



Research Article

HEPATOPROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF *FICUS MOLLIS* ON PARACETAMOL INDUCED LIVER DAMAGE IN ALBINO RATS

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Article Received on: 27/04/14 Revised on: 07/06/14 Approved for publication: 17/06/14

DOI: 10.7897/2230-8407.0506100

ABSTRACT

The present study was conducted to evaluate the hepatoprotective activity of alcoholic extract of *Ficus mollis* stem bark against paracetamol induced liver damage in Albino rats. The alcoholic extract of stem bark of *Ficus mollis* (100, 200 and 400 mg/kg) was administered orally to the animals with hepatotoxicity induced by paracetamol (3 g/kg). Silymarin (25 mg/kg) was given as reference standard. All the test drugs were administered orally by suspending in 0.5 % Carboxy methyl cellulose solution. The plant extract was effective in protecting the liver against the injury induced by paracetamol in rats. This was evident from significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin. It was concluded from the result that the alcoholic extract of *F. mollis* stem bark possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

Keywords: *Ficus mollis*, Paracetamol, hepatoprotective and hepatotoxicity

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 effects¹. 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 disorders². 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. The Herb of *Ficus mollis* bark is used to relieve in pain of limbs. The bark part of *Ficus mollis* is also used for the treatment of cuts and wounds in the form of ointments, but no report is available about the effect of its aqueous extract against Paracetamol induced hepatic damage. In this study, we evaluated the effect of alcoholic extract of stem bark of *Ficus mollis*, against Paracetamol induced hepatic damage in rats, by determining the activities of Biochemical parameters like SGOT, SGPT, ALP and Bilirubin.

MATERIALS AND METHODS

Animals

Wister albino rats of either sex were used for the study of the crude extracts. Institution Animal Ethics Committee has approved the project (788/a/11/CPCSEA). The animals were kept at 27 ± 2°C, relative humidity 44–56 % and light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. Animals were provided with standard diet (Lipton, India) and the food was withdrawn 18 h before the start of the experiment and water *ad libitum*. All the experiments were performed in the morning according to current guidelines for the care of the laboratory animals and

the ethical guidelines for the investigation of experimental pain in conscious animals³

Plant resources and preparation of crude drug extract

The stem bark of *F.mollis* was collected from Tirupati, Andhra Pradesh, India. And authentication was done by Dr. K. Madhava Chetty, Assistant professor, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh, India. Stem bark were shade dried and defatted with petroleum ether. The defatted material was extracted with 95 % ethanol using soxhlet apparatus and then vacuum dried.

Phytochemical studies

All the extracts were subjected for phytochemical study.⁴

Acute toxicity studies

The acute toxicity study for ethanolic extract of stem bark of *F. mollis* was performed using albino rats. The animals were fasted overnight prior to the experiment and maintained under standard conditions. All the extracts were administered orally in increasing dose and found safe up to dose of 2000 mg/kg for all extracts.

Experimental animal and design

The experiment was conducted according to the modified procedures described previously⁵. PCT was dissolved in 0.5 % CMC for oral administration. Rats were randomly divided into six groups, each consisting of six rats. Group1 served as normal control and was orally given pure water for seven days, and then intraperitoneally injected with 10 ml/kg body weight isotonic 0.9 % NaCl. Group 2 served as hepatotoxicity control and was orally given pure water for seven days and then orally intoxicated with 3 g/kg PCT. Group 3 served as standard, and received Standard drug Silymarin 25 g/kg, orally. Group 3, 4 and 5 were treated with

the ethanol extract of *F. mollis* (with concentrations of 100, 200 and 400 mg/kg respectively) for seven days. After 24 h of PCT intoxication, the rats were euthanized by ether and then sacrificed. The blood was collected by cardiac puncture in heparinized tubes. The liver was immediately taken out and washed with ice-cold saline. The blood and liver samples were assessed for their biochemical, as well as histological observation.

Biochemical determinations

The biochemical parameters like serum enzymes: aspartate aminotransferase (AST), serum glutamate pyruvate transaminase (ALT)⁶, serum alkaline phosphatase (ALP)⁷ and total bilirubin⁸ were assayed using assay kits (Span Diagnostic, Surat, India).

DPPH-scavenging activity

The free radical-scavenging activity of the extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH⁹. Solution of 0.1 mM in ethanol was prepared and 1.0 mL of this solution was added to 3.0 mL of all the extracts solution in water at different concentrations (10–100 µg/mL). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical-scavenging activity. Ascorbic acid was used as a standard drug.

Superoxide Radical Scavenging Activity

Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 µg riboflavin, and 12 mM EDTA and 0.1 mg NBT and 1 ml of sample solution. Reaction was started by illuminating the reaction mixture with different concentrations of plant extract and standard ascorbic acid solution viz. 10, 20, 40, 60, 80 and 100 µg/ml for 5 minutes. Immediately; after illumination, the absorbance was measured at 590 nm. Identical tubes with reaction mixture and 1ml of methanol were kept in the dark along and served as control. The percentage inhibition of superoxide anion generation was calculated from $[(A_0 - A_1)/A_0] \times 100$, where A₀ is the absorbance of the control, and A₁ is the absorbance of the extract/standard. The antioxidant activity of the extract was expressed as IC₅₀. All the tests were performed in triplicate and the graph was plotted with the average of three observations.¹⁰

Scavenging Of Hydrogen Peroxide (H₂O₂)

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (pH-7.4); various concentrations of extract or standard in methanol (1 ml) were added to 2 ml of hydrogen peroxide solution in PBS. After 10 minutes absorbance was measured at 230 nm.¹¹

Hydroxy Radical Scavenging Activity

The scavenging activity for hydroxyl radical was measured according to the modified method. The assay was performed by adding 0.1 ml of 10 mM FeCl₃, 0.1 ml of different dilutions of the extract (10-100 mg/ml) dissolved in distilled water, 0.3 ml of phosphate buffer (50 nM, Ph-7.4) and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37 for 1 h. A 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10 % TCA and 1.0 ml of 0.5 % TBA to

develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation and is calculated.¹²⁻¹⁵

Nitric Oxide Radical Inhibition Assay

The reaction mixture (6 ml) containing sodium nitroprusside (10mM, 4mL) and the extractor standard solution (1 ml) were incubated at 25 C for 150 minutes. After incubation, 0.5 ml of reaction mixture was removed, 1 ml of sulphinalic acid reagent (0.33 % in 20 % glacial acetic acid) was mixed and allowed to stand for 5 minutes for completion of diazotization reaction, 1 ml of naphthyl ethylene diamine dihydrochloride was added and the mixture was and mixture was allowed to stand for 30 minutes in diffused light. The absorbance is measured at 540 nm.¹³⁻¹⁵

Lipid Peroxidation Inhibitory Activity

Egg yolk was separated and washed with acetone until the yellow colour is removed the creamy white powder obtained was egg lecithin. Lipid peroxidation was induced by ferric chloride 10 ml (400 mM) and L-ascorbic acid 10 ml (400 mM) to a mixture containing egg lecithin (3 mg/ml) in phosphate buffer solution and different concentrations of the extracts (100 ml). After incubation of 1 h at 37 C the reaction was stopped by adding 2 ml of 0.25 N hydrochloric acid containing 0.375 %w/v thio barbituric acid, boiled for 15 minutes, cooled, centrifuged and absorbance of supernatant was measured at 532 nm.^{14,15}

Histopathological studies

The liver tissue was dissected out and fixed in 10 % formalin, dehydrated in gradual ethanol (50–100 %), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H–E) dye for photomicroscopic observation, including cell necrosis, fatty change, hyaline regeneration, ballooning degeneration.

Statistical analysis

The data are expressed as mean ± S.E.M. The difference among means has been analyzed by one-way ANOVA Dunnett's test.

RESULTS

Phytochemical study

All extracts subjected for phytochemical study showed the presence of alkaloids, proteins, amino acids, phenolic compounds, glycosides and flavonoids.

Acute toxicity studies

Ethanollic and aqueous extracts did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose.

Effects of extracts on AST, ALT, ALP and total bilirubin

The results of hepatoprotective effect of extracts on PCT-intoxicated rats are shown in Table 1. The elevated levels of serum AST, ALT, ALP, and total bilirubin were significantly reduced in the animals groups treated with various extracts. Treatment with ethanolic extract showed highly significant activity (P < 0.001) with maximum inhibition. So, the ethanol extract treated group was superior to the other extracts but not as effective as the silymarin.

Table 1: Effect of extracts of *F. mollis* on biochemical parameters of liver in rats

Group	Regimen (Dose)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Bilirubin (IU/L)
I	NORMAL	88.67 ± 1.085	64.83 ± 0.600	70.50 ± 0.763	0.246 ± 0.006
II	PARACETAMOL (25 mg/kg)	242.5 ± 2.349	290.5 ± 0.763	209.5 ± 0.75	0.958 ± 0.007
III	STANDARD (25 mg/kg)	105.5 ± 0.763**	85 ± 0.577**	84.50 ± 0.763**	0.295 ± 0.007**
IV	FM Extract (100 mg/kg)	204.7 ± 0.666	250 ± 0.577	179.8 ± 1.249	0.641 ± 0.006
V	FM Extract (200 mg/kg)	152.7 ± 0.763**	199.5 ± 0.666**	145 ± 0.577**	0.570 ± 0.005**
VI	FM Extract (400 mg/kg)	139.8 ± 0.600**	115.3 ± 0.881**	100.5 ± 0.763**	0.415 ± 0.016**

N = 6 animals in each group. **P < 0.001 when compared with Paracetamol. Values are expressed as mean

DPPH-scavenging activity

Table 2 illustrates a significant decrease in the concentration of DPPH radical due to scavenging ability of the extracts. The results indicate that ethanolic extract has the better

scavenging activity that was enhanced with increasing concentration. The IC₅₀ of ascorbic acid was found to be 29.6 ± 7.23 µg/mL.

Table 2: Effects of extracts on superoxide, H₂O₂, Nitric oxide, and lipid peroxidation scavenging levels

S.No	Concentration (µg/ml)	% Reduction in H ₂ O ₂ (%)	% Reduction in Lipid Peroxidation (%)	% Reduction in NO (%)	% Reduction in free radicals (%)	% Reduction in DPPH (%)	% Reduction in N.B.T (%)
1.	10	80.92	8.8	40.1	15	33.15	12.81
2.	20	83.55	16.11	50.3	25.6	43.41	24.82
3.	40	59.53	19.5	55.1	39.7	51.67	37.18
4.	60	77.49	21.7	65.2	48.71	65.58	50.11
5.	80	83.45	25.7	70.1	53.8	75.94	58.00
6.	100	72.65	29.3	80.01	61.5	86.61	74.11
		IC ₅₀ = 33.59 µg/ml	IC ₅₀ = 24.37 µg/ml	IC ₅₀ = 68.75 µg/ml	IC ₅₀ = 49.6 µg/ml	IC ₅₀ = 29.6 µg/ml.	IC ₅₀ = 53.8 µg/ml

Histopathological observations

Histology of the liver sections of the

Group 1: Normal = Normal architecture of liver tissue with mild congestion and sensitivity. Figure 1

Group 2: Paracetamol (3 g/kg) = Ballooning degeneration of hepatocytes with fatty liver tissue areas, indicating acute liver damage. Figure 2

Group 3: Standard treated (25 mg/Kg Silymarin) = mild peripheral necrosis, less percentage of liver damage in comparison with other groups. Figure 3

Group 4: Ethanolic extract of *F. mollis* (100 mg) = Ballooning degeneration in mid and peripheral zones, Cross linkage is observed. Figure 4

Group 5: Ethanolic extract of *F. mollis* (200 mg) = Two parenchyma are coming close. Figure 5

Group 6: Ethanolic extract of *F. mollis* (400 mg) = very less percentage of liver damage in comparison with all other groups. Figure 6

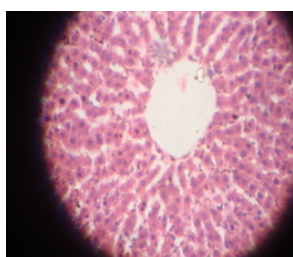


Figure 1: TS normal liver tissue

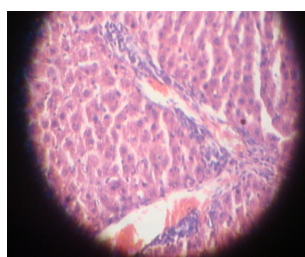


Figure 2: Paracetamol induced liver damage

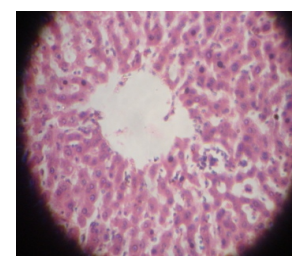


Figure 3: Standard (Silymarin) treated liver tissue

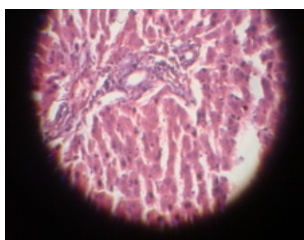


Figure 4: Ethanolic extract 100 mg of *F. mollis* treated liver tissue

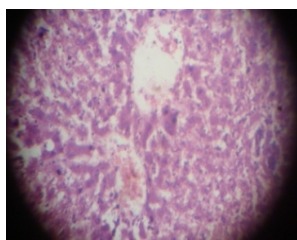


Figure 5: Ethanolic extract 200 mg of *F. mollis* treated liver tissue

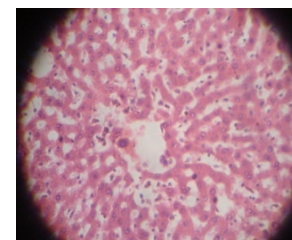


Figure 6: Ethanolic extract 400 mg of *F. mollis* treated liver tissue

DISCUSSION

The hepatotoxin is associated with changes at cellular levels that may lead to deterioration of organ functions. Therefore, any improvement in the treatment of hepatic function could be of potentially a great importance. The possible mechanism of herbal preparation as hepatoprotective agent against paracetamol could be by substantially decreasing lipid peroxidation through the elevation of MDA level in liver homogenate. Generally it is known that most of the paracetamol is excreted by conjugating with glucuronate and sulphate, while metabolized by cytochrome p-450 system to produce a highly toxic N acetyl- p- benzoquinone- imine (NAPQI) which is readily detoxified by enzymatic conjugation with hepatic glutathione (GSH). But, when the detoxification process is disturbed, an active agent NAPQI is produced which in turn binds covalently to tissue macromolecules thereby causing severe hepatic damage. Oral administration extract of alcoholic extract of *Ficus mollis* was standardized, and has a significant hepatoprotective activity in preventive treatments against hepatotoxins induced hepatic damage and comparative normalization of serum enzymes against only Hepatotoxin administered, strongly points out the possibility of *Ficus mollis* being able to condition the hepatocytes so as to protect the parenchymal cells¹⁵.

CONCLUSION

Our aim was to develop Hepatoprotective preparation which could be safe with no interactions and beneficial in hepatoprotection, biochemical studies revealed a dose dependent significant fall in the levels of SGOT, SGPT, ALP, Bilirubin, an increase in the weight of liver in case of seed extract treated animals against paracetamol induced hepatotoxicity. Histopathological studies supplemented the findings by showing mild hepatic degeneration with absence of necrosis in comparison with the model control. Thus indicating the prominent significance of *Ficus mollis* in hepatoprotection against paracetamol induced hepatotoxicity.

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Cite this article as:

C. Hari Kumar, A. Ramesh, G. Krishna Mohan. Hepatoprotective effect of ethanolic extract of *Ficus mollis* on paracetamol induced liver damage in albino rats. Int. Res. J. Pharm. 2014; 5(6):485-488 <http://dx.doi.org/10.7897/2230-8407.0506100>

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