Biomedical & Pharmacology Journal Vol. **1**(2), 383-386 (2008)

Hepatoprotective effect of leaves of *Erythrina indica* Lam.

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(Received: July 25, 2008; Accepted: September 05, 2008)

ABSTRACT

To examine liver protective effect of leaves of *Erythrina indica* (*EI*) against CCl_4 induced liver toxicity in rats.

Ethanol, chloroform and Ethyl acetate extracts of leaves of *EI* (250 mg/kg, p.o.) were administered to the male Wister rats for 10 days. Hepatotoxicity was induced by CCl_4 (0.5 ml/ kg, on 10th day). The activity was assessed by studying bio chemical parameters (SGOT, SGPT, ASAT, ALAT, ALP, albumin, Bilirubin, TGL, Total protein and albumin) and histopathological studies of the liver.

Extracts together with CCl_4 treated rats showed significant restoration of liver function biochemical parameters. Further the activity was evidenced by the histopathological observation indicating that absence of necrosis and fatty infiltration as shown in the rats treated with CCl_4 alone.

Ethanol, Chloroform and Ethyl acetate extracts of leaves of *Erythrina indica* possess significant hepatoprotective activity.

Key words: *Erythrina indica*, carbon tetra chloride, Hepatoprotective, Rats.

INTRODUCTION

The liver is the largest internal organ in the human body. It plays a major role in metabolism and detoxification. It also performs and regulates a wide variety of high-volume biochemical reactions requiring very specialized tissues[1]. Liver disease is a serious health problem. In the traditional system of medicine liver diseases had been successfully treated by using medicinal plants and their formulations. However, there is no satisfactory therapy for serious liver disease; mostly the herbal drugs increase the rate of natural healing process of liver. Hence the search for effective liver protective drug persist.

Erythrina indica Lam (Papilionaceae) is a middle sized tree, widely distributed in India and is used in traditional medicine on account of its Diuretic, anticonvulsant, anti inflammatory, hepatoprotective, anthelmintic and laxative effects[2,3]. Though, no scientific study has been reported on hepatoprotective activity of leaves of *Erythrina indica*, we prompted to study hepatoprotective activity of leaves of *Erythrina indica* against CCl_4 induced liver toxicity in rats. In the present study, the hepatoprotective activity was assessed by some Bio chemical parameters (SGOT (Serum glutamic oxalo acetic transaminase), SGPT (Serum glutamic pyruvic transaminase), ASAT (Aspartate amino transaminase), ALAT (Alanine amino transaminase), TGL (Triglyceride), ALP (Alkaline Phosphatase), Serum albumin, Bilirubin and total protein) and histopathological surveillance of liver.

MATERIAL AND METHODS

Plant collection and authentication

The plant material was collected in the Madurai district, Tamilnadu, India during the month of march 2005. It was authenticated by Dr. Stephen, Department of Botany, The American College, Madurai. A voucher specimen has been kept in our laboratory (EI1) for future reference.

Preparation of extract

The dried, coarsely powdered leaves were subjected to single extraction in a soxhlet extractor [4] using ethanol (90%), chloroform and ethyl acetate for 18-20h. The extracts were then concentrated to dryness under reduced pressure and controlled temperature to yield a semi solid

mass, which was preserved in a refrigerated conditions. Preliminary phytochemical analysis [4,5] were carried out to find out the phytoconstituents present in the crude extracts.

Animals

Male Wister Albino rats (100-150gm) were collected from the animal house of our institute and housed in standard metallic cages under room temperature (20±1^{0}C) and relative humidity 55±1^{0}Cwith 12 h light / dark cycle. The animals were provided with standard pellet diet (M/s Hindustan Lever Ltd, Mumbai, India.) with free access to water *adlibidum.* The present study was approved by institutional animal ethics committee (Approval no. 509/02/C/CPCSEA).

Chemicals

Silymarin was obtained from Sisco Laboratories, Mumbai, India. Thio barbituric acid was obtained from Sigma chemical Co (St. Louis, MO, USA). Bio chemical estimations were done by span diagnostic kits. All the chemicals used in the study were of analytical grade.

hepatoprotective activity

The method described by De et al [6] was employed for evaluating hepatoprotective activity. The animals were divided into six groups each group consist of six animals. The group I animals received 10% aqueous tween 80 (per oral (p.o.)), Group II animals received 10% aqueous tween 80 (p.o.), Group III animals received Silymarin (200 mg / kg), group IV animals received ethanol extract of *EI* (250 mg/kg p.o.), group V animals received ethyl acetate extract of *EI* (250 mg/kg, p.o.), group VI animals received chloroform extract of *EI* (250 mg/kg, p.o.). The treatment was continued for 10 days. On 10th day CCl_4 (0.5 ml / kg, i.p.) was given to groups II, III, IV, V and VI. 24 hrs after CCl_4 administration, blood was withdrawn under light anesthesia. The blood was centrifuged at 3000 rpm and 4°C to obtain sera. The serum was used for the estimation of marker enzymes of liver.

Bio chemical analysis

Total protein was estimated by Biuret method[7], Albumin (ALB) was estimated by BCG method[8], SGOT, SGPT were measured as kinetic reaction using IFCC method, the absorbance of reaction was determined at 340 nm by spectrophotometer[9]. ASAT, ALAT and ALP were estimated by the method of Bergmeyer[10], Serum level of Total bilirubin was estimated by the method of Waters *et al,* 1970[11]. Lipid peroxide level was estimated by the method of Ohkawa *et al.,* [12].

Histopathalogical studies

The animals were sacrificed by cervical dislocation, fresh liver tissues were trimmed approximately to 2 µm thickness, fixed in 10 % buffered formalin, embedded in paraffin then stained with hematoxylin and eosin and observed under original magnification 100x.

Statistical analysis

The statistical analysis were carried out by One Way Analysis of Variance (ANOVA) followed by student 't' test, P> 0.05 was considered significant. All the values are reported as Mean ± SEM.

RESULTS

Phytochemical results

Phytochemical analysis showed the presence of alkaloids, flavonoids, phytosterols, tannins, saponins and glycosides in all the three extracts (ethanol, ethyl acetate and chloroform).

Biochemical results

Administration of Ethanol, Ethyl acetate and Chloroform extract of leaves of *Erythrina indica* (250 mg/kg) significantly (P<0.05) restored CCl_4 induced increase in serum GOT, GPT, ASAT, ALAT, ALP and Bilirubin (table1) and CCl_4 induced decrease in serum TGL, Total protein and Albumin (table 2). It was also observed that increased lipid peroxide level and weight gain in the liver treated with CCl_4 alone and significant recovery in drug treated animals (Table.3). All the parameters observed in *EI* treated animals were comparable to those observed in the animals treated with known hepatoprotective agent Silymarin (200 mg/kg).

Histopathological results

Liver of normal rat showed central vein with radiating columns of hepatocyte. liver of rat treated with CCl_4 alone showed intense centrilobular necrosis and fatty infiltration. Liver of rat treated with

Table 1: Effect of various extracts of leaves of *Erythrina indica* on the biochemical parameters of CCl_4 intoxicated rats

Treatment	Dose	GOT U/L	GPT U/L	ASAT U/L	ALAT U/L	ALP KA units	Bilirubin U/L
Normal	-	52.6± 1.06	63.2± 2.16	52.15± 1.27	17.65± 2.05	238.25± 1.27	0.41± 0.95
CCl_4	0.5 ml/kg	110.7± 0.63	119.5± 1.52	102.2± 2.31	54.16± 3.10	421.34± 1.52	1.512± 0.86
CCl_4(0.5 ml/kg)+Sily.	200 mg/kg	59.7± 0.87*	72.15± 2.36*	54.31± 0.96*	25.19± 1.96*	290.18± 1.06*	0.405± 0.29*
CCl_4 (0.5 ml/kg)+EEI	250 mg/kg	69.1± 1.81*	75.8± 1.67*	63.51± 0.76*	31.49± 1.05*	325.34± 2.07*	0.613± 0.85*
CCl_4 (0.5 l/kg)+EAEI	250 mg/kg	78.5± 1.26*	81.6± 0.96*	71.06± 1.56*	35.07± 0.57*	342.26± 0.86*	0.835± 0.92*
CCl_4 (0.5 ml/kg)+CEI	250 mg/kg	80.2± 1.26*	83.5± 0.96*	75.29± 1.56*	33.16± 0.57*	357.6± 0.86*	0.925± 0.92*

Sily, EEI, EAEI and CEI – silymarin, Ethanol, Ethyl acetate and Chloroform extract of EI respectively. Mean ± S.E.M, n= 6, *P<0.05 (Compared to control) were considered significant, *P<0.05 (Compared to Standard) were considered significant.

Table 2: Effect of various extracts of leaves of *Erythrina indica* on the biochemical parameters of CCl_4 intoxicated rats

Treatment	Dose	sTGL mg/ml	Total protein g/dL	Albumin U/L
Normal	-	61.19±0.57	5.76±0.59	2.31±0.67
CCl_4	0.5 ml/kg	29.13±0.59	2.96±0.58	1.25±0.82
CCl_4(0.5ml/kg)+Silymarin	200 mg/kg	57.92±0.25*	4.81±0.69*	2.949±0.79*
CCl_4 (0.5 ml/kg)+EEI	250mg/kg	51.49±0.36*	3.69±0.93*	2.09±1.69*
CCl_4 (0.5 ml/kg)+EAEI	250mg/kg	46.76±0.25*	2.95±0.47*	1.763±1.87*
CCl_4 (0.5 ml/kg)+CEI	250mg/kg	42.51±0.25*	2.67±0.47*	1.826±1.87*

EEI, EAEI and CEI –Ethanol, Ethyl acetate and Chloroform extract of EI respectively.
Mean ± S.E.M, n= 6, *P<0.05 (Compared to control) were considered significant
*P<0.05 (Compared to Standard) were considered significant

Table 3 : Effect of various extracts of leaves of *Erythrina indica* on the biochemical parameters of CCl_4 intoxicated rats

Treatment	Dose	Liver weightg/100gm body weight	Lipid per oxidation n mol/ml
Normal	-	1.62±0.19	19.16±1.06
CCl_4	0.5 ml/kg	4.16±0.07	35.19±1.26
CCl_4 (0.5ml/kg)+Sily.	200 mg/kg	2.07±1.06	18.27±0.97*
CCl_4(0.5 ml/kg)+EEI	250 mg/kg	2.90±1.02	23.34±0.65*
CCl_4(0.5ml/kg)+EAEI	250 mg/kg	3.26±0.11	25.59±0.65*
CCl_4 (0.5 ml/kg)+CEI	250 mg/kg	3.47±0.16	28.67±0.65*

Sily., EEI, EAEI and CEI – Silymarin, Ethanol, Ethyl acetate and Chloroform extract of EI respectively.
Mean ± S.E.M, n= 6,
*P<0.05 (Compared to control) were considered significant
*P<0.05 (Compared to Standard) were considered significant

CCl_4 and Silymarin showed almost normal architecture of liver. Livers of rat treated with Ethanol, Chloroform and Ethyl acetate extract of leaves of *Erythrina indica* and CCl_4 also showed almost normal architecture of liver there is no evidence for the presence of necrotic cells or fatty infiltration.

DISCUSSIONS

Carbon tetra chloride induced liver toxicity model is well accepted method for evaluating liver protective effect of herbal drugs. Hepatotoxic effect of CCl_4 is mediated through $CCl_3^{\cdot}$. $CCl_3^{\cdot}$ is released by the action of cytochrome p 450, a primary site of action of CCl_4. The free radicals $CCl_3O^{\cdot}$ and/ or $CCl_3OO^{\cdot}$ is reported to alter microsomal membrane and poly unsaturated fatty acid of endoplasmic reticulum, decrease protein synthesis and cause accumulation of triglyceride and fatty liver.

The present study, revealed that administration of CCl_4 markedly elevated serum GOT, GPT, ASAT, ALAT, ALP activities. Lipid peroxide level also high in CCl_4 treated group compared to test drug treated animals. This indicate that liver injury has occurred possibly by membrane lipid per oxidation through free radical formed as result of CCl_4 metabolism. Histopathological observations also revealed that the significant recovery from CCl_4 injury as indicated by the absence of necrosis and fatty infiltration in the drug treated animals than liver treated with CCl_4 alone.

Since antioxidant drugs were reported to possess hepatoprotective activity, we conclude that liver protective effect of leaves of *Erythrina indica* may be due to its anti oxidant property[13]. Further study is needed for the identification of active constituent responsible for the activity.

REFERENCES

1. Maton, Anthea; Jean Hopkins, Charles William McLaughlin, Susan Johnson, Maryanna Quon Warner, David LaHart, Jill D. Wright., *Human Biology and Health*. Englewood Cliffs, New Jersey, USA: Prentice Hall (1993).
2. Kirtikar, K.R. and Basu, B.D., In; Indian medicinal plants, 2nd Edn., vol.II, International Book Distributors., Dehradun, 1264-1265.
3. Nadkarni KM, Nadkarni AK, Indian Materia Medica, Mumbai Popular Prakashan, **1**: 508 (1992).
4. Harborne JB., Phytochemical methods., 3rd Edn., Chapman and Hall, London, 91 (1988).
5. Trease GE, Evans WC, Pharmacognosy, ELBS Publication, Delhi, India, 13th Edn, 171 (1989).
6. De S, Sheila VJ, Ravishankar B, Bhavasar GC: Fitoterapia**.,** **67**: 106 ((1996)).
7. T. Peters Jr. Proposals for standardization of total protein assays. *Clin Chem*. **14**(12): 1147-59 (1968).
8. D. Webster. A study of the interaction of bromocresol green with isolated serum globulin fractions. *Clin Chem Acta*. **53**(1): 109-15 (1974).
9. IFCC method for the measurement of catalytic concentration of enzymes, *Journal of clinical chemistry and clinical bio chemistry.,* **24**: 481-495 (1986).
10. Bergmeyer HU, Methods of enzymatic analysis, Vol.2 IInd Edn, Weinheim:Verlag chemic, 735-739, 760-764,860-864 (1974).
11. Waters MI, Gerade RW, *Microchem.*, **15**: 231 (1970).
12. Ohkawa H, Ohishi N, Yagi K., Assay for lipid per oxidation in animal tissues by thio barbituric acid reaction. *Anal Biochem* **95**: 351-358 (1979).
13. Lin CC, Ming HY, Isac SL, Lin MJ., Evaluation of hepatoprotective and antioxidant activity of Boehmeria nivea var and B. nivea var. tenacissima. *J. Ethnopharmacol* **60**: 9-17 (1998).