Hepatoprotective effect of leaves of *Erythrina indica* Lam.

M. JESUPILLAI and M. PALANIVELU

Department of Pharmaceutical Chemistry, Arulmigu Kalasalingam College of Pharmacy, Krishnan koil - 626 190 (India).

(Received: July 25, 2008; Accepted: September 05, 2008)

**ABSTRACT**

To examine liver protective effect of leaves of *Erythrina indica* (*EI*) against CCl₄ induced liver toxicity in rats.

Ethanol, chloroform and Ethyl acetate extracts of leaves of *EI* (250 mg/kg, p.o.) were administered to the male Wister rats for 10 days. Hepatotoxicity was induced by CCl₄ (0.5 ml/kg, on 10th day). The activity was assessed by studying bio chemical parameters (SGOT, SGPT, ASAT, ALAT, ALP, albumin, Bilirubin, TGL, Total protein and albumin) and histopathological studies of the liver.

Extracts together with CCl₄ treated rats showed significant restoration of liver function biochemical parameters. Further the activity was evidenced by the histopathological observation indicating that absence of necrosis and fatty infiltration as shown in the rats treated with CCl₄ alone.

Ethanol, Chloroform and Ethyl acetate extracts of leaves of *Erythrina indica* possess significant hepatoprotective activity.

**Key words:** *Erythrina indica*, carbon tetra chloride, Hepatoprotective, Rats.

**INTRODUCTION**

The liver is the largest internal organ in the human body. It plays a major role in metabolism and detoxification. It also performs and regulates a wide variety of high-volume biochemical reactions requiring very specialized tissues¹. Liver disease is a serious health problem. In the traditional system of medicine liver diseases had been successfully treated by using medicinal plants and their formulations. However, there is no satisfactory therapy for serious liver disease; mostly the herbal drugs increase the rate of natural healing process of liver. Hence the search for effective liver protective drug persist.

*Erythrina indica* Lam (Papilionaceae) is a middle sized tree, widely distributed in India and is used in traditional medicine on account of its Diuretic, anticonvulsant, anti inflammatory, hepatoprotective, anthelmintic and laxative effects²³. Though, no scientific study has been reported on hepatoprotective activity of leaves of *Erythrina indica*, we prompted to study hepatoprotective activity of leaves of *Erythrina indica* against CCl₄ induced liver toxicity in rats. In the present study, the hepatoprotective activity was assessed by some Bio chemical parameters (SGOT (Serum glutamic oxalo acetic transaminase), SGPT (Serum glutamic pyruvic transaminase), ASAT (Aspartate amino transaminase), ALAT (Alanine amino transaminase), TGL (Triglyceride), ALP (Alkaline Phosphatase), Serum albumin, Bilirubin and total protein) and histopathological surveillance of liver.

**MATERIAL AND METHODS**

**Plant collection and authentication**

The plant material was collected in the Madurai district, Tamilnadu, India during the month of March 2005. It was authenticated by Dr. Stephen, Department of Botany, The American College, Madurai. A voucher specimen has been kept in our laboratory (EI1) for future reference.

**Preparation of extract**

The dried, coarsely powdered leaves were subjected to single extraction in a soxhlet extractor [⁴] using ethanol (90%), chloroform and ethyl acetate for 18-20h. The extracts were then concentrated to dryness under reduced pressure and controlled temperature to yield a semi solid...
mass, which was preserved in a refrigerated
conditions. Preliminary phytochemical analysis[4,5]
were carried out to find out the phytoconstituents
present in the crude extracts.

Animals

Male Wister Albino rats (100-150gm) were
collected from the animal house of our institute and
housed in standard metallic cages under room
temperature (20±1°C) and relative humidity
55±10%C with 12 h light / dark cycle. The animals were
provided with standard pellet diet (M/s Hindustan
Lever Ltd, Mumbai, India.) with free access to water
ad libitum. The present study was approved by
institutional animal ethics committee (Approval no.
509/02/C/CPCSEA).

Chemicals

Silymarin was obtained from Sisco
Laboratories, Mumbai, India. Thio barbituric acid
was obtained from Sigma chemical Co (St. Louis,
MO, USA). Bio chemical estimations were done by
span diagnostic kits. All the chemicals used in the
study were of analytical grade.

hepatoprotective activity

The method described by De et al [6]
was employed for evaluating hepatoprotective
activity. The animals were divided into six groups
each group consist of six animals. The group I
animals received 10% aqueous tween 80 (per oral
(p.o.)), Group II animals received 10% aqueous
tween 80 (p.o.), Group III animals received
Silymarin (200 mg / kg), group IV animals received
ethanol extract of EI (250 mg/kg p.o.), group V
animals received ethyl acetate extract of EI (250
mg/kg, p.o.), group VI animals received
chloroform extract of EI (250 mg/kg, p.o.). The
treatment was continued for 10 days. On 10th day
CCI₄ (0.5 ml / kg, i.p.) was given to groups II, III,
IV, V and VI. 24 hrs after CCI₄ administration,
blood was withdrawn under light anesthesia. The
blood was centrifuged at 3000 rpm and 4°C to
obtain sera. The serum was used for the
estimation of marker enzymes of liver.

Bio chemical analysis

Total protein was estimated by Biuret
method⁷, Albumin (ALB) was estimated by BCG
method⁸, SGOT, SGPT were measured as kinetic
reaction using IFCC method, the absorbance of
reaction was determined at 340 nm by
spectrophotometer⁹. ASAT, ALAT and ALP were
estimated by the method of Bergmeyer¹⁰. Serum
level of Total bilirubin was estimated by the method
of Waters et al, 1970¹¹. Lipid peroxide level was
estimated by the method of Ohkawa et al., ¹².

Histopathological studies

The animals were sacrificed by cervical
dislocation, fresh liver tissues were trimmed
approximately to 2 µm thickness, fixed in 10 %
buffered formalin, embedded in paraffin then stained
with hematoxylin and eosin and observed under
original magnification 100x.

Statistical analysis

The statistical analysis were carried out by
One Way Analysis of Variance (ANOVA) followed by
student ‘t’ test, P> 0.05 was considered significant.
All the values are reported as Mean ± SEM.

RESULTS

Phytochemical results

Phytochemical analysis showed the
presence of alkaloids, flavonoids, phytosterols,
tannins, saponins and glycosides in all the three
extracts (ethanol, ethyl acetate and chloroform).

Biochemical results

Administration of Ethanol, Ethyl acetate
and Chloroform extract of leaves of Erythrina indica
(250 mg/kg) significantly (P<0.05) restored CCl₄
induced increase in serum GOT, GPT, ASAT, ALAT,
ALP and Bilirubin (table1) and CCl₄ induced
decrease in serum TGL, Total protein and Albumin
(table 2). It was also observed that increased lipid
peroxide level and weight gain in the liver treated
with CCl₄ alone and significant recovery in drug
treated animals (Table.3). All the parameters
observed in EI treated animals were comparable to
those observed in the animals treated with known
hepatoprotective agent Silymarin (200 mg/kg).

Histopathological results

Liver of normal rat showed central vein with
radiating columns of hepatocyte. liver of rat treated
with CCl₄ alone showed intense centrlobular
necrosis and fatty infiltration. Liver of rat treated with
### Table 1: Effect of various extracts of leaves of *Erythrina indica* on the biochemical parameters of CCl₄ intoxicated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>GOT U/L</th>
<th>GPT U/L</th>
<th>ASAT U/L</th>
<th>ALAT U/L</th>
<th>ALP KA units</th>
<th>Bilirubin U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>-</td>
<td>52.6±</td>
<td>63.2±</td>
<td>52.15±</td>
<td>17.65±</td>
<td>238.25±</td>
<td>0.41±</td>
</tr>
<tr>
<td>CCl₄</td>
<td>0.5 ml/kg</td>
<td>110.7±</td>
<td>119.5±</td>
<td>102.2±</td>
<td>54.16±</td>
<td>421.34±</td>
<td>1.512±</td>
</tr>
<tr>
<td>CCl₄(0.5 ml/kg)+Sily.</td>
<td>mg/kg</td>
<td>59.7±</td>
<td>72.15±</td>
<td>54.31±</td>
<td>25.19±</td>
<td>290.18±</td>
<td>0.405±</td>
</tr>
<tr>
<td>CCl₄(0.5 ml/kg)+EEI</td>
<td>mg/kg</td>
<td>69.1±</td>
<td>75.8±</td>
<td>63.51±</td>
<td>31.49±</td>
<td>325.34±</td>
<td>0.613±</td>
</tr>
<tr>
<td>CCl₄(0.5 ml/kg)+EAEI</td>
<td>mg/kg</td>
<td>78.5±</td>
<td>81.6±</td>
<td>71.06±</td>
<td>35.07±</td>
<td>342.26±</td>
<td>0.835±</td>
</tr>
<tr>
<td>CCl₄(0.5 ml/kg)+CEI</td>
<td>mg/kg</td>
<td>80.2±</td>
<td>83.5±</td>
<td>75.29±</td>
<td>33.16±</td>
<td>357.6±</td>
<td>0.925±</td>
</tr>
</tbody>
</table>

Sily, EEI, EAEI and CEI – silymarin, Ethanol, Ethyl acetate and Chloroform extract of EI respectively. Mean ± S.E.M, n= 6, *P<0.05 (Compared to control) were considered significant, *P<0.05 (Compared to Standard) were considered significant.

### Table 2: Effect of various extracts of leaves of *Erythrina indica* on the biochemical parameters of CCl₄ intoxicated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>sTGL mg/ml</th>
<th>Total protein g/dL</th>
<th>Albumin U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>-</td>
<td>61.19±0.57</td>
<td>5.76±0.59</td>
<td>2.31±0.67</td>
</tr>
<tr>
<td>CCl₄</td>
<td>0.5 ml/kg</td>
<td>29.13±0.59</td>
<td>2.96±0.58</td>
<td>1.25±0.82</td>
</tr>
<tr>
<td>CCl₄(0.5ml/kg)+Silymarin</td>
<td>200 mg/kg</td>
<td>57.92±0.25*</td>
<td>4.81±0.69*</td>
<td>2.949±0.79*</td>
</tr>
<tr>
<td>CCl₄(0.5 ml/kg)+EEI</td>
<td>250 mg/kg</td>
<td>51.49±0.36*</td>
<td>3.69±0.93*</td>
<td>2.09±1.69*</td>
</tr>
<tr>
<td>CCl₄(0.5 ml/kg)+EAEI</td>
<td>250 mg/kg</td>
<td>46.76±0.25*</td>
<td>2.95±0.47*</td>
<td>1.763±1.87*</td>
</tr>
<tr>
<td>CCl₄(0.5 ml/kg)+CEI</td>
<td>250 mg/kg</td>
<td>42.51±0.25*</td>
<td>2.67±0.47*</td>
<td>1.826±1.87*</td>
</tr>
</tbody>
</table>

EEI, EAEI and CEI – Ethanol, Ethyl acetate and Chloroform extract of EI respectively. Mean ± S.E.M, n= 6, *P<0.05 (Compared to control) were considered significant

### Table 3: Effect of various extracts of leaves of *Erythrina indica* on the biochemical parameters of CCl₄ intoxicated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Liver weight/100gm body weight</th>
<th>Lipid per oxidation n mol/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>-</td>
<td>1.62±0.19</td>
<td>19.16±1.06</td>
</tr>
<tr>
<td>CCl₄</td>
<td>0.5 ml/kg</td>
<td>4.16±0.07</td>
<td>35.19±1.26</td>
</tr>
<tr>
<td>CCl₄(0.5ml/kg)+Silymarin</td>
<td>200 mg/kg</td>
<td>2.07±1.06</td>
<td>18.27±0.97</td>
</tr>
<tr>
<td>CCl₄(0.5 ml/kg)+EEI</td>
<td>250 mg/kg</td>
<td>2.90±1.02</td>
<td>23.34±0.65</td>
</tr>
<tr>
<td>CCl₄(0.5ml/kg)+EAEI</td>
<td>250 mg/kg</td>
<td>3.26±0.11</td>
<td>25.59±0.65</td>
</tr>
<tr>
<td>CCl₄(0.5 ml/kg)+CEI</td>
<td>250 mg/kg</td>
<td>3.47±0.16</td>
<td>28.67±0.65</td>
</tr>
</tbody>
</table>

Sily., EEI, EAEI and CEI – Silymarin, Ethanol, Ethyl acetate and Chloroform extract of EI respectively. Mean ± S.E.M, n= 6, *P<0.05 (Compared to control) were considered significant

*P<0.05 (Compared to Standard) were considered significant
CCl$_4$ and Silymarin showed almost normal architecture of liver. Livers of rat treated with Ethanol, Chloroform and Ethyl acetate extract of leaves of *Erythrina indica* and CCl$_4$ also showed almost normal architecture of liver there is no evidence for the presence of necrotic cells or fatty infiltration.

**DISCUSSIONS**

Carbon tetra chloride induced liver toxicity model is well accepted method for evaluating liver protective effect of herbal drugs. Hepatotoxic effect of CCl$_4$ is mediated through CCl$_3$. CCl$_3$ is released by the action of cytochrome p 450, a primary site of action of CCl$_4$. The free radicals CCl$_3$O and/or CCl$_3$OO$^-$ is reported to alter microsomal membrane and poly unsaturated fatty acid of endoplasmic reticulum, decrease protein synthesis and cause accumulation of triglyceride and fatty liver.

The present study, revealed that administration of CCl$_4$ markedly elevated serum GOT, GPT, ASAT, ALAT, ALP activities. Lipid peroxide level also high in CCl$_4$ treated group compared to test drug treated animals. This indicate that liver injury has occurred possibly by membrane lipid per oxidation through free radical formed as result of CCl$_4$ metabolism. Histopathological observations also revealed that the significant recovery from CCl$_4$ injury as indicated by the absence of necrosis and fatty infiltration in the drug treated animals than liver treated with CCl$_4$ alone.

Since antioxidant drugs were reported to possess hepatoprotective activity, we conclude that liver protective effect of leaves of *Erythrina indica* may be due to its anti oxidant property[13]. Further study is needed for the identification of active constituent responsible for the activity.

**REFERENCES**