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Hepatoprotective activity of *Cleome viscosa* against Carbon tetrachloride induced hepatotoxity in rats

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ABSTRACT - The present study was conducted to evaluate the hepatoprotective activity of aqueous seed extract of *Cleome viscosa* (CV) against carbon tetrachloride (CCl_4) induced liver damage in wistar rats. The aqueous seed extract of CV (200 mg/kg) was administered orally to the animals with hepatotoxicity induced by CCl_4. Silymarin (200mg/kg) was given as reference standard. The seed extract was effective in protecting the liver against the injury induced by CCl_4 in animals. This was evident from significant reduction in serum enzyme aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase and lipid peroxidase and increase in reduced glutathione (GSH). Various pathological changes like steatosis, centrilobular necrosis and vacuolization observed in CCl_4 treated rats, which were prevented to a moderate extent in groups, treated with *Cleome viscosa* and silymarin. It was concluded from the study that aqueous seed extract of CV possesses hepatoprotective activity against CCl_4 induced hepatotoxicity in rats.
KEYWORDS: Carbon tetrachloride; *Cleome viscosa*; Hepatoprotective activity; Silymarin.

INTRODUCTION

Liver disease remains one of the serious health problems. Herbs play a major role in the management of various liver disorders. A number of plants possess hepatoprotective property (1). *Cleome viscosa* (CV) is a common weed used extensively in the Indian traditional system. It is an annual, sticky herb belonging to the family Capparaceae. The plant distributed throughout the plains in India. The aqueous extract of the seeds of this plant has traditionally been used for the treatment of various liver disorders and as analgesic. It potentiates the barbiturate sleeping time in rats and also had a mild laxative effect (2). The leaves of CV were reported to possess hepatoprotective activity in rats (3), based on the above, the present study has been undertaken to investigate the hepatoprotective activity of aqueous extract of the seeds of CV against CCl_4 induced hepatic damage in rats.

MATERIALS AND METHODS

Animals

AMale Wistar Albino rats weighing between 150 – 250 gm were used. The animals were obtained from animal house, IRT Perundurai medical college, Erode, India. On arrival the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30 – 70 %. A 12:12 light:day cycle was followed. All animals were allowed to free access to water and fed with standard commercial rat chaw pallets (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (Regd no: 688/2/C-CPCSEA) and were in accordance with the guidelines of the CPCSEA.

Preparation of seed extract

The seeds of *Cleome viscosa* were collected from mature plants during the month of October from the outskirts of Erode city. The plant was authenticated by the botanist of Botanical Survey of India, Agricultural University, Coimbatore. The collected seeds were sun dried for 7 days and ground to course powder using a blender. The powdered seeds were soaked in sufficient quantity of purified water for maceration, after maceration the meristrum was collected, filtered and then evaporated to obtain dry extract and it was used for the study.

Drugs and chemicals

CCl_4 was obtained from S.D. Fine-chem. Ltd. Boisar, and Silymarin from Indena Spa. Milan, Italy. All other chemicals were obtained from local sources and were of analytical grade.

Methodology

A total of 24 animals were equally divided into 4 groups of six each. Group – I served as normal control received 0.3% carboxy methyl cellulose (1 ml/kg p.o.) once daily for 7 days. Group – II received equal mixture

of CCl_4 and olive oil (50 % v/v, 0.5 ml/kg i.p.) once daily for 7 days (4). Group – III received equal mixture of CCl_4 and olive oil and CV extract (200 mg/kg p.o.) simultaneously for 7 days. Group – IV received equal mixture of CCl_4 and olive oil and standard drug silymarin (200 mg/kg p.o.) (5), simultaneously for 7 days. On 8^{th} day the blood was collected by direct cardiac puncture under light ether anaesthesia and serum was separated for different biochemical analysis. All animals were sacrificed by cervical decapitation and immediately, the livers were dissected out, washed in the ice cold saline and homogenate was prepared in 0.05 M sodium phosphate buffer (pH 7.0) and centrifuged. The supernatant was used for the estimation of γ-glutaryl transpeptidase, lipid peroxidase and reduced glutathione (GSH).

Enzyme assays

The activities of serum hepatic marker enzymes namely aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) (6,7) were assayed in serum using standard kits from Lupin Laboratories and pointe scientifics. The results were expressed as units/litre (U/L). γ – glutamyl transpeptidase activiy was assayed by the method of Tate and Meister (8). GSH was estimated in the liver homogenate using DTNB by the method of Buetler (9). The absorbance was read at 412 nm and the results were expressed as mg GSH/g of wet tissue. The lipid peroxidation in the liver was determined by the method of Ohkawa (10).

Histopathological examination

A portion of liver tissues were collected in 10 % formaldehyde solution for histopathological studies. They were processed in an automatic tissue processor and embedded in paraffin wax. Sections of 5µm were cut on a rotary microtome by serial sectioning until the entire thickness of the liver was sectioned. Staining was done by haematoxylin and eosin and later the microscopic slides of the liver cells were photographed.

Statistical analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA). P values <0.05 were considered significant.

RESULTS

The results of transminase are in table 1 and 2. A significant ($P<0.05$) increase in level of serum AST, ALT and ALP was found due to CCl_4 treatment in group -II. Whereas in group -III (CCl_4 + extract) rats showed a significant ($P<0.05$) decrease in AST, ALT and ALP when compared to group -II. This is comparable with group- IV (Silymarin). There was significant increase ($P<0.05$) in the γ-glutaryl transpeptidase in group- II (CCl_4) as compared to Group-I (Normal control). As significant decrease ($P<0.05$) was found in group III (CCl_4 + extract) as compared to group II and it was comparable with group- IV and group- I. There was marked decreased in GSH of group- II when compared to group- I. The GSH level significantly increased ($P<0.05$) in group- III when compared to group- II. There was a significant increase in lipid peroxidase in group- II. Group- III showed marked decrease in lipid peroxidase and it was comparable with Group- I and Group- IV.

Histopathological Examination

In control animals, liver sections showed normal hepatic cells with well preserved cytoplasm, nucleus and nucleolus and central vein (Figure 1). In CCl_4 treated animals, the sections showed fatty changes in centrilobular necrosis, steatosis and fatty vacuolaization were seen with acute inflammatory cells infiltration sinusoids mainly in central zone (Figure 2). In CCl_4 and CV treated animals, the sections showed mild fatty change and mild sinusoidal congestion (Figure 3). In CCl_4 and silymarin treated animals, the sections showed mild sinusoidal congestion and mild central venous congestion (Figure 4).

DISCUSSION

The CCl_4 is one of the most commonly used hepatotoxins in experimental study of liver disease (11). The lipid peroxidative degradation of bio-membrane is one of the principle causes of hepatotoxicity of CCl_4 (12,13). This is evident from an elevation in the serum marker analysis namely AST, ALT and ALP. *Cleome viscosa* (CV) significantly reduced this serum enzyme in group – III. Simultaneous administration of CV and CCl_4 produced significant recovery of the liver damage induced by CCl_4.

The hepatotoxic effect of CCl_4 are largely due to its active metabolite trichloromethyl radical (14), which binds to the macromolecule and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxide which in tern gives toxic aldehyde that causes damage to liver (15). This was evidenced by increase in level of lipid peroxidation in CCl_4 group and there was significant

Table-1 : Effect of Cleome viscosa on serum enzyme in CCl_4 induced hepatic damage in rats

Groups	Drugs	AST (U/L)	ALT (U/L)	ALP (U/L)
I	Vehicle Control	107.6 ± 9.5	59.5 ± 4.1	249.5 ± 18.2
II	CCl_4 (0.5ml/kg i.p.)	378.9 ± 23.7 [a]	254.9 ± 19.3 [a]	586.9 ± 31.6 [a]
III	CCl_4 + CV (200mg/kg p.o.)	117.6 ± 6.7 [b]	66.2 ± 6.1 [b]	258.4 ± 16.2 [b]
IV	CCl_4 + Silymarin (100mg/kg p.o.)	111.7 ± 8.7 [b]	62.3 ± 5.1 [b]	255.3 ± 24.1 [b]

Values are in mean ± SEM. Number of animals in each groups = 6 ; [a] P<0.05 Vs group I. [b] P<0.05 Vs group II

Table-2 : Effect of CleomeViscosa on Liver γ -glutamyl transpeptidase, Glutathiones and Lipid Peroxidase in CCl_4 induced hepatic damage in rats

Groups	Drugs	γ -Glutamyl transpeptidase µmol/mg tissue	Glutathione mg/gm tissue	Lipid peroxide Mol/mg tissue
I	Vehicle Control	44.06 ± 5.1	46.8 ± 4.8	136.9 ± 11.1
II	CCl_4 (0.5ml/kg i.p.)	198.8 ± 13.8 [a]	20.9 ± 2.1 [a]	289.5 ± 18.2 [a]
III	CCl_4 + CV (200mg/kg p.o.)	59.5 ± 4.8 [b]	35.6 ± 4.6 [b]	165.5 ± 15.6 [b]
IV	CCl_4 + Silymarin (100mg/kg p.o.)	46.7 ± 3.2 [b]	41.3 ± 4.1 [b]	155.5 ± 11.5 [b]

Values are in mean ± SEM : Number of animals in each groups = 6 ; [a] P<0.05 Vs group I.
[b] P<0.05 Vs group II

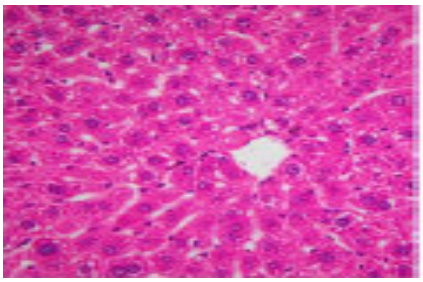

Figure 1 : Liver tissue of control rats showing normal histology

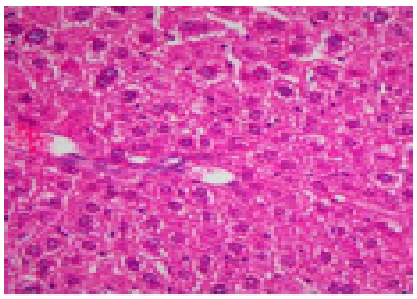

Figure 2 : Liver tissue of CCl_4 treated rats showing centrilobular necrosis, steatosis and fatty vacuolaization were seen with acute inflammatory cells infiltration sinusoids mainly in central zone

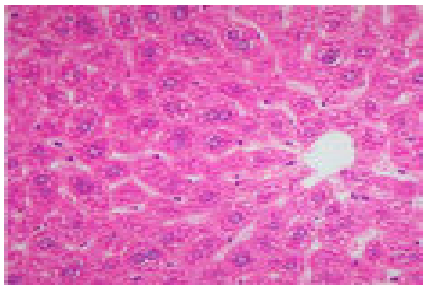

Figure 3 : Liver tissue of rats with CCl_4 and CV treated animals, the sections showed mild fatty change and mild sinusoidal congestion

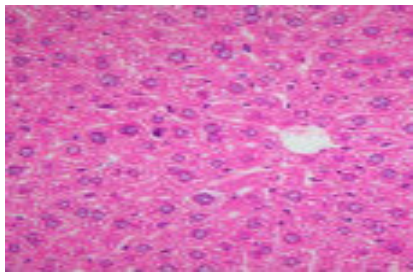

Figure 4 : Liver tissue of rats with CCl_4 and silymarin the sections showed mild sinusoidal congestion and mild central venous congestion

decrease in lipid peroxidation in CCl_4 and CV treated groups.

The estimation of γ-GTP level is valuable screening test with high negative, predictive nature for liver disease (16). In present study γ-GTP activity was elevated in CCl_4 treated rats. Increased activity of γ-GTP indicate a severe damage to tissue membrane during CCl_4 toxicity, because of γ-GTP which is a membrane bound enzyme (17). Administration of CV with CCl_4 to rats showed reduction in γ-GTP activity thus, it could indicate and reflect the membrane stabilizing activity of CV. This indicated that CV resulted in improving in liver function.

GSH plays a protective role in tissue by detoxification of xenobiotics. The tripeptide reduced GSH is essential to maintain structural and functional integrity of the cell. The significant decrease in liver GSH in CCl_4 treated rats may be due to enhanced substrate utilization by glutathione peroxidase (18). Administration of CV during severe liver damage condition has elevated GSH levels, which is turn helps in maintaining the liver tissue damage.

Comparative histopathological study of the liver from different groups of rats corroborated the hepatoprotective efficacy of CV. various pathological changes like steatosis, centrilobular necrosis and vacuolization seen in group II rats were prevented to a moderate extent in groups III and IV. All the effects of CV were comparable with silymarin. The results of our study indicated that the aqueous seed extract of *Cleome viscosa* could protect liver against CCl_4 induced hepatotoxicity.

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