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EVALUATION OF HEPATOPROTECTIVE EFFECT OF *HUGONIA MYSTAX* LEAVES IN CARBON TETRACHLORIDE INDUCED LIVER DAMAGE IN RATS

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Abstract

The investigation was aimed to evaluate the hepatoprotective activity of ethanol extract of *Hugonia mystax* leaves (HMEE) against CCl_4 induced liver damage in rats by determining different *in vivo* antioxidant effect (tissue GSH and lipid peroxidation), physical (wet liver weight and liver volume) and biochemical parameters (SGOT, SGPT, ALP and Bilirubin).

The extract at the dose of 200mg/kg and 400 mg/kg produced significant effect by decreasing the activity of biochemical parameters (SGOT, SGPT, ALP and bilirubin) and tissue lipid peroxidation while it significantly increased levels of tissue GSH in a dose dependent manner. Histopathology observation and physical parameters (wet liver weight and liver volume) also confirm these findings. The effects of extract were comparable to that of standard drug silymarin. These results suggest that HMEE possess hepatoprotective activity against CCl_4 induced liver damage in rats.

Key words : *Hugonia mystax*, hepatoprotective, carbon tetrachloride.

Introduction

Liver plays a major role in intense metabolic activities like detoxification and excretion of many exogenous and endogenous compounds (Reddy *et al*., 1993). Liver toxicity is a major health problem of worldwide proportions. Modern medicine has little to offer to alleviate hepatic diseases and there are not many drugs available to treat liver disorders. Herbal drugs are playing an important role in health care programmes worldwide and different vegetal species have been described for providing active principles that protect against liver damage in experimental animal models (Liu *et al*., 1993, 1995 and Scott, 1999).

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 *et al*., 2012), anti-inflammatory activity (Rajeswari *et al*., 2013), *in vitro* cytotoxic effect (Anandakumar *et al*., 2011), *in vitro* 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 antioxidant and hepatoprotective effects (Tiwari, 2001). Hence, the objectives of the present investigation were to study the effect of ethanol extract of *Hugonia mystax* leaves (HMEE) on physical, biochemical, *in vivo* antioxidants and histopathological parameters against CCl_4 induced liver damage in rats.

Materials and Methods

Plant material

The leaves of plant *Hugonia mystax* were collected from fields of Tirupati, Andhra Pradesh, India. It was identified and authenticated by Dr. K. Madhava Chetty,

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plant taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh (India). 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 70% ethanol extract of *Hugonia mystax* leaves (HMEE). So, 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 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 423 given by CPCSEA.

CCl_4 induced hepatotoxicity (Pradeep *et al*., 2005 and Suja *et al*., 2004)

Healthy wistar albino rats were divided into 5 groups of 6 animals each. Group-I and Group II received distilled water (1 ml/kg) for 5 days. Group III received 100 mg/kg silymarin (standard drug) orally for 5 days. Group IV and Group V received 200 mg/kg and 400 mg/kg HMEE (orally), respectively for 5 days. Group-I received liquid paraffin (1ml/kg) s.c., on 2nd and 3rd day. Group-II, III, IV and V received CCl_4: liquid paraffin (1:1) at a dose of 2ml/kg s.c., on 2nd and 3rd day, after 30 min of vehicle, 100 mg/kg silymarin, 200 mg/kg and 400 mg/kg of HMEE administration. On 6th day, blood samples were collected by retro-orbital plexus method. Rats were sacrificed by cervical dislocation under mild ether anaesthesia and liver tissue was collected.

Biochemical studies

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

Histopathology

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

***In vivo* tissue GSH estimation**

Tissue Glutathione (GSH) measurements were performed using the modification of Ellamn procedure (Aykae *et al*., 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.

***In vivo* lipid peroxidation estimation**

The degree of lipid peroxide formation was assayed by monitoring thiobarbituric reactive substance formation (Buege *et al*., 1978). Take 1.0 ml of biological sample (0.1-2.0 mg of membrane protein or 0.1-2.0 µmol of lipid phosphate) to this adds 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 mins and absorbance of sample was determined at 535 nm against a blank that contain all the reagents minus lipid.

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. P values less than 0.05 was considered to be statistically significant ($p<0.05$).

Results

Acute toxicity

The acute toxicity studies showed that the HMEE is safe at 2000 mg/kg. Therefore as per CPCSEA guideline 423, $1/10^{th}$ (200 mg/kg) and $1/5^{th}$ dose (400 mg/kg) were selected for further hepatoprotective studies.

***In vivo* GSH**

There was a marked depletion of GSH level in CCl_4 treated group. Silymarin 100 mg/kg increased tissue GSH by 96.81%. Treatment with HMEE showed dose dependent increase in the levels of GSH. However, both doses of HMEE have shown lesser increase GSH level than standard silymarin (fig. 3).

***In vivo* lipid per oxidation**

CCl_4 enhanced the lipid peroxidation. The treatment with HMEE significantly reduced the lipid per-oxidation in a dose dependant manner. Silymarin 100 mg/kg showed 50.10% inhibition, whereas 400 mg/kg of HMEE showed 47.40% inhibition, which was almost near to standard silymarin (fig. 4).

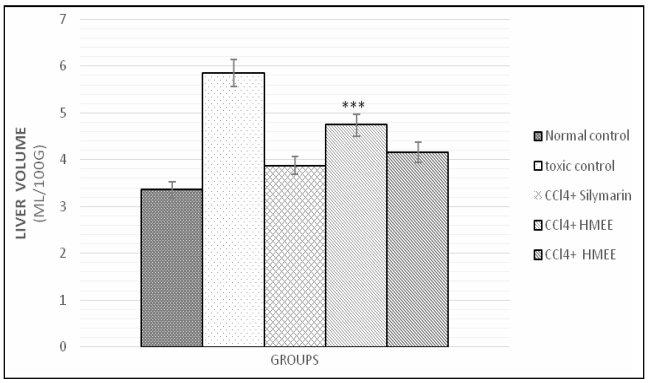


Fig. 1 : Effect of HMEE on liver volume levels in CCl_4 induced hepatotoxicity in rats.

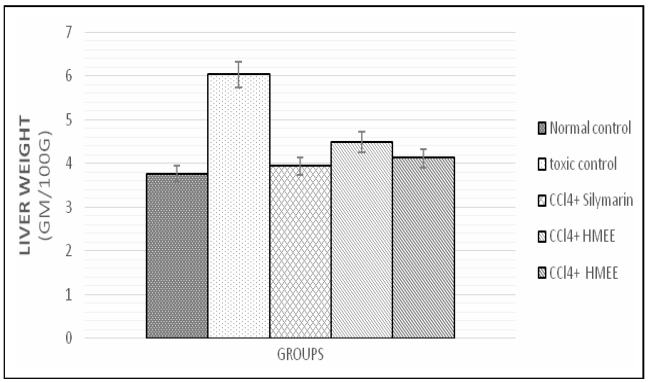


Fig. 2 : Effect of HMEE on liver weight level in CCl_4 induced hepatotoxicity in rats.

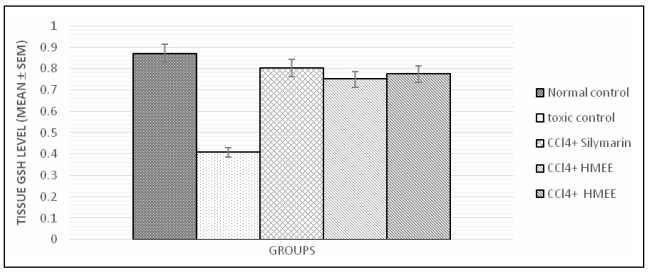


Fig. 3 : Effect of HMEE on tissue GSH levels in CCl_4 induced hepatotoxicity in rats.

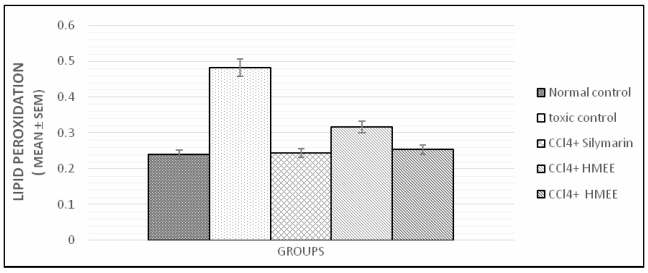


Fig. 4 : Effect of HMEE on lipid peroxidation level in CCl_4 induced hepatotoxicity in rats.

Table 1 : Effects of HMEE on biochemical markers in CCl_4 induced hepatotoxicity in rats.

Treatment	Biochemical parameters (Mean ± SEM)				
	SGOT IU/L	SGPT IU/L	ALP IU/L	Total bilirubin mg/dl	Direct bilirubin mg/dl
Normal Control (1ml dist. Water p.o.)	143.8 ± 11.03	53.98 ± 3.89	172.28 ± 12.135	0.74 ± 0.067	0.19 ± 0.04
Intoxicated control CCl_4 + Liq. Paraffin (1:1) (2 ml/kg s.c.)	423.51 5.33	312.6 ± 9.67	452.03 ± 23.928	2.48 ± 0.16	1.33 ± 0.1294
CCl_4 + Standard (Silymarin) (2 ml/kg s.c.+100 mg/kg p.o.)	166.1 ± 7.02***	56.80 ± 5.81***	195.36 ± 10.34***	1.07 ± 0.08***	0.244 ± 0.031***
CCl_4+HMEE (2 ml/kg s.c.+ 200 mg/kg p.o.)	365.75 ± 11.878^{ns}	235± 22.51**	269± 17.71***	1.54 ± 0.079***	0.34 ± 0.013***
CCl_4+ HMEE (2 ml/kg s.c + 400 mg/kg. p.o.)	351.5 ± 22.583*	219 ± 23.140**	203.17 ± 3.198***	1.495 ± 0.035***	0.245 ± 0.129***

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 CCl_4 treatment.

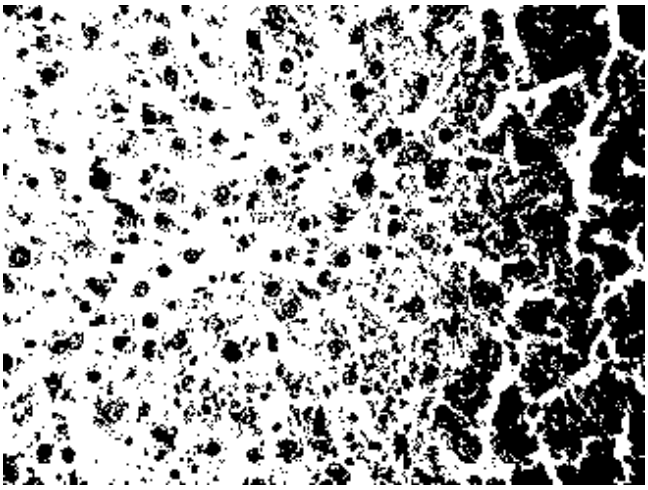
CCl_4-induced hepatotoxicity

Increased levels of liver weight (6.03gm/100gm) (fig. 2), liver volume (5.85 ml/100gm) (fig. 1), SGPT (312.6 IU/l), SGOT (423.51 IU/l), ALP (452.03 IU/l), total bilirubin (2.48 mg/dl) and direct bilirubin (1.33 mg/dl) was observed in CCl_4 treated group. The pretreatment with HMEE (200mg/kg and 400mg/kg p.o.) has brought back the elevated levels of biomarker enzymes of hepatitis in a dose dependant manner (table 1). Treatment with 400 mg/kg of HMEE produced the hepatoprotective activity comparable to that of silymarin 100 mg/kg p.o.

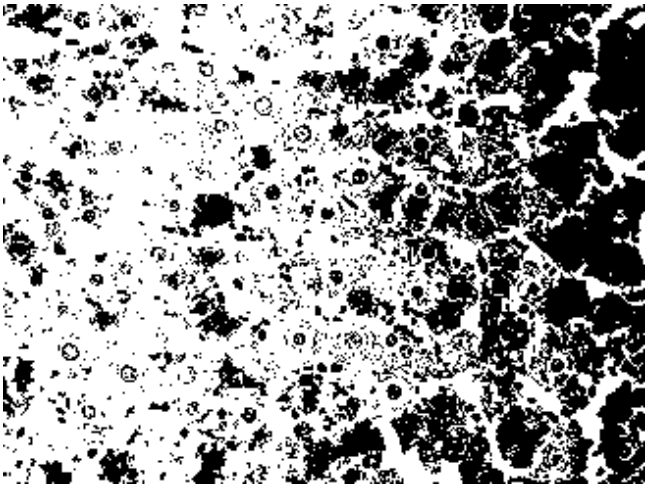
Histopathology

CCl_4 treatment showed congested sinusoids and extensive degeneration of hepatocytes. Treatment with HMEE had shown dose dependant improvement in the liver architecture as indicated by the histopathological observations that there was mild congested sinusoids and fatty change to a lesser extent.

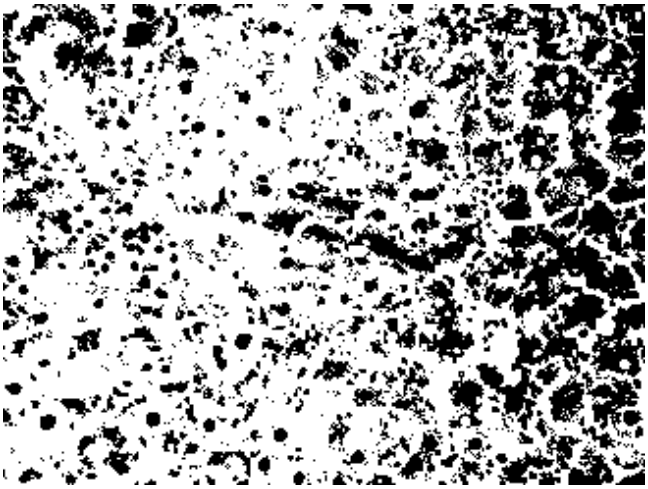
Histopathological studies:



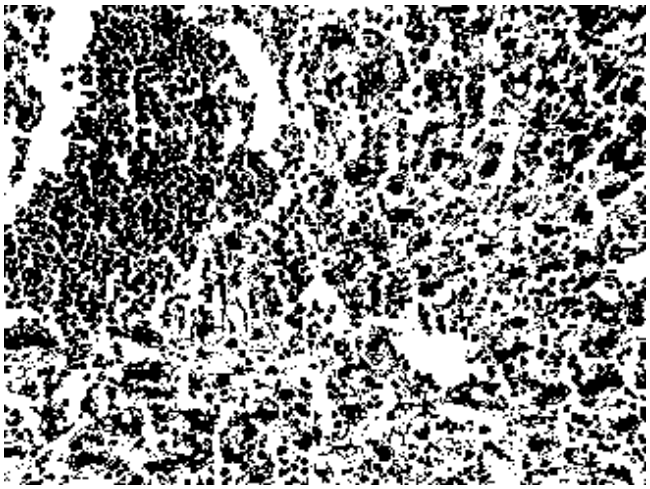
Normal control: Central vein, portal tract and kupffer cells look normal. (H & E 40X)



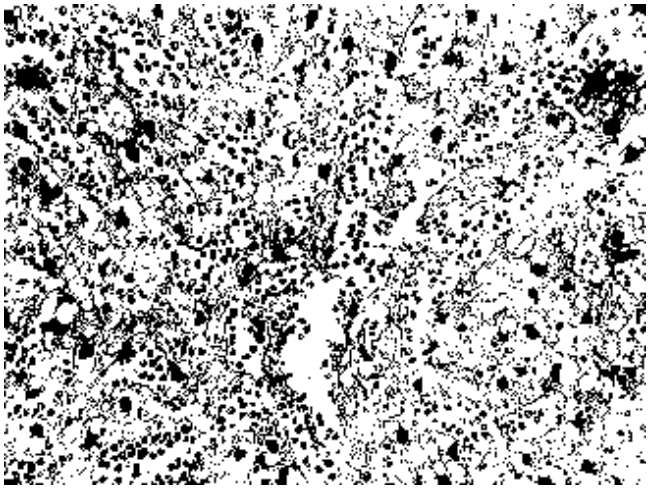
CCl_4+100 mg/kg silymarin: Minimal degeneration of hepatocytes, reduced hemorrhages and sinusoidal congestion. The Liver shows recovery architecture(H & E 40X)



CCl_4+ 400 mg/kg HMEE: Recovery of liver architecture seen. Minimal congestion of sinusoids and minimal infiltration of mononuclear cells. Normal hepatocytes. (H & E 40X)



Intoxicated group: severe hemorrhages in the sinusoidal spaces, congested sinusoids. Degeneration of hepatocytes.(H & E 40X)



CCl_4+ 200 mg/kg HMEE: No hemorrhages, moderate sinusoidal congestion, minimal vacuoles in hepatocytes. (H & E 40X)

The results of physical, biochemical, *in vivo* antioxidant and histopathological studies revealed that the HMEE possess hepatoprotective activity against CCl_4 induced hepatotoxicity in albino rats.

Discussion

Subcutaneous administration of CCl_4 for two days elevated the SGPT, SGOT, ALP, total and direct bilirubin. These findings of intoxicated control are in conformity with the earlier reports (Singh *et al*., 1998). Pretreatment with HMEE (200 mg/kg and 400 mg/kg p.o.) for 5 days significantly reduced the elevated biochemical markers in a dose dependent manner. Treatment with 400 mg/kg of HMEE produced the hepatoprotective activity comparable to that of silymarin 100 mg/kg p.o.

CCl_4 is metabolized to trichloromethyl $CCl_3^{\cdot}$ radical due to the catalytic activity of CYP 450 $2E_1$ enzyme,

which is further converted to trichloromethyl peroxide radical. This trichloro methyl peroxy radical is the main culprit in causing hepatotoxicity. This particular radical forms a covalent bond with sulphydryl group of membrane GSH, protein thiols and unsaturated fats or lipids. This covalent bonding of free radicals with cellular macro molecules initiates the cascade of reactions leadings to lipid peroxidation (Kyung *et al*., 2004; Jeong *et al*., 1999 and Wang *et al*., 2004). The lipid peroxidation inturn alter the membrane permeability and initiates chain of reaction leading to tissue damage and necrosis.

It was observed that the pretreatment with HMEE may be preventing the formation of trichloro methyl peroxy radical. It may be due to tissue GSH levels were not depleted and lipid peroxidation was minimized, this may be the possible mechanism of hepatoprotection offered by HMEE. However, our studies do not confirm whether test extract block CYP 450 $2E_1$ enzyme and thereby inhibit the formation trichloromethyl $CCl_3^{\cdot}$ radical.

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

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