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HEPATOPROTECTIVE EFFECT OF *ERYTHRINA VARIEGATA* AGAINST CARBON TETRACHLORIDE (CCL_4) INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

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ABSTRACT

The present study to screen the hepatoprotective effect of ethanolic extract of whole plant of *Erythrina variegata* (EEEV) in male Wistar albino rats by using CCl_4 induced hepatoptoxicity in rats. The EEEV at safe doses of 200 and 400mg/kg, p.o were selected from acute oral toxicity study. The Silymarin (100mg/kg, p.o) was used as standard drug and administered 3 times at 12h intervals and then CCl_4 (1ml/kg) was administered to all the groups except normal control for 2 days. The hepatoprotective activity was assessed by using various biochemical parameters like SGOT, SGPT, ALP, TP and total bilirubin along with histopathological studies were observed after 36h of CCl_4 treatment. The EEEV at the doses of 200 and 400mg/kg protected from CCl_4 induced liver toxicity in Wistar albino rats as assessed by the biochemical changes. The ethanolic extract of whole plant of *Erythrina variegata* possesses significant protection against CCl_4 induced hepatocellular injury.

Key Words: *Erythrina variegata*, Hepatoprotective, CCl_4, Silymarin, Hepatotoxicity.

INTRODUCTION

Every year about 20,000 deaths are found due to liver disorders [1]. Thus to maintain a healthy liver is a crucial factor for overall health and well beings [2]. Thus, liver diseases remain one of the serious health problems and its disorders are numerous with no effective remedies [3-5]. There is no rational therapy available for treating liver disorders and management of liver diseases is still a challenge to the modern medicine. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders [3]. The use of natural remedies for the treatment of various hepatic diseases has a long history and medicinal plants and their derivatives are still used all over the world [1].

The genus Erythrina comprises of about 110 species of trees and shrubs. The name "coral tree" is used as a collective term for these plants. Coral tree is indigenous to the Old World tropics, possibly originally from India to Malaysia, but is native of ancient westward to Zanzibar and eastward to eastern Polynesia (the Marquesas). It is typically found on sandy soil in littoral forest, and sometimes in coastal forest up to 250m (800ft) in elevation.

Studies on phytochemical of *Erythrina variegata* species (Family: Fabaceae) have demonstrated alkaloids and flavonoids as major constituents. Different parts ***of*** *E. Variegata* have used in traditional medicine as nervine sedative, febrifuge, anti-asthmatic and antiepileptic. In the some experiments, it has potential effects for treatment of some diseases like convulsion, fever, inflammation, bacterial infection, insomnia, helminthiasis, cough, ulcer, cuts, liver disorders and wounds [6-11]. From the source of literature documentation and relevant traditional approaches on plant drugs, the present investigation was carried out to investigate hepatoprotective activity of ethanol extract of *Erythrina variegata* whole plant against CCl_4-induced liver damage in rats to support the claim. Hence, the present study was designed to verify the claims of the native practitioners.

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MATERIALS AND METHODS

Plant material

The whole plant of *Erythrina variegata* was collected from Tirumala hills, Tirupati, Andhra Pradesh. India. It was identified and authenticated by Prof. *Madhava Chetty, K.*, Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our laboratory for future reference.

Preparation of plant extract

The collected plant was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 100g of powdered materials were extracted with ethanol (90%) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is suspended in tween 80 and used for the experiment. The percentage yield of prepared extract was around 9.5%w/w.

Animals Used

Albino rats (180–200 g) of either sex were maintained in a 12 h light/dark cycle at a constant temperature 25 ◦C with free access to feed (Sai durga feeds and foods, Bangalore) and water. All animals were fasted prior to all assays and were allocated to different experimental groups each of 6 rats. Moreover the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

Acute Toxicity Study

The acute toxicity ethanolic extracts of *Erythrina variegata* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected for further study [12].

Carbon tetrachloride induced hepatotoxicity in rats

The liver protective effect was evaluated using the carbon tetrachloride (CCl_4) model described by *Rao and Mishra* [13]. Wistar albino rats (150-200g) were divided into five groups and were subjected to the following treatments

Group-I served as normal control; received vehicle only.

Group-II served as untreated group; received only CCl_4, to assist assessing the severity of toxicity produced by carbon tetrachloride administration.

Groups III-V served as treated groups; received EEEV at the dose of 200 and 400mg/kg, p.o. and standard drug Silymarin at a dose of 100mg/kg, p.o. were administered orally to rats of the respective groups three times at 12h intervals.

Carbon tetrachloride diluted with liquid paraffin (1:1) was administered in dose of 1ml/kg, p.o. for 2 days to all animal groups except for normal control. After 36h of carbon tetrachloride treatment, blood was collected from all groups of rats by puncturing the retro-orbital sinus. Serum was separated by centrifugation at 2500rpm at 37^0C for 15min and analyzed for various biochemical parameters.

Biochemical estimation

The separated serum was subjected to estimate SGOT and SGPT by *Reitman and Frankel* method [14], alkaline phosphatase (ALP) by *Kind and King* method [15], and bilirubin by *Malloy and Evelyn* method [16].

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Tukey-Kramer multiple comparison tests, the p values less than 0.05 were considered as significance.

RESULTS

Acute toxicity study

In the acute toxicity study, the animals treated with the EEEV at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioral changes, body weight changes, or macroscopic findings at any time of observation. There was no mortality in the above-mentioned dose at the end of the 14 days of observation.

Effect of EEEV on CCl_4 – induced hepatotoxicity

The results of EEEV on Carbon tetrachloride-induced hepatotoxicity were represented in Table 1. The animals treated only with CCl_4 exhibited a significant increase (P<0.001) the levels of SGOT, SGPT, ALP, and total bilirubin as well as decrease in the levels of TP when compared to the normal control group after 36h of CCl_4 treatment, indicating hepatocellular damage. The EEEV at tested doses (group-III & IV) produced a significant reduction (P<0.001) in the CCl_4 induced elevated levels of SGOT, SGPT, ALP, and total bilirubin as well as increases the TP when compared to the animals treated only with CCl_4 (group-II) after 36h of CCl_4 treatment. Overall, EEEV at tested doses significantly reduced the levels of hepatic enzymes and total bilirubin.

Table 1. Effects of EEEV on alternation of hepatic enzyme and serum bilirubin in rat after 36h. of CCl_4 treatment

Groups (n=6)	Biochemical Parameters				
	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	TP (gm/dl)	Total Bilirubin (mg/dl)
Group-I (Normal Control)	28.24 ± 2.04***	17.14 ± 1.32***	178.59 ±2.34***	8.12 ± 0.25***	0.84 ± 0.04***
Group-II (CCl_4: 1ml/kg)	60.42 ± 1.22	36.49 ± 1.41	418.95 ± 2.54	2.19 ± 0.14	3.54 ± 0.15
Group-III (EEEV: 200mg/kg)	42.16 ± 1.05***	30.19 ± 1.14***	241.39 ±2.46***	3.32 ± 0.17***	1.32 ± 0.14***
Group-IV (EEEV: 400mg/kg)	36.32 ±2.04***	25.12 ± 1.21***	215.64 ±2.14***	5.64 ± 0.22***	0.92 ± 0.02***
Group-V (Silymarin:100mg/kg)	31.33 ± 2.05***	19.84 ± 1.05***	189.24 ±2.46***	6.79 ± 0.22***	0.79 ± 0.02***

Values are expressed as mean ± SEM of 6 rats in each group. *** $p<0.001$, as compared to CCl_4-treated group. SGOT = Serum glutamate oxaloacetate transaminase, SGPT = Serum glutamate pyruvate transaminase, ALP = Alkaline phosphatase, TP = Total proteins

Figure 1. Effects of EEEV on alternation of Serum Glutamate Oxaloacetate Transaminase in rat after 36h. of CCl_4 treatment

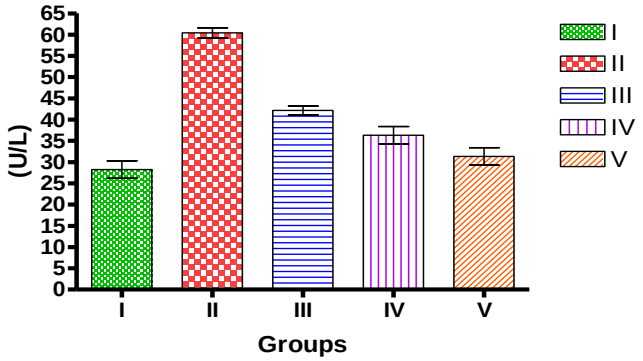


Figure 2. Effects of EEEV on alternation of Serum Glutamate Pyruvate Transaminase in rat after 36h. of CCl_4 treatment

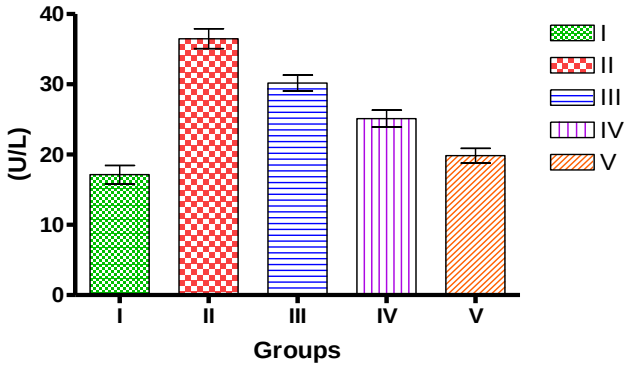


Figure 3. Effects of EEEV on alternation of Alkaline Phosphatase in rat after 36h. of CCl_4 treatment

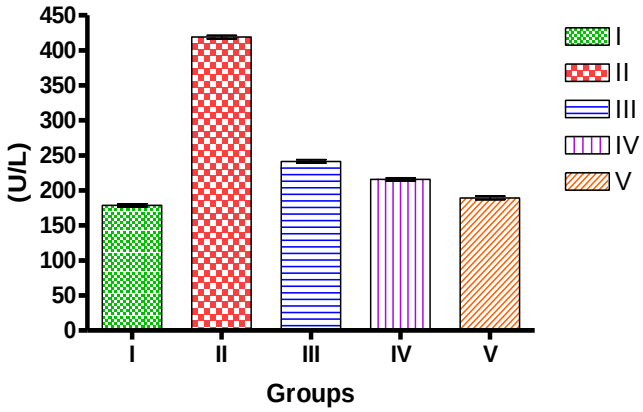


Figure 4. Effects of EEEV on alternation of Total proteins in rat after 36h. of CCl_4 treatment

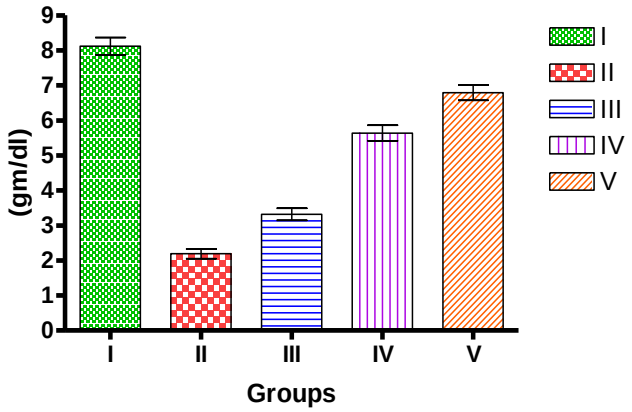
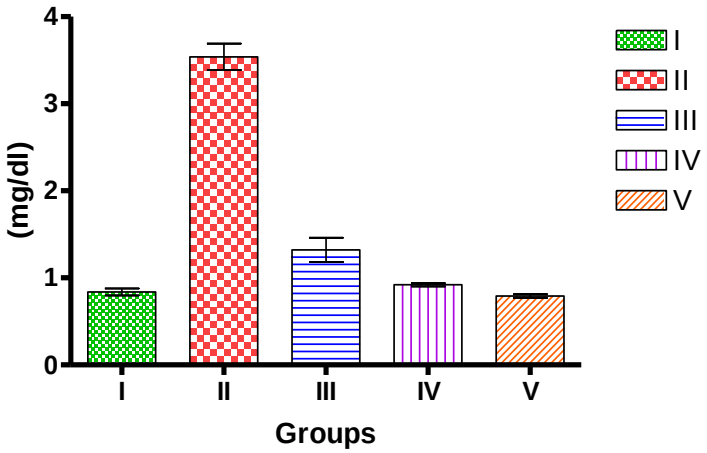


Figure 5. Effects of EEEV on alternation of Total Bilirubin in rat after 36h. of CCl_4 treatment



DISCUSSION AND CONCLUSION

Herbal medicines play a major role in the treatment of liver disorders. A number of medicinal plants and their formulations are widely used for the treatment of these disorders [17,18]. However, there were not enough scientific investigations on the hepatoprotective activities conferred to these plants. One of the plants from Indian flora is *Erythrina variegate*. The present studies were performed to investigate the hepatoprotective activity of ethanolic extract of whole plant *Erythrina variegata* in rats against carbon tetrachloride as hepatotoxin to prove its claims in folklore practice against liver diseases.

Screening of hepatoprotective effect, Carbon tetrachloride (CCl_4) is one of the most commonly used hepatotoxins in the experimental study of liver diseases [19]. CCl_4 is potent hepatotoxin producing centrilobular hepatic necrosis. It is accumulated in hepatic parenchyma cells and metabolized to trichloromethyl free radicals (CCl_3.) by liver cytochrome P-450 dependent monooxygenases. This CCl_3 free radical combined with cellular lipids and proteins in the presence of oxygen to produce lipid peroxides [20]. Thus, antioxidant or free radical generation inhibition is important in protection against CCl_4 induced liver lesion [21]. The flavonoids constituents possess free radical scavenging properties [22]. In general, the extent of liver damage is assessed by histopathological evaluation and levels of hepatic enzymes such as ALP, SGOT, SGPT and also bilirubin release in circulation [23-25].

Administration of hepatotoxins CCl_4 elevated the serum levels of SGOT, SGPT, ALP, and bilirubin as well as decreases total serum proteins (TP) significantly [26,27]. The rise in serum enzymes level and bilirubin has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages [28].

In our investigation, the biochemical changes were observed after 36h. of CCl_4 treatment. Thereby, it was found that the animal groups which are pretreated with EEEV at the dose of 200 and 400mg/kg (groups-III and IV) as well as silymarin at the dose of 100mg/kg (group-V) for three times at 12h. intervals, resulted in significantly decreases the hepatic enzymes such as SGOT, SGPT, ALP and also total bilirubin; as well as increases the total serum proteins (TP) as compared to animals treated only with CCl_4 (group-II). These results give us the suggestion that, the animals which are pretreated with EEEV as well as silymarin, showed a protection against the injurious effects of CCl_4 that may results from the interference with cytochrome P-450. These biochemical restoration may be due to the inhibitory effects on cytochrome P-450 or/and promotion of its glucuronidation [29,30]. Silymarin is a known hepatoprotective drug. It is reported to have a protective effect on the plasma membrane of hepatocytes [31].

It is well documented that the phytoconstituents comes under the category of flavonoids, alkaloids, glycosides, carotenoids, phenols, coumarins, lignans, essential oil, lipids, monoterpenes, xanthenes and organic acids are reported to have hepatoprotective activity [32]. The hepatoprotective activity of *Erythrina variegata* may be attributed due to presence of these constituents. This study supports the traditional claims and the EEEV could be added in traditional preparations for the various liver diseases.

It is concluded from the data, that the ethanolic extract of whole plant of *Erythrina variegata* possesses significant hepatoprotective activity and may prove to be effective for the treatment of liver disorders. However, longer duration studies on chronic models are necessary to elucidate the exact mechanism of action so as to develop it as a potent hepatoprotective drug.

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