

IN VIVO ANTI-OXIDANT AND HEPATOPROTECTIVE EFFECTS OF HUGONIA MYSTAX IN PARACETAMOL INDUCED HEPATOTOXICITY IN RATSDEVENDRA S SHIRODE^{1,2*}, BRIJENDRA B JAIN³, MAHENDRA KUMAR C B⁴¹Department of Pharmacology, Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune - 411 044, Maharashtra, India. ²Jawaharlal Nehru Technological University (JNTU), Hyderabad, Telangana - 500 085, India. ³YSPM's-Yashoda Technical Campus, Satara, Maharashtra, India.⁴St. Mary's College of Pharmacy, Secunderabad - 500 025, Telangana, India.

Email: dssdypcop@gmail.com

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ABSTRACT

Objectives: The objective of the present work is to study the *in vivo* anti-oxidant and hepatoprotective effects of *Hugonia mystax* in paracetamol (PCM) induced hepatotoxicity in rats.

Methods: The *in vivo* anti-oxidant activity of 70% ethanol extract of leaves of *H. mystax* (HMEE) was assessed by determining the tissue glutathione and lipid peroxidation (LPO) levels. HMEE 200 and 400 mg/kg p.o. doses and silymarin p.o. 100 mg/kg were administered to the PCM challenged rats. The effect of HMEE and silymarin on physical (liver weight and liver volume) and biochemical parameters (serum enzymes [serum glutamic oxaloacetic transaminase (SGOT) and serum glutamate-pyruvate transaminase (SGPT)], alkaline phosphate [ALP], and bilirubin) were measured. Furthermore, histopathological changes in the liver were studied.

Results: The HMEE showed *in vivo* anti-oxidant activity. Pre-treatment with HMEE for 7 days significantly reduced the elevated biochemical parameters (SGOT, SGPT, ALP, and bilirubin levels). The hepatic damage in animal pretreated with HMEE was minimal with distinct preservation of structures and architectural frame of the hepatic cells.

Conclusion: These findings demonstrate the protective nature of HMEE against PCM induced hepatotoxicity in rats.

Keywords: *Hugonia mystax*, Hepatoprotective, *In vivo* anti-oxidant, Paracetamol.

INTRODUCTION

Many therapeutic agents cause liver damage, which manifest clinically as hepatitis or only as laboratory abnormality (e.g. increased plasma aspartate transaminase activity). Mild drug-induced abnormalities of liver function are not uncommon, but the mechanism of liver injury is often uncertain. Hepatotoxicity caused by toxic doses of paracetamol (PCM) is clinically important (about 30,000 patients are admitted to the UK hospitals every year with PCM poisoning) [1]. No reliable drugs are available in the allopathic system to cure liver disease. Herbal medicines derived from plant extracts are increasingly being utilized. Hence, the present study was planned to exploit the safety and efficacy of *Hugonia mystax*.

H. mystax, family Linaceae, is a rambling scandent scrub with yellow tomentose twigs and branchlets horizontal provided with a pair of strong hooks. Leaves are simple, alternate, elliptic-obovate glabrous, and penninerved [2]. Literature review mentioned that the roots are astringent, bitter, sweet, febrifuge, and anthelmintic. They are useful in fevers, verminosis, and vitiated conditions of vata, externally as a paste for inflammation [3]. Bark of the root is also employed as an antidote to poison [4]. The modern literature revealed that the plant is reported to possess anti-microbial activity [5-7], anti-inflammatory activity [8], *in vitro* cytotoxic effect [9], and *in vitro* anthelmintic activity [10].

Preliminary phytochemical analysis of HMEE revealed the presence of flavonoids, tannins, and saponins. There are reports that the polyphenolic compounds are possessing anti-oxidant and hepatoprotective effects [11]. Hence, the objective of the present investigation was to study the effect of ethanol extract of *H. mystax* leaves (HMEE) on physical, biochemical, *in vivo* anti-oxidants, and histopathological parameters against PCM induced liver damage in rats.

METHODS**Plant material and preparation of HMEE**

The leaves of plant *H. mystax* were collected from the fields of Tirupati, Andhra Pradesh. It was identified and authenticated by Dr. K. Madhava Chetty, plant taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh. An herbarium specimen was preserved in the college herbal museum. The leaves were shade dried at room temperature and pulverized. The ethanol extract was prepared by using 70% ethanol in a soxhlet apparatus after de-fatting with petroleum ether and chloroform. Preliminary phytochemical investigation showed the presence of flavonoid, tannin, and saponins in 70% HMEE. Hence, HMEE was selected for the study of hepatoprotective activity.

Animals

Wistar albino rats (150-220 g) and mice (18-25 g) of either sex were used for the study. Approval from the Institutional Animal Ethical Committee (1555/PO/a/11/CPCSEA) for usage of the animal in the experiment was obtained as per the Indian CPCSEA guidelines.

Acute toxicity studies

The acute toxicity was determined on albino mice by fixed dose method of OECD Guide line no 420 given by CPCSEA [12].

Experimental designs**PCM induced hepatotoxicity [13]**

Albino rats were randomly assigned into five groups of six animals each. Group I and Group II received normal saline (1 ml/kg) for 7 days. Group III received 100 mg/kg silymarin (standard drug) orally for 7 days. Group IV and Group V received 100 mg/kg and 200 mg/kg HMEE (orally). On 5th day, 30 minutes after the administration of normal saline, 100 mg/kg silymarin, 100 mg/kg, and 200 mg/kg of HMEE to Groups II,

III, IV, and V, respectively, PCM 2 g/kg was given orally. After 48 hrs of PCM feeding, blood samples were collected under mild ether anesthesia and were sacrificed by cervical dislocation and liver tissue was collected.

Biochemical studies

Blood was obtained from all the animals by puncturing retro-orbital plexus. The collected blood was centrifuged (2000 rpm for 10 minutes) to get clear serum and was used to estimate the various biochemical markers such as serum glutamate-pyruvate transaminase (SGPT) [14], serum glutamic oxaloacetic transaminase (SGOT) [15], alkaline phosphate (ALP) [16], and bilirubin (total and direct) [17].

Histopathology

The liver was dissected out and stored in 10% formalin solution. The liver was processed for the histopathological investigations.

In vivo tissue glutathione (GSH) estimation

Tissue GSH measurements were performed using a modification of Ellman procedure [18]. Liver tissue samples were homogenized in ice cold trichloroacetic acid (TCA) (1 g tissue in 10 ml 10% TCA) in an ultrasonic tissue homogenizer. The mixture was centrifuged at 3000 rpm for 10 minutes. Then 0.5 ml of supernatant was added to 2 ml of (0.3 M) disodium hydrogen phosphate solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml in 1% sodium acetate) was added and the absorbance was recorded at 412 nm.

In vivo lipid peroxidation (LPO) estimation

The degree of lipid peroxide formation was assayed by monitoring the formation of thiobarbituric reactive substance [19]. 1.0 ml of biological sample (0.1-2.0 mg of membrane protein or 0.1-2.0 μ mol of lipid phosphate) was added to 2.0 ml of TCA-thiobarbituric acid and HCL solution mixed thoroughly. The solution was heated for 1 hr and cooled. Then the precipitate was removed by centrifugation at 1000 rpm for 10 minutes and the absorbance of the sample was determined at 535 nm against a blank that contain all the reagents minus lipid.

Statistical analysis

Results were expressed as mean \pm standard error of the mean (n=6). Statistical analysis was performed with one-way ANOVA followed by Turkey-Kramer multiple comparisons test. $p < 0.05$ was considered to be statistically significant.

RESULTS

Acute toxicity

No mortality was observed up to 2000 mg/kg of dose in mice. Therefore, 1/10th and 1/5th (200 mg/kg and 400 mg/kg) doses were selected for the study.

In vivo tissue GSH and LPO

In the present study, depletion of GSH level in liver was observed in PCM intoxicated group, while pretreatment of HMEE enhanced the GSH level by 61.04% and 76.02% at the doses of 200 mg/kg and 400 mg/kg, respectively. Silymarin 100 mg/kg increased tissue GSH by 97.28% (Fig. 1). PCM enhanced the LPO. The treatment with HMEE significantly reduced the LPO in a dose dependent manner. Silymarin 100 mg/kg showed 51.94% inhibition, whereas 200 mg/kg of HMEE showed 50.92% inhibition, which is comparable to silymarin (Fig. 2).

PCM induced hepatotoxicity

Administration of PCM in intoxicated control group elevated the serum levels of SGOT, SGPT, ALP, and bilirubin (direct and total) significant ($p < 0.05$). In the present study, HMEE at doses of 200 mg/kg and 400 mg/kg not only prevented the rise in serum level of SGOT, SGPT, ALP, and bilirubin (total and direct) (Table 1) but also improved the liver weight (Fig. 3) and liver volume (Fig. 4) in a dose dependent manner. The histopathological observations (Fig 5) of PCM induced hepatotoxicity also were normalized by the treatment of HMEE. These assessments indicate that the HMEE possess hepatoprotective activity against PCM induced hepatotoxicity.

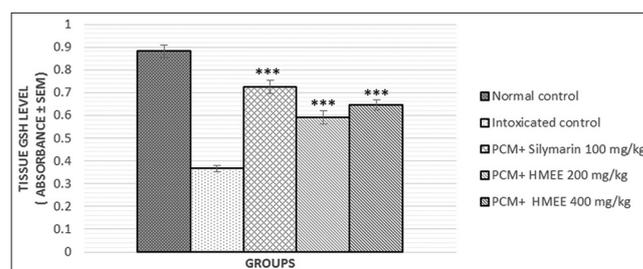


Fig. 1: Effect of ethanol extract of leaves of *Hugonia mystax* on tissue glutathione level in paracetamol induced hepatotoxicity in rats

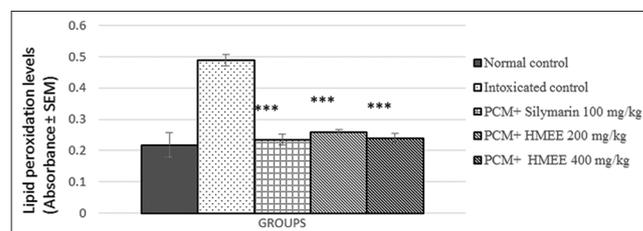


Fig. 2: Effect of ethanol extract of leaves of *Hugonia mystax* on lipid peroxidation levels in paracetamol induced hepatotoxicity in rats

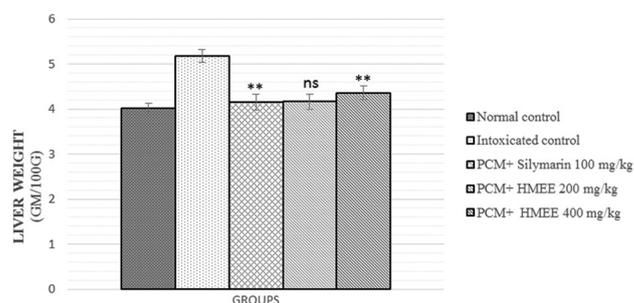


Fig. 3: Effect of ethanol extract of leaves of *Hugonia mystax* on liver weight in paracetamol induced hepatotoxicity in rats

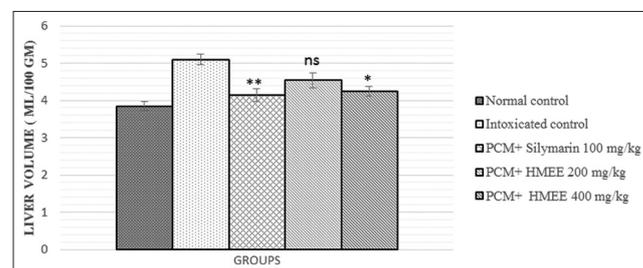


Fig. 4: Effect of ethanol extract of leaves of *Hugonia mystax* on liver volume level in paracetamol induced hepatotoxicity in rats

Histopathological studies

- Group I: Normal control showing mild sinusoidal congestion or NAD (no abnormality detected) (H and E, $\times 40$).
- Group II: Intoxicated control is showing severe fatty degeneration, ballooning of hepatocytes, infiltration of lymphocytes, and congested sinusoids.
- Group III: PCM \pm 100 mg/kg silymarin showing normal liver architecture with minimal hepatocytes degeneration and normal central vein.
- Group IV: PCM \pm 200 mg/kg HMEE is showing moderate fatty changes in hepatocytes and degeneration of hepatocytes.
- Group V: PCM \pm 400 mg/kg HMEE is showing recovery of liver

Table 1: Effect of HMEE on biochemical markers in paracetamol induced hepatotoxicity

Treatment	Biochemical parameters (mean±SEM)				
	SGOT (U/L)	SGPT (U/L)	ALP (IU/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
Normal control (1 ml vehicle)	138.43±5.10	58.38±3.58	163.18±5.91	0.745±0.13	0.221±0.024
Positive control (intoxicated) PCM (2 g/kg p.o.)	379±20.003	332.66±18.226	419.33±26.69	1.138±0.116	0.585±0.1411
PCM±standard (Silymarin) (2 g/kg p.o.±100 mg/kg p.o.)	146.61±5.83***	73.64±2.77***	166.33±5.52***	0.766±0.03 ^{ns}	0.256±0.023**
PCM±HMEE (2 g/kg p.o.±200 mg/kg p.o.)	241±28.537***	198.16±34.45***	291.16±11.09***	0.785±0.05 ^{ns}	0.282±0.02**
PCM±HMEE (2 g/kg p.o.±400 mg/kg p.o.)	235±10.783***	157.25±9.738***	217.16±12.98***	0.752±0.02 ^{ns}	0.25±0.259**

PCM: Paracetamol, HMEE: Ethanol extract of leaves of *H. mystax*, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamate-pyruvate transaminase, ALP: Alkaline phosphate, SEM: Standard error of the mean

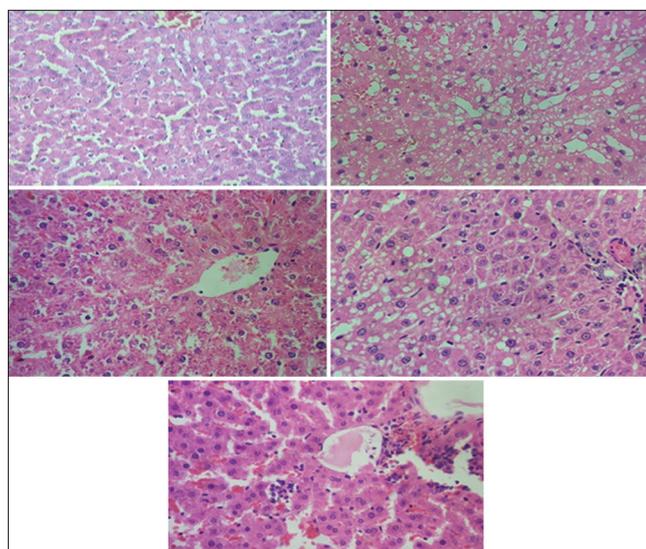


Fig. 5: Histopathological studies of the rat liver in paracetamol induced hepatotoxicity (H and E, ×40)

architecture with minimal infiltration of mononuclear cells and congestion of sinusoids. Central vein is normal (H and E, ×40).

DISCUSSION

There are reports that toxic dose of PCM induced hepatotoxicity is due to activation of PCM to a toxic electrophile N-acetyl p-benzoquinone amine (NAPQI) by a number of isoenzyme of CYP-450 namely CYP 2E₁, CYP 1A₂, CYP 2A₆, CYP 3A₄, and CYP 2D₆. Normally, PCM is eliminated from the body as sulfate and glucuronide to the extents of 95% before oxidation. However, 5% of PCM undergoes bioactivation by above mentioned isoenzymes of CYP to a highly reactive NAPQI [20].

After the over dosage of PCM, routes of sulfation and glucuronidation saturates. As a consequence, oxidation of PCM by CYP-450 isoenzymes are increased leading to the increased concentration of NAPQI. This NAPQI further loses one electron resulting into the toxic radical. This radical interact covalently with membrane macromolecules and damage the membrane. However, this radical is countered by inbuilt tissue anti-oxidants systems such as GSH. Excessive concentration of NAPQI radicals over powers the inbuilt protecting mechanisms thereby causing the membrane damage and necrosis, which releases the biochemical marker enzymes into circulation.

In tissues, SGPT and SGOT are found in higher concentration in cytoplasm [21]. In liver injury, the transport function of the hepatocytes is disturbed resulting in the leakage of plasma membrane [22], thereby serum level of SGPT and SGOT are increased due to cellular leakage and loss of functional integrity of cell membranes in liver [23]. In the HMEE treated group, the reduced levels of elevated SGPT and SGOT may be

a result of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by an overdose of PCM.

ALP is normally excreted via bile by the liver. In liver injury due to hepatotoxin, there is a defective excretion of bile by the liver which is reflected in their increased levels in serum [24]. HMEE significantly prevented the rise in serum level of bilirubin together with the suppression of the activity of ALP in the serum. This suggests the possibility of the HMEE being able to stabilize biliary dysfunction of rat liver during PCM induced hepatotoxicity in rats.

Overall, it is apparent from the results that treatment with HMEE prevents the formation of one electron reduced metabolite of NAPQI. Further, this may be helpful in retaining the membrane GSH contents, reduced LPO, and prevents the tissue damage. In this model, the present study does not rule out the possibility of involvement of the first step of CYP catalyzed activation i.e. formation of NAPQI.

CONCLUSION

In conclusion, the present study demonstrates that HMEE possess hepatoprotective activity. In addition, the hepatoprotective property may be attributed to the polyphenolic compounds of plant, namely tannins and flavonoids. Further investigation is going on to isolate, characterize, and screen the active principles that possess anti-oxidant and hepatoprotective property.

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REFERENCES

1. Jafri MA, Jalis Subhani M, Javed K, Singh S. Hepatoprotective activity of leaves of *Cassia occidentalis* against paracetamol and ethyl alcohol intoxication in rats. *J Ethnopharmacol* 1999;66(3):355-61.
2. Kritikar KR, Basu BD. *Indian Medicinal Plants*. Dehradun: Bishen Singh Mahendra Pal Singh; 1999. p. 412-3.
3. Vaidyaratnum PS. *Indian Medicinal Plants*. Arya Vaidyasala, Kottakkal: Orient Longman; 1995. p. 183-4.
4. Nadkarni AK. *Indian Materia Medica*. Mumbai: Bombay Popular Prakashan; 2002. p. 655-6.
5. Vimalavady A, Kadavul K, Tangavelou AC. Phytochemical screening and antimicrobial activity on the fruits of *Hugonia mystax* L. (Lineaceae). *IJPSR* 2012;3(4):1178-83.
6. Vimalavady A, Kadavul K, Tangavelou AC. Phytochemistry and antimicrobial activity of *Hugonia mystax* L. (Lineaceae). *Int J Pharm Pharm Sci* 2012;4(1): 381-4.
7. Vimalavady A, Kadavul K. Phytochemical screening and antimicrobial activity on the leaves of *Hugonia mystax* Linn. (Lineaceae). *Indian I Nat Prod Resour* 2012;3(2): 161-5.
8. Rajeswari G, Murugan M, Mohan VR. Anti-inflammatory activity of leaf and bark of *Hugonia mystax* L. (Lineaceae). *J Horm Res Pharm* 2013;2(2):80-3.

9. Anandkumar S, Karmegram N. *In vitro* cytotoxic evaluation of *Hugonia mystax* Linn leaf and stem bark extracts. *Int J Bot* 2011;7(4):300-4.
10. Mohankumar M, Laliitha V. *In vitro* anthelmintic activity of *Hugonia mystax* leaves Linn in Indian adult earthworm. *J Pharm Phytochem* 2015;3(5):19-21.
11. Tiwari KA. Imbalance in antioxidant defence and human disease. *Curr Sci* 2001;81:1179-86.
12. Veeraraghavan P. Expert consultant, CPCSEA, OECD Guideline No. 420; Oct, 2000.
13. Chattopadhyay RR. Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract: Part II. *J Ethnopharmacol* 2003;89(2-3):217-9.
14. Bradley DW, Maynard JE, Emery G, Webster H. Transaminase activities in serum of long-term hemodialysis patients. *Clin Chem* 1972;18(11):1442.
15. Rej R, Fasce CF Jr, Vanderlinde RE. Increased aspartate aminotransferase activity of serum after *in vitro* supplementation with pyridoxal phosphate. *Clin Chem* 1973;19(1):92-8.
16. McComb RB, Bowers GN Jr. Study of optimum buffer conditions for measuring alkaline phosphatase activity in human serum. *Clin Chem* 1972;18(2):97-104.
17. Pearlman FC, Lee RT. Detection and measurement of total bilirubin in serum, with use of surfactants as solubilizing agents. *Clin Chem* 1974;20:447-53.
18. Aykaç G, Uysal M, Yalçın AS, Koçak-Toker N, Sivas A, Oz H. The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione, glutathione peroxidase and glutathione transferase in rats. *Toxicology* 1985;36(1):71-6.
19. John BA, Steven AD. *Microsomal Lipid Peroxidation*. London: Moury Kleiman Co.; 1978. p. 302.
20. Janbaz KH, Saeed SA, Gilani AH. Protective effect of rutin on paracetamol- and CCl₄-induced hepatotoxicity in rodents. *Fitoterapia* 2002;73(7-8):557-63.
21. Wells FE. Tests in liver and biliary tract disease. In: Gowenlock HA editor. *Varley's Practical Clinical*. Florida: CRC Press; 1988.
22. Zimmerman HJ, Seef LB. Enzymes in hepatic disease. In: Goodly EI, editor. *Diagnostic Enzymology*. Philadelphia: Lea and Febiger; 1970.
23. Drotman RB, Lawhorn GT. Serum enzymes as indicators of chemically induced liver damage. *Drug Chem Toxicol* 1978;1(2):163-71.
24. Rao RR. Mechanism of drug induced hepatotoxicity. *Indian J Pharm* 1973;5:313-8.