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HEPATOPROTECTIVE ACTIVITY OF THE STEMS OF *ECBOLIUM VIRIDE* (FORSSK.) ALSTON. AGAINST PARACETAMOL-INDUCED HEPATOTOXICITY

S.P.Preethi Priyadharshni*, B.Ganga Rao, K.Balaram Kumar

A.U.College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India.

ABSTRACT

Hepatoprotective activity of *Ecbolium viride* a medicinal herb commonly used in folklore system for wound healing and also against jaundice, was evaluated against Paracetamol (Acetaminophen) induced hepatic damage in male albino rats. Paracetamol (2gm/kg body weight) induced hepatic damage was well manifested by significant increase in the activities of Alanine aminotransferase, Aspartate aminotransferase, Alkaline phosphatase and total bilirubin in serum. Consequent to paracetamol induced hepatic injury, the Serum level was increased. The oral administration of varying doses of different solvent extracts like ethyl acetate and methanolic extracts of *Ecbolium viride* (200 and 400mg/kg body weight) for the period of 10 days reversed these altered parameters to normal levels indicating the antioxidative and hepatoprotective efficacy of *Ecbolium viride* against paracetamol induced liver injury.

Key Words: *Ecbolium viride*, Hepatoprotective activity, Hepatotoxicity, paracetamol, Silymarin.

INTRODUCTION

Medicinal plants play a key role in the human world population health care. About 80% of world's population relies on the use of traditional medicine which is predominantly based on plant materials (Shaik A *et al.*, 2012). Liver damage is very common since liver has to detoxicate lot of toxic substances. Most of the hepatotoxic chemicals damage liver cells, primarily by producing reactive species which form covalent bond with the lipids of the tissue (Deshwal N *et al.*, 2011). Hepatotoxicity in most cases is due to free radical. Free radicals generated by the metabolism of toxicants initiate the toxicity cascade (Kumar *et al.*, 2010). Paracetamol (PCM) also known as Acetaminophen, taken in overdose can cause severe hepatotoxicity and nephrotoxicity. PCM is activated and converted by cytochrome P450 enzymes to toxic

metabolites NAPQI (N-acetyl-p-benzoquinoneimine) that causes oxidative stress and glutathione (GHS) depletion (Sah AK *et al.*, 2012). In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity (Hemamalini *et al.*, 2012). The medicinal action of plants are unique to particular plant species or group of plants and are consistent with this concept as the combination of secondary products in a products in a particular plant is taxonomically distinct (Dar *et al.*, 2012). *Ecbolium viride* belongs to the family Acanthaceae commonly referred to as Blue Fox Tail or Blue Justicia in English, the Indian name includes Neel Kantha. It is an indigenous Indian plant that grows naturally along the Eastern part of India. It is described in Ayurvedic literature as "Neelasahachara" (Vasudevan Nair *et al.*, 1985). Aqueous extracts of dried roots of the plant are used for menorrhoea, rheumatism and jaundice. (Kirtikar KR and Basu BD, 1987). The plant Decoction has been reported to possess many ritual uses

Corresponding Author

S.P.Preethi Priyadharshni
Email: preetihelia@gmail.com

such as in jaundice, menorrhoea, rheumatism (Chopra *et al.*, 1956; Chetty MK *et al.*, 2008), Roots possess anti-inflammatory activity (Lalitha and Sethuraman, 2010), anti-helminthic and also to treat premenstrual colic (Sharma R *et al.*, 2010). Leaves are used in gout and dysuria; decoction of leaves for stricture (Khare, 2007). The roots and leaves are used against tumours (Yusuf AM, 2009) and ethanolic (50%) extract of the plants are used to treat cardiovascular disease (Gamble, 1993; Asolkar, L.V *et al.*, 1992). Crude extracts of plant exhibited a significant antimicrobial activity and in the treatment of some diseases as broad-spectrum antimicrobial agents (Stamatis *et al.*, 2003). The tribes (Tripura and Paliyar) use roots for the treatment of jaundice (Nair RT, 2007). The evaluation of the stem of *E.viride* in the treatment of liver disease has not been reported in the laboratory animals. The present studies were performed to assess the hepatoprotective activity in rats against paracetamol as hepatotoxin to prove its claim in the folklore practices against liver disorders.

PLANT MATERIAL AND METHODS

The stem of *Ecbolium viride* were collected in 2011 from Thalakona and Tirumala, Chittoor district, Andhra Pradesh. Botanical identification of the plants was done by Dr. K. Madhava Chetty, Department of Botany, S.V. University, Tirupati. Specimens of *Ecbolium viride* (Voucher number: 1821) were conserved in S.V. University herbarium, Tirupathi.

Preparation of plant extracts

The Fresh plant of *Ecbolium viride* were shade dried and *Ecbolium viride* (stem) (2kg) stem were separated and powdered by using a Wiley mill and were extracted successively with ethyl acetate and methanol (95%) in the soxhlet apparatus, each for 18 hours. The extracts were concentrated to dryness in the rota vapour till free from the solvents. Later the weight of the extracts were noted and kept in a desiccator. The ethyl acetate extract yield was 22% w/w and methanolic extract obtained was 39% w/w.

Preliminary phytochemical investigation

The ethyl acetate (EAEV) and methanolic (MEEV) extracts were subjected for preliminary phytochemical screening to show the presence of steroid, alkaloid, glycoside, tannin, triterpenoid, carbohydrates reducing sugar and fatty acids. (Peach, 1955; Kokate, 1991).

Drugs and Chemicals

Silymarin- Merck Chemical Company, Mumbai, India., Estimation kits-Span Diagnostics, Surat, India. All other chemicals were obtained from local sources (Sai chemicals, Visakhapatnam) and were of analytical grade.

Experimental Animals

Albino Wistar rats weighing 200-250g were selected for the study. The animals were divided into

different groups of ten animals each. They were maintained at standard laboratory conditions at ambient temperature of $25 \pm 2^\circ\text{C}$ and 44-55% relative humidity with 12 hr light and 12 hr dark cycle. They were fed with commercial pellet diet (Rayan's Biotechnologies Pvt. Ltd, Hyderabad, India) and water *ad libitum*. The experimental protocol was approved by Institutional Ethics Committee and by the regulatory body of the Government [Regd. No. 516/01/A/CPCSEA].

Acute oral toxicity studies

Acute toxicity study was performed for the extract as per Stair case method (Ghosh MN, 2005). For the hepato protective studies, the amount of dose administered was adjusted on the basis of observation during the toxicity studies and accordingly extracts at two dose levels i.e., 200 and 400 mg/kg orally were administered.

Hepatoprotective activity

Hepatoprotective effect of ethyl acetate and methanolic extracts of stem of *E.viride* was demonstrated by using paracetamol induced hepatotoxicity in rats.

Paracetamol induced hepatotoxicity in rats

Among the various hepatotoxins, Paracetamol is the most commonly used drug for medication to all ages of human beings with dose difference. Large doses of paracetamol will cause dose dependent necrosis in rats, mice and man (Mitchell, 1973) hence the paracetamol induced model was selected for the present study. In this study the ethyl acetate and methanolic extracts of *E.viride* (stem) were studied (Shenoy AK *et al.*, 2002) against paracetamol induced hepatotoxicity. An increase in biochemical parameters (SGPT, SGOT, ALP and TB) values above normal level is the index of hepatic damage and restore of the elevated levels, near to normal values indicates the hepatoprotective activity of the selected plants.

Experimental Design

In the dose response experiment, albino rats were randomly assigned into 7 groups of 6 individuals each.

The treatment protocol was planned to study the effect of ethyl acetate extract (EAEV) and methanolic extract (MEEV) in curative aspect of paracetamol induced hepatotoxicity (Shenoy AK *et al.*, 2002). The treatment protocol is summarized and given below.

Group I -normal control, 5% w/v gum acacia suspension orally, 1ml/kg once daily for 10 days. Group II- Paracetamol as toxicant 2g/kg orally once daily for 3 days followed by 1ml/kg 2%W/V gum acacia suspension from 4th day to 10th day. Group III- PCM 2g/kg orally for 3days followed by silymarin 100mg/kg (Setty SR *et al.*, 2007) orally from 4th day to 10th day. Group IV- PCM 2g/kg orally for 3days followed by EAEV 200mg/kg orally from

4th day to 10th day. Group V- PCM 2g/kg orally for 3days day. Group VI- PCM 2g/kg orally for 3days followed by MEEV 200mg/kg orally from 4th day to 10th day. Group VII- PCM 2g/kg orally for 3days followed by MEEV 400mg/kg orally from 4th day to 10th day.

On the 0th day (one day before the dosing) and 11th day blood was collected from each animal for serum analysis. Animals were sacrificed on the 11th day under mild ether anesthesia. Animals were sacrificed and blood was collected directly through retro-orbital plexus. Serum was separated after coagulating at 37 °C for 30 min and centrifuged at 2500 rpm for 15-20 min. The serum was used for the estimation of biochemical parameters, namely, serum glutamic pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP) and total bilirubin (T.B) by using autoanalyser (Tiwari BK *et al.*, 2009) and The liver samples were dissected out, blotted off blood, washed with saline and also stored it in 10% formalin and preceded for histopathology to evaluate the details of hepatic architecture in each group.

Histopathology

After draining the blood, liver samples were excised, washed with normal saline and processed separately for histological observations. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. Paraffin sections were taken at 5 mm thickness, processed in alcohol-xylene series and were stained with alum hematoxylin and eosin. The sections were examined microscopically for histopathological changes (Galigher *et al.*, 1971).

Statistical analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's test. Results were expressed as mean \pm SEM from six rats in each group. P values < 0.05 were considered significant.

RESULTS

Preliminary phytochemical investigation

Preliminary phytochemical investigation of EAEV and MEEV revealed the presence of alkaloids, phytosterols, phenolic compounds, proteins and amino

followed by EAEV 400mg/kg orally from 4th day to 10th acids, flavonoids, tannins and carbohydrates in MEEV extract and EAEV extract showed the presence of alkaloids, carbohydrates, flavonoids, phenolic compound, tannins, proteins and amino acids.

Effect of ethyl acetate and methanolic extracts of stem of *E.viride* against paracetamol-induced hepatotoxicity in rats

In paracetamol induced hepatotoxicity animal model, pretreatment with silymarin (100 mg/kg, p.o.) and methanolic extract of stem of *E.viride* at a dose of 400 mg/kg reduced SGOT, SGPT, ALP, total bilirubin significantly ($P < 0.001$), as compared with paracetamol intoxicated group and at 200 mg/kg reduced SGOT, SGPT, ALP and TB significantly ($P < 0.001$), as compared with paracetamol intoxicated group. The methanolic extract of stem of *E.viride* at 400 mg/kg reduced SGOT and total bilirubin significantly ($P < 0.001$), as compared with paracetamol intoxicated group; ALP significantly ($P < 0.01$), as compared with paracetamol intoxicated group; SGPT, significantly ($P < 0.05$), as compared with paracetamol intoxicated group. However, methanolic extract of 200mg/kg reduced SGOT and ALP significantly ($P < 0.01$), as compared with paracetamol intoxicated group; reduced TB significantly ($P < 0.05$), as compared with paracetamol intoxicated group; dose has not decreased the SGPT level of the serum enzymes significantly (Figure 1).

Histopathology

In histopathological study of paracetamol induced hepatotoxicity model liver section of normal liver showed central vein and cords of hepatocytes; paracetamol intoxicated group rat liver section showed hepatocellular degeneration with fatty changes. Silymarin treated group rat liver section showed normal central vein with mild hepatocytic changes. methanolic extract (200 mg/kg) treated group rat liver section showed less fatty changes vacuolated; methanolic extract (400 mg/kg) treated group rat liver section showed normal central vein. ethyl acetate extract (200 mg/kg) treated group rat liver section showed dilated central vein with less hepatic changes; ethyl acetate extract (400 mg/kg) treated group rat liver section showed mild hepatocytes with fatty changes (Figure 2).

Table 1. Effect of EAEV and MEEV on Paracetamol induced hepatotoxicity in rats (Biochemical parameters) on 0 day(curative)

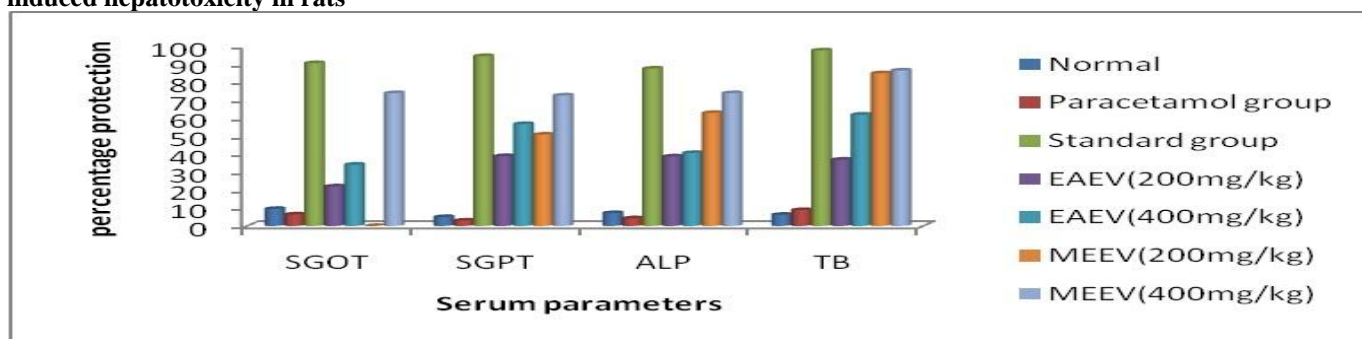
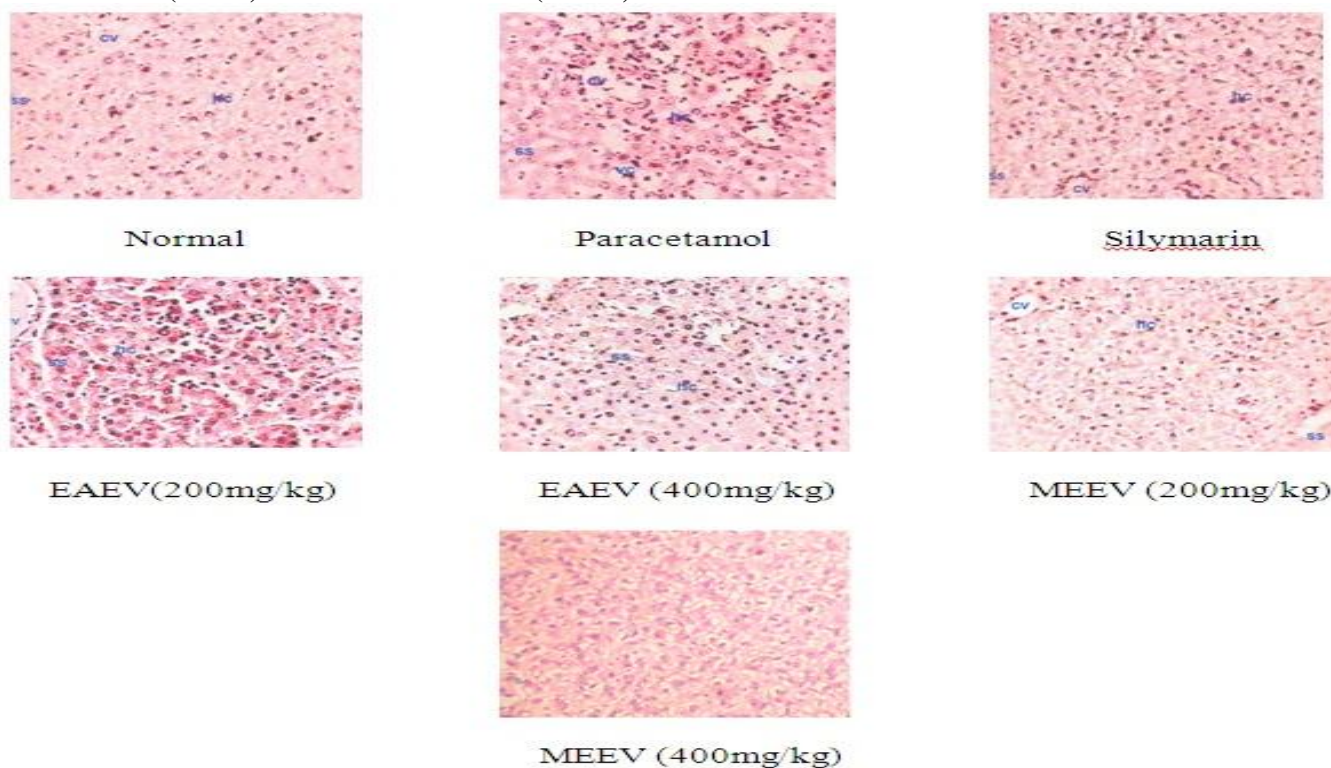
Group	Treatments	SGOT(IU/L)	SGPT(IU/L)	ALP(IU/L)	TB(mg/dl)
1	5%gum acacia suspension(1ml/kg.p.o)	74.67 \pm 0.33	28.47 \pm 1.54	181.22 \pm 2.20	0.918 \pm 0.08
2	Paracetamol	77.40 \pm 0.78	28.22 \pm 0.9	182.18 \pm 0.67	0.818 \pm 0.03
3	Silymarin(100mg/kg.p.o)	70.22 \pm 0.73	29.26 \pm 0.47	179.45 \pm 0.37	0.839 \pm 0.09
4	EAEV(200mg/kg.p.o.)	87.81 \pm 0.48	40.58 \pm 3.00	189.19 \pm 1.24	0.914 \pm 0.07
5	EAEV(400mg/kg.p.o.)	83.78 \pm 2.47	35.78 \pm 1.19	187.25 \pm 2.53	0.868 \pm 0.06
6	MEEV(200mg/kg.p.o.)	81.61 \pm 0.85	30.78 \pm 0.79	181.39 \pm 0.62	0.883 \pm 0.06
7	MEEV(400mg/kg.p.o.)	84.55 \pm 1.20	32.19 \pm 0.48	182.00 \pm 0.61	0.883 \pm 0.07

All values are expressed as Mean \pm SEM, N=6.

Table 2. Effect of EAEV and MEEV on Paracetamol induced hepatotoxicity in rats (Biochemical parameters) on 11th day (Curative)

Group	Treatments	SGOT(IU/L)	SGPT(IU/L)	ALP(IU/L)	TB(mg/dl)
1	5% gumacacia suspension(1ml/kg.p.o)	70.17 ±0.31	40.99 ±0.58	180.61±1.02	0.178±0.09
2	Paracetamol	347.2 ±0.67	230.88±2.49	431.58±0.47	4.12 ±0.02
3	Silymarin(100mg/kg.p.o)	95.6 ±0.47***	40.80 ±0.61***	209.78±0.53***	0.908±0.01***
4	EAEV(200mg/kg.p.o.)	295.58±0.55**	176.86±0.71 ^{ns}	306.19±3.62**	3.009±0.04*
5	EAEV(400mg/kg.p.o.)	259.39±2.74***	136.42±0.55*	314.86±1.93**	2.067 ±1.0***
6	MEEV(200mg/kg.p.o.)	186.19±2.47***	139.78±2.73***	259.61±1.27***	1.217±0.02***
7	MEEV(400mg/kg.p.o.)	148.22±2.20***	92.78 ±0.31***	239.69±0.79***	1.287±0.05***

Values are the mean ± S.E.M. of six rats/treatment;***Significance P<0.001 compared to paracetamol treated groups; **Significance P<0.01 compared to paracetamol treated groups; *Significance P<0.05 compared to paracetamol treated groups.

Fig 1. Percentage protection of EAEV and MEEV on various serum biochemical parameters against paracetamol induced hepatotoxicity in rats**Fig 2. Histopathological changes occurred in the liver after paracetamol intoxication and the treatment with ethyl acetate extract (EAEV) and methanolic extract (MEEV) of *E.viride*.**

DISCUSSION

Liver plays an important role in the metabolism of drug and nutrients. Because of its central role in drug metabolism, it is the most vulnerable tissue for drug toxicity. According to the reports published by USFDA, more than 900 drugs, toxins, and herbs have been reported to cause liver injury, and drugs account for 20%-40% of all instances of hepatic failure (Soni *et al.*, 2011).

The hepatoprotective effects of both ethyl acetate and methanolic extracts of the stem of *E.viride* were studied in rats by using paracetamol induced hepatotoxicity models at the doses of 200 and 400 mg/kg bw. Liver damage was assessed by biochemical studies (SGOT, SGPT, ALP, total bilirubin) and histopathological examinations.

Paracetamol induced hepatotoxicity is one of the well known and commonly used animal model for studying the hepatoprotective property of the plant. Administration of paracetamol at a dose of (2 g/kg/day), p.o. results in hepatic damage. The toxic metabolic N-acetyl-p-benzoquinoneimine is an oxidative product of paracetamol formed by the action of P-450 and it reacts with reduced glutathione (GSH) to yield non-toxic 33-GS-yl-paracetamol. Depletion of GSH causes the remaining quinone to undergo covalent bonding with cellular sulphhydryl groups of protein and leads to cell death. Histopathology of the liver shows necrosis of centrilobular hepatocytes characterized by nuclear pyknosis, eosinophilic cytoplasm and large excessive hepatic lesions (Maiti K *et al.*, 2010).

Pretreatment with sylimarin (100mg/kg.p.o.), ethyl acetate and methanolic extracts (200mg/kg and 400mg/kg) of stem of *E.viride* for 10days has significantly reduced the elevated serum enzyme level. Both ethyl acetate and methanolic extracts reduced the histological changes caused by paracetamol, which further confirmed its hepatoprotective activity against hepatic damage. The possible mechanism of action may be associated with the antioxidant property of stem of *E.viride*.

These results showed that methanolic extract (200mg/kg and 400mg/kg) and ethyl acetate extract (400mg/kg) of stem of *E.viride* possessed significant protection against experimentally induced hepatotoxic model.

CONCLUSION

In the present pharmacological evaluation, the stem extract (ethyl acetate & methanolic) of *Ecbolium viride* plant was extensively investigated for its hepatoprotective potential against Paracetamol induced hepatotoxicity. At the end of this study, a strong conclusion can be drawn that, the methanolic extract of stems of *Ecbolium viride* possess hepatoprotective activities more or less depending on the dose levels.

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