Journal of Scientific and Innovative Research 2013; 2(6): 1111-1115
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2013; 2(6): 1111-1115

Received: 23-10-2013
Accepted: 07-12-2013

Ashok Kumar Gupta
Department of Pharmacology, College of Pharmacy, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh-244001, India

Mansi Verma
Assistant Professor, Department of Pharmacology, College of Pharmacy, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh-244001, India

Sawan Kumar
Department of Pharmacology, College of Pharmacy, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh-244001, India

Correspondence:
Ashok Kumar Gupta
Department of Pharmacology, College of Pharmacy, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh-244001, India

Ficus infectoria shows protective effect against paracetamol induced liver damage in rats

Ashok Kumar Gupta, Mansi Verma, Sawan Kumar*

Abstract

Ficus infectoria (*F. infectoria*) is a plant species that belongs to the Moraceae family. The present study is aimed to determining the hepatoprotective effect of methanolic extract of *F. infectoria* leaves and bark using paracetamol induced liver injury in rats. Rats were divided into five groups and received 1% CMC (1 ml/kg, normal group and toxic), 100 mg/kg of silymarin (standard group) and plant extract (100, 220, and 400 mg/kg) orally once daily for 7 days. Hepatotoxic induction performed using single dose of 2 g/kg paracetamol (PCM) except normal group on day 5th.On day 8th, blood and liver tissues were collected for the estimation of biochemical parameters and histological observation. The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Bonferroni Multiple Comparisons Test. A value of $P<0.05$ was considered statistically significant. Paracetamol increases the serum levels of SGOT, SGPT, Bilirubin (total and direct), ALP and decrease in serum levels of GSH. Treatment with Silymarin and different dose of methanolic extract of *F. infectoria* altered levels of biochemical parameters and showed significant hepatoprotective activity. From the microscopial analysis, sign of necrosis was found to be present in Paracetamol (PCM) treated group whereas maintenance of hepatic structure was observed in silymarin and plant extract treated. In conclusion, methanolic extract of *F. infecrtoria* exerts shows potential effects against paracetamol induced liver injury in rats. Thus, plant may be used as a safe and effective alternative for the management of liver disease.

Keywords: *Ficus infectoria*, Liver damage, Paracetamol, Silymarin.

1. Introduction

Medicinal plants are gaining importance in the fields of research. Medicinal plants originate from almost every part of the globe. Such plants serve the primary healthcare needs of up to 80 % of people in developing countries where there is increasing awareness of and demand for medicinal plants for healthcare and dietary supplements that often help to save lives.[1] *Ficus infectoria* is one of such medicinal plant which is used in different illness conditions.

Ficus infectoria (*F. infectoria*) belongs to the Family Moraceae. Its common name is White Fig. It is locally known as Pilkhan. It is a large spreading tree, with occasional aerial root, found throughout the plains and lower hills. They are also found in Bangladesh, Nepal, Pakistan, Sri Lanka, Southwest China & Indochina.[2, 3] The stem bark contains Methyl ricinolate, Caffeic acid, Bergenin, β-sitosterol, lanosterol. Also contains fructose, sucrose, bergapten, bergaptol and flavonoids.[4] Decoction's of bark is used for washing ulcers, as a gargle in salivation; also used for menstrual disorders and leucorrhoea.[2] The bergapten and bergaptol isolated from the extract had shown antibacterial and antifungal activities.[5] Methanolic extract of *F. infectoria* possessed hyperglycemic properties in diabetic conditions.[6]

Paracetamol is a mild analgesic and antipyretic agent which is safe and effective when taken in low doses. Ingestion of high doses leads to acute liver failure accompanied by centri-lobular degeneration and necrosis in the liver of both man and experimental animals. Toxicity of paracetamol is thought to be produce by N-acetyl-p-benzoquinineimine, a reactive electrophilic metabolite of a cytochrome P-450 mediated reaction.[7]

The present study is designed to investigate the hepatoprotective effect of the methanolic leaf and bark (mixture) extract of *F. infectoria*.

2. Materials and Methods

2.1 Collection of Plant material-

The fresh leaves and bark of *F. infectoria* plant were collected in the month of October from Sultanpur district, Uttar Pradesh, India. The plant material was authenticated by Dr. Tariq Husain (Head & Scientist, Biodiversity & Angiosperm Taxonomy), National Botanical Research Institute (NBRI) Lucknow, India (Accession No. 097837). A specimen sample of the same was preserved in the herbarium section of the College of Pharmacy, Theerthanker Mahaveer University, Moradabad for further reference.

2.2 Preparation of plant extracts-

The leaves and bark of *F. infectoria* were cleaned and shade dried in open air for 8-10 days then pulverized to dry power using electric grinder. About 80 gm of the dried leaf and bark powder (mixture) was extracted with hot solvents of methanol for 24 hours with each solvent, using the Soxhlet apparatus at a temperature of 30 to 35˚C. The extract was concentrated by vacuum rotary evaporator and stored in a refrigerator at 4˚C.

2.3 Preliminary phytochemical screening-

A portion of freshly prepared methanolic extract was subjected to various phytochemical analysis to test the presence or absence of phytoconstituents such ascarbohydrate, glycoside, alkaloid, protein, amino acid, phytosterol, tannin & flavonoids etc.[8, 9]

2.4 Chemicals and drugs-

The chemicals and drug used were Paracetamol tablets IP 500mg (Glaxo Smith Kline,India),Silymarin (Microlabes limited, India), Carboxy Methyl Cellulose, CMC (Loba Chemie, Mumbai),Methanol (Rankem, New Delhi) and ERBA diagnostics kit (ERBA diagnostics Mannheim GmbH, Germany).All the Chemicals and drugs used in this study were analyticaland pharmaceutical grade.

2.5 Maintenance of animals and approval of protocol-

30 Wistar albino rats of either sex weighing between 150 and 200 g were used in this study. These rats were procured from the Central Animal House Facility, Teerthanker Mahaveer University, Moradabad. They were housed in well ventilated stainless-steel cages at room temperature (24 ± 2) ºC in hygienic condition under natural light and dark schedule and were fed on standard laboratory diet. Food and water were given *ad libitium*. Permission for the use of animal and animal protocol was obtained from the Institutional Animal Ethical Committee (IAEC) of Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg. No. 1205/c/08/CPCSEA, Dated:- 21/4/2008).

2.6 Paracetamol-induced hepatotoxicity-

The protective activity against Paracetamol induced hepatotoxicity of *F. infectoria* leaf and bark methanolic extract was determined by the method as reported by Tmwer *et.al*.[10] Hepatotoxicity was induced by orally administered paracetamol (2g/kg orally). The animals were divided into five groups of 6 animals each. Group I served as control group received vehicle (1% CMC orally) for 7 days. Group II served as toxic control received 1% CMC (1ml/kg orally) for 7 days and administration of Paracetamol (2g/kg orally) on 5th day. Group III served as standard received Silymarin at a dose of 100 mg/kg., once a day for 7 days and administration of Paracetamol (2g/kg orally) on 5th day. Group IV served as test-1 received leaf and bark methanolic extract at a dose of 200 mg/kg., once a day for 7 days and administration of paracetamol (2g/kg orally) on 5th day. Group V served as test-2 received leaf and bark methanolic extract at a dose of 400 mg/kg., once a day for 7 days and administration of paracetamol (2g/kg orally) on 5th day.

At the end of experiment, on the 7th day after 2 hour of respective treatments all animals were anaesthetized and blood was obtained from retro-orbital plexus using fine glass capillary and collected in plain sterile microcentrifuge tubes for biochemical estimations. The blood serum was separated by centrifugation at 7000 rpm for15 min.

2.7 Biochemical parameters-

Serum collected was assayed for different biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT)[11], serum glutamate pyruvate transaminase (SGPT)[11], serum alkaline phosphates (ALP)[12], bilirubin (total & direct)[13], and liver glutathione[14] were determined through standard method and by using commercially available kits (ERBA diagnostics Mannheim GmbH, Germany).

2.8 Histopathological assessment-

The animals under ether anaesthesia were sacrificed on 7th day and all the liver separated, washed with 0.9% saline and collected in 10% formalin solution for histopathological studies.[15]

2.9 Statistical analysis-

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Bonferroni Multiple Comparisons Test by using Graph Pad InStat (File version 3.0.10.0). The values were expressed as mean ± Standard Error Mean (SEM) for six rats in each group and $P<0.05$ were considered significant.

3. Results

3.1 Phytochemical screening-

Preliminary phytochemical studied showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponin and phytosterols in methanolic extract of *F. infectoria* (Table 1).

3.2 Hepatoprotective activity-

PCM treated rats showed significant increase in the serum levels of SGOT, SGPT, Bilirubin (total and direct), ALP and decrease in serum levels of GSH (Figure 1, Figure 2 and Table 2).

Pre-treatment with methanolic *F. infectoria* extract restored the depleted GSH (6.33 ± 0.42 at 200mg/kg and 7.05 ± 0.21 at 400mg/kg) concentration near normalcy and brought down the elevated levels of SGOT (73.81 ± 1.78 at 200mg/kg and 62.31 ± 3.09 at 400mg/kg), SGPT (83.52 ± 0.61 at 200mg/kg and 61.94 ± 1.00 at 400mg/kg), ALP (74.63 ± 1.42 at 200mg/kg and 7.05 ± 0.21 at 400mg/kg) and Bilirubin (2.42 ± 0.09 at 200mg/kg and 2.13 ± 0.06 at 400mg/kg for Direct bilirubin and 2.51 ± 0.15 at 200mg/kg and 2.41 ± 0.08 at 400mg/kg for Total bilirubin). The treatment with Silymarin significantly reduced the SGOT, SGPT, Bilirubin (total and direct), ALP and increased the levels of GSH while extract of *F. infectoria* plant also showed significant hepatoprotective activity.

Table 1: Phytochemical constituents of methanol extract of *F. infectoria*.

Phytochemical constituents	Extract (9.60 % w/w)
Alkaloids	+++
Carbohydrates	++
Flavonoids	+++
Glycosides	++
Tannins	+
Proteins	+
Saponin	++
Phytosterols	++

Value in parenthesis is the extractive yield. + = slight presence; ++ = medium presence; +++ = heavy presence.

Table 2: Effect of the methanolic extract (leaf and bark mixture) of *F. infectoria* on different biological parameters.

Group	Dose	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	GSH (IU/L)	Direct Bilirubin mg/dl	Total Bilirubin mg/dl
Normal Control	-	46.10 ±1.34	48.19 ± 0.63	52.10 ± 1.44	8.32 ± 0.50	1.06 ± 0.05	1.52 ± 0.02
PCM Only	2 g/kg	115.4 ± 2.85	124.7 ± 1.02	83.13 ± 1.51	5.20 ± 0.41	3.42 ± 0.10	6.41 ± 0.09
Silymarin + PCM	100 mg/kg	54.28 ± 1.19***	56.00 ± 1.09***	55.44 ± 1.23***	7.73 ± 0.22***	1.76 ± 0.03***	1.89 ± 0.02***
Extract + PCM	200 mg/kg	73.81 ± 1.78***	83.52 ± 0.61**	74.63 ± 1.42**	6.33 ± 0.42ns	2.42 ± 0.09***	2.51 ± 0.15**
Extract + PCM	400 mg/kg	62.31 ± 3.09***	61.94 ± 1.00***	63.04 ± 1.13***	7.05 ± 0.21**	2.13 ± 0.06***	2.41 ± 0.08***

Results expressed as mean ± SEM and ***P<0.001 as compared to toxic control, **P<0.01 compared to toxic control & ns (non-significant) as compared to toxic control

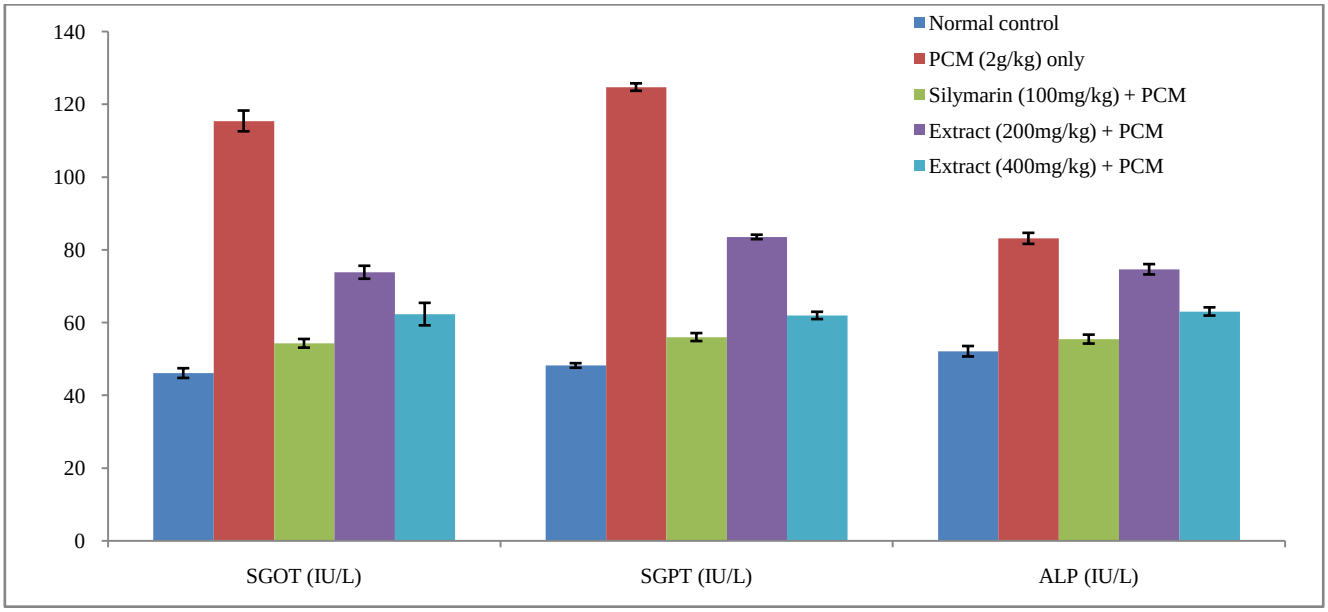

Figure 1: Graph showing variation in different parameter levels

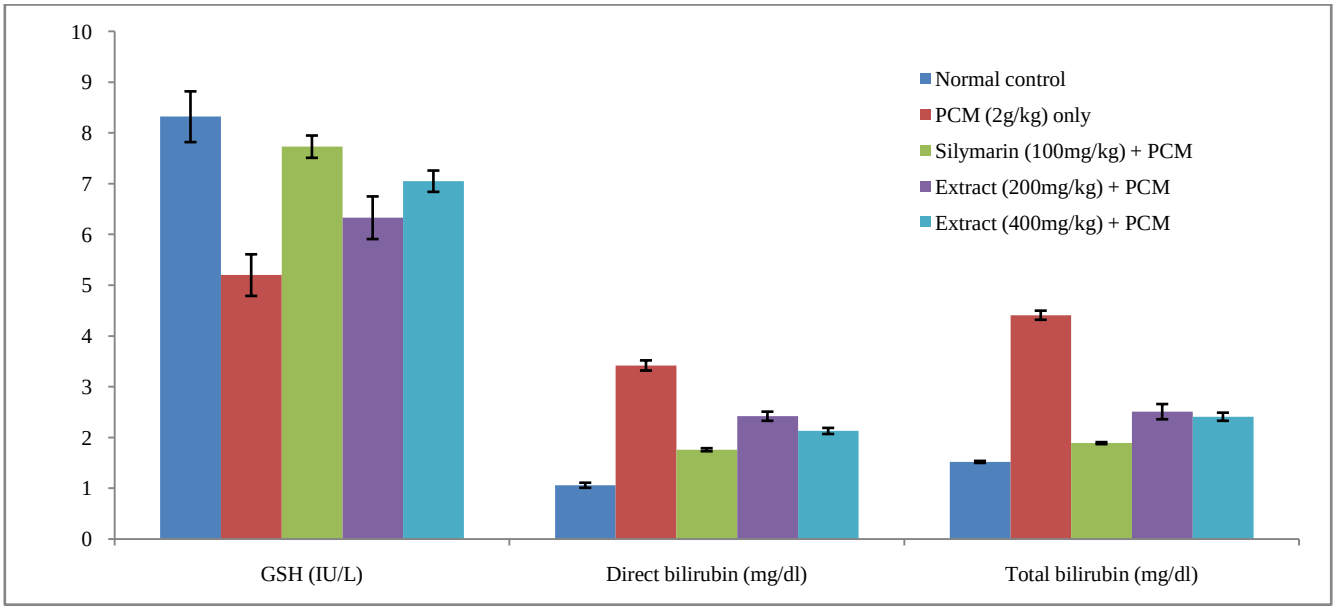

Figure 2: Graph showing variation in different parameter levels

3.3 Histopathological Study-

Histopathological assessment of the paracetamol induced liver toxicity pre-treated with plant extract exhibited strong connection with serum biochemical parameters. Histological observations of the liver tissue of the normal animals showed Hepatocytes (A), Sinusoid (B), Kupffer Cell (C), Portal Vein (D) and Hepatic Artery (E) (Figure 3). The liver sections of normal animals rats did not show any histological changes in the hepatocytes. There is no change in the normal architecture. Sign of necrosis were found to be present in Paracetamol (PCM) treated group. Cell swelling, congestion and degeneration were also found to be present in paracetamol (PCM) treated group. In Silymarin standard treated group sign of cellular damage decreased significantly. No necrosis was observed. In extract treated group, (200 mg/kg) the level of protection was found to be less significant as compared to toxic treated group. In extract treated group, (400 mg/kg) the level of protection was found to be more significant as compared to toxic group.

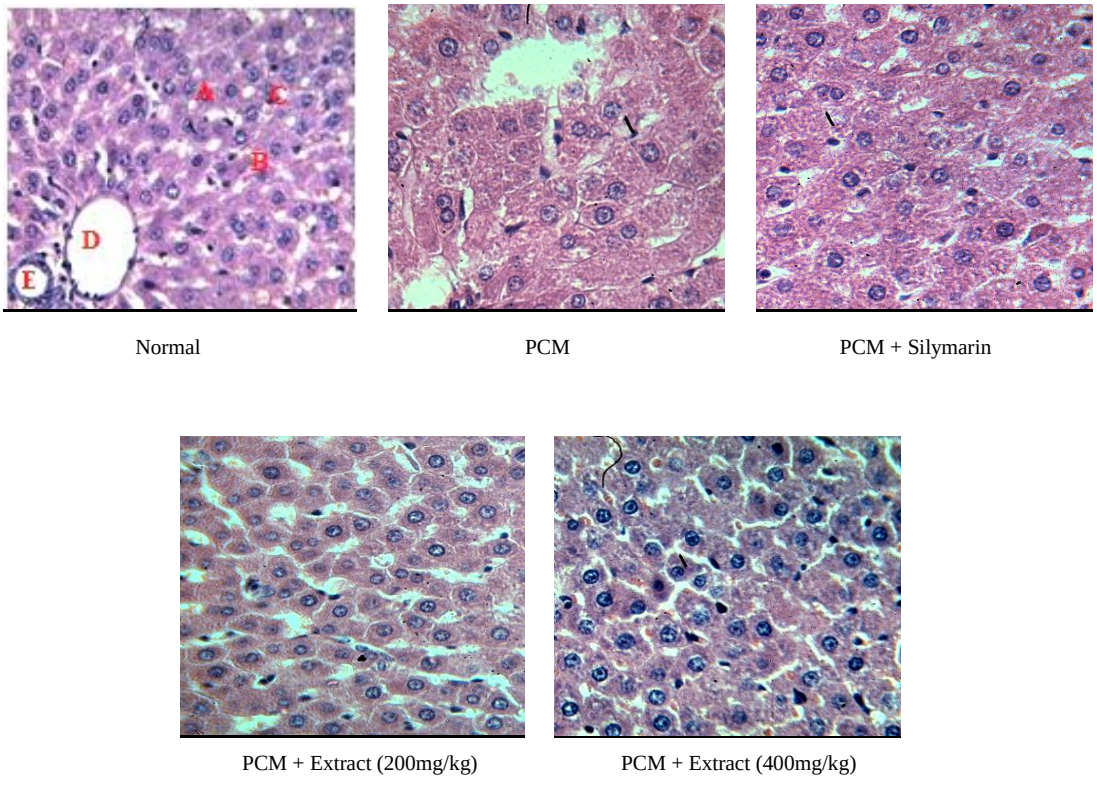

Figure 3: Histological observations of the liver tissue

4. Discussion

It is well documented that PCM are biotransformed under the action of microsomal cytochrome P-450 of liver to reactive metabolites.[16] Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity. So in the present study, paracetamol was employed as toxic agent and the protective effect of *F. infectoria* against the paracetamol induced hepatotoxicity was studied. The extent of toxicity was estimated by histopathological studies and biochemical enzyme markers like SGOT, SGPT, Bilirubin (total and direct), ALP and GSH.

Hepatocytes are the main component that regulates various metabolic activities of liver. Distortion of this organ will result in disorder of body metabolism. The enzyme cytochrome P450 metabolizes paracetamol, forming a important alkylating metabolite known as N-acetyl-p-benzoquinone imine (NAPQI).[17] NAPQI is then irreversibly conjugated with the sulfhydryl groups of glutathione.[18] NAPQI responsible for the depletion of glutathione and initiates covalent binding to cellular proteins that cause the dysfunction and death of hepatocytes, leading to liver necrosis.[19]

The liver sections of the rats treated with PCM intoxicated groups showed hepatic cells with severe injury characterzised by inflammatory infilteration and necrosis in many areas. Thus, it clearly states that, toxicity is due to either of the above mechanisms such as depletion of glutathione store or free radical generation or lipid peroxidation.[18] Pretreatment with silymarin and extract edxhibited significant liver damage, which is evident by the presence of more and less normal hepatocytes and reduced inflammatory infilteration and necrosis.

In our experiments it is observed that tissue GSH levels in the paracetamol group is decreased. This clearly indicates that there is a significant hepatic damage due to paracetamol. This is further evident from the fact that there is elevation in the levels of various biochemical markers of hepatic damage like SGPT, SGOT, bilirubin, and ALP. Treatment with Silymarin and *F. infectoria* has increased tissue GSH level and the elevated levels of above mentioned biochemical markers to the near healthy levels. The treatment has also demonstrated the reduced hepatic damage.

Preliminary phytochemical studies reveal the presence of Flavonoides in methanolic extract of *F. infectoria*. Flavonoides are hepatoprotectives.[20] The observed hepatoprotective activity of *F. infectoria* may be attributed to the presence of flavonoids. From the above preliminary study, we conclude that the methanolic extract of leaf and bark (mixture) of *F. infectoria*, is proved to be one of the herbal remedies for liver ailment.

5. Conclusion

It can be concluded that the data obtained in the present study suggests that the methanolic extract of leaf and bark of *F. infectoria* has significant hepatoprotective activity against PCM induced hepatic damage in rats. It is also needed further research to isolate the compound and exact mechanism responsible for hepatoprotective activity of the plant.

Conflict of interest statement

We declare that there is no conflict of interest.

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