INVESTIGATION OF HEPATOPROTECTIVE ACTIVITY OF FICUS RETUSA (MORACEAE)

N. Jaya Raju1*, N. Sreekanth2

1Dept. of Pharmacy, College of Public Health & Medical Sciences, Jimma University, Jimma, Ethiopia
2Dept. of Pharmacy, J.N.T. University, Hyderabad, Andhra Pradesh, India

Received on: 19/12/2010 Revised on: 20/02/2011 Accepted on: 05/02/2011

ABSTRACT
Ficus retusa (Moraceae) is distributed throughout the Western peninsula and also found in Chota Nagpur, Bihar, Central India, Andamans, Sundribuns, Malaya islands and Australia. In Sanskrit, it is known as ‘Kantalaka’, ‘Kshudra’ and in Telugu it is well known as ‘Yerrajuvvi’. Ficus leaves of ethyl acetate and methanolic extracts (100, 200 and 400 mg/kg) were prepared and tested for its hepatoprotective effect against carbon tetrachloride-induced rats. Alteration in the levels of biochemical markers of hepatic damage like SGPT, SGOT, ALP, bilirubin were tested in both treated and untreated groups. Carbon tetrachloride has enhanced the SGPT, SGOT, ALP and bilirubin levels. Treatment with ethyl acetate extract of Ficus retusa leaves has brought back the altered levels of biochemical markers to the near normal levels in the dose-dependent manner. SGOT (213.1 ± 1.25, 198.2 ± 3.45, 180.6 ± 2.05), SGPT (195.1 ± 2.22, 168.4 ± 1.36, 112.6 ± 3.24), ALKP (215.6 ± 4.60, 202.3 ± 2.45, 195.1 ± 3.20) and T.B (1.24 ± 0.01, 1.05 ± 1.08, 0.96 ± 0.13) levels respectively. Though both the extracts were recorded with significant hepatoprotective activity with same “p” value (p<0.001). Which were significant protective activity in groups treated with FR and silymarin. It was concluded from the study that ethyl acetate and methanolic extracts of FR possess hepatoprotective activity against CCl4 induced hepatotoxicity in rats.

KEYWORDS: Hepatoprotective, Ficus retusa, Carbontetrachloride, Silymarin.

*Corresponding author
N. Jaya Raju, Dept. of Pharmacy, College of Public Health & Medical Sciences, Jimma University, Jimma, Ethiopia. Email: Jayaraju123@yahoo.com

INTRODUCTION
Liver damage is always associated with cellular necrosis, increase in lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, ALP and bilirubin levels are elevated1,2. However there are several herbs/herbal formulations claimed to possess beneficial activity in treating hepatic disorders. Ficus retusa (Moraceae) is distributed throughout the Western peninsula and also found in Chota Nagpur, Bihar, Central India, Andamans, Sundribuns, Malaya islands and Australia. In Sanskrit, it is known as ‘Kantalaka’, ‘Kshudra’ and in Telugu it is well known as ‘Yerrajuvvi’. It is also called as “Indian Laurel Fig”. Root bark and the leaves boiled in oil form good applications for wounds and bruises. Adventitious roots, dried and powdered and mixed with salt are applied to decaying or aching tooth. Bark is used in treatment of liver diseases3-5.

Ficus (Moraceae) species are used in folk medicine for the treatment of various diseases, such as biliousness, ulcers, vomiting, vaginal complaints, fever, diabetes, inflammations, Wound, lycopodium, ulcer itching, diuretic, liver diseases and leprosy, liver6. Ficus retusa is a rapidly growing tree in India but originated from Ceylon. Roots are adventitious, occasionally hanging. Bark is gray and smooth. Branchlets are brown and glabrous7,8. The golden yellow leaves of Ficus retusa contain high amounts of flavonoids and carotenoids, triterpenoids, fatty alcohol, steroids, coumarins, flavane, 4-hydroxybenzoate and isoflavones9-11.

MATERIALS AND METHODS
Plant Material Collection
Ficus retusa (Moraceae) leaves were collected from the Andhra University region of Visakhapatnam, Andhra Pradesh in the month of January 2009 and Authenticated by the taxonomist, Dept of Botany, Andhra University and the specimen Voucher No JNTUCP/2009/F75 has

International Journal of Research in Ayurveda & Pharmacy, 2(1), Jan-Feb 2011 166-169
been preserved in the Department.

**Animals**

Wistar albino rats of either sex weighing between 200-250 gm were obtained from M/s. Mahavir Enterprises, Hyderabad, Andhra Pradesh, India. The animals were housed under standard environmental conditions (temperature of 22 ± 1°C with an alternating 12 h light–dark cycle and relative humidity of 60 ± 5 %), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics committee and by the Regulatory body of the government (Regd no. 516/01/a/CPCSEA). They were fed with standard laboratory diet supplied by M/s. Rayans biotechnologies Pvt. Ltd., Hyderabad, Andhra Pradesh, India. Food and water was allowed *ad libitum* during the experiment.

**Acute toxicity studies**

Acute toxicity studies were performed for extracts of selected plant according to the toxic classic method as per guidelines. None of these extracts showed mortality even at a dose of 1000mg/kg and therefore considered safe.

Toxicological studies were conducted in mice (*N=6*) for all the extracts as per the Irvin’s method at the doses of 100, 300 and 1000 mg/kg, no mortality was observed.

**Materials**

All the materials used for this experiment are of Pharmacopoeial grade. Carbon tetrachloride (E. Merck), silymarin (Sigma Chemical Co.,) and olive oil were purchased from the local supplier. Diagnostic kits for the estimation of SGOT, SGPT, SALKP and serum bilirubin with diazotized sulphanic acid to produce lipid peroxidation were manufactured by Ranbaxy Diagnostics Ltd., New Delhi, India. Water represents the double distilled water; standard orogastric cannula was used for oral drug administration.

**Carbon tetrachloride-induced hepatotoxicity**

The animals were divided into nine groups of six animals each. Group-I served as normal control received 5% acacia mucilage (1 ml/kg.p.o) daily once for 7 days. Group-II served as toxic control and received CCỊ₄ (1 ml/kg i.p) daily once for 7 days. Group-III was treated with the standard drug Silymarin (50 mg/kg .p.o) and followed by CCỊ₄ (1 ml/kg i.p) daily once for 7 days. Groups IV-VI were treated with ethyl acetate extract of *Ficus retusa* at doses of 100, 200 & 400mg/kg p.o, in acacia mucilage respectively followed by CCỊ₄ (1 ml/kg i.p) daily once for 7 days. Groups VII-IX were treated with methanol extract of *Ficus retusa* at doses of 100, 200 & 400mg/kg p.o, in acacia mucilage respectively followed by CCỊ₄ (1 ml/kg i.p) daily once for 7 days. After completion of treatment blood was collected, serum was separated and used for determination of biochemical parameters.

**Collection of blood samples**

All the animals were sacrificed on 7th day under light ether anesthesia. The blood samples were collected separately in sterilized dry centrifuge tubes by puncture retro-orbital plexes and allowed to coagulate for 30 min at 37 °C .The clear serum was separated at 2500rpm (Microcentrifuge) for 10min and subjected to biochemical investigation viz., serum glutamic oxaloacetate transaminase (SGOT), serum glutamic Pyruvate transaminase (SGPT), Alkaline phosphatase (ALP) and Total Bilirubin (TB ).

**Assessment of liver function**

The Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated by UV kinetic method in which both SGOT and SGPT were assayed based on enzyme coupled system; where keto acid formed by the aminotransaminase reacts in a system using NADH. The coenzyme is oxidized to NAD and the decrease in absorbance at 340 nm for SGOT malate dehydrogenase (MDH) reduces to malate with simultaneous oxidation of NADH to NAD. The rate of oxidation of NADH is measured, where as SGPT the pyruvate formed in the reaction is converted to lactate by lactate dehydrogenase. Estimation of Alkaline phosphate (ALKP) involves hydrolysis of P-nitrophenyl phosphate by alkaline phosphatase to give P-nitrophenol, which gives yellow color in alkaline solution. The increase in absorbance due to its formation is directly proportional to alkaline phosphate (ALKP) activity. Estimation of total bilirubin (TB) involved the reaction of bilirubin with diazotized sulphonic acid to form an azocompound, the color of which is measured at 546 nm .All the estimations were carried out using standard kits in semi auto analyzer Screen Master 3000.

**Statistical analysis**

Results of biochemical estimation were reported as mean ±SEM for determination of significant inter group difference was analyzed separately and one-way analysis of variance (ANOVA) was carried out. Dunnet’s test was used for individual comparisons.

**RESULTS AND DISCUSSION**

CCỊ₄ is accumulated in hepatic parenchyma cells and metabolically activated by cytochrome P450-dependent monoxygenases to form a trichloromethyl radical (CCỊ₃). The CCỊ₃ radical alkylates cellular proteins and other macromolecules with simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage . Thus, antioxidant or free radical generation inhibition is...
important in protection against CCl₄-induced liver lesions. Hepatotoxic compounds such as CCl₄ are known to cause marked elevation in serum enzymes and bilirubin levels. It causes a marked decrease in total protein levels. Silymarin is used as standard hepatoprotective compound since it is reported to have a protective effect on the plasma membrane of hepatocytes.

Serum levels of SGOT, SGPT, SALKP and total bilirubin were significantly increased (p<0.001) in carbon tetrachloride (1ml/kg,i.p) treated Group-2 rats. Group-3 rats treated with Silymarin(50mg/kg,p.o) produced significant reduction (p<0.001) in SGOT, SGPT, SALKP and total bilirubin levels when compared to Group-2 rats. The activity of the extracts is found to be dose dependant. The result of this investigation indicated that the methanolic extract of Ficus retusa showed recovery against the toxic effects of CCl₄ thereby confirming the protective effect of the extracts of Ficus retusa. The hepatoprotective activity of Ficus retusa could be due to the presence of bioflavonoids which have hepatoprotective properties. The result of this investigation indicated that the methanolic extract of stem heart wood of Ficus retusa possessed hepatoprotective activity against CCl₄ induced liver damage in rats. Attempts are being made to isolate and characterize the active principle to which the hepatoprotective activity can attribute.

CONCLUSION
The efficacy of any hepatoprotective drug is essentially dependent on its capability of either reducing the harmful effects or in maintaining the normal hepatic physiological mechanism, which have been imbalanced by a hepatotoxin. Oral administration of 100, 200 and 400mg/kg of methanol of stem heart wood of Ficus retusa produced significant decrease in SGOT, SGPT, SALKP and total bilirubin levels. The activity of the extract is found to be dose dependant. In CCl₄ induced toxic hepatitis, toxicity begins with the changes in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures. Administration of ethyl acetate and methanolic extracts of Ficus retusa showed recovery against the toxic effects of CCl₄ thereby confirming the protective effect of the extracts of Ficus retusa.

ACKNOWLEDGEMENT
The authors are very much grateful to Prof. Venkiah, Dept. of Botany, Andhra University for providing the plant material to carry out this work.

REFERENCES
Table 1: Effect of ethyl acetate and methanol extracts of *F. retusa* on biochemical estimation of SGOT, SGPT, SALKP and total bilirubin of CCI4 induced toxicity in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (IU/ml)</th>
<th>SGPT (IU/ml)</th>
<th>SALKP (IU/ml)</th>
<th>Total Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>160.5 ± 0.62</td>
<td>96.95 ± 1.34</td>
<td>179.5 ± 0.99</td>
<td>0.82 ± 0.06</td>
</tr>
<tr>
<td>CCl4 1ml/kg</td>
<td>295.5 ± 0.39</td>
<td>269.5 ± 1.8</td>
<td>296.5 ± 1.45</td>
<td>2.02 ± 0.03</td>
</tr>
<tr>
<td>Silymarin 50mg/kg</td>
<td>174.8 ± 1.88***</td>
<td>107.5 ± 1.45***</td>
<td>187.7 ± 2.25***</td>
<td>0.89 ± 0.04***</td>
</tr>
<tr>
<td>FREE 100 mg/kg</td>
<td>213.1 ± 1.25*</td>
<td>195.1 ± 2.22*</td>
<td>215.6 ± 4.60*</td>
<td>1.24 ± 0.01*</td>
</tr>
<tr>
<td>FREE 200 mg/kg</td>
<td>198.2 ± 3.45**</td>
<td>168.4 ± 1.36**</td>
<td>202.3 ± 2.45***</td>
<td>1.05 ± 1.08***</td>
</tr>
<tr>
<td>FREE 400 mg/kg</td>
<td>180.6 ± 2.65***</td>
<td>112.6 ± 3.24***</td>
<td>195.1 ± 3.20***</td>
<td>0.96 ± 0.13***</td>
</tr>
<tr>
<td>FRME 100 mg/kg</td>
<td>250.2 ± 1.04*</td>
<td>225.2 ± 2.06*</td>
<td>235.1 ± 4.36*</td>
<td>1.38 ± 0.32*</td>
</tr>
<tr>
<td>FRME 200 mg/kg</td>
<td>231.0 ± 3.48**</td>
<td>202.5 ± 1.28**</td>
<td>220.2 ± 1.40**</td>
<td>1.22 ± 0.45**</td>
</tr>
<tr>
<td>FRME 400 mg/kg</td>
<td>210.3 ± 1.26***</td>
<td>165.2 ± 3.03***</td>
<td>205.6 ± 3.20***</td>
<td>1.10 ± 1.07***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for six observations

P: *<0.05, **<0.01, ***<0.001 Compared to respective control group-1
FREE- *Ficus retusa* ethyl acetate extract, FRME- *Ficus retusa* methanol extract

Fig 1: Comparative study of hepatoprotective activity of ethyl acetate and methanol extracts of *F. retusa*

Source of support: Nil, Conflict of interest: None Declared