Screening of liver protective effect of ethanolic leaf extract of *Callicarpa lanata* (EECL) in ethanol induced hepatotoxicity in wister rats

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ABSTRACT

**Background:** The ethanolic extract of *Callicarpa lanata* (EECL), belonging to the family Verbenaceae, were studied for hepatoprotective activity in Wister rats with liver damage induced by ethanol.

**Methods:** Ethanol treated rats showed significant increase in the levels of serum enzyme activities, total bilirubin and reduction in total proteins reflecting the liver injury caused by ethanol. EECL, at a dose of 400 and 200mg/kg body weight exhibited hepatoprotective effect by lowering the Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Alkaline Phosphate (SALP), Gama Glutamyl Transpeptidase (GGTP), total Bilirubin to a significant extent and also significantly increased the levels of total protein in a dose dependent manner.

**Results:** The results were highly significant at dose level of 400mg/kg body weight (p <0.01) and significant at dose level of 200mg/kg body weight (p <0.05). The effects of EECL at both levels were comparable with standard drug silymarin. The hepatoprotective activity was also supported by histopathological studies of liver tissue.

**Conclusions:** In-vivo hepatoprotective activity of ethanolic leaf extract of *Callicarpa lanata* (EECL) against Ethanol induced acute liver injury in rats showed significant results in a dose dependent manner.

**Keywords:** *Callicarpa lanata*, Ethanol, Hepatotoxicity, Hepatocytes, Silymarin

INTRODUCTION

Number of deleterious metabolic changes in the liver are induced by alcohol. Steatosis, alcoholic hepatitis and cirrhosis resulting in weight and volume changes are caused by excessive use of alcohol for a long-time.1,2 Minimum 80% of heavy drinkers had been reported to develop steatosis, 10-35% alcoholic hepatitis, and approximately 10% liver cirrhosis.3 In recent studies, the animal models suggest that liver injury in chronic alcoholics is due to oxidative stress that leads to fibrosis and impaired liver functions and increased apoptosis.4

The medicinal use of many plants (as hepatoprotectives) like Andrographis paniculata, Azadirachta indica, Cassia fistula, Elephantopus scaber, Hibiscus rosasinensis, Indoneesiella echioides, Phyllanthus debilis, Picrorrhiza kurroa has been reported in the literature.5-7
Callicarpa lanata. Family: Verbenaceae, an plant known worldwide as Tomex tomentosa L. Callicarpa tomentosa L. is widely used in tropical countries as a source of ethno medicines. It is recommended in a wide range of ailments including fever, Hepatic obstruction, hepatic eruption, skin diseases and used as a was for aphthae in the mouth. 

Callicarpa lanata leaves contain β- sitosterol, maslinic, oleanolic and ursolic acids and their methyl ester acetates, lupeol acetate and β-amyrin acetate; heartwood contains β-sitosterol and oleanolic acid. Bark contains methyl betulinate, baurenol and β-sitosterol acetate. 

Callicarpa lanata EtOH (50%) extract of root and stem is antiviral and antifungal and plant extract is anti-inflammatory in rats. The seeds are antiperiodic, antipyretic, antispasmodic, tonic, anthelmintic and febrifuge; useful in colic, malaria, fever, hydrocele and skin diseases. Decoction of the seed is used in kidney troubles and blood pressure. It is also useful against consumption and asthma. Seeds are also useful for swellings and restraining haemorrhage. The oil from the seeds is used in convulsions and paralysis. Pod is astringent to the bowels and anthelmintic; cures urinary discharges, leucorrhoea, piles and wounds. Decoction of the pod is used in bleeding piles. 

No scientific report is available relating the hepatoprotective potentials of Callicarpa lanata in ethanol induced liver damage. Therefore, the present work was planned to study the protective effect of ethanolic Callicarpa lanata (EECL) extract in acute ethanol-induced hepatotoxicity in rats.

METHODS

Plant material

The leaf Callicarpa lanata, were collected from Thiruvallur (dt) Tamil Nadu and dried. The shade dried leaves were powdered.

Preparation of plant extract

About 500 g of dried powder was extracted with ethanol by continuous hot percolation, using soxhlet apparatus. The concentrated crude extracts were lyophilized in to powder and used for the study. The extracts were dried under vacuum. The yield was 250g with respect to 2Kg of dried powder and used for oral administration.

Preliminary phytochemical screening of Callicarpa lanata leaves

Preliminary phytochemical tests have been conducted for the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, tannins, protein and amino acids, saponins, gums and mucilage, sterols, fixed oils, fats and flavonoids.

Experimental animals

Wistar male albino rats weighing 150-200g were used for the study. The rats were procured from Sri Venkateswara Enterprises, Bangalore, India. The animals were grouped and housed in polycrystalline cages (38 x 23 x 10cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25±20°C) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by Hindustan Lever Ltd., Bangalore, India and fresh water ad libitum. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the University Animals Ethical Committee (UAEC).

Drugs and chemicals

Silymarin was purchased from Micro Labs. India. SGPT, SGOT, SALP, GGTP, Total Bilirubin and Total Protein kits were procured from Span Diagnostics, India. All other chemicals were of analytical grade and purchased from local suppliers.

Experimental protocol

In order to study the liver protective effect of Callicarpa lanata in rats, dose levels of 400 and 200mg/kg bw p.o were used respectively. 40% v/v ethanol (2ml/100g bw p.o.) was used as hepatotoxic chemical and Silymarin (100 mg/kg bw p.o) was used as a standard drug in this study. Rats were divided into five groups as per following protocol.

- Vehicle control (n=6, Treated with normal saline only for 21 days).
- Hepatotoxic control (n=6, Treated with alcohol for 21 Days).
- Standard group (n=6, Treated with alcohol + silymarin for 21 days).
- Treatment group I (n=6, Treated with alcohol + EECL 400mg/kg bw p.o for 21 days).
- Treatment group II (n=6, Treated with alcohol + EECL 200 mg/kg bw p.o for 21 days).

At the end of the treatment, rats were sacrificed by cervical dislocation, blood samples were collected by direct cardiac puncture. The serum was used for the evaluation of marker enzymes. Liver was dissected out and washed with iced cold saline and a homogenate was prepared in 0.1N Tris HCL buffer (pH 7.4). The homogenate was used for the assay of antioxidant marker enzymes.

Biochemical estimation

The levels of SGPT, SGOT, SALP, GGTP, total bilirubin and total protein were estimated in the serum using standard kits from Span India ltd, Surat, India. The liver homogenate was centrifuged by using high speed cooling centrifuge and supernatant was used for the assay of lipid.
peroxidation (LPO), super oxide dismutase (SOD), Catalase (CAT), reduced glutathione (GSH) and Glutathione peroxidase (GPX).\textsuperscript{18,21}

**Histopathological studies**

The livers were excised quickly and fixed in 10\% formalin and paraffin embedded. Sections of about 4-6 μm were stained with haemotoxylin and eosin (H&E) for Histological evaluation. In brief 4-6 μm thick sections of paraffin embedded rat liver were de-waxed with distilled water for 2 min. Then the section was stained with haemotxylin for 5 min at room temperature. After 15 min, the section was counterstained with eosin for 2 min, dehydrated with alcohol, washed with xylene and blocked by eosin. Hemotoxylin and eosin stained studies were observed under microscope.\textsuperscript{22,23}

**Statistical analysis**

Data for hepatoprotective activity were expressed as Mean±SEM from six rats in each group. Hepatoprotective activity were analyzed statistically using one way analysis of variance (ANOVA), followed by Dunnett’s ‘t’ test. P value of <0.01 was considered as highly significant and <0.05 as significant.

**RESULTS**

Preliminary phytochemical screening of *Callicarpa lanata* Leaf revealed the presence of various compounds; glycosides, alkaloids, flavonoids, tannins, phenols, saponins and sterols. Exposure of rats to alcohol for 21 days showed significantly (p <0.05) elevated levels of serum biochemical markers like SGPT, SGOT, SALP, GGTP, total bilirubin and total protein. The protective effect of Ethanolic extract of *Callicarpa lanata* Leaf on SGPT, SGOT, SALP, GGTP, total bilirubin and total protein in 40\% v/v ethanol treated rats showed significant (p<0.01; p<0.05, respectively) decline as compared to Hepatotoxic control group. The degree of hepatoprotection by Ethanolic extract of *Callicarpa lanata*. (EECL) (400 and 200mg/kg bw p.o.) was observed statistically near value with the standard drug (Table 1). When comparing EECL 400 mg/kg were highly significant than EECL 200 mg/Kg dose. The hepato-protective effects were seen in dose dependant manner.

### Table 1: Results of ethanolic leaf extract *Callicarpa lanata* (EECL) on liver homogenates parameters against ethanol induced injury in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg, po)</th>
<th>LPO (mmol/mg)</th>
<th>SOD (U/mg)</th>
<th>CAT (U/mg)</th>
<th>GPX (U/mg)</th>
<th>GST (U/mg)</th>
<th>GSH (mmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Control</td>
<td>5 ml</td>
<td>0.49±0.09</td>
<td>115.13±22.13</td>
<td>28.5±6.45</td>
<td>4.11±1.02</td>
<td>1.04±0.96</td>
<td>0.34±0.06</td>
</tr>
<tr>
<td>Hepatotoxic</td>
<td>2 ml/100g</td>
<td>4.14±1.04</td>
<td>39.25±10.12</td>
<td>10.34±5.14</td>
<td>1.98±0.95</td>
<td>0.55±0.09</td>
<td>0.01±0.03</td>
</tr>
<tr>
<td>Standard</td>
<td>100</td>
<td>0.50±0.12**</td>
<td>98.32±15.95</td>
<td>27.11±3.68</td>
<td>3.82±1.35**</td>
<td>0.98±0.09**</td>
<td>0.29±0.12**</td>
</tr>
<tr>
<td>Treatment I</td>
<td>400</td>
<td>1.66±0.14**</td>
<td>89.20±12.87</td>
<td>21.2±3.15**</td>
<td>2.52±1.09**</td>
<td>0.87±0.59**</td>
<td>0.21±0.03**</td>
</tr>
<tr>
<td>Treatment II</td>
<td>200</td>
<td>2.51±0.12*</td>
<td>56.12±16.12</td>
<td>17.71±3.15*</td>
<td>1.04±0.98*</td>
<td>0.50±0.89*</td>
<td>0.16±0.10*</td>
</tr>
</tbody>
</table>

n=6; values were expressed Mean±S.E.M.; Group II was compared to Group I. Groups III to V were compared to group II. *p <0.01 vs. ethanol group; highly significant; ** p <0.05 vs. Ethanol group: significant. Data were analyzed by One-way ANOVA followed by Dunnett’s ‘t’ test.

### Table 2: Results of the effects of ethanolic leaf extract *Callicarpa lanata* (EECL) on the biochemical markers of ethanol induced hepatic injury in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg, po)</th>
<th>SGPT u/l</th>
<th>SGOT u/l</th>
<th>SALP u/l</th>
<th>Total bilirubin (mg/dl)</th>
<th>GGTP u/l</th>
<th>TP mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>5 ml</td>
<td>80±0.74</td>
<td>121±0.97</td>
<td>138±1.72</td>
<td>0.70±0.03</td>
<td>124±0.95</td>
<td>6.5±0.06</td>
</tr>
<tr>
<td>Hepatotoxic</td>
<td>2 ml/100g</td>
<td>372±0.58</td>
<td>356±2.95</td>
<td>410±0.08</td>
<td>2.42±0.17</td>
<td>254±2.65</td>
<td>7.4±0.08</td>
</tr>
<tr>
<td>Standard</td>
<td>100</td>
<td>105±1.08**</td>
<td>231±1.46**</td>
<td>198±1.06**</td>
<td>1.10±0.03**</td>
<td>117±1.04**</td>
<td>5.8±0.02**</td>
</tr>
<tr>
<td>Treatment I</td>
<td>400</td>
<td>139.88±8.22**</td>
<td>206.28±4.24**</td>
<td>182.46±8.22**</td>
<td>1.68±4.62**</td>
<td>106±1.06**</td>
<td>4.88±8.12**</td>
</tr>
<tr>
<td>Treatment II</td>
<td>200</td>
<td>168.28±6.76**</td>
<td>166.84±4.26**</td>
<td>298±8.44**</td>
<td>2.06±2.20**</td>
<td>93±1.05**</td>
<td>3.9±0.04**</td>
</tr>
</tbody>
</table>

n=6; values were expressed Mean±S.E.M; Group II was compared to Group I. Groups III to V were compared to group II. *p <0.01 vs. ethanol group; highly significant; ** p <0.05 vs. ethanol group: significant. Data were analyzed by One-way ANOVA followed by Dunnett’s ‘t’ test.
Standard drug silymarin also maintained the similar result. GSH test, showed much decreased level of glutathione in ethanol induced rats. Treatment with *Callicarpa lanata* showed significantly improved level. Similar activity was also observed with the standard drug silymarin. Rats treated with 40% v/v ethanol caused a significant (p<0.01) decline in the hepatic antioxidants such as SOD and CAT in comparison to normal control animals. In liver weight study, the liver weight of alcohol treated rats were highly increased (Table 3).

In histological examinations, hepatocytes of the normal control group showed a normal cellular architecture of the liver. Whereas the liver section of rats treated with toxicant showing intense centrilobular necrosis and vacuolization.

**Table 3: Results of ethanolic leaf extract *Callicarpa lanata* (EECL) on average liver weight of treated animals against ethanol.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg, po)</th>
<th>Liver weight (g) /100g of body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>5ml</td>
<td>2.51±0.42</td>
</tr>
<tr>
<td>Hepatotoxic</td>
<td>2ml /Kg</td>
<td>4.96±0.73</td>
</tr>
<tr>
<td>Standard</td>
<td>100</td>
<td>2.64±0.18**</td>
</tr>
<tr>
<td>Treatment I</td>
<td>400</td>
<td>2.70±0.16**</td>
</tr>
<tr>
<td>Treatment II</td>
<td>200</td>
<td>3.2±0.24*</td>
</tr>
</tbody>
</table>

n=6, Values were expressed as mean±SEM. *P <0.01 Vs ethanol, highly significant; **P <0.05 significant. Data were analyzed by One way ANOVA followed by Dunnett’s ‘t’ test.

**DISCUSSION**

There are many factors which are responsible for the liver damage or injuries such as chemicals and drugs. In the present study ethanol was used to induce hepatotoxicity, since it is clinically relevant. Ethanol produces a constellation of dose related deleterious effects in the liver.24

The majority of ethanol is metabolized in the liver and individuals who abuse alcohol by routinely drinking 50-60 g (about 4 to 5 drinks) of ethanol per day are at risk for developing alcoholic liver disease.25 In addition, both acute
and chronic ethanol administration cause enhanced formation of cytokines, especially TNF-alpha by hepatic Kupffer cells, which have a significant role in liver injury.26-28 Besides the development of fatty liver (steatosis), another early sign of excessive ethanol consumption is liver enlargement, both of which are common findings in alcoholics and heavy drinkers.29,30

Figure 5: Ethanolic leaf extract Callicarpa lanata (EECL) (200mg/kg).

Elevated levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) are indications of hepatocellular injury.31 In the present study EECL at a dose levels of 400mg/kg, po caused a significant inhibition in the levels of SGOT and SGPT towards the respective normal range and this is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by ethanol. On the other hand, suppression of elevated SALP and reduction of raised bilirubin level and an increase in the total plasma protein content suggests the improvement of biliary dysfunction in rat liver during hepatic injuries with toxicants.32

These results indicate that EECL preserved the structural integrity of the hepatocellular membrane and liver cell architecture damaged by ethanol which was confirmed by histopathological examination viz., Figure 1 shows vehicle control, liver section of vehicle control group, showed normal structure of hepatic lobules, whereas black spots are glycogen, white spots indicate the presence of vacuoles, a prominent blood vein with normal appearance. Figure 2 shows Silymarin (100mg/Kg), liver section of Standard group, showed normal structure of hepatic lobules, whereas black spots are glycogen, white spots indicate the presence of vacuoles, a prominent blood vein with normal appearance. Figure 3 gives information Ethanol (40% v/v) 2ml/Kg; liver section of Hepatotoxic control group, showed degeneration of hepatic parenchyma, moderate congestion and microvesicular changes. Figure 4 illustrates Ethanolic leaf extract Callicarpa lanata (EECL) (400mg/kg); liver section of treatment group I, showed marked recovery in hepatic cells with nuclei, cytoplasm, central vein and portal triad. Figure 5 represents ethanolic leaf extract Callicarpa lanata (EECL) (200mg/kg); liver section of treatment group II, showed recovering ruptured cells, necrosis, walls of blood vein and less vacuolisation with disappearance of nuclei.

CONCLUSION

In conclusion, Ethanolic extract of Callicarpa lanata is effective against oxidative liver damage induced by alcohol administration. So, this study will give the pharmacological support to use of folk medicine in the management of alcohol intoxicated hepatic damage.

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