ORIGINAL ARTICLE

In Vitro Hepatoprotective Activity of *Erythrina stricta* Roxb. Against Lipopolysaccharide-Induced Cytotoxicity in Human Hepatocellular Carcinoma Cell Line

Josef Yakin, Faruk Alam

Department of Pharmacy, Faculty of Pharmaceutical Science, Assam down town University, Guwahati, Assam, India

Abstract

Introduction: The use of medicinal plants is deeply rooted in traditional medicine and has significantly shaped the evolution of modern pharmaceuticals. One such plant, *Erythrina stricta* Roxb. belonging to the Fabaceae family, has attracted interest due to its therapeutic benefits. This research explores the hepatoprotective effects of the plant's ethanolic and ethyl acetate fractions. **Materials and Methods:** This *in vitro* study assessed the hepatoprotective effects of *E. stricta* Roxb. ethanolic extract and its ethyl acetate fraction on lipopolysaccharide-induced hepatotoxicity in HepG2 cells. Cell survival was evaluated through the MTT assay. Antioxidant defense parameters, including superoxide dismutase, glutathione, and catalase, along with lipid peroxidation marker malondialdehyde (MDA), were measured. Hepatic enzyme biomarkers aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were analyzed. Phosphorylated protein kinase C (pPKC) levels were quantified by enzyme-linked immunosorbent assay. **Results:** Ethanolic and Ethyl acetate fractions of *E. stricta* Roxb. improved HepG2 cell viability and significantly restored antioxidant enzyme levels. Ethyl acetate fraction markedly decreased MDA levels and normalized liver enzyme activities. Notably, the Ethyl acetate fraction downregulated pPKC expression, indicating anti-inflammatory activity through protein kinase C signaling modulation. **Conclusion:** *E. stricta* Roxb. exhibits hepatoprotective, antioxidant, and anti-inflammatory properties *in vitro*. The Ethyl acetate fraction, rich in phytochemicals, may serve as a promising natural therapeutic agent for liver diseases involving oxidative stress and inflammation.

Key words: Antioxidant, enzyme-linked immunosorbent assay, *Erythrina stricta*, hepatoprotective, HepG2

INTRODUCTION

Hepatotoxicity, resulting from chemically induced liver damage, poses a significant global health challenge due to the liver's central role in metabolism and detoxification. Clinical manifestations range from elevated liver enzymes to fulminant hepatic failure, often stemming from prescription drugs, environmental toxins, alcohol, or bacterial components, such as lipopolysaccharide (LPS). LPS activates the Toll-like receptor 4/nuclear factor-κB pathway, triggering oxidative stress and pro-inflammatory cytokine release, which contribute to hepatocyte injury.[1,2]

Conventionally, synthetic drugs, such as N-acetylcysteine for acetaminophen overdose have limitations, including side effects and cost, spurring interest in plant-derived hepatoprotective agents. Phytochemicals – especially flavonoids, alkaloids, and phenolic acids have demonstrated efficacy in mitigating LPS-induced hepatotoxicity by scavenging free radicals and modulating inflammatory responses.[3,4]

Members of the *Erythrina* genus notably exhibit hepatoprotective and antioxidant properties. Extracts of *Erythrina variegata* and *Erythrina suberosa* have demonstrated hepatoprotective effects in an animal model by enhancing antioxidant activity and reducing markers of lipid peroxidation against chemical-induced liver damage.[5,6]

Address for correspondence:
Dr. Faruk Alam, Faculty of Pharmaceutical Science, Assam down town University, Panikhaiti, Guwahati-781026, Assam, India.
Phone: +91-8638458422.
E-mail: faruk.alam@adtu.in

Received: 26-07-2025
Revised: 16-09-2025
Accepted: 25-09-2025

Erythrina stricta Roxb. a medium-sized tree from the Fabaceae family native to Southeast Asia, is used in ethnomedicine for treating liver disorders, inflammation, and fever. While phytochemical screenings of its bark and leaves have identified alkaloids, flavonoids, and phenolic compounds known for antioxidant and anti-inflammatory activities, its hepatoprotective potential remains underexplored scientifically.[7-9]

Given that other *Erythrina* species demonstrate hepatoprotection through antioxidant and anti-inflammatory pathways, and acknowledging the traditional use of *E. stricta* for liver ailments, it is logical to investigate its effect in LPS-induced hepatotoxicity models. Evaluating its potential could validate traditional knowledge and pave the way for new therapeutic agents that are safer and more cost-effective than current pharmacotherapies.

The primary objective of this study is to investigate the *in vitro* hepatoprotective activity of *E. stricta* Roxb. using the HepG2 cell line. The study focused on evaluating the protective effects of the ethanol extract (Ethanolic extract of *Erythrina stricta* [EES]) and its ethyl acetate fraction (Ethyl acetate fraction of *Erythrina stricta* [EAES]) of *E. stricta* Roxb. against LPS-induced hepatotoxicity. Specific objectives include assessing cell viability using the MTT assay, analysing the activities of key antioxidant enzymes, including superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT) and along with measuring oxidative stress markers, such as malondialdehyde (MDA) levels. Evaluating the activityofhepatic enzyme biomarkers, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). In addition, enzyme-linked immunosorbent assay (ELISA) was performed to quantify phosphorylated protein kinase C (pPKC) levels, to explore potential involvement in intracellular signaling pathways. The ethanol extract provides a broad profile of phytochemicals, while the ethyl acetate fraction was expected to concentrate semi-polar constituents, such as flavonoids and alkaloids. It is hypothesized that the two extracts will demonstrate hepatoprotective effect by minimizing lipid peroxidation, strengthening antioxidant defense system, and influencing the expression of pPKC.

MATERIALS AND METHODS

Extraction and fractionation

Fresh leaves of *E. stricta* Roxb. were collected from Rangia, District- Kamrup, Assam, India. The plant specimen was authenticated at the Department of Botany, Gauhati University (Accession No. GUBH202379). The air-dried leaves were coarsely powdered and extracted with 95% ethanol using a Soxhlet apparatus, according to the established protocol outlined for phytochemical extraction and fractionation.[10] Subsequently, the ethanol extract in crude form was then subjected to liquid–liquid partitioning using petroleum ether followed by ethyl acetate in a separatory funnel to obtain respective fractions. All extracts and fractions were evaporated under reduced pressure at 40–50°C using a rotary evaporator and the resulting concentrates were kept in airtight containers at 4°C until further analysis.[11]

Cell viability assay (MTT assay)

HepG2 cells (procured from NCCS, Pune, India) were cultured in Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (100 µg/mL) under a humidified environment containing 5% CO_2 at 37°C. Cells were seeded in 96-well plates at a density of 4×10^5 cells/well and allowed to adhere overnight. The following day, cells were treated with various concentrations (10–400 µg/mL) of EES and EAES for 24 h. For the control condition, cells were treated with 0.1% DMSO. After the incubation period, 20 µL of MTT reagent (2.5 mg/mL in phosphate-buffered saline [PBS]) was introduced to each well and maintained at incubated condition for an additional 4 h at 37°C. The resulting formazan crystals were dissolved by adding 500 µL of DMSO to each well. Absorbance was recorded at 570 nm employing a microplate reader, and the percentage of viable cells was calculated relative to the non-treated control group.[12]

Measurement of SOD activity

The activity of SOD in HepG2 cells was determined according to a reported method.[13] Briefly, after treatment, the cells were collected and gently washed with PBS and lysed using a suitable lysis buffer. The cell lysates were centrifuged, and the supernatants were collected for enzyme assay. The assay relies on the inhibition of formation of NADH–phenazine methosulfate–nitroblue tetrazolium formazan. One unit of SOD activity was expressed as the quantity of enzyme required to cause 50% inhibition of chromogen formation under the assay conditions. The absorbance was read spectrophotometrically at 560 nm, and the values were shown as units per mg of protein.[13-15]

Estimation of GSH level

The level of GSH in HepG2 cells was quantified following a reported method,[16] which involves the formation of a yellow-colored complex between GSH and 5,5'-dithiobis-(2-nitrobenzoic acid). After treatment, the cells were disrupted and the resulting lysate was centrifuged, to obtain the clear supernatant for further assessment. The absorbance of the resulting solution was recorded at 412 nm using a spectrophotometer. GSH concentration was calculated in terms of ng of GSH per mg of protein. Total protein content in the cell lysates was determined in accordance with the

reported procedure, using bovine serum albumin as the standard.[14]

Estimation of CAT activity

CAT activity in HepG2 cells was determined as per the reported method.[17] Briefly, cell lysates were incubated with hydrogen peroxide (H_2O_2), and the rate of H_2O_2 decomposition was measured spectrophotometrically at 240 nm. The decrease in absorbance corresponds to the catalytic breakdown of H_2O_2. CAT activity was expressed as kilounits per minute per milligram of protein (kU/min/mg protein).[17]

Measurement of MDA level

The level of lipid peroxidation in HepG2 cells was evaluated by measuring thiobarbituric acid (TBA) reactive substances, based on a previously established protocol.[18] Following treatment, the cells were lysed, and the resulting lysate were reacted with TBA reagent. The mixture was then heated to develop a pink colored complex, which was quantified spectrophotometrically at 532 nm. MDA concentrations were determined using a standard curve and expressed as micromoles of MDA per mg of protein (µmol/mg protein).[18,19]

AST, ALT, and ALP activity

The activities of AST, ALT, and ALP were determined to evaluate hepatocellular damage. HepG2 cells were seeded into 96-well plates at a concentration of 2×10^6 cells/well and incubated at 37°C in a humidified 5% CO_2 atmosphere until confluent. Cells were incubated in DMEM containing with 2% FBS for a duration of 24 h. Treatments included LPS (25 µg/mL), Silymarin (positive control), and varying concentrations (0–100 µg/mL) of EES and EAES. After 24 h of treatment, AST, ALT, and ALP activity levels were determined using commercial colorimetric assay kits (Elabscience: AST E-BC-K236-M, ALT E-BC-K235-M, ALP E-BC-K091-M) as per the guidelines outlined in the product manual. Enzyme activities were expressed in international units per liter (IU/L).[20,21]

LPS-induced phospho-PKC activity in HepG2 cells

The EAES fraction, demonstrating superior antioxidant and hepatoprotective activity, was selected to evaluate LPS-induced modulation of pPKC. HepG2 cells (NCCS, Pune) were cultured in DMEM medium with an addition of 10% FBS and maintained at 37°C in 5% CO_2. Cells (>95% viability) were inoculated into a 96-well plates and treated with LPS (25 µg/mL), with or without the addition of EAES fraction (0–100 µg/mL) for 24 h. Levels of Phospho-PKC were determined employing a commercially sourced Sandwich ELISA kit (GENLISA, KBH15203) following the supplier's protocol. Results were expressed in ng/ml.[22]

Statistical analysis

All data are presented as the mean ± standard error of the mean based on a minimum of three independent experiments. Statistical analyses were conducted using one-way analysis of variance, followed by Tukey's multiple comparison test with the aid of GraphPad Prism software (version 8.0.2, 2019). Differences were considered statistically significant at $p<0.05$.

RESULTS

Cell viability assay (MTT assay)

The percentage cell viability with respect to the normal control cell lines (HepG2) at different concentrations of EES and EAES were determined [Figure 1]. The normal control cells showed 100% cell viability in HepG2 cells. The EES at concentration 10 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL and 400 µg/mL showed 95.41 ± 3.16%, 91.37 ± 2.38%, 89.77 ± 3.01%, 85.05 ± 4.02%, 80.01 ± 4.91% and 74.14 ± 4.21% cell viability, respectively. The EAES at concentration 10 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL and 400 µg/mL showed 95.50 ± 2.76%, 92.54 ± 2.36%, 89.27 ± 2.80%, 85.59 ± 2.77%, 81.12 ± 4.18% and 77.65 ± 2.79% cell viability, respectively.

Measurement of SOD activity

Figure 2a and b represent the status of SOD activities in HepG2 cell lines with and without treatment of 25 µg/mL LPS and activities were 66.98 ± 1.77 mU/min/mg protein and 125.78 ± 1.16 mU/min/mg protein, respectively. SOD activity was significantly increased in HepG2 cell line when different concentration of Silymarin, EES and EAES (10, 25, 50, 75 and 100 µg/mL) were introduced in the cultures, respectively.

Estimation of GSH level

Figure 3a and b represent the GSH content in HepG2 cell lines with and without treatment of 25 µg/mL LPS and levels

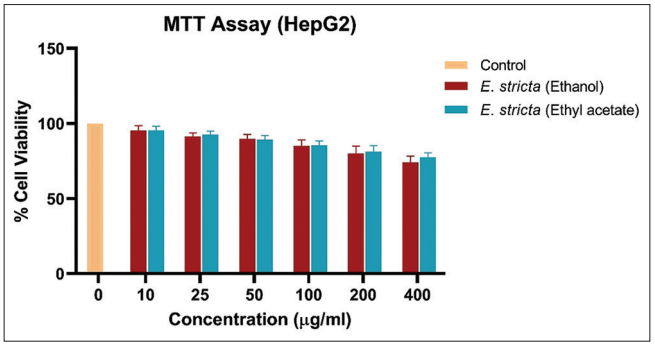

Figure 1: Effect of *Erythrina stricta* fractions (EES, EAES) on cell viability. Data presented as mean ± SEM of three variables (n = 3). EES: Ethanolic *Erythrina stricta*, EAES: Ethyl acetate *Erythrina stricta*, SEM: Standard error of mean

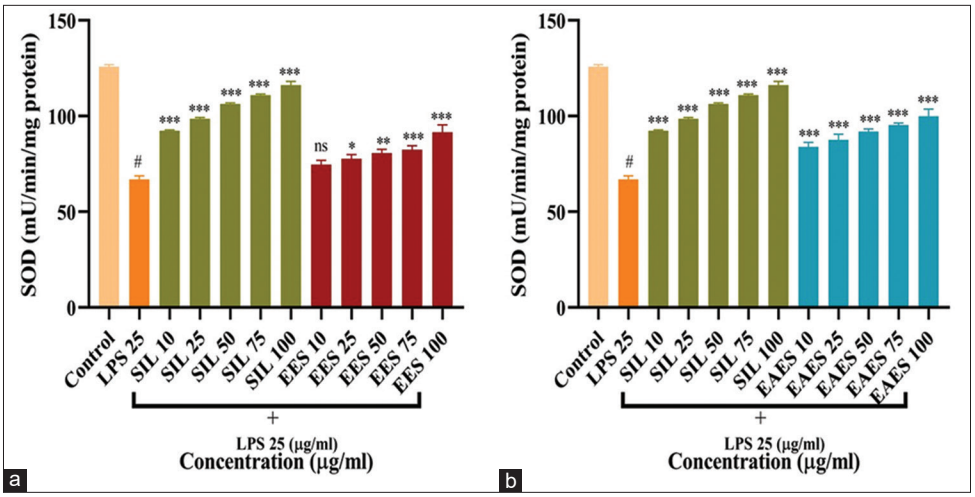

Figure 2: Effect of *Erythrina stricta* extracts on SOD activity in LPS-induced HepG2 cells *in vitro*: (a) EES; (b) EAES. The data are expressed as the mean ± SEM ($n = 3$). One-way analysis of variance analysis was utilized. $^{\#}P < 0.05$ compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and ns - non-significant compared with LPS group. EES: Ethanolic extract of *Erythrina stricta*, EAES: Ethyl acetate fraction of *Erythrina stricta*, LPS: Lipopolysaccharide, SIL: Silymarin, SOD: Superoxide dismutase, SEM: Standard error of mean

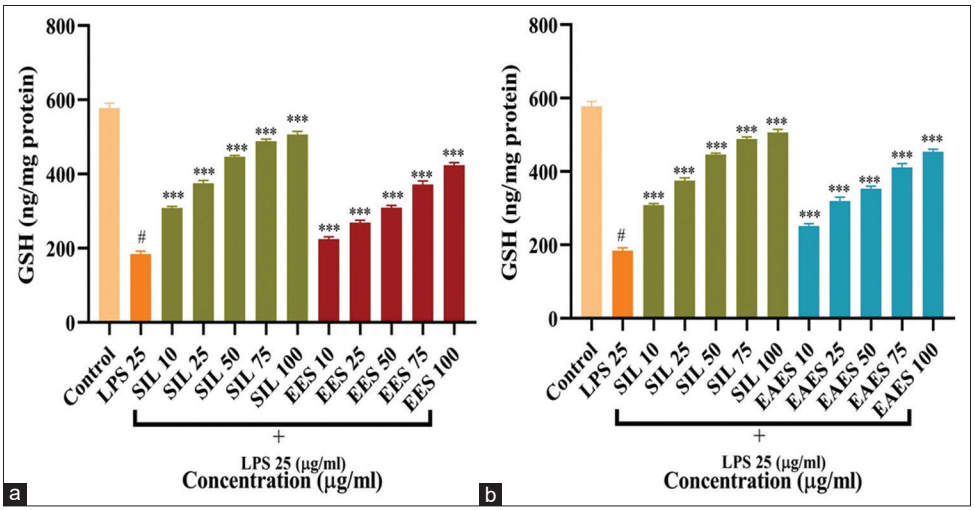

Figure 3: Effect of *Erythrina stricta* extracts on GSH activity in LPS-induced HepG2 cells *in vitro:* (a) EES, (b) EAES. The data are expressed as the mean ± SEM ($n = 3$). One-way analysis of variance analysis was utilized. $^{\#}P < 0.05$ compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and ns - non-significant compared with LPS group. EES: Ethanolic extract of *Erythrina stricta*, EAES: Ethyl acetate fraction of *Erythrina stricta*, LPS: Lipopolysaccharide, SIL: Silymarin, GSH: Reduced glutathione, SEM: Standard error of mean

were 184.01 ± 8.04 ng/mg protein and 577.94 ± 13.04 ng/mg protein, respectively. GSH levels were significantly increased in HepG2 cell lines when different concentration of Silymarin, EES and EAES (10, 25, 50, 75 and 100 µg/mL) were introduced in the cultures, respectively.

Estimation of CAT activity

Figure 4a and b represent the status of CAT activities in HepG2 cell lines with and without treatment of 25 µg/mL LPS and levels were 0.33 ± 0.01 kU/min/mg protein and 1.79 ± 0.03 kU/min/mg protein, respectively. CAT activities were significantly increased in HepG2 cell lines when different concentration of Silymarin, EES and EAES (10, 25, 50, 75 and 100 µg/mL) were introduced in the cultures, respectively.

MDA measurement

Figure 5a and b represent the status of MDA in HepG2 cell lines with and without treatment of 25 µg/mL LPS and levels were 23.87 ± 0.19 uM/mg and 4.89 ± 0.17 uM/mg, respectively. MDA levels were significantly reduced in HepG2 cell lines when different concentration of Silymarin,

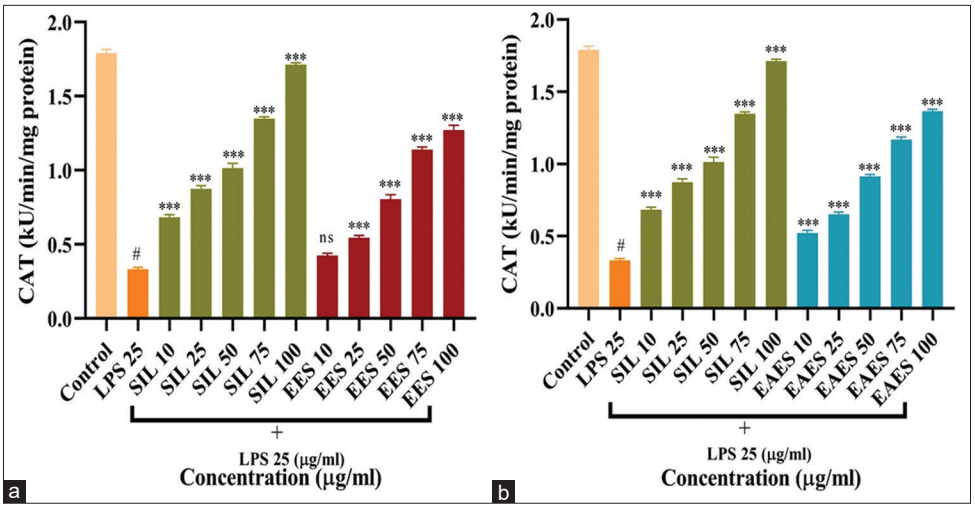

Figure 4: Effect of *Erythrina stricta* extracts on CAT activity in LPS-induced HepG2 cells *in vitro*: (a) EES, (b) EAES. The data are expressed as the mean ± SEM ($n = 3$). One-way analysis of variance analysis was utilized. $^{\#}P < 0.05$ compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and ns - non-significant compared with LPS group. EES: Ethanolic extract of *Erythrina stricta*, EAES: Ethyl acetate fraction of *Erythrina stricta*, LPS: Lipopolysaccharide, SIL: Silymarin, CAT: Catalase, SEM: Standard error of mean

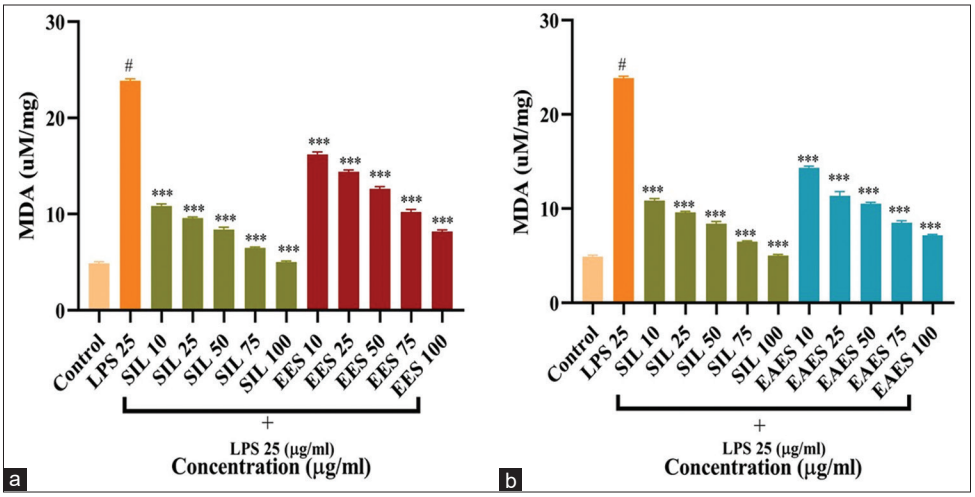

Figure 5: Effect of *Erythrina stricta* extracts on MDA activity in LPS-induced HepG2 cells *in vitro*: (a) EES, (b) EAES. The data are expressed as the mean ± SEM ($n = 3$). One-way analysis of variance analysis was utilized. $^{\#}P < 0.05$ compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and ns - non-significant compared with LPS group. EES: Ethanolic extract of *Erythrina stricta*, EAES: Ethyl acetate fraction of *Erythrina stricta*, LPS: Lipopolysaccharide, SIL: Silymarin, MDA: Malondialdehyde, SEM: Standard error of mean

EES and EAES (10, 25, 50, 75 and 100 µg/mL) were introduced in the cultures, respectively.

AST, ALT, and ALP activity

Figures 6a and b, 7a and b, 8a and b represents the level of AST, ALT and ALP, respectively. A significant increase in the levels of AST, ALT and ALP as compared to control was observed in 25 µg/mL LPS exposed HepG2 cells. These cells, when treated with different concentrations of Silymarin, EES and EAES (10, 25, 50 and 100 µg/mL) showed a significant restoration in altered biochemical parameters toward the normal and were dose dependent manner.

LPS-induced phospho-PKC activity in HepG2 cells

When the pPKC of EAES was estimated by Sandwich ELISA, the fraction exhibited a significant and profound outcome by the inhibition of phosphorylation of PKC. Figure 9 represents the status of pPKC level in HepG2 cell with or without treatment of 25 µg/mL LPS and levels were 32.90 ± 6.98 ng/mL and 14.13 ± 4.94 ng/mL, respectively.

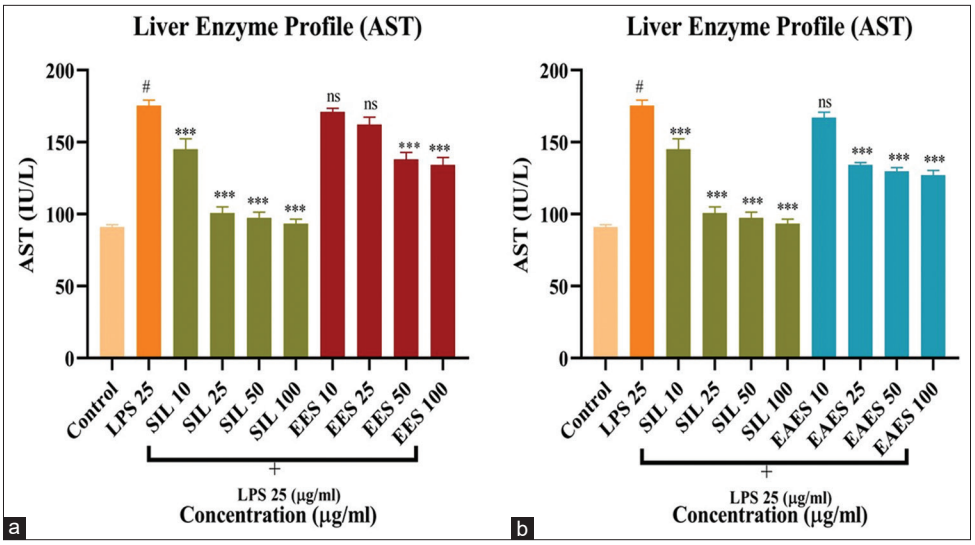

Figure 6: Effect of *Erythrina stricta* extracts on AST activity in LPS-induced HepG2 cells *in vitro*: (a) EES, (b) EAES. The data are expressed as the mean ± SEM ($n = 3$). One-way analysis of variance analysis was utilized. $^{\#}P < 0.05$ compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and ns - non-significant compared with LPS group. EES: Ethanolic extract of *Erythrina stricta*, EAES: Ethyl acetate fraction of *Erythrina stricta*, LPS: Lipopolysaccharide, SIL: Silymarin, AST: Aspartate aminotransferase, SEM: Standard error of mean

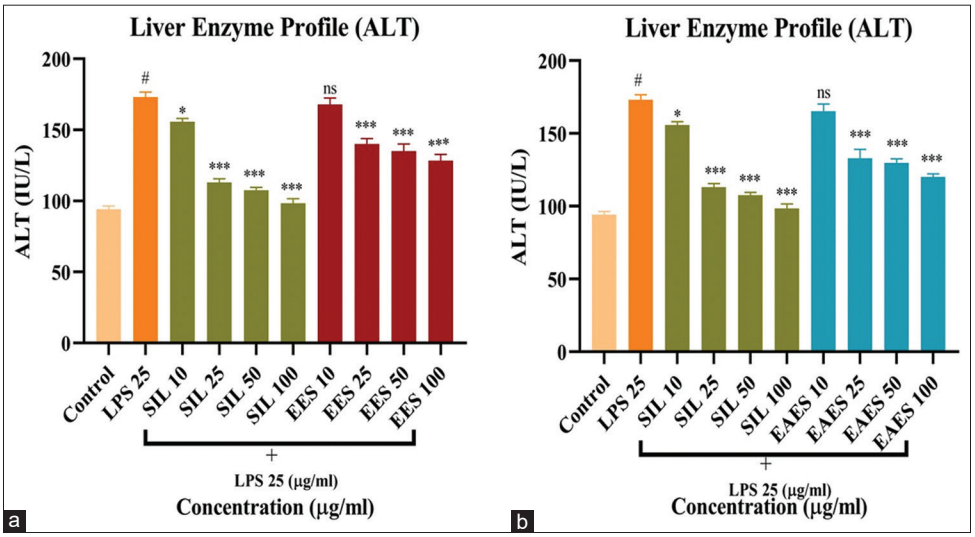

Figure 7: Effect of *Erythrina stricta* extracts on ALT activity in LPS-induced HepG2 cells *in vitro*: (a) EES; (b) EAES. The data are expressed as the mean ± SEM ($n = 3$). One-way analysis of variance analysis was utilized. $^{\#}P < 0.05$ compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and ns - non-significant compared with LPS group. EES: Ethanolic extract of *Erythrina stricta*, EAES: Ethyl acetate fraction of *Erythrina stricta*, LPS: Lipopolysaccharide, SIL: Silymarin, ALT: Alanine aminotransferase, SEM: Standard error of mean

DISCUSSION

The present study highlights the hepatoprotective effects of *E. stricta* Roxb. extracts, specifically the ethanolic extract (EES) and ethyl acetate fraction (EAES), against LPS-induced toxicity in HepG2 cells.

The results of the MTT assay showed that both EES and EAES supported high cell viability, indicating their biocompatibility and potential protective effects. At lower concentrations (10–50 µg/mL), both the fractions maintained cell viability above 89%, suggesting minimal cytotoxicity. Even at moderate concentrations (100–200 µg/mL), cell survival remained high, reinforcing the protective potential of the extracts. However, at higher concentrations (400 µg/mL), there was a minor decline in cell viability, particularly in the EES extract as compared to the EAES fraction, indicating a dose-dependent effect. The EAES fraction exhibited a more stable viability profile, indicating that it might be appropriate for uses that requiring minimal cytotoxic effects.

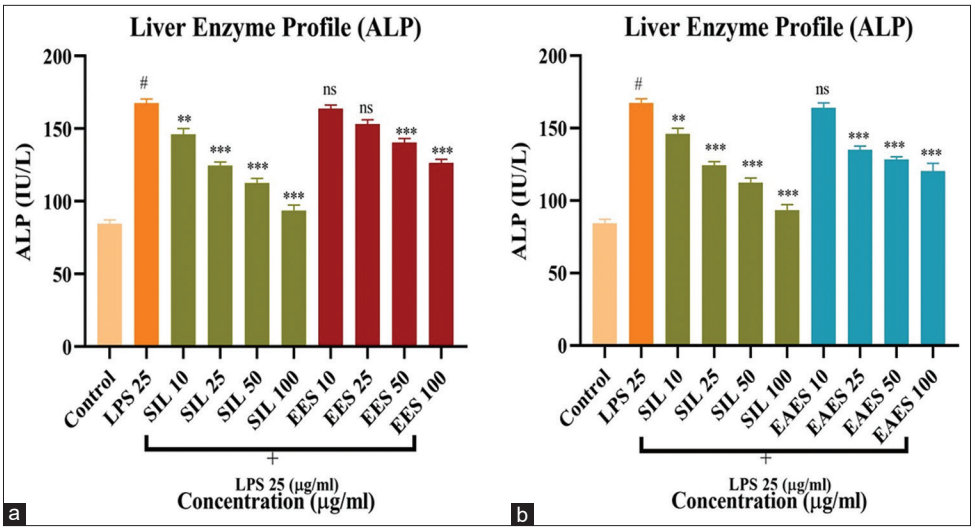

Figure 8: Effect of *Erythrina stricta* extracts on ALP activity in LPS-induced HepG2 cells *in vitro:* (a) EES; (b) EAES. The data are expressed as the mean ± SEM ($n = 3$). One-way analysis of variance analysis was utilized. $^{\#}P < 0.05$ compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and ns - non-significant compared with LPS group. EES: Ethanolic extract of *Erythrina stricta*, EAES: Ethyl acetate fraction of *Erythrina stricta*, LPS: Lipopolysaccharide, SIL: Silymarin, ALP: Alkaline phosphatase, SEM: Standard error of mean

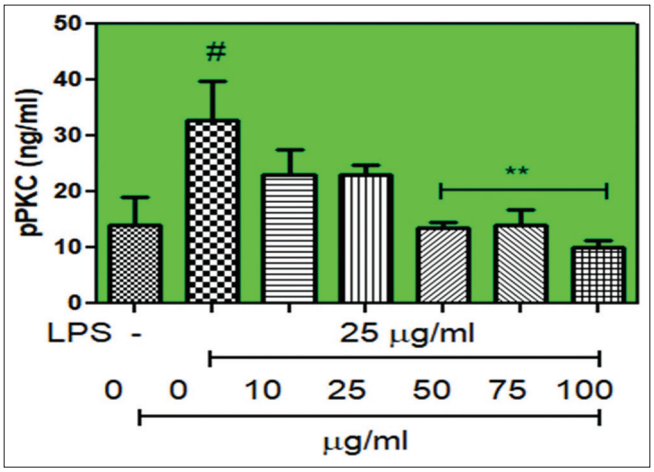

Figure 9: Effect of *Erythrina stricta* (EAES) on pPKC expression in LPS-induced HepG2 cells by ELISA. Data are mean ± SEM ($n = 4$). One-way analysis of variance analysis was utilized. $^{\#}P < 0.05$ compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and ns - non-significant compared with LPS group. EAES: Ethyl acetate fraction of *Erythrina stricta*, LPS: Lipopolysaccharide, pPKC: Phosphorylated protein kinase C, ELISA: Enzyme-linked immunosorbent assay, SEM: Standard error of mean

Oxidative stress plays a major role in hepatic injury. To assess *E. stricta* Roxb. extracts on oxidative stress markers, such as SOD, GSH, CAT and MDA. Following LPS exposure, SOD and GSH levels were markedly restored, identifying oxidative stress-induced cellular damage. Controlled treatment with EES and EAES did indeed significantly recover those levels especially at 75 and 100 µg/mL suggested that they induced a potent antioxidant response. The antioxidant capability of the EAES fraction was marginally superior to that of the EES extract, indicating the existence of strong free radical-scavenging substances.

Reduced CAT activity following LPS exposure also indicated compromised antioxidant defense mechanisms. However, treatment of EES and EAES restored CAT activity, particularly at concentrations of 50 and 75 µg/mL, highlighting their role in enhancing cellular antioxidant protection and protecting hepatocytes from oxidative damage. Lipid peroxidation evaluated through MDA was notably increased in cells treated with LPS, indicating cellular stress and damage to membranes. Both EES and EAES significantly lowered MDA levels, with the EAES fraction showing a stronger effect, suggesting its involvement in mitigating lipid peroxidation and oxidative damage.

Liver function enzyme analysis indicated that LPS exposure significantly elevated AST, ALT, and ALP levels, marking hepatic stress and injury. However, co-treatment with *E. stricta* Roxb. (EES and EAES) extracts effectively reduced these enzyme levels, suggesting notable hepatoprotective activity. The most effective concentrations (50 and 100 µg/mL) exhibited liver-protective properties comparable to the standard drug, Silymarin. These findings suggest that *E. stricta* Roxb. extracts may contribute to restoring liver function and preserving hepatocyte integrity.

The EES and EAES fractions showed considerable hepatoprotective and antioxidant properties, as indicated by a comparative assessment of the extracts; nonetheless, the EAES fraction was more successful in restoring oxidative stress markers, such as SOD, GSH, CAT, and MDA, while also preserving cell viability. Targeted use of specific extract concentrations may enhance efficacy and

reduce possible risks, as observed by the observed dose-dependent effects.

Moreover, the phosphorylation status of Protein Kinase C (pPKC) was assessed through a Sandwich ELISA to investigate how *E. stricta* Roxb. fraction influence PKC signaling in HepG2 cells. The findings indicated a significant impact of the EAES fraction on inhibiting PKC phosphorylation. In the control group, the baseline pPKC level was recorded at 14.13 ± 4.94 ng/mL, whereas LPS treatment significantly increased pPKC levels to 32.90 ± 6.98 ng/mL. This increase aligns with the established role of LPS in activating PKC signaling pathways, essential for inflammatory reactions and cellular stress mechanisms. Nonetheless, treatment with the EAES fraction significantly decreased pPKC levels, indicating its anti-inflammatory and cytoprotective effects. The observed decrease in pPKC following treatment indicates that the EAES fraction effectively hinders PKC phosphorylation, potentially disrupting inflammatory pathways triggered by LPS.

These results are consistent with earlier studies highlighting the important function of PKC in inflammation, as inhibiting its phosphorylation has been shown to decrease inflammation. These findings indicate that the EAES fraction of *E. stricta* Roxb. may serve as a probable treatment for conditions linked to increased PKC activation and inflammation. Further extensive studies, including mechanistic evaluations and *in vivo* validations, will be essential to completely assess its therapeutic potential.

CONCLUSION

Our findings confirm the hepatoprotective and antioxidant effects of *E. stricta* Roxb. extracts, especially of the EAES, against LPS-induced cytotoxicity in HepG2 cells. The extracted fractions revealed high viability on cells at moderate concentration values, which shows their cytoprotective potential. The extracts also showed restoration of oxidative stress markers, such as SOD, GSH, MDA and CAT which fortifies the cellular defense against oxidative damage. Striking decline of elevated liver enzymes AST, ALT and ALP indicate their role in alleviating the hepatic stress and protecting liver.

The EAES fraction of *E. stricta* Roxb. remarkably decreased PKC phosphorylation, indicating its involvement in regulating inflammatory signaling pathways. The reduction in pPKC levels highlights its anti-inflammatory function, which can be crucial in liver damage caused by inflammation. The dose-dependent effects highlighted the need to optimize extract concentrations for therapeutic uses.

In conclusion, *E. stricta* Roxb. extract, especially EAES fraction, is a strong candidate for hepatoprotection and antioxidant activity. These findings may support their application as a natural treatment for liver conditions associated with inflammation and oxidative stress. Future research should focus on *in vivo* validation of these effects and isolation and characterization of bioactive compounds responsible for the observed activity.

ACKNOWLEDGMENT

The authors are thankful to Assam down town University, for providing laboratory and the basic facilities for carrying out experimental work.

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Source of Support: Nil. **Conflicts of Interest:** None declared.