EAS Journal of Pharmacy and Pharmacology
Abbreviated Key Title: EAS J Pharm Pharmacol
ISSN 2663-0990 (Print) & 2663-6719 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-1 | Issue-6 | Nov-Dec-2019 | DOI : 10.36349/EASJPP.2019.v01i06.010

Research Article

Effect of *Hugonia Mystax* Leaves on Physical and Biochemical Parameters in Ethanol Induced Liver Damage in Rats

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Abstract: The present investigation was aimed to evaluate hepatoprotective effects of ethanol extract of leaves of *Hugonia mystax* (HMEE) against ethanol induced hepatotoxicity in rats. The HMEE at the doses of 200 and 400 mg/kg and silymarin 100 mg/kg were administered to the ethanol challenged rats. The effect of HMEE and silymarin on physical (wet liver weight, liver volume) and biochemical parameters (SGOT, SGPT, ALP, direct and total Bilirubin) were measured in ethanol induced hepatotoxicity in rats. Treatment with HMEE (200mg/kg and 400mg/kg) reduced the elevated levels of above mentioned physical parameters and biochemical markers of hepatotoxicity. The hepatoprotective properties may be attributed to the polyphenolic compounds like flavonoids, saponins and tannins that are present in the HMEE.

Keywords: *Hugonia mystax*, hepatoprotective, Physical parameters, Biochemical parameters, Ethanol.

INTRODUCTION

Liver is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction (Ward and Daly, 1999). Continuous use of agents like paracetamol, anti-tubercular drugs, chemicals used as food preservatives and agrochemicals are threatening the integrity of liver. Further addiction of alcohol and other drugs aggravated the problem and malnutrition also an important cause of liver damage. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders (Karan *et al.*, 1999; Chaterrjee, 2000).

Hugonia mystax is a rambling scandent scrub belongs to Linaceae family. Leaves are alternate and elliptic-obovate glabrous (Kirtikar & Basu, 1999). 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 (Vaidyaratnum, 1995). Bark of the root is also employed as an antidote to poison (Nadkarni, 2002). The pharmacological data reveals that the plant possess antimicrobial activity (Vimalavady *et al.*, 2012), anti-inflammatory activity (Rajeswari *et al.*, 2013), cytotoxic effect (Anandakumar *et al.*, 2011), anthelmintic activity (Mohankumar *et al.*, 2015).

Preliminary phytochemicals analysis of HMEE revealed the presence of flavonoids, tannins and saponins. There are reports that the polyphenolic compounds are possessing hepatoprotective effects (Tiwari, 2001). Hence, the objectives of the present investigation was to evaluate hepatoprotective effects of HMEE against ethanol induced hepatotoxicity in rats.

METHODOLOGY

Plant Material & Preparation of HMEE

The leaves of plant *Hugonia mystax* were collected, identified and authenticated by Dr. K. Madhava Chetty, plant taxonomist, Dept of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh. 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 saponins flavonoid and tannin in 70% ethanol extract of

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Journal homepage:
http://www.easpublisher.com/easjpp/

Article History
Received: 29.11.2019
Accepted: 08.12.2019
Published: 23.12.2019

Hugonia mystax leaves (HMEE). So, HMEE was selected for the further study of hepatoprotective activity.

Experimental Animals

Wistar albino rats weighing between 150-220g and mice weighing between 18-25 g of either sex were used for the study. Approval from the institutional animal Ethical committee (1554/PO/a/11/CPCSEA) for usage of 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 (Veeraraghavan, 2000). No mortality was observed upto 2000 mg/kg of dose in mice. Therefore $1/10^{th}$ and $1/5^{th}$ (200 mg/kg and 400 mg/kg) doses were selected.

Hepatoprotective study [Kapoor *et al*., 1994; Gulati *et al*., 1995.]

Healthy wistar albino rats were divided into 5 groups of 6 animals each.

Group I: -

served as normal control group, received distilled water (5 ml/kg body weight, p.o) as vehicle for 21 days.

Group II: -

Intoxicated group/ethanol treated group, received 40 % ethanol (2 ml/100g body weight, p.o.) for 21 days.

Group III: -

standard group/silymarin treated group, received silymarin (100 mg/kg body weight, p.o.) and 40 % ethanol (2 ml/100 g p.o.) for 21 days.

Group IV: -

HMEE treated group, received HMEE (200 mg/kg body weight, p.o.) and 40 % ethanol (2 ml/100 g p.o.) for 21 days.

Group V: -

HMEE treated group, received HMEE (400 mg/kg body weight, p.o.) and 40 % ethanol (2 ml/100g p.o.) for 21 days.

Biochemical studies

Blood was obtained from all the animals by puncturing retro-orbital plexus. Collected blood was centrifuged (2000 rpm for 10 mins) to get clear serum and was used to estimate various biochemical markers like SGPT (Bradley *et al*., 2003), SGOT (Rej *et al*., 1973), ALP (McComb *et al*., 1972), Bilirubin (total and direct), (Pearlman *et al*., 1974).

Statistical Analysis

Results were expressed as mean ± SEM (n=6). Statistical analysis was performed with one way ANOVA followed by Turkey-Kramer multiple comparisons test.

RESULTS AND DISCUSSION

The mean value of serum liver enzymes SGPT, SGOT, ALP and bilirubin (Total and direct) were significantly increased ($P<0.05$) in ethanol intoxicated group. Administration of silymarin (100 mg/kg p.o.) and HMEE (200 mg/kg p.o and 400 mg/kg p.o.) significantly decreased activities of serum SGPT, SGOT, ALP and bilirubin (Total and direct) levels towards near normal. (Table no 1). The groups treated with silymarin and HMEE (at high dose 400 mg/kg) showed significant restoration of liver weight and liver volume near to normal control group (figure 1 & 2).

Table No. 1-Effect of BLEE on Biochemical markers in Ethanol induced hepatotoxicity

Treatment	Biochemical parameters Mean ± SEM				
	SGOT U/L	SGPT U/L	ALP IU/L	Total Bilirubin mg/dl	Direct Bilirubin mg/dl
Negative control (1ml dist. Water p.o.)	103.6 ± 11.41	81.83 ± 9.170	119.83 ± 12.983	0.52 ± 0.05	0.256 ± 0.033
Ethanol (Intoxicated control)(40 % ethanol, 2 ml/100 g p.o)	371.055 ± 12.119	226.5 ± 19.019	242.17 ± 19.835	1.24 ± 0.17	0.702 ± 0.042
Ethanol + Silymarin (2 ml/100 g p.o+ 100 mg/kg. p.o.)	111.16± 15.085***	87.83± 9.77***	124± 13.42***	0.61± 0.04***	0.282± 0.035***
Ethanol + HMEE (2 ml/ 100g p.o+ 200 mg/kg. p.o.)	344.66± 17.742^{ns}	105.33± 8.758***	172.83± 23.377^{ns}	0.642± 0.053***	0.408± 0.026***
Ethanol + HMEE (2 ml/ 100 g p.o.+ 400 mg/kg. p.o.)	266.5± 25.373**	85 ± 8.524***	148.5 ± 11.918*	0.578± 0.05***	0.388± 0.028***

Values are the mean ± S.E.M. of six rats/ treatment.

Significance ***P<0.001, **P<0.01, *P<0.05, ^{ns}P>0.05, compared to ethanol intoxicated group.

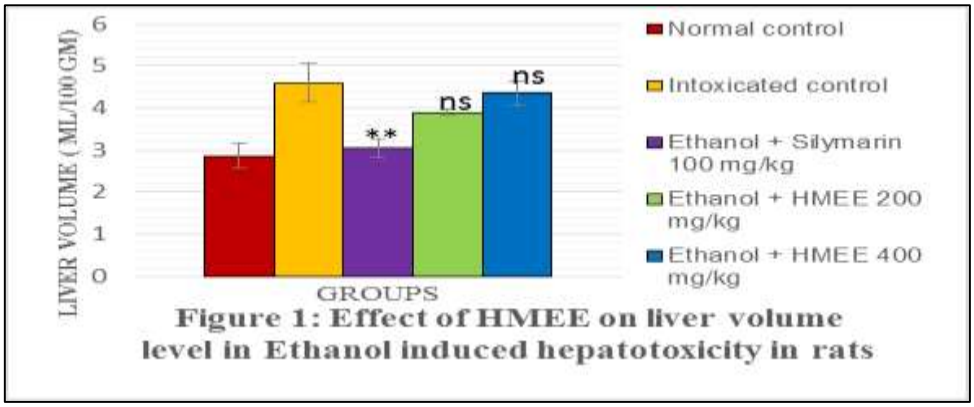

Figure 1: Effect of HMEE on liver volume level in Ethanol induced hepatotoxicity in rats

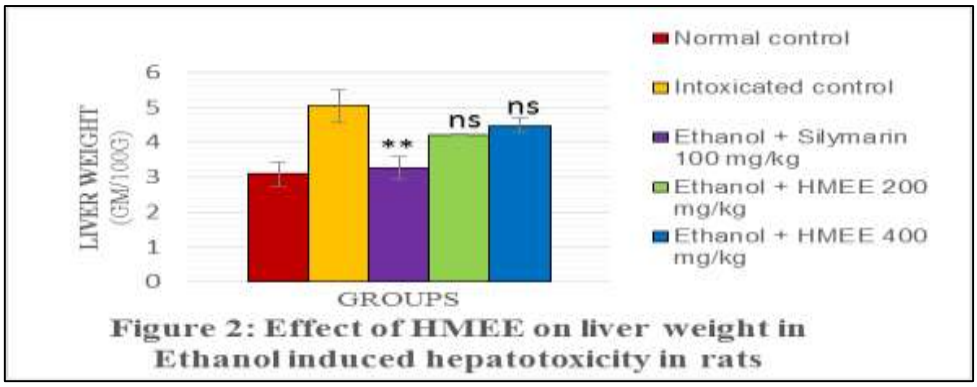

Figure 2: Effect of HMEE on liver weight in Ethanol induced hepatotoxicity in rats

In the ethanol induced hepatotoxicity model, various mechanisms/pathways are responsible to cause hepatotoxicity in rats. first mechanism; chronic alcohol increase the release of endotoxin from gut bacteria and membrane permeability which activate Kupffer cells to release eicosanoids, TNFα and free radicals, which is critical for producing a hypermetabolic state in parenchymal cells. This leads to hypoxia in pericentral regions of the liver lobule where toxic free radicals are formed upon reintroduction of oxygen, causing cell death (Adachi *et al.,* 1994; 1995).

Second mechanism; ethanol is converted in acetyl aldehyde in presence of alcohol dehydrogenase. The Kupffer cells and the endothelial cells of the liver as well as the hepatocytes contain xanthine dehydrogenase that is readily converted into xanthine oxidase [Brass *et al.,* 1991]. Xanthine dehydrogenase/xanthine oxidase catalyze the acetyl aldehyde into acetate which further leads to formation of reactive oxygen species in presence of cytochrome p450 2E1.

Overall, Cytochrome P450 dependent microsomal ethanol-oxidizing system, catalase and non – enzymatic ethanol oxidation (Kennedy and Tipton, 1990) and the involvement of free radical species are responsible for hepatotoxicity (Albano *et al.,* 1988). Ethanol induced hepatic hypoxia also has been invoked as a possible cause of the potentiation of hepatotoxicity (Gulati *et al.,* 1995).

The increase in the activity of serum enzymes levels associated with SGPT and SGOT has been observed in ethanol treated groups, which shows an enhanced permeability, injury and necrosis of hepatocytes (Goldberg and Watts, 1965). Elevation of ALP and Bilirubin in intoxicated group indicated the obstructive biliary process. HMEE showed reduction in levels of SGPT, SGOT, ALP and Bilirubin (total and direct) which indicate improvement in cellular leakage of enzymes and biliary excretion process. These results were also confirmed by physical parameters.

CONCLUSION

HMEE possess significant hepatoprotective activities. It may be due to presence of flavonoids and saponins. Further study is needed to isolate and characteristics the Phytochemicals for hepatoprotective activity.

Acknowledgement

The authors are thankful to Dr. D. Y. Patil college of Pharmacy, Akurdi, Pune for providing all the facilities to carry out this research work.

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