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Cassia roxburghii seeds protect Liver against Toxic effects of Ethanol and Carbontetrachloride in rats

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Abstract: Hepatitis is a major health problem in human due to various chemicals including therapeutic agents and other environmental toxins and this can produce illnesses like jaundice which sometimes may lead to even death. Natural products are the best source of remedies for the treatment of diseases including hepatoprotectives. Thus identification of a potential therapeutic agent for the protection of liver from the hepatotoxins will provide a useful way for the prevention of these liver related illnesses. In our present study we selected seeds of *Cassia roxburghii* DC., which is used in the ethnomedicine for various liver disorders for the screening of its hepatoprotective activity using alcohol and carbon tetra chloride induced liver injury in rats. Hepatotoxicity was induced in male Wistar rats by ethanol and carbon tetrachloride, i.p. The methanolic extract of *Cassia roxburghii* was administered at the doses of 250 mg/kg and 500 mg/kg. The Hepatoprotective effect of these extracts was evaluated by liver function biochemical parameters and histopathological studies of liver, compared with silymarin. The methanolic extract of *Cassia roxburghii* reversed the toxicity produced by ethanol - CCl_4 in dose dependent manner. The extract at the doses of 250 mg/kg and 500 mg/kg are comparable to the effect produced by Liv 52, a standard hepatoprotective against hepatotoxins. Liver sections from *Cassia roxburghii* seed and Liv 52 treated groups clearly showed a histological picture that closely approximates that of the control group. The study identified a plant with potential hepatoprotective constituents which will to be isolated and characterized in future.

Keywords: *Cassia roxburghii*; Hepatotoxicity; Hepatoprotective.

Introduction

Liver – a major metabolic organ affected by various chemicals and toxins daily and identification of a successful hepatoprotective agent will provide a useful tool for the treatment of hepatic diseases. In absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders[1] and quite often claimed to offer significant relief. Among those *Cassia roxburghii* is one of the medicinal plants used in ethnomedicine for the treatment of various liver ailments. To the best of our knowledge there is no scientific report is available in support of the hepatoprotective activity of methanolic extract of the seeds of *Cassia roxburghii*. Therefore, to justify the traditional claims we have assessed the hepatoprotective effect of *Cassia roxburghii seeds* using ethanol - CCl_4 -intoxicated rats.

Experimental

Acute Toxicity Studies (Up and Down Procedure)[2]

Swiss albino mice of either sex weighing between 18-25gm were procured from KMCH College of Pharmacy animal house, Coimbatore. The animals are randomly selected, marked to permit individual identification and kept in their cages for at least 5 days prior to dosing and to allow them for acclimatization to the laboratory conditions. The temperature in the experimental animal room was maintained at 25˚C (±1˚C). Three animals were treated with 2000 mg/kg as single treatment per oral as

per the limit test of OECD Guidelines 425 and the animals were observed continuously for first 4 hrs and finally overnight mortality was observed.

Hepatoprotective Activity[3]

Experimental design

In-vivo hepatoprotective activity of the extracts was determined in ethanol and CCl_4 challenged rats. All the study was approved by Institutional Animal Ethical Committee (Proposal no. KMCRET/Ph.D/04/2007). The prepared extract was mixed with water and the resultant mixture was used for the acute toxicity studies and hepatoprotective screening.

Experimental Animals

Wistar albino rats of either sex weighing between 180-200gm were procured from KMCH College of Pharmacy animal house, Coimbatore. The rats were divided into 5 groups containing 6 animals each under standard laboratory conditions and had a free access to commercial pellet diet and water *ad libitum*. The room temperature was maintained at 25± 1˚C. Amongst the groups, Group 1 served as saline control, Group 2 to 5 served as the model for alcohol- CCl_4 induced hepatotoxicity. The effects of *Cassia roxburghii* and Liv 52 were studied in the above models. All the experiments were performed in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals[4] .

Experimental method[3]

Group 1: Rats were given normal saline (1m1/kg/day, p.o.) for 21 days.

Group 2to 5 were given 40%v/v ethanol (2ml/100g body wt. p.o) for 21 days. On 20th day they were injected with CCl_4 (1:1 in groundnut oil, 0.1 ml/kg body wt. sc) and were sacrificed 48hours later and biochemical estimation were performed. In addition to this, the following treatment was given to the animals of group 3, 4 and 5 from day 15 to 21 of study period.

Group 3 : Ethanol$_+$ CCl_4 + methanolic extract of *Cassia roxburghi* (250 mg/kg, p.o)

Group 4 : Ethanol$_+$ CCl_4 + methanolic extract of *Cassia roxburghi* (500 mg/kg, p.o)

Group 5 : Ethanol$_+$ CCl_4 + Liv 52 (1 ml/kg, p.o)

At the end of the treatment on 22nd day irrespective of all the groups, rats were sacrificed under light ether anesthesia. The blood was collected by sino-orbital puncture from all the groups; serum was separated by centrifugation at 2000rpm for 20mins and was utilized for the biochemical studies. Liver tissues were fixed in paraffin wax for histopathological estimation.

Biochemical Study

The following biochemical parameters were estimated using E-Coline estimation kit supplied by E.Merck (India) Ltd.

1. Serum glutamate oxaloacetate transaminase (SGOT) or Aspartate amino transferase (ASAT)[5],
2. Serum glutamate pyruvate transaminase (SGPT) or Alanine amino transferase (ALAT) [5] ,
3. Alkaline phosphatase (ALP) [6]
4. Total bilirubin (TB)[7]
5. Total protein (TP) [8]
6. Albumin[8]
7. Total cholesterol (TC) [8]

Histopathological Study

The rats were sacrificed under light ether anesthesia and the livers were removed and washed with normal saline. Small pieces of liver tissues were collected in 10% formal saline buffer for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5-6 microns in thickness were made and stained with haematoxylin and eosin[9] for histopathological examination.

Statistical Analysis

The data were expressed as mean ±SEM (Standard error mean). The statistical significance between groups was analyzed using‘t’ test and P value of 0.05 or less was considered significant.

Results

The oral administration of methanolic extract of *Cassia roxburghii* seeds caused neither any behavioral changes nor mortality up to 2000 mg/kg. So the LD50 of *Cassia roxburghii* seed was thus found to be more than 2000 mg/kg. A sub lethal dose of CCl_4 and ethanol challenged rats showed a significant increase in the serum levels of transaminases SGOT (503.9±8.38), SGPT (339.3±13.4), Alkaline phosphatase (491.0±5.85), Total bilirubin (0.67±0.21) and a significant reduction in the serum levels of total protein (4.34±0.21), albumin (2.1±0.39) and total cholesterol (29.75±0.38) indicating the liver injury caused by ethanol-carbon tetra chloride combination. Whereas animals treated with methanolic extract at doses of 250 and 500mg/kg of *Cassia roxburghii* exhibited a decrease in SGOT, SGPT, ALP and TB along with a significant increase in TP, Albumin and TC .It is evident from the Table 1 that the methanolic extract of *Cassia roxburghii* reversed the toxicity produced by ethanol- CCl_4 in dose dependent manner. The methanolic extract of *Cassia roxburghii* at the doses of 250 mg/kg and 500 mg/kg are comparable to the effect produced by Liv 52, a standard hepatoprotective against hepatotoxicants. Treatment with Liv 52 also reversed the hepototoxicity under the present investigational conditions. Histopathology of liver from normal control group showed normal arrangement of hepatocytes with clearly brought out nuclei, central vein and portal triad. Sinusoids at the periphery or the lobule are fused into a reticulum. The hepatocytes are arranged in a series of branching and anastomosing perforated laminae to form a labyrinth, between which were sinusoidal spaces. The

cytoplasm of the hepatocytes was clearly eosinophilic with prominent nuclei. Microscopical examination of alcohol-CCl_4 treated liver section showed various degrees of pathological changes starting from cloudy swelling, hydropic changes and necrosis of hepatic cells as well as clearly brought out centrilobular fatty changes with clear space representing fatty material or lipid.

Liver sections from *Cassia roxburghii* seed and Liv 52 treated groups clearly showed a histological picture that closely approximates that of the control group. The plate-like arrangements of the hepatocytes were seen, the sinusoidal spaces were also visible, but not as prominent as in the control group. The cytoplasms of the hepatocyte were clearly eosinophilic as in the control group.

Discussion and Conclusion

Up to the present time, the etiology and treatment of most liver diseases are not known. The liver is the commonest site affected during the toxic manifestation of many drugs. Liv. 52 (an indigenous drug) has been shown to protect the liver against carbon tetrachloride- induced hepatotoxicity and ethanol-induced hepatic damage[10,11,12] .The mechanism of this protective effect of Liv. 52 remain to be elucidated.

Toxicity in liver due to ethanol and CCl_4 is attributed to the toxic metabolites formed, responsible for the initiation of CCl_4 dependent lipid per oxidation, the nature of which is not yet unambiguously determined. The most likely candidate is the trichloromethyl radical[13,14].

Alcoholic liver disease is a common consequence of prolonged and heavy alcohol intake. This disease encompasses a wide spectrum of lesions, the most characteristic being alcoholic steatosis (fatty liver), alcoholic hepatitis, alcoholic fibrosis and cirrhosis[15]. To simulate the human model of alcoholic liver injury, carbon tetrachloride was included in the study to cause hepatocellular necrosis in alcohol fed rats.

The ability of aliphatic alcohols to potentiate carbon tetrachloride (CCl_4) hepatotoxicity in experimental animals is well established[16,17,18,19] . The hepatotoxic effects of carbon tetrachloride are largely due to its active metabolite, the free radical[20]. These activated radicals bind covalently to the macromolecules and induce per oxidative degradation of membrane lipids of endoplasmic reticulum rich in polysaturated lipid fatty acids. This lipid per oxidative degradation of biomembranes is one of the principle cause of hepatotoxicity[21]. This eventually leads to hepatocelluar necrosis and is reflected in our experiment by marked change in various enzymatic parameters of alcohol and carbon tetrachloride treated rats.

Administration of methanolic extract of *Cassia roxburghii* seed showed significant hepatoprotective activity, which was comparable to a herbal preparation Liv52, reputed for its hepatic stimulant activity. Phytochemical reports revealed that the methnolic extract of the plant was found to contain higher concentration of flavonoids and glycosides[22]. The qualitative phytochemical investigation on the methanolic extract of *Cassia Roxburghii* also showed positive for flavonoids by zinc chloride, alkaline reagent and shinoda test. Further, it has been reported that the flavonoid constituents of the plant possess antioxidant properties[23] and was found to be useful in the treatment of liver damage[24].

Further studies are in progress for the isolation and characterization of the flavonoids from the seeds of *Cassia roxburghii* for this activity.

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Table 1: Effect of methanolic extract of *Cassia roxburghii* seeds (MECR) on biochemical parameters against ethanol + CCl_4 induced hepatic injury

Group	SGOT (Units/ml)	SGPT (Units/ml)	ALP (Units/ml)	TB (mg/dl)	TP (g/dl)	Albumin (g/dl)	TC (mg/dl)
Normal	87.6±6.6	58.1±7.7	347.8±14.15	0.29±3.6	6.75±0.27	3.95±0.061	78.9±3.16
Ethanol+ CCl_4	503.9±8.38	339.3±13.4	491.0±5.85	0.67±0.21	4.34±0.21	2.1±0.39	29.75±0.38
MECR (250mg/kg, p.o)	89.8±6.54*	63.8±4.65*	348.6±8.25*	0.32±0.010*	6.06±0.19*	3.77±0.17*	75.6±1.84*
MECR (500mg/kg, p.o)	94.8±6.6*	67.6±4.7*	342.8±13.4*	0.42±1.84 *	5.45±0.61*	4.61±1.87*	70.2±5.85*
Liv-52 (1ml/kg,p.o)	97.5±7.28*	69.6±4.66*	347.5±6.67*	0.28±0.008*	5.75±0.21*	4.07±0.16*	76.4±2.13*

Values are Mean ± SEM; where n=6; *denotes statistical significance ('t' test) in comparison to ethanol - CCl_4 group at P<0.001 respectively

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