Evaluation of hepatoprotective activity on the leaves of *Ficus benjamina* Linn.

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**ABSTRACT**

The present study was conducted to evaluate the hepatoprotective activity of ethanolic extract of *Ficus benjamina* Linn. against CCl\(_4\) induced liver damage in rats. The ethanolic extract of *Ficus benjamina* Linn. (250 and 500mg/kg) and isolated compounds (500mg/kg) was administered orally to the animals with hepatotoxicity induced by CCl\(_4\) (1.5 gm/kg). Silymarin (100mg/kg) was given as reference standard. All the test drugs were administered orally by suspending in liquid paraffin solution. The plant extract and both isolated compound was effective in protecting the liver against the injury induced by CCl\(_4\) in rats. This was evident from significant reduction in serum enzymes SGPT, SGOT, ALP, Serum bilirubin and liver weight. It was concluded from the result that the ethanolic extract of *Ficus benjamina* possesses hepatoprotective activity against CCl\(_4\) induced hepatotoxicity in rats.

**Key words:** *Ficus benjamina*, Hepatoprotective, CCl\(_4\), SGPT, SGOT, Bilirubin.

**INTRODUCTION**

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects\(^1\). In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders\(^2\). In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. *Ficus benjamina* Linn (Moraceae) is widely cultivated in Hawai‘i. In warmer regions the tree is grown as a specimen, street tree or as a hedge, pot or in the ground. *Ficus benjamina* has been claimed to be useful as
traditionally medicine for the treatment of certain skin disorders, stomachic, hypotensive and anti-dysentery. The fruit extract of F. benjamina possessed antitumor activity and significant antibacterial activity. Twigs are used as insect repellent by keeping them under the beds. Leaf juice is used as flea and bug repellent. Latex is applied on boils and published data reported Leaves, bark and fruits are use as antimicrobial, antibacterial, antitumor, anti-inflammatory, antinociceptive, antipyretic, cytotoxic activity.\textsuperscript{3,4,5} The study was conducted to establish the use of \textit{Ficus benjamina} Linn. as hepatoprotective against CCl\textsubscript{4} induced hepatotoxicity in rats.

**MATERIALS AND METHODS**

**Collection and authentication:** Plant materials were collected from Jhansi, U. P., during the month of Nov. 2010. Authentication of plant materials is done by Dr. P. B. Singh (Research Officer), National Vrkshayurveda Research Institute (NVRI), Jhansi, with accession no. 5315.

**Preparation of Extraction:** The shade dried plant leaves of Ficus benjamina Linn. (600gm) was coarsely powdered using grinder and continuous extracted in a soxhlet apparatus at 50 °C with 2500 ml Petroleum ether than 2500ml ethanol. The extract was filtered through a paper filter (Whatman, No.1) and evaporated under reduced pressure by the rotary evaporator. The obtained extracts were stored in amber coloured glass bottle for further processing.

**Acute oral toxicity studies**

Acute oral toxicity was performed by using OECD guidelines – 423 (Organisation of Economic Co-Operation Development) – Fixed Dose Procedure. The purpose of this study is to allow selection of the appropriate starting dose for the main study.

Acute oral toxicity of \textit{Ficus benjamina} Linn. was performed in Wistar Albino Rats. The rats were kept for 4 hr of fasting prior to the experiment and body weight of the rats should be noted. Usually rats weighting 180-250 gm were used for acute toxicity studies. The dose was given to every rat orally according to body weight. The test for acute toxicity was performed at 5, 50, 300, and 2000mg/kg oral dose of Ethanolic extract of \textit{Ficus benjamina} linn. leaves. Food was given for a 1-2 hours after the administration of drug.

During the first 4 hr. after the drug administration, animals were continuously observed for gross behavioral changes & then observation is continued for 24 hr & 72 hr in regular intervals for 14 days. The parameter such as hyperactivity, grooming, convulsions, sedation, hypothermia, change in fur colour, mortality, moribund stage or death were observed.

**Table No.1 : Assessment of Acute toxicity studies.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>No. of mice</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>24 hrs</td>
</tr>
<tr>
<td>Extracted dose</td>
<td>5 mg</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>50 mg</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>300 mg</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2000 mg</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>
LD$_{50}$ of Ethanolic extract of *Ficus benjamina* linn leaves were done as per OECD guidelines (Revised draft 423). The animal did not show any signs of toxicity and behavioral changes after 24 hrs and 72 hrs.

**Drugs and Chemicals:** All the chemicals were analytical grade. CCl$_4$ was obtained from Pharmacognoy lab, Institute of Pharmacy BU Jhansi. Silymarin was obtained from Allied Chemicals & Pharmaceuticals (P) Ltd. New Delhi. It is a poly herbal formulation which produces hepatoprotective activity against CCl$_4$.

**Animals:** Healthy Wistar Albino Rats weighing about (180-250gm) of either sex was obtained from animal house, Institute of Pharmacy, Bundelkhand University, Jhansi. The animals were housed under the uniform laboratory condition & fed with commercial diet & provide with water ad libitum, during the experiment. The animal were procured from Gwalior & permitted for study under the Institutional Animal Ethical Committee. All protocols of the study were approved by the Institutional Animal Ethical Committee with reference number BU/PHARM/IAEC/10/006. The IAEC is approved by committee for the purpose of control and supervision of experiments on animals (CPCSEA) with registration number 716/02/a/CPCSEA.

**Preparation of plant extract, hepatotoxin & standard drug**

a) **Preparation of extract solution:** The Ethanolic extract of plant *Ficus benjamina* Linn. leaves were dissolved in Polyethylene glycol for oral administration. The solution of ethanolic extract was prepared at a dose of 250 and 500 mg/kg body weight.

b) **Hepatotoxin:** CCl$_4$ was suspended in liquid paraffin (CCl$_4$: liquid paraffin 1:2; 1.5ml/kg i.p) body weight orally.

c) **Standard Drug:** Silymerine at a dose of 100 mg/kg bodyweight administered by oral route. It is a polyherbal formulation used as a standard drug.$^6$

**Evaluation of hepatoprotective activity**

**Carbon tetrachloride Induced Hepatotoxicity:**

1) **Experimental Design:** The general principle involved in the evaluation of Hepatoprotective activity is to induce liver toxicity or infection with the help of hepatotoxin in the liver of experimental animals. The magnitude of the protective activity is measured as both in-vivo & in-vitro by estimating the following parameters:

a) **Morphological Parameters:** It includes the change occurring in weight, volume of Liver & also change in weight of rats are studied.

b) **Functional Parameters:** The Metabolism of barbiturate by Liver damage is studied by recording mean sleeping time using Phenobarbitone or Thiopental.

c) **Histological Parameters:** These include the necrosis induced, type and the extent of degradation & fibrosis in Liver tissue.
Biochemical Parameters: These are the most reliable parameters in the in-vivo study & include the estimation of different enzymes like SGOT or Aspartate transferase & Alanine transferase or SGPT & Serum alkaline phosphate (SALP). It also includes estimation serum bilirubin (SBLN) & estimation of Hydroxyproline fat & protein content of Livers.

MATERIALS AND METHODS

Carbontetrachloride induced hepatotoxicity
Rats were divided into seven groups of six animals each. The rats of control group (I) received three doses of 5% gum acacia mucilage (1ml/kg, per oral.) at 12 hour intervals (0 hour, 12 hour and 24 hour). The rats of Carbon tetrachloride group (II) received three doses of vehicle at 12 hour intervals and a single dose of Carbon tetrachloride (1.5 ml/kg i.p.) diluted in liquid paraffin (1:2) 30 minutes after the administration of 1st dose of vehicle. The rats of standard group (III) received three doses of Silymarin (100mg/kg) at 0 hour, 12 hour and 24 hour. Carbon tetrachloride was administered (1.5ml/kg i.p.) 30 minutes after the first dose of silymarin. While the rats of test group (IV, V) received three doses of test extract at the dose of 250 and 500mg/kg body weight per oral and test group (VI, VII) received three doses of test isolated compound V2, V2 at the dose of 500mg/kg body weight per oral at 0 hour, 12 hour and 24 hour. Carbon tetrachloride was administered (1.5ml/kg i.p.) 30 minutes after the first dose of test extract. After 36 hour of administration of carbon tetrachloride, blood was collected and serum was separated and used for determination biochemical parameters.

Table No. 2: Assessment of Hepatoprotective activity by serum enzyme level in blood.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Treatment</th>
<th>SGPT(IU/L)</th>
<th>SGOT(IU/L)</th>
<th>ALP(IU/L)</th>
<th>Serum bilirubin</th>
<th>Liver Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal control (Vehicle treated)</td>
<td>127.83±1.72</td>
<td>105.17±1.47</td>
<td>200.33±3.56</td>
<td>1.15±0.03</td>
<td>5.95±0.04</td>
</tr>
<tr>
<td>2.</td>
<td>Hepatotoxic Control (CCl4 Treated)</td>
<td>287.33±9.266*</td>
<td>252.50±8.54**</td>
<td>448.14±14.64***</td>
<td>4.66±0.01**</td>
<td>9.59±0.02**</td>
</tr>
<tr>
<td>3.</td>
<td>Standard Silymarin</td>
<td>120.17±3.71**</td>
<td>99.0±3.03****</td>
<td>189.50±4.50</td>
<td>1.33±0.02**</td>
<td>6.75±0.07**</td>
</tr>
<tr>
<td>4.</td>
<td>Extract 250 mg</td>
<td>145.83±15.30**</td>
<td>136.17±5.26**</td>
<td>258.0±11.04**</td>
<td>1.45±0.13*</td>
<td>6.46±0.47**</td>
</tr>
<tr>
<td>5.</td>
<td>Extract 500 mg</td>
<td>130.50±2.88**</td>
<td>95.83±4.40**</td>
<td>208.0±5.04**</td>
<td>1.13±0.02*</td>
<td>5.99±0.07**</td>
</tr>
<tr>
<td>6.</td>
<td>Isolated compound V2 500mg</td>
<td>128.67±2.16**</td>
<td>112.0±5.96**</td>
<td>217.50±6.01**</td>
<td>1.26±0.05**</td>
<td>5.81±0.02**</td>
</tr>
<tr>
<td>7.</td>
<td>Isolated compound V3 500mg</td>
<td>131.17±2.63**</td>
<td>107.0±4.73**</td>
<td>212.0±4.73**</td>
<td>1.08±0.11*</td>
<td>5.82±0.13*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, as compared to control.
Figure no.1: Effect of Ficus benjamina Linn. on CCl₄ induced hepatotoxicity in rats enzymes.

![Graph showing the effect of Ficus benjamina Linn. on CCl₄ induced hepatotoxicity in rats enzymes.](image)

Figure no.2: Effect of Ficus benjamina Linn. on CCl₄ induced hepatotoxicity in rats Serum bilirubin

![Graph showing the effect of Ficus benjamina Linn. on CCl₄ induced hepatotoxicity in rats Serum bilirubin.](image)

Figure no.3: Effect of Ficus benjamina Linn. on CCl₄ induced hepatotoxicity in rats liver.
Effect of Ficus benjamina on CCl₄ induced hepatotoxicity in rats liver

![Graph showing liver weight comparison between different groups](image)

Photograph of liver slide

Photograph 1: Liver of rat treated with vehicle.  Photograph 2: Liver of rat treated with CCl₄.
Photograph 3: Liver of rat treated with Silymarin.  Photograph 4: Liver of rat treated with FB (250 mg/kg).

Photograph 5: Liver of rat treated with FB (500 mg/kg).  Photograph 6: Liver of rat treated with v2 (500 mg/kg).
Photo 7: Liver of rat treated with v3 (500 mg/kg).

**Photograph 1:** Normal Histopathology of rat liver showed normal histology of liver. **Photograph 2:** Histopathology of CCl4 treated rat liver showed severe macro vascicular fatty changes, severe inflammation and fatty degeneration. **Photograph 3:** Histopathology of Silymarin treated rat liver showed normal hepatocytes, no evidence of hepatic damage. **Photograph 4:** Histopathology of ethanolic extract (250mg/kg) treated rat liver showed mild fatty degeneration and mild chronic inflammation and also shows mild focal rearrangements of cells. **Photograph 5:** Histopathology of ethanolic extract (500mg/kg) treated rat liver showed normal hepatocytes, no evidence of hepatic damage. **Photograph 6:** Histopathology of isolated compound V2 treated rat liver showed normal hepatocytes, no evidence of hepatic damage. **Photograph 7:** Histopathology of isolated compound V3 treated rat liver showed normal hepatocytes, no evidence of hepatic damage.

**Assessment of liver function**

Blood was collected from all the groups by puncturing the retro-orbital plexus and was allowed to clot at room temperature and serum was separated by centrifugation at 2500rpm for 10 min. The serum was used for estimation of biochemical parameters to determine the functional state of the liver. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated by a UV kinetic method based on the reference method of International federation of Clinical Chemistry in which both SGOT and SGPT were assayed based on enzyme-coupled system; where keto acid formed by the aminotransferase reacts in a system using NADH. The coenzyme is oxidized to NAD and the decrease in absorbance at 340 nm is measured. For SGOT malated dehydrogenase is used to reduce oxaloacetate to malate where as for SGPT the pyruvated formed in the reaction is converted to lactate by lactate dehydrogenase. Alkaline phosphatase (ALP) was estimated by method described by Comb and Bowers, 1972. involving hydrolysis of p-nitrophenol which gives strong yellow colour in alkaline solution. The increase in absorbance due to its formation is directly proportional to ALP activity; while total bilirubin (TBL) by Jendrassik and Grof, 1938. which involves the reaction of...
bilirubin with diazotized sulphanilic acid to form an azo compound, the colour of which is measured at 546 nm.

**Histopathological studies**
The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was fixed in Boucin’s solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12h, then embedded in paraffin using conventional methods and cut into 5µm thick sections and stained using haematoxylin-eosin dye and finally mounted in di-phenyl xylene. The sections were then observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

**Statistical Analysis:** The result was expressed as mean ± S.P. and % protection by test drug extract hepatotoxin induced charges. The present protection was calculated by considering the difference in enzyme levels between rats treated with hepatotoxin & control. A 100% protection indicates that there is complete inhibition of CCl₄ induced increase in the level of the biochemical parameters, statistical evaluation was done by unpaired t- test to compare each group treated with hepatotoxin.

**RESULTS**
Preliminary Phytochemical studies revealed the presence of phenolics compound and flavonoids were noticed in ethanolic extract of *Ficus benjamina* linn. leaves. Therefore, there is possibility that ethanolic extract of *Ficus benjamina* may possess hepatoprotective activity.

In our experiment it is observed that the level of hepatic biochemical markers i.e. SGOT, SGPT, ALP & Bilirubin is increased in comparison to the control group, shown in table. This clearly indicates that there is significant hepatic damage due to the CCl₄. The toxic effect of CCl₄ was controlled in animals treated with ethanolic extract of *Ficus benjamina* 250 and 500 mg/kg/day and isolated compound V2 and V3 by way of restoration of the markers levels in the liver.

From the bar diagram representation for the enzyme level of SGOT, SGPT & SALP shown in figure. It is concluded that ethanolic extract of *Ficus benjamina* have almost equivalent, properly to reduced the elevated level biochemical markers when compared with Silymerine. The elevated bilirubin level similar to Silymerine shown in Fig. No.2.

**Hepatoprotective activity**
The results of Carbon tetrachloride induced hepatotoxicity were shown in table-1. In the Carbon tetrachloride control group, the significant acute hepato cellular damage, and biliary obstruction was indicated by the elevated level of SGPT, SGOT, ALP and TBL But the group which received the test drug of ethanolic extract at the dose of 250mg/kg body weight p.o showed a significant decrease in the elevated levels of SGPT, SGOT, ALP, TBL these biochemical parameters are comparable with the standard silymarin hepatoprotective drug. Therefore, the silymarin and the ethenolic extract restored the altered level of enzymes significantly. Histopathological liver sections of control groups showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and central vein. Disarrangement of normal hepatic cells with necrosis and vacuolization are observed in Carbon tetrachloride intoxicated liver. The liver
sections of the rat treated with 250,500 mg/kg bodyweight p.o of ethenolic extract and isolated compound V2 and V3 of Ficus benjamina followed by carbon tetrachloride intoxication showed less vacuole formation and absence of necrosis and overall less visible changes observed were comparable with standard Silymarin, supplementing the protective effect of the test drug and the standard hepatoprotective drug.

**DISCUSSION**

The present studies were performed to assess the hepatoprotective activity in rats, against Carbon tetrachloride as hepatotoxin to prove its claim in folklore practice against liver disorder. The changes associated with Carbon tetrachloride induced liver damage of the present study appeared similar to the acute viral hepatitis.\(^\text{11}\) Carbon tetrachloride is a widely used experimental hepatotoxicant, is biotransformed by the cytochrome P-450 system to produce the trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb Ca\(^2+\) haemostasis and finally result in cell death.\(^\text{12}\) Animals of Group II (received Carbon tetrachloride) significantly lost their body weight and showed reduced food consumption as compared to control group. Animals of Group III, IV, V, VI and VII (received Carbon tetrachloride plus 250 and 500mg/kg body weight of test extract,250 and 500mg/kg body weight isolated compound V2, V3 and standard drug Silymarin 100mg/kg body weight) showed a significant increase in body weight and food consumption when compared to Carbon tetrachloride group animals. These findings suggested the extract administered has significantly neutralized the toxic effects of Carbon tetrachloride and helped in regeneration of hepatocytes.\(^\text{13}\) Estimating the activities of serum marker enzymes, like SGPT, SGOT, ALP can make the assessment of liver function when liver cell plasma membrane is damaged, a variety of enzyme normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepato cellular damage.\(^\text{14}\) The tendency of these enzymes to return to near normally in extract administered group is a clear manifestation of antihepatotoxic effects of the extract. The levels of total protein and albumin were reduced due to the Carbon tetrachloride-induced hepatotoxicity. The reduction is attributed to the initial damage produced and localized in the endoplasmic reticulum which result in the loss of P-450 leading to fatty liver.\(^\text{12}\) Inhibition of bile acids synthesis from cholesterol which is synthesis in liver or derived from plasma lipids, leading to increase in cholesterol levels were also resulted due to Carbon tetrachloride intoxication suppression of cholesterol levels by the extract suggest the bile acid synthesis inhibition was reversed. Reduction in the levels of SGPT and SGOT towards the normal value is an indication of regeneration process. Reduction in ALP levels with concurrent depletion of raised bilirubin levels suggests the stability of the biliary function during injury with Carbon tetrachloride. This hepat protective effect exhibited by the ethenolic extract of *Ficus benjamina* at the dose level of 500mg/kg body weight and isolated compound V2 and V3 500mg/kg body weight was comparable with the standard drug, A Silymarin. Histopathological liver sections also revealed that the normal liver architecture was disturbed by hepatotoxin in Carbon tetrachloride group, whereas in the liver sections of the rat treated with the ethenolic extract and intoxicated with Carbon tetrachloride the normal cellular architecture was retained and it in comparable with the standard Silymarin group, hence confirming the significant hepatoprotective effect of extract of *Ficus benjamina* at the dose of 500 mg/kg body weight and isolated compound V2 and V3 500mg/kg body weight . In accordance with these results, it may be confirmed due to the
presence of phytoconstituents such as flavonoids, alkaloids and glycosides which are present in the ethanolic extract could be considered as, responsible for the significant hepatoprotective activity. In conclusion, it can be said that the ethanolic extract of *Ficus benjamina* exhibited a hepatoprotective effect against Carbon tetrachloride induced hepatotoxicity. Efforts are in progress to isolate and characterize the active principle, which is responsible for the hepatoprotective efficacy of this valuable medicinal plant.

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