ISSN 0976 – 3333

Available Online at www.ijpba.info.

International Journal of Pharmaceutical & Biological Archives 2010; 1(4): 399-403

ORIGINAL RESEARCH ARTICLE

Hepatoprotective Effect of C*leome Viscosa L.* Seeds in Paracetamol Induced Hepatotoxic Rats

Ashok Kumar Mobiya*, Ashutosh Kumar Patidar, G. Selvam, M. Jeyakandan

College of Pharmacy, Dr. Shri R.M.S. Institute of Science and Technology, Bhanpura, Dist.-Mandsaur (M.P.) 458775 India

Received 02 Oct 2010; Revised 12 Oct 2010; Accepted 20 Oct 2010

ABSTRACT

In present study, Hepatoprotective activity of the ethanolic extract of *Cleome Viscosa L.* seeds was studied on paracetamol treated albino rats. The hepatoprotective effect was evaluated on the basis of liver function parameters viz. serum total bilirubin, total protein, alanine transaminase, aspartate transaminase and alkaline phosphatase activities. Treatment with *Cleome Viscosa L.* seeds extract had shown significant hepatoprotective effect also supported by histopathological studies on liver, the result were in comparison with the standard drug silymarin.

Keywords: *Cleome Viscosa L.*, Paracetamol, Silymarin, Hepatoprotective

INTRODUCTION

Liver, the most versatile but complex internal organ of human body, plays vital role in metabolic activities. Its importance also lies in its impetus in management of internal enviourment and biochemical conversion of endogenous and exogenous chemical to harmless and excretable compounds. Therefore being a vital organ, its protection has a special status in therapeutics.[1]Prolonged drug therapy, excessive use of the some of the commonly used medicines like paracetamol, diclofenac etc., alcoholism, exposure to certain xenobiotic, polutants and certain disease state have been reported to affect liver functioning. The major clinical manifestation of liver disorder is jaundice. Despite of the excellent regeneratation capacity of this organ, a slight injury or toxicity may lead to fatal complications. Therefore damage to the liver inflicted by hepatotoxic agents is of grave consequences.Unavailability of rational therapy in modern medicine and no or very less positive influence of synthetic drugs in liver damage have urged researchers in this field to look for herbal drugs with better hepatoprotective action. Traditional medicines are effective in certain disease and are based on their age-old use in folklore system of medicine. Natural products of plant origin with hepatoprotective and antioxidants properties play an important role in treatment of liver toxicity.[2]

***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 is known as Hurhur (Hindi) in Indian traditional medicine. Traditionally, this plant is used in various disorders such as diarrhea, fever, inflammation, liver diseases, bronchitis, skin diseases and malarial fever. The juice is useful in piles, lumbago and earache.[3]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. We knows that paracetamol induced hepatotoxicity and it's confirmed by practical observation and for these hepatotoxic agents, *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. Hence an attempt was made to screen the ethanolic extract of cleome viscose

***Corresponding Author:** Ashok Kumar Mobiya, **Email-** mobprajapati@gmail.com, **Contact No:** +917697154004

linn. Seeds for hepatoprotective effect against paracetamol induced liver damage in albino rats with camparison to silymarin as standard drug.

MATERIALS AND METHODS

Plant Materials

The seeds of *Cleome Viscosa L.* were collected from local field of Bhanpura, Dist. Mandsaur (M.P.) and authenticated by Dr. Rakesh Gupta, Department of Dravyaguna, SDPS Ayurved Medical College, Bhanpura. Voucher specimen was deposited to herbarium of SDPS Ayurved Medical College vide specimen no. SDPS/10/PS/114.

Preparation of Plant Extract

The plant material was shade dried and powdered mechanically. The powdered plant first defatted with petroleum ether and than extracted with ethanol by using Soxhlet extractor. The extract so obtained was concentrated in vaccum using rotary flash evaporator.[4]

Drugs and Chemicals

Paracetamol was obtained from Suvidhinath Lab, Baroda and standard drug silymarin was procured from Microlabs Ltd., Bangalore, Karnataka.

Animal Studies

Three month old wister albino rats (150-180 gm) of either sex were used for this study. The animals were kept in polyethylene cage at 24±2ºC under photoperiod of 12:12 hour light dark cycle. All the animals were fed with standard pelletted diet (Hindustan Lever, Kolkata) and water *ad libitum.* The study was approved by Institutional Animal Ethical Committee (IAEC) and was in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA).

Acute Toxicity Study

The study was carried out in order to determine the therapeutic and toxic dose of the seeds of *Cleome Viscosa L.*. To establish LD_{50} the method described by turner was employed. Wistar strain of albino rats (150-180 gms) was divided into five group each containing 10 animals among which one was control. The control group received 0.5 ml of 0.2% CMC while other received one each of test sample in the dose of 1800 mg/kg body wt. suspended in 0.2% CMC.[5]

Assessment of Hepatoprotective Effect

In order to assess hepatoprotective action of *Cleome Viscosa L.* extract in albino rats, the rats were divided into the following groups each containing 4 rats (n=4):

Group 1: Control rats: which were fed normal diet and water.

Group 2: Paracetamol treated rats: paracetamol 500 mg/kg body weight p.o. on daily basis for 7 days.

Group 3: Reference rats: treated with silymarin 100 mg/kg and paracetamol 500 mg/kg body weight p.o. on daily basis for 7 days.

Group 4: Extract treated rats: received ethanolic extract of *Cleome Viscosa L.* seeds 300 mg/kg body weight p.o. and paracetamol 500 mg/kg body weight p.o. on daily basis for 7 days.

Group 5: Extract treated rats: treated with ethanolic extract of *Cleome Viscosa L.* seeds 450 mg/kg body weight p.o. and 500 mg/kg body weight p.o. on daily basis for 7 days.

After 24 hours of the last treatment, the rats were anaesthetized with ether and blood samples from each animal of all groups were collected by retro-orbital plexus puncture in sterlized centrifuge tubes. The blood samples were then allowed to coagulate at 30ºC for 45 minutes. Serum portion was separated from each sample by centrifugation at 25000 g at 30ºC for 10 minutes and subjected to biochemical investigation to assess liver function on the basis of total bilirubin, serum aminotransaminases (alanine and aspartate) and alkaline phosphatase.[6] Total protein was estimated as per the method of Lawry and Farr.[7]

Histopathological Studies

After collecting blood samples, the animals from all groups were sacrificed by cervical dislocation and liver was removed liver was then cut into small pieces and fixed in 10% neutral formalin solution for 2 days, followed by dehydration through graded alcohol and xylene. The portions were then embedded in parafin wax following the standard microtechnique. Section were made at multiple levels and stained routinely with hematoxylin and eosin. Mounted slides were examined for histopathological changes in liver and their micrographs were taken.[8]

Statistical Analysis

The result are expressed as means±standard deviation (S.D.) and values were calculated for each group. A one way analysis of variance (ANOVA) followed by Dunnet's test for significance analysis using Graph Pad Prism software. The minimum level of significance was set of $P<0.05$.

RESULTS AND DISCUSSION

Biochemical Analysis

Administration of paracetamol had resulted in hepatotoxicity, as evident by significant rise in biochemical parameters. The ethanolic extract of *Cleome Viscosa L.* seeds at 300 mg/kg body weight and 450 mg/kg body weight p.o. exhibited statisticaly significant reduction in the elevated levels of enzymes selected for the study along with total bilirubin content when compared to paracetamol treated group. A comparable increase in total protein content (1.27±0.024 gm/dl) was observed in extract treated group at a dose of 450 mg/kg body weight p.o. with respect to paracetamol treated group (0.72±0.03 gm/dl). The results are summarized as **Table 1**.

TABLE 1:

Effect of ethanolic extract of *Cleome Viscosa Linn.* seeds on biochemical parameters

MEAN ± S.D.

GROUPS	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TOTAL BILIRUBIN (mg/dl)	TOTAL PROTEIN (gm/dl)
Control	45.20 ±1.24	144.6±0.42	156.2±1.58	0.53 ±0.05	7.37±0.18
PCM Control (500 mg/kg)	243.5±2.70*	396.8±0.43*	388.4±9.4*	3.60±0.15*	4.49±0.09
PCM+Extract (300 mg/Kg)	134.5 ± 3.6#	258.2±1.46#	202.2±6.2#	2.6 ±0.17#	5.56±0.12
PCM+Extract (450 mg/Kg)	114.0± 0.99#	230.5±2.12#	170.6±2.2#	1.62±0.04#	6.30±0.16
PCM+Silymarin(100 mg/Kg)	109.9± 1.11#	204.2±1.27#	164.8±1.24#	1.15±0.08#	7.08±0.23

Histopathological Analysis

In rats of control group, the liver architecture was normal and the cells were arranged radially (**Fig. 1**). The liver dissected from paracetamol treated rats showed vacuole formation and fatty degeneration. Some of the cells found to have damaged cell walls (**Fig. 2**). In rats treated with the lower dose of extract i.e. 300 mg/kg body weight p.o. along with paracetamol, the damage was less marked and vacuole formation was observed. The liver cells were observed to be well organized around the central vein along with fat depositions (**Fig. 3**). In rats treated with higher dose of extract i.e. 450 mg/kg body weight p.o., the liver appeared normal (**Fig. 4**). Similar texture and cell arrangement were observed in the liver section of rats treated with silymarin (**Fig. 5**). These changes in the liver architecture were coincided with the corresponding changes in the enzyme levels and hence hepatoprotective effect of *Cleome Viscosa L.* seeds was confirmed. Paracetamol toxicity is caused by excessive use or overdose of the antipyretic-analgesic drug paracetamol. Mainly causing liver injury, paracetamol toxicity is one of the most common causes of poisoning and acute liver failure worldwide. With progressive disease, signs of liver failure may develop; these include low blood sugar, low blood pH, easy bleeding, and hepatic encephalopathy. Some will spontaneously resolve, although untreated cases may result in death. Since the changes associated with paracetamol induced liver damage are similar to that of acute viral hepatitis,[9] paracetamol mediated hepatotoxicity was chosen as the experimental model. Damage to the liver or hepatotoxicity results not from paracetamol itself, but from one of its metabolites, *N*-acetyl-*p*-benzoquinoneimine (NAPQI). NAPQI depletes the liver's natural antioxidant glutathione and directly damages cells in the liver, leading to liver failure. Hepatocellular necrosis leads to elevation of serum marker enzymes, which are released from the liver into blood.[10] The increased levels of ALT, AST, ALP and TB on exposure to paracetamol indicated considerable hepatic injury in present study. The effectiveness of any drug with hepatoprotective action is necessarily dependent on its capability of either reducing the deleterious effect or in maintaining the normal hepatic physiology, which have been altered by a hepatotoxin. In present study, paracetamol-induced liver necrosis was inhibited significantly by *Cleome Viscosa L.* seed extract, which confirms its protective action against experimentally induced liver damage in rats. AST, ALT, ALP and TBL are the most sensitive tests employed in the diagnosis of hepatic disease.[11] The elevated levels of these parameters due to paracetamol toxicity were

significantly reduced by the treatment with ethanolic extract of *Cleome Viscosa L.* seeds.

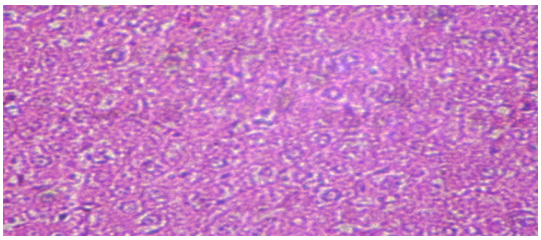

Fig. 1 Liver section from control group showing normal liver histopathology (Central vein, hepatocytes and portal vein)

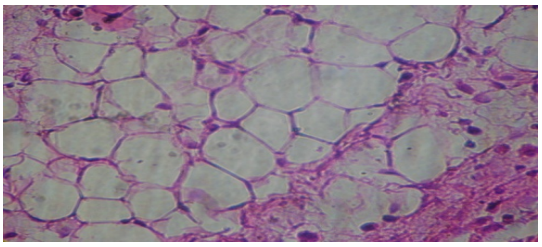

Fig.2 Liver section from paracetamol treated group showing changes of fatty degenerations as well as necrosis of hepatocytes

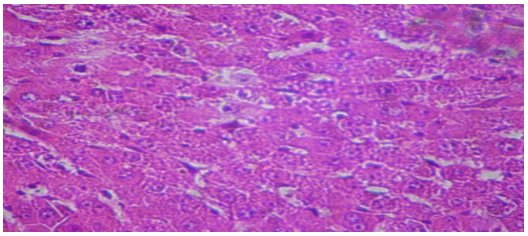

Fig. 3 Liver section from *Cleome Viscosa L.* seeds extract treated group (300 mg/kg) showing some necrotic region and regenerative heap

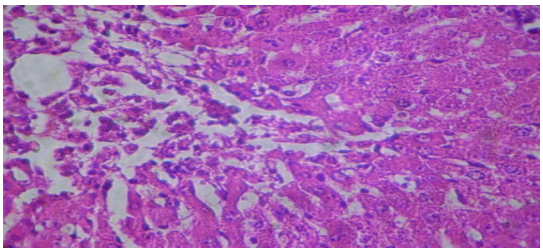

Fig. 4 Liver section from *Cleome Viscosa L.* seeds extract treated group (450 mg/kg) showing central vein, hepatocytes cells with normal architecture

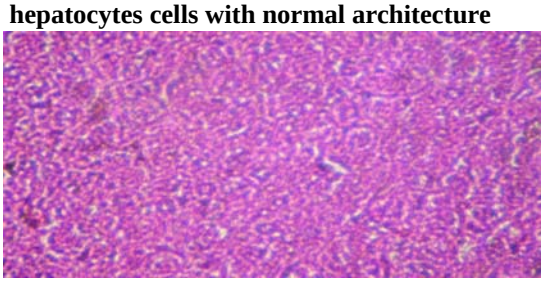

Fig. 5 Liver section from silymarin treated group (100 mg/kg) showing central vein, hepatocytes cells with normal architecture

Therefore it can be concluded from this investigation that leaves of *Cleome Viscosa L.* seeds exhibited hepatoprotective activity and this may be due to its rich contents of flavonoids. Hepatoprotective activity of flavonoids is well documented earlier.[12,13] Further work in the direction of characterization and standardization of *Cleome Viscosa L.* extract is in progress. Administration of paracetamol at higher dose resulted in enhanced levels of liver function associated enzymes. It was also found to affect hepatospecific biochemical parameters and induce significant cytotoxicity in liver cells. Pronounced increase in these parameters may occur due to metabolites of paracetamol resulted from its biotransformation. This may have resulted in oxidative injury to cellular components of liver, very similar to jaundice. In the present study, ethanolic extract of *Cleome Viscosa L.* seeds, rich in flavonoid content, had shown significant protective effect in hepatic cellular injury caused by paracetamol. The result were also supported by histopathological studies of rat liver as evident from regeneration of hepatocytes upon treatment with *Cleome Viscosa L.* extract.

REFERENCES

1. Ravishankar SB, Bhavsar GC. Plants with hepatoprotective activity. Indian Drugs 1993; 30: 363-365.
2. Vaidya AB, Sirsat SM, Doshi JC, Antarkar DS. Selected medicinal plants and formulation as hepatobiliary drug: An overview. Indian J. Clin. Pharmacol. Ther 1996; 17: 7-11.
3. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Vol 1. New Dehli:Periodical Experts;1975: 183-185.
4. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 5 ed. India: Nirali Prakashan Publications 1997; 109-137.
5. Turner RA. Screening method in pharmacology. 1 ed. New York: Academic press Inc.; 1971: 302-304.
6. Gaur K, Panda BB, Nema RK, Jain CP. Hepatoprotective activity of *Jatropha gossypifolia* against carbon tetrachloride induced hepatic injury in rats. Asian J. Pharm. Clini. Res 2009; 1(2): 50-54.
7. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurements with folin phenol reagent. J Biol Chem 1951; 193: 265-275.

8. Galighar AE, Kozloff EN. Essentials of Practical Microtechnique. Philadelphia: Lea and Febiqu 1971; 210(2): 77.
9. Suja SR, Latha PG, Pushpgandhan P, Rajshekharan S. Evaluation of hepatoprotective effect of *Helminthostachys zeylamica (L)* Hook against carbon tetrachloride induced liver damage vulgaris J.. Agric. Food Chem 2001; 42 (2): 248-251.
10. Shnoy AK, Somayaji SN, Bairy KL. Hepatoprotective activity of ethanolic extract of *Ginkgo biloba* against carbon tetrachloride induced hepatic injury in rats. Indian J Pharmacology 2002; 46(2):167-174.
11. Harper HA. In Review of Physiological Chem. Los Atlos (California): Lange Medical Publishers 1961.
12. Choski S, Patel SS and Saluja AK. Silymarin: A promising herbal hepatoprotective drug. Indian Drugs 2000; 37: 566-569.
13. Chin YH. Antioxidant activity of extract from Polygonum aviculare L. Biol Res 2006; 39:281-288.

ACKNOWLEDGEMENT

Authors express their sincere gratitude to Subhash Sojatia, Chairman, Dr. Shri. R.M. Sojatia Foundation, Bhanpura, Dist. Mandsaur (M.P.), India for providing all facilities to carry out the work.