HEPATO PROTECTIVE ACTIVITY OF INDIAN MEDICINAL PLANT
ON CCL4 INDUCED LIVER DAMAGE IN WISTAR RATS

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ABSTRACT
Plants and plant extracts have been used since the dawn of civilization
by mankind. The uses of ethno botanical preparations for various
reasons justified or not, are still continued by various cultures all over
the world. Considering structural and biological diversity of terrestrial
plants, they offer a unique renewable resource for the discovery of
potential new drugs and modern medicine has developed a rational
strategy for drug discovery which involves the study of plants and
plant materials based on their ethno botanical usage (Cordell et al.,
1991). Natural products are sources of active compounds that may be
useful in the development of new and potent drugs. Ficus religiosa L.,
Moraceae, is widely planted in the tropics. The chemical constituents
of F. religiosa include tannin, saponin gluanol acetate, β-sitosterol,
leucoanthocyanidin, and leucoanthocyanin. These are used for the
treatment of pain, inflammation, impotence, menstrual disturbances, and urine related
problems, and as uterine tonic. The present study aimed to evaluate hepatoprotective effects
of F. religiosa linn on ccl4 induced liver injury in Wistar rats.
KEY WORDS: Ficus religiosa, Ethanolic extract, Hepatoprotective activity, CCl4 induced liver damage, Ursocol.

INTRODUCTION

The use of natural remedies for the treatment of liver diseases has a long history, starting with the Ayurvedic treatment, and extending to the Chinese, European and other systems of traditional medicines. The 21st century has seen a paradigm shift towards therapeutic evaluation of herbal products in liver diseases by carefully synergizing the strengths of the traditional systems of medicine with that of modern concept of evidence-based medicinal evaluation, standardization of medicinal products and randomized placebo controlled clinical trials to support clinical efficiency. The liver is the largest glandular organ in the body, and has more functions than other organ of the body. A person’s entire blood supply passes through the liver several times a day. The liver has a pivotal role in the human metabolism. Liver produces and secretes bile; it also produces prothrombin and fibrinogen, blood clotting factors and heparin, a mucopolysaccharide sulfuric acid ester that helps keep blood from clotting within the circulatory system. The liver converts sugar into glycogen.

Liver diseases have become one of the major causes of morbidity and mortality in man and animals all over globe and hepatotoxicity due to drugs appears to be the most common contributing factors. Among the many diseases that can affect the liver the most common is viral hepatitis and it can also be caused by drugs, bacteria, mushrooms, parasites like amoebas or giardiasis. About 20,000 deaths found every year due to liver failure. One in twelve persons suffer from some form of chronic liver disease worldwide, while more than 50 million people have advanced chronic liver disease in India. The most common indication for liver transplant is advanced chronic liver disease, including viral diseases such as Hepatitis B and C, alcohol abuse and acute liver failure.

Biotransformation of exogenous compounds is one of the major functions performed by the liver. This function is expressed by the hepatocytes, which represents about 65% of the total cell population and 90% of the volume of the organ. Hepatocytes are richly endowed with drug metabolizing enzymes, which are conveniently divided into two groups. Phase-I reactions are generally oxidative, reductive and hydrolytic processes; they provide the necessary functional group for phase-II reactions, which are generally conjugations. If the overall effect of these enzymes is to convert chemicals to more water soluble forms which
can be secreted readily by the body, it appears that a number of compounds are metabolized to toxic metabolite.

In vivo studies make it difficult to distinguish the primary effects of compounds to those induced secondarily because liver functions are under the influence of various endogenous and exogenous factors, which result in complex interactions with other organs. Moreover, most of our understanding regarding liver injury induced by drugs and other chemicals at a mechanistic level remains confined to experimental models. Since the rates and the routes of drug metabolism can vary greatly, particularly when comparisons involved laboratory animals and man, data obtained in animals cannot be extrapolated with a certainty to the human situation.

These drawbacks of in vivo animal studies explain why many investigators have turned to simpler experimental models for studying drug metabolism and response of the organ or liver cells to potentially toxic agents. Conventional drugs used in the treatment of liver diseases viz, are sometimes inadequate and can have serious side effects; therefore it is necessary to search for alternative drugs for the treatment of liver disease in order to replace currently used drugs of doubtful efficacy and safety.

Steroids vaccines and antiviral drugs that are employed as therapy for liver diseases have potential adverse effects especially when administered for long periods. There is worldwide trend for use of traditional herbal drugs for the treatment of liver diseases. Several leads from plant sources have been found as potential hepatoprotective agents with diverse chemical structures.

**OBJECTIVE OF THE STUDY**

The overall objective of proposed study is to explore the application of traditional medicinal plants of India. The study is to be carried out to understand the effectiveness of the plant as Hepatoprotective drug. The following aspects of the plant extract are to be carried out to access the potential biochemical activities, histopathological studies. The specific objectives aimed in the present work are as follows:

Preparation of ethanolic extract of Ficus religiosa (Peepal) using Soxhlet Apparatus process.

1) To investigate preliminary phytochemical constituents of ethanolic extract of Ficus religiosa.
2) To determine hepatoprotective activity of Ficus religiosa linn.
3) To estimate serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and total bilirubin.
4) To perform Histopathology of liver.

MATERIALS AND METHODS\(^{[10-16]}\)

Preparation of plant extract
The roots were shade dried and powdered; the coarse powder was subjected to extraction with ethanol in soxhlet apparatus.

Selection of Animals
30 Male Wistar Rats (8-10 weeks), weighing 150 – 200g, were obtained from the Central Animal Facilities of NIP, DESHMUKHI. The animals were housed in well ventilated cage and large spacious hygienic cages during course of the experimental period. The animals were allowed free access to standard laboratory pellets and drinking water ad libitum. The study protocol was approved by CPSCEA, IAEC meeting held at Nizam Institute of Pharmacy & Research Center, Deshmukhi.

Chemicals, Reagents and Drug
1) Standard drug Ursocol 300mg was obtained from NIP, DESHMUKHI.
2) CCL4, hepatotoxin was obtained from NIP, DESHMUKHI.
3) Herbal plant (FICUS RELIGIOSA LINN, PEEPAL) was obtained from O.U, HYDERABAD.
4) Estimation kits for AST, ALT, SBR, T.P, DIR, SPAN diagnostic kits were used.
5) All the other solvents and chemicals used for extraction and physiochemical investigation were as of analytical grade purchased from NIP, DESHMUKHI.

Determination of acute oral toxicity
Acute toxicity of ethanolic extract of Ficus religiosa was done according to OECD guidelines No.423. Low and high dose was selected for the treatment.

Principle
It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step; sufficient information is obtained on the acute toxicity of the test
substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses.

**Method**
The overnight fasted mice were divided into four groups, each group consisting of two animals. The ethanolic extract of Ficus religiosa was given in various doses (5, 50, 300, 2000 mg/kg) by gastric incubation with syringe. After administration of the extract, the animals were observed continuously for the first two hours and at 24 hours to detect changes in behavioural responses and also for tremors, convulsions, salivation, diarrhoea, lethargy, sleep, coma and also were monitored up to 14 days for toxic symptoms and mortality.

**Phytochemical analysis**
**Preliminary qualitative tests**
The extract was subjected to preliminary qualitative phytochemical investigation. The various tests and reagents used.

**Detection of carbohydrates**
Extract was dissolved in 5ml of distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

A) Molisch’s Test
Filtrates were treated with 2 drops of alcoholic alpha-naphthol solution in a test tube and 2 ml Concentrated sulphuric acid was added carefully along the sides of the test tubes. Formation of violet ring at the junction indicates the presence of carbohydrates.

**Detection of alkaloids**
Extract was dissolved individually in dilute hydrochloric acid and filtered. The filtrate was tested carefully with alkaloid reagents.

A) Mayer’s Test
Filtrate was treated with Mayer’s reagent (potassium mercuric iodide), formation of a yellow cream precipitate indicate the presence of alkaloids.

**Detection of glycosides**
Extract was hydrolyzed with dilute hydrochloric acid, and the hydrolysate was subjected to glycosides test.
A) Legal’s test
The extract was treated with sodium nitroprusside in pyridine and methanolic alkali. The formation of pink to red color indicates the presence of cardiac glycoside.

Detection of saponins
A) Froth’s Test
The extract was diluted with distilled water to 20ml shaken in a graduated cylinder for 15mins. The formation of 1cm layer of foam indicates the presence of saponins. The extract was treated with chloroform and filtered separately. The filtrate was treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layer turns red, sterols are present. If lower layer turns golden yellow triterpenes are present.

Detection of fixed oils and fats
A) Gelatin test
To the extract, 1% gelatin solution containing solution chloride was added. The formation of white precipitate indicates the presence of tannins.

B) Lead acetate Test
The extract was treated with few drops of lead acetate solution; formation of yellow precipitate indicates the presence of flavonoids.

C) Vanillin hydrochloric Test
The extract was treated with few drops of vanillin hydrochloride reagent. The formation of red color indicates the presence of tannins.

Detection of proteins and amino acids
A) Millon’s Test
The extract was treated with 2ml of Millon’s reagent. Formation of white precipitate, which turns to red upon heating, indicates the presence of proteins.

B) Ninhydrin Test
To the extract, 0.25% ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates presence of amino acids.
Evaluation of Hepato-protective activity\cite{17-20}

CCl4 induced hepatotoxicity in rats models was used for evaluation of hepatoprotective activity for plant extract. The experimental design was as follows (Table No.1).

Table No 1: Five groups of Wistar Rats, six in each. (30 Rats).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control group.</td>
</tr>
<tr>
<td>Group II</td>
<td>CCl4 for the induction liver damage.</td>
</tr>
<tr>
<td>Group III</td>
<td>Ursocol 25mg/kg + CCl4 group</td>
</tr>
<tr>
<td>Group IV</td>
<td>F. religiosa 300mg/kg + CCl4 group</td>
</tr>
<tr>
<td>Group V</td>
<td>F. religiosa 400mg/kg + CCl4 group</td>
</tr>
</tbody>
</table>

Table No. 1 Experiment design for assessment of hepatoprotective activity of Ficus religiosa.

**Physical parameters**

1) Liver weight

**Biochemical estimations**

**Parameters:** The following parameters have been considered to determine the required pharmacological activity.

1) Aspartate amino transferase
2) Alanine amino transferase
3) Alkaline phosphatase
4) Total bilirubin
5) Total protein.

**Procedure for Estimation of Biochemical Parameters**

a. Estimation of SGOT (Serum glutamate oxaloacetic transaminase)

This reagent kit was intended for in-vitro quantitative determination of SGOT (AST) activity in serum/plasma.

**Working reagent preparation**

Add reagent 2 to reagent 1 in ratio 1:4 i.e. 1ml of reagent 2 + 4ml of reagent 1.

**Assay procedure**

The working reagent was allowed to attain 370C before performing the test. 1 ml of working reagent was mixed with 100 µl of test solution and the absorbance was recorded.
Calculation

General formula for converting absorbance change into international units (IU) of activity is

\[ \text{AST activity (IU/L)} = \frac{(\Delta A/	ext{min})}{\text{kinetic factor}} \]

Where A/minute = change in the absorbance per minute

Kinetic factor = 1768

b. Estimation of SGPT (Serum glutamate pyruvate transaminase)

This reagent kit was intended for in-vitro quantitative determination of SGPT (ALT) activity in serum/plasma.

Working reagent preparation

Add reagent 2 to reagent 1 in 1:4 ratio i.e. 1ml of reagent 2 + 4 ml of reagent 1.

Assay procedure

The working reagent was allowed to attain 370°C before performing the test. 1ml of working reagent was mixed with 100 µl of test solution and the absorbance was recorded.

Calculation

General formula for converting absorbance change into international units (IU) of activity is

\[ \text{ALT activity (IU/L)} = \frac{(\Delta A/	ext{min})}{\text{kinetic factor}} \]

Where A/minute = change in the absorbance per minute

Kinetic factor = 1768

c. Estimation of ALP

Summary

ALP is an enzyme found in high concentrations in the liver, biliary, tract epithelium and in the bones. Increased levels are associated mainly with liver and bone disease.

Assay procedure

The working reagent was allowed to attain 370°C before performing the test. 1ml of working reagent was mixed with 20 µl of test solution and the absorbance was recorded.

Calculation

General formula for converting absorbance change into international units (IU) of activity is

\[ \text{ALP activity (IU/L)} = \frac{(\Delta A/	ext{min})}{\text{kinetic factor}} \]

Where A/minute = change in the absorbance per minute
Kinetic factor = 2712

d. Estimation of Bilirubin

This reagent kit was intended for in-vitro quantitative determination of direct and indirect bilirubin from serum/plasma.

Table No 2: Assay procedures.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Total bilirubin</th>
<th>Direct bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipette into test tubes marked</td>
<td>Blank</td>
<td>Test</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>100 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>Working reagent</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Serum/plasma</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>Normal saline</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>

Mix well, incubate for 5 minutes at 370C and read absorbance at 546 to 630 nm against reagent blank.

Calculation

T. Bilirubin (mg/dl) = Absorbance of test for total bilirubin/Absorbance of sample blank for Total bilirubin x factor.

D. Bilirubin (mg/dl) = Absorbance of test for total bilirubin/Absorbance of sample blank for Total bilirubin x factor

e. Estimation of Total Protein

This reagent kit was intended for in-vitro qualitative determination of total protein from serum/plasma.

Table No 3: Assay procedure.

<table>
<thead>
<tr>
<th>Pipette into tubes marked</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>1000 µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>10 µl</td>
<td>-</td>
</tr>
<tr>
<td>Test</td>
<td>-</td>
<td>-</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

Incubate for 10 min. at 370C read absorbance of the standard and each test at 578 nm (550-580 nm) against reagent blank.

Calculation

Total Protein (g/dl) = Absorbance of test / absorbance standard x 6.5.
f. Estimation of Albumin (Bromocresol Green, End point assay)

This reagent kit was intended for in-vitro quantitative determination of total protein from serum/plasma.

### Table No 4: Assay procedure.

<table>
<thead>
<tr>
<th>Pipette into tubes marked</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>1000 µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Reagent 2</td>
<td></td>
<td>-10 µl</td>
<td></td>
</tr>
<tr>
<td>Serum/plasma</td>
<td></td>
<td></td>
<td>10 µl</td>
</tr>
</tbody>
</table>

Incubate for 1 min. at 300C, read absorbance of the standard and each test at 630nm (550-580nm) against reagent blank.

### Calculation

\[
\text{Albumin (g/dl)} = \frac{\text{Absorbance of test}}{\text{absorbance standard}} \times 4 \\
\text{Globulins = total protein – albumin.}
\]

### Histopathology

**Procedure**

At the end of the study, all the surviving animals of the respective groups were sacrificed by an overdose of chloroform anaesthesia. After exsanguinations of the animal’s liver was removed immediately and washed with ice cold saline. The tissue samples were fixed with 10% formaldehyde, dehydrated in a graded series of ethanol, and embedded in paraffin wax before sectioning. The tissue was cut into sections approximately 5µm thick, dewaxed, and rehydrated. The sections were then stained with haematoxylin-eosin dye and studied for histopathological changes using a light microscope. Each sample was observed at a magnification of 100X.

### RESULTS

**Preliminary Phytochemical Screening**

The ethanolic root extract of Ficus religiosa was selected for the present study. About 500mg of the dried roots were powdered and extracted with ethanol. The nature of extract is as follows:

### Table No 5: The nature and extractive value of roots of Ficus religiosa.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Extracts</th>
<th>Color Yield</th>
<th>(%) w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EEFR</td>
<td>Dark brown and semisolid</td>
<td>12.6</td>
</tr>
</tbody>
</table>
Phytochemical Investigation

The ethanolic root extract of Ficus religiosa was subjected to different preliminary chemical tests to determine the chemical constituents present in the extract, the results of which are tabulated as below (Table No. 6).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Test</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Proteins</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Amino acids</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Flavonoids</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) present, (-) absent

Pharmacological Activity

Acute oral toxicity

Acute oral toxicity was carried out according to OECD guidelines; EEFR was safe up to 2000mg/kg and were lethal at 5000mg/kg dose. Hence the dose selected for study was 300mg/kg as low dose and 400mg/kg as high dose.

Physical Parameter

Effect of wet liver weight

CCl4 induced hepatotoxicity (100mg/kg) caused enlargement of liver which was evident from increase in wet liver weight. EEFR at dose of 300mg/kg and 400mg/kg and Ursocol (300mg) for 17 days showed significant restoration of wet liver weight near to normal when compared to control group.

Table No 7: Effect of Ficus religiosa extract on liver weight.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Group</th>
<th>Wet liver weight (gm/kg) Mean ±S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>5.5 ± 0.14</td>
</tr>
<tr>
<td>2</td>
<td>CCl4</td>
<td>7.45 ± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>Ursocol 25mg/kg</td>
<td>5.76 ***±0.03</td>
</tr>
<tr>
<td>4</td>
<td>EEFR 300mg/kg</td>
<td>6.92*** ±0.04</td>
</tr>
<tr>
<td>5</td>
<td>EEFR 400mg/kg</td>
<td>5.40*** ±0.12</td>
</tr>
</tbody>
</table>

Values are Mean ± S.E.M. (n=6); ONE way ANOVA followed by Turkey multiple comparisons test.

Significance values are***p<0.001, **p<0.01, *p<0.05 CCl4 group vs all groups.
Biochemical parameters

Effect of Ficus religiosa extract on serum biomarker enzymes in CCl4 induced hepatotoxicity

Rat treated with carbon tetrachloride developed a significant hepatic damage observed as elevated serum level of hepato specific enzymes like ALP, ALT, and AST when compared to the normal control. After treatment with Ursocol and extract in respective groups had showed good protection against CCl4 toxicity to liver. Turkey multi comparision test indicates a significant reduction in elevated serum enzyme level with extract treated group to toxic control group ethanolic extracts at a dose of 400mg/kg showed the most protective effect in reducing the elevated serum enzyme level (P < 0.001) followed by 300mg/kg (P < 0.01).

Effect of Ficus religiosa extracts on total protein, albumin, and total bilirubin in Carbon tetrachloride induced hepatotoxicity

CCl4 treatment has considerably reduced the serum total protein and albumin levels. Treatment with Ursocol and extracts showed significant increase in the total protein and albumin level as compared to the CCl4 group. Ethanolic extracts at a dose of 400mg/kg showed significant increase in total protein and albumin level (p<0.001) followed by 300mg/kg (p<0.01).

Effect of Ficus religiosa extracts on Total and Direct bilirubin in Carbon Tetrachloride induced hepatotoxicity

Elevation of direct and total bilirubin level after administration of CCl4 indicate its hepatotoxicity. Treatment with Ursocol and extract significantly reduced level of direct and total bilirubin level when compared to the toxic control indicating hepatoprotective effect of plant extract.

Table No 8: Effect of Ficus religiosa extracts on serum biomarker enzymes in CCl4 induced hepatotoxicity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Biochemical Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALP(IU/L)</td>
</tr>
<tr>
<td>Control</td>
<td>75.6±1.5</td>
</tr>
<tr>
<td>CCl4</td>
<td>478.7±4.6</td>
</tr>
<tr>
<td>Ursocol 25mg/kg</td>
<td>137.8±2.5***</td>
</tr>
<tr>
<td>EEFR 200mg/kg</td>
<td>233.9±7.0***</td>
</tr>
<tr>
<td>EEFR 400mg/kg</td>
<td>261.6±11.1***</td>
</tr>
</tbody>
</table>

Values are Mean ± S.E.M. (n=6); ONE way ANOVA followed by Turkey multiple comparisions test.
Significance values are ***p < 0.001, ** p < 0.01, * p < 0.05 and ns p > 0.05 CCl4 group vs all groups.

Table No 9: Effect of Ficus religiosa extract on serum parameters Total protein, Albumin and Bilirubin.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Biochemical Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total Protein g/dl</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.15±0.18</td>
</tr>
<tr>
<td>CCl4</td>
<td>2.51±0.17</td>
</tr>
<tr>
<td>Ursocol 25mg/kg</td>
<td>5.27±0.08***</td>
</tr>
<tr>
<td>EEFR 300mg/kg</td>
<td>5.44±0.07***</td>
</tr>
<tr>
<td>EEFR 400mg/kg</td>
<td>6.12±0.58***</td>
</tr>
</tbody>
</table>

Values are Mean ■ S.E.M. (n=6); ONE way ANOVA followed by Turkey multiple comparisons test.

Significance values are ***p < 0.001, ** p < 0.01, * p < 0.05 CCl4 group vs all groups.

Fig No. 1: Effect of Ficus religiosa extracts on wet liver in CCl4 induced hepatotoxicity.

Values are Mean ■ S.E.M. (n=6); ONE way ANOVA followed by Turkey multiple comparisons test.

Significance values are ***p<0.001, **p<0.01, *p<0.05 CCl4 group vs all groups.
Fig No.2: Effect of Ficus religiosa extract on ALP in CCl4 induced hepatotoxicity.

Values are Mean ± S.E.M. (n=6); ONE way ANOVA followed by Turkey multiple comparisons test.

Significance values are ***p<0.001, **p<0.01, *p<0.05 CCl4 group vs all groups.

Fig No. 3: Effect of Ficus religiosa extract on serum biomarker enzymes in CCl4 induced hepatotoxicity.

Values are Mean ± S.E.M. (n=6); ONE way ANOVA followed by Turkey multiple comparisons test.

Significance values are ***p<0.001, **p<0.01, *p<0.05 CCl4 group vs all groups.
Fig No. 4: Effect of Ficus religiosa extract on AST in CCl4 induced hepatotoxicity.

Values are Mean ± S.E.M. (n=6); ONE way ANOVA followed by Turkey multiple comparisons test.

Significance values are***p<0.001, **p<0.01, *p<0.05 CCl4 group vs all groups.

Fig No. 5: Effect of Ficus religiosa extract on Total Protein in CCl4 induced hepatotoxicity.

Values are Mean ± S.E.M. (n=6); ONE way ANOVA followed by Turkey multiple comparisons test.

Significance values are***p<0.001, **p<0.01, *p<0.05 CCl4 group vs all groups.

Fig No. 6: Effect of Ficus religiosa extract on Total Albumin in CCl4 induced hepatotoxicity.
Values are Mean ± S.E.M. (n=6); ONE way ANOVA followed by Turkey multiple comparisons test.
Significance values are ***p<0.001, **p<0.01, *p<0.05 CCl4 group vs all groups.

**Fig No. 7:** Effect of Ficus religiosa extract on total bilirubin in CCl4 induced hepatotoxicity.
Values are Mean ± S.E.M. (n=6); ONE way ANOVA followed by Turkey multiple comparisons test.
Significance values are ***p<0.001, **p<0.01, *p<0.05 CCl4 group vs all groups.

**Fig No. 8:** Effect of Ficus religiosa extract on direct bilirubin in CCl4 induced hepatotoxicity.
Values are Mean ± S.E.M. (n=6); ONE way ANOVA followed by Turkey multiple comparisons test.
Significance values are ***p<0.001, **p<0.01, *p<0.05 CCl4 group vs all groups.
Histopathological Studies
The protective effect of Ficus religiosa extracts were further confirmed by histopathological examination of liver.

1) Normal Control Group
Sections studied show structure of liver. Architecture is normal. Central vein, sinusoids and hepatocytes are normal.

2) Carbon Tetrachloride Group
Sections showed marked necrosis and inflammatory cell infiltration in the centrizonal area, inflammatory cells were also observed in the portal triad. Development of fibrosis septae was observed between central veins and portal triad.

3) URSOCOL + CCl4 Group
Sections studied shows structure of liver. The architecture is maintained. There is mild congestion and portal triaditis and inflammatory cells were observed in the centrizonal area.

4) EEFR (300 mg/kg) + CCl4 Group
Sections studied show structure of liver parenchyma with partial architecture showing regeneration of hepatocytes.

5) EEFR (400 mg/kg) + CCl4 Group
Sections studied showed greater reduction of the necrosed area and sparse inflammatory cell infiltration around the central vein showing regeneration of hepatocytes.

Fig No. 9: Normal control. (H & E X 100)
Fig No. 10: Toxicant control. (H & E X 100)
DISCUSSION

Hepatoprotective activity

In living system liver is considered to be highly sensitive to toxic agents. It’s also one of the hardest working and can even re-grow its own tissue. It can work when a large portion of it is removed or diseased. It participates in a variety of metabolic activities perhaps by virtue of presence of number of enzymes and thus may self expose too many toxicants, chemicals and drugs which could injure it. In our hepatoprotective study carbon tetrachloride was used as a hepatotoxicant to induce liver damage, since it was reported that a single dose of this hepatotoxin could produce centrilobular hepatic necrosis and that chronic administration led to cirrhosis and hepatocarcinoma. Derivatives which are apparently responsible of structural proteins and enzymes inactivation. The toxic effect of carbon tetrachloride is attributed to the latter metabolite. Carbon tetrachloride may increase the synthesis of fatty acids and decrease the release of hepatic lipoproteins. The increased intracellular concentration of calcium,
increase in nuclear volume and enlargement of nucleoli and also inhibits mitochondrial activity which leads to cell death.

Physical Parameters

Wet Liver weight

Wet liver weight was increased in toxic control group as compared to normal. CCl4 induced hepatotoxicity produce fatty changes and also it is observed that there is a fall in serum lipids in another series of experiments. In this case water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting total liver mass. It is reported that liver mass is important parameter in ascertaining the hepatoprotective effect of the drugs. Treatment with Ficus religiosa extract significantly reduced the wet liver weight of animals where EEFR (300 and 400 mg/kg) showed decreased liver weight (p < 0.001) hence it possesses statistically significant hepatoprotective activity.

Biochemical Parameters

Estimation of serum marker enzymes

Estimation of serum marker enzyme is a useful quantitative marker of the extent and type of hepatocellular damage. During hepatic damage, cellular enzymes like AST, ALT and ALP present in the liver cells leak into the serum, resulting in increased concentrations. SGPT or ALT is a cytosolic enzyme primarily present in hepatocytes. The level of SGPT in serum increases due to leakage of this cellular enzyme into plasma by CCl4 induced hepatic injury. SGOT or AST ia a mitochondrial enzyme present in liver parenchymal cells released from heart, liver, skeletal muscle, and kidney. ALP is an enzyme in cell lining the biliary duct of liver. ALP levels rised in plasms because of large bile duct obstruction by CCl4. After treatment with Ficus religiosa extract, there was a significant reduction observed in ALT, AST, and ALP showing dose dependant activity where EEFR showed less activity. The enzyme levels were almost restored to the normal which is an indicative of hepatoprotective activity.

Direct and Total bilirubin

Bilirubin is a break down product of heam. The liver is responsible for clearing the serum bilirubin. In this mechanism bilirubin is taken up into hepatocytes, conjugated and secreted into the bile, which is excreted into intestine. Total bilirubin includes both direct and indirect bilirubin. In the progressive liver injury from necrosis to cirrhosis, reduces bilirubin metabolism (reduction in hepatocyte uptake, impaired conjugation of bilirubin and reduced
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hepatocyte secretion of bilirubin). Decreased level of serum bilirubin after treatment with Ficus religiosa extract indicates the effectiveness in normalizing the functional state of liver.

**Total protein and total albumin**
The liver produces most plasma proteins in the body. Being a part of cell membrane and as an enzyme, protein participate the intricately balanced subcellular fractions. Proteins play a major role in the synthesis of microsomal detoxifying enzymes and help to detoxify the toxicants which enter into animal body. CCl4 induced liver toxicity decreases the total protein and albumin level in serum due to damage to the tissues. After treatment with Ficus religiosa extract there was increase in serum protein and albumin level indicating plant’s hepatoprotective activity.

**Histopathological Studies**
Histopathological examination of liver section of vehicle control group showed normal cellular architecture with distinct hepatic cells, sinusoidal space. In the liver section of rats intoxicated with carbon tetrachloride there is disarrangement and degeneration of normal hepatic cells characterized by congestion, vacuolar degeneration, necrosis, and inflammatory cell collections with intense centrilobular necrosis and destruction of central vein. While rats treated with Ursocol showed prominent central vein less disarrangement and degeneration of hepatocytes. The liver section of rats treated with two different doses (300 and 400mg/kg) of Ficus religiosa extract showed regeneration of central vein and less disarrangement of hepatocytes in EEFR extract when compared with CCl4 intoxicated rats. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes. Phytochemical evaluation of Ficus religiosa revealed the presence of vitamins, minerals, amino acids, flavonoids, sterol, triterpenes, and tannin in EEFR extract. Hence a reduction in the levels of these enzymes demonstrates membrane stabilizing activity of the plant.

**CONCLUSION**
Evaluation of the hepatoprotective activity was also done by estimating the serum levels of marker enzymes like AST, ALT, and ALP, serum Bilirubin, Total protein & Total albumin. Where the results were found to be significant. The histopathological studies supported the results of biochemical tests, showing less damage in the cytoarchitecture of the liver. In case of carbon tetrachloride treated groups there will be rise in serum marker enzymes such as
SGPT, SGOT, ALP, serum bilirubin, and decrease in the level of Total protein, Total albumin where as the animals treated with the root extract of Ficus religiosa significantly reduced the elevated levels of above mentioned serum marker enzymes and increase in the levels of Total protein, Total Albumin. Hence, at this point it is concluded that the extract of Ficus religiosa offers hepatoprotection. It can be concluded that Ficus religiosa have potential hepatoprotective activity and attenuates the hepatotoxic effects of CCl4 by membrane stabilizing effect.

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