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HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF *CLEOME GYNANDRA* L. AGAINST CARBON TETRACHLORIDE AND PARACETAMOL - INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for liver injury. The present study was conducted to evaluate the hepatoprotective activity of methanolic extract of leaves of *Cleome gynandra* in wistar rats. The studies were conducted using the two popular inducing agents Paracetamol (2 g/kg, p.o.) in 1% NaCMC and Carbon tetrachloride (1 ml/kg). Silymarin (100 mg/kg, p.o.) was used as reference drug in the respective models. The effect was estimated by measuring the enzymatic levels and histo- pathological studies. The methanolic extract of leaves of *Cleome gynandra* has shown very significant hepatoprotection against both Paracetamol and CCl_4 - induced hepatotoxicity study models in wistar rats. This was evidenced by marked reduction in marker enzymes in serum. Histopathological studies also confirmed the hepatoprotective nature of the extract.

Keywords: *Cleome gynandra*, hepatoprotective, paracetamol, carbon tetrachloride, flavanoids, triterpenes.

INTRODUCTION:

Liver is the vital organ of metabolism and excretion. It is continuously and variedly exposed to xenobiotics because of its strategic placement in the body. Hepatic injury is associated with distortion of metabolic functions, thus liver ailments remain as one of the serious health problems [(1)]. Drug induced liver injury is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and regulatory agencies. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all liver failure [(2)]. More than 900 drugs have been concerned in causing injury to liver and it is the most familiar reason for a medicine to be withdrawn from the market. According to the United States Acute Liver Failure Study Group, drug-induced liver injury accounts for more than 50% of acute liver failure, including liver damage caused by overdose of acetaminophen and idiosyncratic liver injury triggered by other drugs. Chemicals frequently cause subclinical injury to liver that can be detected by estimating liver enzyme levels. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. However, there are not enough drugs available for the treatment of liver disorders.

Recently, many folk remedies from plant origin are being evaluated for its possible antioxidant and hepatoprotective effects against different chemical-induced liver damage in experimental animals [(3, 4)]. *Cleome gynandra* belongs to the family Capparaceae, is used as a medicinal plant and can be found in all over the world. It grows as a weed in paddy fields and road sides, in India it never cultivates but grows everywhere. It has remarkable nutritional and antioxidant properties. It is an erect annual herb grows upto 200-500 mm tall stems has glandular hairs, Leaves are palmately compound with 3-5 leaflets. The leaves are used as ingredients in other mashed foods, and dried leaves are incorporated in weaning foods. The leaves are bitter so cooked with other vegetables. Leaves are rich in vit.C, vit.A, calcium and iron. The plant is found to be used as immunomodulatory, anti-oxidant, analgesic, anti-carcinogenic etc[5, 6].

MATERIALS AND METHODS

Plant material

Leaves of *Cleome gynandra* was collected during flowering season from Utukur village, Kadapa district, Andhra Pradesh, India. The plant was authenticated by Dr. Madhava Chetty, Taxonomist, S.V. University, Tirupathi, India. The collected leaves were washed immediately and dried at 50°C for a week, powdered mechanically, sieved (10/44) and stored in air-tight containers.

Preparation of Extracts

About 2000 g of the powdered material was subjected to soxhlation and exhaustively extracted with methanol for 48 h. The solvent was distilled off at low temperature under reduced pressure using rotary flash evaporator (buchi, flawil, Switzerland). The semisolid mass obtained was dried in an oven at 40^0C, powdered, labeled as MECG and stored in desiccator.

Chemicals

Carbon tetrachloride was procured from S.D. Fine Chemicals Ltd. (India). Silymarin was obtained as gift sample from Ranbaxy (Devas, India). Standard kit of SGPT, SGOT, ALP and bilirubin were obtained from Jain Scientific Industries, Moradabad, India. All other reagents used were of analytical grade.

Phytochemical investigation

Phytochemical tests were carried out to find out the presence of phytoconstituents viz flavanoids, saponins, glycosides, carbohydrates, phenols etc and the results are shown in Table: 1

Experimental Animals

Wistar rats (150-200 g) were used in this experiment. They were housed in standard cages by maintaining a temperature of 22± 2ºC at 12:12 hours light dark cycle. The animals were provided with pellet diet and water *ad libitum*. The experimental procedures were carried out in strict compliance with the ethical guidelines for investigations of experimental pain in conscious animal framed by the Institutional Animal Ethical Committee rules and regulations in this institute.

Acute toxicity studies

Acute oral toxicity studies were conducted to determine the LD_{50} cut off value (mg/kg body weight) as per the OECD Guideline - 425.

Biochemical estimation of markers of oxidative stress

SOD activity was determined according to previous report[7]. CAT activity was determined from the rate of decomposition of H_2O_2 by the reported method[8]. GPX activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3 [9]. Glutathione reductase activity was assayed according to previous reports [10]. Protein content in the tissue was determined by earlier method reported [11], using bovine serum albumin (BSA) as the standard.

Histopathological study

The liver was removed and stored immediately in 10 percent formalin. The tissue was subsequently put in paraffin. Thin (5µm) sections were drawn using a microtone and then stained with hematozylin and eosin and mounted in neutral di-styrene-dibutyl propylene (DPX) medium and examined using photo microscopy [12].

Assessment of hepatoprotective activity

A toxic dose or repeated doses of a known hepatotoxin such as carbon tetrachloride, paracetamol, thioacetamide, rifampicin, alcohol, D-galactosamine, allyl-alcohol etc., are administered to induce liver damage in experimental animals [13]. If the hepatotoxicity produced by the toxin is prevented or reduced, then the test substance is considered as an effective hepatoprotective agent. In the present investigation, rats (n=6) were randomized into following groups and the pharmacological investigation was carried using carbon tetrachloride and paracetamol as inducing agents and the test MECG at dose levels of 200 and 400 mg/kg as hepatoprotective agent.

1) Group I -1% w/v NaCMC per orally for 21 days.
2) Group II - CCl_4 (1 ml/kg) administered by i.p + 1% w/v NaCMC per orally for 21 days.
3) Group III - Paracetamol (2 g/kg) in 1% NaCMC per orally for 21 days.
4) Group IV- CCl_4 (1 ml/kg) administered by i.p + MECG (200mg/kg) in 1% w/v NaCMC per orally for 21 days.
5) Group V- CCl_4 (1 ml/kg) administered by i.p + MECG (400mg/kg) in 1% w/v NaCMC per orally for 21 days.
6) Group VI- Paracetamol (2 g/kg) and MECG (200mg/kg) in 1% w/v NaCMC per orally for 21 days.
7) Group VII- Paracetamol (2 g/kg) and MECG (400mg/kg) in 1% w/v NaCMC per orally for 21 days.
8) Group VIII- CCl_4 (1 ml/kg) administered by i.p and Silymarin (100 mg/kg) in 1% w/v NaCMC per orally for 21 days.
9) Group IX- Paracetamol (2 g/kg) and Silymarin (100 mg/kg) in 1% w/v NaCMC per orally for 21 days.

Treatment with plant extract was started after 24 hrs of administration of inducing agents. After 21 days of such treatment, rats were sacrificed by cervical dislocation. Blood was collected and serum was separated by allowing the blood samples to coagulate for 30 min at 37ºC followed by centrifugation (3000 rpm for 15 min) and subjected for determination of biochemical parameters like total bilirubin, SGPT, SGOT and ALP[14]. Liver was dissected out, washed with ice cold Phosphate Buffer Saline (PBS) (0.1 M, pH 7.4) and 10% tissue homogenate used for different biochemical analysis. A part of the liver was used for histopathological studies.

STASTICAL ANALYSIS

The results are expressed as Mean ± SEM of six animals from each group. The data were evaluated by one-way ANOVA followed by Tukey's multiple comparison tests. *P values <0.05 was considered statistically significant.

RESULTS

Preliminary phytochemical screening

The various phytoconstituents present in different extracts were given in Table 1. MECG showed significant amounts of flavanoids and triterpenes.

Acute toxicity studies

The MECG did not exhibit any toxic effects up to 4000 mg/kg body weight on oral administration. Body weight before and after administration were noted and any changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous system, somatomotor activity, behavioral pattern were observed, sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were seen. The onset of toxicity and signs of toxicity were not seen in the rats up to 72 hr of observation period. This indicates the safety of extract.

Biochemical parameters

Rats treated with carbon tetrachloride and paracetamol showed a significant hepatic damage as observed from elevated levels of hepato-specific enzymes as well as severe alteration in different liver parameters. SGPT, SGOT, and total bilirubin in serum were increased in carbon tetrachloride and paracetamol intoxicated control animals. Treatment with the methanolic extract of *Cleome gynandra* caused significant protection against paracetamol and CCl_4–induced increase in serum enzyme levels and bilirubin in a dose responsive manner. Similarly, LP, SOD, CAT and GSH contents were estimated from liver homogenate and MECG showed significant protection against both paracetamol and CCl_4 induced liver damage.

Histopathological Studies

Histhopatological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central veins. Disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration were observed in paracetamol and CCl_4 intoxicated animals. The liver sections of the rats treated with methanolic extract of *Cleome gynandra* and standard drugs followed by paracetamol and CCl_4 intoxication showed a sign of protection as it was evident the absence of necrosis and vacuoles.

DISCUSSION

Carbon tetrachloride and paracetamol are the well known hepato-destructive agents that are widely used to induce acute-toxic liver injury in laboratory animals. The changes associated with CCl_4-induced hepatic damage are similar to that of acute viral hepatitis. The hepatotoxicity of CCl_4 has been reported to be due to its biotransformation by cytochrome P-450 system to produce trichloroethylene free radicals. These free radicals may again react with oxygen to form trichloroethylene peroxy radicals, which exert their action on lipids membrane of endoplasmic reticulum to evoke lipid peroxidation[15]. Overdose of paracetamol causes a potentially fatal, hepatic centrilobular necrosis. The hepatotoxicity of paracetamol has been attributed to the formation of a toxic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) by the action of cytochrome P4502E1 [16]. In the present investigation, CCl_4 and paracetamol administration resulted in elevated activities of AST, ALT and ALP in serum against their respective control values. Similarly, serum bilirubin level was also found to be increased significantly as a result of CCl_4 and paracetamol toxicity. On the other hand, total serum protein level was lowered in response to CCl_4 and paracetamol administration when compared with control. Abnormally higher activities of serum ALT, AST and ALP after CCl_4 and paracetamol administration are an indication of the development of hepatic injury, which is responsible for leakage of cellular enzymes into the blood. When liver plasma membrane gets damaged, a variety of enzymes normally located in the cytosol are released into the circulation [17].

Oral administration of various doses of MECG to CCl_4 and paracetamol intoxicated rats resulted in gradual normalization of the activities of AST, ALT and ALP. This evidently suggests the protective effect of the extract in improving the functional integrity of liver cells. Serum bilirubin is considered as an index for the assessment of hepatic function and any abnormal increase indicates hepatobiliary disease and severe disturbance of hepatocellular architecture. CCl_4 and Paracetamol administration resulted in increased serum bilirubin level, thereby suggesting severe hepatic injury and confirming the hepatotoxic nature of CCl_4 and paracetamol. Treatment with MECG significantly decreased the elevated level of total bilirubin in serum towards normalcy indicating its hepatoprotective efficacy.

Hepatic lipid peroxidation (LP), expressed as TBARS (thiobarbituric acid reacting substances), increased significantly in CCl_4 and paracetamol toxicity. While, the activities of protective enzymes such as Superoxide dismutase (SOD) and catalase (CAT) and glutathione and glycogen content in liver tissue were lowered after paracetamol administration. Enhanced LP and reduced activities of SOD and CAT is an indication of generation of free radical stress as a mark of hepatic damage due to CCl_4 and paracetamol toxicity. Marked reductions in the activities of these free radical scavenging enzymes, SOD and CAT, associated with CCl_4 and paracetamol toxicity were significantly reversed to normal on oral feeding of MECG in a dose dependent manner conferring the antilipid peroxidative ability to the extract. CCl_4 and Paracetamol induced damage of hepatocytes is also a reason behind decreased glycogen content of liver tissue. Significant increase in hepatic glycogen level was observed after administration of the extract indicating improvement in hepatic status. Histopathological examination of liver sections of the normal control group showed normal cellular architecture with distinct hepatic cells. However, distinct hepatic necrosis was noted after CCl_4 and paracetamol administration with destruction of hepatic cells. MECG treatment to such CCl_4 and paracetamol intoxicated rats showed recovery of the hepatocytes from necrosis. This also suggests that the plant extract has a tremendous potential to reverse the changes induced by paracetamol toxicity back to normal.

The curative efficacy of MECG was dose dependent as evidenced by gradual reversal of the altered values of various biochemical markers back to normal following oral administration. This may, probably be through promotional activation of antioxidative enzymes and regeneration of hepatocytes that restore the structural and functional integrity of liver. The protective effects due to treatment with *Cleome gynandra* extract strongly indicated the possibility of the extract being able to prevent and/or mitigate any leakages of marker enzymes into circulation, condition the hepatocytes to accelerate regeneration of parenchymal cells, and preserve the integrity of the plasma membranes and hence restore these enzymes levels [18]. Thus, the present investigation confirms the hepatoprotective action of *Cleome gynandra* against paracetamol induced hepatotoxicity in rats.

Table - 1: Qualitative phytochemical analysis of methanolic extract of *Cleome gynandra* (MECG)

Constituent	MECG
Alkaloids	+
Mayer`s test	+
Dragendorff`s test	-
Wagner`s test	-
Hager`s test	
Carbohydrates	+++
Molish`s test	++
Fehling`s test	++
Benedict`stest	
Glycosides	+++
Libermann-Burchard test	+++
Salkowski test	-
Borntrager`s test	
Saponins	+++
Foam test	
Phenolic compounds	++
Ferric chloride test	+++
Shinoda test	+
Lead acetate test	-
Alkaline reagent test	

+++ High, ++ Moderate, + Slight, - Negative

Table: 2- Methanolic extract of *Cleome gynandra* (MECG) on serum enzymatic activity in CCl_4 and Paracetamol induced liver damage in rats (n = 6)

GROUP	SGOT (IU/L)	SGPT (IU/L)	ALP (KA Units)	Total Bilirubin (mg/dL)
Group I	97.37±5.89	76±5.0	165.2±7.31	0.54±0.005
Group II	420.5±12.28##	301±14##	332.9±10.5###	4.25±0.4###
Group III	409±9.7##	286.4±9.3##	321.9±8.5###	3.9±0.7###
Group IV	135.5±14.5*	145.8±7.5**	245.9±2.9***	1.4±0.08**
Group V	117.9±12.5***	101.5±8.7**	205.7±9.5**	0.8±0.05***
Group VI	142.5±9.6***	147.8±9.6***	251.7±9.6***	1.7±0.07**
Group VII	119.5±11.5***	107.9±9.5**	215.6±4.9***	0.9±0.07**
Group VIII	107±9.7***	89.5±8.6**	187.5±5.17**	0.7±0.04***
Group IX	110.7±4.6***	91.2±4.8**	192.1±6.5**	0.9±0.05***

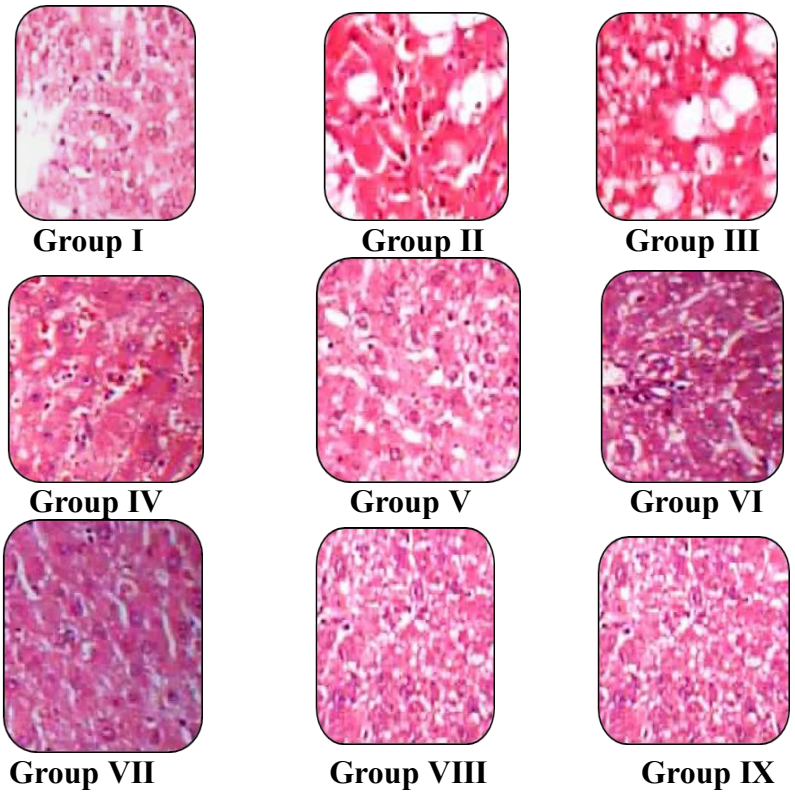
The results are expressed as Mean ± SEM of six animals from each group. # indicates p<0.05, ## indicates p<0.01 and ### indicates p<0.001 when compared to normal. * indicates p<0.05, ** indicates p<0.01 and *** indicates p<0.001 when compared to CCl_4 and paracetamol intoxicated groups were evaluated by one-way ANOVA followed by Tukey's multiple comparison tests.

TABLE: 3- Methanolic extract of *Cleome gynandra* (MECG) on LP, SOD, CAT, GSH and Glycogen content in liver in rats (n = 6)

GROUP	LP (moles of MDA formed/mg protein)	SOD (Units of activity/mg protein)	CAT (µ moles of H2O2 decomposed /mg protein)	GSH (µg/mg protein)
Group I	28.16 ±0.81	15.68 ± 1.22	116.24 ± 2.25	4.41 ± 0.16
Group II	618.29 ±12.4##	8.6 ± 0.67##	50.87 ± 3.69##	1.92 ± 0.62##
Group III	712.9 ±24.5#	8.39 ± 0.64##	61.22 ± 4.18###	2.08 ± 0.88###
Group IV	280.32 ± 4.22**	11.81 ± 1.06***	81.55 ± 1.68**	3.72 ± 0.12*
Group V	164.3 ±6.28***	12.54 ± 1.02**	93.26 ± 2.46***	3.96 ± 0.14**
Group VI	267.9 ±11.2**	11.04 ±1.72***	96.48 ± 1.99***	2.94 ± 0.09***
Group VII	151.9 ± 3.8**	12.84 ±1.04**	102.22 ± 1.44**	3.61 ± 0.05**
Group VIII	118.6 ± 2.81**	13.52 ± 0.92	106.24 ± 1.85**	3.82 ± 0.09**
Group IX	94.8 ±6.5**	12.96 ± 0.64**	107.12 ± 1.08**	4.01 ± 0.06**

The results are expressed as Mean ± SEM of six animals from each group. # indicates p<0.05, ## indicates p<0.01 and ### indicates p<0.001 when compared to normal. * indicates p<0.05, ** indicates p<0.01 and *** indicates p<0.001 when compared to CCl_4 and paracetamol intoxicated groups were evaluated by one-way ANOVA followed by Tukey's multiple comparison tests.

Figure 1: Microphotographs (10 x 40) of liver section taken from rats.



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