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

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**A B S T R A C T**

*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 (*P* < 0.05), SOD (*P* < 0.05) 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.

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**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 (indicanes 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].
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 ingestion 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/PCSEA/UI).

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 hepatoprotective 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-Elvenhem 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 Upadhay [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
sections of 5–10 μ size. These sections were then stained with haematoxyline and eosin dye. The degree of liver damage was examined by a pathologist of R. S. Diagnostic Centre, Lucknow. The degree of necrosis was expressed as the mean of 10 high power fields (HPFs), chosen at random and classified on a scale of 0–5 (no hepatocyte necrosis, 0; necrosis in few hepatocytes, 1; necrosis in more than 10% but less than 24% of hepatocytes, 2; necrosis in more than 25% but less than 30% of hepatocytes, 3; necrosis in more than 40% but less than 49% of hepatocytes, 4; and necrosis in more than 50% of hepatocytes, 5) as per Silva et al. [19].

2.9. Statistical analysis

A result of biochemical estimation has been expressed as mean ± Standard Error of Mean (SEM). The values were subjected to One Way Analysis of Variance (ANOVA) using Graph Prism version 3.0. The variance in a set of data has been estimated by Tukey multiple compare test. The values of P < 0.05 were statistically significant.

3. Results

3.1. Acute toxicity study

Mice administered with 50% methanolic extract of E. indica up to 2000 mg/kg did not show any abnormal behavior, during initial 4 h after drug administration. No mortality was observed during 14 days after treatment. Thus two doses (100 and 200 mg/kg p.o.) were employed for further pharmacological studies.

3.2. Effect of E. indica on body weight, liver weight and relative liver weight

The results are presented in Fig. 1 Body weight (initial and final) of rats treated with rifampicin and isoniazid decreased significantly from control 214 ± 2.6 to 164 ± 3.9 g (P < 0.001).

However, administration of E. indica extract in doses of 100 mg and 200 mg/kg body weight significantly increased the weight of INH + RIF treated rat to 184 ± 7.11 (P < 0.05) and 204 ± 5.36 (P < 0.001) respectively as well as 100 mg/kg Silymarin significantly increased the body weight to 210 ± 2.2 (P < 0.001) when compared to control group I.

Liver weights and relative liver weight of rats (Fig. 2) treated with (INH + RIF) was increased significantly (P < 0.001) from control group I. Administration of 100 and 200 mg/kg of E. indica extract significantly reduced from P < 0.05 to P < 0.001.

3.3. Effect of E. indica extract on liver marker levels

The results of hepatoprotective effects of methanolic extract of E. indica on INH + RIF intoxicated rats are shown in Table 1. Administration of INH + RIF at a dose of 50 mg/kg body weight p.o. each significantly (P < 0.001) elevated SGPT, SGOT, ALP, LDH and, Serum Bilirubin (SBL) activities while TP and ALB were significantly decreased (P < 0.001) when compared to control group I.

Treatment of methanolic extract of E. indica at a dose of 100 and 200 mg/kg, 1 h prior to INH + RIF administration significantly reversed the elevation of transaminases and ALP activities towards normal. Serum bilirubin (SBL) and LDH were significantly reduced (P < 0.05 to P < 0.01) by administration of E. indica at a dose of 100 mg/kg and 200 mg/kg as compared to hepatotoxic group II rats. The protection was better on dose 200 mg/kg and a significant increase (P < 0.01) was observed in the levels of TP and albumin in the serum, against the hepatotoxic control group II. Administration of silymarin significantly reversed (P < 0.001) the altered liver markers levels when compared to hepatotoxic group II.

3.4. Effect of E. indica extract on anti-oxidant parameters

Activities of hepatic SOD, CAT, GSH and LPO are presented in Table 2. The GSH, SOD and CAT content had significantly decreased (P < 0.05 to P < 0.001) in E. indica treated groups when compare to group II whereas antitubercular drugs intoxicated group II had shown significant decrease (P < 0.001) in these parameters compared to control group I. The results in Table 2 showed clear significant change in the antioxidant levels of MDA in antitubercular drugs intoxicated rats as (P < 0.001) compared to control group I. Treatment with E. indica at the doses of 100 and 200 mg/kg significantly prevented this elevation in levels in LPO were (P < 0.01 and P < 0.001) respectively. In different doses level of E. indica 200 mg/kg has shown maximum protection which was almost comparable to those of the normal control and silymarin.
3.5. Histopathological observations

Microscopic examination on normal liver section shows intact parenchymal cells. Mucosal glands are seen compactly arranged, consisting of cells with vesicular nuclei with nucleoli and abundant eosinophilic cytoplasm. Basement membrane is thick and intact (Fig. 3A). The degeneration and necrosis of liver cells, presence of pycnotic nuclei, granular cytoplasm and increase in intercellular spaces with inflammatory collections and loss of cellular boundaries indicated with yellow circle (Fig. 3B). In rats group treated with E. indica extract in two different doses (Fig. 3C and D), shows marked changes at the periphery, granular cytoplasm and decrease in intercellular spaces (indicated by circle) as compared to hepatotoxic control rats. Liver sections show minimal degenerative changes of hepatocytes with minimal swelling.

The consequences of histopathologic grading were presented in Table 3. The treatment with extracts showed that there is a significant reduction in tissue damage along with minimal evidence of inflammation. Group V treated with silymarin 100 mg/kg, body weight as reference drug (Fig. 3E) shows intact parenchymal cells. Mucosal glands are seen compactly arranged without any abnormality or any degenerative changes of hepatocytes.

Table 1
Effect of E. indica on biochemical parameters.

| Groups and treatments | SGPT (IU/L) | SGOT (IU/L) | ALP (IU/L) | SBL (mg/dL) | LDH (U/L) | TP (g/L) | ALB (g/L) |
|-----------------------|-------------|-------------|------------|-------------|-----------|----------|----------|
| Normal control        | 4606 ± 1.70 | 86.16 ± 1.01| 84.459 ± 3.389 | 0.71 ± 0.01 | 386.5 ± 6.27 | 6.98 ± 0.345 | 2.814 ± 0.049 |
| INH + RIF (50 mg/kg each) | 152.51 ± 3.67# | 158.33 ± 1.03# | 225.36 ± 3.190# | 1.72 ± 0.247# | 601.83 ± 10.8# | 2.98 ± 0.170# | 1.39 ± 0.09# |
| E. indica 100 mg/kg | 129.66 ± 5.61*** | 127.16 ± 7.24*** | 211.2 ± 5.472* | 1.10 ± 0.04* | 543.42 ± 17.93* | 3.97 ± 0.33* | 2.01 ± 0.04* |
| E. indica 200 mg/kg | 102.03 ± 5.08*** | 106.83 ± 1.27*** | 134.8 ± 12.351*** | 0.99 ± 0.03*** | 426 ± 10.80*** | 5.54 ± 0.96*** | 2.3 ± 0.14*** |
| Silymarin 100 mg/kg | 83.15 ± 6.13*** | 88.61 ± 2.43*** | 93.2 ± 1.58*** | 0.81 ± 0.02*** | 391.83 ± 15.09*** | 5.67 ± 0.22*** | 2.87 ± 0.05*** |

Values are expressed as mean ± SEM of 6 rats in each group.
P values: "<0.05, "<0.01, "<0.001 compared with group II (INH + RIF).
P values: "<0.01 compared with respective normal control group I.

Table 2
Effect of E. indica on SOD, CAT, GSH and LPO.

| Groups and treatments | SOD (Units/mg tissue protein) | CAT (μ mol H2O2/mg tissue protein) | GSH (μg/mg tissue protein) | LPO (μ mole/mg tissue protein) |
|-----------------------|-------------------------------|-----------------------------------|----------------------------|--------------------------------|
| Normal control        | 4.92 ± 0.431                  | 17.08 ± 0.21                     | 5.21 ± 0.072               | 27.44 ± 1.42                   |
| INH + RIF (50 mg/kg each) | 1.76 ± 0.077#                   | 8.03 ± 0.34*                     | 1.90 ± 0.16*               | 82.6 ± 0.16*                   |
| E. indica 100 mg/kg | 2.69 ± 0.752*                   | 10.20 ± 0.58**                   | 2.15 ± 0.083               | 63.12 ± 5.99**                  |
| E. indica 200 mg/kg | 3.712 ± 0.056***               | 12.59 ± 0.42***                  | 2.48 ± 0.90**              | 42.6 ± 1.53***                 |
| Silymarin 100 mg/kg | 4.11 ± 0.97***                 | 14.013 ± 0.47***                 | 3.45 ± 0.11***             | 36.4 ± 1.63***                 |

Values are expressed as mean ± SEM of 6 rats in each group.
P values: "<0.001 compared with respective normal control group I.
P values: "<0.05, "<0.01, "<0.001 compared with group II (INH + RIF).

Table 3
Histopathologic grading of liver necrosis in experimental rats.

| Groups | Treatments | Histopathological grading |
|--------|------------|---------------------------|
| Group I | normal control | 0.0 ± 0.0 |
| Group II | INH + RIF (50 mg/kg each) | 3.39 ± 0.98# |
| Group III | E. indica 100 mg/kg | 2.54 ± 1.04 |
| Group IV | E. indica 200 mg/kg | 1.56 ± 0.95* |
| Group V | Silymarin 100 mg/kg | 1.28 ± 0.78* |

Values are expressed as mean ± SEM of 6 rats in each group.
P values: "<0.001 compared with respective normal control group I.
P values: "<0.05 compared with group II (INH + RIF).

Fig. 3. Effect of methanolic extracts of E. indica against antitubercular drugs (INH + RIF) induced histopathological changes in experimental groups of rats (H&E staining @ 40X). (A) Normal rats, (B) Liver section of anti-tubercular drugs (INH + RIF) induced rat liver, (C and D) Liver section of rats treated with methanolic extract of E. indica 100 and 200 mg/kg, (E) Liver section of silymarin treated rats.
Histological examination of liver tissues in rats supplemented with *E. indica* extract at the dose of 200 mg/kg body weight showing nearly normal tissue architecture, absence of inflammatory cells in the central areas showing significant hepatoprotective effect.

4. Discussion

In the present investigation, *E. indica* was evaluated for the hepatoprotective activity using antitubercular drugs (INH + RIF) induced liver toxicity in rat. Drug-induced liver toxicity is a potentially serious adverse effect of the currently used antitubercular chemotherapeutic regimens containing isoniazid and rifampicin.

The combination of these two anti-tubercular drugs-induced hepatotoxicity, manifested mainly as hepatocellular steatosis and centrilobular necrosis, possibly associated with cholestasis, and it has been suggested that toxic isoniazid metabolites bind covalently to cell macromolecules in both animal and human case studies [20].

The conversion of monocetyl hydrazine, a metabolite of isoniazid, to a toxic metabolite via cystochrome P450 leads to hepatotoxicity. Rifampicin induces cystochrome P450 enzyme causing an increased production of toxic metabolites from acetyl hydrazine (AcHz). Rifampicin can also increase the metabolism of isoniazid to isonicotinedialdehyde and hydrazine, both of which are hepatotoxic. The plasma half-life of AcHz (metabolite of I) is shortened by Rifampicin and AcHz is quickly converted to its active metabolites by increasing the oxidative elimination rate of AcHz, which is related to the higher incidence of liver necrosis caused by INH and RIF in combination [21].

In addition to these mechanisms; oxidative stress induced hepatic injury is one of the important mechanisms in hepatotoxicity produced by antitubercular drugs [22]. The combination of INH and RIF was reported as higher rate of inhibition of biliary secretion, an increase in liver cell lipid peroxidation and cystochrome P450 was thought to be involved the synergistic effects of Rif on INH. The serum levels of a number of studied hepatic enzymes behave as diagnostic indicators for hepatic injury. The present study revealed an increased level of SGPT, SGOT, LDH and ALP in serum of the INH + RIF induced animals certainly indicate liver damage. An increase in the levels of these marker enzymes in serum was due to the leakage of the enzymes from liver as a result of tissue damage [21].

On concurrent treatment with methanolic extract of *E. indica* at dose of 100 and 200 mg/kg respectively, the serum marker enzyme levels were near to normal indicating protection against liver damage (Table 1). This protective effect could be possibly due to the reduction in the tissue damage brought by the methanolic extract of *E. indica*. The results were compared with the standard silymarin. It is a general perception that, the serum bilirubin levels are elevated in hepatic injury. A marked elevation was observed in serum bilirubin (SBL) levels of INH + RIF induced rats, whereas total protein (TP) and albumin levels in the serum were markedly decreased. A reduction in synthesizing proteins was seen following intoxication of the liver with hepatotoxicants. As seen in the silymarin treated group and methanolic extract of *E. indica*, all studied parameters were restored to normal condition from the abnormal ones.

The body has an effective defense mechanism to prevent and neutralize the free radical induced damage. This is proficient by a set of endogenous antioxidant enzymes such as SOD, and catalase. Suppression of the anti-oxidant system in anti-tubercular drugs intoxicated rats has been reported earlier [22].

The significantly reduced activities of SOD and CAT observed point out the hepatic damage in the rats administered with antitubercular drugs but on treatment with 100, and 200 mg/kg of *E. indica* groups showed significant increase in the level of these enzymes due to the ability of the administered compounds to scavenge reactive oxygen species.

Increase in the level of lipid peroxides in liver reflected the hepatocellular damage. The depletion of antioxidant defenses and/or raise in free radical production deteriorates the prooxidant-antioxidant balance, leading to oxidative stress-induced cell death [23]. Depletion of reduced glutathione (GSH) is known to result in enhanced lipid peroxidation and excessive lipid peroxidation can cause increased glutathione consumption [24], as observed in the present study which indicates the antioxidant activity of the *E. indica*. Furthermore, on treatment with different doses of *E. indica* (100 and 200 mg/kg) significantly reduction in the level of lipid peroxidation and an important cause of destruction and damage to hepatocellular membranes, and elevation in the level of GSH in liver. The increase in hepatic GSH level in the rats treated with *E. indica* may be due to de novo GSH synthesis or GSH regeneration. The hepatoprotective effect of the *E. indica* was further assessed by the histopathological examinations which could be only possible by the overall protective character of the extract. On phytochemical screening, *E. indica* revealed the presence of flavonoids, steroidal alkaloids, saponin, triterpenes and glycosides as the major chemical constituents. Hence, it is possible that the mechanism of hepatoprotection of *E. indica* may be due to its antioxidant property [25] present in these phytochemicals. Our finding also supports the previous hepatoprotective study carried out on *E. indica* [3]. Also reducing the oxidative stress imposed by antitubercular drugs as well as others like analgesic and healing property [26], which may prevent hepatic damage. This study involved the determination of hepatoprotective activity of the *E. indica* extract against hepatotoxicity induced by antitubercular drugs. Further investigations are required for the identification of active constituents responsible for the hepatoprotection.

5. Conclusion

The methanolic extract of *E. indica* has shown dose dependent activity against INH + RIF induced hepatic damage in experimental rats. The hepatoprotective effect of the *E. indica* was further corroborated by the histopathological examinations. Further investigation of these promising protective effects of *E. indica* against antitubercular drug induced hepatic injury may have a considerable impact on developing clinically feasible strategies to treat patients with hepatotoxicity.

Conflicts of interest

All authors have none to declare.

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