

Hepatoprotective Activity of *Cucumis trigonus* Roxb. Fruit against CCl₄ Induced Hepatic Damage in Rats

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Abstract

In India, a number of medicinal plants and their formulations are used to cure hepatic disorders in traditional systems of medicine. No systemic study has been done on protective effect of *Cucumis trigonus* Roxb. (Cucurbitaceae) to treat hepatic diseases. Protective action of *C. trigonus* fruit extracts was evaluated in this study in animal model of hepatotoxicity, which was induced by carbon tetrachloride. Forty two healthy female albino Wistar rats weighing between 180 and 200 g were divided into seven groups of 6. Group 1 was normal control group; Group 2, the hepatotoxic group was given CCl₄; Group 3 was administered standard drug (Liv-52); Groups 4-7 received pet. ether, chloroform, alcohol and aqueous fruit extract (300 mg/kg) with CCl₄. The parameters studied were alanine transaminase, aspartate transaminase, alkaline phosphatase and serum bilirubin activities. The hepatoprotective activity was also supported by histopathological studies of liver tissue. Results of the biochemical studies of blood samples of CCl₄ treated animals showed significant increase in the levels of serum enzyme activities, reflecting the liver injury caused by CCl₄. Whereas blood samples from the animals treated with chloroform and aqueous fruit extracts showed significant and alcohol extract showed highly significant decrease in the levels of serum markers, indicating the protection of hepatic cells. The results revealed that alcoholic fruit extract of *Cucumis trigonus* could afford highly significant protection against CCl₄ induced hepatocellular injury.

Keywords: Hepatoprotective; Hepatotoxicity; CCl₄; *Cucumis trigonus*; Liver.

Introduction

Liver disease is a worldwide problem. Liver is an organ of paramount importance as it plays an essential role in maintaining the biological equilibrium of vertebrates (1). Additionally, it is the key organ of metabolism and excretion is continuously and variedly exposed to xenobiotics because of its strategic placement in the body. The toxins absorbed from the intestinal tract

gain access first to the liver resulting in a variety of liver ailments. Thus liver diseases remain one of the serious health problems (2). Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects (3-5).

Therefore, many folk remedies from plant origin are evaluated for its possible antioxidant and hepatoprotective effects against different chemical-induced liver damage in experimental animals. CCl₄-induced hepatotoxicity model is frequently used for the investigation of hepatoprotective effects of drugs and plant

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extracts. The changes associated with CCl₄-induced liver damage are similar to that of acute viral hepatitis (6).

Cucumis trigonus known as 'Bitter gourd' is a plant belonging to the Cucurbitaceae family and is indigenous to india, ceylon, malaya, north australia, afghanistan and persia (7). In Indian traditional system of medicine the fruit pulp of the plant is used as expectorant, liver tonic, stomachic and purgative. The fruit pulp is useful in leprosy, jaundice, diabetes, bronchitis and amentia (8). No systematic study has been done on protective efficacy of *Cucumis trigonus* to treat hepatic diseases. Therefore, the protective action of *Cucumis trigonus* fruit extracts was evaluated in an animal model of hepatotoxicity induced by carbon tetrachloride.

Experimental

Plant extract

The fruits of *Cucumis trigonus* Roxb. were collected in August 2008 from Beed, Maharashtra State, India. The plant was authenticated by Dr Harsha Hegde Research officer, regional medical research centre, belgaum, karnataka, india and the voucher specimen has been deposited in the herbarium of the Department of Pharmacognosy and Phytochemistry, K.L.E.S's College of Pharmacy, Belgaum, Karnataka, India. The collected fruits were shade dried at room temperature. The two hundred grams of dried powdered fruits of *Cucumis trigonus* were extracted by continuous hot extraction process using soxhlet apparatus with petroleum ether, chloroform and alcohol. The powder was finally macerated with chloroform-water IP (*As per Indian Pharmacopeia*). The extracts were filtered and concentrated under reduced pressure and low temperature (40°C) on a rotary evaporator.

Phytochemical analysis

The extracts of the plant material were screened for various classes of natural products using standard qualitative methods as described by Harborne (9).

Experimental animals

Female albino Wistar rats weighing between 180 and 200 g were obtained from animal

house, Department of Livestock Production, Government Veterinary College, Hebbal, Bangalore, India. Animals were maintained on a standard laboratory diet. Food and water were given *ad libitum*. They were housed in standard stainless-steel cages at a 12 h cycle of light and dark. Room temperature was kept at 22 ± 2°C and humidity maintained at 50%. All the chemicals used were of the analytical grade from standard companies.

Treatment of animals

Rats were randomly divided into 7 groups with 6 animals in each group. Group 1 served as negative control and was administered a single daily dose of distilled water by oral gavage for seven days. Liver damaged was induced by administration of CCl₄ (2 mL/kg, IP as 50 : 50 solution in olive oil) on 1st, 4th and 7th day to the animal of remaining group. Group 2 received only CCl₄, group 3 received CCl₄ and standard reference Liv-52 4 mL/kg. p.o. for 7 days. The drug control groups (4, 5, 6 and 7) were given the plant extracts orally in doses of 300 mg/kg/0.2 mL (in distilled water), respectively, one hour after the administration of carbon tetrachloride, for 7 days (10, 11). Twenty four h after CCl₄ injection animals were anaesthetized by light ether anaesthesia and blood was collected from the vena cava, and the serum was separated for subsequent use for different enzyme measurements. The rats were then decapitated and the livers were carefully dissected and cleaned of extraneous tissues. Part of the liver tissue was immediately transferred to 10% formalin for histopathological assessments.

Assessment of liver damage

Liver damage was assessed by the estimation of serum activities of AST, ALT, ALP and total bilirubin according to the method of Reitman, Kind and Mally by using commercially available test kits (12-14).

The livers were removed from the animals and the tissues were fixed in 10% formalin for at least 24 h. Then, the paraffin sections were prepared (Automatic tissue processor, Autotechnique) and cut into 5 µm thick sections using a rotary microtom. The sections were then stained with Haematoxylin-Eosin dye and

Table 1. Effect of different extracts of *C. trigonus* on serum activities of AST, ALT, ALP and Bilirubin of CCl₄ intoxicated rats.

Group	Treatment	AST	ALT	ALP	Bilirubin
1.	Control	104.0 ± 0.258	51.17 ± 0.307	118.2 ± 0.307	0.330 ± 0.002
2.	CCl ₄	935.5 ± 0.670 [†]	642.5 ± 0.619 [†]	198 ± 0.004 [†]	1.198 ± 0.004 [†]
3.	Liv 52	127.2 ± 0.401 [*]	63.00 ± 0.447 [*]	127.0 ± 0.447 [*]	0.416 ± 0.004 [*]
4.	Pet.ether extract	663.7 ± 1.358	205.8 ± 0.909 [*]	191.0 ± 0.894 [*]	0.886 ± 0.008 [*]
5.	Chloroform extract	67.5 ± 0.500 [*]	77.50 ± 0.500 [*]	142.8 ± 0.477 [*]	0.571 ± 0.007 [*]
6.	Alcohol extract	141.8 ± 0.401 [*]	69.17 ± 0.401 [*]	131.7 ± 0.333 [*]	0.443 ± 0.006 [*]
7.	Aqueous extract	280.5 ± 0.428 [*]	87.17 ± 0.542 [*]	157.5 ± 0.562 [*]	0.670 ± 0.002 [*]

Values are mean ± SEM, N = 6, [†]p ≤ 0.001 vs. normal control, ^{*}p ≤ 0.001 vs. CCl₄ control
Units of AST, ALT and ALP activity are U/L and Bilirubin is mg/dL.

studied for histopathological changes, such as necrosis, fatty changes, ballooning degeneration and lymphocyte infiltration. Histological damages were scored as: Ø, absent; +, mild; ++, moderate; +++, severe; +++++, extremely severe (15).

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA), followed by the Tukey's test for individual comparisons using SPSS software and p ≤ 0.01 was regarded as significant.

Results

The yield of dried extract was pet. ether (40-60°C) 10.4 (%), chloroform 2.25 (%), alcohol 1.8 (%) and aqueous 11.7 (%). The alcohol and chloroform extracts were found to be positive for the presence of steroids, triterpenoids, saponins and glycosides. Aqueous extract was found positive for the presence of carbohydrates, steroid and triterpenoids. However pet. ether extract was found positive for the presence of fats and oils.

Administration of CCl₄ to rats caused a significant elevation in serum activities of AST, ALT, ALP and serum bilirubin after 24 h. Treatment of rats with 300 mg/kg dose of the *Cucumis trigonus* alcoholic, chloroform and aqueous extracts (p.o.) markedly prevented CCl₄- induced elevation of AST, ALT, ALP and bilirubin. However, 300 mg/kg of the petroleum ether extract did not prevent elevation of the enzymes. Serum bilirubin (total) levels were also significantly enhanced by CCl₄ treatment but total bilirubin was remarkably reduced by treatment

with 300 mg/kg of the alcoholic, chloroform and aqueous extracts (p.o.). Liv-52 with a dose of 4 mL/kg also significantly prevented CCl₄-induced elevation of serum AST, ALT, ALP and bilirubin activities (Table 1).

Histopathological examinations of the liver sections of the rats treated with CCl₄ showed centrilobular necrosis, fatty changes, congestion and infiltration of lymphocytes around the central veins. Centrilobular necrosis, which is a more severe form of injury, was markedly prevented by treatment 300 mg/kg doses of alcohol, chloroform and aqueous extract but not 300 mg/kg dose of the petroleum ether extract (Table 2).

Discussion

In Indian system of medicine certain herbs are claimed to provide relief against liver disorders. The claimed therapeutic reputation has to be verified in a scientific manner. In the present study one such drug *Cucumis trigonus* was taken for the study. The chloroform, alcohol and aqueous extract of *Cucumis trigonus* possess significant hepatoprotective activity. However highly significant effect was seen with alcoholic extract against CCl₄ damage. The petroleum ether extract did not protect rat liver against CCl₄ damage.

Our investigation on the extracts showed the presence of steroids, triterpenoids and cardiac glycosides in the alcoholic extract. According to these results, it maybe hypothesized that steroids and triterpenoids, which are present in the alcoholic extract, could be considered responsible for the hepatoprotective activity.

Table 2. Effect of *C. trigonus* extract on histopathological damages induced by CCl₄ in rats.

Microscopic observation	Control	CCl ₄	Liv-52	Pet.erther extract	Chloroform extract	Alcohol extract	Aqueous extract
Fatty changes	+	+++	+	++	+	+	+
Degeneration in hepatic cord	Ø	+++	+	++	+	+	++
Deformation in hepatocytes	Ø	++++	+	+++	+	+	+
Focal necrosis	Ø	Ø	Ø	Ø	+	Ø	+
Centrilobular necrosis	Ø	++++	Ø	+++	Ø	Ø	Ø
Congestion in central vein	Ø	+++	+	++	+	+	+
Congestion in sinusoids	+	+++	+	+++	++	+	+
Infiltration of lymphocytes	Ø	++	+	+	+	+	+

Ø, absent; +, mild; ++, moderate; +++, severe; +++++, extremely severe; rats were injected with determined concentrations of the *C. trigonus* extracts (p.o.) for seven consecutive days before injection of 2 ml/kg CCl₄ (IP). Histopathological damages were assessed as explained under materials and methods.

CCl₄ metabolism begins with the trichloromethyl free radical (CCl₃·) by the action of the mixed function of the cytochrome P₄₅₀ oxygenase system. This free radical, which is initially formed as relatively unreactive, reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical (CCl_{3OO}·). Both radicals are capable of binding to proteins or lipids, or abstracting a hydrogen atom from an unsaturated lipid, thus, initiating lipid peroxidation (16-19). Lipid peroxidation may cause peroxidative tissue damage in inflammation. Therefore, inhibition of the cytochrome P₄₅₀-dependent oxygenase activity could cause a reduction in the level of toxic reactive metabolites and a decrease in tissue injury. On the other hand, an elevation of plasma AST, ALT, ALP and bilirubin activities could be regarded as a sign of damage to the liver cell membrane.

Many compounds known to be beneficial against carbon tetrachloride-mediated liver injury exert their protective action by toxin mediated lipid peroxidation either via a decreased production of CCl₄ derived free radicals or through antioxidant activity of the protective agent themselves (20).

Conclusions

In conclusion, the results indicated that under the present experimental conditions, alcoholic extract of *Cucumis trigonus* fruit showed hepatoprotective effects against CCl₄ induced liver damage in rats.

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References

- (1) Venkateswaran S, Pari L, Viswanathan P and Menon VP. Protective effect of Livex, a herbal formulation against erythromycinestolate-induced hepatotoxicity in rats. *J. Ethnopharmacol.* (1997) 57: 161-167.
- (2) Karan M, Vasisht K and Handa SS. Antihepatotoxic activity of *Swertia chirata* on carbon tetrachloride-induced hepatotoxicity in rats. *Phytother. Res.* (1999) 13: 24-30.
- (3) Latha U, Rajesh MG and Latha MS. Hepatoprotective effect of an ayurvedic medicine. *Indian Drugs* (1999) 36: 470-473.
- (4) Dhuley JN and Naik SR. Protective effect of Rhinax, an herbal formulation against CCl₄-induced liver injury and survival in rats. *J. Ethnopharmacol.* (1997) 56: 159-164.
- (5) Mitra SK, Seshadri SJ, Venkataranganna MV, Gopumadhavan S, Venkatesh Udupa UV and Sarma DN. Effect of HD-03-a herbal formulation in galactosamine-induced hepatopathy in rats. *Ind. J. Physiol. Pharmacol.* (2000) 44: 82-86.
- (6) Rubinstein D. Epinephrine release and liver glycogen levels after carbon tetrachloride administration. *Am. J. Physiol.* (1962) 203: 1033-1037.
- (7) Naik, VR, Agshikar NV and Abraham JS. *Cucumis trigonus* Roxb. II. Diuretic activity. *J. Ethnopharmacol.* (1981) 3: 15-19.
- (8) Arya VS. (ed.) *Indian Medicinal Plants, a Compendium*

- of 500 Species. Orient Longman Ltd., Madras (1994) 235-36.
- (9) Harborne JB. *Phytochemical Methods*. Chapman & Hall, New York (1973) 1-150.
- (10) Kujawska M, Jodynis-Liebert J, Ewertowska M, Adamska T, Matlawska I and Bylka W. Protective effect of *Aquilegia vulgaris* (L.) on carbon tetrachloride-induced oxidative stress in rats. *Indian J. Exp. Biol.* (2007) 45: 702-11.
- (11) Shefalee KB, Paulomi J, Mamta BS and Santani DD. Investigation into hepatoprotective activity of *Citrus limon*. *Pharm. Biol.* (2007) 45: 303-11.
- (12) Reitman S and Frankel S. *In-vitro* determination of transaminase activity in serum. *Am. J. Clin. Pathol.* (1975) 28: 56.
- (13) Kind PRN and King D. *In-vitro* determination of serum alkaline phosphatase. *J. Clin. Pathol.* (1972) 7: 322.
- (14) Mally HT and Evelyn KA. Estimation of serum bilirubin level. *J. Biol. Chem.* (1937) 191: 481.
- (15) Akram J, Mohammad JK, Zahra D and Hossein N. Hepatoprotective activity of *Cichorium intybus* L. leaves extract against carbon tetrachloride induced toxicity. *Iranian J. Pharm. Res.* (2006) 1: 41-46.
- (16) Brattin WJ, Glende Jr. EA and Recknagel RO. Pathological mechanisms in carbon tetrachloride hepatotoxicity. *J. Free Rad. Biol. Med.* (1985) 1: 27-38.
- (17) Gosselin RE, Smith RP and Hodge HC (eds.). Carbon tetrachloride. *Clinical Toxicology of Commercial Products*. Williams and Wilkins, Baltimore (1984) 101-107.
- (18) Recknagel RO, Glende Jr. EA, Dolak JA and Waller RL. Mechanisms of carbon tetrachloride toxicity. *Pharmacol. Ther.* (1989) 43: 139-154.
- (19) Lee KJ and Jeong HG. Protective effect of platycodi radix on carbon tetrachloride-induced hepatotoxicity. *Food Chem. Toxicol.* (2002) 40: 517-525.
- (20) Hewawasam RP, Jayatilaka KAPW, Pathirana C and Mudduwa LKB. Hepatoprotective effect of *Epaltes divaricata* extract on carbon tetrachloride induced hepatotoxicity in mice. *Indian J. Med. Res.* (2004) 120: 30-34.

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