



HEPATOPROTECTIVE EFFECTS OF DIFFERENT FRACTIONS OF ERYTHRINA INDICA IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The study was undertaken to investigate the hepatoprotective effects of the different fractions (Petroleum ether, ethyl acetate and chloroform) of ethanolic extract of *Erythrina Indica*. The different fractions of extract were administered intraperitoneally as a single dose of 150 mg/kg body weight to alloxan induced diabetic rats exhibited correction of altered biochemical parameters viz., SGOT and SGPT levels in diabetic rats. The effect of plant fractions were compared with standard drug Metformin. Thus, this study indicates that various fractions (Petroleum ether, ethyl acetate and chloroform) of the ethanolic extract of *Erythrina Indica* have favorable effect in hepatoprotective activities.

Keywords: *Erythrina Indica*, Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Alloxan.

INTRODUCTION

Over the last few decades the reputation of herbal remedies has increased globally due to its therapeutic efficacy and safety. A scientific investigation of traditional herbal remedies for diabetes may provide valuable lead for the development of alternative drug and therapeutic strategies. In recent years, numerous traditional medicinal plants were tested for their antidiabetic, hypolipidemic and hepatoprotective potential in the experimental animals¹.

The study of the effect of *Erythrina Indica* on lipid profile in hyperlipidemic individuals showed that it significantly reduces the lipid level (cholesterol and triglyceride)². There was a significant rise in SGOT and SGPT levels in diabetic rats, which could relate to excessive accumulation of amino acids (glutamate and alanine) in the serum of diabetic animals as a result of amino acids mobilization from protein stores³ medicinal plants and herbs are of great importance to the health of the individuals and communities. The presence of active constituents viz. Alkaloids, glycosides, phenyl coumarin, saponins, tannins, triterpenes, alkaloids, flavonoids and potential therapeutic activities like antihelminthiasis, sedative, anti-inflammatory, nematocidal, worm infection and hyperlipidemic⁷⁻⁹ have been all ready reported in leaf extract of *Erythrina Indica*⁴⁻⁶. Bioactive richness of the active constituents of leaves of *Erythrina Indica* has promoted us to undertake the present study.

MATERIALS AND METHODS

The fresh leaves of *Erythrina Indica* were collected from in and around Chennai and Identified by Dr. Sasikala, Department of Pharmacognosy, captain Srinivasamoorthy drug research institute of ayurveda, Chennai.

Preparation and fractionation of crude extracts: The crude extract was obtained through cold extraction process. The coarse powder was submerged in ethanol and allowed to stand for 10 days with occasional shaking and stirring. When the solvent became concentrated the alcohol content was filtered through cotton and then through filter paper (What man filter paper no. 1). Then the solvent was allowed to evaporate using rotary evaporator at temperature 40-45°C. Thus, the highly concentrated crude extract was obtained. That was then fractionated using petroleum ether, ethyl acetate and CHCl₃. The solvents of these fractions were evaporated by rotary evaporator and then dried under mild sun. The dried fractions of extract were then preserved in the freeze for the experimental uses¹⁰.

Drugs and chemicals used: The standard drug Metformin was the generous gift samples from Dr. Reddy's laboratories Ltd. Hyderabad, India. Alloxan was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. SGPT and SGOT wet reagent diagnostic kits were purchased from AMP Medizintechnik GmbH; Austria. Dimethylsulfoxide (DMSO) was purchased from Loba Chemie, Bombay, India and used to dissolve Metformin and the different fractions of extract of *Erythrina Indica*

Preparation of dosage of active drug and plant extract: Metformin: Metformin was in microcrystalline form and freely soluble in water. The dosage was prepared in solution form using sterilized water in such a concentration that, each 0.1 ml of solution contained Metformin according to the dose of 150 mg/kg body weight since Metformin is effective in such dose.

Erythrina indica: The fractionated extracts of *Erythrina Indica* were dissolved in 99% DMSO to prepare the solution where each 0.1 ml contained *Erythrina Indica* according to the dose of 150 mg/kg body weight¹¹ 0.1 ml



of each solution was administered intraperitoneally to every 100 gm body weight of the rats during treatment to achieve required dose of fractions of plant extract.

Selection of animals: A total number of 30 long-Evans male rats weighing about 150-180 gm, age 2 months were purchased from animal house of Saastra college of pharmaceutical education and research, Nellore Prior to commencement of the experiment all the rats were acclimatized to the new environmental condition for a period of one week. During the experimental period the rats were kept in a well ventilated animal house at room temperature of 25°C and were supplied with standard pellets and fresh drinking water. All the rats were kept in cages with wide square mesh at bottom to avoid coprophagy and maintained with natural 12 hour light and dark cycle¹².

Grouping of experimental rats: 30 long- Evans male rats were randomly assigned into 6 groups, 5 rats in each group.

Group 1	Normal Control
Group 2	Diabetic Control
Group 3	Diabetic + Metformin (150mg/kg body wt.)
Group 4	Diabetic + Petroleum ether fraction (150mg/kg body wt.)
Group 5	Diabetic + Ethyl acetate fraction (150mg/kg body wt.)
Group 6	Diabetic + Chloroform fraction (150mg/kg body wt.)

Experimental induction of diabetes and collection of serum: Group 1 animals were used for normal control receives only vehicle (DMSO). Groups 2-6 animals were allowed to fast for 12 hrs and were induced diabetic by injection intraperitoneally a freshly prepared solution of alloxan (110 mg/kg body wt.) in normal saline after base line glucose estimation was done. The alloxan treated animals were allowed to feed over night to overcome drug induced hypoglycemia. After 48 hours blood glucose content was measured by using Bioland G- 423 test meter using blood sample from the tail vein of the rats.

When the condition of diabetes was established (blood glucose levels above 11.1 m mol/L) the drug (Metformin) and fractions of plant extract were administered to the respective groups of animals selected for the study. After completing the 24 hours blood glucose level testing the rats were sacrificed and 7 ml of blood was collected directly from heart of each rat by syringes, centrifuged at 4000 rpm for 10 minutes and the serum was obtained. The serum was then used for the experimental analysis¹³

Hepatoprotective activity test (SGOT and SGPT test): In this case, the blood serum was used for testing of the SGOT (Serum Glutamic Oxaloacetic Transaminase) and SGPT (Serum Glutamic Pyruvic Transaminase) levels. The concentrations were analyzed by taking absorbance by UV spectrophotometer (Shimidzu UV- 1200, Tokyo, Japan) using commercial wet reagent diagnostic kits (AMP Medizintechnik GmbH; Austria) according to manufacturer's protocol.¹⁴⁻¹⁵

Statistical analysis: The results were expressed as mean±SEM using Graph Pad Prism (version 4.0) computer

program (Graph pad Software San Diego, CA, USA). We used a one-way analysis of variance (ANOVA), followed by Scheffe's post-hoc test or students paired or unpaired t-test where appropriate. The statistical method applied in each analysis was described in each figure. Results were considered to be significant when p values were less than 0.05 (p<0.05).

RESULTS

The effect of the different fractions of ethanolic extract of *Erythrina Indica* on the SGOT and SGPT levels were investigated in the alloxan-induced diabetic rats using Metformin as a standard agent.

Effect of different fractions *Erythrina Indica* on enzymes (SGOT, SGPT) in diabetic rats: In case of the effect of different fractions of *Erythrina Indica* it was observed that the petroleum ether, ethyl acetate and CHCl₃ fractions reduced SGOT level to 32%, 37% and 24% respectively, than the diabetic control group. On the other hand, it was observed that the petroleum ether, ethyl acetate and CHCl₃ fractions of *Erythrina Indica* reduced SGPT level to 57%, 73% and 74% respectively, than the diabetic control group (Table 1).

Table 1: Effect of different fractions of erythrina indica ethanolic extract on SGPT and SGOT level

Group	SGPT (Unit/ml)	SGOT (Unit/ml)
Normal Control	20.3	18.2
Diabetic Control	24.6	42.8*
D + Metformin	14.9	29.2
D + Ethyl acetate	18.2	16.0
D + Chloroform	18.1	10.3 ψ
D + Pet-ether	14.2*	14.1

* Indicates significant difference (p<0.05) from normal control group.

ψ indicates significant difference (p<0.05) from the diabetic control. Data are expressed as means ± sem.

DISCUSSION

Flavonoids are known for their diverse biological activities including hypolipidemic activity resulting from their antioxidant activity¹⁶. The increase in the activities of SGOT and SGPT is found in diabetic rats. The higher levels of SGOT and SGPT may give rise to a high concentration of glucose. In other words, the gluconeogenic action of SGOT and SGPT plays the role of providing new supplies of glucose from other sources such as amino acids. Following intraperitoneal administration of different plant fractions significantly reduced the SGOT and SGPT levels (Table 1). The obtained results suggest that *Erythrina Indica* possesses hepatoprotective capacity due to flavonoids and sulfurated compounds and other components such as saponins, tannins, triterpenes and alkaloids⁷⁻⁹.



CONCLUSION

In conclusion, the administration of plant fractions produced significant reduction of SGOT and SGPT level. It causes rapid induction of hepatoprotective effect in diabetic rats. Thus, in the light of our pharmacological studies the study of various fractions of *Erythrina Indica* extract might offer a natural key in hepatoprotective activity. Further chemical and pharmacological investigations are in progress to elucidate in detail the active principles and the exact mechanism of actions.

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