

## **Short Communication**

### **Hepatoprotective Activity of Ethanol Extract of *Cleome rutidosperma* Aerial Parts against Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats**

**Anuradha. Khuntia\*, Sujit Kumar Mohanty, M.Sai Harika**

C. E. S. College of Pharmacy, Chinnatekur, Kurnool-518003, Andhra Pradesh, India

#### **\*Corresponding author**

Anuradha. Khuntia

Email: [anuphchemist@gmail.com](mailto:anuphchemist@gmail.com)

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**Abstract:** *Cleome rutidosperma*, Linn (Family-Cleomaceae) is claimed to relieve earache, pain. However thorough literature survey indicated that different plants of the same genus available in tropical parts of India have various reported biological activities like antifungal activity, antibacterial activity, analgesics, antipyretic, anthelmintic activity, antimycobacterial activity and cytotoxic activity and CNS depressant activity etc. Hepatoprotective activity has been reported on some other plants of *Cleome* genus. Thus with an assumption that, the plant *Cleome rutidosperma* might have similar hepatoprotective activity, the ethanolic extract of aerial parts of *Cleome rutidosperma* was investigated for the said activity. It was found that the ethanol extract of a dose of 400 mg/kg body weight exhibited moderate protective effect by lowering the serum levels of alanine aminotransferase (ALT) or Serum Glutamate Pyruvate Transaminase (SGPT), aspartate aminotransferase (AST) or Serum Glutamate Oxaloacetate Transaminase (SGOT) and cholesterol to a significant extent. The hepatoprotective activity was also supported by histopathological studies of liver tissue. Since results of biochemical studies of blood samples of carbon tetrachloride treated rats showed significant increase in the levels of serum enzyme activities, reflecting the liver injury caused by CCl<sub>4</sub> and blood samples from the animals treated with the methanol extracts of *Cleome rutidosperma* showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells, the extracts of four above plants could afford significant dose-dependent protection against CCl<sub>4</sub> induced hepatocellular injury.

**Keywords:** *Cleome rutidosperma*, Hepatoprotective activity, Carbon tetrachloride

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#### **INTRODUCTION**

Carbon tetrachloride (CCl<sub>4</sub>), a well known toxicant, is metabolically activated by cytochrome P450 to form CCl<sub>3</sub> free radicals, which initiate lipid peroxidation in the cell. CCl<sub>4</sub> induces liver necrosis, and the Kupffer cells may possibly phagocytose the necrotic cell remnants. In addition, CCl<sub>4</sub> metabolites react with polyunsaturated fatty acids to propagate a chain reaction leading to lipid peroxidation / covalently bind with lipids and proteins; leading to the destruction of cell membrane and liver damage. Hepatotoxicity or liver damage by CCl<sub>4</sub> can be measured by the analysis of several biochemical parameters including serum enzymes (ALT, AST, ALP), bilirubin and protein etc. The level of serum enzymes, bilirubin, TBARS increased in blood due to the administration of CCl<sub>4</sub> leading to cell membrane damage and necrosis. Serum enzymes are more specific to liver, and are a better marker for detecting liver injury. Carbon tetrachloride gives trichloromethyl radicals, which upon reacting with reactive oxygen species (ROS) yield trichloromethyl peroxide radicals that form covalent bond with membrane lipids and disrupt the membrane integrity. About 20,000 deaths are found every year due

to the liver disorders. More than 160 photochemicals isolated from different plant species showed hepatoprotective activity. Herbal drugs contain a variety of chemical constituents which showed potent liver protective activity [1].

*Cleome rutidosperma* (Capparidaceae) is a low growing herb, up to 70 cm tall, found in waste herb grounds and grassy places with trifoliate leaves and small, violet blue flowers, which turn pink as to West Africa, although it has become naturalized in various parts of tropical America as well as Southeast Asia. According to traditional use, the different parts of the plants of *Cleome* genus are used as stimulant, antiscorbutic, anthelmintic, vesicant, rubefacient and carminative. The antiplasmodial, analgesic, locomotor antimicrobial, diuretic, laxative activities of *Cleome rutidosperma* were reported earlier. *Cleome rutidosperma* is traditionally used in the treatment of paralysis, epilepsy, convulsions, spasm, pain and skin disease. The popular use of the roots, however, refers mainly to its analgesic, anti-inflammatory and anthelmintic activity. Such plants are used as a source of traditional medicine by most of the population in developing

countries as it is passed on from generations to generations. Most of the plants used in traditional medicine have solid scientific support with regard to their efficacy. Although no work regarding evaluation of hepatoprotective activity has been reported on *Cleome rutidosperma*, similar activity have been reported on some other plants of *Cleome* genus [10]. Thus with an assumption that, the plant *Cleome rutidosperma* might have similar hepatoprotective activity, so the ethanolic extract of aerial parts of *Cleome rutidosperma* was investigated for the said activity.

## MATERIAL AND METHODS

### Plant collection & Extraction

The plant material (whole plant) was collected from Salipur, Cuttack District of Odisha, India during September 2009 and was authenticated at Botanical Survey of India, Shibpur, Howrah, West Bengal, India. Avoucher specimen, (C.R.-1) has been kept in our research laboratory for future reference. The fresh aerial parts were washed under running tap water to remove adhered dirt, followed by rinsing with distilled water, shade dried and pulverized in a mechanical grinder to obtain coarse powder. The powder was extracted with 90% ethanol using Soxhlet apparatus. The solvent was removed under reduced pressure, which gave a greenish-black colored sticky residue (yield 11.6% w/w on dried material basis).

### Animals

Swiss albino rats weighing between 80 and 90 gm were used in this evaluation. These rats aged between 2 and 2.5 months. They were housed in well ventilated stainless-steel cages at room temperature ( $24 \pm 2^\circ\text{C}$ ) in hygienic condition under natural light and dark schedule and were fed on standard laboratory diet. Food and water were given.

### Experimental Design for Hepatoprotective Activity

The rats were divided randomly into three groups of six rats each. Group A was treated as control and was injected with 0.2 ml/kg (B wt) of liquid paraffin once daily. In group B liver damage was produced by injection of Carbon tetrachloride (1 ml/kg Bwt, i.p) in liquid paraffin once daily. Group C received ethanolic extract of plant at a dose of (400 mg/kg B wt) once daily. The doses were given to the respective rats for seven days.

### Assessment of Hepatoprotective Activity

In the present study the hepatoprotective activity was evaluated biochemically and histopathologically. Clinical signs and body weights were recorded. Blood was collected from the animals, by cardiac puncture, for haematological and biochemical analysis. A portion of blood sample was dispensed into EDTA anticoagulant bottle for haematological analysis. The blood samples

from euthanized rats were dispensed into plain tubes and allowed to stand for 3 hours to ensure complete clotting. The clotted blood samples were then centrifuged at 300 rpm for 10 minutes. The clear sera were aspirated and stored frozen for serum biochemical analysis. The indices determined included Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), total and direct bilirubin, creatinine, urea, uric acid, Alkaline Phosphatase (ALP). All haematological and biochemical analysis mentioned above were carried out in Tarinipatholab, Salipur, Cuttack, Odisha, India.

The histopathological studies for the dissected liver were carried in Prasanti Laboratories, Cuttack, Odisha, India. For the studies, sections of livers of each rat were immersed immediately in 250 ml of neutralized 10% (v/v) formalin. The tissues were kept in the fixative for 12 hours, dehydrated with serial ethanol cycles (70% to absolute) and then embedded in paraffin. The paraffin-embedded tissue was cut into 5 micrometre sections. The tissue sections are deparaffinised and stained with haematoxylin-eosin. Microscopic examinations were done at the magnification of X 400.

## RESULT

The effect of ethanol extract of *Cleome rutidosperma* on serum transaminase, alkaline phosphatase, bilirubin levels in  $\text{CCl}_4$  intoxicated rats are summarized in table 1. There was a significant ( $p < 0.001$ ) increase in serum GOT, GPT, ALP and bilirubin levels in  $\text{CCl}_4$  intoxicated group compared to the normal control group. Ethanolic extract of *Cleome rutidosperma* at the dose of 400 mg/kg orally significantly decreased the elevated serum marker enzymes to almost normal level (table-1). The extract also reduced the level of bilirubin compared to the untreated group [11].

The histological appearance of the hepatocyte reflects their damage conditions. Exposure of hepatocytes to toxic agents such as  $\text{ccl}_4$  leads to histopathological changes from the normal histological appearance. The hepatocytes of rats treated with a single dose of 1.25 ml  $\text{CCl}_4/\text{Kg}$ , showed centrilobular necrosis and extensive fatty change was observed on the mid zonal or entire lobe at 24 hours after treatment. Effective hepatoprotective agents will protect the hepatocytes from the histopathological changes caused by toxic agents. Liver tissues of rats treated with  $\text{ccl}_4$  and silymarin showed no necrosis or fatty deposition but had only minimal portal inflammation reflecting good protection of the known hepatoprotective drug silymarin. Histological changes in the liver of rats treated with 20.7 mmol/kg of ethanolic extract and  $\text{ccl}_4$  showed a significant recovery except cytoplasmic vascular degenerations around portal tracts, mild inflammations around portal tracts, mild inflammation and foci of lobular inflammations.

**Table 1: Effect of ethanol extract of *Cleome rutidosperma* on serum transaminase, alkaline phosphatase, bilirubin levels in CCl<sub>4</sub> intoxicated rats**

Parametres	Normal Control	Ccl <sub>4</sub> Control	EECR (400mg/kg)	Silymarin (25mg/kg)
SGOT(U/L)	56.7±9.2	178.3±14.8 <sup>000</sup>	90.2±11.7***	75.3±7.7***
SGPT(U/L)	23.2±2.2	60.5±5.9 <sup>000</sup>	28.9±4.4*	27.08±3.68**
Bilirubin (mg/dl)	0.12±0.04	0.44±0.05	0.19±0.04	0.14±0.03*
ALP(U/L)	141.8±8.2	382.9±12.9 <sup>000</sup>	214.5±10.3***	163.2±10.9***

Effect of ethanolic extract of *Cleome rutidosperma* on some serum biochemical parametres of CCl<sub>4</sub> intoxicated rats Values are MEAN ± S.E.M. No. of rats in each group is 6. CCl<sub>4</sub> control group compared with normal control group <sup>0</sup>p<0.001. Experimental groups compared with CCl<sub>4</sub> control group \* p<0.05, \*\* p<0.01, \*\*\* p<0.001

## DISCUSSION

Liver injury induced by CCl<sub>4</sub> is the best characterized system of xenobiotic-induced hepato-toxicity and is commonly used models for the screening of anti-hepatotoxic and/or hepatoprotective activities of drugs. The changes associated with CCl<sub>4</sub> induced liver damage are similar to that of acute viral hepatitis. It has been established that carbon tetrachloride accumulates in hepatic parenchymal cells and gets metabolically activated by cytochrome P-450 dependant monooxygenases from trichloromethyl free radical (CCl<sub>3</sub>). These free radicals alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxides, leading to liver damage. Lipid peroxidation will initiate pathological changes such as depression of protein synthesis [4,5].

In the assessment of liver damage by CCl<sub>4</sub> hepatotoxin the determination of enzyme levels SGPT and SGOT is more specific to the liver and a better parameter for detecting liver damage and largely used. Necrosis and liver damage release the enzyme into circulation; therefore it can be measured in serum. High levels of SGOT indicate liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. SGPT catalyzes the conversion of alanine to pyruvate and glutamate and is released in similar manner. Therefore SGPT is more specific to liver and a better parameter for detecting liver injury [6].

Serum ALP and bilirubin levels are also related to the status and function of hepatic cells. Increase in Serum ALP is due to increased synthesis, in presence of increasing biliary pressure. The site specific oxidative damage of some of the susceptible amino acids of proteins is regarded as the major cause of metabolic dysfunction during pathogenesis. Hypoalbuminaemia is the most frequent in the presence of advanced chronic liver diseases. Hence decline in total protein content can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism, which accelerates the

regeneration process and the production of liver cells. Treatment with CCl<sub>4</sub> increases the levels of total lipids, total triacylglycerols and total cholesterol in liver [7].

Phytoconstituents such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including hepatoprotective and antioxidant activity [2, 3]. There has been growing interest in the analysis of certain flavonoids, terpenoids and steroids stimulated by intense research into their potential benefits to human health. Presence of these phytoconstituents in *Cleome rutidosperma* may be responsible for the observed hepatoprotective activity [8, 9].

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