Vol 2|Issue 2| 2012 |106-110.

International Journal
of
Pharmacy Review & Research
www.ijprr.com

e-ISSN: 2248 – 9207
Print ISSN: 2248 – 9193

HEPATOPROTECTIVE EFFECT OF *MALACHRA CAPITATA (L.)* AGAINST CARBON TETRA CHLORIDE-INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

N. Sriram[*1], K. Vinoth Kumar[2], Shubhrajit Mantry[3], S. Sakthivel[4], Pandith Shiva Prasad[5]

[*1,5]Smt.Sarojini Rammulamma College of pharmacy, Shashabgutta, Mahabubnagar, A.P, India.
[2]Seven Hills college of Pharmacy, Tirupati, A.P. India.
[3]Kottam institute of Pharmacy, Thimmapur, Itikyal, Mahabubnagar (Dist) A.P, India.
[4]P.R.R.M. College of Pharmacy, Kadapa, Andhra Pradesh, India.

ABSTRACT

The present study to investigate the phytoconstituents, acute oral toxicity and hepatoprotective activity of aqueous extract of *Malachra capitata (L.)* (AMC) using CCl_4 induced hepatotoxicity in male Wistar albino rats. The AMC at doses of 100, 200 and 400mg/kg, p.o and the standard drug Liv.52 (40mg/kg, p.o) were administered for 7 days in CCl_4 intoxicated rats. The hepatoprotective activity was assessed by using various biochemical parameters like SGOT, SGPT, alkaline phosphatase (ALP) and acid phosphatase (ACP), also total bilirubin and urea. The biochemical changes and histopathological studies were observed on 4^{th} and 8^{th} day. AMC at tested doses significantly decrease ($P<0.001$) the elevated levels of the hepatic enzymes, total bilirubin and urea in a dose dependent manner after 3days whereas it's subsequent return towards near normal after 7days indicating the recovery of hepatic cells. The AMC afforded significant protection against CCl_4 induced hepatocellular injury.

Key words: Hepatotoxicity, CCl_4, Hepatic Enzymes, *Malachra capitata (L.)*, Hepatoprotective, AMC.

INTRODUCTION

The liver regulates many important metabolic functions, detoxification, and secretory functions in the body. Hepatic injury is associated with distortion of these metabolic functions [1]. Thus, liver diseases remain one of the serious health problems and its disorders are numerous with no effective remedies. Despite, considerable progress in the treatment of liver diseases by oral hepatoprotective agents, search for newer drugs continues because the existing synthetic drugs have several limitations [2-4]. So, the search for new medicines is still ongoing. Because liver performs many vital functions in the human body and damage of liver causes unbearable problems. [5, 6]. Keeping this fact in view, the present study was undertaken to investigate the hepatoprotective activity of *Malachra capitata (L.)* leaves against carbon tetrachloride-induced hepatic damage in albino rats.

Malachra capitata (L.) is a herb belongs to family: Malvaceae. Description: Mostly erect, coarse, annual or perennial herb 1-2 m tall, throughout densely whitish- or yellowish-tomentose with stellate hairs and usually also moderately to copiously hispid with simple or stellate hairs to 2 mm long; roots long-petioled; stipules lanceolate, 5-15 mm long; blades orbicular to ovate, 2-10 cm long, palmately sinuate to 3-, 5-, or 7-lobed, lobes mostly obtuse, crenate to serrate, the base obtuse or truncate; flowers in axillary, pedunculate, bracteate heads, bracts 1-2 cm long, stipitate and subtended by paired, filiform bracteoles, conduplicate, suborbicular to ovate, obtuse or acute, entire or once or twice dentate, obtuse to cordate at base, prominently veined and whitish basocentrally; involucral bracts wanting; calyx tubular-campanulate, 4-8 mm long, 5-lobed to below middle, lobes ovate-lanceolate, white with brownish or reddish nerves; petals yellow, obovate, 10-15 mm long, slightly exceeding staminal column; mericarps 3-3.5 mm long, muticous, reddish veined, puberulent; seed obovoid-cuneate, about 2.5 mm long, black, whitish-pubescent about hilum. The root of the *Malachra capitata* (L.) is traditional remedies for the many disease condition such as pain, hepatic

[*]**Corresponding Author N. Sriram** E mail: srirampharma@gmail.com

cirrhosis, inflammation, diarrhea, convulsion, dementia, pyrexia, ulcer, healing of wounds [7-9].

However, there are no ethnomedicinal information and scientific findings for the above said traditional claim for hepatic disorders. Therefore, to justify the traditional claims the present study was undertaken to find out if aqueous extract of *Malachra capitata (L.)* leaves demonstrates the hepatoprotective activity against CCl_4-induced liver damage in rats. Hence, the present study was designed to verify the claims of the native practitioners.

MATERIALS AND METHODS

Plant collection

The Plant material of *Malachra capitata (L.)* roots was collected from Tirunelveli District, in the Month of August 2011. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of plant extract

The roots of the *Malachra capitata* (L.) are properly washed in tap water and then rinsed in distilled water. The rinsed roots are dried in an oven at 35^{o}C for 4 days. The dried roots of *Malachra capitata* was crushed to obtain powder. These powdered samples are then stored in airtight polythene bags protected from sunlight until use. The aqueous extract of each sample was prepared by soaking 10g of powdered sample in 200ml distilled water for 12h. The extracts are then filtered using Whatmann filter paper. Percentage yield of aqueous extract of *Malachra capitata* was found to be 10.5 % w/w.

Preliminary phytochemical screening

The phytochemical examination of aqueous extract of *Malachra capitata (L.)* roots was performed by the standard methods [10]**.**

Animals used

Male albino rats (150-220g) were obtained from the animal house and maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

Acute Toxicity Study

The acute toxicity of aqueous extract of *Malachra capitata* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study [11].

Carbon tetrachloride-induced hepatotoxicity in rats

The liver protective effect was evaluated using the carbon tetrachloride (CCl_4) model described by Visweswaram et al. [12]. Wistar albino rats (150-220gm) were divided into six groups of six rats each and were subjected to the following treatments: Group-I served as normal control received distilled water (1 ml/kg, p.o) for 7days. Group II -VI received 0.75 ml/kg CCl_4 administered orally as single dose. After 36 hours, Groups III-VI received AMC with doses of 100, 200 and 400mg/kg, p.o and the standard drug Liv.52 with dose of 40mg/kg, p.o, respectively once daily for 7days. The blood was collected by puncturing the retro-orbital sinus of three rats from each group on 4th day of treatment and 8th day after the treatment respectively. From the collected blood samples, serum was separated to assess various biochemical parameters.

Biochemical estimation

The separated serum was subjected to estimate SGOT and SGPT by Reitman and Frankel method, alkaline phosphatase (ALP) and acid phosphatase (ACP) by Kind and King method**,** bilirubin by Malloy and Evelyn method and urea by Bousquet method [13-16]. The rats were then sacrificed by bleeding and the liver was carefully dissected, cleaned of extraneous tissue, and part of the liver tissue was immediately processed for histopathological investigation.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett's test p values less than 0.05 were considered as significance.

RESULTS

The results of preliminary phytochemical screening of the aqueous extract of *Malachra capitata (L.)* revealed that presence of alkaloids, flavonoids, carbohydrates, glycosides, tannins, terpeniods and absence of saponins and steroids.

Acute toxicity study

Acute toxicity study in which the animals treated with the AMC at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioral changes, body weight changes, or macroscopic findings at any time of observation. There was no mortality in the above-mentioned dose at the end of the 14 days of observation.

Effect of AMC on CCl_4 – induced hepatotoxicity

The results of AMC on carbon tetrachloride-induced hepatotoxicity were represented in Table 1 and Table-2. The CCl_4 only treated animals exhibited a significant increase (P<0.001) the levels of SGOT, SGPT, alkaline phosphatase (ALP) and acid phosphatase (ACP) and also total bilirubin and urea when compared to the normal control group on both 4th and 8th day, indicating hepatocellular damage.

The AMC at tested doses (group III-V) produced a significant reduction (P<0.001) in the CCl_4-induced elevated levels of SGOT, SGPT, alkaline phosphatase (ALP) and acid phosphatase (ACP), also total bilirubin and urea when compared to the CCl_4 only treated animals (group-II) after 3days of treatment and reduced furthermore to the normalcy on 8th day although the lowest dose (100 mg/kg) tested could produced significant reduction even after 3days of treatment (Table 1). Overall, AMC at tested doses significantly reduced the levels of hepatic enzymes, total bilirubin and urea in a dose dependent manner. After 7 days, the hepatic enzymes levels were almost restored to the normal after treating with AMC at the dose of 400mg/kg, p.o.

A standard drug, Liv.52 at a dose of 40 mg/kg (group-VI) administered orally produced a significant reduction (p<0. 001) compared to CCl_4 only treated animals (group-II) on both 4th and 8th day and these protective effects almost close to AMC 400mg/kg, p.o.

Table 1. Effect of AMC on CCl_4-induced alteration of hepatic enzymes, serum bilirubin and urea in rat liver after 3 days

Design of Treatment	Biochemical parameters					
	SGOT(U/ml)	**SGPT**(U/ml)	**ALP** (KA Units)	**ACP**(KA Units)	**Bilirubin**(mg/dl)	**Urea**(mg/dl)
Group-I: Normal control (DW-1 ml/kg; p.o)	48.19 ± 1.26	62.21 ± 2.06	19.60 ± 1.92	4.22 ± 0.47	0.84 ± 0.04	35.47 ± 1.33
Group-II: CCl_4 (0.75 ml/kg; p.o)	182.24 ± 1.32^{*c}	155.38 ± 2.25^{*c}	47.00 ± 1.12^{*c}	5.73± 0.43^{*c}	3.17 ± 0.05^{*c}	95.42 ± 1.36^{*c}
Group-III:AMC (100 mg/kg; p.o)	127.37 ± 1.26*	134.36 ± 2.43*	33.42 ± 1.71*	5.13 ± 0.04*	1.52 ± 0.01*	73.22 ± 1.13*
Group-IV:AMC (200 mg/kg; p.o)	77.22 ± 1.42*	94.35 ± 1.43*	28.47 ± 1.15*	4.55 ± 0.14*	1.33 ± 0.04*	55.17 ± 1.43*
Group-V:AMC (400mg/kg; p.o)	68.30 ± 1.22*	72.44 ± 2.32*	28.42 ± 1.05*	4.24 ± 0.43*	1.14 ± 0.02*	49.64 ± 0.32*
Group-VI: Liv.52 (40 mg/kg; p.o)	60.14 ± 1.42	67.64 ± 2.93*	23.80 ± 1.25*	4.14 ± 0.04*	1.02 ± 0.03*	41.16 ± 0.95*

Values are Mean ± SEM of 6 animals each in a group.
^{*c} P<0.001 , when compared group I *Vs* group-II,
*P<0.001, when compared group II *Vs* group III, IV, V and VI
AMC = aqueous extract of *Cordia subcordata*, CCl_4 = Carbon tetrachloride
DW=distilled water

Table-2: Effect of AMC on CCl_4-induced alteration of hepatic enzymes, serum bilirubin and urea in rat liver after7 days

Design of Treatment	Biochemical parameters					
	SGOT (U/ml)	**SGPT** (U/ml)	**ALP** (KA Units)	**ACP** (KA Units)	**Bilirubin** (mg/dl)	**Urea** (mg/dl)
Group-I: Normal control (DW-1 ml/kg; p.o)	48.32 ± 1.26	58.21 ± 0.26	15.62 ± 0.22	5.22 ± 0.27	0.74 ± 0.02	37.21 ± 1.52
Group-II: CCl_4 (0.75 ml/kg; p.o)	157.40 ± 1.33^{*c}	117.44 ± 1.31^{*c}	45.42 ± 0.31^{*c}	5.42 ± 0.13^{*c}	2.52 ± 0.34^{*c}	91.47 ± 0.34^{*c}
Group-III: AMC (100 mg/kg; p.o)	105.16 ± 1.15*	96.12 ± 0.15*	27.14 ± 0.14*	3.62 ± 0.14*	1.42 ± 0.15*	65.22 ± 1.12*
Group-IV: AMC (200 mg/kg; p.o)	64.23 ± 0.31*	72.02 ± 0.32*	20.03± 0.24*	3.22 ± 0.34*	1.14 ± 0.22*	47.13 ± 1.12*
Group-V: AMC (400 mg/kg; p.o)	55.14 ± 1.14*	62.42 ± 0.14*	14.54 ± 0.01*	3.25 ± 0.12*	1.22 ± 0.01*	38.60 ± 1.41*
Group-VI: Liv.52 (40 mg/kg; p.o)	55.14 ± 2.21*	62.12 ± 1.33*	17.34 ± 0.32*	3.19 ± 0.02*	0.92 ± 0.32*	35.26 ± 1.22*

Values are Mean ± SEM of 6 animals each in a group. ^{*c} P<0.001 , when compared group I *Vs* group-II,
*P<0.001, when compared group II Vs group I, III, IV, V and VI
AMC= aqueous extract of *Cordia subcordata*, CCl_4 = Carbon tetrachloride.DW=distilled water.

DISCUSSION AND CONCLUSION

The present studies were performed to assess the hepatoprotective activity of aqueous extract of *Cordia subcordata* leaves in rats against carbon tetrachloride as hepatotoxin to prove its claims in folklore practice against liver disorders. It is well documented that carbon tetrachloride-induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of drugs or medicinal plants' extracts, by in vivo and in vitro techniques [17-21]. Carbon tetrachloride (CCl_4) is a potent hepatotoxin producing centrilobular hepatic necrosis. It is accumulated in hepatic parenchyma cells and metabolized to CCl_3 by liver cytochrome P450-dependent monooxygenases [22].

Usually, the extent of hepatic damage is assessed by histopathological evaluation and the level of hepatic enzymes ALT, AST and ALP release in circulation [**23].** The administration of CCl_4 resulted in a significant increase in the serum SGOT, SGPT, alkaline phosphatase (ALP) and acid phosphatase (ACP) and also total bilirubin and urea within 36 hours [24, 25]. The rise in serum levels of AST, ALT, ALP and ACP has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages [26].

In our study, the biochemical changes were observed after each 3 and 7 days. Thereby, it was found that, the administration of AMC at doses of 100, 200 and 400mg/kg, p.o for 3 days resulted in significantly decreases the CCl_4-induced elevated levels of the hepatic enzymes SGOT, SGPT, alkaline phosphatase (ALP) and acid phosphatase (ACP) in a dose dependent manner. These results indicating the production of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extracts. Whereas, the AMC extracts at tested doses decreases the CCl_4-induced elevated level of hepatic enzymes in rats, and its subsequent return towards near normalcy after 7days. Reduction in the levels of SGOT and SGPT towards the normal value is an indication of regeneration process. Reduction of ALP levels with concurrent depletion of raised bilirubin level suggests the stability of the biliary function during injury with CCl_4.

Bilirubin is the conventional indicator of liver diseases [27]. The rise in the levels of serum bilirubin is the most sensitive and confirms the intensity of jaundice [28]. These biochemical restorations may be due to the inhibitory effects on cytochrome P450 or/and promotion of its glucuronidation [29]. The marked elevation of bilirubin and urea level in the serum of group II CCl_4 intoxicated rats were significantly decreased in the groups III-V AMC treated animals after 3days. Whereas, after 7 days of treatment, bilirubin and urea level in the serum CCl_4 intoxicated rats subsequently return towards near normalcy in the groups III-V AMC treated animals. These results further substantiate *Cordia subcordata* as a potent hepatoprotective agent.

It has been reported that Liv.52 protects liver from the hepatotoxicity of carbon tetrachloride [30-31]. An appreciable protective effect was observed even after 3 days compared with 7 days treatment using marketed product (Liv.52). The extent of production by extracts appeared to depend on the duration of treatment. Overall, these results suggest that the AMC could protect the liver against damage induced by CCl_4 when comparable with Liv.52 [32-36].

The attributivity of the observed alterations of SGOT, SGPT, alkaline phosphatase (ALP) and acid phosphatase (ACP), serum ALT were confirmed by histopathological studies of liver sections which reveal that the normal liver architecture was disturbed by hepatotoxin (CCl_4) intoxication. In the liver sections of the rats treated with AMC extract for 7 days, the normal cellular architecture was retained as compared to Liv.52, thereby further confirming the potent hepatoprotective effect of *Cordia subcordata* leaves.

Further research is needed to isolate and purify the active principle involved in hepatoprotection of this plant as well as to confirm the mechanisms responsible for hepatoprotective activity. The present finding provides scientific evidence to the ethnomedicinal use of *Cordia subcordata* in treating hepatic disorders.

REFERENCES

1. Wolf PL. *Indian Journal of Clinical Biochemistry*, 14, 1999, 59–90.
2. Handa SS, Sharma A and Chakraborti KK. *Fitoterapia*, 57, 1986, 307-45.
3. Venkateswaran S, Pari L, Viswanathan P, Menon VP. *J Ethnopharmacol*, 57, 1997, 161–167.
4. Latha U, Rajesh MG, Latha MS. *Ind Drugs*, 36, 1999, 470–473.
5. Lupper S. *Altern Med Rev*, 4, 1999, 179–189.
6. Mitra SK, Venkataranganna MV, Sundaram R and Gopumadhavan S. *J. Ethnopharmacol*, 63, 1998, 181-186.
7. Jigna P, Rathish N and Sumitra P. Preliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity. *Indian J. Pharmac*, 37 (6), 2005, 408-409.
8. Okwu DE. Evaluation of chemical composition of indigenous species and flavoring agents. *Global J. Pure & Appl. Sci*, 7 (3), 2001, 455-459.
9. Kawale MV and Choudhary AD. Phytochemistry of Phylanthus niruri. *Bioinfolets*, 5(2), 2009, 8-9.
10. Harbone JP. Phytochemical Methods, A Guide to modern technique of plant analysis, (Chapmann and Hall, London), 1973, pp. 1-271.
11. OECD, 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals organisation for economical co-operation and development, Paris, June, 2000.
12. Visweswaram D, Rajeswara Rao P, Satyanarayana S. *Indian J Pharmacol*, 26, 1994, 301 – 303.

13. Reitman S, Frankel S. *Am J Clin Path*, 28, 1957, 56-62.
14. Kind PRN, King EJ. *J. Clin. Path*, 7, 1954, 132-136.
15. Malloy HT, Evelyn KA. *J Biol Chem*, 119, 1937, 481-485.
16. Bousquet BF, Julien R, Bon R, Dreux C. *Ann Biol Clin*, 29, 1971, 415.
17. Venkidesh R, Subhash C Mandal, Dilipkumar Pal, Mohana Lakshmi S, Saravanakumar A. Hepatoprotective activity of *smilax chinensis l.* In carbon tetrachloride induced hepatotoxicity in Rats. *International Journal of Biological & Pharmaceutical Research.* 2010; 1(2): 72-75.
18. Satheesh Kumar P, Kishor Kumar V. *In-Vitro* Antioxidant activity of methanolic extract of *Atalantia monophylla* Linn bark. *International Journal of Biological & Pharmaceutical Research*, 3(1), 2012, 122-125.
19. Kiso Y, Tohkin M, Hikino H. *Planta Med*, 49, 1983, 222–225.
20. Allis JW, Ward TR, Seely JC, Simmons JE. *Fundamental Appl Toxicol*, 15, 1990, 558–570.
21. Cornelius CE. Animal Models in Liver Research. San Diego: Academic Press; 37, 1993, 341.
22. Recknagel RO, Glende EA, Jr Dolak JA, Waller RL. *Pharmacol Ther*, 43, 1989, 139–154.
23. Plaa G, Charbonneau M. In: Hayes, A.W. (Ed.), Principles and Methods of Toxicology. Raven Press, New York, 1994, pp 841–846.
24. Wachstein M. *Gastroenterol*, 37, 1959, 525- 37.
25. Max Wachstein, Elizabeth Meisel and Carmen Falcon. *Am J Pathol*, 40(2), 1962, 219–241.
26. Sallie R, Tredger JM, William R. *Biopharm Drug Disp*, 12, 1991, 251-259.
27. Girish S, Achliya Sudhir, Wadodkar G, Avinash Dorle K. *J Ethnopharmacol*, 90, 2004, 229-232.
28. Cavin C, Mace K, Offord EA, Schilter B. *Food Chem Toxicol*, 39, 2001, 549–556.
29. Drotman RB, Lawhorn GT. *Drug Chem Toxicol*, 1, 1978, 163-171.
30. Karandikar SM, Joglekar GV, Chitale GK and Balwani JH. *Acta Pharmacol Toxicol*, 20, 1963, 274-280.
31. Meena Kataria and Singh LN. *Indian J Experimental Biol*, 35, 1997, 655-657.
32. Elumalai A, Chinna Eswaraiah M, Sindhura S, Rajendra D, Manikanta KVC and Rajkumar CH. Acute toxicity studies and antipyretic activity of a Poly herbal formulation. *International Journal of Biological & Pharmaceutical Research*, 3(1), 2012, 130-132.
33. Amzad Hossaina M, Raj Nagoorub M, Abdullah Bin Gansau J. New flavone from the leaves of local medicinal plant *Corydyline terminal is* Kunth. *International Journal of Biological & Pharmaceutical Research*, 3(2), 2012, 223-226.
34. Arshed Iqbal Dar, Saxena RC, Bansal SK, Bharti Matadeen, Rahul Saxena. Protective effect of *Citrullus colocynthis* L. against polluted water induced hepatotoxicity in albino rats. *International Journal of Biological & Pharmaceutical Research*, 3(2), 2012, 240-244.
35. Moronkola1 DO and Kunle OF. Essential oil compositions of leaf, stem bark and root of *Alstonia boonei* De Wild (Apocyanaceae). *International Journal of Biological & Pharmaceutical Research*, 3(1), 2012, 51-60.
36. Chaudhary GD, Poonia P, Kamboj P, Kalia AN. Hepatoprotective potential of *lawsonia inermis* l. (seeds). *International Journal of Phytopharmacology.* 3(1), 2012, 66-73.