



Evaluation of hepatoprotective activity of aerial parts of *Eremurus himalaicus* Baker.

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Abstract: The aim of the present study was to evaluate the hepatoprotective activity of methanolic and aqueous extract of *Eremurus himalaicus* against paracetamol-induced hepatotoxicity. Activity was measured by monitoring the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin. Silymarin was used as positive control. The results of the paracetamol-induced liver toxicity experiments showed that mice treated with methanolic and aqueous extract of *E. himalaicus* (200 mg/kg) showed a significant decrease in the activity of ALT, AST and ALP and the level of bilirubin, which were all elevated in the paracetamol treated group. The current study confirmed the hepatoprotective effects of methanolic and aqueous extract of *E. himalaicus* against the paracetamol induced hepatotoxicity.

Key words: Paracetamol; hepatoprotective; *Eremurus himalaicus*.

INTRODUCTION

Liver is the main metabolic site of the body where the biotransformation of xenobiotic takes place. Being the major drug-metabolizing and drug detoxifying organ in the body, liver is exposed to high concentrations of toxicants and toxic metabolites making it susceptible to injury (Sheila *et al.*, 1993; Lal *et al.*, 2007).

Paracetamol is a widely used analgesic antipyretic which produces acute liver damage in high doses. At therapeutic doses, paracetamol is safely bio transformed and eliminated as non-toxic conjugates of sulfate and glucuronic acid, and a small portion is converted to NAPQI (N-acetyl-p-benzoquinone imine) which is detoxified by glutathione (GSH) and eventually eliminated in the urine or bile (Sabina EvanPrince, 2013). However, during overdose of paracetamol, the glucuronidation and sulfation routes become saturated and the NAPQI thus formed binds covalently to liver proteins leading to the lipid peroxidative degradation of glutathione which ultimately results in the necrosis of hepatocytes characterized by pyknosis, and eosinophilic cytoplasm followed by large hepatic lesion (Sing Robin *et al.*, 2012). Hepatotoxicity induced by acetaminophen results in prominent elevations of biochemical marker markers like SGOT, SGPT, ALP, bilirubin levels and reactive oxygen species (ROS) (Raju N Jaya *et al.*, 2010).

E. himalaicus (Himalayan desert candle) is native of temperate Himalayas, grows from Afghanistan to Himachal Pradesh at an altitude of 2100-3300m (Wendelbo and Furse, 1969). *E. himalaicus* of great horticultural potential and its young leaves are used as vegetable (Shilaji, 2011). Besides this, *E. himalaicus* is used in anaemia, as a digestive and galactagogue and also possesses antibacterial property. An herbal formulation with *E. himalaicus* Baker has been used in the treatment of migraine with insomnia. (Tramboo Ahlam, 2013).

MATERIALS AND METHODS

Plant material and preparation of extract

The plant material was collected from Harwan area of district Srinagar, J&K, India, identified and authenticated

by Prof. A. R. Naqshi, Department of Botany, University of Kashmir. Specimen voucher number is KUAA12. After collection and authentication, aerial parts of the plant were air dried and powdered. The extracts were prepared by cold percolation method using methanol and water as solvent. The extract was evaporated to dryness under reduced pressure and controlled temperature (40-50°C) The extract was then kept in desiccator to remove remaining moisture, if present, and finally stored in air tight containers at 4°C for further use.

Procurement of animals

Swiss albino mice of either sex weighing 25-30g were used for the study. The albino mice were obtained from animal house of Indian Institute of Integrative Medicine - Jammu, Jammu and Kashmir, India. This Institution is approved by CPCSEA, Government of India, (Approval No.801/03/ca/CPCSEA), for carrying out animal studies and the protocol for the present study was approved by Institutional Animal Ethical Committee [Approval no. F-IAEC (Pharm. Sc.) Approval/2013/07]. The animals were housed in polypropylene cages and maintained under standard environmental conditions: 25±2°C, 12:12 h light: dark cycle and 45-55% humidity, with free access to food and water *ad libitum*. All experiments were carried out during the life period (8.00-18.00 h).

Acute oral toxicity study

The acute toxicity study was performed as per Organization for Economic Cooperation and Development (OECD) guidelines no 425. Swiss Albino mice were used for this study. Since the plant is locally used as vegetable, only limit test was performed as per the guidelines.

Evaluation of hepatoprotective activity

Mice were divided into five groups;

Group I: 0.5% carbomethoxy cellulose (CMC) (control group).

Group II: 1gm/kg paracetamol (toxic group).

Group III: 50mg/kg silymarinin in addition to paracetamol (standard group).

Group IV: Methanolic extract of aerial parts of *E. himalaicus* (200mg/kg, in 0.5% CMC) in addition to paracetamol.

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Group V: Aqueous extract of aerial parts of *E. himalaicus* (200mg/kg, in 0.5%) in addition to paracetamol.

Biochemical parameters

After seven days of the treatment, mice of each group were anaesthetized with ether, and blood was collected directly from the heart. It was centrifuged at 2,000g for 10 min at 4°C to separate the serum and kept at 4 °C to assay the activities of serum enzymes. Glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT), Alkaline phosphatase (ALP) and Serum bilirubin were determined by ultraviolet spectrophotometric method.

Parameters	Control	Paracetamol	Silymarin	MeOH ext	Aq ext
ALT (U/L)	23.156±0.8	37.372±0.48###	24.476±0.75**	29.872±0.83**	31.332±1.25**
AST (U/L)	56.502±0.93	148.336±2.4###	59.364±0.629***	67.316±5.17**	71.226±3.8**
ALP (U/L)	69.658±1.55	92.674±1.46###	74.714±1.19**	77.118±3.06**	79.132±2.78**
Total bilirubin (µmol/L)	2.014±0.034	6.972±0.48###	2.346±0.106***	3.72±0.256**	3.98±0.062**

Results are expressed as mean of 6 values ± SEM. Data was statistically analysed by ANOVA followed by Tukey's test. *P<0.05, **P<0.01, ***P<0.001, P>0.05 are considered as significant, highly significant, extremely significant and insignificant respectively compared with paracetamol treated. ###P<0.001 is considered as extremely significant compared with control.

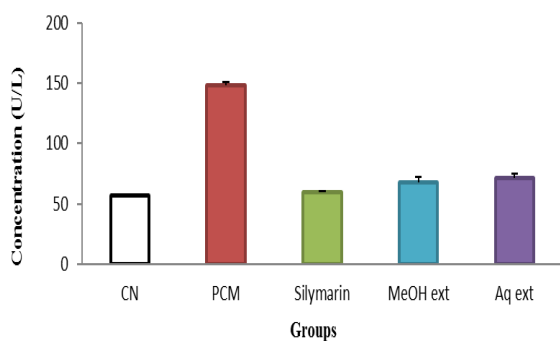


Figure 1: Graphical representation of effect of methanolic and aqueous extract of *E. himalaicus* on serum AST.

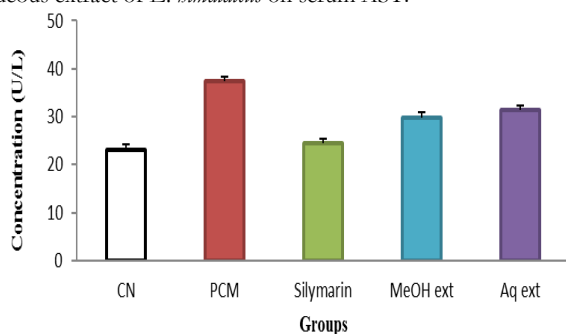


Figure 2: Graphical representation of effect of methanolic and aqueous extract of *E. himalaicus* on serum ALT.

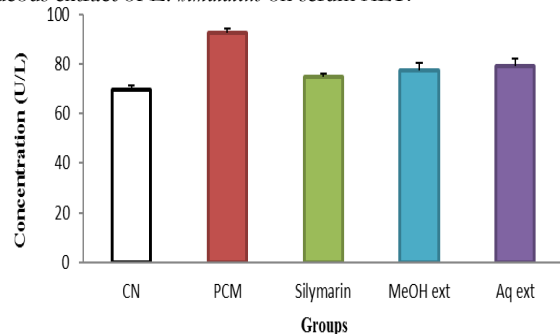


Figure 3: Graphical representation of effect of methanolic and aqueous extract of *E. himalaicus* on serum ALP.

RESULTS

Serum levels of ALT, AST, ALP and bilirubin were significantly increased in paracetamol treated group (1mg/kg p.o) i.e. Group 2. Silymarin treated mice (50mg/kg p.o) i.e. Group 3 showed a significant reduction in the elevated levels of ALP, ALT, AST and total bilirubin. In the test groups i.e. Group 4 and Group 5 treated with methanolic and aqueous extract of *E. himalaicus* at the dose of 200mg/kg p.o there was a significant reduction in AST, ALT, ALP and total bilirubin levels when compared to Group-2 mice.

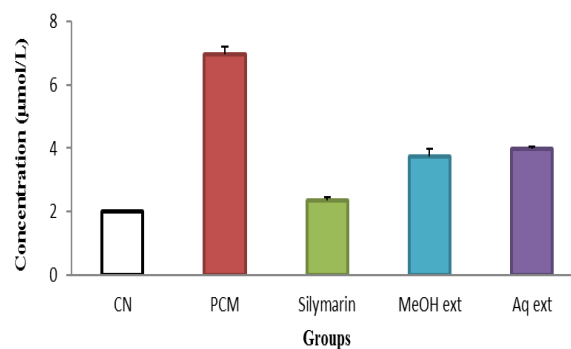


Figure 4: Graphical representation of effect of methanolic and aqueous extract of *E. himalaicus* on serum bilirubin.

DISCUSSION

In the current study paracetamol administration resulted in elevated levels of total serum bilirubin, ALT, AST and ALP in the paracetamol treated group compared to control group indicating hepatic injury, which is responsible for raised serum parameters. The increased activities of serum hepatic enzymes; AST, ALT, ALP in paracetamol treated animal groups may be attributed to cellular leakage and loss of functional integrity of cell membrane in liver (Abraham, 2005). Bilirubin concentration has been used to evaluate chemically induced hepatic injury. Besides various normal functions, liver excretes the breakdown product of haemoglobin namely bilirubin into bile. It is well known that necrotizing agents like paracetamol produce sufficient injury to hepatic parenchyma to cause large increases in bilirubin content (Plaa Gabriel *et al.*, 1982). Silymarin treated group shows a significant protection against the increase in serum levels of biochemical parameters of hepatotoxicity. Methanolic and aqueous extract of *E. himalaicus* showed similar protection against raised serum parameters.

All these findings demonstrate the hepatoprotective activity of aerial parts of *E. himalaicus*, However, as the study was carried out using crude extracts, thus further studies are needed to ascertain the main phyto-constituents responsible

for activity and also to elucidate the molecular mechanism responsible for the pharmacological action.

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