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INVESTIGATION OF HEPATOPROTECTIVE ACTIVITY OF *FICUS RETUSA* (MORACEAE)

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ABSTRACT

Ficus retusa (Moraceae) is distributed through out Western peninsula and also found in Chota Nagpur, Bihar, Central India, Andamans, Sundribuns, Malaya islands and Australia. In Sanskrit, it is known as 'Kantalaka', 'Kshudra' and in Telugu it is well known as 'Yerrajuvvi'. Ficus leaves of ethyl acetate and methanolic extracts (100, 200 and 400 mg/kg) was prepared and tested for its hepatoprotective effect against carbon tetrachloride induced in rats. Alteration in the levels of biochemical markers of hepatic damage like SGPT, SGOT, ALP, bilirubin were tested in both treated and untreated groups. Carbon tetrachloride has enhanced the SGPT, SGOT, ALP and bilirubin levels. Treatment with ethyl acetate extract of *Ficus retusa* leaves has brought back the altered levels of biochemical markers to the near normal levels in the dose dependent manner SGOT (213.1± 1.25, 198.2± 3.45, 180.6 ± 2.05), SGPT(195.1±2.22, 168.4±1.36, 112.6±3.24), ALKP(215.6±4.60, 202.3±2.45, 195.1±3.20) and T.B(1.24±0.01, 1.05±1.08, 0.96±0.13) levels respectively. Though both the extracts were recorded with significant hepatoprotective activity with same "p" value ($p<0.001$). Which were significant protective activity in groups treated with FR and silymarin. It was concluded from the study that ethyl acetate and methanolic extracts of FR possess hepatoprotective activity against CCl_4 induced hepatotoxicity in rats.

KEYWORDS: Hepatoprotective, *Ficus retusa,* Carbontetrachloride, Silymarin.

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INTRODUCTION

Liver damage is always associated with cellular necrosis, increase in lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, ALP and bilirubin levels are elevated[1,2]. However there are several herbs/herbal formulations claimed to possess beneficial activity in treating hepatic disorders.

Ficus retusa (Moraceae) is distributed through out Western peninsula and also found in Chota Nagpur, Bihar, Central India, Andamans, Sundribuns, Malaya islands and Australia. In Sanskrit, it is known as 'Kantalaka', 'Kshudra' and in Telugu it is well known as 'Yerrajuvvi'. It is also called as "Indian Laurel Fig". Root bark and the leaves boiled in oil form good applications for wounds and bruises. Adventitious roots, dried and powdered and mixed with salt are applied to decaying or aching tooth. Bark is used in treatment of liver diseases[3-5].

Ficus (Moraceae) species are used in folk medicine for the treatment of various diseases, such as biliousness, ulcers, vomiting, vaginal complains, fever, diabetes, inflammations, Wound, lucoderma, ulcer itching, diuretic, liver diseases and leprosy, liver[6]. *Ficus retusa* is a rapidly growing tree in India but originated from Ceylon. Roots are adventitious, occasionally hanging. Bark is gray and smooth. Branchlets are brown and glabrous[7-8]. The golden yellow leaves of *Ficus retusa* contain high amounts of flavonoids and carotenoids, triterpenoids, fatty alcohol, steroids, coumarins, flavane,4-hydroxybenzoate and isoflavones[9-11].

MATERIALS AND METHODS

Plant Material Collection

Ficus retusa (Moraceae) leaves were collected from the Andhra University region of Visakhapatnam, Andhra Pradesh in the month of January 2009 and Authenticated by the taxonomist, Dept of Botany, Andhra University and the specimen Voucher No JNTUCP/2009/F75 has

been preserved in the Department.

Animals

Wistar albino rats of either sex weighing between 200-250 gm were obtained from M/s. Mahavir Enterprises, Hyderabad, Andhra Pradesh, India. The animals were housed under standard environmental conditions (temperature of 22 ± 1°C with an alternating 12 h light–dark cycle and relative humidity of 60 ± 5 %), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics committee and by the Regulatory body of the government (Regd no. 516/01/a/CPCSEA). They were fed with standard laboratory diet supplied by M/s. Rayans biotechnologies Pvt. Ltd., Hyderabad, Andhra Pradesh, India. Food and water was allowed *ad libitum* during the experiment.

Acute toxicity studies

Acute toxicity studies were performed for extracts of selected plant according to the toxic classic method as per guidelines. None of these extracts showed mortality even at a dose of 1000mg/kg and therefore considered safe.

Toxicological studies were conducted in mice (N=6) for all the extracts as per the Irvin's method[12] at the doses of 100, 300 and 1000 mg/kg, no mortality was observed.

Materials

All the materials used for this experiment are of Pharmacopoeial grade. Carbon tetrachloride (E. Merck), silymarin (Sigma Chemical Co.,) and olive oil were purchased from the local supplier. Diagnostic kits for the estimation of SGOT, SGPT, SALKP and serum bilirubin were purchased from local supplier (Sai chemicals) manufactured by Ranbaxy Diagnostics Ltd., New Delhi, India. Water represents the double distilled water; standard orogastric cannula was used for oral drug administration.

Carbon tetrachloride-induced hepatotoxicity

The animals were divided into nine groups of six animals each. Group-I served as normal control received 5% acacia mucilage (1 ml/kg.p.o) daily once for 7 days. Group-II served as toxic control and received CCl_4 (1 ml/kg i.p) daily once for 7 days[13] Group-III was treated with the standard drug Silymarin (50 mg/kg .p.o) and followed by CCl_4 (1 ml/kg i.p) daily once for 7 days[14] Groups IV-VI were treated with ethyl acetate extract of *Ficus retusa* at doses of 100, 200 & 400mg/kg p.o. in acacia mucilage respectively followed by CCl_4 (1 ml/kg i.p) daily once for 7 days. Groups VII-IX were treated with methanol extract of *Ficus retusa* at doses of 100, 200 & 400mg/kg p.o, in acacia mucilage respectively followed by CCl_4 (1 ml/kg i.p) daily once for 7 days. After completion of treatment blood was collected, serum was separated and used for determination of biochemical parameters.

Collection of blood samples

All the animals were sacrificed on 7th day under light ether anesthesia. The blood samples were collected separately in sterilized dry centrifuge tubes by puncture retro-orbital plexes and allowed to coagulate for 30 min at 37 °C .The clear serum was separated at 2500rpm (Microcentrifuge) for 10min and subjected to biochemical investigation viz.., serum glutamic oxaloacetate transaminase (SGOT), serum glutamic Pyruvate transaminase (SGPT), Alkaline phosphatase (ALP) and Total Bilirubin (TB).

Assessment of liver function

The Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated by UV kinetic method in which both SGOT and SGPT were assayed based on enzyme coupled system; where keto acid formed by the aminotransaminase reacts in a system using NADH.The coenzyme is oxidized to NAD and the decrease in absorbance at 340 nm for SGOT malate dehydrogenase (MDH) reduces to malate with simultaneous oxidation of NADH to NAD.The rate of oxidation of NADH is measured, where as SGPT[15] the pyruvate formed in the reaction is converted to lactate by lactate dehydrogenase.Estimation of Alkaline phosphate (ALKP)[16] involves hydrolysis of P-nitrophenyl phosphate by alkaline phosphatase to give P-nitrophenol, which gives yellow color in alkaline solution. The increase in absorbance due to its formation is directly proportional to alkaline phosphate (ALKP) activity. Estimation of total bilirubin (TB)[17] involved the reaction of bilirubin with diazotized sulphanic acid to form an azocompound, the color of which is measured at 546 nm .All the estimations were carried out using standard kits in semi auto analyzer Screen Master 3000.

Statistical analysis

Results of biochemical estimation were reported as mean ±SEM for determination of significant inter group difference was analyzed separately and one-way analysis of variance (ANOVA) was carried out[18]. Dunnet's test was used for individual comparisons[19].

RESULTS AND DISCUSSION

CCl_4 is accumulated in hepatic parenchyma cells and metabolically activated by cytochrome P450-dependent monooxygenases to form a trichloromethyl radical (CCl_3). The CCl_3 radical alkylates cellular proteins and other macromolecules with simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage[20]. Thus, antioxidant or free radical generation inhibition is

important in protection against CCl_4-induced liver lesions[21]. Hepatotoxic compounds such as CCl_4 are known to cause marked elevation in serum enzymes and bilirubin levels. It causes a marked decrease in total protein levels. Silymarin is used as standard hepatoprotective compound since it is reported to have a protective effect on the plasma membrane of hepatocytes[22].

Serum levels of SGOT, SGPT, SALKP and total bilirubin were significantly increased ($p<0.001$) in carbon tetrachloride (1ml/kg.i.p) treated Group-2 rats. Group-3 rats treated with Silymarin(50mg/kg.p.o) produced significant reduction ($p<0.001$) in SGOT, SGPT, SALKP and total bilirubin

In Groups:4-6 treated with ethyl acetate extract of *Ficus retusa* at doses of 100, 200 and 400mg/kg; p.o respectively, there is significant decrease in SGOT, SGPT, SALKP and total bilirubin levels when compared to Group-2 rats. The activity of the extracts is found to be dose dependant. The results were given in **Table 1** and **Fig 1**.

Carbontetrachloride (1ml/kg.i.p) intoxication in normal rats produced elevated levels of serum biochemical parameters significantly SGOT (295.5 ± 0.39), SGPT (269.5 ± 1.8), ALKP (296.5 ± 1.45), T.B (2.02 ± 0.03) indicating acute hepatocellular damage and biliary obstruction.

When compared to the CCl_4 toxic control group, the group treated with the ethyl acetate extracts of Ficus *retusa,* at doses of 100, 200 and 400mg/kg; p.o in CCl_4 intoxicated rats exhibited a significant reduction ($p<0.01$) of SGOT (213.1± 1.25, 198.2± 3.45, 180.6 ± 2.05), SGPT (195.1±2.22, 168.4±1.36, 112.6±3.24), ALKP (215.6±4.60, 202.3±2.45, 195.1±3.20) and T.B (1.24±0.01, 1.05±1.08, 0.96±0.13) levels respectively.

In Groups: 7-9 treated with methanol extract of *Ficus retusa* at doses of 100, 200 and 400mg/kg; p.o respectively, there is significant decrease in SGOT, SGPT, SALKP and total bilirubin levels when compared to Group-2 rats. The activity of the extracts is found to be dose dependant.

When compared to the CCl_4 toxic control group, the group treated with the methanol extracts of Ficus *retusa,* at doses of 100, 200 and 400mg/kg; p.o in CCl_4 intoxicated rats exhibited a significant reduction ($p<0.001$) of SGOT (250.2±1.04, 231.0±3.48, 210.3±1.26), SGPT (225.2±2.06, 202.5±1.28, 165.2±3.03), ALKP (235.1±4.36, 220.2±1.40, 205.6±3.20) and T.B (1.38±0.32, 1.22±0.45, 1.10±1.07) levels respectively.

Though both the extracts were recorded with significant hepatoprotective activity with same "p" value ($p<0.001$). The ethyl acetate extract was found to be more potent than methanol extract because of effect on percentage reduction in elevated levels of biochemical parameters and effect was dose dependant.

CONCLUSION

The efficacy of any hepatoprotective drug is essentially dependent on its capability of either reducing the harmful effects or in maintaining the normal hepatic physiological mechanism, which have been imbalanced by a hepatotoxin.

Orally administered doses of 100, 200 and 400mg/kg of methanol of stem heart wood of *Ficus retusa* produced significant decrease in SGOT, SGPT, SALKP and total bilirubin levels. The activity of the extracts is found to be dose dependant. In CCl_4 induced toxic hepatitis, toxicity begins with the changes in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures[23]. Administration of ethyl acetate and methanolic extracts of *Ficus retusa* showed recovery against the toxic effects of CCl_4 thereby confirming the protective effect of the extracts of Ficus *retusa.*

The hepatoprotective activity of *Ficus retusa* could be due to the presence of bioflavonoids which have hepatoprotective properties[24-26]. The result of this investigation indicated that the methanolic extract of stem heart wood of *Ficus retusa* possess hepatoprotective activity against CCl_4 induced liver damage in rats. Attempts are being made to isolate and characterize the active principle to which the hepatoprotective activity can attribute.

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Table 1: Effect of ethyl acetate and methanol extracts of F. *retusa* on biochemical estimation of SGOT, SGPT, SALKP and total bilirubin of CCl_4 induced toxicity in rats

Groups	SGOT (IU/ml)	SGPT (IU/ml)	SALKP (IU/ml)	Total Bilirubin (mg/dl)
Control 1ml/kg	160.5 ± 0.62	96.95 ± 1.34	179.5 ± 0.99	0.82 ± 0.06
CCl_4 1ml/kg	295.5 ± 0.39^{+}	269.5 ± 1.8^{+}	296.5 ± 1.45^{+}	2.02 ± 0.03^{+}
Silymarin 50mg/kg	174.8 ± 1.88***	107.5 ± 1.45***	187.7 ± 2.25***	0.89 ± 0.04***
FREE 100 mg/kg	213.1 ± 1.25*	195.1 ± 2.22*	215.6 ± 4.60*	1.24 ± 0.01*
FREE 200 mg/kg	198.2 ± 3.45**	168.4 ± 1.36**	202.3 ± 2.45***	1.05 ± 1.08***
FREE 400 mg/kg	180.6 ± 2.05***	112.6 ± 3.24***	195.1 ± 3.20***	0.96 ± 0.13***
FRME 100 mg/kg	250.2 ± 1.04*	225.2 ± 2.06*	235.1 ± 4.36*	1.38 ± 0.32*
FRME 200 mg/kg	231.0 ± 3.48**	202.5 ± 1.28**	220.2 ± 1.40**	1.22 ± 0.45**
FRME 400 mg/kg	210.3 ± 1.26***	165.2 ± 3.03***	205.6 ± 3.20***	1.10 ± 1.07***

Values are mean ± SEM for six observations
P: $^{+}$<0.001 Compared to respective control group-1
P: *<0.05, **<0.01, ***<0.001 Compared to respective control CCl_4 group-2
FREE-*Ficus retusa* ethyl acetate extract, FRME- *Ficus retusa* methanol extract

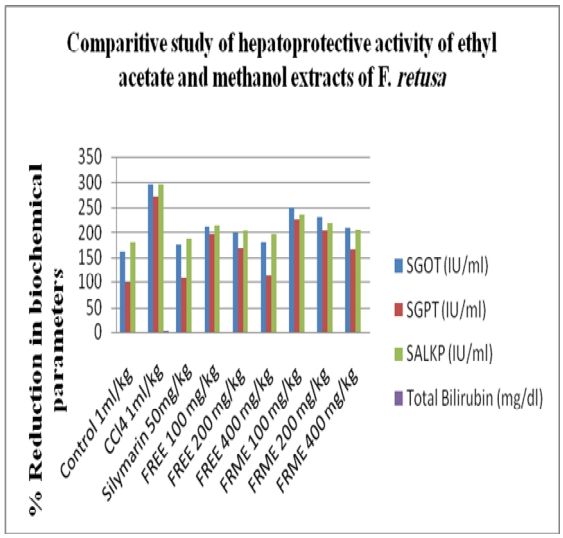

Fig 1: Comparitive study of hepatoprotective activity of ethyl acetate and methanol extracts of F. *retusa*

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