

Evaluation of hepatoprotective activity of *Capparis brevispina* DC. stem bark

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Abstract

The effect of the ethanol extract of the stem bark of *Capparis brevispina* DC. (CB) was studied against paracetamol (overdose) induced hepatotoxicity in Wistar rats. Significant hepatoprotective effects were obtained against liver damage induced by overdose of paracetamol (Acetaminophen) as evident from decreased serum levels of glutamate pyruvate transaminase (SGPT), glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (SAKP) and bilirubin (SB) in the CB treated groups (250, 500 mg/kg), compared to the intoxicated controls. The hepatoprotective effect was further confirmed by histopathological 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. CB 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.

Keywords: *Capparis brevispina*, Free radical scavenging activity, Hepatoprotection, Histopathology of liver, Paracetamol.

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is likely that when bioactive compounds are found in one species, more species of the same genus may contain active compounds of a similar nature⁴. Hence the present paper deals with the hepatoprotective activity of ethanol extract of stem bark of *C. brevispina*.

Materials and Methods

Plant material

The stem bark of *C. brevispina* was collected from Aryankavu, Kollam, Kerala, during January 2007. The plants were authenticated by the taxonomist of the Institute. A voucher specimen has been deposited at the Herbarium (TBGT 57012 dated 19 January 2007).

Preparation of plant extract

The stem bark of *C. brevispina* was washed thoroughly in tap water, shade-dried and powdered. The powder (100 g) was successively extracted with 1000 ml of ethanol overnight with constant stirring. The filtrate was then concentrated and the solvent was evaporated under reduced pressure in a rotary evaporator. The yield of the extract was found to be 4.13 % (w/v). This crude extract was

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, anaemia, arthritis and gout. They contain considerable amounts of the antioxidant

bioflavonoid, rutin. *Capparis decidua* Edgew. is astringent and used in cardiac troubles and biliousness. *C. spinosa* Linn. is used in splenic, renal and hepatic complaints. It has antitubercular property. Its root bark is used in Ayurvedic preparations like Liv. 52, used to treat acute viral hepatitis and cirrhosis¹. The potent hepatoprotective effects of *C. spinosa* have already been reported².

C. brevispina DC., 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 berries³. It



Capparis brevispina (Habit)



Branches with flowers

referred to as CB. 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 - 270 g) and Swiss albino male mice (18 - 20 g), 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 of the Institute's Animal Ethics Committee.

Behavioural and toxicological effects

Three groups of ten mice were administered with graded doses of the CB extract (500, 1000 and 2000 mg/kg, p.o.). One group was maintained as control and was given 0.5% Tween-80. All

the animals were observed continuously for 1h for any gross behavioural changes and death, if any, and then, intermittently for the next 6h, and then again at 24h after dosing with CB extract.

In vitro antilipid peroxidation studies

The antilipid peroxidant effect of CB was studied *in vitro*, following the modified method of Yoshiyuki *et al*⁵ and Masao *et al*⁶. Briefly, 0.5 g of the rat liver tissue was sliced and homogenized with 10 ml of 15 mM KCl-Tris-HCl buffer (pH 7.2). The reaction mixture was composed of 0.25 ml of liver homogenate, 0.15 ml of Tris-HCl buffer (pH 7.2), 0.1 mM ascorbic acid (AA), 4 mM FeCl₂ and 0.05 ml of various concentrations of CB extract (25, 50 and 100µg/ml). The mixture (in quadruplicate) was incubated at 37°C for 1h in capped tubes. Then, 0.1 N HCl, 0.2 ml of 9.8% sodium dodecyl sulphate (SDS), 0.9ml of distilled water

and 2 ml of 0.6% thiobarbituric acid (TBA) were added to each tube and vigorously shaken. The tubes were placed in a boiling water bath at 100°C for 30 min. After cooling, the flocculent precipitate was removed by adding 5 ml of *n*-butanol and they were centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant was measured at 532 nm.

Paracetamol induced hepatotoxicity

Paracetamol (Acetaminophen; Sigma Chemical Company, U.S.A) was suspended in 0.5% gum acacia and administered p.o., at a dose of 2.5 g/kg. This dose is known to cause liver damage in rats⁷. 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 paracetamol intoxicated control group received a daily dose of 0.5% gum acacia for 4 days and 2 ml of paracetamol suspension (2.5 g/kg, p.o.) on day 3. Groups III and IV animals received a daily dose of CB extract p.o., for 4 days (250 and 500 mg/kg) and 2ml of paracetamol suspension (2.5 g/kg, p.o.) on day 3, 30 min after CB administration. Group V animals received a daily dose of Silymarin (Sigma Chemical Company, USA) at a dose of 100 mg/kg p.o., for 4 days and 2 ml of paracetamol suspension (2.5g/kg, p.o.), 30 min after Silymarin administration. The animals were sacrificed 48 h after paracetamol administration by mild ether anaesthesia. From all the six groups, blood samples were collected separately from carotid artery into sterilized dry centrifuge 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 transaminase⁸ (SGPT), serum glutamate oxaloacetate transaminase⁸ (SGOT), serum alkaline phosphatase⁹ (SAKP) and serum bilirubin¹⁰ (SB) 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 photomicroscopic observations of the liver histological architecture.

Statistical analysis

Statistical comparison between control and treated groups was made using Analysis of Variance, followed by multiple comparisons¹¹.

Results

Behavioural and toxicological effects

In the toxicity study, no mortality occurred during 24 h of observation with the three doses of CB (500, 1000, 2000 mg/kg p.o.), administration. The LD₅₀ of mice was thus observed to be greater than 2000 mg/kg (data not shown).

In vitro antilipid peroxidation studies

In FeCl₂-AA treatment, the rat liver homogenate was induced with

ascorbic acid /Fe²⁺ (FeCl₂-AA) to cause non-enzymatic lipid peroxidation and the action of CB on the system was determined. There was significant increase of malondialdehyde (MDA) in FeCl₂-AA treated rat liver homogenate, compared to normal control without FeCl₂-AA, whereas the ethanolic extract of the stem bark of *C. brevispina* significantly reduced the accumulation of lipid peroxides *in vitro* in a dose dependent manner up to 100 µg/ml (Table 1).

Paracetamol induced hepatotoxicity

Administration of paracetamol (2.5 g/kg, p.o.) caused a significant increase in serum enzymes, namely SGOT, SGPT, SAKP and SB in rats, as compared to normal rats. However, pretreatment with the plant extract, CB prior to paracetamol administration caused a significant reduction in the values of SGOT, SGPT, SAKP and SB dose-dependently up to 250 mg/kg dose, which is almost comparable to the Silymarin (100 mg/kg) treated groups. Further increase in the dose of CB (500 mg/kg) was not effective

in reducing the serum enzyme levels any further (Table 2).

Histopathological studies

The hepatoprotective effect of CB was further confirmed by histopathological examination of the liver samples from the respective groups. Histological architecture of paracetamol treated liver sections showed degeneration of hepatic cells with centrilobular necrosis and karyolysis. However, administration of CB (250 mg/kg) almost normalized these defects in the histological architecture of the liver resembling that of Silymarin treated groups, showing its potent hepatoprotective effects (Figs 1-4).

Discussion

The present study reports the potential hepatoprotective activity of *C. brevispina* against hepatic injury produced by paracetamol, the known hepatic toxin in rats. Paracetamol is a well known analgesic and antipyretic agent, which is safe in therapeutic doses, but deliberate paracetamol overdose is the

Table 1: Inhibitory effect of ethanol extract of *Capparis brevispina* stem bark (CB) on FeCl₂ – ascorbic acid (AA) – induced lipid peroxidation in rat liver homogenate *in vitro*

Treatments	CB 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	1.667 ± 0.010	40.01
FeCl ₂ – AA + CB	50	1.598 ± 0.005*	42.49*
FeCl ₂ – AA + CB	100	1.471 ± 0.001*	47.07*

Values are the mean ± SDE, n = 3, ANOVA * P ≤ 0.05 vs FeCl₂ – AA Control

Table 2: Effect of ethanol extract of *Capparis brevispina* stem bark (CB) on rat serum parameters after overdose paracetamol administration

Groups	SGPT (IU/l)	SGOT (IU/l)	SAKP (KA Units/100 ml)	SB (mg %)
Normal control (10% Tween – 80)	43.5 ± 2.3	41.5 ± 1.2	69.0 ± 1.1	0.338 ± 0.001
Paracetamol control (paracetamol, 2.5g/kg)	200.5 ± 1.3	202.5 ± 2.3	245.5 ± 2.3	0.884 ± 0.003
Paracetamol (2.5g/kg) + CB (250 mg/kg)	56.0 ± 2.1**	60.0 ± 1.1**	70.0 ± 2.6**	0.390 ± 0.001**
Paracetamol (2.5g/kg) + CB (500 mg/kg)	91.0 ± 1.7**	96.5 ± 2.7**	101.0 ± 1.2**	0.559 ± 0.007**
Paracetamol (2.5g/kg) + Silymarin (100 mg/kg)	66.0 ± 2.3**	58.0 ± 1.1**	49.5 ± 1.3**	0.325 ± 0.002**

Values are the mean ± SDE, n = 6, ANOVA **P ≤ 0.01 compared to paracetamol control

most common cause of drug induced liver diseases, resulting in fatal hepatic necrosis in man, rats and mice. Paracetamol is metabolised primarily in the liver. It is mostly converted to inactive compounds via Phase II metabolism by conjugation with sulfate and glucuronide, which are excreted by the kidneys. Only a small, yet significant amount is metabolised *via* the hepatic Cytochrome P₄₅₀ enzyme system, which is responsible for the toxic effects of paracetamol due to a highly-reactive intermediary metabolite, *N*-acetyl-*p*-benzo-quinone imine (NAPQI)¹²⁻¹⁴, which at therapeutic dose, is quickly detoxified by combining irreversibly with the sulfhydryl groups of glutathione or administration of a sulfhydryl compound such as *N*-acetylcysteine, to produce a non-toxic conjugate that is eventually excreted by the kidneys¹⁵. As the dose of paracetamol increases, the sulfate and glucuronide pathways become saturated, and more paracetamol is shunted to the Cytochrome P₄₅₀ system to produce NAPQI. As a result, hepatocellular supplies of glutathione become exhausted and NAPQI is free to react with cellular membrane molecules, resulting in widespread hepatocyte

damage and death, leading to acute hepatic necrosis¹².

An obvious sign of hepatic injury is the leaking of cellular enzymes into the plasma¹⁶, due to the disturbances caused in the transport functions of hepatocytes¹⁷. 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 enzymes is a useful quantitative marker of the extent and type of hepatocellular damage.

Pretreatment of the rats with 250 and 500 mg/kg, *p.o.*, of CB extract for 4 days before paracetamol administration resulted in a significant protection of paracetamol-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 hepatocytes¹⁸.

The hepatoprotective effect of CB was further confirmed by histopathological examination of the liver. The histological observations proved the

effect of CB in preventing hepatocellular necrosis or mononuclear infiltration, as reported by Dhuley and Naik¹⁹. CB administration resulted in bringing about an almost normal histological architecture of the liver.

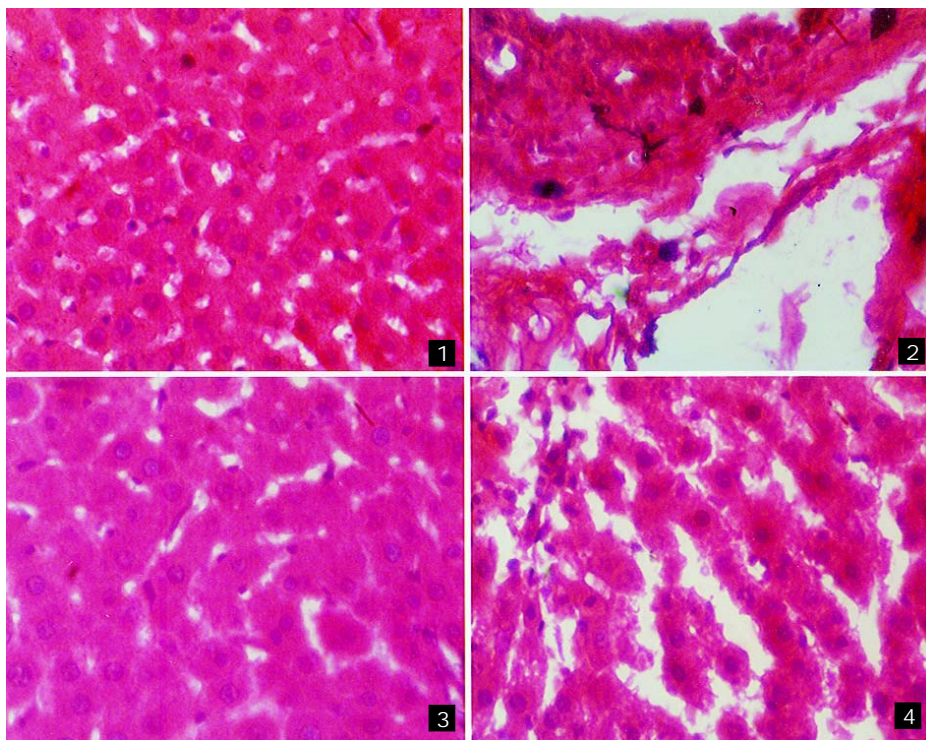
Lipid peroxidation is a complex and natural deleterious process. The effects of free radicals on human beings have recently been considered as their close relation to toxicity, diseases and aging^{20, 21}. Liver is under constant threat of oxidants, especially hydrogen peroxide. The peroxidative property of hydrogen peroxide can be justified as the formation of free radicals with Fe²⁺/ascorbic acid.

Fe²⁺/ascorbic acid forms an important tool for the study of *in vitro* lipid peroxidation in terms of thiobarbituric acid reactive substances (TBARS) formation. These free radicals could further attack the phospholipid of membrane causing lipid peroxidation. The fact remains that the generation of free radical imposes depletion of anti-oxidants such as glutathione (reduced). However, oxidative stress results in toxicity when the rate at which the free radicals are generated exceeds the cell's capacity for their removal. Malondialdehyde (MDA)

is one of the end products in the lipid peroxidation process²². The amount of malondialdehyde formed was quantified by reaction with thiobarbituric acid (TBA) and used as an index of lipid peroxidation. In the present study, the significantly elevated level of lipid peroxides observed in the liver tissue treated with FeCl₂-ascorbic acid indicate excessive formation of free radicals and activation of the lipid peroxidation system resulting in hepatic damage. The significant decline in the lipid peroxide content in the liver tissue treated with FeCl₂-AA + CB indicated the anti-lipid peroxidative effect of CB.

C. spinosa a closely related species of *C. brevispina* has been reported to have hepatoprotective activity²³ due to the presence of flavonoids like quercetin and quercitroside²⁴. P-methoxybenzoic acid isolated from the ethanolic extract of aerial parts of *C. spinosa* was found to exhibit antihepatotoxic activity against paracetamol induced hepatotoxicity². Previous phytochemical studies on *C. spinosa* have shown the presence of alkaloids, lipids, polyphenols, flavanoids and indole and aliphatic glucosinolates²⁵. The presence of several quercetin and kaempferol glycosides, as well as of hydroxycinnamic acids, have also been demonstrated in *Capers*²⁶. It has been also reported that Liv.52, a powerful and popular hepatic stimulant²⁷ contains 24 % *C. spinosa* that improves the functional efficiency of the liver²⁸.

Preliminary phytochemical studies in our laboratory have indicated the presence of flavanoids in *C. brevispina* stem bark²⁷. In the present study, it is likely that the flavonoids of CB, may be responsible for the



Figs 1-4: 1. Liver of normal control rat showing hepatic cells with normal nuclei and cytoplasm ($\times 350$); 2. Section of paracetamol treated (paracetamol control group) rat liver, showing gross necrosis of hepatocytes with nuclear pyknosis, congestion, vascular degeneration and karyolysis ($\times 350$); 3. Section of CB (250 mg/kg) + paracetamol treated rat liver, showing marked improvement over paracetamol control group ($\times 350$); 4. Section of Silymarin (100 mg/kg) + paracetamol treated rat liver, showing almost normal architecture of liver ($\times 350$).

hepatoprotective activities of CB. Flavonoids, consumed in large amounts in the daily diet, are known to protect the liver²⁷. Further, phytochemical studies are in progress to isolate, characterize and identify the specific active flavonoids in this plant responsible for liver protection.

Conclusion

In conclusion, the results of the present study demonstrate that the stem bark of *C. brevispina* possesses potent hepatoprotective action on paracetamol (overdose) induced hepatic damage in rats besides significant free radical scavenging activities.

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