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HEPATOPROTECTIVE EFFECT OF *FICUS DALHOUSIAE*MIQ.ON PARACETAMOL INDUCED HEPATOTOXCITY IN RATS

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ABSTRACT

Ficus dalhousiae (family Moraceae) is used to treat cardiac, liver and skin disorders. This study aims to investigate possible hepatoprotective activities of ethanolic extract of leaf and bark of *Ficus dalhousiae* against paracetamol-induced hepatotoxicity.Hepatotoxicity was induced in Wistar male rats by oral administration of paracetamol at a dose 2 g/kg body weight, on 7th day after the administration of ethanolic extract of leaf and bark of *Ficus dalhousiae* and silymarin (100 mg/kg). Both extracts of this plant were administered orally at doses of 200 mg/kg and 400 mg/kg body weight daily for 7 days. Several serum markers, serum glutamate oxoloacetate transaminase (SGOT), serum glutamate pyruvate transaminase(SGPT), alkaline phosphatase (ALP), bilirubin, total protein and total cholesterol were measured to assess the effect of the extract on paracetamol induced hepatic damage. The study also included gross pathological and histopathological examination of liver.Blood samples from rats treated with ethanolic extract of leaf and bark of *Ficus dalhousiae* (200 mg/kg body weight and 400 mg/kg body weight) had significant reduction ($p<0.05$) in serum markers and improvement in biochemical parameters in paracetamol administered animals, indicating the effect of the extract in restoring the normal functional ability of hepatocytes. Between both plant parts, bark extract was found to posses' good therapeutic efficacy($p<0.001$) in ameliorating the toxic effects of paracetamol-induced toxicity in wistar albino rats. Silymarin (100 mg/kg,p.o.) was used as a reference drug.The ethanolic extract of leaf and bark of *Ficus dalhousiae* exhibits protective effects against paracetamol-induced hepatotoxicity.

Key words: *Ficus dalhousiae*; Paracetamol; Silymarin; Hepatotoxicity

INTRODUCTION

Liver is the vital organ of metabolism and excretion. Liver diseases such as jaundice, cirrhosis and fatty liver are very common worldwide. About 20,000 deaths in a year are due to liver disorders[1]. Of this hepatocellular carcinoma is one of the most common tumors in the world and nearly 2,50,000 new cases reported each year.[2] The liver damage may be caused by xenobiotics, malnutrition, anaemia, environmental toxins, viral infection, alcohol consumption and hepatotoxic drugs such as certain antibiotics, chemotherapeutic agents, paracetamol, high doses of CCl_4, thioacetamide.[3] These hepatotoxic agents can react with the basic cellular components and consequently induce all types of liver lesions[4]

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It is therefore essential to search an alternative drug for the treatment of liver disease to put back the currently used drugs of tentative efficacy and safety.[5] In absence of a reliable liver protective drug in the modern system of medicine, a number of medicinal preparations in Ayurveda, the Indian system of medicine, are recommended for the treatment of liver disorders. In India numerous medicinal plants and their formulations were used for treating liver disorders in the traditional system of medicine as well as ethanobotanical practice.[6]Herbal drugs play an important role in management of various disorders and many of this speedup the healing processes of the liver which are considered to be effective andsafe alternative treatments for hepatotoxicity. In such perspective, one such drug is *Ficus dalhousiae*Miq., which has ethno pharmacological significance to be used for hepatic disorders [7].

Ficus dalhousiae is the species of *Ficus* of the family Moraceae,a small spreading tree with young softly pubescent branches growing in rock crevices. It is a species endemic to peninsular India[8] and a very rare species.[9]Miquel in 1847[10] first described this species as

Urostigma dalhousiae based on Wight's collection from India and later[11] he renamed it as *Ficus dalhousiae*.As its population size is very small, it is included under the very rare category in the threatened plants list. In traditional system of medicine the fruits are used in the treatment of heart diseases and leaves and bark are used in the treatment of liver and skin diseases. Bark paste is used in treatment of cracked feet[12,13].Taking into consideration the traditional claim of *Ficus dalhousiae* has been used in liver diseases, the present study aims to investigate hepatoprotective effect of *Ficus dalhousiae* against paracetamol induced hepatic injury in rats.

MATERIALS AND METHODS

Collection and preparation of extracts

The plant*Ficus dalhousiae*leaves and stem barkwere collected in the month of March 2013, from Tirupathi Hills, Andhra Pradesh, India, after the authentication of the plant by Prof. Dr. P.Jayaraman, Plant Anatomy Research Centre (PARC),Tambaram, Chennai. India, a voucher specimen of the plant is being maintained in the herbarium of Department of Pharmacognosy, Ratnam Institute of Pharmacy, Nellore, Andhra Pradesh, India.The ethanolic extract of leaves and bark of *Ficus dalhousiae* (EELFD & EEBFD) was prepared by cold maceration usingethanol as a solvent for a period of 7days with intermittent stirring and on the final day of procedure the extract was filtered and concentrated under reduced pressure to yield a green semisolid mass.

Animals

Wister albino rats weighing 150-200 g wereprocured from animal houseof Ratnam Institute of Pharmacy, Nellore, AndhraPradesh, India. The experimental protocol was approved and Ethical clearance for the handling of experimental animals was obtained from Institutional Animal Ethics Committee*(CPCSEA Reg.No. 1/200)* as per the guidance of the committee for the purpose of control and supervision of Experiments on animals (CPCSEA)and used for the studies. The animals were housed individually in polypropylene cages,under standard laboratory conditions (12:12 hour light and dark cycle; at an ambient temperature of 25 ± 3^{o}C; 35- 60% of relative humidity). The animals were fed with standard rat pellet diet and water *ad libitum* during the experiment.

Hepatoprotective activity

Paracetamol induced hepatotoxicity model was adopted for the study[14]. The rats were divided into 7 groupsof 6 animals each.

Group I: Animals (Control) were received 1ml/kg p.o0.5% SCMC for 7 days

GroupII: Animals (Hepatotoxic) were received 1 ml/kg p.o 0.5% SCMC for 7 days

Group III: Animals (Standard) were received Silymarin 100 mg /kgp.o for 7 days.

Group IV: Animals were received ethanolic extract of leaves 200mg/kg p.o for 7 days.

Group V: Animals were received ethanolic extract of leaves 400mg/kg p.o for 7 days.

Group VI: Animals were received ethanolic extract of bark 200mg/kg p.o for 7 days.

Group VII: Animals were received ethanolic extract of bark 400mg/kg p.o for 7 days.

On the 7th day, paracetamol suspension was given orally,2 g/kg body weight, to all the rats except those inGroup I. At the end of the experimental period, the rats were fasted overnight and sacrificed by ether. Blood andliver samples were collected for biochemical and histological studies.

Biochemical studies

Blood was obtained from all animals by puncturing the retro-orbital plexus. Blood samples were allowed to clotfor 45 min at room temperature. Serum was separated by centrifugation at2.5 x g at 30°C for 15 min and assayed for SGOT, SGPT, ALP[15], total bilirubin (TB)[16], total protein(TP)[17]and total cholesterol(TC)[18].

Histopathological studies

Paraffin sections (7 µm thick) of buffered formalin–fixed liver samples were stained (nuclei in blue and cytoplasm in pink) with hematoxylin-eosin to identify the histological changes under the microscope.

Statistical analysis

For determination of significant inter-group differencesof each parameter one-way analysis of variance(ANOVA) was carried out. Dunnet test was used for individualcomparisons after significant ANOVA results.The differences with $P < 0.05$ were considered statisticallysignificant. GraphPad prism 5 software (GraphPadSoftware, Inc. California, USA) was used for the statisticalanalysis.

RESULTS

Serum biochemical parameters

Administration of paracetamol to rats by oral route caused liver damageas indicated by a significant increase in serum enzyme SGOT,SGPT,ALP activity,total bilirubin and total cholesterol levels ($p<0.001$) compared to control rats. Elevated levels of these enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. Due to hepatic damage there was decreased production of proteins, so the total proteinlevels of paracetamol group was less than control groupco-administration of rats with EELFD and EEBFD of both doses(200&400mg) remarkably ($p<0.05$) restored paracetamol induced elevated serum level of SGOT, SGPT, ALP,TB, and TC towards normal value respectively, except that EELFD of 200mg restored the ALP levels non-significantly. The decreased levels of TP in paracetamol treated groups were re-established to normal. These increased and decreased levels of various serum marker enzymes and other parameters were depicted in Table 1.Restoration potential of large dose($p<0.01$) of both leaf and bark extract is more in contrast to that of low dose ($p<0.05$) of the same. Relatively, it was evidenced that EEBFD of both doses shows good therapeutic efficacy when compared to EELFD in normalizing the aberrated levels of serum marker enzymes.

Pathological studies

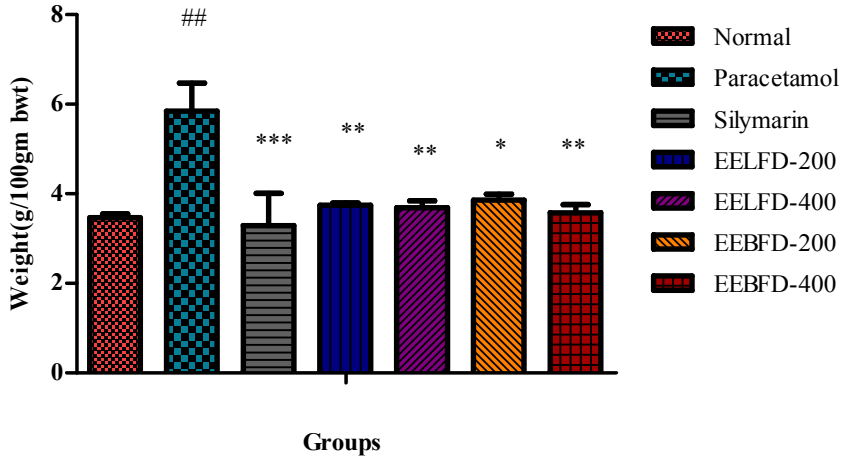
Gross pathological investigation of liver tissue reveals that organ weight increases in paracetamol treated rats when compared to extract pre treated rats (Figure 1). Histopathological studies of rat liver tissue from the normalgroup (Group I) showed normal hepatic cells with central vein and sinusoidal dilation (Figure 2a). In the paracetamol group (Group II), severe hepatotoxicity was observed in the form of severe necrosis and disappearance of nuclei and congestion (Figure 2b). Histopathological analysis showed that the pathological lesions caused by paracetamol were very minimal in groups pretreated with EELFD and EEBFD (Group IV-VII). Normal hepatocytes with regenerating hepatocytes and sinusoidal spaces were observed in groups treated with extracts of both parts of the plant at a dose of 200 and 400 mg/kg body weight, respectively (Figures 2d-2g). Liver tissue from paracetamol plus silymarin group (Group III) had normal hepatic cells with portal vein and sinusoidal spaces (Figure 2c**).**

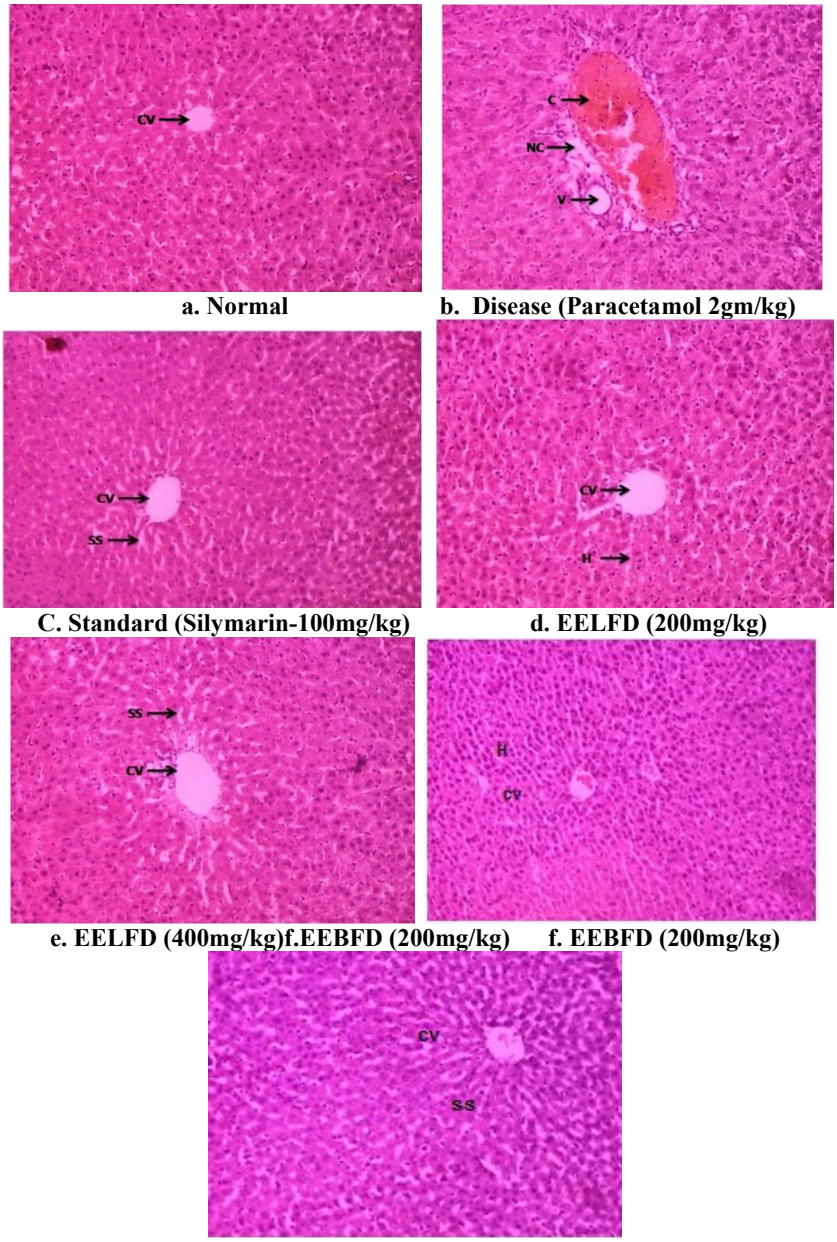
Table 1.Effect of silymarin, EELFD and EEBFD on various biochemical parameters in paracetamol induced acute liver injuryin rats

Treatment design	Dose/kg (po)	Parameters					
		SGOT(IU/L)	SGPT(IU/L)	ALP(IU/L)	TB(mg/dL)	TP(g/dL)	TC(mg/dL)
I. Normal	1ml of 0.5%SCMC	88.39±4.27	37.58±2.75	107.8±5.33	0.323±0.04	7.60±0.25	94.67±7.37
II. Paracetamol	2gm	144.4±3.64 †††	226.2±2.47 †††	184.4±4.05 †††	3.413±0.42 †††	2.89±0.20 †††	172.3±4.79 †††
III. Silymarin	100mg	99.71±4.82 ***	56.69±3.02 ***	126.6±2.51 ***	0.611±0.07 ***	6.19±0.25 ***	107.8±4.01 ***
IV. EELFD	200mg	126.0±3.46 *	202.2±3.10 *	168.3±3.74 ns	2.24±0.23 **	4.14±0.44 *	139.76±4.60 **
V. EELFD	400mg	108.20±3.60 ***	107.7±5.34 ***	163.6±4.08 **	0.718±0.12 **	5.72±0.29 ***	108.0±4.85 ***
VI. EEBFD	200mg	111.7±3.93 ***	198.30±7.07**	162.8±2.41 **	0.956±0.06 ***	4.68±0.25 **	139.0±6.20 **
VII. EEBFD	400mg	102.5±4.73 ***	101.6±6.93 ***	132.2±2.49 ***	0.64±0.03 ***	6.02±0.32 ***	*103.7±4.35* ***

The values are expressed as mean ± SEM. Statistical significance test was done by ANOVA followed by Dunnet's test.;†infers Paracetamol group compared to Normal group;* infers test and standard groups compared to paracetamol group;† & *-p< 0.05; †† & **-p<0.01;††† & ***-p<0.001; ns-non significant.

Figure 1: Effect of silymarin, EELFD and EEBFD on liverweight in paracetamol induced Acute liver injury in rats





a. Normal **b. Disease (Paracetamol 2gm/kg)**

C. Standard (Silymarin-100mg/kg) **d. EELFD (200mg/kg)**

e. EELFD (400mg/kg)f.EEBFD (200mg/kg) **f. EEBFD (200mg/kg)**

g. EEBFD (400mg/kg)

Figure 2.Histography of hepatic tissues of paracetamol treated rats showing hepatic cells (H), central vein (CV), congestion (C), necrotic cells (NC) and sinusoidal spaces (SS)

DISCUSSION

Paracetamol induced hepatotoxicity is the most commonly used screening method for testing the hepatoprotective nature of plant extracts. The study of serum markers such as SGOT, SGPT, ALP and bilirubin, and total protein and total cholesterol has been found to be of great value of assess to clinical and experimental liver damage[19]. In the present investigation, the rats suffered significant hepatic damage from treatment with paracetamol, as indicated by elevated levels of serum markers (Table 1). A rise in SGOT is usually accompanied by an increase in SGPT, which plays a vital role in the conversion of amino acids to keto acids[20]. Pretreatment with EELFD and EEBFD, both at 200 mg/kg body weight and 400 mg/kg body weight, significantly attenuated elevated levels of serum markers. This suggests that ethanolic extract of leaf and bark of the plant stipulates the hepatocytes so as to protect the integrity of the membrane from paracetamol-induced leakage of serum markers into circulation. These changes can be considered a functional improvement of hepatocytes and may be caused by accelerated regeneration of parenchyma cells. Serum ALP and bilirubin are related to hepatic cell damage[14]. Increase in serum ALP is due to increased synthesis in the presence of increasing biliary pressure[21]. Reduction of serum protein in paracetamol treated group may be due to formation of protein adduct. Toxic metabolites lead to

covalent modification of cellular target protein, cell death andorgan damage[22].Alteration of bio membrane lipid profile disturbs its fluidity and increases micro viscosity of the membrane as a result of cholesterol increasing, which leads to cellular rigidity. Intoxication of rats with paracetamol may have altered membrane structure and function as suggested by the increases in cholesterol[23].The normalized recovery of all serum enzyme levels and biochemical parameters may be due to the presence of flavonoids and their antioxidanteffects, which may protect the hepatic cell damage induced by paracetamol. Compared to EELFD,EEBFD of both doses show better efficacy in alleviating serum enzyme levels indicating that bark part may possess good free radical scavenging properties than leaf part of plant.Paracetamol induced a significant increase in liver weight,which is due to blocking of secretion of hepatic triglycerides into the plasma.[24]Silymarin (100mg/kg),EELFD and EEBFD at both doses(100 &200mg/kg) prevent the increase of liver weight. Histopathological studies of rats administered paracetamol showed severe necrosis and disappearance of nuclei. This could be due to the formation of highly reactive metabolites, because of excessive administration of paracetamol. All these histopathological changes were significantly reduced in rats treated with ethanolic extract of leaf and bark of *Ficus dalhousiae* and by the standard silymarin.

CONCLUSION

In conclusion, it was said that the plant *Ficus dalhousiae* posses good hepatoprotective activity and among leaf and bark, ethanolic extract of bark evidenced more antioxidant properties and restores all the abberated parameters in paracetamol induced hepatotoxic rats significantly than that of leaf extract.Further investigation is to be carried out for its efficacy by chronic treatment and on molecular basis.

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