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In vivo investigation of hepatoprotective activity of *Cleome viscosa* L. in albino rats

Raghavan Rajaraman, Renuka Saravanan and B. Dheeba and Sivakumar Ramalingam*

Department of Chemistry and Biosciences, SRC- SASTRA University, Kumbakonam, Tamil Nadu, South India

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ABSTRACT

This study was aimed to investigate the phytochemical constituents and hepatoprotective activity of Cleome viscose seeds extract on CCl_4 induced hepatotoxicity. Hepatotoxicity was induced in normal albino rats. CCl_4 was administered orally at a dosage of 20mg/kg body weight in physiological saline for 1 day. The rats were divided into five groups comprising of four rats each. Group I Normal control. Group II Disease control CCl_4 (20 mg /kg b.w). Group III Animals treated with the aqueous extract of Cleome viscosa seeds (150mg/Kg body weight orally) for 14 days. Group IV Animals treated with the aqueous extract of Cleome viscosa seeds (300mg/Kg body weight orally) for 14 days. Group V Animals treated with standard drug Silymarin (20mg/Kg body weight orally) for 14 days. After completed the experiment, the liver markers, lipid profile and antioxidants were analysed. Phytochemical screening of aqueous extract of Cleome viscosa seeds indicates the presence of flavonoids, tannins, glycosides, phenol, steroids, alkaloids, quinone, saponin and coumarin. Supplementation of Asteracantha longifolia significantly restored the liver markers, lipid profile and antioxidant markers on in CCl_4 induced Wistar albino rats. The results of our study showed that Cleome viscosa seeds possess significant hepatoprotective and antioxidant activity, probably due to its phytochemicals.

Keywords: *Cleome viscose*, Phytochemicals, Carbon tetrachloride, Antioxidant

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INTRODUCTION

Liver diseases are mainly caused by toxic chemicals, over doses of drugs (paracetamol, carbon tetra chloride, anti cancer drugs, (paracetamol, carbon tetra chloride, anticancer drug ,antibiotic and oral contraceptives), excessive consumption of alcohol, infections and auto immune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages[1]. Liver damage induced by carbon tetrachloride is the most commonly used model for the Screening of hepatoprotective drugs [2]. The rise in serum levels of Glutamic Pyruvate Transaminase (SGPT), Glutamic Oxaloacetic Transaminase (SGOT) and cholesterol following carbon tetrachloride has been attributed to the damaged structural integrity of the liver cells. These components are cytoplasmic in location and released into circulation after Cellular damages [3]. Carbon tetrachloride also plays a significant role in Inducing triacylglyceral accumulation, depletion of GSH, depression of protein synthesis and loss of enzymes activity [4].

Herbal medicines have been used in the treatment of liver diseases for a long time so the maintenance of a healthy liver is essential for the overall well being of an individual. Liver injury induced by toxins is more common nowadays. Herbal remedies are focused in the pharmaceutical industry to evolve a safe route for liver disorders.

Cleome viscosa L. (Capparidaceae) is a widely distributed herb with yellow flowers and long slender pods containing seeds. The whole plant is sticky in nature and has a strong odour resembling asafoetida. It is found throughout the greater part of India, often in waste places and and Tamil it is known as "Naikkadoku" in Indian traditional medicine. Traditionally, this plant is used to treat diarrhoea, fever, inflammation, liver diseases, bronchitis, skin diseases and malarial fever. The juice is useful in piles, lumbago and earache. Now a days, paracetamol is a most commonly used drug but nobody aware about its adverse effect when it used as long therapy or in large dose. *Cleome Viscosa* L. seeds extract used as a hepatoprotective agent. Literature survey indicates that no synthetic studies have been carried out on the clinical evaluation of hepatoprotective effect of *Cleome Viscosa* L. against paracetamol induced hepatotoxicity [5]. The aim of the present work is to investigate the hepatoprotective activity of *Cleome viscose* seeds extract.

MATERIALS AND METHODS

IDENTIFICATION AND AUTHENTICATION:

Plant sources selected for the present study is *Cleome viscosa* Linn. aerial seeds were collected from in and around Kumbakonam (Thanjavur Dt.), identified and authenticated by the Department of Botany, Government Arts College (autonomus), Kumbakonam, Tamil Nadu.

Preliminary Phytochemical Screening of various extracts: Harborne [6]**.**

Experimental animals

Healthy adult wistar strain of albino rats, weighing 100-120g were used as experimental models. Animals were kept in well ventilated cages and fed with standard rat chow pellet and water *ad libitum.* All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (Approval No: 790/03/ac/CPCSEA).

Experimental design

The rats were divided into five groups comprising of four rats each.
Group I Normal control
Group II Disease control CCl_4 (20 mg /kg b.w)
Group III Animals treated with the aqueous extract of *Cleome viscosa* seeds
(150mg/Kg body weight orally) for 14 days
Group IV Animals treated with the aqueous extract of *Cleome viscosa* seeds
(300mg/Kg body weight orally) for 14 days
Group V Animals treated with standard drug Silymarin (20mg/Kg body weight orally) for 14 days.

After the experimental period, animals were sacrificed by cervical decapitation. Blood was collected and serum was separated by centrifuged at 3000rpm for 10mins. Liver were dissected out and washed in ice-cold saline. Liver tissue was homogenized in 0.1M phosphate buffer, pH7.4 and used for studying various parameters. Liver tissues were also used for histopathalogical studies.

BIOCHEMICAL ESTIMATION

Assay of aspartate transaminase [7] Estimation of alanine transaminase [7] Estimation of serum alkaline phosphatase [7]. Estimation of serum bilirubin [8]

Estimation of protein [9] Estimation of serum / tissue triglycerides [10] Cholesterol was estimated in serum and tissue by the method of Parekh and Jung [11]. Estimation of lipid peroxides [12] Assay of glutathione peroxidase [13]. Assay of superoxide dismutase [14]. Assay of reduced glutathione [15]. Estimation of glutathione reductase [16].

Statistical analysis

Results were statistically analysed by Mean ± S.E. to consignificance between groups were estimated using students T test. $P<0.05$ is estimated as significance valued in the treated groups when compared to control.

RESULTS AND DISCUSSION

Table1. Represents the phytochemicals present in the methanolic and aqueous extract of *Cleome viscosa* seeds alkaloids, glycosides quinines phenol and coumarin were present in both the extract.

Table :1 Phytochemical analysis of various aqueous extracts of *Cleome viscosa.* Linn

S.No	Components	Observation	
		Methanol extract	Aqueous Extract
1	Terpenoids	-	-
2	Flavonids	-	-
3	Steroids	+	-
4	Glycosidase	+	+
5	Alkaloids	+	+
6	Quinine	+	+
7	Phenol	+	+
8	Tannins	+	+-
9	Saponins	-	+
10	Coumarin	+	+
11	Lignin	-	-

(+) Presence; (-) Absence
Our results are similar to [17].

Phytochemical screening of fresh and dry seeds showed the presence of flavonoids, glycosides, tannins, phenol and coumarin present in extracts except methanolic extract. Resins and phenols are present in moderate amounts. Sterols are present only in chloroform extracts. Terpenoids is present only in fresh leaf extracts and quinones only in benzene and aqueous extracts of dry leaf.

Flavonoids are present in fresh and dry leaf extracts of the plant but were absent in the seed extracts. They are a group of polyphenolic compounds that have potent antimicrobial, anti inflammatory actions. Flavonoids are free radical scavengers which prevent oxidative cell damage and have strong anti-cancer activity. The antioxidant, anti-inflammatory, antimutagenic and antimicrobial activities of the plant may be due to the presence of Flavanoids [18].

Alkaloids are a group of naturally occurring chemical compounds and chief class of plant secondary metabolites. They are bitter to taste and are toxic to other organisms and hence act in inhibiting microbial growth. The antibacterial and antifungal properties of the plant may be due to the presence of alkaloids. [19]

The serum marker enzymes were found to be increased in hepatotoxic induced rats as compared with normal groups. Administration of plant extract restored the activities of the marker enzymes to near normal level (Table 2).

In the present study it was observed that the animals treated with CCl_4 resulted in the significant hepatic damage as shown by the elevated levels of marker enzymes. These changes in the marker level will reflect in hepatic structural integrity. The rise in the SGPT plays a vital role in the conversion of alanine to pyruvate and glutamate (amino acids to ketoacid). The pre-treatment with extract significantly attenuated the elevated levels of the serum markers. SGPT is more specific to liver, and is thus a better parameter for detecting the liver injury [20].

Table 2 Effect of aqueous extract of hepatic marker enzymes in CCl_4 induced hepatotoxic rats

Particulars	AST(U/L)	ALT(U/L)	ALP(U/L)
Group 1	40.45±1.25	34.50±1.32	96.25±1.93
Group 2	113.50±1.44	97.25±1.38	309.50±2.50
Group 3	72.75±1.11	58.50±1.44	218±4.48
Group 4	47.50±1.04	38.00±0.20	115.25±4.48
Group 5	52.25±1.25	40.50±0.65	118.75±1.11

Data are expressed as mean ±S.E.M (n=6)
P<0.05 when compared with normal control and disease control
P<0.05 statistically significant when compared with CCl_4 treated group

Serum SGOT ,SGPT and ALP, are the most sensitive markers employed in the diagnosis of hepatic damage because these are cytoplasmic in location and are released in to the circulation after cellular damage [21].

Similar observation was observed by [22]. Hepatocellular necrosis leads to evation of the serum marker enzymes, which are released from liver into blood

Table 3: Effect of aqueous extract of *Cleome viscosa* on the level of protein and Bilirubin in CCl_4 induced hepatotoxic rats. A significant decrease with concomitant decrease in bilirubin level was observed in group2 animals upon administration of plants extract the level of protein bilirubin were restored to near normal (Table 3).

The levels of total protein was reduced due to the cc induced hepatotoxicity. The reduction is attributed to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of P_{450} leading to its functional failure with a decrease in protein synthesis and accumulation of triglyceride leading to fatty liver[23].

Bilirubin assay is a sensitive test to substantiate the functional integrity of the liver and severity of necrosis **[**24**]**.Bilirubin also measure the binding, conjugation and excretory capacity of hepatocytes and is proportional to the erythrocyte degradation rate. [25] increase in serum Bilirubin level may be found in hepatocellular damage, haemolytic jaundice (or) hepatitis ccl_4 injury causes significant degeneration of hepatocytes and blockade of the bile ducts which result in to significant increase in the serum total bilirubin and direct Bilirubin levels [26] .

Table 3: Effect of aqueous extract of *Cleome viscosa* on the level of protein and Bilirubin in CCl_4 induced hepatotoxic rats

Particulars	Protein(g/dl)	Bilirubin(mg/dl)
Group 1	7.24±0.02	0.77±0.01
Group 2	3.93±0.03	3.84±0.05
Group 3	5.05±0.04	1.05±0.01
Group 4	6.97±0.02	1.00±0.04
Group 5	6.48±0.009	1.06±0.01

Data are expressed as mean ±S.E.M (n=6)
P<0.05 when compared with normal control and disease control
P<0.05 statistically significant when compared with CCl_4 treated group

In present study, administration of *cleome viscosa* L Czern during cc toxicity decrease the cholesterol (TC) and triglycerides (TGL) on serum and it reaches approximately to the normal value. These depicts that the test drug has the potency of activating of TC and TGL activity (Table 4).

Intoxication with CCl_4 also, resulted in inhibition of bile acids synthesis from cholesterol which is synthesized in liver (or) derived from plasma lipids, leading to an increase in cholesterol levels. Suppression of cholesterol levels by CCl_4 suggested the inhibition of the synthesis of bile acids from cholesterol [27].

A number of hepatotoxic agents cause accumulation of fatty deposits predominantly triglycerides in the parenchyma cells of liver. This accumution of triglycerides may be as result of an imbalance between the rate of synthesis and the rate of release of triglycerides by the parenchymal cells into systemic circulation [28]. Proposed that a block of the secretion of hepatic triglycerides into plasma is the major mechanism underlying the fatty liver induced in rats by CCl_4.

Table 4: Effect of aqueous extract of *Cleome viscosa* on the level of cholesterol and triglycerides in CCl_4 induced hepatotoxic rats

Particulars	Cholesterol(mg/dl	Triglycerides(mg/dl)
Group 1	163.75±1.75	183.75±1.93
Group 2	67.75±1.31	76.00±1.58
Group 3	113.50±1.71	128.73±1.47
Group 4	163.25±1.75	178.25±1.49
Group 5	140.50±0.65	157.75±1.11

Data are expressed as mean ±S.E.M (n=6)
P<0.05 when compared with normal control and disease control
P<0.05 statistically significant when compared with CCl_4 treated group

Table 5: Effect of aqueous extract of *Cleome viscosa* on LPO, GSH and antioxidant enzyme activity in CCl_4 induced hepatotoxic rats. GSH is a critical determine of tissue susceptibility to oxidative damage and the depletion of hepatic GSH has been shown to be associated with enchanced toxicity to chemicals inducing CCl_4. The significant impairment of heptic GSH status associated with a substantial hepatocellular damage induced by CCl_4

suggested the derminant role of hepatic GSH in the development of ccl_4 toxicity a similar observation was made by [29].

Malondialdehyde (MDA) is a major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fattyacid. MDA, a secondary product of lipid peroxidation is used as an indicator of tissues damage involving a series of chain reactions. It reacts with thiobarbituric acid , producing red coloured products .lipid peroxidation has been implicated in the pathogenesis of increased membrane rigidity, osmotic fragility, reduced erythrocyte survival and perturbation in lipid fluidity. It has been hypothesized that one of the principal cause of CCl_4 induced hepatotoxicity is lipid peroxidation of hepatocytes membranes by free radical derivatives of CCl_4 [30].

Hepatotoxic effect of CCl_4 is due to be of the mechanism of generation and antioxidant property is claimed to be one of the mechanism of hepatoprotective drugs. The preliminary phytochemical reports revealed that the ethanolic extract of the leaves were found to contain higher concentration of flavonoids and alkaloids .it has been repoted that the flavonoid possess antioxidant properties by free radical scavenging . Flavonoids are phenolic compounds widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant and free radical scavenging abilities [20].

Table 5: Effect of aqueous extract of *Cleome viscosa* on LPO, GSH and antioxidant enzyme activity in CCl_4 induced hepatotoxic rats

Particulars	**LPO** (nM of MDA formed/g of tissue)	**Glutathione peroxidase** (µg of GSH hydrolysed /g tissue/minutes)	**SOD** (mg of epinephrine oxidized /g tissue)	**Glutathione reductase** (µg of GSH produced/ g tissue/minute)	**Reduced glutathione** (µg/g tissue)
Group 1	21.83±1.83	0.84±0.02	9.64±0.58	3.82±0.02	26.33±0.15
Group 2	169.17±0.57	0.24±0.01	213.83±2.92	1.04±0.02	10.41±0.18
Group 3	118.33±0.43	0.60±0.02	103.03±1.91	1.07±0.02	14.00±0.09
Group 4	54.33±1.82	0.82±0.01	43.48±0.52	2.21±0.02	20.27±0.09
Group 5	87.17±1.26	0.81±0.01	65.20±0.34	2.76±0.03	22.04±0.16

Data are expressed as mean ±S.E.M (n=6)
P<0.05 when compared with normal control and disease control
P<0.05 statistically significant when compared with CCl_4 treated group

An increase in lipid peroxidation and a suppression of antioxidant capacity because of its consumption plays an important role in the genesis of hepatic damage .lipid peroxidation is autocatalytic process, which is a common consequence of cell death [31].

SOD scavenges the superoxide radicals by dismutation .the the group 2 animals showed a decrease in the SOD levels which may be due to the increases lipid peroxidation or the inactivation of enzyme by cross linking with malondialdehyde. The enzyme inactivation leads to accumulation of superoxide radicals which may further stimulate the per oxidation process [32].

Gpx is a selenium dependent enzyme has high potency in scavenging reactive free radicals . When Gpx activity in liver increase, the glutathione level is decreased. Inhibition of Gpx by goldthioglucose (GTG) has been found to increase the susceptibility of hepatocytes to CCl_4 toxicity, indicating that a component of CCl_4 toxic effect involves formation of species that are detoxified by Gpx enzymes. So the level of Gpx reduced to normal by the plant extract. [33].

This study confirmed that the seeds of *C.viscosa* possess various phytoconstituents (Alkaloids, glycosides, tannins, phenol and coumarin) which may contribute its antioxidant and hepatoprotective activity. This study paired a way for the future investigations to analyse & to isolate a novel pharmacologically active compound from the seeds of *C.viscosa* which is used for treating infectious disease and metabolic disorder.

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