Evaluation of Hepatoprotective activity of *Capparis sepiaria* leaves

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ABSTRACT

The effect of the ethanolic extract of the leaves of *capparis sepiaria* Linn was studied against carbon tetrachloride induced hepatotoxicity in Wister rats. Significant hepatoprotective effects were obtained in liver damage induced by carbon tetrachloride as evident from decreased serum levels of glutamate pyruvate transaminase (SGPT),glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP) and bilirubin (SB) in the *capparis sepiaria* **(**CS) treated groups (100,200 mg/kg), compared to the intoxicated controls. The hepatoprotective effect was further confirmed by histopathalogical studies of the liver, which showed improved architecture, absence of nuclear pycnosis, hepatocyte congestion and necrosis, when compared with the liver of the toxin group of animals. *capparis sepiaria* **(**CS) extract also showed significant free radical scavenging activity *In Vitro*. Thus the present study provides a scientific rationale for the traditional use of this plant in the management of liver disorders.

KEY WORDS: *Capparis sepiaria*, free radical scavenging activity, hepataprotection, histopathology of the liver, carbon tetrachloride.

INTRODUCTION

Capers, comprising of species of the genus *capparis* Linn. (family-capparaceae) are reported to have many medicinal properties. They reduce flatulence and have anti-rheumatic effects in ayurvedic medicine; capers are recorded as hepatic stimulants and protectors, improving liver function. They have been used to treat arteriosclerosis, as diuretics, kidney disinfectants, vermifuges, and tonics. Infusions and decoctions from caper root bark have been traditionally used for dropsy, anemia, arthritis and gout. They contain considerable amounts of the antioxidant bioflavonoid, rutin. *Capparis sepiaria* edgew is an astringent and used in cardiac troubles and biliousness. *Capparis spinosa* Linn is used in splenic, renal and hepatic complaints. It also has antitubercular property. Its root bark is used in ayurvedic preparations like liv. 52, used to treat acute viral hepatitis and cirrhosis[1]. The potent hepatoprotective effects of *C.spinosa* have already been reported.[2]

Capparis sepiaria a closely related species is a dense armed handsome shrub, distributed throughout India and hence known as the Indian Caper. It has coriaceous leaves, white flowers and ovoid to ellipsoid berries[3]. It is likely that when bioactive compounds are found in

one species, more species of the same genus may contain active compounds of a similar nature.[4]. Hence the present paper deals with the hepatoprotective activity of ethanolic extract of leaves of of *Capparis sepiaria*.

Materials and Methods

Plant Material

The healthy leaves with petiole of *c. sepiaria* were collected from Sirumalai, Kodai road, Tamil nadu. The plants were authenticated by the taxonomist of the American College, Madurai. A voucher specimen has been deposited at the herbarium.

Preparation of Plant Extract

The leaves with petiole of *Capparis sepiaria*, were washed thoroughly in tap water, shade-dried and powdered. The powder (100g) was successively extracted with 1000ml of ethanol overnight with constant stirring. The filtrate was then concentrated and the solvent was evaporated. The yield of the extract was found to be 5.12 % (W/V). This crude extract was referred to as *Capparis sepiaria* (CS). For administration, the crude extract was suspended in 10% tween-80 to required concentrations and used for the experiments.

Experimental Animals

Wistar albino male rats (150-270g) and Swiss albino male mice (18-20g), obtained from the institute's animal house were used for the present study. They were housed under standard laboratory conditions and were fed commercial rat feed (Lipton India Ltd., Mumbai, India) and boiled water, ad libitum. All animal experiments were carried out according to NIH guidelines, after getting the approval (Ref.No:8620/E4/3/03) of the Institute's Animal Ethics Committee.

Behavioral and Toxicological Effects: (OECD guidelines)

Three groups of ten mice were administered with graded doses of the CS extract (500,1000and 2000 mg/kg,) one group was maintained as control and was given 0.5% tween-80. All the animals were observed continuously for 1h for any gross behavioral changes and death, if any, and then, intermittently for the next 6h, and then again at 24h after dosing with CS extract.

In Vitro Antilipid Peroxidation Studies

The antilipidperoxide effect of CS was studied *In Vitro*, following the modified method of Yoshiyuki *et al*[5] and Masao *et al*[6].Briefly 0.5g of the rat liver tissue was homogenized with 10 ml of 15mM KCl-Tris-HCl buffer (pH 7.2). the reaction mixture was composed of 0.25 ml of liver homogenate , 0.15ml of KCl-Tris-HCl buffer (pH 7.2) 0.1mM ascorbic acid (AA), 4mM $FeCl_2$ and 0.05 ml of various concentrations of CS extract (25,50 and 100 µg/ml). The mixture (in quadruplicate) was incubated at 37^0 C for 1 hr in capped tubes. Then , 0.1N HCl , 0.2ml of 9.8 % sodium dodecyl sulphate (SDS) , 0.9ml of distilled water and 2ml of 0.6% of thiobarbituric acid (TBA) were added to each tube and shaken vigorously , the tubes were placed in a boiling water bath bath at 100^0 C for 30 min , after cooling , the flocculent precipitate was removed by adding 5ml of n butanol and they were centrifuged at 3000rpm for 20min . The absorbance of supernatant was measured at 532nm.

Carbon Tetrachloride Induced Hepatotoxicity

Carbon Tetrachloride was suspended in 0.5% gum acacia and administered p.o. at a dose of 0.3ml/kg. This dose is known to cause liver damage in rats[7]. Healthy rats were divided into six groups (six per group). Group I, the normal control group received a single daily dose of 10% tween-80 p.o., for 4 days. Group Ⅱ, the carbon tetrachloride intoxicated control group received a daily dose of 0.5% gum acacia for 4 days and 0.3ml/kg (Carbon Tetrachloride mixed with an equal volume of liquid paraffin) on day 3, 30 min after CS administration. Group V animals received a daily dose of Silymarin (Sigma Chemical Company, U.S.A) at a dose of 100 mg/kg p.o., for 4 days 0.3ml/kg (Carbon Tetrachloride mixed with an equal volume of liquid paraffin and, p.o.,), 30 min after Silymarin administration by mild ether anesthesia. From all the six groups, blood samples were collected separately from carotid tubes and allowed to coagulate for 30 min at 37˚C; the clear serum was separated at 2500 rpm for 10 min and subjected to the liver function tests.

Liver Function Tests

Biochemical parameters like serum enzymes, serum glutamate pyruvate transaminase (SGPT)[8] serum glutamate oxaloacetate transminase (SGOT) [8], serum alkaline phosphatase [9] (ALP) and serum bilirubin (SB) [10] were assayed according to the standard methods.

Histopathological Studies

After blood draining, liver samples were excised from the control and treated groups of animals and washed with normal saline separately. They were fixed in 10% buffered formalin for 24 hours. The formalin-fixed liver samples were stained with haematoxylin-eosin for photo microscopic observations of the liver histological architecture.

Statistical Analysis

Statistical comparison between control and treated groups was made using analysis of variance, followed by multiple comparisons.[11]

Results

Behavioral and Toxicological Effects

In the toxicity study, no mortality occurred during 24 h of observation with three doses of CS (500, 1000, 2000 mg/kg p.o.), administration. The LD_{50} of mice was thus observed to be greater than 2000 mg/kg.

***In Vitro* Antilipid Peroxidation Studies**

In $FeCl_2$-AA treatment, the rat liver homogenate was induced with ascorbic acid / Fe^{2+} ($FeCl_2$-AA) to cause non-enzymatic lipid peroxidation and the action of CS on the system was determined. There was significant increase of malondialdehyde (MDA) in $FeCl_2$-AA treated rat lever homogenate, compared to normal control without FeCl2-AA, whereas the ethanolic extract of the leaves of *Capparis sepiaria* significantly reduced the accumulation of lipid peroxides *In Vitro* in a dose dependent manner up to 100 µg/ml. (Table 1).

Carbon Tetrachloride Induced Hepatotoxicity

Administration of 0.3ml/kg (Carbon Tetrachloride mixed with an equal volume

of liquid paraffin and, p.o.,), of CCl_4 resulted in significant rise in level of enzymes viz. SGPT, SGOT, ALP, SB, and the levels of SB and HP (hydroxyl proline) were increased following CCl_4 treatment as evident from the values of G5 (control). The group G2 and G3 treated with extract alone (100mg/kg&200 mg/kg) and G4 stachydrine alone treated (25mg/kg) showed almost same results of normal, there by indicating that the extract did not produce any hepatotoxicity. The increased parameters observed in control (G5) were brought to normal levels as in normal control in both treatment group G6 (100mg/kg) and G7 (200mg/kg) and didn't show any alterations in the liver function. The increased parameters observed in control (G5) were brought to normal in the (G8) stachydrine treated (25mg/kg) and didn't show any alterations in liver functions. These results were comparable to the standard drug silymarin (20mg/kg) treated group (G9).

Histopathological Studies

The hepatoprotective effect of CS was further confirmed by histopathological examination of the liver samples from the respective groups. Histological architecture of Carbon tetrachloride treated liver sections showed degeneration of hepatic cells with centrilobular necrosis and paralysis. However, administration of CS (100 mg/kg) almost normalized these defects in the histological architecture of the liver resembling that of Silymarin treated groups, showing its potent hepatoprotective effects. (Plate 11).

Discussion

The present study reports the potential hepatoprotective activity of *Capparis sepiaria* against hepatic injury produced by Carbon Tetrachloride, the known hepatic toxin in rats. CCl4 is a commonly used hepatotoxin used for production of experimental liver toxicity. Its metabolites such as trichloromethyl radical (CCl3*) and trichloromethyl peroxy radical (CCl3O2*) are involved in the pathogenesis of liver. CCl4 causes changes around the central vein in the liver and other oxidative damages with the leakage of marker enzymes like SGPT, SGOT and ALP in the serum, increase in serum SB levels and decrease in serum Total Protein. Seven days pretreatment with the test extract (100 mg/kg p.o.) protected the animals significantly ($p<0.01$) from CCl4 induced hepatotoxicity as shown in table and figures which clearly indicates the hepatoprotective activity of the alcohol extract of *Capparis sepiaria*. Different components including β-sitosterol and alkaloids have been isolated from the plant .[12, 13, 14.]

An obvious sign of hepatic injury is the leaking of cellular enzymes into the plasma, when liver cell plasma is damaged; a variety of enzymes located in the cytosol is released into the blood, thereby causing increased enzyme levels in the serum. The estimation of serum marker of the extent and type of hepatocellular damage.

Pretreatment of the rats with 100 and 200 mg/kg, p.o., of CS extracts for 4 days before Carbon tetrachloride administration resulted in a significant protection of Carbon tetrachloride - induced elevation of serum marker enzymes and bilirubin, which is almost comparable to the effect of the positive control, silymarin. Silymarin is a known hepatoprotective compound obtained from *silybum marianum* gaertn. It is reported to have a protective effect on plasma membrane of hepatocytes.[15]

The hepatoprotective effect of CS was further confirmed by histopathological observations proved the effect of CS in preventing hepatocellular necrosis or mononuclear infiltration, as reported by Dhuley and Naik *et al* [16] CS administrations resulted in bringing about an almost normal histological architecture of the liver.

Lipid per oxidation is a complex and natural deleterious process. The effects of the free radicals on human beings have recently been considered as their close relation to toxicity, diseases and aging.[17, 18] Liver is under constant threat of oxidants, especially hydrogen peroxide. The per-oxidative property of hydrogen peroxide can be justified as the formation as free radicals with Fe^{2+} /ascorbic acid.

Fe^{2+} /ascorbic acid form an important tool for the study of invitro lipid per oxidation in terms of thiobarbituric acid reactive substances (TBARS) formation. The free radical could further attack the phospholipids of membrane causing lipid per oxidation, the fact remains that the generation of free radical imposes depletion of antioxidants such as glutathione (reduced). However oxidative stress results in toxicity when the rate at which the free radicals are generated exceeds the cell capacity for their removal. Malondialdehyde (MDA) some of the end products in the lipid per oxidation process.[19] The amount of Malondialdehyde formed was quantified by reaction with thiobarbituric acid (TBA) and used as an index of lipid per oxidation.In the present study, the significantly elevated levels of lipid peroxides observed in the liver tissue treated with $FeCl_2$ –ascorbic acid indicate excess formation of free radicals and activation of lipid per oxidation system resulting in hepatic damage. The significant decline in the lipid peroxide content in liver tissue treated with $FeCl_2$-AA along with CS indicated the anti lipid peroxidative effect of CS.

C.spinosa a closely related species of *C. sepiaria* has been reported to have hepatoprotectivce activity, due to presence of flavonoids like quercetin and quercitroside. p-methoxybenzoic acid isolated from the ethaniolic extract of aerial parts of *C. spinosa* was found to exhibit antihepatotoxic activity against paracetamol induced hepatotoxicity .[20] Previous phytochemical studies on *C.spinosa* have shown the presence of alkaloids, lipids, polyprenols flavonoids and indole and aliphatic glucocinolates [21] The presence of several quercetin and kaempferol glycosides,as well as of hydroxycinnamic acids, have also been demonstrated in capers[22], it has been also reported that Liv 52, a powerful and powerful and popular hepatic stimulant [23] contains 24% *C.spinosa* that improves the functional efficacy of liver.[24]

Preliminary phytochemical studies in our laboratory have indicated the presence of flavonoids in *C. sepiaria* leaves [12,13,14] , it is likely hat the flavonoids of CS , may be responsible for the hepatoprotective activities of CS. flavonoids, consumed in large amounts in the daily diet , are helpful to protect the liver .Further , phytochemical studies are in progress to isolate , characterize and identify the specific active flavonoid in this plant responsible for liver protection .

Conclusion

In conclusion, the results of the present study demonstrate that the leaves of *Capparis sepiaria* posses potent hepatoprotective action on carbon tetra chloride induced hepatic damage in rats

besides significant free radical scavenging activities.

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TABLE 1- INHIBITORY EFFECT OF ETHANOLIC EXTRACT OF *CAPPARIS SEPIARIA*. (CS) ON $Fecl_2$– ASCORBIC ACID (AA)-INDUCED LIPID PER OXIDATION IN RAT LIVER HOMOGENATE *IN VITRO*

Treatements	CS Concentration (µg/ml)	MDA(n mole/mg protein)	MDA inhibition (%)
Normal Control	-	1.349 ±0.003	-
$FeCl_2$-AA Control	-	2.779±0.007	-
$FeCl_2$-AA +CB	25	1.632 ±0.110	40.01
$FeCl_2$ -AA +CB	50	1.56± 0.005*	42.49*
$FeCl_2$ -AA +CB	100	1.02± 0.001*	47.07*

Values are the mean ±SDE n=3, ANOVA p≤0.05*, vs. $Fecl_2$- AA control

TABLE NO.2- RESULT OF BIOCHEMICAL ANALYSIS

GROUPS N=5	SGOT ± SEM (U/L)	SGPT ± SEM (U/L)	SAKP ± SEM (U/L)	SB ± SEM (mg/dl)	HP ± SEM µgm
G1	239.6±3.736	129±3.43	157.6±1.88	0.68±0.02	66.4±1.59
G2	242.67±1.75	129.36±1.94	161.6±1.72	0.314±0.178	65.66±1.5
G3	243.27±1.52	130.26±2.10	162.6±1.68	0.652±0.18	66.2±1.25
G4	243.82±10.6	130.68±1.98	163.2±1.72	0.681±0.178	66.8±1.23
G5	312.35±1.39	165.56±2.23	439.2±5.39	1.302±0.1	132.2±2.72
G6	242.2±2.29	133.7±3.28	313.2±5.25	0.604±0.0023	65.84±1.15
G7	232.9±1.78	136.4±1.02	175.2±1.5	0.4902±0.018	66.4±0.654
G8	234.12±5.44	134.6±3.5	313.8±2.52	0.62±0.016	67.35±0.790
G9	241.24±1.5	136.504±0.875	315.4±5.06	0.698±0.01	65.4±1.209

Values are mean ± SD,$P<0.001$. (N=5), students‘t’ test

G5 is significantly different from G1

G5 to G9 are significantly different from G5

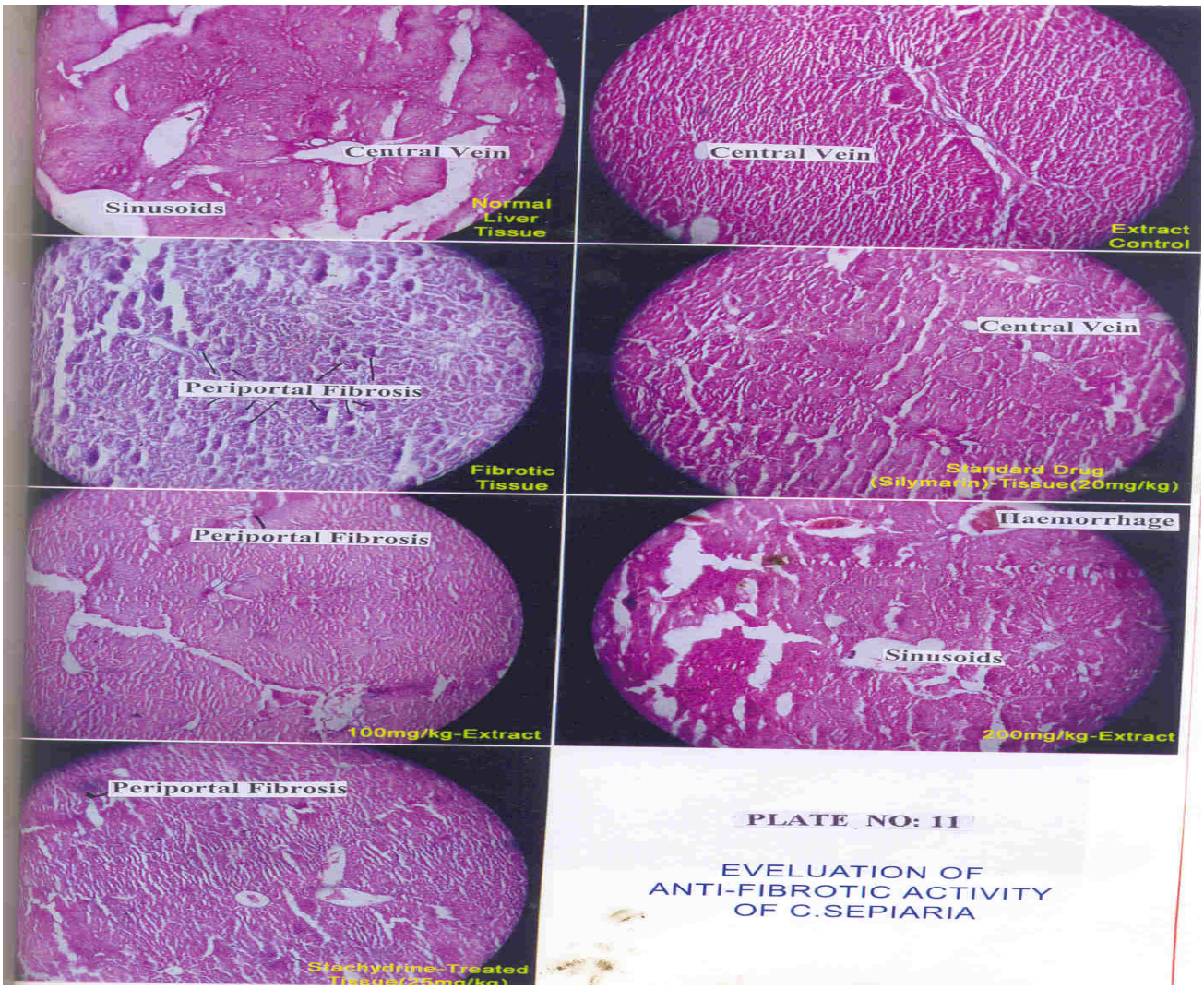

PLATE NO: 11-Evaluation of Anti-Fibrotic Activity of *C.sepiaria* (Linn)

- First section shows - Liver of normal hepatic cells with normal sinusoids and central vein
- Second section shows-extract control, undiseased.
- Third section shows carbon tetrachloride treated rat liver, showing gross necrosis of hepatocytes with nuclear pycnosis, congession, and vasculardegeneration and karyolysis diseased liver image.
- Fourth shows standard silymarin treated (100mg/kg) +carbontetrachloride treated rat liver showing almost normal architecture of liver.
- Fifth and sixth section shows 100 & 200mg/kg extract + carbon tetrachloride treated rat liver showing marked improvement over silymarin treated group.
- Seventh shows stachydrine treated 25mg/kg + carbon tetrachloride treated rat liver showing marked improvement over silymarin treated group.