms are shown as δ 176 (C4), 164 (C3), 161 (C8), 156 (C2), 148 (C5), 147 (C6), 145 (C4), 136 (C1), 122 (C5,6), 120 (C2,3), 116 (C9), 115 (C10), 103 (C7), and 98 (C-OCH$_3$) ppm (Figure 2 C). Based on the spectral data the isolated compound was found to be 3,8,4-trihydroxy-7-methoxy flavone (Figure 2 D).

**Figure 1:** Spectrum of compound A (A) FTIR (B) $^1$H NMR (C) $^{13}$C NMR (D) Chemical structure of 8-Hydroxy-3-methoxy flavone

**Figure 2:** Spectrum of compound B (A) FTIR (B) $^1$H NMR (C) $^{13}$C NMR (D) Chemical structure of 3,8,4 – trihydroxy-7-methoxy flavone
Isolated compounds C

The infra-red (IR) spectral analysis of the isolated compound C revealed the presence of phenolic (OH) group (Figure 3 A). The $^1$H NMR spectrum offers significant insights into the proton environment of a compound. The presence of singlets at δ 11.87, δ 11.28, and δ 11.13 (Figure 3 B) strongly indicated the existence of protons located within highly shielded environments. Shielded protons are likely associated with hydroxyl groups or other electron-donating functional groups. Additionally, a singlet observed at δ 8.02 signified a proton occupying a distinct chemical environment. Furthermore, the presence of multiplet and doublets at various chemical shifts and coupling constants suggested the coexistence of diverse types of protons within the molecular structure. On the other hand, the $^{13}$C NMR spectrum provides valuable information concerning the compound’s carbon resonances. The spectrum displayed distinct carbon signals spanning from δ 177.23 to δ 17.43, indicative of a wide array of carbon environments within the compound. The presence of carbon resonances in high chemical shift regions, such as δ 177.23, (Figure 3 C) suggested the possible existence of carbonyl or ester functionalities. Conversely, signals at lower shifts, for example, δ 17.43, are indicative of aliphatic carbons. Based on the spectral data the isolated compound was found to be 3,5,7-trihydroxy-2-(4-hydroxy-3-((4,5,6-trihydroxy-2-methyltetrahydro-2H-pyran-3-yl)oxy)phenyl)-4H-chromen-4-one (Figure 3 D).

LC-MS analysis

The LC-MS analysis of isolated compounds (A–C) represented the single purified peaks (Figure 4).

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Figure 3: Spectrum of compound C (A) FTIR (B) $^1$H NMR (C) $^{13}$C NMR (D) Chemical structure of 3,5,7,4'-tetrahydroxy-5'-rhamnose-flavone

Figure 4: LC-MS spectrum of isolated compounds A, B, and C representing the single purified peaks
Acute toxicity studies

Investigation of acute toxicity was conducted following standard protocols. When administered orally to experimental rats at a dose of 50 mg/kg, the isolated compounds exhibited no signs of toxicity. However, at higher doses of 300 and 2000 mg/kg, toxic effects were observed, leading to morbidity. Rats receiving 50 mg/kg dose underwent bi-daily examinations over two weeks, during which no toxic symptoms were observed. Consequently, oral LD_{50} was determined to be 50 mg/kg, representing a safer dosage for compounds A, B, and C. For further research, a 1/10th fraction of this LD_{50}, i.e., 5 mg/kg, was selected as the preferred dose.

Effects of isolated compounds (A-C) on paracetamol-induced hepatotoxicity

The paracetamol treatment caused a substantial increase (p < 0.01) in AST, ALT, ALP, GGTP, and TB levels with a substantial decrease in TP and TA levels as seen in toxic group compared to control and pretreatment groups.

When compared to toxic group, pretreatment with isolated compounds A, B, and C, at a dose of 5 mg/kg resulted in a significant decrease (p < 0.01) in AST, ALT, ALP, GGTP, and TB levels (Table 1 and 2) and a significant elevation (p < 0.01) in TP and TA levels. Standard drug silymarin demonstrated significantly similar results. Among the isolated compounds A - C, compounds B showed a significant decrease in serum transaminases, and bilirubin and a significant increase in TP and TA levels.

Effect of isolated compounds (A-C) on oxidative stress

Toxicity-related changes in MDA, SOD, GPX, and CAT activities. When compared to normal group, paracetamol-induced oxidative stress in the liver manifested as a significant increase (p < 0.01) in MDA level and a significant decrease (p < 0.01) in CA, GPX, and SOD activities. Furthermore, isolated compounds (A-C) significantly enhanced antioxidant status, as evidenced by elevated SOD, GPX, and CAT activities (p < 0.01) and reduced MDA level as compared to toxic group that received paracetamol alone (Table 3). Among the isolated compounds, compound B produced a better effect when compared with A and C in improving SOD, GPX, and CAT activities and reducing MDA levels.

### Table 1: Effect of isolated compounds of fruits of *Cordia obliqua* on serum enzymes AST, ALT, and ALP in paracetamol-induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment dose (mg/Kg)</th>
<th>AST (IU/mL)</th>
<th>ALT (IU/mL)</th>
<th>ALP (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control group</td>
<td>54.25±2.50</td>
<td>35.75±1.70</td>
<td>40.50±1.35</td>
</tr>
<tr>
<td>II</td>
<td>Toxic group</td>
<td>115.90±4.70</td>
<td>93.20±3.60</td>
<td>132.20±4.90</td>
</tr>
<tr>
<td>III</td>
<td>Silymarin (75)</td>
<td>61.40±2.80*</td>
<td>41.30±1.90*</td>
<td>53.20±2.10*</td>
</tr>
<tr>
<td>IV</td>
<td>Compound A (5)</td>
<td>70.80±3.40*</td>
<td>50.10±2.35*</td>
<td>62.25±2.50*</td>
</tr>
<tr>
<td>V</td>
<td>Compound B (5)</td>
<td>65.75±2.95*</td>
<td>47.45±2.08*</td>
<td>57.40±2.25*</td>
</tr>
<tr>
<td>VI</td>
<td>Compound C (5)</td>
<td>66.30±3.05*</td>
<td>48.75±2.20*</td>
<td>60.70±2.40*</td>
</tr>
</tbody>
</table>

*p < 0.01, significantly different from toxic positive control group

### Table 2: Effect of isolated compounds of fruits of *Cordia obliqua* on serum enzymes TP, TB GGTP and TA in paracetamol-induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment dose (mg/Kg)</th>
<th>TP (g/dL)</th>
<th>TB (mg/dL)</th>
<th>GGTP (mg/dL)</th>
<th>TA (g/dL)</th>
</tr>
</thead>
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<tr>
<td>I</td>
<td>Control group</td>
<td>5.35±0.70</td>
<td>1.95±0.15</td>
<td>105.90±3.84</td>
<td>3.98±0.24</td>
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<tr>
<td>II</td>
<td>Toxic group</td>
<td>3.20±0.18</td>
<td>4.55±0.82</td>
<td>177.30±5.88</td>
<td>2.05±0.12</td>
</tr>
<tr>
<td>III</td>
<td>Silymarin (75)</td>
<td>4.80±0.50*</td>
<td>2.63±0.33*</td>
<td>124.70±4.20*</td>
<td>2.95±0.20*</td>
</tr>
<tr>
<td>IV</td>
<td>Compound A (5)</td>
<td>4.18±0.40*</td>
<td>3.18±0.47*</td>
<td>135.55±4.60*</td>
<td>2.40±0.14*</td>
</tr>
<tr>
<td>V</td>
<td>Compound B (5)</td>
<td>4.45±0.47*</td>
<td>2.85±0.40*</td>
<td>129.40±4.38*</td>
<td>2.55±0.19*</td>
</tr>
<tr>
<td>VI</td>
<td>Compound C (5)</td>
<td>4.36±0.42*</td>
<td>3.05±0.48*</td>
<td>130.15±4.44*</td>
<td>2.47±0.17*</td>
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</tbody>
</table>

*Indicates p < 0.01 significantly different from toxic positive control group
Table 3: Effect of isolated compounds of fruits of *Cordia obliqua* on oxidative stress and antioxidant markers in paracetamol-induced hepatotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment dose (mg/Kg)</th>
<th>SOD (U/mg Protein)</th>
<th>CAT (U/mg Protein)</th>
<th>GPX (U/mg Protein)</th>
<th>MDA (nmol/mg Protein)</th>
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<tbody>
<tr>
<td>I</td>
<td>Control group</td>
<td>130.25± 3.30</td>
<td>280.45± 6.25</td>
<td>1.30±0.15</td>
<td>3.95</td>
</tr>
<tr>
<td>II</td>
<td>Toxic group</td>
<td>71.30± 1.25</td>
<td>188.50±4.25</td>
<td>0.44±0.03</td>
<td>7.45±0.40</td>
</tr>
<tr>
<td>III</td>
<td>Silymarin (75)</td>
<td>104.70±3.20*</td>
<td>254.35±5.75*</td>
<td>0.92±0.07*</td>
<td>4.55±0.32*</td>
</tr>
<tr>
<td>IV</td>
<td>Compound A (5)</td>
<td>94.20±2.45*</td>
<td>230.40±5.20*</td>
<td>0.63±0.05*</td>
<td>5.15±0.45*</td>
</tr>
<tr>
<td>V</td>
<td>Compound B (5)</td>
<td>98.70±2.60*</td>
<td>242.30±5.35*</td>
<td>0.72±0.08*</td>
<td>4.85±0.34*</td>
</tr>
<tr>
<td>VI</td>
<td>Compound C (5)</td>
<td>97.85±2.55*</td>
<td>237.45±5.20*</td>
<td>0.60±0.04*</td>
<td>5.04±0.38*</td>
</tr>
</tbody>
</table>

*P < 0.01 statistically significantly compared to toxic group

(A) Normal control, Section of normal liver tissue with portal triad showing portal vein (V), portal artery (arrow) and hepatic ducts (arrowhead).

(B) Toxic control, Section of liver tissue of animal treated with PCM showing necrosis (N), fatty vacuole (F) and central vein (v).

(C) Silymarin, Section of normal liver tissue with portal triad showing portal vein (V), portal artery (arrow) and hepatic ducts (arrowhead).

(D) Isolated compounds A, Section of the liver tissue of PCM + 5 mg/kg b.wt, po CC-1 treated animals showing normal arrangement of hepatocytes around the portal vein (V), absence of necrosis and moderate accumulation of fatty vacuoles (F).

(E) Isolated compounds B, Section of the liver tissue of PCM + 5 mg/kg b.wt, po CC-2 treated animals showing normal arrangement of hepatocytes around the portal vein (V), portal artery (arrow) and hepatic ducts (arrowhead).

(F) Isolated compounds C, Section of the liver tissue of PCM + 5 mg/kg b.wt, po PE-2 treated animals showing normal arrangement of hepatocytes around the portal vein (V), absence of necrosis and moderate accumulation of fatty vacuoles (F).

Figure 8: Histopathology of liver tissues
Histopathological features

Histology of liver sections of normal rats (Group I) showed a normal liver picture with brought-out central vein, preserved cytoplasm and prominent nucleus and nucleolus (Figure 8 A). The liver sections of paracetamol-induced hepatotoxic rats (Group II toxic group) showed hepatic cells with serum toxicity characterized by inflammatory cell collection, scattered inflammation across liver parenchyma, focal necrosis and swelling up of vascular endothelial cells (Figure 8 B). Silymarin (Group-III) exhibited protection from paracetamol-induced changes in the liver (Figure 8 C). Isolated compounds A-C pretreatment at a dose of 5 mg/kg (groups IV to VII) appeared to significantly ameliorated paracetamol toxicity as revealed by the hepatic cells with preserved cytoplasm. Pretreatment also caused a marked decrease in inflammatory cell aggregation (Figure 8 D to G).

DISCUSSION

Many important compounds with therapeutic potential have been discovered from medicinal plants, and so exploiting potential of natural medicines is therefore essential [10]. This study aimed to isolate, characterize, and investigate the therapeutic effects of new phytocconstituents isolated from ethanolic (COE) and aqueous (COA) extracts of Cordia oblique Wild in paracetamol-induced hepatotoxicity in Wistar rats. The COE were subjected to phytochemical screening and results indicated the presence of fatty acids, anthraquinones, glycosides, saponins, alkaloids, tannins, flavonoids, coumarins, phenolics, triterpenes, and sterols. Column chromatography was used to separate phytoconstituents from COE. The polarity of anticipated phytocconstituents, reviews from relevant literature, findings of phytochemical screening, and TLC investigations were all taken into consideration while choosing the solvent system for column chromatography. Beginning with hexane and ethyl acetate, the polarity of column elution process was gradually increased for efficient elution. Thin layer chromatography was conducted for all fractions, and the fractions with similar Rf values were bulked. The fractions were then subjected to preparative TLC employing ethyl acetate: hexane (25:75 v/v) as the solvent. The isolated compounds (A, B and C) were purified by recrystallization and distillation and identified as flavonoids from spectral data. Three isolated compounds from COE are been reported for the first time in this study.

The liver is the most crucial target for drug toxicity because it is extremely vulnerable to chemically induced damage [17]. Hepatotoxicity, which is shown by an increase in liver enzymes, is connected to compromised liver functions brought on by exposure to drugs or another non-infectious agent. Cordia oblique has hepatoprotective, antioxidant, antimicrobial, antihyperlipidemic, anti-diabetes, anti-cancer, and anti-inflammatory activities [7]. Furthermore, it is not toxic and well tolerated at a dose of 2000 mg/kg as shown in this study. Thus, it has the potential to be developed into modern medicine for the treatment of numerous ailments. A group of enzymes found in hepatic cells are usually used as indicators of liver damage. In liver injury, levels of these enzymes (alanine transaminase, ALT; aspartate transferase, AST; and alkaline phosphatase, ALP) usually rise.

Results of this study showed that, in comparison to control group, paracetamol overdose as seen in the toxic group was manifested by elevated serum transaminases and bilirubin levels. Paracetamol administration also caused a substantial increase in AST, ALT, ALP, GGTP, and TB levels with a substantial decrease in TP and TA levels. Treatment with silymarin or isolated compounds 5 days before and 2 days after paracetamol administration revealed a reduction in AST, ALT, ALP, GGTP, and TB levels and elevated TP and TB levels. A raised AST level indicates liver damage and catalyzes the conversion of alanine to pyruvate and glutamate, which are eliminated in like-manner. In this way, ALT is a better parameter for detecting liver injury since it is more specific to the liver. Increased hepatic enzyme activity indicates cellular leakage and a compromise of functional integrity of liver cell membranes. Hyperbilirubinemia found in paracetamol-induced hepatotoxic group in this study may be due to inappropriate bile absorption, metabolism, and discharge by damaged liver cells. Compared to control group, paracetamol-induced oxidative stress in the liver manifested as a significant increase in MDA levels and a significant decrease in CAT, GPX, and SOD activity [4]. Lipid peroxidation has a more significant impact on the liver, which results in production of high molecular mass proteins aggregated with membrane. As a result, rise in MDA is a sign of lipid peroxidation. Excessive production of free radicals and reactive oxygen species during metabolism of paracetamol leads to exhaustion of natural body antioxidant system and increased lipid peroxidation [4]. A protective enzyme called SOD converts oxygen superoxide anion to hydrogen peroxide. Hydrogen peroxide is changed by CAT into water and oxygen. By
combining reduced glutathione, GPX decreases peroxide levels. When co-administered with paracetamol, the isolated compounds (A-C) considerably enhanced antioxidant status by elevating SOD, GPX, and CAT activities and reducing MDA levels as compared to toxic group.

Histopathological findings of paracetamol-treated rats alone showed hepatic cells with serum toxicity characterized by inflammatory cell collection, scattered inflammation across liver parenchyma, focal necrosis and swelling up of vascular endothelial cells. Treatment with the isolated compounds (A-C) and silymarin significantly ameliorated paracetamol-induced toxicity, as revealed by preserved cytoplasm of hepatic cells and decrease in inflammatory cells. The hepatoprotective and free radical scavenging potential of the isolated compounds A, B, and C in paracetamol induced hepatotoxicity rat model may be due to the presence of phenolic compounds and flavonoids, which have antioxidant and hepatoprotective properties.

CONCLUSION
The isolated compounds from Cordia obliqua (A-C) reduce the harmful effects of paracetamol-induced hepatotoxicity in Wistar rats. Its hepatoprotective activity against paracetamol-induced damage is due to the presence of flavonoids and phenolic substances in the extracts. This study provides information on the phytochemical collection of Cordia oblique and acts as a facilitator for upcoming bioassays. Isolated compounds (B and C) are promising lead molecules to develop new and more potent drugs.

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Conflict of Interest
No conflict of interest associated with this work.

Contribution of Authors
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REFERENCES
8. Sivagnanam S, Devarasu P. Hepatoprotective activity of Cordia obliqua against paracetamol-induced liver toxicity


