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Effect of ethanol extract of *Hugonia mystax* on Rifampicin induced hepatotoxicity in rats

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ABSTRACT

Ethanol extract of leaves of *Hugonia mystax* (HMEE) was tested for its effect on rifampicin induced hepatotoxicity in albino rats. HMEE at the doses of 200 and 400 mg/kg and silymarin 100 mg/kg were administered to the rifampicin 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 rifampicin induced hepatotoxicity in rats. Similarly, hepatic tissues were subjected to histopathological observations and *in vivo* antioxidant activity (tissue Glutathione and lipid peroxidation levels). Treatment with HMEE (200mg/kg and 400mg/kg) reduced the elevated levels of above mentioned physical parameters and biochemical markers of hepatotoxicity. Histopathological findings and *in vivo* antioxidant studies also confirmed that HMEE possess hepatoprotective effects. The hepatoprotective and *in vivo* antioxidant properties may be attributed to the polyphenolic compounds like flavonoids, Saponins and tannins that are present in the HMEE.

KEY WORDS: *Hugonia mystax,* hepatoprotective, *in vivo* Antioxidant, Rifampicin.

1. INTRODUCTION

Liver is central to the metabolism of virtually every foreign substance. Continuous use of agents like paracetamol, tetracycline, anti-tubercular drugs, oral contraceptives of hormonal origin, chemicals used as food preservatives and agrochemicals are threatening the integrity of liver. About 20,000 deaths found every year due to liver disorders (Handa, 1986). Rifampicin, an antitubercular drug, is largely considered to enhance toxicity of other anti-tubercular drugs (such as isoniazid and pyrazinamide) due to potent induction on cytochrome P450 (Yew and Leung, 2006). Conventional or synthetic drugs for treatments of liver diseases are sometimes inadequate and can have serious adverse effects. In view to develop potent hepatoprotective plant against rifampicin induced hepatotoxicity in albino rats, the effect of HMEE was investigated in albino rats.

Hugonia 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 (Kirtikar and Basu, 1999). Literature review mentioned that the roots are astringent, bitter, sweet, febrifuge and anthelmentic. 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 modern literature revealed that the plant is reported to possess antimicrobial activity (Vimalavady, 2012), anti-inflammatory activity (Rajeswari, 2013), *in vitro* cytotoxic effect (Anandakumar, 2011), *in vitro* anthelmintic activity (Mohankumar, 2015).

Preliminary phytochemicals analysis of HMEE revealed the presence of flavonoids, tannins and saponins. There are reports that the polyphenolic compounds are possessing antioxidant and hepatoprotective effects (Tiwari, 2001). Hence the objectives of the present investigation were to study the effect of HMEE on physical, biochemical, *in vivo* antioxidants and histopathological parameters against rifampicin induced liver damage in rats.

2. MATERIALS AND METHODS

The leaves of plant *Hugonia mystax* were collected from fields of Tirupati, Andhra Pradesh. It was identified and authenticated by Dr. K. Madhava Chetty, plant taxonomist, Dept.of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh. A 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 HMEE. So, HMEE was selected for the study of hepatoprotective activity.

2.1. Animals: Wistar albino rats (150-220g) 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 animal in the experiment was obtained as per the Indian CPCSEA guidelines.

2.2. Acute Toxicity Studies; The acute toxicity was determined on albino mice by fixed dose method of OECD Guide line no 420 given by CPCSEA. The acute toxicity studies revealed that the ethanol extract is safe at 2000 mg/kg. Hence extract was treated as non-toxic and $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of the 2000 mg/kg was selected for hepatoprotective activity.

2.3. Experimental designs (Bafna, 2004; Kulkarni, 2007): The animals were divided into five groups of six animals each.

Group I: Normal control group - The animals in this group received distilled water (1 ml/100 gm, p.o.) as vehicle four times at 12 hr intervals.

Group II: Positive control (toxic) group - The animals in this group received rifampicin (1 gm/kg, p.o. in 5 % gum acacia) at day one.

Group III: Standard group – The animals in this group received Silymarin (100 mg/kg, p.o.) four times at 12 hr intervals. Rifampicin (1g/kg,p.o.) was administered 30 minutes after the administration of first dose of Silymarin (100 mg/kg, p.o.) .

Group IV and Group V: HMEE groups - The animals in this groups received HMEE (200 mg/kg p.o. and 400 mg/kg p.o. respectively) four times at 12 hr intervals. Rifampicin (1g/kg,p.o.) was administered 30 minutes after the administration of first dose of. HMEE

Forty-eight hours after rifampicin administration, blood was collected from retro orbital plexus. Collected blood was centrifuged (2000 rpm for 10 mins) to get clear serum and various biochemical parameters like SGPT (Bradley, 2003), SGOT (Rej, 1973), ALP (McComb, 1972), Bilirubin (total and direct), (Pearlman, 1974) were estimated.

The liver was dissected out and processed for glutathione (GSH), LPO, wet liver weight, liver volume and histopathological investigations**.**

2.4. *In vivo* tissue GSH estimation: Tissue Glutathione (GSH) measurements were performed using the modification of Ellamn procedure (Aykae, 1985). Liver tissue samples were homogenized in ice cold trichloroacetic acid (1gm tissue in 10 ml 10% TCA) in an ultra trux tissue homogenizer. The mixture was centrifuged at 3000 rpm for 10 mins. Then 0.5 ml of supernatant was added to 2ml of (0.3M) disodium hydrogen phosphate solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4mg/ml in 1% sodium acetate) was added and absorbance was measured at 412 nm.

***2.5. In vivo* lipid peroxidation estimation:** The degree of lipid peroxide formation was assayed by monitoring thiobarbituric reactive substance formation (Buege, 1978). 1.0 ml of biological sample (0.1-2.0 mg of membrane protein or 0.1-2.0 μmol of lipid phosphate) was mixed with 2.0 ml of TCA-TBA-HCL solution and mixed thoroughly. Solution was heated for 1 hr and cooled. Then precipitate was removed by centrifugation at 1000 rpm for 10 min. and absorbance of sample was determined at 535 nm against a blank that contain all the reagents minus lipid.

2.6. Statistical analysis: Results were expressed as mean ± SEM (n=6). Statistical analysis was performed with one way ANOVA followed by Turkey-Kramer multiple comparison test. P values less than 0.05 was considered to be statistically significant ($p<0.05$).

3. RESULTS AND DISCUSSION

Rifampicin is rapidly metabolized to deacetyl rifampicin, which binds to RNA polymerase and it leads to inhibition of RNA synthesis; thus inhibiting the nucleic acid and protein synthesis. It induces fatty liver and finally cirrhosis. This causes fatal liver damage acute hepatic failure, which is accompanied by increase in the activity of some serum enzymes (Bafna and Mishra, 2004; Shankar, 2005).

In the rifampicin induced liver damage in albino rats, the increased levels of SGOT and SGPT in serum are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lowhorn, 1978). Alkaline phosphatase (ALP) is the prototype of these enzymes that reflect the pathological alteration in biliary flow (Ploa and Hewitt, 1989). ALP is membrane bound protein enzyme, which are released unequally depending on the pathological phenomenon (Szezeklik, 1961). Rifampicin induced elevation in this enzymatic activity in serum is in line with high level of serum bilirubin content.

Animals treated with only rifampicin showed a significant ($p<0.01$) increase in the level of biochemical parameters (SGPT, SGOT, ALP and Bilirubin), physical parameters (wet liver weight, liver volume), liver peroxide content (LPO) and a significantly ($p<0.01$) decrease tissue glutathione (GSH), when compared with normal group of animals(figures 1-4). Treatment with HMEE showed significant improvement in liver damage in all above parameters in dose dependent manner (Table 1). Histopathological studies also confirmed these findings (figure 5). Group I, Normal control showing Hepatic globular structure, normal central vein and portal tract, Group II (Intoxicated group: Rifampicin) showing severe necrosis of hepatocytes, disturbed Liver architecture & Infiltration of inflammatory cells, Group III (Rifampicin + Std drug 100 mg/kg) showing normal liver architecture, Minimal degeneration of hepatocytes and sinusoidal congestion, Group IV (Rifampicin + HMEE 200 mg/kg) showing mild

fibrous tissue proliferation around blood vessels & minimal sinusoids congestion, Group V (Rifampicin + HMEE 400 mg/kg) showing maintained liver architecture, minimal congestion of sinusoids and fatty changes.

The hepatoprotective activity of HMEE against rifampicin induced liver damage may be due to inhibitory effects on formation of the active metabolic, 25-desacetyl rifampicin which in turn reduces drug metabolizing enzymes. The HMEE showed significant reduction in biochemical markers, which suggest the stability of the biliary functions during injury and it indicate the sign of regeneration process of liver.

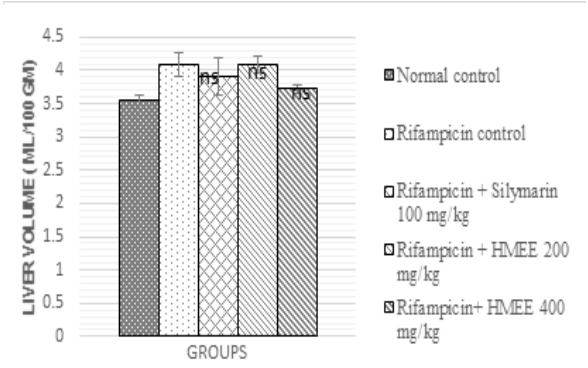


Figure.1.Effect of HMEE on liver volume level in Rifampicin induced hepatotoxicity in rats

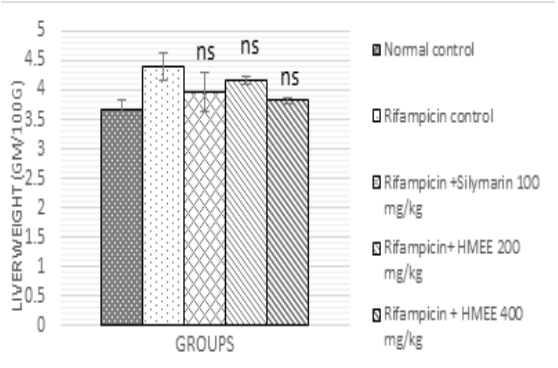


Figure.2.Effect of HMEE on liver weight in Rifampicin induced hepatotoxicity in rats

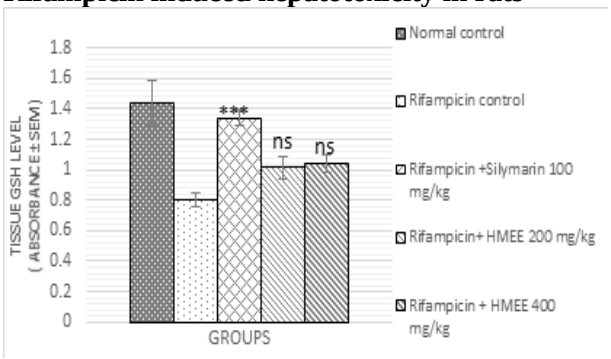


Figure.3.Effect of HMEE on Tissue GSH level in Rifampicin induced hepatotoxicity in rats

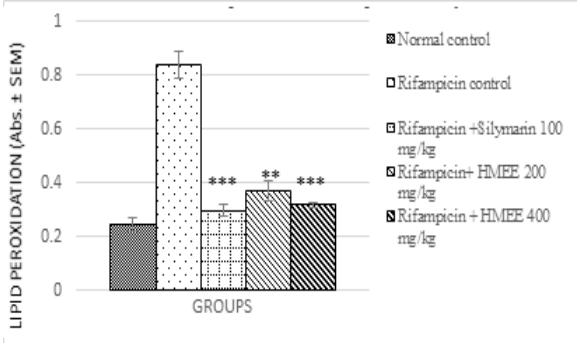
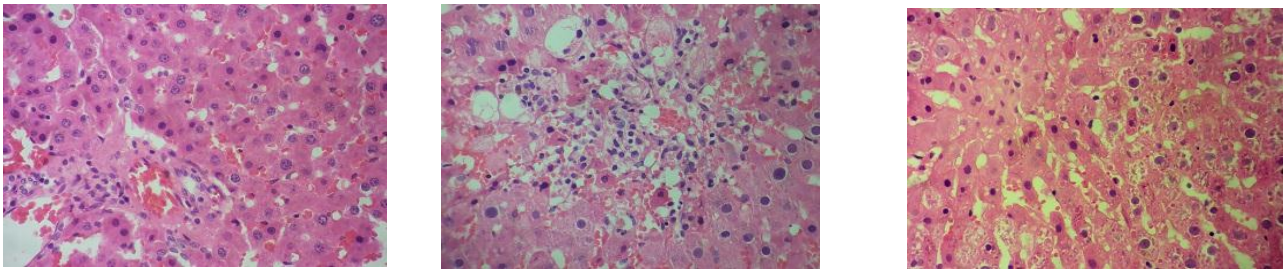
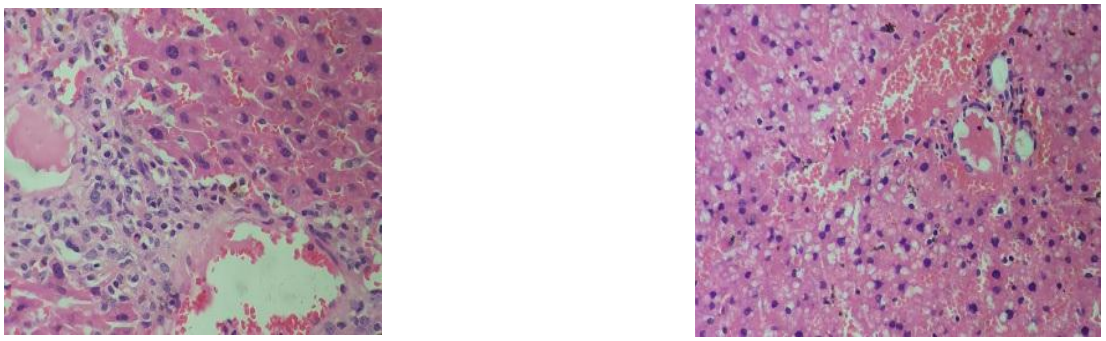


Figure.4.Effects of HMEE on Lipid peroxidation levels in Rifampicin induced hepatotoxicity



Group I; Normal control **Group II Intoxicated group: Rifampicin Group III (Rifampicin** + Std drug 100 mg/kg)



Group IV (Rifampicin + HMEE 200 mg/kg) **Group V. (Rifampicin + HMEE 400 mg/kg)**

Figure 5. Histopathological studies

Table.1.Effect of HMEE on Biochemical markers in rifampicin 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 (1ml dist. water p.o.)	90 ± 5.10	59 ± 5.26	116.5 ± 13.576	0.62 ± 0.026	0.19 ± 0.019
Rifampicin control (1ml dist.water p.o.+ Rifampicin 1 gm/kg p.o.)	619 ± 29.57	693 ± 79.38	680.17 ± 24.898	2.56 ± 0.216	1.485 ± 0.06
Rifampicin + Silymarin (1 gm/kg p.o.+ 100mg/kg, p.o.)	145± 6.875***	110± 7.30***	190.16± 7.298***	0.728± 0.037***	0.2± 0.018***
Rifampicin + HMEE (1 gm/kg p.o.+ 200mg/kg p.o.)	155± 11.793***	112.83± 5.373***	207.5± 8.172***	1.3± 0.08***	0.54± 0.05***
Rifampicin +HMEE (1 gm/kg p.o.+ 400mg/kg p.o.)	127± 6.314***	93 ± 2.978***	186.5 ± 2.54***	0.75± 0.037***	0.465± 0.061***

Values are the mean ± S.E.M. of six rats/ treatment.Significance ***P<0.001, compared to Rifampicin treatment

4. CONCLUSION

It can be concluded from this study that administration of HMEE minimize the effect of rifampicin induced liver damage in rats. In addition, the hepatoprotective property may be attributed to the polyphenolic compounds present in the plant extract, namely tannins, flavonoids and saponins. Further investigation is going on to isolate, characterize and screen the active principles that possess antioxidant and hepatoprotective property in the plant extract.

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