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Hepatoprotective Activity of *Ficus religiosa* Leaf Extract in Rats

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ABSTRACT

Ficus religiosa was investigated for its possible protective effect against paracetamol and CCl_4-induced hepatic damage. IV administration of a sub-lethal dose of paracetamol (500 mg/kg) produced liver damage in rats as manifested by the rise in serum level of transaminases (AST and ALT). Ttreatment of rats with *Ficus religiosa* (200 mg/kg) prevented the paracetamol-induced rise in serum enzymes. The hepatotoxic dose of CCl_4 (1.5 ml/kg; orally) also raised the serum AST and ALT levels. The same dose of *Ficus religiosa* (200 mg/kg) was able to prevent the CCl_4-induced rise in serum enzymes. These results indicate that *Ficus religiosa* possesses hepatoprotective activity.

Key words: *Ficus religiosa*, paracetamol, CCl_4, Hepatoprotective.

1. INTRODUCTION

Liver is the most important organ in the body. It plays a pivotal role in regulating various physiological processes. It is also involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles. It helps in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. In addition, it aids metabolism of carbohydrate, protein and fat, detoxification, secretion of bile and storage of vitamins. The role played by this organ in the removal of substances from the portal circulation makes it susceptible to first and persistent attack by offending foreign compounds, culminating in liver dysfunction.

Liver diseases remain one of the major threats to public health and are a worldwide problem. They are mainly caused by chemicals like acetaminophen (in large doses), excess consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages[1,2]. Acetaminophen, a mild analgesic and antipyretic drug, developed in the last century, causes serious liver necrosis in humans and in experimental animals if taken in large doses[3,4]. While alcohol is one of the main causes of end stage liver disease worldwide, alcoholic liver disease is the second most common reason for liver transplantation in the United States[5]. Due to increased frequency of drinking and change of diet construction, such as the increase of fat content, the incidence of liver diseases has increased in China, becoming another important risk factor for morbidity and mortality in addition to viral hepatitis. The spectrum of alcoholic liver disease ranges from fatty liver to alcoholic hepatitis and ultimately fibrosis and cirrhosis.

A large number of medicinal plants, natural products and dietary components have been evaluated as potential nephroprotective agents. The *Ficus religiosa* **(**family-Moraceae) is widely planted in the tropics. The tree is very long lived and one tree near Bombay is reported to be over 3,000 years old. The barks of *Ficus religiosa* species contains tannin, saponin gluanol acetate, *β*sitosterol, leucopelargonidin– 3 – O – *β* – D -glucopyranoside, leucopelargonidin – 3 – O – *α* –L - rhamnopyranoside, lupeol, ceryl behenate, lupeol acetate, *α*-amyrin acetate, leucoanthocyanidin, and leucoanthocyanin[6]. Some reported pharmacological activity of *F. religiosa* like fruit extracts exhibited antitumor activity in the potato disc bioassay[7,8]. Aqueous extract was decreased the fasting blood glucose and exaggerated activity of superoxide dismutase SOD in streptozotocin induced type II diabetic rats[9], anthelmintic activity of the methenolic extract[10-12].

2. MATERIALS AND METHODS

Wistar rats weighing 130-165g were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) under 12 h light/dark cycle and controlled temperature (24 ± 2°C) and fed with commercial pellet diet and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least one week before the commencement of experiment. The experimental protocol was approved by the Institutional Animal Ethical Committee,Truba institute of Pharmacy, Bhopal Madhya Pradesh, India.

2.1 Collection & Authentification of plant

The plant material was collected from local area of Bhopal in March 2011 and was authenticated at the Department of Botany, Hari Singh Gour University Sagar. A voucher specimen number or the herbarium number was Bot/Her/Bl763 has been deposited.

2.2 Preparation of the extract

The preparation of extract was carried out according to the published method [13]. Briefly, the stem bark of *F. religiosa* was shade dried and powdered. Approximately 0.95 kg of powdered drug material was extracted using 99% pure ethanol in the ratio of 1:2 (w/v) in air tight container. The extract obtained (EBFR) was dried in a steam bath and the dried mass was weighed and recorded. The percentage of yield was calculated. The weight of dried crude extract obtained was approximately 0.16 g which commemorated with the percentage yield of 17.16%.

2.3 Phytochemical Screening

Standard phytochemical methods were used to test for the presence of saponins, alkaloids, tannins, anthraquinones, cardiac glycosides, cyanogenetic glycosides, amino acid & protein and flavonoids.

2.4 Animal treatment in CCl_4 induced Hepatotoxicity

Rats were divided into five groups of six animals in each group. Table 1 shows the dose schedule of carbon tetrachloride and test samples against CCl_4 intoxication.

Group 1 and 2 was treated for seven days with 2٪ accasia, group 3rd treated with silymarin and group 4th treated with aqous extract and group 5th treated with methanolic extact. On seventh day 30 min after dosing group 2nd,3rd,4th,5th was treated with CCl_4 1.5ml/kg (1:1 ccl_4 in olive oil) orally. The animals were sacrificed after 36 h after administration of acute dose of CCl_4. The blood was collected by carotid artery. Biochemical parameter aspartate aminotransferase (AST), alanine aminotransferase (ALT) were tested in Awasti Diagnostic Center, Bhopal using Span diagnostic kits.

2.5 Animal treatment in paracetamol induced hepatotoxicity

This model is used to produce experimental liver damage only, since rat are resistant to paracetamol induced hepatotoxicity. Paracetamol administered orally as a single dose of 500mg/kg in rate produce hepatotoxicity. Paracetamol is administered as a single dose of 500mg/kg. After 48hrs, they treated with the test drug for 7th days. At the end of experiment the blood was collected from carotid artery. Biochemical parameter aspartate aminotransferase (AST), alanine aminotransferase (ALT) were tested in Awasti Diagnostic Center, Bhopal using Span diagnostic kits.

3. RESULTS AND DISCUSSION

Liver is the main organ responsible for the biosynthesis, uptake & degradation of protein and enzyme. Liver function may therefore be reflected to some extent on the level and/ or the activities of these biochemical compounds. The immune system is increasingly found to be involved in the development of several chronic illness, for which allopathic medicines has provided limited tools for treatment & especially prevention.

It is well established that CCl_4 induces hepatotoxicity by metabolic activation; therefore it selectively causes toxicity in liver cells maintaining semi normal metabolic function. CCl_4 is biotransformed by cytochrome P450 system in the endoplasmic

Table 1: Groups & Doses schedule for CCl_4 (Carbon tetrechloride) induced hepatotoxicity

Sl. No.	Group	Treatment
1	I	Control received 2% acacia for 7 days (o.d.)
2	II	2% acacia for 7 days + ccl_4 1.5ml/kg (1:1 ccl_4 in olive oil) orally
3	III	Silymarin (100mg/kg/day,P.O.) 7 days +CCl_4 on 7 day
4	IV	Aq. Extract (200mg/kg/day) fine suspension of 2% acacia + CCl_4 on 7 day
5	V	Methanol Extract (200mg/kg/day) fine suspension of 2% acacia + CCl_4 on 7 day

Table 2: Groups & Doses schedule for Paracetamol induced hepatotoxicity

Sl. No.	Group	Treatment
1	I	Control received 2% acacia for 5 days (o.d.)
2	II	2% acacia for 5 days + Paracetamol (500mg/kg) for one day only
3	III	Silymarin (100mg/kg/day,P.O.) 5 days + Paracetamol (500mg/kg) for one day only
4	IV	Aq. Extract (200mg/kg/day) fine suspension of 2% acacia + Paracetamol (500mg/kg) for one day only
5	V	Methanol Extract (200mg/kg/day) fine suspension of 2% acacia + Paracetamol (500mg/kg) for one day only

Table 3: Effect of *ficus religiosa* on serum biochemical parameter in Paracetomol & CCl_4 induced hepatic toxicity in rat

Group	Dose	SGOT(U/L)	SGPT(U/L)
Group 1 (vehicle)	1ml/kg	98.5±4.7	72.86±6.19
Group 2 (CCl_4)	1.5ml/kg	141.13±9.30	119.30±17.2
Group 3 (std.+ CCl_4)	100mg/kg	73.65±3.10**	57.22±2.81**
Group 4 (aqs.+ CCl_4)	200mg/kg	115±5.87*	98.00±8.9**
Group 5 (mtoh.+ CCl_4)	200mg/kg	103.89±5.7**	77.32±10.7**

Groups from II to V received CCl_4 30 min after treatment on 7th day.

The data obtained were analyzed by one way ANOVA followed by Dunnett Multiple Comparisons Test. Each value represent the mean ± SEM; n=6. **p< 0.01*p< 0.05,vs. Group II.

Table 4: Effect of *ficus religiosa* on serum biochemical parameter in Paracetamol induced hepatotoxicity

Group	Dose	SGOT(U/L)	SGPT(U/L)
Group 1 (vehicle)	1ml/kg	98.5±4.7	72.86±6.19
Group 2 (pcm)	500mg/kg	133.2±3.2	111.31±8.7
Group 3 (std.+ pcm)	100mg/kg	69.76±2.9**	59.65±3.25**
Group 4 (aqs.+ pcm)	200mg/kg	119.15±4.39*	95.11±5.71^{ns}
Group 5 (mtoh.+ pcm)	200mg/kg	111.3±3.1**	79.88±4.5**

Groups from II to V received PCM 48 hrs before treatment on day.

The data obtained were analyzed by one way ANOVA followed by Dunnett Multiple Comparisons Tests. Each values represent the mean ± SEM; n=6. **p< 0.01*p< 0.05, ns p> 0.05 vs. Group II.

reticulum to produce trichloromethyl free radical (CCl_3). Trichloromethyl free radical when combined with cellular lipids and proteins in the presence of oxygen form trichloromethylperoxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethylperoxyl free radical leads to elicit lipid peroxidation. The destruction of Ca2+ homeostasis, finally results in cell death. Hepatoprotective activity of any drug is the ability of its constituents to inhibit the aromatase activity of cytochrome P450 thereby favoring liver regeneration. In the present study it was observed that the administration of CCl_4 decreased the levels of proteins and increased the levels of serum marker enzymes significantly (P<0.001) which is an evidence of existence of liver toxicity when compared to normal animals.

Paracetamol is a commonly and widely used analgesic antipyretic agent. Hepatotoxic doses of paracetamol deplete the normal levels of hepatic glutathione. The hepatic cytochrome P450 enzyme system metabolizes paracetamol, forming a minor yet significant alkylating metabolite known as NAPQI (*N*-acetyl-*p*-benzo-quinone imine). NAPQI is then irreversibly conjugated with the sulfhydryl groups of glutathione. NAPQI is primarily responsible for the toxic effects of paracetamol.

Production of NAPQI is primarily due to, two isoenzymes of cytochrome P450: CYP2E1 and CYP1A2. The P450 gene is highly polymorphic, however, and individual differences in paracetamol toxicity were believed to be due to a third isoenzyme, CYP2D6. CYP2D6 metabolises paracetamol into NAPQI to a lesser extent than other P450 enzymes, its activity may contribute to paracetamol toxicity in extensive and ultrarapid metabolisers, and when paracetamol is taken at very large doses. In the liver, the cytochrome P450 enzymes CYP2E1 and CYP3A4 were primarily responsible for the conversion of paracetamol to NAPQI which undergoes conjugation with glutathione. Conjugation depletes glutathione, a natural antioxidant. This in combination with direct cellular injury by NAPQI, leads to cell damage and death. Excess production of paracetamol metabolite causes the initial hepatic damage and subsequent activation of inflammatory mediator TNF-α which in turn contribute to tissue necrosis.

4. CONCLUSION

In the present study, it was seen that administered of ccl_4 & paracetamol elevates the level of serum marker enzymes SGPT, SGOT, ethanolic extract of *ficus religiosa* & silymarin treated group exhibited lower level of SGPT, SGOT level in ccl_4 & paracetamol treated group. The stabilization of serum SGPT, SGOT levels by *ficus religiosa* is a indication of the improvement of the functional status of liver cells.

The biochemical examination clearly level that the hepatic cells are normal in ethanolic extract of ficus religiosa treated group (200mg/kg p.o.) in contrast group which received CCl_4 & paracetamol. Thus ethanolic extract of *ficus religiosa* can be considered to be an effective hepatoprotective in nature, as it normalize the damage caused by CCl_4 to hepatic function. Thus the ethanolic extract of *ficus religiosa* seems to possess hepatoprotective activity.

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