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Research Article

HEPATOPROTECTIVE ACTIVITY OF *CAPPARIS SEPIARIA* STEM AGAINST CARBON TETRACHLORIDE-INDUCED HEPATOTOXICTY IN RATS

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ABSTRACT

The hepatoprotective effect of the alcohol extract of *Capparis sepiaria* Linn. (Capparaceae) stem against carbon tetrachloride (CCl_4)-induced toxicity was studied in albino rats. The rats were given daily pretreatment with alcohol extract of *C. sepiaria* (100 mg/kg) and the standard silymarin (25 mg/kg) orally for 7 days. The toxicant used on 7^{th} day was CCl_4 at a dose of 1.25 ml/kg as 1:1 mixture with olive oil. The extract produced significant ($p<0.01$) reduction in the elevated levels of aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin (TB) and rise of decreased total protein level when compared with the toxic control.

Key-words: hepatoprotective, *Capparis sepiaria*, carbon tetrachloride, silymarin

INTRODUCTION

Herbal drugs play a major role in the treatment of hepatic disorders. In the absence of reliable liver protective drugs in modern medicine, in India a number of medicinal plants and their formulations are used to cure hepatic disorders in traditional systems of medicine[1]. To evaluate the hepatoprotective effect of drugs, liver injury is induced in animals using chemicals like carbon tetrachloride, paracetamol and alcohol. Some plants of the genus *Capparis* (Capparaceae) were reported to have hepatoprotective activity[2, 3,4.] Hence it was decided to evaluate whether this activity could be related to this genus. In this study *Capparis sepiaria* Linn. stem was selected for investigation of its hepatoprotective activity against carbon tetrachloride (CCl_4)- induced hepatotoxicity[5].

C. sepiaria is a much branched, thorny, woody climber growing to a height of 3-4 m. It is distributed in dry parts of India, in Deccan peninsula and Andamans. In Ayurveda**,** *C. sepiaria* is used as stomachic, tonic, appetizer; removes *kapha* and *vata,* cures fever, tumours, used in inflammation, wound healing and diseases of muscles; also acts as blood purifier. The ground root is used as a cure for snake bite, the plant is also useful in skin diseases[6, 7]. In anticancer treatment, *C. sepiaria* is used along with paste of *Cassia fistula* Linn.(Caesalpinaceae) and *Flacourtia ramontchi* L'Her. (Flacourtiaceae) for 'kaphaja' tumours [8]**.** The leaves are used in treatment of oral ailments in Dharwad district, Karnataka, India[9].

Root extract was found to possess anti-inflammatory and analgesic activity[10] which may be related to the inhibition of enzymes responsible for prostaglandin biosynthesis, increased vascular permeability or inhibition of degranulation of mast cells[11, 12, 13].

Leaves of *C.sepiaria* contain four pentacyclic triterpenoid alcohols, taraxasterol, α- and β-amyrin, β-sitosterol[14] and erythrodiol[15]. Betulin and a triterpenoid alcohol were isolated from the leaves[16]. Root bark, stem, leaves and flowers contain alkaloids, glycosides, terpenes, sterols and flavonoids. An alkaloid was isolated from the alcohol extract of roots[10]. This paper reports the anti-hepatotoxic effect of *C. sepiaria* stem in CCl_4- induced liver injury in rats.

MATERIALS AND METHODS

Plant Collection and Extraction

C. sepiaria stems were collected from Plant Resource Centre, Bhubaneswar in the month of December 2006 and were identified and authenticated by Dr. P.C.Panda, Senior Scientist, Regional Resource Centre, Bhubaneswar. A voucher specimen (TSN-CS13/12/06) was deposited in the Phytochemistry and Pharmacognosy Division, College of Pharmaceutical Sciences, Andhra University.

Dried plant material was packed in a Soxhlet apparatus and extracted exhaustively with 90% alcohol. The extract was concentrated and dried using Rotary Flash Evaporator to give a brown semi-solid residue.

Chemicals

All chemicals used were of analytical grade and obtained from Qualigens Chemical Company (Mumbai, India). The Olive oil was from Figaro Co. (Madrid, Spain). The kits for the estimation of aspartate transaminase (AST), alanine transaminase (ALT), Alkaline phosphatase(ALP), Total Bilirubin(TB) and Total Protein were purchased from Ranbaxy Fine

Chemicals Limited (Diagnostics Division, Himachal Pradesh, India). The standard drug silymarin was a gift sample from Micro Labs (Bangalore, India).

Animal Treatment

Wistar albino rats (150-200 g) obtained from Mahaveer Enterprises, Hyderabad, were maintained on 12 h light/dark cycle and allowed food and water *ad libitum*. Study was conducted after obtaining ethical committee clearance from the Institutional Animal Ethics Committee of Al-Ameen College, Bangalore. No. AACP/IAEC/M-76/2006.

Animals were randomly divided into four groups of six animals each. Group I served as vehicle control and received 4% acacia suspension. Group II served as toxic control and received 4% acacia suspension. Group III served as standard group and received silymarin (25 mg/kg).Group IV served as test group and received alcohol extract of *C.sepiaria* stem (100 mg/kg). All treatments were given orally for 7 days successively. On 7^{th} day, liver toxicity was induced in rats with 1:1(v/v) mixture of CCl_4 in olive oil at a dose of 1.25 ml/kg, p.o in all groups except vehicle control.

Biochemical Analysis

Twenty-four hours after CCl_4 treatment (Day 8), blood was collected from the retro orbital plexus of all the rats. Serum was separated by centrifugation at 2500 rpm at 37 ºC for 15min and analyzed for ALT[17], AST[18], ALP[19], TB[20] and Total Protein[21].

Statistical analysis

All the results were expressed as mean ± SEM. One-way Analysis of Variance (ANOVA) was used for the statistical analysis of data. Dunnett's multiple comparison test was used for determining the significance. A probability value of $p<0.05$ was considered as significant.

RESULTS

The LD_{50} of alcoholic extract of *C.sepiaria* has been reported as 750mg/kg i.p. in mice (22); hence the test dose selected for the study was 100mg/kg b.w. p.o. ($1/10^{th}$ of LD_{50}).The administration of CCl_4 resulted in significant rise ($p<0.01$) in ALT, AST, ALP and TB levels along with significant decrease in total protein ($p<0.01$) levels when compared with Group I (vehicle control)(Table I). The oral administration of alcohol extract of *C.sepiaria* and silymarin reduced the CCl4 induced increase in the AST, ALT and TB levels ($p<0.01$), reversed the depletion of total protein significantly ($p<0.05$ and $p<0.01$ respectively) but no significant decrease was observed in ALP levels when compared with GroupII (CCl_4 treated group) (Table I).

In the Histopathological studies, the liver sections of rats treated with vehicle showed normal hepatic architecture (figure 1), whereas that of CCl_4 treated group showed total loss of hepatic architecture with intense peripheral central vein necrosis, fatty changes, congestion of sinusoids, kupffer cell hyperplasia, crowding of the central vein and apoptosis (figure 2). In the case of rats treated with silymarin (figure 3) and those pre-treated with *C.sepiaria* alcohol extract (figure 4), the liver showed normal hepatic architecture with only moderate accumulation of fatty lobules and mild degree of cell necrosis, clearly indicating the protection offered by standard drug silymarin and the plant extract.

Table1. Effect of alcohol extract of *C.sepiaria* on various biochemical parameters in rats with carbon tetrachloride-induced hepatotoxicity

Groups	SGOT (U/L)	SGPT (U/L)	ALP(U/L)	TB(mg/dl)	Total Protein(g/dl)
I (Vehicle Control)	81.77±2.48	40.0±1.93	190.4±6.46	0.052±0.01	10.88±0.23
II (CCl_4 Control)	353.7±2.48[a]	261.5±5.50[a]	257.7±4.65[a]	0.28±0.03[a]	9.18±0.09[a]
III (*C.sepiaria* ext+ CCl_4)	170.9±8.46[b]	152.7±6.8[b]	246.8±7.95	0.07±0.06[b]	9.80±0.15[c]
IV (Silymarin + CCl_4)	120.7±6.13[b]	99.02±7.71[b]	250±6.21	0.06±0.01[b]	9.89±0.16[b]

Values are mean ± SEM, N= 6, [a] $p \leq 0.01$ compared with Group I. [b] $p \leq 0.01$, [c] $p \leq 0.05$ compared with Group II

Photomicrographs representing effect of plant extract against carbon tetrachloride-induced hepatotoxicity in rats

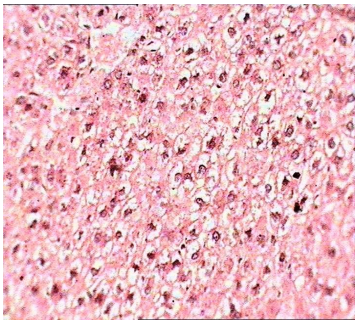

Fig1- vehicle treated

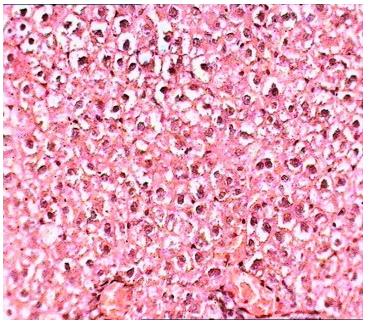

Fig2- CCl_4 treated

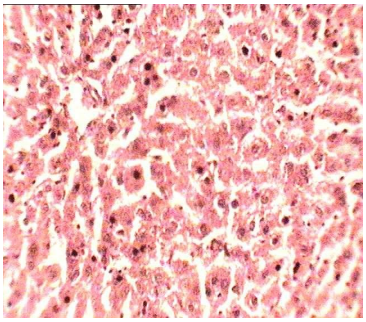

Fig 3- pre-treated with silymarin + CCl_4

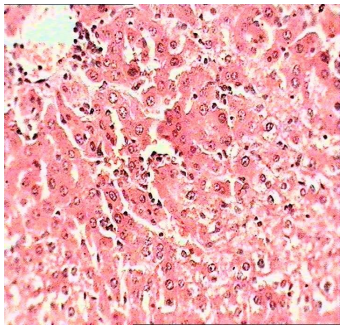

Fig 4- pre treated with plant extract + CCl_4

DISCUSSION

CCl_4 is a commonly used hepatotoxin used for production of experimental liver toxicity. Its metabolites such as trichloromethyl radical (CCl_3*) and trichloromethyl peroxy radical (CCl_3O_2*) are involved in the pathogenesis of liver. As shown in figure 2, CCl_4 causes changes

around the central vein in the liver and other oxidative damages with the leakage of marker enzymes like AST, ALT and ALP in the serum, increase in serum TB levels and decrease in serum Total Protein.

Seven days pretreatment with the test extract (100 mg/kg p.o.) protected the animals significantly ($p<0.01$) from CCl_4 induced hepatotoxicity as shown in table and figures which clearly indicates the hepatoprotective activity of the alcohol extract of *C.sepiaria* stem.

Different components including β-sitosterol and alkaloids have been isolated from the plant (Aditya Chaudhary, 1970; Chaudhari, 2004). Earlier works have reported the presence of sterols, alkaloids, saponins and glycosides in the alcohol extract. Reports of β- sitosterol and alkaloids as hepatoprotective agents have been published (Al-Quaawi, 2004; Vijayan, 2003). Hence the hepatoprotective activity of the *C.sepiaria* alcohol extract in the present study may be due to the presence of these compounds.

The results of the present study suggest that alcohol extract of *C.sepiaria* stem at a dose of 100 mg/kg, p.o. showed significant hepatoprotective activity which may be related to this genus.

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