



Original

Anti-Hepatotoxic Activity of *Mucuna Capitata* Roxb. Extract Against Paracetamol Induced Hepatotoxicity

Bhaskara Reddy Nallamilli*, Sucharita .P, Rama Krishna .K, Ravi Kishore .B

VJ'S College of Pharmacy, Sy.No.721, DBV Raju Township, Diwancheruvu, Rajahmundry-533103, Andhra Pradesh, India.

ARTICLE INFO

Received 10 Oct. 2013
Received in revised form 19 Oct. 2013
Accepted 20 Oct. 2013

Keywords:

Anti-Hepatotoxic activity,
Mucuna Capitata Roxb.,
Paracetamol,
Silymarin.

Corresponding author: Department of Pharmacology, VJ'S College of Pharmacy; Sy.No: 721, DBV Raju Township, Diwancheruvu, RAJAHMUNDRY-533103 E. G. Dist., Andhra Pradesh, INDIA.
E-mail address:
bhaskarareddypharma@gmail.com

ABSTRACT

Herbal formulations available with a wide range of indications like protective to liver, hepatic regulator, as treatment for hepatic dysfunction, for hepatic regeneration as well as liver stimulant and tonic. Despite the widespread use, there is a lack of scientific evidence on their efficacy and safety. **Aim:** The present study investigates the possible anti-hepatotoxic activity of *Mucuna Capitata* Roxb (*MCR*) against paracetamol induced hepatotoxicity in albino wistar rats. **Methods:** Alcoholic extract (80% Ethanol) of the *MCR* whole plant was used in the study. A comparison was also made between the actions of *MCR* extract and a known hepatoprotective drug silymarin. Preliminary phytochemical screening was carried. Bio-chemical parameters like SGOT, SGPT, SALP, Total bilirubin, Total protein and histopathological studies were used to evaluate the anti-hepatotoxic activity. Acute toxicity studies were carried out according to OECD (420) guidelines, 200mg and 400mg/kg BW doses were selected for the present study. **Results:** Effect of *MCR* found to be dose dependent and reduces the elevated levels of SGOT, SGPT, SALP, Total bilirubin and Total protein. 400mg/kg B.W. shows highly significant anti-hepatotoxic activity ($P \leq 0.001$ compared to Group-II). In histopathological studies, 400mg/kg B.W showed no significant necrosis. **Conclusion:** From the results, it is evident that *MCR* has significant anti-hepatotoxic activity against paracetamol induced hepatotoxicity in albino Wistar rats.

© 2013 British Biomedical Bulletin. All rights reserved

Introduction

Liver is the largest organ/gland in the body contributing about 2% (i.e. abt.1.5 Kg in man) of the total body weight. It is extremely active organ, performs many different functions and yet it is discrete organ, and many of its functions interrelated with one another. This becomes especially evident in abnormalities of the liver, because many of its functions are disturbed simultaneously¹⁻³. It's continuous exposure to the various endogenous and exogenous substances, it is overpowered, results in liver damage. Liver damage or failure is always associated with hepatocytes necrosis and elevated levels of biochemical parameters like SGOT, SGPT, SALP and Total bilirubin levels⁴. The death rate increases by each year due to hepatic disorders^{5,6}. In fact, drug-induced liver toxicity is the leading cause of acute liver failure according to FDA. In the modern medicine, they are very few to reliable in the treatment of hepatotoxicity. Traditional medicine becomes significant source of pharmaceutical drugs, leads to increasing demand for phytodrugs and some herbals have proven hepatoprotective activity e.g. *Andrographis paniculata*, *Glycyrrhiza glabra*, *Phyllanthus niruri* etc^{7,8}.

Paracetamol is a known analgesic and anti-pyretic, which produces hepatotoxicity at higher doses⁸, caused by reaction metabolite N-acetyl-p-benzo quinonemine (NAPQI), which causes oxidative stress and depletion of glutathione⁹. Induction of cytochrome P-450 or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity^{10,11}.

The present study aimed to investigate the potential anti-hepatotoxic activity of *Mucuna Capitata Roxb.* against paracetamol induced hepatotoxicity.

Materials and Methods

Chemicals

Silymarin, a standard drug was obtained from Micro Labs, Bangalore as a gift sample. Paracetamol procured from Merck & Co. Animal feed pellets were procured from Lipton's India Ltd., Hyderabad. All other chemicals and solvents used in the study were of analytical grade.

Collection and Extraction of Plant Material

The whole herb of *MCR* were collected from G.Kothapalli, East Godavari Dist, Andhra Pradesh, India during the month of December and they were authenticated by taxonomist Dr. J.Sunitha, Govt. Arts College, Rajahmundry. The whole plant was washed, dried under shade and powdered to coarse consistency in cutter mill. The powder was passed through 40# mesh particle size and stored in an air tight container at room temperature. A voucher herbarium specimen was preserved in the crude drug herbarium of Govt. College (A), Rajahmundry with reference number GACPH0189.

The powdered material of whole plant was extracted with alcohol (80% Ethanol) in soxhelt apparatus by simultaneous extraction for 48 hrs. The extract was filtered and concentrated to dryness by means of evaporation to avoid the decomposition of the natural metabolites¹². The percentage yield of alcoholic extract of the powdered roots was determined. The extract was preserved in a refrigerator till further use. Preliminary phytochemical analysis was carried out to confirm the presence of Alkaloids, Glycosides, Flavanoids, Tannins, Carbohydrates, Phenolic compounds, Proteins, Amino acids, Fixed oils and Saponins in the extracts using various standard methods¹³⁻¹⁵.

Preparation of Dose for Dried Extract and Standard drug

The alcoholic extract was formulated as suspension in distilled water using Tween-80 as suspending agent. The strength of the suspension was according to the dose administered and was expressed as weight of the dried extract^{16,17}. Silymarin 25mg/kg BW was used as a reference standard drug for evaluating anti-hepatotoxic activity which was made into suspension in distilled water using Tween-80 as suspending agent.

Experimental Animals

Healthy albino Wistar rats (150-200g) of either sex were used for the study obtained from college animal house. Animals were housed in colony cages at ambient temperature of $25\pm 20^{\circ}\text{C}$, 12hr light/dark cycle and $50\pm 5\%$ relative humidity with free access to food and water ad libitum during week acclimatization on a commercial pellet diet, which was finely ground before being administered to animals. After a week acclimatization period the animals were divided into 5 groups containing 6 in each namely (Group I, II, III, IV & V). Food but not water was deprived overnight and during the experiment. All the experiments were carried out during the light period (9:00-16:00h). The institutional animal ethical committee approved the study protocol. The experimental procedures were carried out in strict compliance with the Institutional Animal Ethics committee regulations (Regd no. 1283/c/09/CPCSEA). Protocol approval reference number is PBRI/IAEC/11/PN-129.

Acute oral toxicity studies

The acute oral toxicity study of the extracts was carried as per the OECD (420) guidelines. Administration of the extracts stepwise up to the dose 2000mg/kg BW caused no considerable signs of toxicity (i.e. mortality or behavioral changes) in the tested animals. $1/10^{\text{th}}$ and $1/5^{\text{th}}$ of the upper limit

dose (i.e. 200 and 400mg/kg B.W) was selected as the levels for examination of anti-hepatotoxic activity of the MCR extract.¹⁸

Experimental Design

Albino Wistar rats were divided into 5 groups each containing 6 animals. Group I served as normal control group received only vehicle, Group II served as paracetamol control treated with paracetamol (2g/kg BW p.o), Group III served as Standard treated with silymarin (25mg/kg BW p.o), Group IV served as test treated with alcoholic extract (200mg/kg BW p.o) + and Group V served as test treated with alcoholic extract (400mg/kg BW p.o). All the groups were fasted for 24hrs prior to the treatment of paracetamol except Group I. The vehicle or drug treatment was carried out orally for continuously for 7 days with concurrent administration of paracetamol on 6th and 7th days. On 8th day blood was collected by retro-orbital plexus under mild ether anesthesia and serum was separated for determination of biochemical parameters. The livers of the animals were isolated and kept in 10% formalin solution for histopathological studies^{19,20}.

Collection of Blood and separation of serum

Blood was collected by retro-orbital plexus under mild ether anesthesia. The serum samples were allowed to clot and the serum was separated by centrifugation at 15000 rpm for 5min to determine the biochemical parameters.

Biochemical analysis

The serum was assayed for determination of SGPT, SGOT using calorimetric method^{21,22}. Estimation of Serum alkaline phosphatase determine by Kind and King's method²². Estimation of total bilirubin (TB) involved the reaction of bilirubin with diazotized sulphanic acid to form an azocompound, the color of which is measured at 546nm. All the estimations were carried out

using standard kits in semi auto analyzer Screen Master 3000²⁴. Total Protein (TP) estimated by using Lowry OH method²⁵.

Histopathological studies

Processing of liver for the histopathological study was performed by following Modified Luna's Method 1960 and staining was carried out using Hemotoxylin and Eosin Protocol^{26,27}.

Statistical Analysis

The results of the study were expressed as mean±S.E.. Data was analysed by using one way ANOVA followed by Turkey Kramer test post t-test for multiple comparisons. Values with $P \leq 0.001$ were considered significant compared with Group 2 and $P \leq 0.01$ compared with Group 1.²⁸

Results and Discussion

The extractive value of the ethanolic extract of *MCR* whole plant found to be 18.5%. The extract of *MCR* was found to be nontoxic up to the dose of 2g/kg BW and did not cause any behavioral changes or death of tested animals. The phytochemical screening indicates the presence of carbohydrates, alkaloids, glycosides, flavonoids, and fixed oils. From the results of biochemical parameters of the animals it was found that alcoholic extract have significantly reduced the elevated levels of SGOT, SGPT, SALP, TB and TP. Results of the present study were given in the Table.1.

Serum enzyme levels show the status of the liver. The elevated levels of the serum enzymes indicted about cellular leakage and loss of functional integrity of the cell membrane in the liver. High level of SGOT indicates liver damage such as due to viral hepatitis. SGPT catalyses the conversion of alanine to glutamate and is released in a similar manner. Therefore, SGPT is more specific to the liver and a better parameter for detecting liver damage. SALP and TB levels

also related to the status and function of hepatic cells. Thus, lowering effect of enzyme content in serum is definite indication of anti-hepatotoxic action of the drug.

In the histopathological studies, it was clearly found that necrosis, degeneration, disarrangement of the hepatocytes along with inflammation in sinusoids, dilated blood vessels and sinusoidal spaces are flooded with inflammatory cells and RBC was observed in paracetamol treated animals. High protection was observed with 400mg/kg BW dose of *MCR* extract, no significant necrosis although signs of inflammation was present. Central and portal veins were also found to be normal with normal sinusoidal space. Both doses showed mild to moderate improvement in toxicity. Representative photographs of histopathological changes showing effect of the test material were shown in figure 1.

Conclusion

The biochemical and histopathological studies confirmed that the anti-hepatotoxicity activity of alcoholic *MCR* extract against paracetamol induced hepatotoxicity.

Acknowledgement

We express our profound sense of gratitude and heartfelt thanks to Pinnacle Biomedical Research Institute, Bhopal for supporting our research work to conduct.

References

1. Arthur C .Guyton, John E. Hall. The liver as an organ. Text book of medicinal physiology, 11th Edition, Elsevier: 2006.859.
2. Sembulingam K, Prema Sembulingam. Liver and gallbladder. Essentials of medicinal physiology, 5th Edition, Jaypee: 2010.236.
3. Ross and Wilson. The digestive system. Anatomy and Physiology in health and illness, 10th Edition, Churchill livingstone Elsevier: 2007. 305-306.

4. Mascolo N, Sharma R, Jain SC, Capasso F. *J Ethnopharmacol* 1998; 22:211.
5. Handa SS, Sharma A. Hepatoprotective activity of andrographolide against galactosamine & paracetamol intoxication in rats. *Indian J Med Res* 1990 Aug; 92:284-92.
6. Sharma B, Sharma UK. Hepatoprotective activity of some indigenous plants. *Int J PharmTech Res* 2010; 2(1): 568-572.
7. Meganathan M, Madhana MG, Sasikala P, Mohan J, Gowdhaman N, Balamurugan K, et al. Evaluation of antioxidant effect of Omega 3-fatty acid against paracetamol induced liver injury in albino rats. *Global J Pharmacol* 2011; 5(1): 50-53.
8. S.K.Sharma, M. Ali and J. Gupta, *Phytochemistry and Pharmacology*, 2002; 2: 253-270.
9. Boyd EH, Berezky GM, Liver necrosis from paracetamol. *Br. J. Pharmacol* 1996; 26:606-614.
10. Moron M, Depierre JW, Mannervik B, Levels of glutathione, glutathione reductase, glutathione transferase activities in rat lung and liver. *Biochim. Biophys. Acta* 1979; 582: 67-68.
11. Gupta AK, Chitme H, Dan SK, Misra N. Hepatoprotective activity of Raulwolfia serpentine rhizome in paracetamol intoxicated rats. *J. Pharmacolo. Toxicolo.* 2006a; 1: 82-88.
12. Sudhankar M, Rao ChV, Lakshman Rao A, Raju DB. Antinociceptive and anti-inflammatory effects of the standardized oil of Indian Callistemon lanceolatus leave in experimental animals. *Acta Pharm Turc* 2004; 46:131-9.
13. Trease and Evans-Pharmacognosy, William C Evans, 16th Edition
14. Kokate C.K, Practical Pharmacognosy, 4th Edition, New Delhi, 1999; 149-56
15. Khandelwal K.R, Practical Pharmacognosy, 2nd Edition, 2000; 149-56
16. Gennaro AR , Remington: The science and Practice of Pharmacy, vol.1, Philadelphia: Lippincott Williams & Wilkins; 200;20(1):743-5
17. Bhaskara Reddy Nallamilli, K Veer Reddy, Lakshmi Madhavi R, Lakshmi Prasanna M, Chanikya dora T. Antihyperlipidemic activity of *Malus domestica* and *Namilly vitaceae* fruit peel extract in hyperlipidemic rats. *BioMedRx* 2013,1(5),480-483.
18. Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), OECD Guidelines for the testing of chemicals, revised draft guidelines 423: Acute oral toxic class method, revised document. India: Ministry of Social justice and Empowerment; 2000.
19. Bhaskara Reddy N, et al., Hepatoprotective activity of Cichorium intybus (Linn.) root extract against carbon tetrachloride induced hepatotoxicity in albino Wister rats, *Drug Invention Today* (2013), <http://dx.doi.org/10.1016/j.dit.2013.08.005> (article in press).
20. Nadeem M., Dandiya P.C, Pasha K.V, Imran M., Alani D.K, Vohora S.B, Hepatoprotective activity of some herbal formulations in India. *Indian Drugs* 1997; 33; 392-6
21. Reitman S & Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Amer. J. Clin. Pathol.* 28: 56-63, 1957.
22. S. Hajzer, Oddelenie klinickej biochemie, Fakultna nemocnica s poliklinikou, Kosice, ÖSSR, Comparison of Direct Spectrophotometric Determinations of Bilirubin with Candidate Reference Method in Sera of Newborns *J. Clin. Chem. Clin. Biochem.* Vol. 27, 1989, pp. 445-449
23. Kind Pr, King Ej. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. *J Clin Pathol.* 1954 Nov; 7(4):322-326.
24. Willard R, Faulkner, Samuel Meites. Selected methods for the small clinical chemistry laboratory, 1982, 9:113-118.
25. Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall (1951) Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* 193:265-275
26. Luna L.G, Manual histology staining methods of armed forces institute of pathology, 3rd edition, New York, 1968; 1-31.
27. Special stains and HE, Gary W. Gill, CT (ASCP), Chapter-20, 177-184.

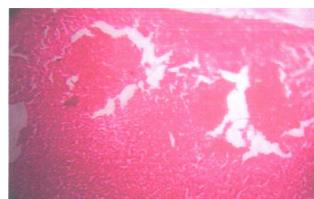
Table 1. Reduction of various biochemical parameters due to treatment with MCR against Paracetamol induced hepatotoxicity in rats

S. No	Parameter	Group-I	Group-II	Group-III	Group-IV	Group-V
1	SGOT (IU/L)	245.5±11.54	675.5±33.71*	483.5±12.88***	400±13.54***	290±6.81***
2	SGPT (IU/L)	258.5±16.07	834.5±72.86*	612±27.43***	510.6±13.5***	376.6±27.35***
3	SALP (IU/L)	528.5±3.82	690±4.63*	368±4.75***	531.6±5.71***	513.6±2.42***
4	TB (mg/dl)	1.079±0.145	3.609±1.13*	1.037±0.16***	1.2±0.12***	1.13±0.0394***
5	TP (g/dl)	6.4±8.48	3.8±1.50*	6.2±13.54***	5.76±9.79***	6.3±9.25***

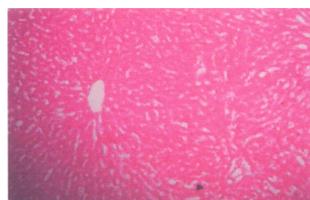
*Note: Results are expressed as Mean ± SEM, N=6. Values are statistically significant at *P≤0.01 Compared with Group I, ***P≤0.001 compared with Group II. (ANOVA)*



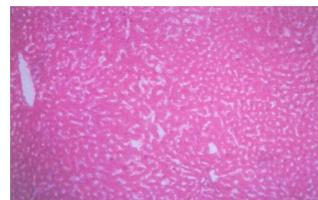
Group I (Control)



Group II (Paracetamol)

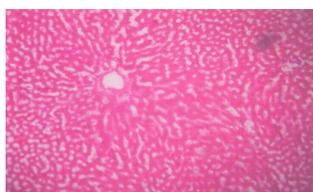


Group III (Silymarin+Paracetamol)



Group IV (200mg test

extract+Paracetamol)



Group IV (400mg test extract+Paracetamol)

Figure. 1. Representative photographs of histopathological changes showing effect of the test material on the rats intoxicated with paracetamol.