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Original Research Article (Experimental)

Evaluation of hepatoprotective potential of *Erythrina indica* leaves against antitubercular drugs induced hepatotoxicity in experimental rats

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ABSTRACT

Background: *Erythrina indica* Lam. traditionally used in the treatment of laxative, diuretic, worm infestation, liver ailment and joints pain.
Objective: To evaluate the antihepatotoxic potential of *Erythrina indica* against isoniazid (INH) and rifampicin (RIF) induced hepatotoxicity in rats.
Methods and material: Liver toxicity was induced by antitubercular drugs (INH+ RIF) at dose level of 50 mg/kg each, p.o for 28 days. 50% methanolic extract of *Erythrina indica* (100 and 200 mg/kg) were administered orally once daily for 28 days. The hepatoprotective activity was assessed using various biochemical parameters SGOT, SGPT, ALP, bilirubin, total protein, albumin and LDH. Meanwhile, *in vivo* antioxidant activities as SOD, CAT, GSH and, LPO were measured in liver homogenate also histological examinations were carried out to assess hepatoprotective activity.
Statistical analysis used: The values were subjected to one way analysis of variance (ANOVA) followed by Tukey multiple compare test. Results were considered statistically significant when $P < 0.05$.
Results: Obtained results demonstrated that the treatment with *Erythrina indica* (*E. indica*) significantly prevented drug induced increase in serum levels of hepatic enzymes. Furthermore, *Erythrina indica* significantly reduced the lipid peroxidation ($P < 0.01$ tp $P < 0.001$) in the liver tissue and restored activities of defense antioxidant enzymes GSH (2.15 ± 0.08 to 2.48 ± 0.99; $P < 0.05$), SOD (2.69 ± 0.752 to 3.712 ± 0.056; $P < 0.05$ to $P < 0.01$) and CAT (10.20 ± 0.58 to 12.59 ± 0.42; $P < 0.05$ to $P < 0.001$) towards normal. Histopathology of liver tissue showed that *Erythrina indica* attenuated the hepatocellular necrosis, regeneration and repair of cells toward normal.
Conclusion: The results of this study strongly indicate the protective effect of *Erythrina indica* against liver injury which may be attributed to its hepatoprotective activity, and there by scientifically support its traditional use.

1. Introduction

Erythrina indica Lam. (Family: Papilionaceae) is a medium sized tree widely distributed throughout India. Traditionally, its leaves are used as laxative, diuretic, emmenagogue, galactagogue and also used in the treatment of worm infestation, liver ailment and joints pain [1–3]. Phytochemically, *E. indica* contain alkaloids (N-norprotosinomenine, protosinomenine, erysodienone, 3-erythroidine, erysopine, erythraline, etc.), sterols (campesterol, β-sitosterol, β-amyrin), isoflavones (indicanines D and E) and flavonoids include apigenin, genkwanin, iso-vitexin, swertisin, saponarin [4–6].

Earlier scientific investigation of *E. indica* showed that the crude extract has anti-osteoporotic, cytotoxic, cardiovascular, central nervous system effect, anthelmintic, analgesic, antiulcer, antioxidant, and diuretic activity [7].

Liver is the most important organ concerned with the biochemical activities in the human body. It regulates many important metabolic functions and hepatic injury is associated with alteration of these metabolic functions [8].

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Severe liver diseases are one of the most serious health problems in the world today and are characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis, hepatocellular carcinoma and their prevention and treatment options still remain limited. Although viral infection is one of the main causes of hepatic injury, xenobiotics, hepatotoxins, excessive therapy, environmental pollutants, and chronic alcohol ingestions can also cause severe liver injury. Many traditional remedies employ herbal drugs for the treatment of liver ailments [9].

To the best of our knowledge there were no scientific reports available in support of traditional claim of hepatoprotective potential of *E. indica*. Therefore, present study was designed to demonstrate the hepatoprotective effects of methanolic extract of *E. indica* leaves against isoniazid and rifampicin induced liver damage in Sprague–Dawley rats. Isoniazid and rifampicin (INH and RIF), being the first line drugs used as anti-tuberculous chemotherapy, are known to be associated with hepatotoxicity [10,11].

2. Materials and methods

2.1. Chemicals and drugs

All the chemicals used were of analytical grade and were procured from Sigma Chemical Co., St. Louis, MO, USA, and Qualigens Fine Chemicals, Mumbai, India.

2.2. Preparation of plant extract

The fresh leaves of *E. indica* were collected from Pallavaram, Chennai, India and was authenticated by National Institute of Herbal Science, Plant Anatomy Research Center, Chennai, Tamilnadu (Voucher specimen no. PARC-2011/955). 500 g of the coarsely powdered and dried material of *E. indica* were packed in muslin cloth and subjected to a Soxhlet extractor for continuous hot extraction with methanol (50%) for 72 h at 30 °C. Thereafter methanolic extract of *E. indica* was filtered and concentrated under reduced pressure and finally vacuum dried at temperature 40 °C and the pressure 760 torr to 1 bar. The yield of the methanolic extract was 12.5% w/w.

2.3. Animals

Male Sprague–Dawley (SD) rats (150–200 g) and Swiss albino mice (25–30 g) were procured from Central Drug Research Institute, Lucknow, India. The animals were housed separately in polypropylene cage at temperature of 22 ± 2 °C and 50–60% relative humidity, with a 12 h light:dark cycle respectively, for one week before and during the commencement of experiment. Animals were allowed to access standard rodent pellet diet (Dayal animal feed, India) and drinking water. Food was withdrawn 18–24 h before the experiment, though water was allowed *ad libitum* and allocated to different experimental groups. The study protocols were approved by Institutional Animal Ethics Committee (IAEC) of Integral University, Faculty of Pharmacy, Lucknow, India (Reg. No. 1213/ac/2008/CPCSEA/IU).

2.4. Toxicity studies

Acute toxicity study was performed for the methanolic (50%) extract of leaves of *E. indica* according to the Organisation for Economic Co-operation and Development guidelines (OECD)-No. 423 (2001) for acute toxic classic method [12]. Three female Swiss albino mice were used for each step in this study. The animals were kept fasting for overnight only on water, after which the extracts were administered intragastrically at the different doses of 50 and 300 mg/kg. Food or water was withheld for a further 1–2 h after drug administration. Mice were closely observed for the initial 4 h after the administrations, and then once daily for 14 days to observe the mortality. If mortality occurred in two out of three animals at any dose, then this dose was assigned as toxic dose. If the mortality occurred in one animal, then this same dose was repeated to confirm the toxic dose. If mortality did not occur, the procedure was repeated for further higher dose, i.e., 2000 mg/kg. One-twentieth and one-tenth of the maximum tolerated dose of the extract tested (2000 mg/kg) for acute toxicity, did not indicate mortality was selected for evaluation of hepatopreventive effect of *E. indica*, i.e., 100 and 200 mg/kg.

2.5. Anti-tubercular drugs induced hepatotoxicity

Male Sprague–Dawley rats (150–200 g) were divided into 5 groups comprising six animals (n = 6) in each group. Group I (NC) received 1% carboxy methyl cellulose (CMC) and served as healthy control. Group II rats were administered in a combination of two antitubercular drugs viz. INH and RIF (50 mg/kg body weight each, p.o.) for 28 days to produce hepatotoxicity [13], while group III (MEEI 100) and IV (MEEI 200) received orally 100 and 200 mg/kg body weight of *E. indica* for 28 days, prior to antitubercular drugs challenge as per group II. Group V received Silymarin (standard) the known hepatoprotective compound at a dose of 100 mg/kg, p.o., daily for 28 days, prior to antitubercular drugs challenge as per group II. All of the above drugs were prepared freshly every day and suspended in 1% CMC for the administration.

The experiment was completed at the end of 28 days of experimental period, the body weight of each rat was taken before sacrifice. The overnight fasted animals were anaesthetized and sacrificed 24 h after the last dose of the drug. Blood was collected by retro-orbital plexus followed by heart puncture and allowed to clot before centrifugation at 2500 × g for 15 min at 4 °C to separate serum. The liver tissue was washed twice with ice cold saline, blotted, dried, and weighed. The relative liver weight was calculated as the percentage ratio of liver weight to the body weight. A small portion of the tissue was fixed in formalin for histological examination. The remaining tissues were stored at −20 °C for not more than 12 h before analysis [14].

2.6. Assessment of hepatoprotective activity

The serum was used for estimating the biochemical parameters viz., glutamic oxaloacetic transaminase (SGOT), glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin (BL), lactate dehydrogenase (LDH), albumin, total protein (TP) and total bilirubin by using standard assay kit method.

2.7. Assessment of antioxidant parameters

Hepatic tissues of rats were homogenized (10%) in phosphate buffer (pH 7.4) with a Potter-Elvenhjem glass homogenizer. The homogenate was centrifuged at 12,000 rpm for 20 min at 4 °C to obtain post mitochondrial supernatant (PMS) and it was used for the estimation of lipid peroxidation (LPO) [15]. The activity of catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH) in the PMS of liver was measured by the methods described by Aebi [16], Kakkar et al. [17], and Upadhyay [18].

2.8. Histopathological studies

For histopathological studies, the slices of liver from each group were preserved in 10% buffered neutral formalin (pH 7.4). The tissues were mounted in the laboratory by embedding paraffin