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Hepatoprotective Activity of *Symplocos racemosa* Roxb. *Bark* Extract in Carbon tetrachloride induced Liver Damage in Rats

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ABSTRACT

Symplocos racemosa Roxb. is a tree in Sanskrit called as Lodhra. It grows in plains and hills of North and East India. In the present work, the crude extracts of *Symplocos racemosa* Roxb. is taken for the hepatoprotective effect on the liver of rats. The bark of plant were dried, powdered was subjected to continuous hot extraction in Soxhlet apparatus. Carbon tetrachloride (CCl4) induced liver toxicity was carried out for the estimation of the liver protective functions of the plant. Wistar albino rats (150-200 g) were divided into five groups and were subjected to the following treatments. (methanolic extract of *Symplocos racemosa* at the dose of 250 and 500mg/kg, p.o. and standard drug silymarin at a dose of 100mg/kg, p.o. were administered orally to rats of the respective groups three times at 12h intervals. Carbon tetrachloride diluted with liquid paraffin (1:1) was administered in dose of 1mL/kg, p.o. for 2 days. Histopathological and biochemical observations were made. The MESR at tested doses (group-III & IV) produced a significant reduction ($p<0.01$) in the CCl4 induced elevated levels of SGOT, SGPT, ALP, γ-GT and total bilirubin as well as increases the TP when compared to the animals treated only with CCl4 (group-II) after 36h of CCl4 treatment. Overall, MESR at tested doses significantly reduced the levels of hepatic enzymes and total bilirubin.

Keywords: ***Symplocos racemosa*** Roxb., Carbon tetrachloride, Liver Damage, SGOT, SGPT, ALP, γ-GT and total bilirubin, Silymarin.

INTRODUCTION

The liver helps in the various metabolic processes of the body like regulating the sugar. The free sugar is made into glucogen and stored in the liver and muscles while during the requirement of energy it converts the glycogen from the liver back into glucose for the utilisation of the energy. In a diabetic subject when there is less regulation of the glucose due to the reduced insulin levels in the circulating blood, the function of regulating the blood glucose is devoid or less this results in the excess of blood glucose in the blood leading to higher glucose levels and the associated complications. Hence liver in addition to the pancreas helps closely monitor and regulate the functions of the body. Even excess of other metabolic products from fat, proteins are also regulated by the liver. In the present work, the crude extracts of *Symplocos racemosa* Roxb. is taken for the hepatoprotective effect on the liver of rats.

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MATERIALS AND METHODS

Plant collection

Symplocos racemosa Roxb.is collected from the plant collectors and taken from the market in Chennai. The plant was authenticated by Dr. Madhava Chetty, S.V.University, Tirupathi.

Preparation of extracts

The bark of plant were dried, separated and made to dry powder of 40 # size. It was then passed through the 40 mesh sieve. A weighed quantity (200g) of the powder was subjected to continuous hot extraction in Soxhlet apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of MESR was found to be 16.5% w/w.

Phytochemical screening

The phytochemical screening of various extracts of the plant is reported earlier.

Animals used

Albino Wistar rats, weighing 150–200 g were used. The selected animals were housed in acrylic cages

in standard environmental conditions (20–25 ◦C), fed with standard rodent diet and water *ad libitum*. The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and the experimental protocols duly approved by the institutional ethical committee.

Carbon tetrachloride induced hepatotoxicity in rats

Carbon tetrachloride (CCl4) induced liver toxicity was carried out for the estimation of the liver protective functions of the plant.The model described by *Rao and Mishra* (Rao and Mishra, 1998) was adopted. Wistar albino rats (150-200 g) were divided into five groups and were subjected to the following treatments; group-I served as normal control; received vehicle only. Group-II received only CCl4, to assist assessing the severity of toxicity produced by carbon tetrachloride administration. Groups III-V received MESR (methanolic extract of *Symplocos racemosa* at the dose of 250 and 500mg/kg, p.o. and standard drug silymarin at a dose of 100mg/kg, p.o. were administered orally to rats of the respective groups three times at 12h intervals. Carbon tetrachloride diluted with liquid paraffin (1:1) was administered in dose of 1mL/kg, p.o. for 2 days to all animal groups except for normal control. After 36h of carbon tetrachloride treatment, blood was collected from all groups of rats by puncturing the retro-orbital sinus. Various biochemical parameters were estimated after collecting the serum separated by centrifugation at 2500 rpm at 37º C for 15 min.

Biochemical estimation

The separated serum was subjected to estimate SGOT and SGPT by *Reitman and Frankel m*ethod, (Reitman and Frankel, 1957), alkaline phosphatase (ALP) by *Kind and King* method (Kind and King, 1954), and bilirubin by *Malloy and Evelyn* method (Malloy and Evelyn, 1937).

Histopathological studies

The rats were sacrificed and the liver was carefully isolated. The tissues of liver were fixed in 10 % formalin and embedded in paraffin wax. Sections of 4-5 microns thickness were made using rotary microtome and stained with haematoxylin-eosin and histological observations were made under light microscope (Luna, 1966; Galigher and Kozloff, 1971).

Fig.1 Histopathological sections of the liver in male albino Wister rat during various treatments

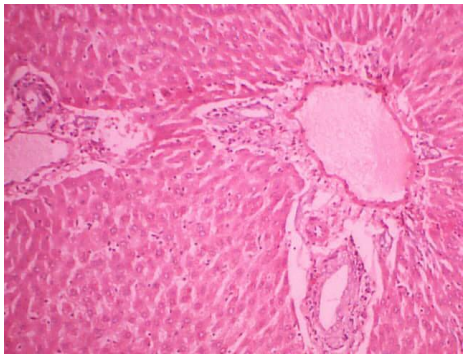

Fig:1a. GROUP I
CCl_4 treated
(Negative control)

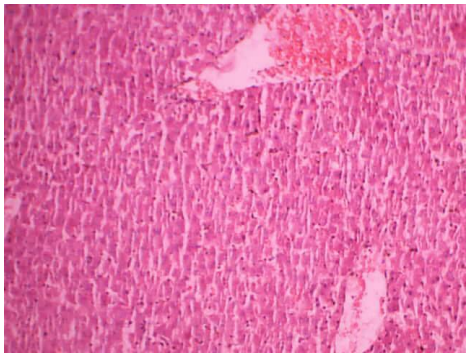

Fig:1b GROUP II
(CCl_4 + MESR 200)

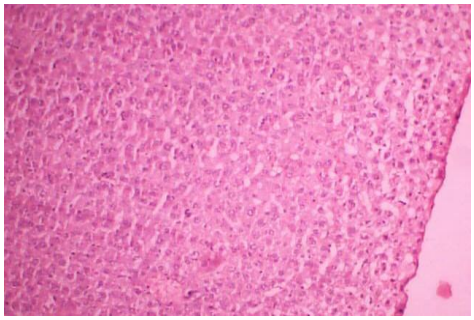

Fig1c GROUP III

(CCl_4 + MESR 400)

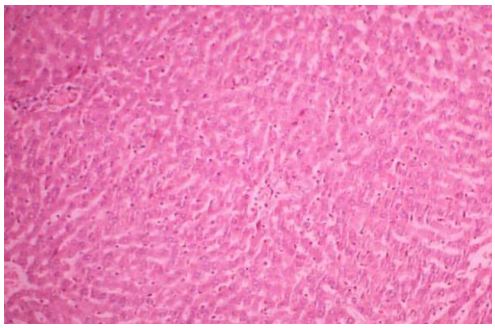

Fig1d GROUP IV

(CCl_4 + standard)

Table.1 Hepatoprotective study of *Symplocos racemosa* Roxb. on albino rats for various biochemical parameters

Groups (n=6)	Biochemical Parameters					
	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Gamma-GT (IU/L)	TP (g/dL)	Total Bilirubin (mg/dL)
Group-I (Normal Control)	22.71±0.03	18.54±0.22	186±3.05	49.83±1.63	9.24±0.19	0.79±0.13
Group-II (CCl4:1mg/Kg)	66.07±0.89	39.54±0.52	412.61±3.53	97.31±1.97	4.17±0.45	4.15±0.13
Group-III (MESR:250mg/Kg)	47.14±1.52***	34.75±0.73***	271±2.11***	67.49±1.37***	2.53±0.74	1.57±0.57***
Group-IV (MESR:500 mg/Kg)	42.57±1.09***	26.61±1.39***	219.87±2.02***	56.73±0.71***	7.32±0.25***	0.91±0.47***
Group-V (silymarin:100 mg/Kg)	36.51±1.54***	22.17±0.87***	199.16±2.52***	54.37±1.62***	7.82±0.76***	0.82±0.19***

Values are expressed as mean SEM of 6 rats in each group. *** $p<0.001$, as compared to CCl4-treated group. SGOT = serum glutamate oxaloacetate transaminase, SGPT = serum glutamate pyruvate tranaminase, ALP = alkaline phosphatase, γ-GT = gamma glutamyl transpeptidase, TP = total proteins

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Tukey-Kramer multiple comparison tests, the *p* values less than 0.05 were considered as significance.

RESULTS

Effect of MESR on CCl4 – induced hepatotoxicity

The results of MESR on carbon tetrachloride induced hepatotoxicity were represented in Table 1. While observation, the animals treated only with CCl4 exhibited a significant increase ($p<0.01$) the levels of SGOT, SGPT, ALP, γ-GT and total bilirubin as well as decrease in the levels of TP when compared to the normal control group after 36h of CCl4 treatment, indicating hepatocellular damage. The MESR at tested doses (group-III & IV) produced a significant reduction ($p<0.01$) in the CCl4 induced elevated levels of SGOT, SGPT, ALP, γ-GT and total bilirubin as well as increases the TP when compared to the animals treated only with CCl4 (group-II) after 36h of CCl4 treatment. Overall, MESR at tested doses significantly reduced the levels of hepatic enzymes and total bilirubin.

Effect of MESR on histolopathological change

Histopathological examination of liver sections of normal control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein. Disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration were observed in CCl4 intoxicated rats. The liver sections of the groups-III and IV rats treated with MESR at the dose of 250 and 500 mg/Kg, p.o) showed a sign of protection as it was evident by the moderate accumulation of fatty lobules, absence of necrosis and vacuoles in a dose dependent manner. Almost similar sign of protection was shown in the liver sections of silymarin at a dose of 100mg/Kg,p.o. treated rats.

DISCUSSION AND CONCLUSION

In diabetes, hyperglycemia is accompanied with dyslipidemia, (Bierman *et al*, 1966; Garber, 2002), i.e. characterized by increase in TC, LDL, VLDL, TG and fall in HDL. Hypercholesteremia and hypertriglyceridemia are primary factors involved in the development of atherosclerosis and coronary heart diseases which are the secondary complications of diabetes. This altered serum lipid profile was reversed towards normal after treatment with the MESR. MESR exhibited hypocholesterolemic and hypotriglyceridemic effects, while increased the levels of HDL in streptozotocin-induced diabetic rats. However, MESR was found to be more effective in reducing the levels of TG and LDL. The elevated atherogenic index, i.e. TC/HDL ratio, which is a useful determinant of cardiovascular risk, (Grover *et al*, 1999), was also shifted towards normal after MESR treatment. Thus, it is reasonable to conclude that MESR could modulate blood lipid abnormalities.

Symplocos racemosa Roxb. could be proved that the traditional use of *plant* as a hypoglycemic agent is justified and that extracts from this plant show a dose-dependent activity which is comparable to the standard hypoglycemic drug glibenclamide.

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