Natural Product Sciences
9(1) : 13-17 (2003)

Hepatoprotective Effect of *Coccinia indica* Against CCl_4 Induced Hepatotoxicity

GM Mohana Rao*, M Vijayakumar, ChV Rao, AKS Rawat, and S Mehrotra

Pharmacognosy and Ethnopharmacology division, National Botanical Research Institute, Rana Pratap Marg, Lucknow INDIA-226001

Abstract – The hepatoprotective effect of the ethanolic extract of *Coccinia indica* fruits in rats treated with carbon tetrachloride. In hepatotoxic rats, liver damage was studied by assessing parameters such as aspartate aminotransferase (AST), alanine aminotransferase (AlT), alkaline phosphatase (AlP) and gamma glutamyl transpeptidase (GGT) in serum, and concentrations of total proteins, total lipids, phospholipids, triglycerides and cholesterol in both serum and liver. The effect of co-administration of ethanolic extract on the above parameters was further investigated. Histopathological study of the liver in experimental animals was also undertaken. Hepatic damage as evidenced by a rise in the levels of AST, AlT, AlP and GGT in serum, and also changes observed in other biochemical parameters in serum and liver showed a tendency to attain near normalcy in animals co-administered with the extract. The normal values for AST (IU/L), AlP (IU/l), protein (g/100 ml) and total lipids (mg/100 ml) in serum (i.e., 20.24, 70.04, 5.72 and 135.54 respectively) were found to alter towards values 32.61, 127.11, 3.83 and 265.91 in hepatotoxic rats. These parameters attained near normal values (i.e., 22.82, 79.30, 5.22 and 151.24 for AST, AlP. protein and total lipids respectively) in ethanolic extract co-administered rats. Profound steatosis, ballooning degeneration and nodule formation observed in the hepatic architecture of CCl_4 treated rats were found to acquire near-normalcy in drug co-administered rats, thus corroborating the biochemical observations. Thus the study substantiates the hepatoprotective potential of ethanolic extract of *Coccinia indica* fruits.

Keywords – hepatoprotection, *Coccinia indica*, carbontetrachloride, histopathology.

Introduction

Liver is the largest and one of the most complex internal organs in the body. It plays an important role in the maintenance of the internal environment through its multiple and diverse functions. It plays a central role in detoxification and excretion of many endogenous and exogenous compounds. Hence, Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem. Herbal drugs are playing an important role in health care programs world wide, and there is a resurgence of interest in herbal medicines for treatment of various ailments including hepatopathy. Various indigenous plants are known to play a vital role in the management of liver disorders but the perusal of literature reveals lack of scientific authenticity of the traditional medicine. Hence the present study was undertaken to fill the lacuna in this regard.

Coccinia indica W. & A. (cucurbitaceae) is a perennial creeping herb with long tapering tuberous roots and grows abundantly in India. The plant occurs both in bitter and non-bitter forms. The green immature fruits of the bitter variety are extremely bitter in taste but they loss their bitterness rapidly during ripening and ultimately become sweet to taste and scarlet in colour. These fruits are reported (Nadkarni 1954, Kirtikar *et al.*, 1933) to be useful in various ailments. The plant is known to possess good hypoglycemic properties (Kumar *et al.*, 1983, Hossain *et al.*, 1992). As there is no reference in literature regarding the hepatoprotective aspects of this plant, in this paper we report the activity of the ethanolic extract of the fruits of the *coccinia indica.* A number of pharmacological and chemical agents act as hepatotoxin and produce variety of liver ailments (Ram *et al.*, 1999). Carbon tetrachloride (CCl_4) intoxication in rats is an experimental model widely used to study necrotic and steatotic changes in hepatic tissue. Accordingly, our experiment was designed to use CCl_4 intoxicated rat liver as a model.

Materials and Methods

Plant materials – The fresh green mature fruits of *Coccinia indica* were collected from the market, Lucknow between the months of July and august 2001. These fruits were identified and authenticated by experts in dept. Pharmacognosy, National Botanical Research Institute, Lucknow. A voucher

*Author for correspondence, E-mail: mmraos@rediffmail.com

specimen has been deposited in the herbarium, NBRI, Lucknow. The collected materials were washed thoroughly in water, chopped and air dried for a week at 35-40°C and pulverized in electric grinder. The powder obtained was successively extracted in petroleum ether (60-80°C), benzene, chloroform, ethanol and distilled water. The extracts were then made to powder by using rotary evaporator under reduced pressure. Fruits of *C. indica* yielded 0.8, 0.9, 2.4, 2.6 and 2.4% w/w powdered extract with petroleum ether, benzene, chloroform, ethanol and distilled water respectively. A pilot study using the various extracts at the dose of 100-mg/kg-body weight revealed the ethanolic extract offering maximum hepatoprotection. Accordingly, powdered ethanolic extract of *C. indica* fruits was prepared in sufficient quantity and stored in refrigerator for further use.

Experimental animals – Twenty-four male albino rats of Sprague-Dawley strain weighing 150-180 g were purchased from Central Drug Research Institute, Lucknow. The animals were housed in polypropylene cages and maintained in controlled temperature (27±2°C) and light cycle (12 h light and 12 h dark). They were fed with Amrut Laboratory Animal Feed [Nav Maharashtra Chakan Oil Mills Ltd, Pune]. Water was supplied *ad libitum.* They were given one-week time to get acclimatized with the laboratory conditions. Initial body weight of each animal was recorded. Ethical clearance for the use of animals was obtained from the committee constituted for the purpose.

Experimental induction of hepatic damage – Liver damage was induced in rats by administering CCl_4 subcutaneously (sc) in the lower abdomen in a suspension of liquid paraffin (LP) in the ratio 1: 2 v/v at the dose of 1 ml CCl_4/kg body weight of each animal. CCl_4 was administered twice a week, on every first and fourth day of all the 13 weeks.

Experimental design – Rats were divided into 3 groups of 8 animals each as follows: Group I animals served as control and received sc administration of LP only at the dose of 3 ml/kg body weight, twice a week for a duration of 13 weeks. Group II animals constituted hepatotoxic rats and received sc administration of LP+CCl_4 twice a week for a total of 13 weeks. Group III animals were the herb-treated animals, received sc administration of LP+CCl_4 as in group II rats along with oral administration of ethanolic extract in a suspension of 1 ml water at the dose of 100 mg/kg body weight daily for 13 weeks. Replenishing a known quantity of fresh food daily at 10.30 a.m. and thereby measuring the food intake of the previous day carried out measurement of daily food consumption. LP, LP+CCl_4 and LP+ CCl_4+ ethanolic extract were administered at the same time between 10-11a.m. Body weight of rats was recorded weekly to assess percentage of weight gain in each group. General well being and behaviour of the animals were observed daily throughout the period of study. The litter in the cage was renewed twice a week to ensure maximum comfort for the animals. Animals were kept starved overnight after 13 weeks. On the next day, after recording the weight in each case, they were sacrificed by decapitation by making an incision on jugular vein to collect blood. The liver tissue was dissected out, blotted off blood, washed in saline and weighed immediately. This was kept in frozen containers and proceeded for bio-chemical estimations.

Biochemical estimations – Serum was separated from the collected blood and subjected to biochemical estimations of different parameters like aspartate aminotransferase (AST, Reitman *et al.*, 1957), alanine aminotransferase (ALT, Reitman *et al.*, 1957), alkaline phosphatase (ALP, Kind *et al* 1957), gamma glutamyl transpeptidase (GGT, Naftalin *et al.*, 1969), total proteins (Lowry *et al.*,1951), total lipids (Fring *et al.*, 1970), triglycerides (Van hendle *et al.*,1957), phospholipids (Varley *et al.*, 1988), and cholesterol. Liver homogenates of all the three groups were also subjected to various biochemical estimations.

Histopathology – A portion of liver tissue in each group was fixed in 10% formasal (formalin diluted to 10% with normal saline) and proceeded for histopathology. After paraffin embedding and block making, serial sections of 5 μ thickness were made, stained with Haematoxylin and Eosin and examined under microscope. A few photomicrographs of representative types were also taken.

Statistical analysis – One-way analysis of variance (ANOVA) followed by Scheffe's test was applied for determining the statistical significance of difference in enzymes, protein and lipid levels between different groups. The level of significance was set at 0.05.

Results

Food consumption and weight gain – We observed that food consumption and weight gain significantly increased in group III animals as compared to other groups. In-group II rats food consumption and weight gain significantly decreased compared to other groups.

Serum marker enzymes – All the marker enzymes, *viz.*, AST, AlT, AlP and GGT registered enhanced activity in CCl_4-treated rats as compared to control group (Table 1). In ethanolic extract co-administered group, the levels of these enzymes were found retrieving to wards normalcy.

Other biochemical parameters – The total protein concentration of the serum and liver was lesser in group II animals, when compared with normal control. (Tables 1

Table 1. Effect of *Coccinia indica* on different biochemical parameters in the serum of rats

Parameter	Group I Control with LP only	Group II LP + CCl_4 only	Group III LP+CCl_4 +EEC
AST (IU /L Serum)	20.24 ± 0.58	32.61 ± 1.34#	22.82 ± 0.76*
ALT (IU/L Serum)	23.62 ± 1.34	60.91 ± 3.68#	31.11 ± 2.61*
ALP (IU/L Serum)	70.04 ± 4.61	127.11 ± 5.24#	79.30 ± 4.04*
GGT (IU/L Serum)	1.92 ± 0.41	23.63 ±0.16#	2.67 ± 0.72*
Total protein (g/100ml)	5.72 ± 0.31	3.83 ± 0.16#	5.22 ± 0.33*
Total lipids (mg/100ml)	135.54 ± 6.16	265.91 ± 9.20#	151.24 ± 6.41*
Triglycerides (mg/100ml)	7.43 ± 0.72	12.14 ± 1.14#	8.91 ± 0.66*
Cholesterol (mg/100ml)	65.93 ± 3.61	98.04 ± 5.02#	68.72 ± 4.01*
Phospholipids (mg/100ml)	120.21 ± 6.91	210.93 ± 14.21#	132.62 ± 9.43*

# P < 0.05and * < 0.05 compared to control group.Values expressed as mean ± SEM for 8 animals.
LP = Liquid paraffin; CCl_4 = Carbon Tetrachloride; EEC = Ethanolic extract of fruits of *C. indica.*

Table 2. Effect of *Coccinia indica* on different biochemical parameters in the liver of rats

Parameter	Group I control with LP only	Group II LP + CCl_4 only	Group II LP + CCl_4 + EEC
Total protein (g/100g)	6.48 ± 0.38	3.91 ± 0.23#	6.68 ± 0.31*
Total lipids (mg/100g)	6289 ± 641	8431 ± 199#	6765 ± 119*
Triglycerides (mg/100g)	612.2 ± 84.1	901.6 ± 56.7#	717.1 ± 43.6*
Cholesterol (mg/100g)	601.9 ± 29.1	865.2 ± 36.1#	661.8 ± 31.5*
Phospholipids (mg/100g)	2409 ± 102.8	1477 ± 62.6#	2215 ± 103.2*

#P<0.05and *<0.05 compared to control group.Values expressed as mean ± SEM for 8 animals. LP = Liquid paraffin; CCl_4 = Carbon Tetrachloride;EEC = Ethanolic extract of fruits of *C. indica.*

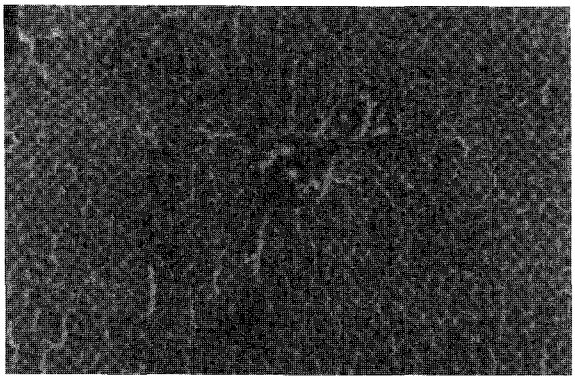

Fig. 1. Liver section from normal rat showing normal liver architecture with hepatic lobules

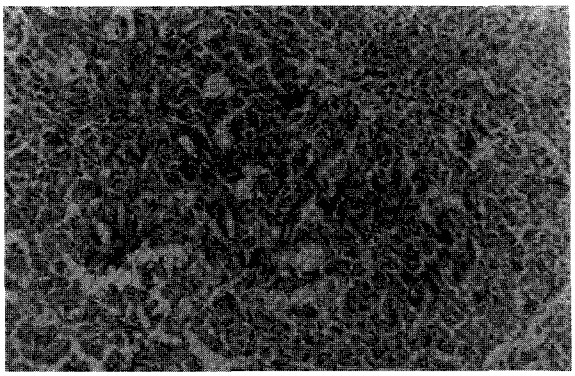

Fig. 2. Liver section from CCl_4 treated rat showing severe steatosis and focal necrosis. Central vein with minimal perivenular fibrosis

and 2) and it attained an almost normal value in group III rats. The level of total lipids, triglycerides and cholesterol in serum as well as liver recorded significant increment in CCl_4 administered rats as compared to those of group I. All these bio-chemical changes showed signs of returning towards the normalcy in group III animals. There was a significant decline in the concentration of phospholipids in liver tissues of CCl_4-treated rats as compared to normal control. In group 3 animals phospholipid concentration attained normalcy.

Histopathological observation – Histopathological study of liver from group I animals showed a normal hepatic architecture (Fig. 1). In CCl_4 treated group, severe hepatotoxicity was evidenced by profound steatosis, centrilobular necrosis, ballooning degeneration, nodule formation and fibrosis (Fig. 2).

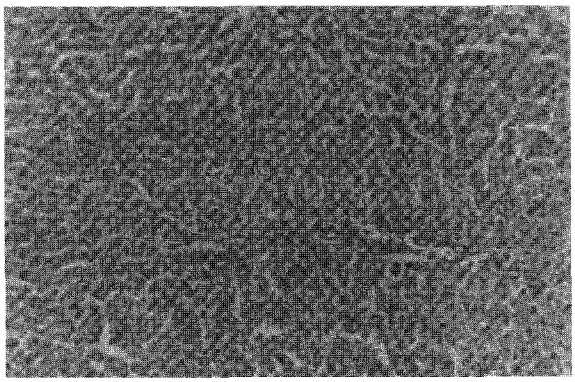

Fig. 3. Liver section from CCl_4 + *C. indica* treated rats showing almost normal liver architecture.

In-group III animals, the liver exhibited an almost normal architecture, barring a little deformity of hepatocytes with pyknosis and clearing of cytoplasm (Fig. 3).

Discussion

The changes associated with CCl_4 induced liver damage are similar to that of acute viral hepatitis. CCl_4, a widely used experimental hepatotoxicant, is biotransformed by the cytochrome P-450 system to produce the trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb Ca^{2+} homeostasis, and finally result in cell death (Reckengel *et al.*, 1989).

Animals of group II (received CCl_4 alone) significantly lost their body weight and showed reduced food consumption as compared to control. Animals of group III which received both (CCl_4 + ethanolic extract) showed a significant increase in body weight and food consumption when compared to group II, and the values were even significantly higher than that of group I rats. These findings suggested that ethanolic extract administration has significantly neutralised the toxic effect of CCl_4 and helped in regeneration of hepatocytes. These observations are in perfect conformity with that of Farooq *et al.*, 1997.

Estimating the activities of serum marker enzymes, like AST, AlT, AlP and GGT, can make assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage (Mitra *et al.*, 1998). The enhanced activities of these serum marker enzymes observed in CCl_4-treated rats in our study correspond to the extensive liver damage induced by the toxin. The tendency of these enzymes to return towards a near normal level in group III rats is a clear manifestation of anti-hepatotoxic effect of ethanolic extract of fruits of *Coccinia indica.*

The site specific oxidative damage of some of the susceptible aminoacids of proteins is now regarded as the major cause of metabolic dysfunction during pathogenesis (Uday B *et al.*, 1999). Hypoalbuminaemia is most frequent in the presence of advanced chronic liver diseases. Hence decline in total protein content can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases. The lowered level of total proteins recorded in the serum as well as liver of CCl_4 treated rats reveals the severity of hepatopathy. The attainment of near normalcy in the total serum and liver of herb treated rats further elucidates the hepatoprotective effect of *C. indica.* Treatment with CCl_4 increases the levels of total lipids, total triacyl glycerols and total cholesterol in the liver (Seakins *et al.*, 1963). Presence of significantly high concentration of total lipids and cholesterol in the serum and liver tissue of group II animals and its recovery towards near normal values in ethanolic extract administered rats coincides with the above observations, thus unearthing the hepatoprotective effect of *C. indica* once again. Hepatotoxins like CCl_4 can interfere with the hepatic phospholipid synthesis (Recknagel *et al.*, 1967). The phospholipid content in serum registered a significant hike and that of liver showed a diminution in CCl_4 administered group, which was retrieved to near normalcy in ethanolic extract treated rats. This observation also indicates the hepatoprotective potential of *C. indica.*

A comparative histopathological study of liver from different groups further corroborated the hepatoprotective efficacy of *Coccinia indica.* Works are in progress here to unravel the antioxidant activity of *C. indica* and also to isolate and purify the active principle involved in hepatoprotection of this promising plant.

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(Accepted January 13, 2003)