Effect of unripe fruit extract of *Ficus glomerata* (Roxb) in CCl_4 and Parcetamol induced Hepatotoxicity in rats

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Summary

In present study, Hepatoprotective activity of the 70% ethanolic extract of unripe fruit of *Ficus glomerata* was studied on paracetamol and CCL_4 treated albino rats. The hepatoprotective effect was evaluated on the basis of estimation of biochemical parameter like SGPT, SGOT, ALP, ACP, TB, DB. The 70% ethanolic extract of fruits of *Ficus glomerata* at the dose of (100mg/kg, 250mg/kg, 500mg/kg) produces a dose dependant significant reduction in biochemical parameter as well as in morphological parameter. Treatment with unripe fruit of *Ficus glomerata* extract had shown significant hepatoprotective effect also supported by histopathological studies on liver, the result was in comparison with the standard drug silymarin. Silymarin was used as the reference standard at 25mg/kg p.o. and it showed effect in both he hepatotoxicity models. The aorgan protective activity which may be attributed to polyphenolic compounds like flavanoids and tannins that are present in the unripe fruits of *Ficus glomerata*.

Key words : *Ficus glomerata*, CCL_4, paracetamol, hepatoprotective, marker enzymes (SGPT, SGOT, ALP, ACP, TB and DB)

Introduction

Liver, the most versatile but complex internal organ of human body, plays vital role in metabolic activities. Its importance also lies in its impetus in management of internal enviourment and biochemical conversion of endogenous and exogenous chemical to harmless and excretable compounds. Therefore being a vital organ, its protection has a special status in therapeutics.[1] Prolonged drug therapy, excessive use of the some of the commonly used medicines like paracetamol, diclofenac etc., alcoholism, exposure to certain xenobiotic, polutants and certain disease state have been reported to affect liver functioning. The major clinical manifestation of liver disorder is jaundice. Despite of the excellent regeneratation capacity of this organ, a slight injury or toxicity may lead to fatal complications. Therefore damage to the liver inflicted by hepatotoxic agents is of grave consequences. Unavailability of rational therapy in modern medicine and no or very less positive influence of synthetic drugs in liver damage have urged researchers in this field to look for herbal drugs with better hepatoprotective action. Traditional medicines are effective in certain disease and are based on their age-old use in folklore system of medicine. Natural products of plant origin with hepatoprotective and antioxidants properties play an important role in treatment of liver toxicity.[2]

One of such plant is *Ficus glomerata* (Roxb) has been used in traditional system of medicine for treating diabetes diarrhoea, liver diseases, piles, asthma, leprosy and diarrhea.[3] The hepatoprotective activity of leaves of *Ficus glomerata* has been reported.[4] Leaves shows anti-bacterial activity[5], stem bark shows anti-tussive potential[6], anti-diuretic activity[7], anti-pyretic potential[8], anti-inflammatory activity of the leaves, bark and unripe fruit[9-11], hepatoprotective activity of the leaves[4], hypoglycemic activity of roots, leaves and fruit[12-14] and anti-filarial activity of the fruits.[15]

However, there is no scientific claims has been made regarding the hepatoprotective activity of unripe fruits of *Ficus glomerata*. In view of this, in the present investigation an attempt will be made to study hepatoprotective role of unripe fruit extract of *Ficus glomerata*.

Material and Methods

The unripe fruits of *Ficus glomerata* were collected from the surrounding fields of Harapanahalli. The identification of plant was made by Professor K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli. A voucher specimen has been deposited at the museum of our college. The unripe fruits were collected in the month of April. The unripe fruits were dried in shade at room temperature. The dried unripe fruits were powdered by using grinder, to coarse powder and this powder was packed into soxhlet column and the extracted 70% ethanol ($60 - 80^0$) for 24 hrs. The extract was concentrated under reduced pressure with the use of rotatory flash evaporator. Further the extracts were concentrated by using hot water bath ($70 - 80^0$). The dried extract was stored in airtight container in refrigerator below 10^0C.

Preliminary phytochemical screening

The preliminary phytochemical screening was carried out on the 70% ethanolic extract of unripe fruit of *Ficus glomerata* for qualitative identification. The tests for common phytochemicals were carried out by standard methods described in practical pharmacognosy by Dr. C.K. Kokate[16] and K.R. Khandelwal.[17]

Determination of acute toxicity (LD_{50})[16]

Method: The acute toxicity of 70% ethanolic extract of unripe fruit of *Ficus glomerata* were determined in albino mice weighing 25 – 30 gm, of single sex, normally female, maintained under standard conditions. The animals were fasted over night prior to the experiment. Fixed dose (OCED Guideline No. 420) method of CPCSEA was adopted for toxicity studies

Animals:

The male albino rats wistar stain 150 – 200 g and albino mice 20 – 30 g were used through the experimentation. The animals were procured from Venkateshwara Associates, Bangalore, Karnataka. After randomization into various groups, animals were acclimatized for period of 10 days under standard husbandry condition as follows; Room temperature 27 ± 3^0C**,** Relative humidity 65 ± 10%, 12 hrs light/dark cycle. All the animals were fed with rodent pellet diet (Gold mohr, Lipton India Ltd.,) and water was allowed ad-libitum under strict hygienic condition. Ethical clearance for performing experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC).**[18]**

Evaluation of hepatoprotective activity

CCl_4 induced hepatotoxicity (Malaya gupta et al., was followed[19])

Albino rat of wistar strain weighing 150 – 200 g were selected and divided into six groups of each containing six animals. Animals were treated as shown above for a period of 10 days. At the end of every 72 hrs. i.e. 4^{th} day, 7^{th} day and 10^{th} day CCl_4 was administered to all group other than group I^{101}. CCl_4 was administered at the concentration of 30% in liquid paraffin (1 ml/kg body weight, i.p.). Group III received standard drug silymarin 25 mg/kg p.o. once in a day and CCl_4 as mentioned above. Whereas group IV, V and VI were treated with test extract dose of (100, 250 and 500 mg/kg p.o.) respectively. During this period of treatment the rat were maintained under normal diet and water. Treatment duration was of 10 days, all the animals were sacrificed 24 hrs. after the last injection of CCl4 i.e. on 11^{th} day. Blood was collected by carotid bleeding under mild ether anesthesia using disposable syringe and needle. After the blood was collected it was allowed to clot at room temperature for 30 min. followed by centrifugation (3000 rpm for 15 min.) and subjected for determination of biochemical parameters.

Liver were dissected out and subjected for morphological study such as wet liver weight and wet liver volume of each animal was determined. The volume of wet liver was measured by displacement method and further the liver were placed in 10% formalin solution for histopathological study.[20]

Paracetamol induced hepatotoxicity (Malaya gupta et al., was followed[19])

Albino rat of wistar strain weighing 150 – 200 g were selected and divided into six groups of each containing six animals. Animals were treated as shown above for a period of 7 days. Paracetamol was administered to all group other than group I daily at the dose of (500 mg/kg p.o.) Group III received standard drug silymarin (25mg/kg p.o.) simultaneously for a period of 7 days. Whereas group IV, V and VI were treated with test extract dose of (100, 250 and 500 mg/kg p.o.) respectively. During this period of treatment the rat were maintained under normal diet and water. Treatment duration was of 7 days, all the animals were sacrificed 18 hrs. fasting of the last dose i.e. on 8^{th} day. Blood was collected by carotid bleeding under mild ether anesthesia using disposable syringe and needle. After the blood was collected it was allowed to clot at room temperature for

30 min. followed by centrifugation (3000 rpm for 15 min.) and subjected for determination of biochemical parameters.
Liver were dissected out and subjected for morphological study such as wet liver weight and wet liver volume of each animal was determined. The volume of wet liver was measured by displacement method and further the liver were placed in 10% formalin solution for histopathological study.[20]

Biochemical parameters includes

***Estimation of serum SGPT*[21]**
SGPT catalyses the transfer of amino group from L-alanine to 2-oxoglutarate with the formation of pyruvate and L-glutamate. The pyruvate so formed is allowed to react with NADH to produce L-lactate. The rate of this reaction is monitored by an indicator reaction coupled with LDH in the presence of NADH (nicotinamide adenine dinucleotide). The oxidation of NADH in this reaction is measured as a decrease in the absorbance of NADH at 340 nm, which is proportional to SGPT activity.

***Estimation of SGOT*[22]**
SGOT catalyses the transfer of amino group from L-aspartate to 2-oxoglutarate forming oxaloacetate and L-glutamate. The rate of this reaction is monitored by an indicator reaction coupled with malate-dehydrogenase (MDH) in which the oxaloacetate formed is converted to malate in the presence of reduced nicotinamide adenine dinucleotide (NADH). The oxidation of NADH in this reaction is measured as a decrease in absorbance of NADH at 340 nm, which is proportional to SGOT activity.

***Estimation of serum alkaline phosphate (ALP)*[23]**
Serum alkaline phosphatase hydrolyses p-nitrophenyl phosphate into p-nitrophenol and phosphate in the presence of oxidizing agent Mg^{2+}. This reaction is measured as absorbance is proportional to the ALP activity.

***Estimation of serum acid phosphate (ACP)*[24]**
Serum acid phosphatase splits α naphthyl phosphate in to α naphthol and phosphate. The α naphthol is quantitated by coupling it with a diazonium salt to form a highly coloured azo dye whose absorbance is measured.

***Estimation of serum bilirubin*[25]**
Bilirubin reacts with diazotised sulphanilic acid in acidic medium to form pink coloured azobilirubin with absorbance directly proportional to bilirubin concentration. Direct bilirubin, being water soluble directly reacts in acidic medium. However, indirect or unconjugated bilirubin is solubilised using a surfactant and then it react similar to direct bilirubin.

***Histopathological studies*[20]**
The animals were sacrificed and the liver of each animal was isolated. The isolated liver was cut in to small pieces and preserved and fixed in 10% formalin for two days. Following this was the washing step where by the liver pieces were washed in running water for about 12 hrs. This was followed by dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 hrs. each. Then the final dehydration is done using absolute alcohol with about three changes for 12 hrs. each.

The cleaning was done by using chloroform with two changes for 15 to 20 minutes each. After clearing the liver pieces were subjected to paraffin infiltration in automatic tissue processing unit.

The liver pieces were washed with running water to remove formalin completely. To remove the water, alcohol of increasing strengths was used since it is a dehydrating agent. Further alcohol was removed by using chloroform and chloroform removed by paraffin infiltration.

Statistical analysis:

The results were expressed as mean ± standard error mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test and $p < 0.01$ was considers significant.

Results

Biochemical parameter in CCl_4 induced hepatotoxicity.

Rats subjected to the CCL_4 challenge alone (positive control group) developed significant liver injury as evident from a significant elevation in the biochemical markers, like SGPT, SGOT, ALP, ACP, TB & DB when compared with negative control group (Table No. 1). Oral administration of the test extract exhibited dose dependent significant reduction in the CCL_4 induced increase in the biochemical levels. However there was decrease in the level of TB & DB were found with the 100mg/kg of the test extract but the result found statistically not significant. Treatment with the reference standard, silymarin (25 mg/kg p.o.) also reversed the hepatotoxicity significantly. Hepatoprotective potency of the test extract at the dose 500mg/kg was found closer to that of standard.

Morphological parameter in CCl_4 induced hepatotoxicity.

Intoxication of rats with CCl_4 results in enlargement of liver which was pale reddish brown in colour. Rats subjected to the CCL_4 challenge alone (positive control group) developed significant increase in the morphological parameter like wet liver weight and wet liver volume when compared with negative control group (Table No. 1). Oral administration of the test extract exhibited dose dependent significant reduction in the CCL_4 induced increase in the morphological parameter. However there was decrease in wet liver weight were found with the 100mg/kg of the test extract but the result found statistically not significant. Treatment with the reference standard, silymarin (25 mg/kg p.o.) also reversed the increase morphological parameter significantly. Organ protective potency of the test extract at the dose 500mg/kg was found closer to that of standard.

Biochemical markers in paracetamol induced hepatotoxicity.

There was marked elevation of biochemical markers, like SGPT, SGOT, ALP, ACP, TB & DB in rats which were intoxicated alone with paracetamol (positive control group) when compared to negative control group (Table No.2). Oral administration of EEFFG has significantly reduced the elevated levels of biochemical markers at all the doses (100mg/kg, 250mg/kg, 500mg/kg) in a dose dependent manner the result are given in (Table No.2). Treatment with the reference standard, silymarin (25 mg/kg p.o.) also reversed the hepatotoxicity significantly.

Morphological parameter in paracetamol induced hepatotoxicity.

Intoxication of rats with paracetamol alone (positive control group) increases wet liver weight, and wet liver volume when compared to negative control group. The intoxication induced changes were attenuated by EEFFG at all doses. However there was decrease in wet liver volume with the 100mg/kg of the test extract but the result found statistically not significant. Treatment with the reference standard, silymarin (25 mg/kg p.o.) also reversed the increase morphological parameter significantly.

Histopathological Studies in CCl_4 induced hepatytoxicity

Histopathological profile of liver from CCL_4 (positive control group)intoxicated rats reveals hepatic globular architecture disrupted, hepatic cells has shown various degree of fatty degeneration like ballooning of hepatocytes, fatty cyst, infiltration of lymphocytes and proliferation of kuffer cells. Congestion of liver sinusoids. Protective effect of test extract was confirmed by histopathological examination of liver section. Administration of test extract at the dose of 500mg/kg that is (Plate-6) showed a significant improvement of the hepatic architecture and areas of Kupper cell proliferation and sinusoid appeared normal on contrary with 100mg/kg and 250mg/kg.

Histopathological Studies in paracetamol induced hepatytoxicity

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TABLE NO. 1
Effect of EEFFG on biochemical and morphological parameter in CCl_4 induced hepatotoxicity.

Groups	SGPT IU/l	SGOT IU/l	ALP IU/l	ACP IU/l	TB mg/dl	DB mg/dl	Volume/ 100gb.w.	Weight/ 100g b.w
-ve control	104.37 ± 3.182	119.39 ± 6.636	303.77 ± 1.82	30.46 ± 0.40	0.368 ± 0.002	0.291 ± 0.003	3.16 ± 0.023	3.06 ± 0.063
+ve control	247.77 ± 9.402	367.57 ± 9.115	522.56 ± 7.14	56.116 ± 0.74	0.625 ± 0.011	0.582 ± 0.013	4.68 ± 0.037	4.23 ± 0.021
Standard silymarin 25 mg/kg	147.60 ± 3.536***	162.91 ± 2.774***	341.42 ± 3.63***	38.69 ± 0.58***	0.417 ± 0.004***	0.377 ± 0.016***	3.23 ± 0.026***	3.18 ± 0.031***
EEFFG 100 mg/kg	216.20 ± 10.388*	334.46 ± 5.286*	493.19 ± 5.19*	51.27 ± 1.49*	0.592 ± 0.005^{ns}	0.541 ± 0.010^{ns}	4.47 ± 0.071*	4.04 ± 0.050^{ns}
EEFFG 250 mg/kg	204.09 ± 5.675**	321.16 ± 5.449**	467.14 ± 4.81***	47.18 ± 0.50**	0.574 ± 0.005**	0.522 ± 0.012*	4.20 ± 0.054***	3.94 ± 0.053**
EEFFG 500 mg/kg	160.03 ± 5.142***	187.14 ± 10.804***	353.70 ± 9.27***	41.55 ± 1.59***	0.447 ± 0.013***	0.431 ± 0.012***	3.96 ± 0.057***	3.50 ± 0.079***

Values are mean ± SEM (n = 6).
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to positive control.

TABLE NO.2
Effect of EEFFG on biochemical and morphological parameter in Paracetamol induced hepatotoxicity.

Groups	**SGPT IU/l**	**SGOT IU/l**	**ALP IU/l**	**ACP IU/l**	**TB mg/dl**	**DB mg/dl**	**Volume/ 100g b.w.**	**Weight/ 100g b.w**
-ve control	107.79 ± 3.408	124.99 ± 4.92	303.77 ± 1.82	30.46 ± 0.40	0.384 ± 0.0032	0.257 ± 0.0065	2.67 ± 0.039	3.20 ± 0.013
+ve control	233.43 ± 2.814	266.20 ± 6.23	522.56 ± 7.14	55.11 ± 0.074	0.563 ± 0.0066	0.571 ± 0.0086	3.90 ± 0.034	4.19 ± 0.020
Standard silymarin 25 mg/kg	129.82 ± 3.387***	157.65 ± 2.85***	341.42 ± 3.64***	38.69 ± 0.58***	0.419 ± 0.0028***	0.310 ± 0.0068***	2.91 ± 0.045***	3.35 ± 0.024***
EEFFG 100 mg/kg	184.06 ± 11.74*	240.43 ± 3.80*	491.86 ± 3.57*	51.27 ± 0.97*	0.528 ± 0.0091*	0.533 ± 0.0031*	3.85 ± 0.028^{ns}	4.061 ± 0.0311*
EEFFG 250 mg/kg	117.21 ± 17.23*	230.60 ± 7.04**	468.42 ± 4.45***	47.18 ± 1.50**	0.503 ± 0.0063**	0.456 ± 0.013***	3.71 ± 0.078*	3.89 ± 0.044**
EEFFG 500 mg/kg	149.00 ± 15.82***	181.47 ± 6.16***	357.32 ± 10.43***	41.66 ± 1.59***	0.447 ± 0.0088***	0.387 ± 0.011***	3.25 ± 0.065***	3.63 ± 0.036***

Values are mean ± SEM (n = 6).
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to positive control.

FIG 1
Effect of EEFFG on Histopathological Studies in CCl_4 induced hepatotoxicity

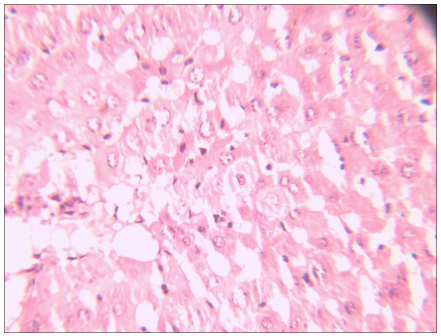

Fig A 100 mg/kg

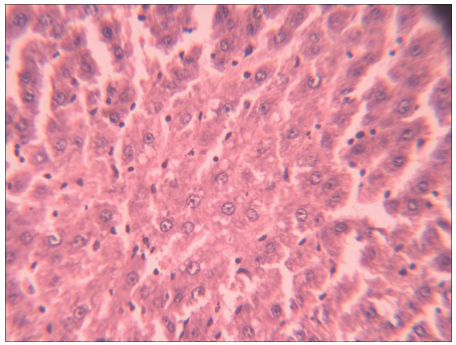

Fig B 250 mg/kg

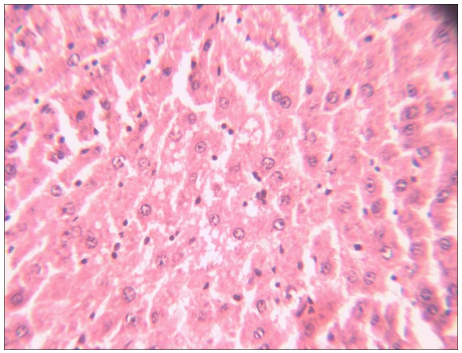

Fig C 500 mg/kg

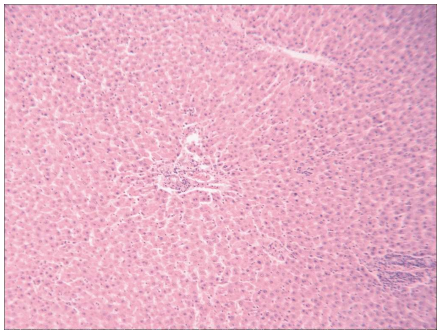

Fig D Control CCl_4

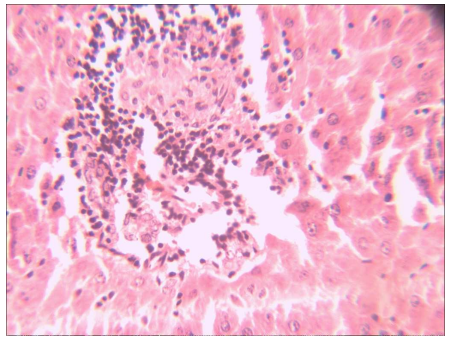

Fig E Hept Control CCl_4

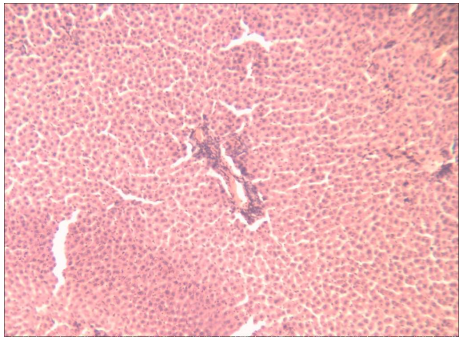

Fig F Standard

FIG-2
Effect of EEFFG on Histopathological Studies in Paracetamol induced hepatotoxicity

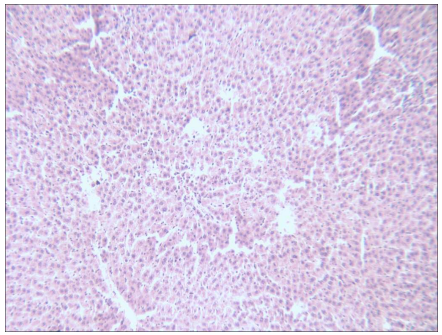

Fig A Control Para

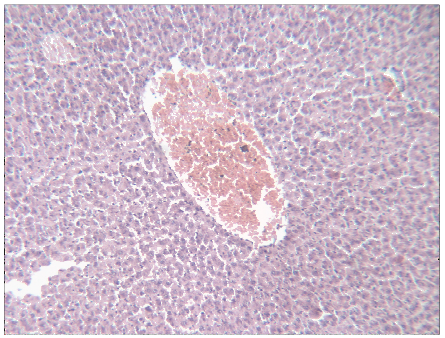

Fig B Hept Control Para

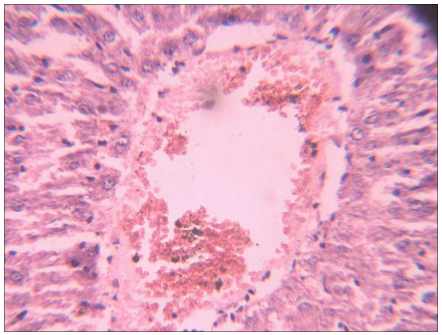

Fig C Para 100

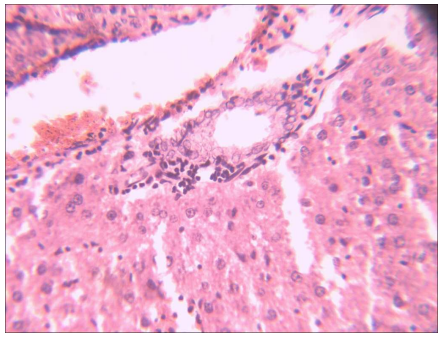

Fig D Para 250

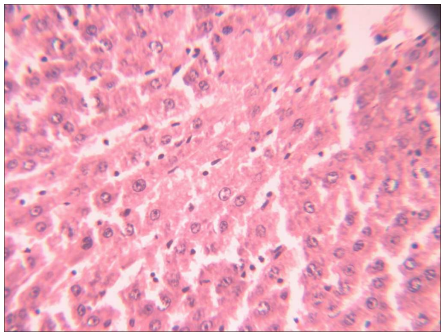

Fig E Para 500

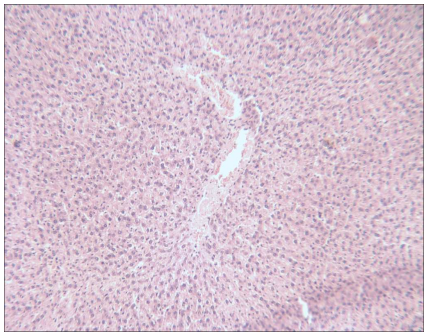

Fig F Standard Para

Discussion

Hepatic system of an organism is involved in metabolic activities of it. In this process it is exposed to various challenges and hence, hepatic system is not only evolved to perform its function but also to protect itself to various challenges like exposure to antibiotics/xenobiotics, chemicals etc. Liver is such an organ that its physiological role and its self protective mechanism are well developed and orchestrated. Inspite of such balanced internal mileiu, hepatic aberration, damage and necrosis commonly occurring due to over exposure to hepatotoxic causes to such an extent that it over powers the mechanism. Inspite of ultra modern advances in medical science, pharmaco-therapeutics treatment with synthetic drugs is not yet realized. However there are several herbs and herbal formulation which are found to be/claimed for treating hepatic disorders.

In the present study one of the local available plant *Ficus glomerata* (Roxb) were selected on the basis of native practitioners and available phytochemical profile of the plant. In the present study the extract of the title plant and were prepared by using soxhlet procedure and further subjected phytochemical screening. It is observed that the extract showed a marked presence of carbohydrate, flavanoids, tannins, glycoside, and steroids. Liver damage induced by CCl_4 and acetaminophen are commonly used models for the screening of hepatoprotective drugs.[26, 27] The rise in the levels of SGPT, SGOT, ALP, ACP and Bilurubin (Direct & total) has been atributed to the damaged structural integrity of liver.[28] Liver cell injury induced by CCl_4 involves initially the metabolism of CCl_4 to trichlromethyl free radical by the mixed function oxidase system of the endoplasmic reticulum. It is postulated that secondary mechanism links CCl_4 metabolism to the wide spread disturbance in hepatocyte function. These secondary mechanisms could involve the generation of toxic product arising directly from CCl_4 metabolism or from peroxidative degeneration of membrane lipids.[29]

These biochemical markers are cytoplasmic in location and are released in circulation after the cellular damage.[30] Both CCl_4 and acetaminophen share a common property to be converted into their respective reactive metabolites N-acetyl-p-benzoquinoneimine (NAPQI) and halogenated free radical (HRF) by hepatic cytochrome P450.[31, 32]

The results of the present investigation clearly demonstrate the various biochemical changes, produced in the serum and in rats by intoxication with CCl4 and acetaminophen, and were reversed by the treatment of extract at different doses and were supported by the histopathological report.

The probable mechanism by which *Ficus glomerata* exerts its protective action against CCl_4 and acetaminophen induced hepatotoxicities could be the stimulation of hepatic regeneration through an improved synthesis of proteins, or with interference with the liberation of microsomal activation to toxicants.

Also postulated that the inhibitor cytochrome P450 (CYPs) can impair the bioactivation of acateamine and CCl_4 into their respective reactive metabolites and thus provide protection against the hepatocellular damage.[33, 34] The extract may stimulate the inhibitor cytochrome P450 (CYPs) and their by providing the protection against the hepatotoxicant.

Moreover it is reported that flavanoids and tannins were reported to posses variety of pharmacological activity including hepatoprotective activity. In the present investigation also preliminary phytochemical investigation on EEFFG gave positive tests for

flavanoids and tannins this could be the reason for significant hepatoprotective property of the test extract.

References

1. Ravishankar SB, Bhavsar GC. Plants with hepatoprotective activity. Indian Drugs 1993; 30: 363-365.
2. Vaidya AB, Sirsat SM, Doshi JC, Antarkar DS. Selected medicinal plants and formulation as hepatobiliary drug: An overview. Indian J. Clin. Pharmacol. Ther 1996; 17: 7-11.
3. Warrier PK, Nambiar VPK, Ramankutty C. Indian Medicinal Plants, Madras. Oriental Longman Ltd., 1996; (3): 34p.
4. Mandal SC, Maity TK, Das J, Pal M, Saha BP. Hepatoprotective activity of *Ficus racemosa* leaf extract on liver damage caused by carbon tetrachloride in rat. Phytotherapy Research 1999; 13(5): 430-32.
5. Mandal SC, Shah BP, Pal M. Studies on antibacterial activity of *Ficus glomerata* Linn. leaf extract. Phytotherapy Research 2000; 14(4): 278-80.
6. Bhaskara Rao R, Murugesan T, Pal M, Saha BP, Mandal SC. Antitussive potential of methanol extract of stem bark of *Ficus glomerata* Linn. Phytotherapy Research 2003; 17(9): 1117-18.
7. Ratnasooriya WD, Jayakody JR, Nadarajah T. Antidiuretic activity of aqueous bark extract of Sri Lankan *Ficus racemosa* in rats. Acta Biology Hung 2003; 54(3-4): 357-63.
8. Rao RB, Anupama K, Swaroop KR, Murugesan T, Pal M, Mandal SC. Evaluation of anti-pyretic potential of *Ficus glomerata* bark. Phytomedicine 2002; 9(8): 731-33.
9. Mandal SC, Maity TK, Das J, Saha BP, Pal M. Antiinflammatory evaluation of *Ficus racemosa* Linn. leaf extract. J Ethnopharmacol 2000; 72(1-2): 87-92.
10. Rachel W Li, Stephen P Myers, David N Leach, David Lin G, Greg Leach. Across-cultural study : Anti-inflammatory activity of Australian and Chinese plants. J Ethnopharmacol 2003; 85(1): 25-32.
11. Shivakumar H, Sankara SLVJ, Vaidya VP. Anti-inflammatory activity of the unripe fruits of *Ficus glomerata*. Indian Drugs 2007; 44(1): 48-50
12. Ajit Kar, Choudhary BK, Bandyopathdhy NG. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. J Ethnopharmacol 2003; 84(1): 105-08.
13. Mandal SC, Mukharjee PK, Das J, Pal M, Saha BP. Hypoglycemic activity of *Ficus racemosa* Linn leaves in streptozotocin induced diabetic rat. Nat Prod Sci 1997; 3(1): 38-41.
14. Patil KS, Varke PD, Chaturvedi SC. Hypoglycemic properties of *Ficus glomerata* fruits in alloxan induced diabetic rat. J Nat Rem 2006; 6(2): 120-23.
15. Vandan Mishra, Nizam U Khan, Singhal KC. Potential antifilarial activity of fruit extracts of *Ficus racemosa* Linn. against Setaria cervi in vitro. Indian J Exp Biol 2005; 43: 346-50.
16. Kokate CK. Practical Pharmacognosy 4th ed. New Delhi: Vallabha prakashan.,1999.169p.
17. Khandelwal KR. Practical Pharmacognosy. 11th ed. Pune: Nirali Prakashan 2004: 149p.

18. Mrs. Prema Veeraraghavan. Expert consultant, CPCSEA, OECD guide line No. 420; Oct 2000.
19. Malaya gupta, Upal Kanti Mazumder, Thangavel Siva Kumar, Periyasamy, Gomathi, Ramanathan Sambath Kumar. Antioxidant and hepatoprotective effects of *Bauhinia racemosa* against paracetamol and carbon tetrachloride induced liver damage in rats. Iranian Journal of Pharmacology and Therapeutics 2004; 3: 12-20.
20. Luna LG. Manual of histology and staining methods of Armed Forces institute of Pathology, 3rd ed. New York, McGraw Hill Book Co., 1986; 1p.
21. Bradley DW., et al. Clin Chem. 1972; 18: 1442 p.
22. Rej R, Fasce CF, Vanderlinde RE. Clin Chem. 1973; 19: 92p.
23. MacComb RB, Bower GN. Clin Chem 1972; 18: 97p.
24. Alex Kaplan, Szabo LL. Clin Chem. 1983; 216p.
25. Pearlman PC, Lee RT. Clin Chem. 1974; 20: 447p.
26. Slater TF. Biochemical mechanism of liver injury.1965; London : Academic press: 1p.
27. Plaa GI, Hewitt WR. Toxicology of the liver. Raven Press Zakim D, Boyer T.D; 1982: 103p.
28. Chenoweth MB, Hake CL. Ann Rev Pharmacol. 1962; 2: 363p.
29. Brattin WJ, Glende EA Jr, Recknagel RO. Pathological mechanisms in carbon tetrachloride hepatotoxicity. J Free Radic Biol Med. 1985; 1: 27-32.
30. Sallie R, Tredger JM, William R. Biopharm Drug Disp 1974; 12: 251p.
31. Packer JE, Slater TF, Wilson RL. Life Sci. 1978; 23:2617p.
32. Van De Straat R, De Vries J, Depets AJJ, Vermueulein NPE. Biochem Pharmacol 1987; 36: 2065.
33. Castro JA, de Ferreyra GC, de Castro CR, Sesame H, de Fenso MO, Gillette JR Biochem Pharmacol 1974; 23: 295.
34. Nelson EB, Montes M, Goldstein M. Toxicology 1980; 17: 73p.