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Hepatoprotective Activity of Leaves of *Sapindus trifoliatus* Linn.

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Effects of ethanolic extract of the leaves of *Sapindus trifoliatus* was studied on CCl_4 induced hepatic injury in albino rats. The study revealed that the leaf extract at the dose levels of 100, 200 and 400 mg/kg body weight significantly reduced serum alanine transaminase, asparatate transaminase, serum alkaline phosphatase, total bilirubin and total proteins.

Key Words: *S. trifoliatus*, Hepatoprotective activity.

INTRODUCTION

Sapindus trifoliatus (Sapindaceae) commonly known as Indian filbert and soap-nut tree. Fruits, leaves, roots and bark of the tree[1] are generally used for therapeutic purposes[2]. The fruit of the plant is reported to have expectorant, emetic, alexipharmic, abortificiant and spermicidal effects[3] and is also used in excessive salivation, epilepsy and chlorosis[4]. However, no specific study has been carried out so far to check the hepatoprotective activity of leaf extract of *Sapindus trifoliatus*.

EXPERIMENTAL

Preparation of ethanolic extract: The leaves of *Sapindus trifoliatus* were collected from the local areas near to Mangalore, Karnataka. The plant of *Sapindus trifoliatus* had been authenticated by botanist Mrs. Usha Nalini, Head of Botany Department, St. Agnes college, Mangalore. The leaves were cleaned, dried and broken down into pieces and powdered into a coarse powder by a mechanical grinder. The powder was then passed through sieve no. 40 and extracted with ethanol in soxhlet extractor exhaustively for 20-24 h. The extract was concentrated to dryness under reduced pressure and controlled temperature using flash evaporator.

Acute toxicity studies: Acute toxicity study was carried out in female albino rats as per staircase method[5] and OECD guidelines 425[6] were followed. The animals tested with oral dose starting from 100 mg/kg body weight upto 2000 mg/kg body weight of ethanolic extract of leaves of

S. trifoiatus. The animals were continuously observed for 2-3 h for general behavioural, neurological, autonomic profile and death for a period of 24 h and for 14 d, after administration of the leaf extract. There was no mortality and no signs of toxicity were found upto 2000 mg/kg/ body weight and found to safe up to 2000 mg/kg body weight. All the experiments were performed within the guidelines of the Institutional ethical committee of KSHEMA. Derlakatte, Mangalore. (KSHEMA/IAEC/032/2004).

Assessment of hepatoprotective activity: Assessment of hepatoprotective activity[7] was carried out on 6 albino rats. The rats of either sex were used and the animals were segregated into 6 group each of 6 rats and maintained on normal pellet and water *ad libitum*.

Group-I served as control receiving 2 mL/kg/ b.w. p.o. (olive oil) for 9 d. Group-II received toxicant CCl_4. Group-III served as standard receiving the drug silymarin at the dose of 25 mg/kg b.w for 9 d. Groups-IV, V and VI received the different doses of ethanolic extract of *S. trifoliatus* 100, 200 and 400 mg/kg/b.w p.o., respectively for 9 d.

Hepatotoxicity in all group except group II was induced by subcutaneous injection of 1:1 (v/v) mixture of CCl_4 in olive oil at the dose of 1 mL/kg on day 7th while group I was treated with olive oil at the dose of 0.5 mL /kg/b.w. subcutaneously on 7th day. After the 9th day animals were sacrified by decapitation. The blood was collected and serum obtained after centrifugation (3000 rpm for 15 min) was used for various biochemical estimation.

Biochemical estimation: Serum was separated from the blood and subjected to various biochemical parameters like aspartate aminotransaminase (AST), alkaline phoshphatase (ALP), alanine transaminase (ALT), total bilirubin and total proteins.

Histopathological study: The rats were scarificed under deep anaesthesia and livers were excised quickly, washed with normal saline and preserved in 10 % buffered neutral formalin solution for histopathological studies. The liver pieces were then embedded in paraffin using conventional methods and cut into 5 µm thick sections and stained using haeomatoxyline-eosin dye and finally mounted in diphenyl xylene. Then the sections were observed under microscope for histopathological changes in liver structure[8].

RESULTS AND DISCUSSION

Carbon tetrachloride intoxication in normal rats elevated the serum levels of alanine aminotransaminase, aspartate amino transaminase, alkaline phosphatase, total bilirubin and reduced the values of serum total proteins significantly. The rats of all the three groups treated with ethanolic extract of *Sapindus trifoliatus* at different dose levels showed significant

reduction in alanine aminotrans-aminase, aspartate aminotransaminase alkaline phosphatase, total bilirubin and also showed a significant elevation in serum total proteins as compared to the CCl_4 treated group.

Histological profile: The microscopic examination of liver of group I showed a normal portal triad, sinusoids and cord arrangement of hepatocytes (Fig. 1). The microscopic examination of liver of group II showed marked to moderately severe fatty change of liver with presence of large fat vacuoles in the cytoplasm pushing the nuclei at the periphery. At places many fat vacuoles are seen united and are forming small fat cysts as well.

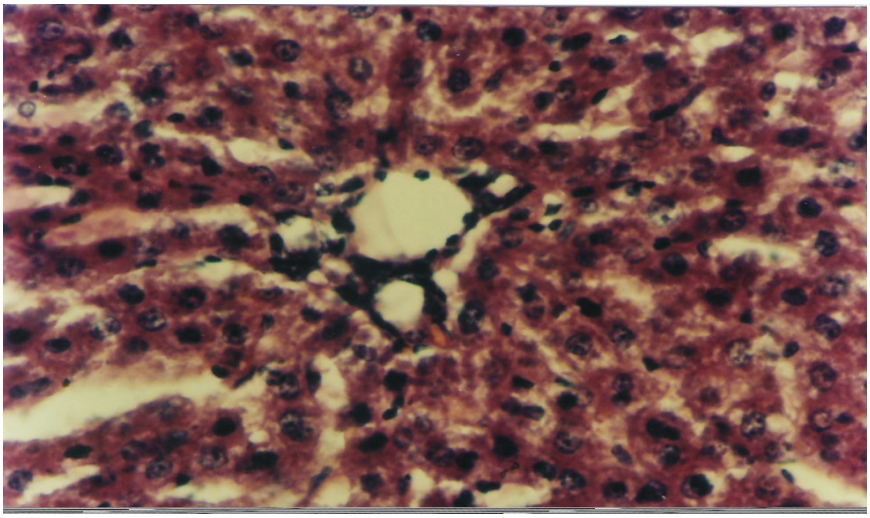

Fig. 1. Histopathological slide of liver (Group-I) [H. and E. Staining, 10 × 45] (Normal liver showing a normal portal triad, sinusoids and cord arrangement of hepatocytes)

Occasional areas in this group are also showing degeneration and necrosis of hepatocytes (Fig. 2). The microscopic examination of liver of group VI showed almost normal appearing hepatocytes and no fatty change, or absence of fatty change, or absence of fatty change in hepatocytes. There is also no inflammation or necrosis. Only occasional fine fat vacuoles could be seen in some hepatocytes. Thus the finding of this group are comparable with the finding of silymarin treated group, suggesting the Hepatoprotection at this dose (Fig. 4). The microscopic examination of liver of group V showed moderate degree of fatty change in the liver. However fair number hepatocytes are seen intermixed with fat laden hepatocytes (Fig. 5). The microscopic examination of liver of group IV revealed that the test drug when used in 100 mg/kg wt. was not able to provide proper protection

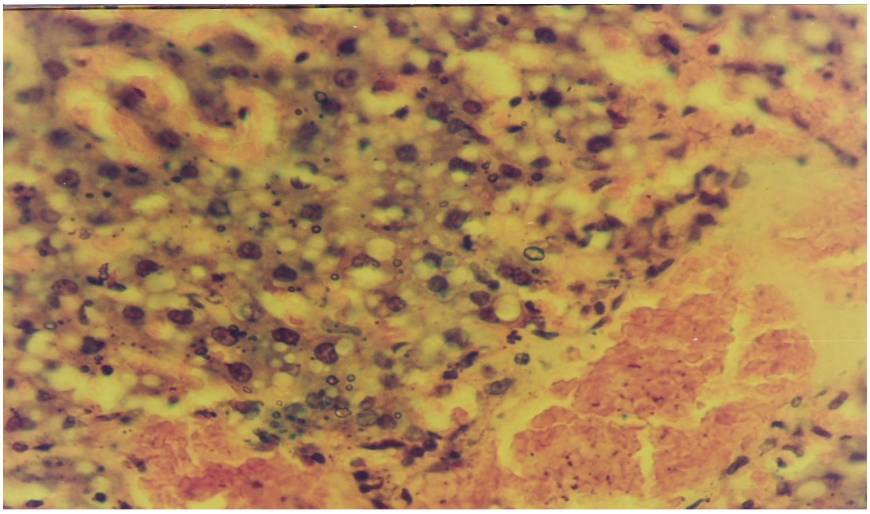

Fig. 2. Histopathological slide of liver (Group-II) [H. and E. Staining, 10 × 45] (CCl_4 treated liver showing marked fatty changes and large foci of degeneration and hepatocyte necrosis)

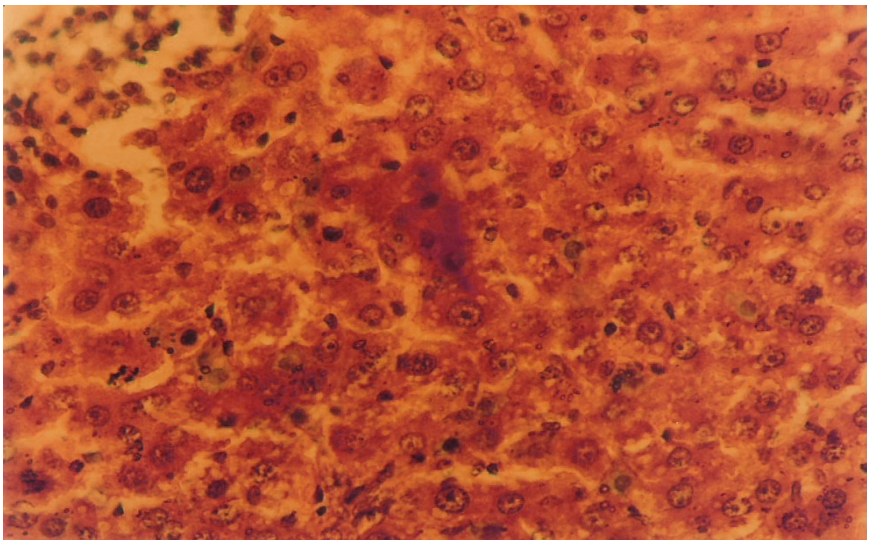

Fig. 3. Histopathological slide of liver (Group-III) [H. and E. Staining, 10 × 45] (Liver exposed to CCl_4 and pretreated with silymarin at the dose of 25 mg/kg body wt, showing normal appearing hepatocytes and no fatty change, or absence of fatty change in hepatocytes, there is also no inflammation or necrosis. Only occasional fine fat vacuoles are seen in some hepatocytes)

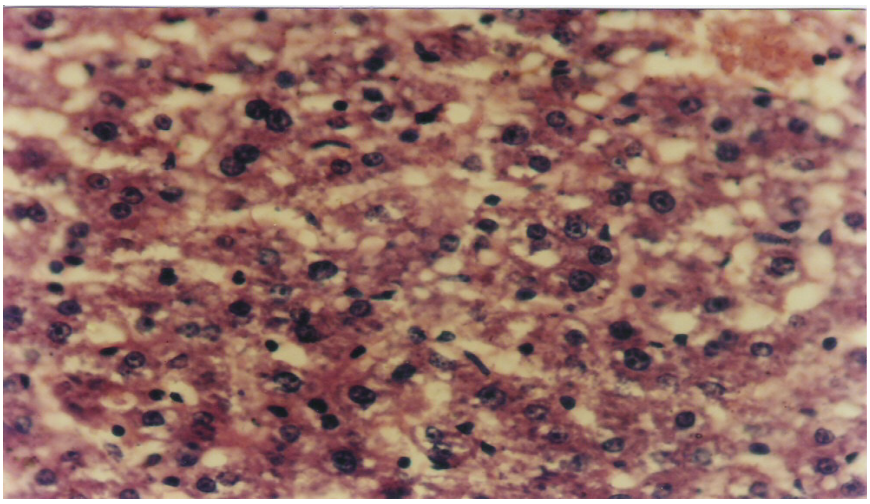

Fig. 4. Histopathological slide of liver (Group-IV) [H. and E. Staining, 10 × 45] (Liver exposed to CCl_4 and pretreated with *S. trifoliatus* at the dose 100 mg/kg body wt. showing moderately severe fatty changes)

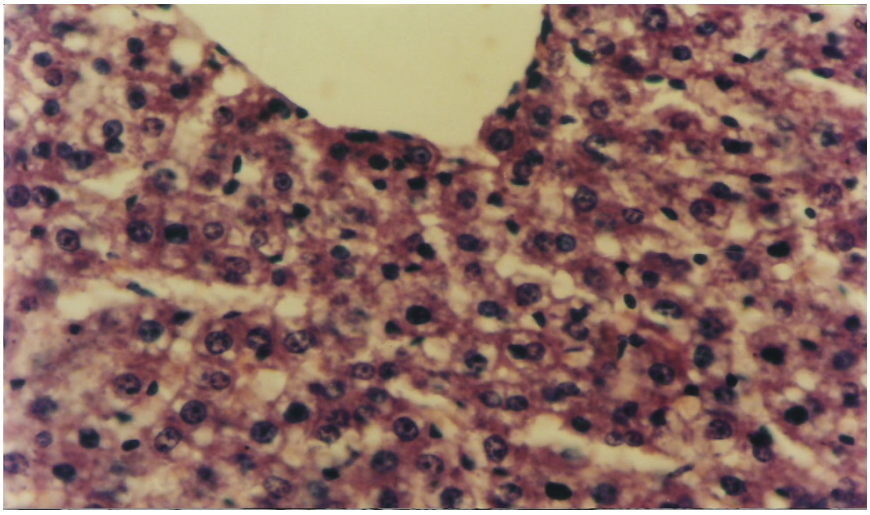

Fig. 5. Histopathological slide of liver (Group-V) [H. and E. Staining, 10 × 45] (Liver exposed to CCl_4 and pretreated with *S. trifoliatus* at the dose 200 mg/kg body wt. showing moderate degree of fatty changes. Fat laden hepatocytes are seen intermix with normal hepatocytes)

from fatty change in liver as the sections of liver at this dose showed moderately severe fatty change (Fig. 4). The microscopic examination of liver of group III revealed almost normal hepatocytes with only occasional fine fat vacuoles and mild inflammation. No significant fatty change or necrosis or marked inflammation was seen, this indicated that silymarin provided significant hepatoprotection from fatty change (Fig. 3).

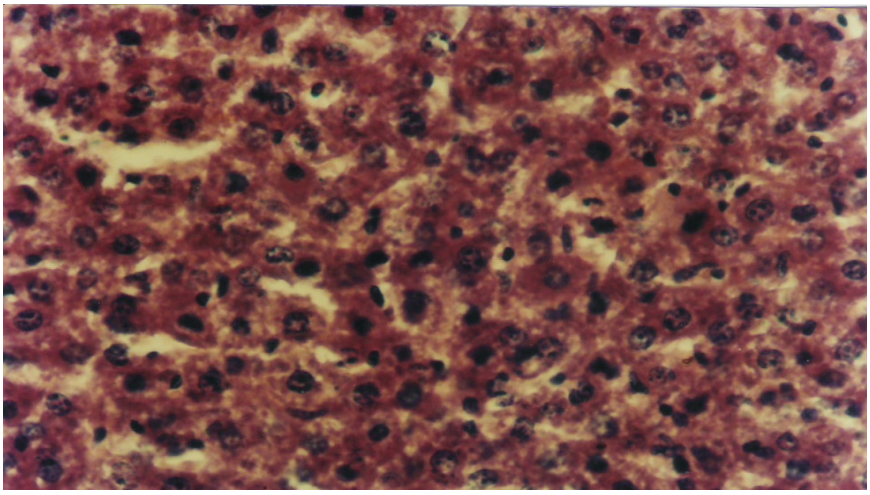

Fig. 6. Histopathological slide of liver (Group-VI) [H. and E. Staining, 10 × 45] (Liver exposed to CCl_4 and pretreated with *S. trifoliatus* at the dose 400 mg/kg body wt., showing almost normal appearing hepatocytes. Fine fat vacuoles are seen only in occasional hepatocytes. Results are comparable with silymarin indicating marked protection of liver from fatty changes)

Carbon tetrachloride has been used to induce hepatotoxicity in experimental animals[9,10]. The toxins like CCl_4 which produce hepatic necrosis are believed to do so by forming highly reactive free redicals or there unstable intermediaries in the process of metabolic degradation. The hepatotoxicity of CCl_4 is due to formation of highly reactive metabolite, the trichloro-free radical CCl_3. A preliminary activation of CCl_4 to CCl_3 results in (i) covalent binding of CCl_3 to neighboring cell components, (ii) lipid peroxidation which damages the membrane structure of the endoplasmic reticulum and inhibits many enzyme activities and (iii) the liberation of diffusible reactive breakdown products that can cause systemic disturbances[11].

Administration of ethanolic extract of leaves of *Sapindus trifoliatus* at all the dose levels *viz.*, 100, 200 and 400 mg/kg/d, showed significant

hepatoprotective activity but the results obtained from the dose level of 400 mg/kg/d statistically comparable with the results obtained from the standard drug silymarin. In support histopathological reports also revealed that there is a marked hepatoprotection in group III, IV, V and VI. Though the ethanolic extract of leaves of *S. trifoliatus* which contains carbohydrates, Proteins, flavonoids, terpenoids and steroids showed significant hepatoprotective activity (Table-1).

TABLE-1
EFFECT OF ALCOHOLIC EXTRACT OF LEAVES OF *Sapindus trifoliatus* ON CCl_4 INDUCED HEPATOTOXICITY IN RATS

Group no.	Group (n = 6)	Total proteins (mg/dL)	Total bilirubin (mg/dL)	AST (U/L)	ALP (U/L)	ALT (U/L)
I	Control	10.72 ± 0.008	0.596 ± 0.042	55.13 ± 0.121	164.23 ± 0.50	106.92 ± 0.09
II	CCl_4 treated	6.73 ± 0.11	1.591 ± 0.004	223.66 ± 0.49	355.90 ± 0.714	359.22 ± 0.29
III	CCl_4 + Silymarin (25mg/kg)	9.19 ± 0.003*	0.7967 ± 0.004*	94.20 ± 0.31*	177.44 ± 0.85*	151.01 ± 0.12*
IV	CCl_4 + *ST* leaf extract (100mg)	8.00 ± 0.003*	1.220 ± 0.068*	131.18 ± 0.21*	210.47 ± 0.54*	233.06 ± 0.35*
V	CCl_4 + *ST* leaf extract (200mg)	8.42 ± 0.009*	1.060 ± 0.0051*	128.11 ± 0.11*	195.28 ± 0.43*	180.82 ± 0.34*
VI	CCl_4 + *ST* leaf extract (400mg)	8.69 ± 0.003*	0.913 ± 0.008*	123.23 ± 0.26*	182.67 ± 1.23*	167.81 ± 0.38*

Values are expressed as mean ± SEM, n=6 rats in each groups ST-*Sapindus trifoliatus*; *Significant ($p < 0.05$) compared to control.

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REFERENCES

1. K.M. Nadkarni and R.N. Chopra, Indian Materia Media, Popular Prakashan, Bombay, Vol. 1, p. 1102 (1976).
2. G. Panday, *Dervyaguna vijanana*, Varanasi KrishnadusAcademy, Vol. 1, p. 191 (1998).
3. R.P. Rastogi and B.N. Mehrotra, Compendium of Indian Medical Plants, CDRI Publication, New delhi, Vol. 2, p. 609 (1999).
4. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants, B.L.M. Basu Publication, Allahabad, Vol. 2, p. 63 (1991).
5. M.N. Ghosh, Fundamentals of Pharmacology, Hilton and Company, Kolkata, edn. 3, p. 190 (2005).
6. OECD 425 Guidelines, OECD Guidelines for Testing Chemicals, 1/20, 1-26 (2001).
7. D. Bhattacharyya, R. Mukherjee, S. Pandit, N. Das and T.K. Sur, *Indian J. Pharmacol.*, **35**, 183 (2003).
8. A. Stevans and I. Wilson, Theory and Practice of Histopathological Techniques, Churchill Living Stone, edn. 4 (1996).
9. L.M. Tierney, S.J. McPhee and Papadakisma, Current Medical Diagnosis and Treatment, McGraw-Hill, edn. 43, p. 638 (2004).
10. T.F. Slater, Biochemical Studies on Liver Injury, Academic Press Inc. Ltd., London, pp. 11-39 (1978).

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