IJPSR (2011), Vol. 2, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 10 June, 2011; received in revised form 09 August, 2011; accepted 29 September, 2011

PROTECTIVE EFFECT OF COCCINIA INDICA LEAF EXTRACT AGAINST ALCOHOL COMBINED WITH CARBON TETRACHLORIDE AND PARACETAMOL INDUCED LIVER DAMAGE IN RATS

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Keywords:

Hepatoprotective effect, *Coccinia indica*, Alcohol, Carbon tetrachloride, Paracetamol

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ABSTRACT

Liver is a major metabolic organ affected by various toxins and chemicals. So the potential therapeutic agent is needed for the treatment of liver diseases. Natural products are the best source of remedies for the treatment of all diseases.The aim of our study was to investigate the effect of leaf extract of *Coccinia indica* against Alcohol combines with CCl_4 and Paracetamol induced hepatotoxicity.The effects of oral treatment with *Coccinia indica* (CI) leaf extracts (100mg/kg and 200mg/kg for 7 days) were studied on hepatic damage induced by alcohol (40% alcohol 2.0ml/100g, p.o. for 21 days) and CCl_4 (0.1ml/kg, s.c. on 20th day) and also with paracetamol (750mg/kg ip.) in rats. Biochemical parameters in serum like glutamate oxaloacetate transaminase (SGOT), total Billirubin (TB), Alkaline phosphatase (ALP) and total proteins (TP) were estimated to assess the liver function. Alcohol – CCl_4 and paracetamol treatment produced an increase in SGOT, ALP, Total billirubin and decrease in total proteins indicating the liver damage. These effects were progressively reduced (SGOT, ALP and Total billirubin) and increased (Total proteins) by treatment doses of (100mg/kg and 200mg/kg) CI leaf extracts. These biochemical observations were supplemented by histopathological examination of liver sections. CI leaf extract protected the liver from alcohol-CCl_4 and paracetamol induced hepatic damage.

INTRODUCTION: Liver has a pivotal role in regulation of physiological processes. It is involved in several biochemical pathways related to growth, nutrient supply, metabolism, secretion and storage. Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, CCl_4, excess consumption of alcohol, high doses of paracetamol and infections). Nearly 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity [4, 6]. Only a small portion of the hepatoprotective plants as well as formulations used in traditional medicines are pharmacologically evaluated for their efficacy.

Several plants were reported as hepatoprotective against hepatotoxicity in animals. Some of the polyherbal formulations are verified for their hepatoprotective action against chemical induced liver damage in experimental animals [5, 8].

Although, rats orally fed with alcohol develop fatty changes in the liver, they do not develop the more severe forms of liver injury seen in humans, namely

hepatitis and cirrhosis possibly due to a short life span[10] Hence to stimulate the human model CCl_4 was included in the study to cause hepatocellular necrosis in alcohol fed rats. Because strong experimental evidence that ethanol may potentiate the hepatotoxic effects of CCl_4 [18, 21].

Paracetamol toxicity is major course of acute liver failure. Paracetamol is quite safe and well tolerated in therapeutic doses. However at toxic dose (750mg/kg), paracetamol produces acute liver failure [3, 7].

Coccinia indica Linn. (CI) (cucurbitaceae) widely distributed throughout India. Different parts of this plant have been reported to exhibit several medicinal properties. Pharmacological properties like anti-inflammatory, anthelmintic and diabetes mellitus [2, 13, 16, 17] have been reported by several workers. Many studies have focused on the hepatoprotective activity of coccinia grandis leaves [12, 14].

To our knowledge no study has investigated the liver protection against Alcohol combines with CCL4 and paracetamol induced hepatotoxicity in rats.

The present investigation has been designed to study the effect of leaf extract of *coccinia indica* against Alcohol combines with CCl_4 and Paracetamol induced hepatotoxicity.

MATERIALS AND METHODS: The leaves of Coccinia Indica were collected during the month of march in Chidambaram,Tamilnadu,India The voucher specimen was deposited in the Department of Botany, Annamalai University, Chidambaram, Tamil Nadu, India. The material was dried in shade, they were powdered and Extracted with methanol. Extract was evaporated under low pressure by using Buchi type evaporator.

Drugs and Chemicals: Alcohol (E.Merck, Germany); Carbon tetrachloride (E.Merck, Bombay, India) Paracetamol (farmsons, Gujarat). All other chemicals were obtained from local sources and were of analytical grade.

Animals: Male wistar albino rats weighing (150-200g) were obtained from Raja Muthiah Medical College, Annamalai University, Chidambaram, Tamil Nadu, India. They were maintained at standard housing conditions and fed with commercial diet and provided with water ad libitum during the experiment. The institutional animals ethical committee (Reg. No 163/1999/CPCSEA) permitted the study.

Acute toxicity Study: Acute toxicity was performed as per OECD -423 guidelines

Experimental Design: Rats were divided in to six groups, each group consisting of six animals

Group-I: Received normal saline 1.0ml/kg/day (po) for 21 days

Group II : Received 40% ethanol (2ml/100g body wt, po) for 21days. On 20th day they were injected with CCl_4 (0.1ml/kg/b.w.s.c).

Group III: Received 40% ethanol (2ml/100g body wt, po) for 21 days. On 20th day they were injected with CCl_4 (0.1ml/kg/b.w.s.c) and simultaneously administered CI extract 100mg/kg/day, po from 15th to 21st day of study.

Group IV: Received 40% ethanol (2ml/100g body wt, po) for 21days. On 20th day they were injected with CCl_4 (0.1ml/kg/b.w.s.c) and simultaneously administered CI extract 200mg/kg/day, po from 15th to 21st day of study.

Group V ; Received alcohol extract of coccinia indica 100mg/kg p.o for 7 days and simultaneously administered paracetamol 750mg/kg on 7th day.

Group VI: Received alcohol extract of coccinia indica 200mg/kg p.o for 7 days and simultaneously administered paracetamol 750mg/kg on 7th day.

Biochemical study: At the end of experimental period all the animals were sacrificed by cervical dislocation. The blood samples were collected by direct cardiac puncture and the serum was used for the assay of marker enzymes viz; Glutamate oxaloacetate transaminase (SGOT) [15], alkaline phosphatase(ALP) [1], total Bilirubin (TB) [11] and total proteins (TP) [9].

Histopathological Examination: Small pieces of liver tissues were collected in 10% formaldehyde solution for histopathological study. The pieces of liver were processed and embedded in paraffin wax.

Sections made were about 4-6 µm in thickness. They were stained with hematoxylin and eosin and photographed.

Statistical Analysis: The results are expressed as mean ± SEM of six animals from each group. The statistical analysis was carried out by one way analysis of variance (ANOVA) p values <0.05 were considered significant.

RESULTS:

Alcohol – CCl4 induced Hepatotoxicity: The oral administration of coccinia indica leaves caused neither any behavioural changes nor mortality upto 2000mg/kg.So the LD50 of coccinia indica leaves was found to be more than 2000mg/kg.

The administration of alcohol-CCl_4 to the animals resulted in a marked increase in total Bilirubin (TB), serum oxaloacetate transaminase (SGOT) and serum alkaline phosphatase (ALP) activities, However, the serum total protein level was decreased indicating the liver injury,Whereas animal treated with 100mg/kg and 200mg/kg CI extracts exhibited a decrease in total bilirubin,serum oxaloacetate transaminase and alkaline phosphatase along with significant increase in total protein activities. The effect was only marginal at a dose of 100mg/kg whereas at 200mg/kg the extract effectively prevented Alcohol-CCl4 induced hepatotoxicity. The results were presented in **table 1**.

TABLE 1: EFFECT OF CI EXTRACT ON BIOCHEMICAL PARAMETERS IN RATS SUBJECTED TO ALCOHOL-CCl4 INDUCED HEPATOTOXICITY

Group	SGOT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Total protein (mg/dl)
I Control	44.67 ± 2.19	176.50 ± 4.23	0.44 ± 0.03	8.94 ± 0.11
II Alcohol – CCl_4	87.33 ± 2.22	320.17±13.14	2.10 ± 0.13	5.79 ± 0.20
III Alcohol - CCl_4 + CI Extract 100mg/kg	69.17 ± 3.48	230.67 ± 9.02	0.91 ± 0.11	6.92 ± 0.26
IV Alcohol - CCl_4 + CI Extract 200mg/kg	55.50 ± 1.65	212.33 ± 8.94	0.64± 0.05	7.05 ± 0.25
One-way ANOVA				
F	78.78	76.59	495.88	36.69
df	23	23	23	23
P	0.001	0.001	0.001	0.001

Values are mean±SEM of six animals in each groups Group II compared with Group I (P<0.001), Group III and IV compared with Group II (P<0.001)

Histologically, control animals showed normal hepatic architecture, the group II animals exhibited intense centrilobular necrosis, vacuolization and macrovesicular fatty changes (**Figure 1**). Moderate accumulation of fatty lobules and cellular necrosis were observed in the animals treated with methanol extracts of 100mg/kg and 200mg/kg.

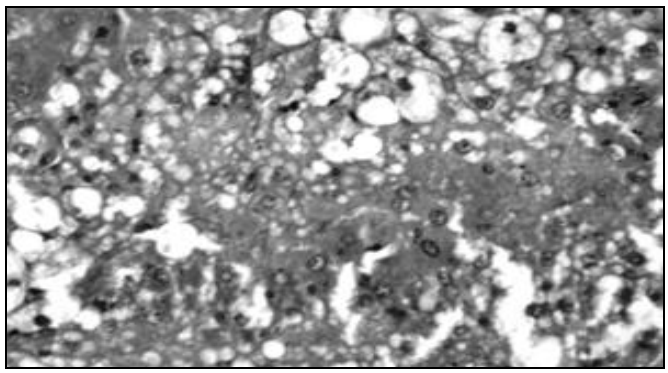

FIG. 1. LIVER TISSUE OF ALCOHOL COMBINES WITH CCL4 RATS SHOWING CENTRILOBULAR NECROSIS VACUOLIZATION, MACROVESICULAR FATTY CHANGES OF HEPATIC CELLS

However, the methanol extract (200mg/kg) treated animals exhibited significant liver protection against Alcohol-CCl_4 induced liver damage, as evident by the presence of normal hepatic cords, absence of necrosis and fatty infiltration (**Figure 2**).

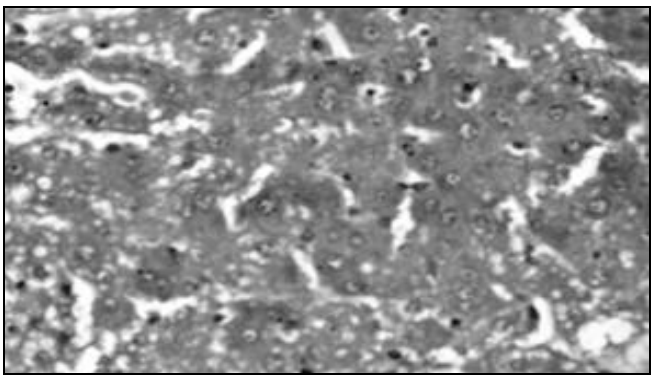

FIG. 2. LIVER TISSUE OF ALCOHOL-CCl4 + 200mg/kg CI EXTRACT TREATED RATS SHOWING NORMAL HEPATIC CELLS AND CENTRAL VEIN

Paracetamol induced Hepatotoxicity: There was significant increase in the levels of the marker enzymes (SGOT, SGPT, ALP) and decrease in the level of total protein in animals treated with paracetamol when compared with the control animals.

For the animals given the extract (200 mg/kg), the levels of these enzymes and total protein were relatively normal when compared with Paracetamol treated group .The results were presented in **table 2**.

TABLE 2: EFFECT OF CI EXTRACT ON BIOCHEMICAL PARAMETERS IN RATS SUBJECTED TO PARACETAMOL INDUCED HEPATOTOXICITY

Group	SGOT(IU/L)	ALP(IU/L)	Total Bilirubin (mg/dl)
I (Control)	44.67±2.19	176.50 ± 4.2	0.44±0.03
II (Paracetamol)	85.33 ± 1.7	266.5 ±5.71	1.9050 ±2.668
III (Paracetamol+CI extract)			
100 mg/kg	70.17 ± 1.81	215.83 ±4.92	1.55 ±2.704
200mg/kg	51.8.3± 1.97	186.33± 2.55	0.55 ± 3.33
One-way ANOVA			
F	90.196	80.388	533.280
df	23	23	23
P	0.001	0.001	0.001

Values are mean ± SEM of 6 animals in each groups Group II compared with Group I (P<0.001), Group III compared with Group II (P<0.001)

Paracetamol administration caused necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis and eosinophillic cytoplasm (**Figure 3**) and the protective effect of *Coccinia indica* (200 mg/kg) was confirmed by Histopathological examination (**Figure 4**).

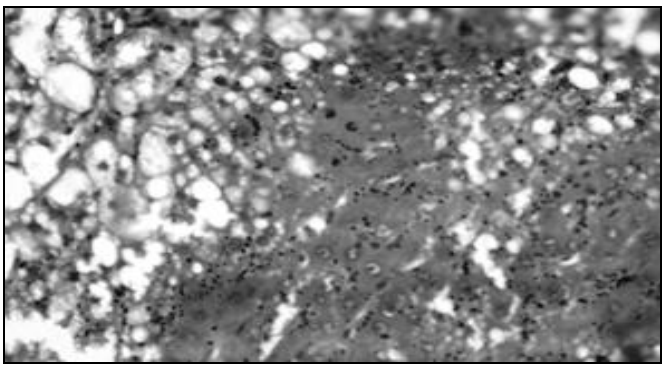

FIG. 3. LIVER TISSUE OF PARACETAMOL TREATED RATS SHOWING NECROSIS OF THE CENTRILOBULAR HEPATIC CELLS CHARA CTERIZED BY NUCLEAR PYKNOSIS AND EOSINOPHILLIC CYTOPLASM

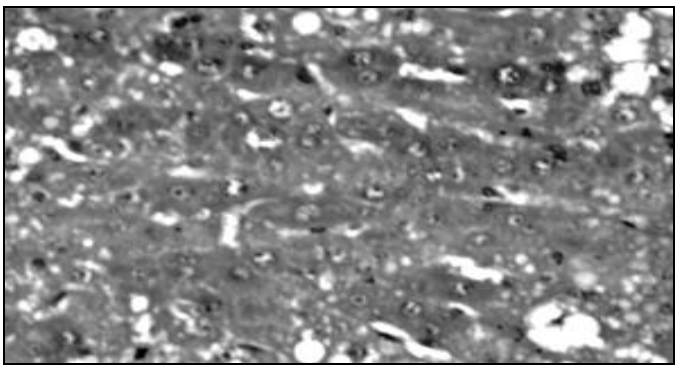

FIGURE 4. LIVER TISSUE OF PARACETAMOL + CI EXTRACT 200mg/kg TREATED RATS SHOWING NORMAL HEPATIC CELLS AND CENTRAL VEIN

DISCUSSION: Alcohol pretreatment stimulates the toxicity of CCl_4 due to increased production of toxic reactive metabolites of CCl_4, namely trichloromethyl free radical by the microsomal mixed function oxidase system. This activated radical binds covalently to the macromolecules and induces peroxidative degradation of membrane lipids of endoplasmic reticulum rich in poly unsaturated fatty acids. This peroxidative degradation of biomembranes is the principle cause of hepatotoxicity. This is evidenced by an elevation in the serum maker enzymes namely SGOT, ALP and TB and reduction in the serum TP. CI extract has decreased the level of serum marker enzymes namely SGOT, ALP, Bilurubin and increased the level of proteins which indicates hepatoprotective activity.

Paracetamol causes hepatic necrosis when administered in large quantities. It has been shown that its toxic metabolite N-acetyl-p-benzoquinone imine covaiently binds to hepatic proteins after the cellular glutathione has been depleted. Sorne of these proteins have been shown to be mitochondrial proteins. possibly involved in oxidative phosphorylation. Hence, it has been suggested that the mitochondrion is a target of Paracetamol –induced hepatotoxicity [19, 20].

The paracetamol induced hepatotoxicity was controlled in CI extract treated groups indicated that the CI extract helped to resist the damage caused by paracetamol.

In order to provide a better understanding of the possible role of the extract of *Coccinia indica* leaves in the hepatoprotective effect observed in this study, we carried out a preliminary phytochemical screening of the extract of the leaves and found it to contain flavonoids.

Earlier report indicated that the flavonoids are phenolic compounds exert multiple biological effects,including antioxidant properties and free radical scavenging abilities. So the hepatoprotective activity of the extract may be due to its antioxidant property exerted by flavanoids in the leaves. These results of our study indicate that treatment with CI extract protectected the liver against Alcohol-CCl_4 and paracetamol induced hepatotoxicity.

ACKNOWLEDGEMENT: The authors are grateful to Dr. G. Revi Kumar, Central Animal House, Raja Muthiah Medical College, Annamalai University, Annamalai nagar, Chidambaram, Mr. R. Suresh Kumar, Department of Horticulture, Annamalai University, Chidambaram, Tamil Nadu, India, for providing all the necessary facilities to carry out this work.

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