

Evaluation of Methanolic Extract of *Cleome chelidonii* for Hepatoprotective Activity against Paracetamol and Ethanol Induced Hepatotoxicity in Rats

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ABSTRACT

Objective: Traditionally *Cleome chelidonii* (Cleomaceae) used by tribal's for the treatment of many liver disorders. The aim of this work was to evaluate the in vitro antioxidant and hepatoprotective activity of methanolic extract of *Cleome chelidonii* against paracetamol and ethanol induced liver toxicity in rats. **Methods:** The in vitro antioxidant activity was evaluated by estimating the lipid peroxidation, superoxide radical, hydroxyl radical and DPPH radical scavenging activities. Hepatotoxicity was induced in rats by oral administration of paracetamol (2 g/kg) and ethanol (2ml/100g) body weight. Blood samples were collected from retro orbital puncture from each rat and the Serum alkaline phosphatase (ALP), Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT) and Total Bilirubin (TBIL) levels were estimated. **Results:** The methanolic extract of *Cleome chelidonii* and ascorbic acid at different concentrations scavenged the superoxide radicals, lipid peroxidation, hydroxyl radicals and DPPH radical in a dose dependent manner. The curative treatment with the methanolic extract of *Cleome chelidonii* at 100, 200 and 400 mg/kg body weight and standard silymarin (100 mg/kg) was found to be prevent the rise in Serum ALP, SGPT, SGOT and TBIL levels due to paracetamol and ethanol treatment. The extract showed better effect in inhibiting the free radicals in in vitro studies which might be partly responsible for its beneficial action against paracetamol and ethanol induced liver damage. **Conclusion:** The free radical scavenging activity of the extract might be responsible for its protective activity against paracetamol and ethanol induced hepatotoxicity in rats.

Keywords: *Cleome chelidonii*, ethanol, hepatoprotective activity, lipid peroxidation, paracetamol

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INTRODUCTION

Liver is one of the major organs responsible for maintenance of metabolic functions, secretion and storage including regulation of various physiological processes. It involves in synthesizing useful principles and detoxicates toxic substances. Hepatotoxicants like alcohol, toxic chemicals, infections etc induce liver diseases mainly through oxidative damage and lipid peroxidation. The regulation of metabolic functions by liver is mainly affected by injury to liver caused by toxins absorbed from the intestine resulting in liver diseases of various kinds. Thus, hepatic disorders are still a global health problem caused by various alcohol consumption, environment toxins and

viruses, remain one of the major fears to public health [1]. Jaundice is one of the serious disorders of the liver; it is the tenth leading cause of death in the world and most common symptom of impaired liver function is jaundice. The major symptom of jaundice is turning of eyes and skin to yellow due to excessive bilirubin in the blood. Jaundice where classified into various types such as hemolytic jaundice, obstructive and toxic or hepatic jaundice [2, 3]. It can be hemolytic jaundice where in, high level of RBC destruction occurs or obstructive jaundice result from blockage in the bile duct. Thus liver diseases are some of the fatal disease in the world wide today. They pose a serious challenge to

international public health. Modern medicines have little to offer for alleviation of hepatic disease and the plant based preparations which are employed for their treatment of liver disorders. But there are not much drug available for the treatment of liver disorders [4,5]. Therefore many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal model [6,7]. The medicinal use of many hepatoprotective plants like *Elephantopus scaber*, *Hibiscus rosasinensis*, *Picrorrhiza kurroa*, *Phyllanthus debilis* and *Andrographis paniculata*, *Azadirachata indica*, *Cassia fistula* has been reported in the literature [8, 9].

Cleome chelidonii (Cleomaceae) (commonly known as mountain bee plant or celandine spider flower) is found throughout in India from the Santal hills, Orissa and Gujarat, southwards. It is mostly found in Tirupathi, Ratnagiri areas. *Cleome chelidonii* mainly contains glucocapparin and glucocleomin as phytochemical constituents. *Cleome chelidonii* was also reported for its anti-oxidant and anti-inflammatory activity [10].



Figure 1: Cleome chelidonii plant

MATERIALS AND METHODS

Plant collection and identification:

The plant material such as whole plant of *Cleome chelidonii* was collected from seshachalam hills of cuddapah and chittoor districts of Andhra Pradesh state. The plants materials were identified and authenticated by Dr. K. Madhava chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, and Triupati. A voucher specimen of *Cleome chelidonii* has been deposited in the Herbarium of the Department of botany for the further reference.

Preparation of methanolic extract of selected plant:

Freshly collected plant material was dried under shade and the dried material was milled to obtain a coarse powder. To the coarse power (1kg) in round bottomed flask and 1 liter methanol (95%) was added and macerated for 24 hours at room temperature. The macerated power was packed in a soxhlet apparatus and subjected to continuous extraction with 3 liter of methanol (95%). The liquid extract was collected and evaporated under reduced pressure until a soft mass obtained. The mass obtained was weighed in each case. The extracts were thoroughly air dried to remove all traces of the solvent.

Phytochemical studies

The methanolic extract was subjected to qualitative chemical tests for the detection of various plant constituents like carbohydrates, glycosides, proteins, alkaloids, phytosterols, flavonoids, tannins and phenolic compounds, saponins, etc [11].

Animals:

In this study albino Wister rats of either sex weighing between 150-250g were used. The rats were housed under standard conditions of constant temperature and lighting (12 hours light/dark cycle). They had access to standard pellet diet and water ad libitum. The institutional animal ethical committee of Vignan institute of pharmaceutical technology, approved by Committee for the purpose of control and supervision of Experiment on Animals (CPCSEA) with registration number 1499/Po/a/11/CPCSEA approved the study.

Chemicals:

All the chemicals and the solvents were of analytical grade. Silymarin was obtained as gift sample from Microlabs, Bangalore, India and paracetamol was supplied by Arabindo Laboratories, Hyderabad, India as a gift sample and Sodium CMC. Standard kits for the estimation of selected biochemical parameters such as SGPT, SGOT, ALP and T. BIL were of Erba Diagnostics, Germany purchased from local suppliers.

Acute oral toxicity studies:

The acute toxicity study was performed to ensure the safety, to know the therapeutic Index (TI) and for the determination of

lethal dose (LD50) value of drugs. In the present study the acute oral toxicity studies are conducted for methanolic extract of *Cleome chelidonii* on swiss albino mice as per organization for economic cooperation and development (OECD) guideline 420.

***In vitro* antioxidant activity:**

The antioxidant activity of methanolic extract of *Cleome chelidonii* was studied by invitro studies and compared with antioxidant ascorbic acid. The inhibition of lipid peroxidation was estimated by Ohkawa et al 1979 [12]. Hydroxyl radical scavenging activity was estimated by Elizabeth and Rao 1990[13]. Superoxide radical scavenging activity was estimated by Mc Cord and Fridovich 1969 [14] and DPPH radical scavenging activity was determined.

Experimental Design:

Hepatoprotective activity:

Paracetamol induced hepatotoxicity model:

The albino rats of either sex (150-250g) were randomly selected and divided into 6 groups, each consisting of six animals. They

were maintained under standard conditions (room temperature at 25±20C, 12 hrs light and 12 hrs dark) and free access to food along with water (upto 2 weeks before the experiment) to adopt laboratory conditions. The animals were deprived of food for 18 hrs and water allowed ad libitum prior to experiment.

The normal control group received vehicles for 10 days p.o. The positive control (toxicant) groups were treated with 2 g/kg paracetamol for 3 days and vehicles were administered 4th to 10th day orally [15]. Other groups received 2 g/kg paracetamol for 3 days orally and silymarin/respective plant extract was administered orally from 4th to 10th day. At the end of the study (11th day) blood samples were collected and subjected for the estimation of biochemical parameters such as SGPT, SGOT, ALP, Serum Total bilirubin. The selected doses of the plant extract and toxicant were suspended in 1% sod CMC prior to administration. The treatment protocol is summarized and given below.

Table 1: Experimental protocol for paracetamol induced hepatotoxicity

S.No	Group	Treatment
1	Group A	Received vehicle 1% sod.CMC for 10 days orally.
2	Group B	Received paracetamol (2g/kg) for 3 days in 1% sod. CMC orally and vehicle from 4 th to 10 th day orally.
3	Group C	Received paracetamol (2g/kg) in 1% sod.CMC for 3 days and silymarin at a dose of 100 g/kg from 4 th day to 10 th day orally.
4	Group D	Received paracetamol (2g/kg) in 1% sod.CMC for 3 days and Methanolic extract of <i>cleome chelidonii</i> at dose of 100mg/kg from 4 th to 10 th day orally.
5	Group E	Received paracetamol (2g/kg) in 1% sod.CMC for 3 days and methanolic extract of <i>cleome chelidonii</i> at a dose of 200mg/kg from 4 th to 10 th day orally.
6	Group F	Received paracetamol (2g/kg) in 1% sod.CMC for 3 days and methanolic extract of <i>cleome chelidonii</i> at a dose of 400mg/kg from 4 th to 10 th day orally.

Ethanol induced hepatotoxicity model:

The albino rats of either sex (weight, 150 to 250 gm) were selected randomly and divided into 6 groups, each consisting of six animals. They were maintained under standard condition (room temperature 25C± 10C with 12 hrs dark/ light cycle) with free access to water and standard pellet diet for 2 weeks. Alcohol at a dose 3.76 g/kg (equivalent to 40% of alcohol 2ml/100g, total volume in two equal doses daily) was administered for 25 days and blood samples were collected on 18th and

26th day to assess the alcohol induced liver damage.

The normal control group received vehicle for 25 days p.o. The positive control group treated with alcohol at a dose of 3.76 g/kg p.o for 25 days [16, 17]. In treatment groups alcohol was administered for 18 days and from 19th day onwards silymarin plant extracts (suspended in 1% sod. CMC) were administered for the remaining days without alcohol. The blood samples (1.5ml) were collected from retro orbital puncture on day 18th and 26th, and subjected for

estimation of biochemical parameters such as SGPT, SGOT, ALP, Serum Total bilirubin. Then on the 26th day the animals were sacrificed and the livers were isolated and

washed with fresh saline. Livers were stored in 10% formalin for histopathology study. The treatment protocol is summarized and given below.

Table 2: Experimental protocol for Ethanol induced hepatotoxicity

SNO	Group	Treatment
1	Group A	Received vehicle 1% sod. CMC p.o.
2	Group B	Received 40% alcohol(2ml/100g) for 18 days p.o.
3	Group C	Received of 40% alcohol (2ml/100g) in divided doses for 18 days and silymarin at dose of 100mg/kg from 19 th to 25 th day p.o.
4	Group D	Received 40% alcohol (2ml/100g) in divided doses for 18 days and methanolic extract of <i>Cleome chelidonii</i> at a dose of 100mg/kg from 19 th to 25 th day p.o.
5	Group E	Received 40% alcohol (2ml/100g) in divided doses for 18 days and methanolic extract of cleome chelidonii at dose of 200mg/kg from 19 th to 25 th day p.o.
6	Group F	Received 40% alcohol (2ml/100g) in divided doses for 18 days and methanolic extract of cleome chelidonii at a dose of 400mg/kg from 19 th to 25 th day p.o.

Statistical analysis

Results were expressed as Mean \pm SEM, (n=6). Statistical analysis was performed with one way analysis of variances (1 way ANOVA) followed by Bonferroni's multiple comparison tests using Graph pad Prism-5 software. P value less than 0.05 was considered to be statistically significant. *= $P < 0.05$, **= $P < 0.01$, ***= $P < 0.001$, and ns = not significant, when Group B compared with Group A and rest of groups compared with Group B.

RESULTS

Preliminary phytochemical study:

The preliminary phytochemical screening of methanolic extract of *Cleome chelidonii* showed the presence of flavonoids, Polyphenolic compounds and tannins.

Acute toxicity studies:

Oral administration of selected methanolic extract of *Cleome chelidonii* at a dose of 2000mg/kg body weight did not show any toxic signs during the observation period of 24 hr. in all the rats tested. The plant material was safe at 200 mg/kg body weight and 1/5th, 1/10th and 1/20th (100 mg/kg, 200 mg/kg and 400 mg/kg) of this

cutoff dose have been selected for further study.

In vitro antioxidant study:

The amount of extract and ascorbic acid needed for 50% scavenging of superoxide radicals was found to be 455.86 μ g and 341.96 μ g respectively. The amount needed for 50% inhibition of lipid peroxide was 608.47 μ g (extract) and 372.30 μ g (ascorbic acid). The quantity needed for 50% inhibition of hydroxyl radicals was 365.28 μ g (extract) and 297.16 μ g (ascorbic acid) and The quantity needed for 50% inhibition of DPPH radicals was 270 μ g (extract) and 124.59 μ g (ascorbic acid) (**Table 3 & Fig. 2**).

Effect of Methanolic extract of *Cleome chelidonii* on biochemical parameters in paracetamol induced hepatotoxicity model: In the present study, the hepatoprotective activity of the selected plant extract *Cleome chelidonii* was evaluated in paracetamol induced liver toxicity by estimating the biochemical parameters like AST, ALT, ALP and T. BIL.

Table 3: Invitro 50% inhibition of Methanolic extract of Cleome chelidonii and Ascorbic acid on Free Radical Scavenging Activity

Extract/ Ascorbic acid	DPPH radical	Superoxide radical	Hydroxyl radical	Lipid peroxide
Ascorbic acid	124.59	341.96	297.16	372.30
Methanolic extract of <i>Cleome chelidonii</i>	270	455.86	365.28	608.47

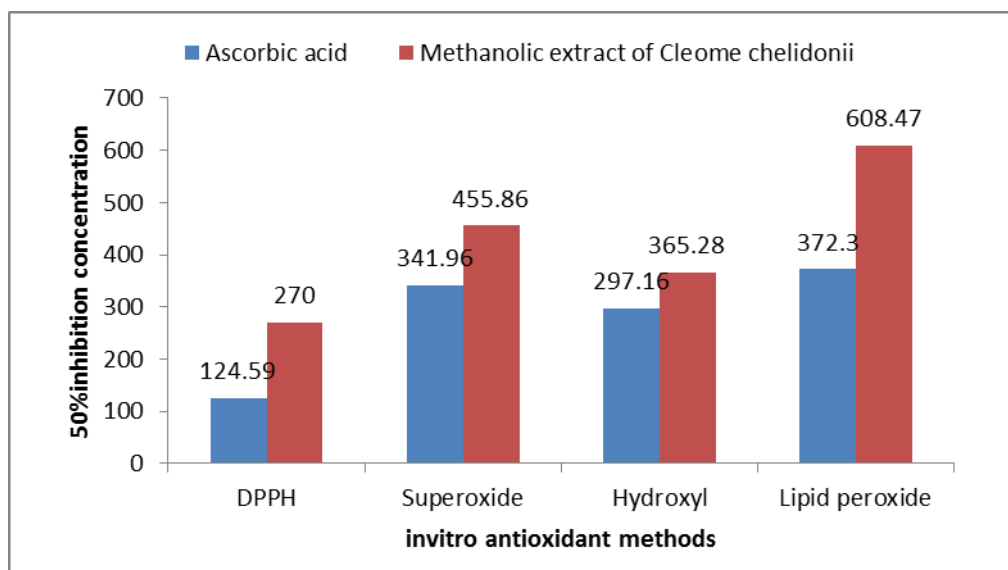


Figure 2: Invitro 50% inhibition of Methanolic extract of Cleome chelidonii and Ascorbic acid on Free Radical Scavenging Activity

Acute administration of paracetamol produced marked elevation of the above serum biochemical parameters in treated rats (Group B) compared to that of the control group (Group A). Curative treatment with the selected plant at a dose of 100

mg/kg, 200 mg/kg and 400 mg/kg produced dose dependent protection in PCM induced rise of the parameters. Silymarin at 100 mg/kg body weight significantly protect the liver from paracetamol toxicity (Table 4 & Fig. 3).

Table 4: Percentage protection produced by extract against paracetamol intoxication

Group/ Treatment	After treatment (11 th day)			
	AST (U/L)	ALT(U/L)	ALP(U/L)	T.BIL (mg/dl)
Silymarin	99.21	99.66	98.86	99.27
100	42.98	57.21	41.45	41.04
200	66.97	71.71	60.67	68.98
400	94.52	92.90	93.84	94.49

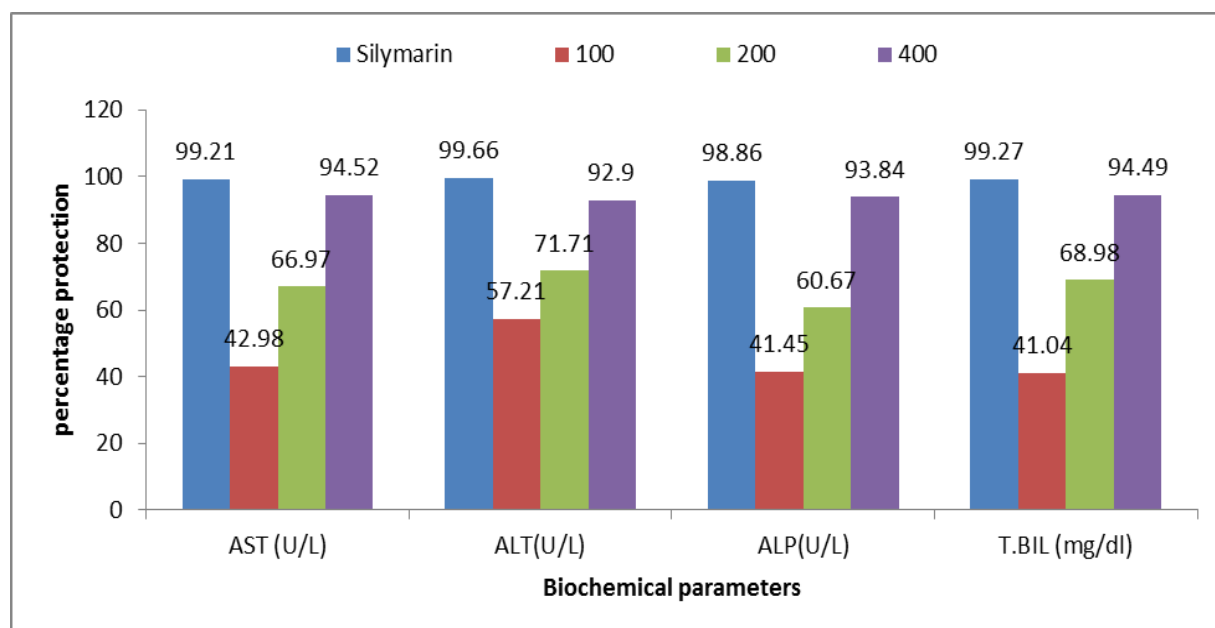


Figure 3: Percentage protection produced by extract against paracetamol intoxication

Effect of Methanolic extract of *Cleome chelidonii* on biochemical parameters in Ethanol induced hepatotoxicity model:

In ethanol treated groups there was a significant increase in selected biochemical parameters such as AST, ALT, ALP and T.BIL. Whereas pretreatment with

Methanolic extract of *Cleome chelidonii* (100 mg/kg, 200mg/kg, 400 mg/kg, p.o) and Silymarin at a dose of 100 mg/kg caused significant protection in selected biochemical parameters. The results were showed in (Table 5 & Fig. 4).

Table 5: Percentage protection produced by extract against alcohol intoxication

Group/ Treatment	After treatment (26 th day)			
	AST (U/L)	ALT(U/L)	ALP(U/L)	T.BIL (mg/dl)
Silymarin	98.20	98.52	98.21	102.63
100	64.01	61.33	57.71	33.50
200	73.69	78.46	77.72	64.02
400	95.63	96.97	93.39	88.42

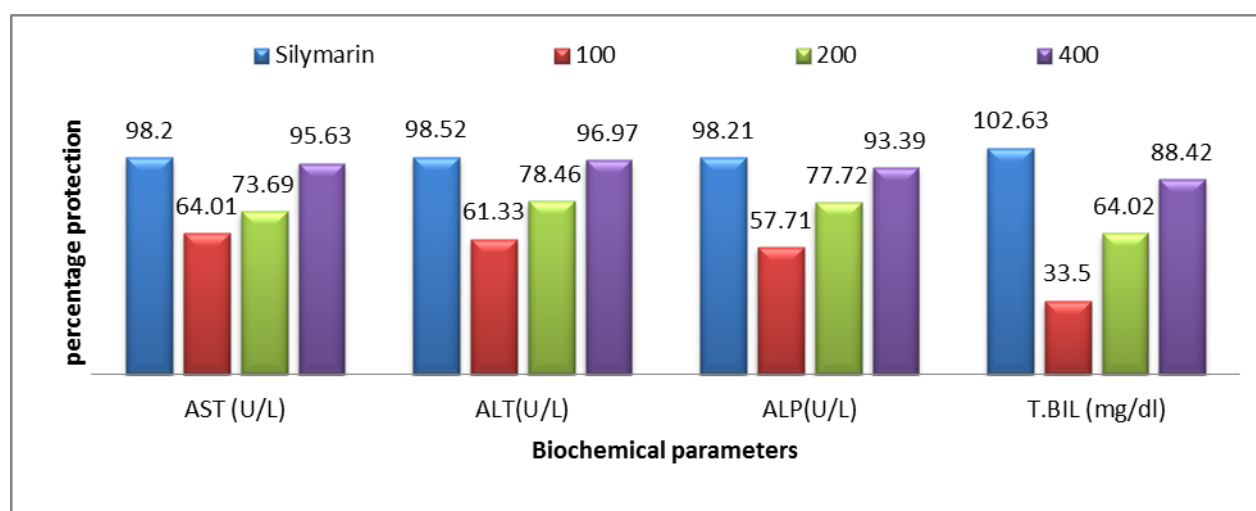


Figure 4: percentage protection produces by extract against alcohol intoxication

Histopathological studies:

Histopathological studies of liver also provided a supportive evidence for biochemical analysis.

Group A: In case of normal control group hepatic globular structure was found to be normal and no abnormality was detected (Fig. 5).

Group B: In case of ETH treated group (toxicant control) up to 75% derangement of cords, fatty and vacuolar degeneration, fatty ballooning, micro vesicular steatosis, cell enlargement, necrosis and cellular infiltration have been observed without any regeneration (Fig. 6).

Group C: In case of 100 mg/kg of silymarin showed less than 25% derangement of cords. Very good regeneration is also observed with normal hepatic feature (Fig. 7).

Group D: In case of 100 mg/kg of *Cleome*

chelidonii showed less than 75% derangement of cord, fatty and vacuolar degeneration, necrosis and less than 25% cellular infiltration and fatty ballooning have been observed without regeneration (Fig. 8).

Group E: In case of 200 mg/kg of *Cleome chelidonii* showed less than 50% derangement of cord, and vacuolar degeneration, necrosis and less than 25% cellular infiltration have been observed. No regeneration was observed (Fig. 9).

Group F: In case of 400 mg/kg of *Cleome chelidonii* showed less than 25% derangement of Cords. Fatty and vacuolar degeneration, necrosis and cellular infiltration have been observed. Pleomorphic cells with good regeneration are also observed with normal Hepatic features (Fig. 10).

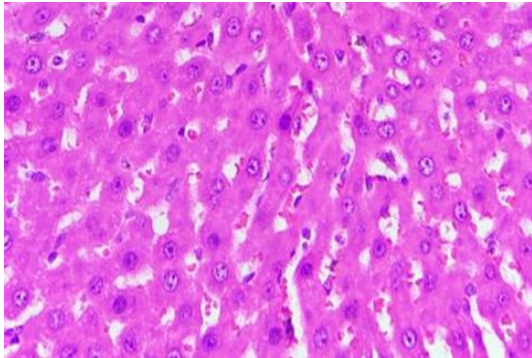


Figure 5: Liver architecture of Group A rats in ETH induced hepatotoxicity model (400X)

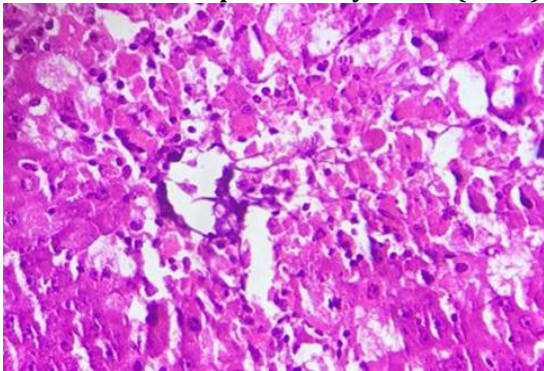


Figure 6: Liver architecture of Group B rats treated with ETH 2ml/100g for 26 days (400X)

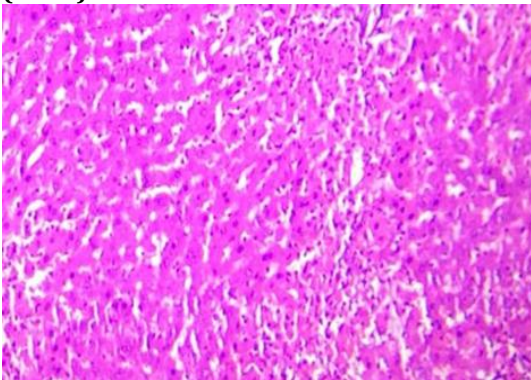


Figure 7: Liver architecture of Group C rats Treated with ETH 2 ml/100g+Silymarin 100mg/kg for 26 days (400X)

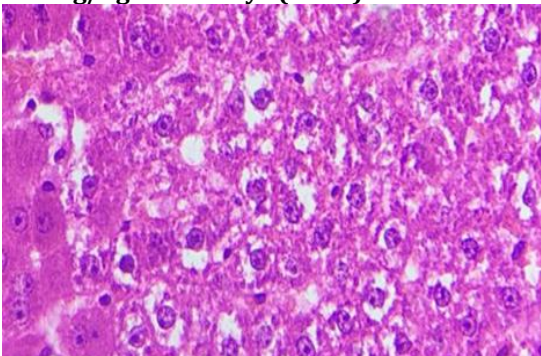


Figure 8: Liver architecture of Group D rats treated with ETH 2ml/100g+Cleome chelidonii 100mg/kg for 26 days (400X)

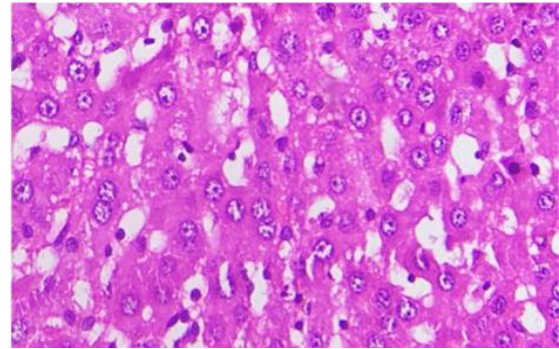


Figure 9: Liver architecture of Group E rats treated with ETH 2ml/100g+Cleome chelidonii 200mg/kg for 26 days (400X)

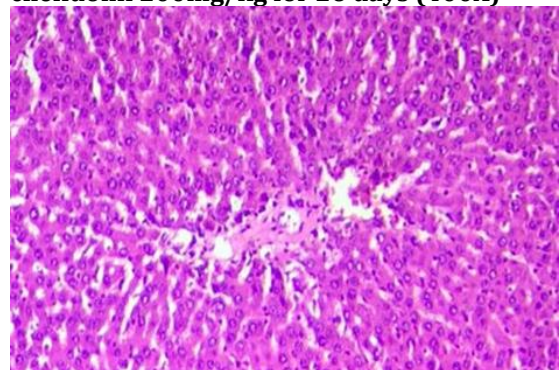


Figure 10: Liver architecture of Group F rats treated with ETH 2ml/100g+Cleome chelidonii 400mg/kg for 26 days (400X)

DISCUSSION

Paracetamol is a well-known antipyretic and analgesic agent, which is safe in therapeutic doses, but can produce fatal hepatic necrosis in man, rats and mice with toxic doses. Paracetamol induced hepatotoxicity was used by several researchers as model for screening hepatoprotective activity. The dose used for induction of hepatotoxicity by different researchers was found to vary. The dose of PCM used was 2 g/kg body weight in albino rats of Wister strain. In the present study 2 g/kg body weight of PCM was used as toxicant [18, 19].

The presence of flavonoids, Polyphenolic compounds and Tannins etc in the selected plant extract might be responsible for the antioxidant activity and the same might be the reason for their beneficial activity against paracetamol induced liver toxicity in curative studies.

The selected plant extract found to show superoxide radical scavenging and hydroxyl radical scavenging activity. Both these free radicals are generated in the hepatotoxicity

produced by ethanol, hence to see further their hepatoprotective effect, the selected herbal drug was also evaluated against ethanol induced hepatotoxicity as ethanol also produce hepatotoxicity by virtue of free radical generation capacity.

In the present study the hepatoprotective activity of the selected plant extract was evaluated in ethanol induced liver toxicity in rats. Administration of ethanol for 26 days produced change of the serum levels of these markers in ETH treated rats (Group B) compared to that of the control group. In cleome chelidonii treated group the hepatoprotective activity was found to be good in curative studies. ETH is activated into reactive intermediate by CYP2E1. Better hepatoprotective activity showed at higher dose (400 mg/kg bd. Wt.) is comparable with that of silymarin.

The histopathological study also supported the biochemical and physical evidence for the hepatoprotection shown by the selected herbal drugs. The normal hepatic cell is a polygonal cell and binucleated with nucleolus and abundant eosinophilic cytoplasm [20]. The above features were found in normal control group. In ETH treated group (Group B) in both prophylactic and curative studies histopathology showed very high derangement of cords, fatty and vacuolar degeneration, necrosis and cellular infiltration with no sign of regeneration. Whereas Cleome chelidonii treated group showed little decrease in these abnormalities with poor regeneration. The treated group showed good reduction in these abnormalities but still fatty and vacuolar degeneration and necrosis is not much corrected by the plant.

The presence of flavonoids, polyphenolic compounds, Tannins might be responsible for the antioxidant activity [21, 22] and same might be the reason for the beneficial activity against ETH induced hepatotoxicity.

CONCLUSION

The lipid peroxidation inhibition activity coupled with free radical scavenging activity of the extract might be responsible for its protective activity against paracetamol and ethanol induced hepatotoxicity in rats.

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