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Original Article

# Hepatoprotective Activity of Methanolic Extract of *Flacourtia sepiaria* ROXB against D-Galactosamine Induced Oxidative Stress in Rats

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ABSTRACT

D-galactosamine-induced liver damage in rats is recognized to be similar to viral hepatitis in humans from both morphological and functional points of view. Galactosamine has considerable liver specificity because hepatocytes have high levels of galactokinase and galactose-1-P-uridylyltransferase while other organs do not. *Flacourtia sepiaria* (F. sepiaria) belonging to the family Flacourtiaceae is a medium sized tree widely distributed in the dry jungles of Bengal, Bihar, Orissa and all districts of the Madras presidency. The plant has been used extensively in the traditional medicine as hepatoprotective. In the present study, Methanolic Extract of *Flacourtia sepiaria* (MEFS) exhibited significant hepatoprotective activity, afforded protection Galactosamine-induced liver damage, which could be at least partly attributed to free radical scavenging activity of tannins and antioxidants in the extract.

**KEYWORDS:** - Hepatoprotective, D-Glucosamine, *Flacourtia sepiaria.*

## 1. INTRODUCTION

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification of the exogenous and endogenous challenges like xenobiotics, drugs, viral infections and chronic alcoholism. Diverse homeostatic mechanisms are affected if liver function is impaired, with potentially serious consequences. About 20,000 deaths occur every year due to liver diseases.

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Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2,50,000 new cases each year. Although viruses are among the important cause of liver diseases, excessive drug therapy, environmental pollution and alcoholic intoxication are not uncommon [1].

D-galactosamine-induced liver damage in rats is recognized to be much like viral hepatitis in humans from both morphological and functional points of view [2]. Galactosamine has great liver specificity because the hepatocytes have high levels of galactokinase and galactose-1-P-uridylyltransferase, while other organs are not affected [3]. Galactosamine causes liver cell injury, with spotty hepatocytes necrosis and prominent portal and parenchyma inflammation [4].

Galactosamine also depletes uridine diphosphate (UDP) by increasing formation of UDP-sugar derivatives, which results in inhibition of RNA and protein synthesis, leading to deterioration of the cell membranes [5, 6]. The possible hepatoprotective mechanisms of (MEFS) extract of Flacourtia sepiaria roxb have not been reported yet. Therefore, in the present study, hepatoprotective effects and possible mechanisms of protection by Flacourtia sepiaria Roxb were examined on the galactosamine-treated rat.

*Flacourtia sepiaria* (F.sepiaria) belonging to the family Flacourtiaceae is a medium sized tree widely distributed in the dry jungles of Bengal, Bihar, Orissa and all districts of the Madras presidency. Various parts are widely used in folk medicine; an infusion of the leaves is given in case of snake bites and its bark triturated with sesame oil is used as a liniment in rheumatism and gout. The ashes of root are also given in kidney diseases and have also been proved to possess anti microbial activity [7, 8]. Xanthine oxidase inhibitory activity has been reported for the aerial parts [9]. However no report regarding hepatoprotective activities of the F.sepiaria is available in the literature. Thus in the light of knowledge that F. sepiaria is widely used in folklore, we intend to evaluate the hepatoprotective activities of the MEFS.

## 2. MATERIALS AND METHODS

**PLANT MATERIAL**

Plant material was collected from hilly regions of Kerala, India. The plant was botanically identified by Dr.V.Chelladurai, Research Officer Botany, (Rtd) CCRAS, Government of India. A voucher Specimen has been kept in the Department of chemistry (NCP/CH/PS/FS01), National College of Pharmacy, Calicut.

**Extraction**

The aerial parts of F.sepiaria were collected, shade dried for 3 weeks, powdered mechanically and sieved through No. 20 mesh sieve. About 800g of the powdered aerial part was first defatted with petroleum ether (PEF, 60º-80ºC) and then consecutively extracted with ethyl acetate (EAEF) and methanol (MEF) by soxhlet extraction (order of increasing polarity). The crude extracts were concentrated by using rotary vacuum evaporator and dried at room temperature. The extract obtained with each solvent was weighed and the percentage yield was calculated in terms of dried weight of the plant material. The percentage yield of methanolic extract was 8.8%W/W.

**CHEMICALS AND INSTRUMENTS:-**

All the materials used for this experiment were of analytical grade. The chemicals used were manufactured by Sigma Chemical Co. Diagnostic kits for the estimations were manufactured by Ranbaxy Diagnostics Ltd., New Delhi, India. Standard or gastric cannula was used for oral drug administration.

**Acute Toxicity studies**:-

The acute toxicity for 70% MEFS was determined on male wistar rats, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose method of OECD guideline No.420 given by CPCSEA was adopted for toxicity studies, selection and acclimatization of animals [10]. Albino rats of wistar strains weighing between 180-220gm were selected from animal experimental laboratory, and used throughout the study. They were housed in micro nylon boxes in a control environment (temp $25\pm2^0$C) and 12 hrs dark/light cycle with standard laboratory diet and water ad libitum. The study was conducted after obtaining institutional animal ethical committee clearance. As per the standard practice, the rat were segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They were fed on healthy diet and maintained in hygiene environment in our animal house.

**METHODOLOGY** [11, 12]

**Treatment Protocol** The acclimatized animals were divided into 5 groups of each 6 animals, designated as

**Group 1:** normal control and receive normal diet and water.

**Group2:** control received 400mg/kg IP D galactosamine for 21 days (400mg/kg)

**Group 3:** Standard control received 25mg/kg of silymarin orally for 21 Days (25mg/kg).

**Group 4:** Served as a treatment control group and was administered MEFS at a dose of 200mg/kg through orally for 21days

**Group 5:** Served as a treatment control group and was administered MEFS at a dose of 400mg/kg through orally for 21 days.

## 3. RESULTS AND DISCUSSION

**Biochemical analysis** [13, 14]

On day 22 after 24 hrs of Galactosamine administration animals in all the groups were humanely sacrificed using Ketamine HCL and 4ml of blood was withdrawn by cardiac puncture and allowed to clot for 30mins at room temperature. The serum was separated by using refrigerated centrifuge and used for the assay of marker enzymes viz AST, ALT, ALP, TP, TB and LDH. The livers were dissected out immediately, washed with ice-cold saline and

10% homogenates in phosphate buffer solution (PH 7.4) were prepared. Liver homogenate was used for the assay of Lipid per oxidation (LPO) while some fraction of homogenates were centrifuged at 7000rpm for 10 min at $4^0$ C using refrigerated centrifuge, and the supernatants were used for the assay of Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx). Some portion of liver from each group was aseptically excised and stored in 10% formalin for histopathological studies.

**Table 1: Effect of methanolic extract of *Flacourtia sepiaria* and Silymarin pre-treatment on biochemical parameters of the rats intoxicated with D-Galactosamine.**

| Group. No. | TREATMENT DOSE (mg/Kg) | AST (IU/mL) | ALT (IU/mL) | ALP (IU/mL) | TP (gm/dl) | TB (mg/dl) | LDH (U/L) |
|---|---|---|---|---|---|---|---|
| I | Normal control 10ml/kg normal saline | 80.70± 3.95 | 48.45± 2.44 | 64.30± 2.12 | 6.20± 0.70 | 0.48± 0.15 | 212.40± 6.52 |
| II | Toxic control 25mg/kg D-galactosamine | 306.22± 8.35*a | 325.30± 7.75*a | 295.20± 6.30*a | 3.40± 0.30*a | 1.22± 0.30*a | 356.30± 7.20*a |
| III | Standard control silymarin 25mg/kg | 155.36± 4.25*b | 160.65± 4.40*b | 196.42± 5.20*b | 5.50± 0.60*b | 0.60± 0.15*b | 250.20± 6.75*b |
| IV | Treatment control MEF 200mg/kg | 240.60± 6.62*b | 222.70± 5.20*b | 268.30± 5.75*b | 4.58± 0.42*b | 0.90± 0.18*b | 285.18± 7.02*b |
| V | Treatment control MEF 400mg/kg | 225.55± 5.68*b | 205.50± 5.05*b | 252.30± 5.30*b | 4.75± 0.48*b | 0.82± 0.16*b | 262.20± 6.80*b |

- Values are expressed as Mean ± SEM.
- Values are found out by using one way ANOVA followed by Newmann keul's multiple range tests.
- *a – values are significantly different from normal control at P< 0.01.
- *b – values are significantly different from toxic control (G2) at p< 0.01.

**Table 2: Effect of methanolic extract of *Flacourtia sepiaria* and silymarin pre-treatment on biochemical liver parameter in D-Galactosamine induced hepatotoxicity.**

| Group. No. | TREATMENT DOSE (mg/Kg) | SOD (U/mg) Protein | CATALASE (U/mg) Protein | GPx (U/mg) Protein | LIPID PEROXIDATION (nmoles OF MDA/g liver weight) |
|---|---|---|---|---|---|
| I | Normal control 10ml/kg Normal saline | 9.12± 0.86 | 0.195± 0.06 | 10.8± 0.90 | 106.76± 4.06 |
| II | Toxic control 25mg/kg D-galactosamine | 2.60± 0.12*a | 0.012± 0.01*a | 2.60± 0.14*a | 160.82± 7.92*a |
| III | Standard control Silymarin 25mg/kg | 7.88± 0.68*b | 0.090± 0.04*b | 8.82± 0.62*b | 112.80± 4.25*b |
| IV | Treatment control MEF 200mg/kg | 7.22± 0.60*b | 0.078± 0.02*b | 7.60± 0.45*b | 136.42± 4.84*b |
| V | Treatment control MEF 400mg/kg | 7.52± 0.64*b | 0.084± 0.03*b | 8.25± 0.55*b | 128.30± 4.48*b |

- Values are expressed as Mean ± SEM.
- Values are find out by using one way ANOVA followed by Newmann keul's multiple range tests.
- *a – values are significantly different from normal control at P< 0.01.

*b – values are significantly different from toxic control (G2) at p< 0.01.

## STATISTICAL ANALYSIS

The Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Newmann Keul's multiple range tests. The values are represented as Mean ± SEM. Probability value of P < 0 .01) Serum Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline phosphatase (ALP), Total bilirubin (TB) and Lactate dehydrogenase and significant decrease in (P< 0.01) Total protein levels were observed in animals treated with galactosamine 400mg/kg (Group II) as compared to normal control group(Group I). Pretreatment with MEFS at a dose 200mg and 400mg/kg, orally, for 21days, decreased the levels of above indices like AST ,ALT , ALP, TB, LDH, and increased levels of TP significantly (P < 0.01) serum AST, ALP, TB, LDH and significant increase in TP at (P<0.01) in group III. SOD, CAT and GPx levels decreased significantly and LPO levels increased in liver homogenates of rats treated with galactosamine 400mg/kg (group II) as compared to normal control group[17,18] (Group I). On the other hand, pretreatment with MEFS at a dose 200mg and 400mg/kg, orally, for 21 days, increased SOD, CAT and GPx levels and decreased levels of LPO significantly (P<0.01) Liver homogenate enzymes such as SOD, CAT, GPx decreased the levels of LPO significantly (P<0.01) in group III as cited in table 1 and table 2.

### Histopathological Observations

Histology of liver sections of normal control animals (Group I) showed normal liver architecture of the central vein, and its associated structure. were preserved cytoplasm and prominent nucleus and nucleolus (Fig.1). The liver sections of galactosamine-treated animals (Group II) showed hepatic cells with serum toxicity characterized by inflammatory cell collection, scattered inflammation across liver parenchyma, focal necrosis and swelling up of vascular endothelial cells [15, 16] (Fig.2). Silymarin (Group-III) exhibited protection from galactosamine-induced changes in the liver (Fig.3). Pretreatment with MEFS at a dose 200mg and 400mg/kg, (group IV and V) appeared to significantly prevent the galactosamine toxicity as revealed by the hepatic cells with were preserved cytoplasms. Pretreatment also caused marked decrease in inflammatory cells (Fig.4 and Fig.5).

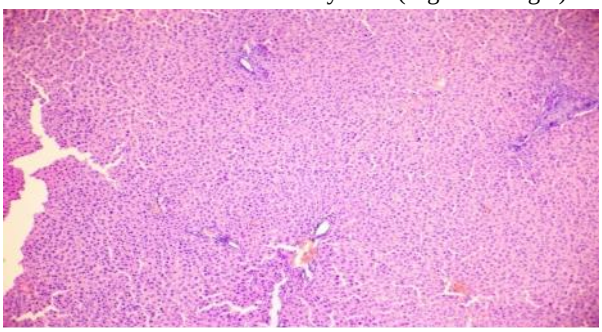

**Fig 1: Liver section of normal control rats showing normal liver lobular architecture with well brought out central vein and prominent nucleus and nucleolus.**

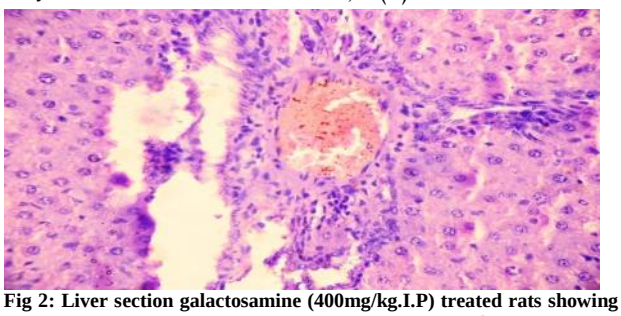

**Fig 2: Liver section galactosamine (400mg/kg.I.P) treated rats showing severe toxicity with congested blood vessels with inflammatory cell collection and endothelial cell swelling.**

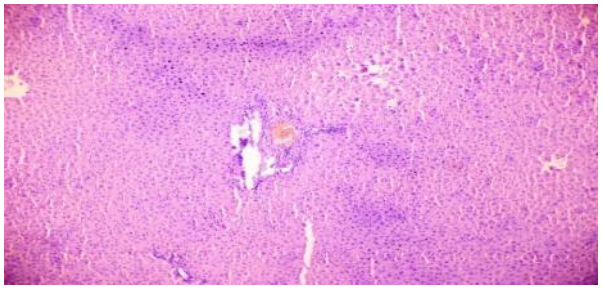

**Fig 3: Liver section of rats treated with silymarin 25mg/kg (p.o) + galactosamine(400mg/kg) showing only a few inflammatory cells around portal tract.**

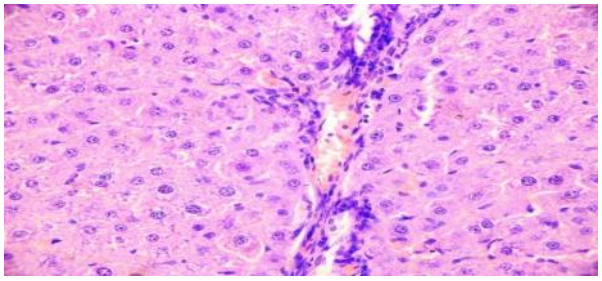

**Fig 4: Liver section of rats treated with MEF (200mg/kg) + galactosamine (400mg/kg) showing only slight inflammation.**

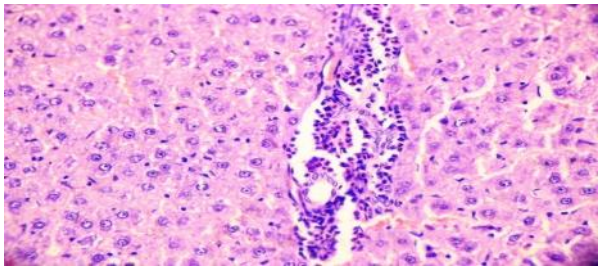

**Fig 5: Liver section of rats treated with MEF (400mg/kg) + galactosamine (400mg/kg) showing only slight inflammation.**

## 4. CONCLUSION

MEFS demonstrated significant protective effect in galactosamine- induced liver damage which could be at least partly mediated via free radical scavenging activity due to the presence of flavonoids, tannins and antioxidants. Although there were many studies showing oral pre-treatment with alcoholic extract of several plants significantly prevented the d-galactosamine-induced alterations in respiration and oxidative phosphorylation of liver mitochondria [17]. Also D-Galactosamine is a well known model of hepatotoxicity that closely resembles acute liver failure (ALF) seen clinically [18], additional studies are needed to better understand the mechanism of action of MEFS which may be responsible for the hepatoprotective activity. As liver being highly specialized tissues regulating wide variety of high volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions [19], the more studies in MEFS may demonstrate clear perception in the scientific justification for the use of MEFS widely in folk medicine for hepatic disorders.

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