CHAPTER VI
PREVENTIVE AND CURATIVE EFFECT OF ELEPHANTOPUS SCABER LINN METHANOLIC EXTRACT AGAINST CARBON TETRACHLORIDE INDUCED HEPATIC FIBROSIS IN RATS.

6.1 Introduction

Hepatic fibrosis is the wound response to chronic hepatic injury, including alcohol abuse, viral infection, and cholestasis. The accumulation of components of the ECM is the main pathologic feature of hepatic fibrosis (Bissel DM et al., 1996). It is characterized by excessive production and deposition of extracellular matrix (ECM) molecules. Hepatic fibrosis, a precursor of cirrhosis, developing in response to chronic hepatocellular injury show general features of a wound repair process and involves the abnormal accumulation of extracellular matrix proteins (ECM), particularly collagens (Khan et al., 2009a, Khan et al., 2009b, Khan et al., 2011). In the injured liver, these ECM components are produced in stellate cells or lipocytes and transform it into myofibroblasts in the space of Disse. This is accompanied by the synthesis of large quantities of the major components of ECM, including collagen, fibronectin, laminin and proteoglycans.

The CCl₄ treated rats is frequently used as an experimental model to study hepatic fibrosis. CCl₄ intoxication generates free radicals that trigger a cascade of events resulting in hepatic fibrosis. CCl₄ a highly toxic chemical agent, causes hepatic injury including hepatocytic necrosis, steatosis, and inflammation. Research for establishing a model of liver fibrosis with CCl₄ began in 1936. Since then many methods to establish a model of liver
fibrosis have been tried. Among them, hepatic fibrosis caused by CCl₄ has been extensively used in experimental models in rats because hepatic responses in rats to chronic CCl₄ stimulation are shown to be superficially similar to human cirrhosis.

Traditional plant drugs have been found to be effective in preventing fibrogenesis and other chronic liver injury (Yao et al., 2005; Wills and Asha, 2006). *E.scaber* has been proved to be an efficient hepatoprotectant (Rajesh and Latha, 2001, Sheeba et al., 2012) but no evidences are available on the antifibrotic property of *E.scaber*.

As many unknowns still remain about the antifibrotic efficacy of *E.scaber*, this study was undertaken with a view to probe how far this medicinal plant is effective in combating fibrosis induced by carbon tetrachloride. Both preventive and curative efficacy of the plant extract was evaluated.

### 6.2 Materials and methods

#### 6.2.1 Preparation of plant extract.

The plant material was collected, prepared for extraction and extraction performed using methanol as explained in section 2.1.1 and 2.1.2. For animal experiment the crude extract was prepared as a suspension in 5% Tween 80.

#### 6.2.2 Animals

Male Wistar rats weighing between 120 to 150g were used for the experimental purpose. The animals were kept as explained in section 2.2
6.2.3 Induction of hepatic fibrosis – Experimental design

(i) Pre-treatment evaluation (preventive model)

Rats were divided into five groups with six animals in each group.

Group I - Animals served as vehicle control and received oral administration of liquid paraffin twice a week at the dose of 3ml/kg body weight on every first and fourth days of the week.

Group II - Animals constituted the toxic control group which received oral administration of LP+CCl₄(1:1 V/V) twice a week at the dose of 3ml/kg body weight orally on every first and fourth days of the week.

Group III – Animals were same as in Group II but received methanolic extract of *Elephantopus scaber* at a dose of 100 mg/ kg body weight.

Group IV – Animals were same as in Group II but received methanolic extract of *Elephantopus scaber* at a dose of 200 mg/ kg body weight.

Group V – Animals were same as in Group II but received silymarin at a dose of 50 mg/ kg body weight.

Oral treatment with *E. scaber* methanolic extract 200 mg/kg and 100 mg/kg doses were started for group III and IV animals respectively. Simultaneously, standard drug silymarin (50 mg/kg) treatment was started for group V animals. In the pre-treatment evaluation *E.scaber* extract and silymarin were given one week before the onset of CCl₄ administration and continued up to 10 weeks. Body weights of the animals were taken initially
and at the end of each week. The rats were sacrificed 48 h after the last dose of CCl₄ administration. Blood and liver samples were collected.

(ii) Post-treatment evaluation (Curative model)

Rats were divided into five groups with six animals in each group.

Group I - Animals served as vehicle control and received oral administration of liquid paraffin twice a week at the dose of 3ml/kg body weight on every first and fourth days of the week.

Group II - Animals constituted the toxic control group which received oral administration of LP+CCl₄ (1:1 V/V) twice a week at the dose of 3ml/kg body weight orally on every first and fourth days of the week.

Group III - Animals were same as in Group II but received methanolic extract of *Elephantopus scaber* at a dose of 100 mg/ kg body weight.

Group IV - Animals were same as in Group II but received methanolic extract of *Elephantopus scaber* at a dose of 200 mg/ kg body weight.

Group V - Animals were same as in Group II but received silymarin at a dose of 50 mg/ kg body weight.

Hepatotoxicity was induced by CCl₄ administration for a period of 10 weeks for groups II, III, IV and V. After 10 weeks of intoxication with CCl₄, oral treatment with *Elephantopus scaber* methanolic extract at 200 mg/kg and 100 mg/kg doses were started for group III and IV animals respectively. Simultaneously, standard drug Silymarin (50 mg/kg) treatment was started for group V animals for two weeks. Body weights of the animals were taken.
initially and at the end of each week. Animals were sacrificed on the 15th day after the last dose of CCl₄ administration. Blood samples and tissue samples were collected.

6.2.4 Biochemical parameters investigated

Blood collected was kept for 30 min at room temperature. Serum was separated by centrifugation at 3000 rpm at room temperature for 15 min. and ALT (EC.2.6.1.2), AST (EC.2.6.1.2), ALP (EC.3.1.3.1), LDH (EC.1.1.1.27), TP and albumin levels in serum were estimated.

The liver tissue was dissected out, blotted off blood, washed in saline and weighed instantaneously. This was kept in frozen containers and proceeded for biochemical estimations.

6.2.5 Determination of hydroxyproline

The hydroxyproline content of the liver was measured. For the estimation 200 mg of liver tissue was homogenized and the homogenate was mixed with 6N HCl and then hydrolysed at 110°C for 16 h. After cooling 100 µl of each sample was made up to 2ml with acetate–citrate buffer. Added 1ml of chloramine-T reagent (0.56% buffered) and kept for 20 min at room temperature. Then added 1ml of freshly prepared Ehrlich’s reagent and incubated at 60°C for 15 min and cooled. Read the absorbance at 560 nm against a reagent blank which contained the complete system without the tissue sample.

6.2.6 Histopathological studies

Portions of liver were put in 10% formalin, embedded in paraffin and processed for histopathological analysis as described in section 2.8. Liver sections were graded numerically based on Knodell’s histological activity
index to assess the degree of histologic injury in hepatic fibrosis. The parameters were graded from score 0 to 4, with 0 indicating no abnormality, 1 indicating periportal with or without bridging necrosis, 2 indicating interlobular degeneration and focal necrosis and 3 with portal inflammation and score 4 indicating fibrosis.

6.2.7 Immunohistochemical studies

Liver samples were collected, washed with phosphate-buffered saline (PBS) and fixed overnight in 10% buffered formalin. Serial sections (5 µm) were prepared after the samples had been dehydrated in graded ethanol solutions, cleared in xylene and embedded in paraffin wax and processed as described in section 2.9.

6.3 Results

6.3.1 Body weight and liver weight

Body weight of CCl$_4$ treated animals declined significantly ($p < 0.05$) by the end of the 10th week of exposure when compared with the normal rats. *E. scaber* extract treatment improved the loss of body and liver weight during CCl$_4$ administration in the preventive group (Fig6.1). In post-treatment animals, *E. scaber* extract enhanced the body weight within a period of two weeks after intoxication with CCl$_4$ for 10 weeks (Fig6.2).
Fig 6.1. Graph showing the body weight pattern of fibrotic rats administered with CCl_4 and *E. scaber* extract in preventive groups. The mean of each of the groups is represented, with error bar indicating the standard deviation. Group I- vehicle control, Group II- Toxic control, Group III- *E. scaber* 200 mg/kg and CCl_4 treated, Group IV- *E. scaber* 100 mg/kg and CCl_4 treated, and Group V- Silymarin 50 mg/kg and CCl_4 treated animals.

Fig 6.2. Graph showing the body weight pattern of fibrotic rats administered with CCl_4 and *E. scaber* extract in curative groups. The mean of each of the groups is represented, with error bar indicating the standard deviation. Group I- vehicle control, Group II- Toxic control, Group III- *E. scaber* 200 mg/kg and CCl_4 treated, Group IV- *E. scaber* 100 mg/kg and CCl_4 treated, and Group V- Silymarin 50 mg/kg and CCl_4 treated animals.
Table 6.1  Final body weight and liver weight of different groups of rats in the preventive and curative treatments.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Body weight (gm) Preventive group</th>
<th>Liver weight (gm) Preventive group</th>
<th>Body weight (gm) Curative Group</th>
<th>Liver weight (gm) Curative Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>vehicle control</td>
<td>260.83 ± 13.19 †</td>
<td>6.9 ± 0.08 †</td>
<td>270.83±13.19</td>
<td>7.3 ± 0.08 †</td>
</tr>
<tr>
<td>Toxic control CCl4 treated</td>
<td>165.71 ± 9.75*</td>
<td>4.4 ± 0.1*</td>
<td>194.17±6.65*</td>
<td>4.8 ± 0.08*</td>
</tr>
<tr>
<td>E. scaber extract (200 mg/kg) + CCl4</td>
<td>230.33 ±10.8†</td>
<td>6.4 ± 0.07†</td>
<td>205±8.37†</td>
<td>6.0 ± 0.08†</td>
</tr>
<tr>
<td>E. scaber extract (100 mg/kg) + CCl4</td>
<td>226.67±13.66†</td>
<td>5.1±0.06†</td>
<td>200.83±9.27†</td>
<td>5.0±0.07†</td>
</tr>
<tr>
<td>Silymarin (50mg/kg) + CCl4</td>
<td>244.67 ±9.22†</td>
<td>6.7 ± 0.08†</td>
<td>210.83±13.19†</td>
<td>6.2 ± 0.08†</td>
</tr>
</tbody>
</table>

*p ≤ 0.05 versus vehicle control.
†p ≤ 0.05 versus CCl4 control.
Values are Mean ± S.D, n = 6.

6.3.2 Liver function tests

AST, ALT, ALP and LDH activities in the serum of CCl4 administered rats were elevated in contrast to the normal rats. In the preventive treatment E. scaber remarkably prevented the rise of AST, ALT, ALP and LDH values as expressed in table 6.2. In the curative treatment animals, it reversed the toxic effect of CCl4 as evidenced by the activities of AST, ALT, ALP and LDH displayed in table 6.3.
Table 6.2 Effect of *E. scaber* methanolic extract and silymarin on AST, ALT, ALP and LDH activities in CCl₄ exposed fibrotic rats in preventive treatment groups

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>LDH (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: vehicle control</td>
<td>143.16 ± 13.37</td>
<td>42.66 ± 6.12</td>
<td>179.66 ±16.53</td>
<td>169.00 ± 6.69</td>
</tr>
<tr>
<td>Group II: CCl₄ control</td>
<td>2221.66± 123.43*</td>
<td>652.66 ±35.56*</td>
<td>837.16±34.97*</td>
<td>525.83±30.88</td>
</tr>
<tr>
<td>Group III: <em>E. scaber</em> extract(200 mg/kg) + CCl₄</td>
<td>495.83 ± 34.55†</td>
<td>199.16 ±32.46†</td>
<td>412.5 ± 46.05†</td>
<td>240.83±20.35</td>
</tr>
<tr>
<td>Group IV: <em>E. scaber</em> extract(100 mg/kg) + CCl₄</td>
<td>945.83 ± 29.39†</td>
<td>425.83± 17.65†</td>
<td>637.5±35.46</td>
<td>421.66±13.66</td>
</tr>
<tr>
<td>Group V: Silymarin treated + CCl₄</td>
<td>720.83 ±46.62†</td>
<td>334.16 ±10.68†</td>
<td>503.33±34.19</td>
<td>313.33±16.93</td>
</tr>
</tbody>
</table>

*p ≤ 0.05 versus vehicle control.
†p ≤ 0.05 versus CCl₄ control.

Values are Mean ± S.D, n = 6

Table 6.3 Effect of *E. scaber* methanolic extract and silymarin on AST, ALT, ALP and LDH activities in CCl₄ exposed fibrotic rats in curative treatment groups

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>LDH (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Vehicle control</td>
<td>141.83 ± 12.49</td>
<td>42.00 ± 5.47</td>
<td>179.50 ±15.90</td>
<td>170.83 ±7.25</td>
</tr>
<tr>
<td>Group II: CCl₄ control</td>
<td>1411.16± 17.38*</td>
<td>437.55 ±35.62*</td>
<td>751.00±26.67*</td>
<td>506.50±14.01*</td>
</tr>
<tr>
<td>Group III: <em>E. scaber</em> extract(200 mg/kg) + CCl₄</td>
<td>204.16±19.92†</td>
<td>90.16±7.85†</td>
<td>328 ± 35.49†</td>
<td>207.33±8.95†</td>
</tr>
<tr>
<td>Group IV: <em>E. scaber</em> extract(100 mg/kg) + CCl₄</td>
<td>414.66 ± 11.99†</td>
<td>201.66± 15.70†</td>
<td>516.33±25.35†</td>
<td>400.00±22.80†</td>
</tr>
<tr>
<td>Group V: Silymarin treated + CCl₄</td>
<td>338.33 ± 19.13†</td>
<td>151.66 ±10.80†</td>
<td>443.8 ±22.04†</td>
<td>310.16±12.00†</td>
</tr>
</tbody>
</table>

*p ≤ 0.05 versus vehicle control.
†p ≤ 0.05 versus CCl₄ control.

Values are Mean ± S.D, n = 6
6.3.2.1 Estimation of aspartate aminotransferase (AST)

_E. scaber_ methanolic extract (200 mg/kg) treatment exerted its protection by 83% and 95% in preventive and curative groups and _E. scaber_ methanolic extract (100 mg/kg) treatment exerted its protection by 61% and 79% in preventive and curative groups in CCl<sub>4</sub> intoxicated rats. Silymarin (50 mg/kg) treated rats exerted its protection by 72% and 92% in preventive and curative groups respectively when compared to the CCl<sub>4</sub> control. Treatment with _E. scaber_ extract showed significant (_p_ ≤ 0.05) change in contrast to the CCl<sub>4</sub> control (Fig 6.3 & 6.4).

**Fig 6.3** Effect of _E. scaber_ methanolic extract on aspartate aminotransferase (AST) activity in the serum of fibrotic rats in preventive treatment groups

* _p_ ≤ 0.05 versus vehicle control.
† _p_ ≤ 0.05 versus CCl<sub>4</sub> control.

Values are Mean ± S.D, _n_ = 6
Fig 6.4  Effect of *E. scaber* methanolic extract on aspartate aminotransferase (AST) activity in the serum of fibrotic rats in curative treatment groups

![Graph showing AST activity in different treatment groups](image)

* *p* ≤ 0.05 versus vehicle control.
† *p* ≤ 0.05 versus CCl₄ control.
Values are Mean ± S.D, n = 6

6.3.2.2 Estimation of alanine aminotransferase (ALT)

*E. scaber* methanolic extract (200 mg/kg) treatment exerted its protection by 74% and 88% in preventive and curative groups and *E. scaber* methanolic extract (100 mg/kg) treatment exerted its protection by 37% and 60% in preventive and curative groups when compared to the CCl₄ control. Silymarin (50 mg/kg) treated rats exerted its protection by 52% and 72% in preventive and curative groups respectively when compared to the CCl₄ control. Treatment with *E. scaber* extract showed significant (*p* ≤ 0.05) change in contrast to the CCl₄ control (Fig 6.5 & 6.6).
Fig 6.5 Effect of *E. scaber* methanolic extract on alanine aminotransferase (ALT) activity in the serum of fibrotic rats in preventive treatment groups

![Graph showing the effect of E. scaber methanolic extract on ALT activity in serum of fibrotic rats in preventive treatment groups.](image)

* p ≤ 0.05 versus vehicle control.
† p ≤ 0.05 versus CCl₄ control.
Values are Mean ± S.D, n = 6

Fig 6.6 Effect of *E. scaber* methanolic extract on alanine aminotransferase (ALT) activity in the serum of fibrotic rats in curative treatment groups

![Graph showing the effect of E. scaber methanolic extract on ALT activity in serum of fibrotic rats in curative treatment groups.](image)

* p ≤ 0.05 versus vehicle control.
† p ≤ 0.05 versus toxic control.
Values are Mean ± S.D, n = 6
6.3.2.3 Estimation of alkaline phosphatase (ALP)

*E. scaber* methanolic extract (200 mg/kg) treatment exerted its protection by 44% and 74% in preventive and curative groups and *E. scaber* methanolic extract (100 mg/kg) treatment exerted its protection by 35% and 41% in preventive and curative groups when compared to the CCl₄ control. Silymarin (50 mg/kg) treated rats exerted its protection by 51% and 54% in preventive and curative groups respectively when compared to the CCl₄ control. Treatment with *E. scaber* extract showed significant (*p* ≤ 0.05) change in contrast to the CCl₄ control (Fig 6.7 & 6.8).

**Fig 6.7** Effect of *E.scaber* methanolic extract on alkaline phosphatase (ALP) activity in the serum of fibrotic rats in preventive treatment groups

![Graph showing ALP activity](image)

* *p* ≤ 0.05 versus vehicle control.
† *p* ≤ 0.05 versus CCl₄ control.

Values are Mean ± S.D, n = 6
Fig 6.8  Effect of *E. scaber* methanolic extract on alkaline phosphatase (ALP) activity in the serum of fibrotic rats in curative treatment groups

![Graph showing ALP activity in different groups](image)

* *p* ≤ 0.05 versus vehicle control.
† † *p* ≤ 0.05 versus CCl4 control.

Values are Mean ± S.D, n = 6

6.3.2.4 Estimation of lactate dehydrogenase (LDH)

*E. scaber* methanolic extract (200 mg/kg) treatment exerted its protection by 80% and 89% in preventive and curative groups and *E. scaber* methanolic extract (100 mg/kg) treatment exerted its protection by 30% and 32% in preventive and curative groups when compared to the CCl4 control. Silymarin (50 mg/kg) treated rats exerted its protection by 58% and 60% in preventive and curative groups respectively when compared to the CCl4 control. Treatment with *E. scaber* extract showed significant (*p* ≤ 0.05) change in contrast to the CCl4 control (Fig 6.9 & 6.10).
Fig 6.9 Effect of *E.scaber* methanolic extract on lactate dehydrogenase (LDH) activity in the serum of fibrotic rats in preventive treatment groups

![Graph showing LDH activity in different groups](image)

* *p* ≤ 0.05 versus vehicle control.
† *p* ≤ 0.05 versus CCl₄ control.

Values are Mean ± S.D, n = 6

Fig 6.10 Effect of *E.scaber* methanolic extract on lactate dehydrogenase (LDH) activity in the serum of fibrotic rats in fibrotic rats in curative treatment groups

![Graph showing LDH activity in different groups](image)

* *p* ≤ 0.05 versus vehicle control.
† *p* ≤ 0.05 versus CCl₄ control.

Values are Mean ± S.D, n = 6
6.3.2.5 Estimation of hydroxyproline

*E. scaber* methanolic extract (200 mg/kg) treatment exerted its protection by 78% and 82% in preventive and curative groups and *E. scaber* methanolic extract (100 mg/kg) treatment exerted its protection by 46% and 54% in preventive and curative groups when compared to the CCl₄ control. Silymarin (50 mg/kg) treated rats exerted its protection by 65% and 74% in preventive and curative groups respectively when compared to the CCl₄ control. Treatment with *E. scaber* extract showed significant (*p* ≤ 0.05) change in contrast to the CCl₄ control (Table 6.4 & 6.5).

6.3.2.6 Estimation of total protein and albumin

Treatment with *E. scaber* methanolic extract (200 mg/kg) exhibited a significant increase in total protein and albumin levels in preventive and curative groups whereas *E. scaber* methanolic extract (100 mg/kg) treatment exhibited a marginal increase in the albumin level in preventive and curative groups when compared to the CCl₄ control. Silymarin (50 mg/kg) treated rats exerted an increase in total protein and albumin in between *E. scaber* methanolic extract of (200 mg/kg) and *E. scaber* methanolic extract of (100 mg/kg) in preventive and curative groups respectively when compared to the CCl₄ control. Treatment with *E. scaber* extract showed significant (*p* ≤ 0.05) change in contrast to the CCl₄ control (Table 6.4 & 6.5).
Table 6.4  Effect of *E. scaber* methanolic extract and silymarin on total protein, albumin and hydroxy proline levels in CCl4 exposed fibrotic rats in preventive treatment groups

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>TOTAL PROTEIN gm%</th>
<th>ALBUMIN gm%</th>
<th>HYDROXY PROLINE nmol/mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: vehicle control</td>
<td>7.23±0.16</td>
<td>3.68±0.15</td>
<td>0.372±0.01</td>
</tr>
<tr>
<td>Group II: CCl4 control</td>
<td>6.57±0.05*</td>
<td>2.34±0.05*</td>
<td>1.55±0.05*</td>
</tr>
<tr>
<td>Group III: <em>E. scaber</em> extract(200 mg/kg) + CCl4</td>
<td>6.83±0.06†</td>
<td>3.30±0.06†</td>
<td>0.529±0.036†</td>
</tr>
<tr>
<td>Group IV: <em>E. scaber</em> extract(100 mg/kg) + CCl4</td>
<td>6.55±0.04</td>
<td>2.88±0.06†</td>
<td>1.01±0.054†</td>
</tr>
<tr>
<td>Group V: Silymarin treated + CCl4</td>
<td>6.68±0.03†</td>
<td>3.10±0.03†</td>
<td>0.785±0.01†</td>
</tr>
</tbody>
</table>

*p* ≤ 0.05 versus normal control.

†*p* ≤ 0.05 versus CCl4 control.

Values are Mean ± S.D, n = 6

Table 6.5  Effect of *E. scaber* methanolic extract and silymarin on total protein, albumin and hydroxy proline levels in CCl4 exposed fibrotic rats in curative treatment groups

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>TOTAL PROTEIN gm%</th>
<th>ALBUMIN gm%</th>
<th>HYDROXY PROLINE nmol/mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: vehicle control</td>
<td>7.19±0.17</td>
<td>3.68±0.15</td>
<td>0.371±0.015</td>
</tr>
<tr>
<td>Group II: CCl4 control</td>
<td>6.53±0.08*</td>
<td>3.01±0.11*</td>
<td>1.366±0.038*</td>
</tr>
<tr>
<td>Group III: <em>E. scaber</em> extract(200 mg/kg) + CCl4</td>
<td>6.7 9±0.04†</td>
<td>3.42±0.03†</td>
<td>0.554±0.034†</td>
</tr>
<tr>
<td>Group IV: <em>E. scaber</em> extract(100 mg/kg) + CCl4</td>
<td>6.66±0.03</td>
<td>3.09±0.04†</td>
<td>0.829±0.030†</td>
</tr>
<tr>
<td>Group V: Silymarin treated + CCl4</td>
<td>6.74±0.02†</td>
<td>3.22±0.04†</td>
<td>0.633±0.0†</td>
</tr>
</tbody>
</table>

*p* ≤ 0.05 versus vehicle control.

†*p* ≤ 0.05 versus CCl4 control.

Values are Mean ± S.D, n = 6
6.3.3 Histopathological studies

In preventive and curative treatment models histopathological study of liver from group I animals showed a normal hepatic architecture (Fig.6.11A and 6.12A). CCl₄ treated group, exhibited a significantly high degree of fibrosis with scores 3.6 and 3.4 in preventive and curative treatment model(Fig. 6.11B and 6.12B).

In E. scaber treated rats, at doses 200 mg/kg and 100 mg/kg body weight, and in silymarin treated groups, liver exhibited significant protection with scores 1.6 ; 2.5 ; 1.9 respectively in preventive treatment groups(Fig.12C-E) and 1.1; 2.0; 1.6 respectively in curative treatment groups (Fig.13C-E).From the results it is clear that E.scaber methanolic extract treatment effectively improved histological scores in both preventive and curative models. Comparing the effectiveness of the preventive and the curative groups, the latter had more marked reduction in histological grading when compared with the former.

6.3.4 Immunohistochemical studies

Immunohistochemical analysis showed that Collagen-III was predominantly expressed along fibrous septa in CCl₄ treated rats. Weak immunostaining of localized Collagen -III was observed in Silymarin and E. scaber extract treated rats, at doses 100 mg/kg and 200 mg/kg body weight in preventive (Fig.6.13) and curative groups (Fig. 6.14).
Fig 6.11. Histopathological features in liver of fibrotic rats in the preventive treatment group. Liver tissue was stained with H & E (100x). (A) Normal rat liver (B) CCl₄ control (C) CCl₄ + E. scaber methanolic extract (200 mg/kg) (D) CCl₄ + E. scaber methanolic extract (100 mg/kg). (E) CCl₄ + Silymarin (50 mg/kg) treated rats.
Fig 6.12. Histopathological features in liver of fibrotic rats in the curative treatment group. Liver tissue was stained with H & E (100x). (A) Normal rat liver (B) CCl₄ control (C) CCl₄ + *E. scaber* methanolic extract (200 mg/kg) (D) CCl₄ + *E. scaber* methanolic extract (100 mg/kg). (E) CCl₄ + Silymarin (50 mg/kg) treated rats.
Fig 6.13 Immunohistochemical localization of Collagen-III in liver of fibrotic rats in the preventive group. Liver tissue was immunostained for Collagen-III followed by staining with hematoxylin (100×). A. Normal control, B. CCl₄ control, C. CCl₄ + methanolic extract 100mg/kg, D. CCl₄ + methanolic extract 200mg/kg, E. CCl₄ + silymarin 50mg/kg.
Fig 6.14 Immunohistochemical localization of Collagen-III in liver of fibrotic rats in the curative group. Liver tissue was immunostained for Collagen-III followed by staining with hematoxylin (100×). A. normal control, B. CCl₄ control, C. CCl₄ + methanolic extract 100mg/kg, D. CCl₄ + methanolic extract 200mg/kg, E. CCl₄ + silymarin 50mg/kg.
6.4 Discussion

Liver fibrosis is a condition of abnormal proliferation of connective tissue due to various types of chronic liver injury often caused by viral infection and chemicals. CCl₄ intoxication for a period of 10 weeks triggers the production of free radicals that will ultimately lead to hepatic fibrosis (Wills and Asha, 2006). Oxidative stress, resulting from an imbalance in the generation of free radicals and antioxidant defense molecules, affects biological macromolecules causing their structural alterations that lead to cell damage and its death (Ryter et al., 2007). This phenomenon is considered to be a major factor in the pathogenesis of a variety of liver diseases (Flora, 2007). In this regard, reduction of oxidative stress may be a good target for prevention of hepatic fibrosis.

Weight loss is one of the major symptoms of hepatotoxicity. Loss of body weight and liver weight is the hallmark of hepatic fibrosis (Kew, 1996). In the present study, the decline in body weight and liver weight observed in CCl₄ treated groups was improved by the administration of *E.scaber* at doses 200 mg/ kg body weight and 100mg / kg body weight in a dose dependent manner. Similar results obtained by the administration of silymarin (50mg/kg body weight), proves the protective effect of *E.scaber* in hepatic fibrosis both in preventive and curative models. In this respect silibinin administration showed improvement in body weight in nitrosodimethylamine (DMN)-induced fibrotic rats. (Ezhilarasan et al., 2012)

The enzymes AST, ALT, ALP and LDH are considered the most sensitive markers of liver injury as they are found in the cytoplasm of liver cells, thus damage of these cells lead to their rapid leakage into the blood circulation (Ramaiah, 2007). The activities of AST, ALT, ALP and LDH of
the extract treated group were significantly decreased as compared to the CCl$_4$ treated group. The tendency of these enzymes to return towards normal level in *E. scaber* extract treated groups may be due to its antifibrotic effect. The effect was more pronounced in the curative study than that in the prophylactic one. In this respect *Lygodium flexuosum* exerts effective protection in carbon tetrachloride induced hepatic fibrosis in rats (Wills and Asha, 2006).

In chronic liver diseases, the serum albumin level is reduced due to protein synthesis disruption in the liver (Mi-Ok and Jeon-Ok, 2010). The liver is the site of albumin and fibrinogen synthesis and also some of the alpha and beta globulins. Decrease in the albumin and total protein content in the CCl$_4$ treated group is due to decreased synthesis of albumin and other proteins. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism, which accelerates their generation process and the production of liver cells (Awang, 1993). Treatment with *E. scaber* methanolic extract increased the level of albumin and other proteins. Researchers showed that *Tinospora crispa* administration improved the synthesis of albumin and other proteins in thioacetamide induced hepatic fibrosis (Farkaad et al., 2011).

Human and animal studies suggest that hepatic immunity is altered in fibrosis and that liver inflammation is the hallmark of early-stage liver fibrosis, ultimately resulting in hepatic stellate cells (HSC) activation and extra cellular matrix (ECM) deposition. Stellate cells are regarded as the primary target cells for inflammatory stimuli in the injured liver. Following liver injury, hepatic stellate cells become activated and express a combination of matrix metalloproteinases (MMPs) and their specific tissue inhibitors (TIMPs) (Benyon and Arthur, 2001). The lack of specific inhibitors of the extracellular matrix limits the prevention and treatment of hepatic fibrosis (Mavier and
Mallot, 1995). Stellate cells transform into myofibroblasts which are responsible for the deposition of collagen fibres in liver. Thus pharmacologic intervention in hepatic fibrosis is targeted to inhibition of stellate cell activation, inhibition of ECM synthesis, or stimulation of matrix protein degradation. Hydroxyproline is an amino acid found almost exclusively in collagens. Determination of the hydroxyproline content in the liver tissue is regarded as a good marker to quantify fibrosis and to evaluate the effectiveness of new antifibrotic agents, (Miao-Xian et al., 2009). Most of the antifibrotic herbal drugs prevent fibrosis by increased removal of deposited collagen, enhanced collagenolytic activity and enhanced apoptosis of hepatic stellate cells. CCl$_4$ administration increased the hydroxyproline content of the liver. The decreased hydroxyproline content by $E$. scaber treatment exhibit its antifibrotic activity.

Histological analysis showed the CCl$_4$ caused prominent hepatic steatosis, necrosis, and formation of regenerative nodules and fibrotic septa between the nodules. Our results suggest that oral administration of methanolic extract of $E$. scaber improved the state of steatosis with a significant reduction in the number of macro- and microvesicular steatosis, and it also apparently suppressed hepatic fibrogenesis by reducing the thickness of bridging fibrotic septa.

In immunohistochemical studies, strong positive staining for Collagen-III was observed in livers of CCl$_4$ treated rats. Marked improvement of rat livers in $E$. scaber extract treated rats co-exposed with CCl$_4$ as shown by immunohistochemical techniques supports the antifibrotic action of the extract.
In short the effectiveness of *E. scaber* methanolic extract was studied both in the preventive and curative treatment of hepatic fibrosis. The loss of body weight of the treated group was regained. Liver enzyme activities were decreased. High degree of fibrosis in CCl₄ treated rats was prevented by extract treatment as evidenced by histopathology and Immuno histochemistry. Hydroxyproline content in the liver was also remarkably reduced by the treatment with the extract. Total protein and albumin levels were increased by the treatment with the extract. The effects of methanolic extract of *E. scaber* were more promising in the curative study than in the prophylactic one for the management of liver fibrosis.

The methanolic extracts were known to possess sequiterpene lactones and sesquiterpenes (DeSilva, 1982), which might be responsible for the protective effect of methanolic extract of *E. scaber* against hepatic fibrosis. In short the methanolic extract of *E. scaber* exerted significant protective activity against hepatic fibrosis by increased removal of deposited collagen, enhanced collagenolytic activity and enhanced apoptosis of hepatic stellate cells.