Asian Journal of Chemistry Vol. 19, No. 4 (2007), 2550-2554

Evaluation of Hepatoprotective Effects of *Coccinia grandis* Linn. Against Carbon Tetrachloride Induced Liver Damage in Wistar Rat

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The fruits of *Coccinia grandis* Linn. are used by the tribes of Karnataka, for the treatment of various hepatic disorders. In the present study, the effect of the methanolic extract of *Coccinia grandis* Linn. fruits on carbon tetrachloride (CCl_4)-induced liver damage in Wistar rats was studied. The results showed that significant hepatoprotective effect was obtained against CCl_4-induced liver damage, by oral administration of *Coccinia grandis* Linn methanolic extract as evident from decreased levels of serum enzymes in the treated groups, compared to the controls. Thus, the present study provides a scientific rationale for the traditional use of this plant in the management of liver diseases.

Key Words: *Coccinia grandis* Linn., Carbon tetrachloride, Hepatoprotecive activity.

INTRODUCTION

Liver, the largest organ in vertebrate body, plays a major role in intense metabolic activities like detoxification and excretion of many exogenous and endogenous compounds[1]. Liver injury, caused by toxic chemicals and certain drugs, has been recognized as a toxicological problem. In the absence of reliable liver protective drugs in modern system of allopathic medical practice, herbal drugs are playing an important role in

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health care programmes worldwide and there is a resurgence of interest in herbal medicines for treatment of various hepatic ailments[2].

Coccinia grandis Linn., (Cucurbitaceae) is an perennial, much branched handsome tendril climber grows abundantly in India, particularly in Bengal, Andhra Pradesh and Karnataka. Surveys conducted in the tribal areas of Karnataka revealed the use of the plant in the treatment of jaundice and snake bite. *Coccinia grandis* has been reported to possess aphrodisiac, anorexient, antipyretic and expectorant activities[3]. The plant has the reputation in Bengal for having a remarkable effect in reducing the amount of sugar in the urine of diabetic patients. It has been considered as the Indian substitute for insulin[4]. Apart from the tribal use of the plant in the treatment of jaundice, no systematic scientific study has been undertaken to evaluate hepatoprotective activity of the plant. Hence, an attempt was made to assess the plant for hepatoprotective activity against CCl_4 induced hepatotoxicity in rats.

EXPERIMENTAL

The fruits of *Coccinia grandis* were collected from the surrounding fields of Harapanahalli in the month of December, 2004 and were authenticated by Prof. K. Prabhu, Department of Botany, T.M.A.E. Science institute, Harapanahalli. A voucher specimen (No.Pharmcog/Scscp-72/2003) was preserved at departmental herbarium. The dried powder (100 g) of fruits was extracted with 1000 mL methanol overnight, with constant stirring. The extract was filtered and the filtrate, concentrated under reduced pressure to yield 900 mg of the crude extract (0.9 %, w/w, with respect to the dried plant material). This crude extract was referred to as CG. The crude extract was suspended in 0.5 % Tween-80, to required concentrations and used for the experiments.

Experimental animals: Wistar albino rats, males (150-200 g) obtained from the Institute animal house of the institute were used for the present study. They were housed under standard conditions and fed commercial rat feed (Lipton India Ltd, Mumbai, India) and boiled water *ad libitum*. All experiments involving animals were done after getting the approval of the Institute's Animal Ethics Committee. [Reg No: 157/1999/CPCSEA/HPR].

CCl_4-induced hepatotoxicity studies: Rats were divided into six groups (six per group). **Group I**, the normal control group animals were administered p.o., a single daily dose of 0.5 % Tween-80 (1 mL) on all 5 d and olive oil (1 mL/kg) s.c., on days 2 and 3. **Group II**, the CCl_4 control group animals were administered a single daily dose of 0.5 % Tween-80 (1 mL) p.o., on all 5 d and on the second and third day, they were administered s.c., CCl_4:olive oil (1:1). **Group III** animals were administered CG (100 mg/kg) p.o., on all 5 d and a single dose of CCl_4 (2 mL/kg) s.c., on

days 2 and 3, 0.5 h after CG administration. **Group IV** animals were administered CG (200 mg/kg) p.o., on all 5 d and a single dose of CCl_4 (2 mL/kg) s.c., on days 2 and 3, 0.5 h after CG administration. **Group V** animals were administered CG (300 mg/kg) p.o., on all 5 d and a single dose of CCl_4 (2 mL/kg) s.c., on days 2 and 3, 0.5 h after CG administration. **Group VI** animals were administered Silymarin, the known hepatoprotective compound (Sigma Chemical Company, USA), at a dose of 100 mg/kg p.o., on all 5 d and a single dose of CCl_4 (2 mL/kg) s.c., on days 2 and 3, 0.5 h after Silymarin administration. On the fifth day, all the animals were sacrificed by mild ether anaesthesia. Blood samples were collected for evaluating the biochemical parameters.

Biochemical estimations: Biochemical parameters like serum enzymes: serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase (SAKP) and serum bilirubin (SB)[5-8] serum bilirubin, cholesterol and total protein[9-11] were assayed according to standard methods.

Statistical analysis: The result of each parameter was reported as Mean ± SEM. The reduction in biochemical parameters was calculated by considering the difference in biochemical parameter between hepatotoxin treated and control group to determine significant inter group differences of all treated groups with control group. Statistical significance was analyzed employing one-way Anova followed by Post-hoc Dunnett's test. Values at $p < 0.001$ were considered significant.

RESULTS AND DISCUSSION

Administration of CCl_4 to rats caused significant increase in serum enzymes like AST, ALT, SAKP and SB, compared to normal control rats. Treatment with CG caused significant reduction of these values (Table-1), dose-dependently, almost comparable to the Silymarin treated group. Preventive action of liver damage by CCl_4 has been widely used as an indicator of liver protective activity of drugs in general since the changes associated with CCl_4-induced liver damage are similar to that of acute viral hepatitis. CCl_4-mediated hepatotoxicity was chosen as the experimental model. It has been established that CCl_4 is accumulated in hepatic parenchyma cells and metabolically activated by cytochrome P450-dependent monooxygenases to form a trichloromethyl radical ($^{\bullet}CCl_3$)[12]. The $^{\bullet}CCl_3$ radical alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage. AST, ALT, SAKP and SB are the most sensitive tests for diagnosis of liver diseases. Hepatotoxic compounds such as CCl_4 are known to cause marked elevation in serum enzymes and bilirubin levels. The present study revealed a significant

increase in the activities of AST, ALT, SAKP and SB within 48 h of exposure to CCl_4, indicating considerable hepatocellular injury. Administration of CG methanolic extract attenuated the increased levels of the serum enzymes, produced by CCl_4 and caused a subsequent recovery towards normalization almost like that of Silymarin treatment as reported by Castro *et al*[13]. This suggested the possibility that CG extract is able to condition the hepatocytes, so as to cause accelerated regeneration of parenchyma cells, thus protecting against membrane fragility and decrease of leakage of the marker enzymes into the circulation. Silymarin is a known hepatoprotective compound. It is reported to have a protective effect on the plasma membrane of hepatocyte. The present finding that treatment with 200 mg/kg of CG for 5 d significantly reduced CCl_4-induced damage in rat liver, therefore, justifies the therapeutic dosage of the drug employed by the tribe.

TABLE-1
EFFECT OF *Coccinia grandis* (CG) FRUIT METHANOLIC EXTRACT ON RAT SERUM ENZYMES AFTER CCl_4 ADMINISTRATION[a]

Groups	AST (IU/l)	ALT (IU/l)	SAKP (KA units/ 100 mL)	SB (IU/l)	Serum cholesterol (mg/dl)	Total protein (mg/dl)
Group-I Normal control	85.6 ± 10.30	34.2 ± 4.30	49.3 ± 2.71	0.25 ± 0.01	157.67 ± 1.05	9.57 ± 0.24
Group-II CCl_4 control	255.2 ± 18.50	180.6 ± 10.20	95.3 ± 10.31	5.00 ± 1.50	272.33 ± 1.94	5.05 ± 0.13
Group-III CCl_4 +CG (100 mg/kg)	200.1 ± 12.00	120.3 ± 9.80	60.3 ± 6.30	3.50 ± 1.30	182.00 ± 2.97	8.71 ± 0.15*
Group-IV CCl_4 +CG (200 mg/kg)	90.0 ± 9.23	41.2 ± 7.91	55.2 ± 7.26	1.70 ± 1.20	265.50 ± 3.24	5.45 ± 0.15
Group-V CCl_4 +CG (300 mg/kg)	91.0 ± 8.21	40.3 ± 6.26	53.1 ± 6.21	1.68 ± 0.99	195.67 ± 1.22	7.85 ± 0.10*
Group-VI CCl_4 + Silymarin (100 mg/kg)	89.3 ± 10.20	39.3 ± 9.70	52.3 ± 4.72	1.30 ± 0.30	200.17 ±1.30	7.98 ± 0.06*

[a] Values are the mean ± S.D., $n = 6$.

*Significance $p \leq 0.001$, compared to CCl_4 control.

ACKNOWLEDGEMENT

One of the authors (BMVS) is thankful to Dr.T. Yunus Pasha, Principal, S.C.S. College of Pharmacy, Harapanahalli for providing the facilities to carry out the research work.

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(*Received*: 24 December 2005; *Accepted*: 27 November 2006) AJC-5285