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Hepatoprotective Activity of Ethanolic Extract of the Stems of Anisochilus Carnosus against Carbon Tetrachloride-induced Hepatotoxicity in Rats

Abstract

Purpose: To evaluate the hepatoprotective activity of ethanolic extract of the stems of Anisochilus Carnosus (EEAC) against carbon tetrachloride (CCl_4) induced hepatotoxicity in rats.

Methods: Hepatotoxicity was induced in albino Wistar rats of either sex by intraperitoneal injection of CCl_4 in olive oil (1:1). Two doses of ethanolic extract of *Anisochilus Carnosus* (200 and 400 mg/kg body weight) were administered to the experimental rats. The hepatoprotective effect of the extract was evaluated by the assay of liver function biochemical parameters like serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, alkaline phosphatase, total bilirubin and total protein.

Results: In ethanolic extract treated animals, the toxic effect of CCl_4 was significantly controlled by the plant extract as compared to the normal and the standard drug silymarin treated group.

Conclusion: Ethanolic extract of stems of *Anisochilus Carnosus* possesses significant hepatoprotective activity.

Keywords: Anisochilus carnosus, Hepatoprotective activity, Hepatotoxicity, Carbon tetrachloride, Silymarin.

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Introduction

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects [1]. Treatment of many liver diseases is symptomatic and often disappointing, since much is still obscure about their etiology [2]. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in ayurveda recommended for the treatment of liver disorders [3]. Many formulations containing herbal extracts are sold in the Indian market for liver disorders, but management of liver disorder by a simple and precise herbal drug is still an intriguing problem. Several Indian medicinal plants have been extensively used in the Indian traditional system of medicine for the management of liver disorder [4]. In view of severe undesirable side effects of synthetic drugs, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.

Anisochilus Carnosus (common name: Kapurli; synonyms: *Lavandula carnosa*, *Plectranthus strobilifer*; Hindi: *Panjiri-ka-patta*; Telugu: *Ritchu-Rodda*, *Omamaku;* Tamil: *Karpuravalli*) belongs to the family Lamiaceae (Mint family). It is an annual herb, found in the Western Ghats. Stems are erect, 30-60 cm tall, robust and branched [5]. The herb has been traditionally used for the treatment of gastrointestinal disorders, cough, cold, fever and ulcer [6, 7]. Anti-ulcer activity of *A. carnosus* leaf extract in pylorus ligated rats has been reported [8]. To the best of our knowledge, the hepatoprotective activity of stems of the herb has not been clinically evaluated so far. In the present study, the hepatoprotective activity of the ethanolic extract of the stems of the herb against carbon tetrachloride induced hepatotoxicity in rats is reported.

Materials and Methods

Plant material

Fresh stems were collected from Sri Venkateswara University campus, Tirumala gardens of Chittor district of Andhra Pradesh, India and authentified by Asst Prof Dr K Madhava Chetty, of the Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. Voucher specimen was deposited at Department of Pharmacognosy for further reference.

The plant material was dried under shade at room temperature, reduced to moderately coarse powder and extracted successively with 95% ethanol using soxhlet apparatus. Resultant ethanolic extract was dried under vacuum. The defatted extract of *A. carnosus* (EEAC) was used for the preliminary phytochemical screening and hepatoprotective studies.

Phytochemical Screening

A preliminary phytochemical screening was carried out for the extract employing the standard procedure revealed the presence of various phytoconstituents including alkaloids, flavonoids and glycosides [9, 10].

Animals and Acute toxicity studies

Wistar albino rats of both sexes (weighing 130-160 g) were used in the present study. They were housed in clean polypropylene cages (38×23×10 cm) with not more than three animals per cage and maintained under standard laboratory condition (temperature 25±2 ºC) with dark and light cycle and provided standard pellet diet and water ad libitum. Experimental protocols for the pharmacological and toxicity studies were reviewed and approved by the Institutional Animal Ethics Committee (IAEC/PRRMCP/2006/07).

Acute toxicity study was performed for the extract as per Stair case method [11]. For the hepatoprotective studies, the amount of dose administered was adjusted on the basis of observation during the toxicity studies and accordingly extracts at two dose levels i.e., 200 and 400 mg/kg orally were administered.

Assessment of hepatoprotective activity

Thirty rats were divided randomly into five groups, each comprising of six animals. **Group I** (Normal control) received oral dose of 0.5% sodium CMC (1ml each) for 10 days. **Group II** (Toxicant control) received CCl_4 (2ml/kg, 50% in olive oil) for inducing hepatotoxicity. **Group III** received standard poly herbal drug 'Liv-52' (5ml/kg; p.o.) (Liv-52 syrup-Himalaya Drug Company, Bangalore, India). **Group IV** received

EEAC 200 mg/kg in a day for 10 days and **Group V** received EEAC 400 mg/kg in a day for 10 days [12, 13]. All the animals were killed on day 11 under light ether anesthesia. The blood samples were collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to centrifuge. The biochemical investigations were performed by using a biochemical semi-auto-analyzer. The biochemical parameters considered were: SGPT, SGOT, ALP, total bilirubin and serum protein [14, 15].

Statistical analysis

All the values are expressed as mean±SEM and data analyzed by one-way ANOVA. At 95% confidence interval, p values less than 0.05 were considered to be significant.

Results

In CCl_4 induced hepatotoxicity, the administration of the toxicant CCl_4 showed a distinct rise in the levels of serum marker enzymes namely SGPT, SGOT, ALP and Total Bilirubin as shown in Group II of Table 1. The drug treatment (EEAC) was carried out at two dose levels 200 and 400mg/kg, both of which along with the standard (Liv-52) treated group showed a significant reduction in the elevated enzyme levels ($p<0.01$). These data suggests a dose dependent hepatoprotective activity of EEAC.

Table 1: Effect of EEAC on CCl_4 induced hepatotoxicity in rats (Biochemical Parameters)

Group	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Total Protein (gm/dl)
I (Control)	23.21±7.60*	34.65±6.73*	86.7±4.26*	0.62±0.03**	6.9±0.11***
II (Toxicant)^{a}	44.99±14.86	54.32±10.38	143.4±3.41	0.76±0.02	6.52±0.13
III (Standard)^{b}	29.05±9.45*	37.92±7.12*	102.3±0.95*	0.68±0.04***	16.12±3.82*
IV (Treatment group EEAC-200mg/kg)^{b}	36.06±12.10*	46.767±8.90*	99.56±0.59*	0.56±0.02*	7.08±0.24***
V (Treatment group EEAC-400mg/kg)^{b}	29.45±9.79*	38.09±7.69*	103±5.38*	0.66±0.02***	13.54±3.07*

All values are expressed as Mean±SEM, N=6; ^{a}as compared to control group, ^{b}as compared to toxicant group. Analysis by One-way ANOVA followed by Dunnet's test; Significant *at $p<0.01$, **at $p<0.05$, ***at $p>0.05$.

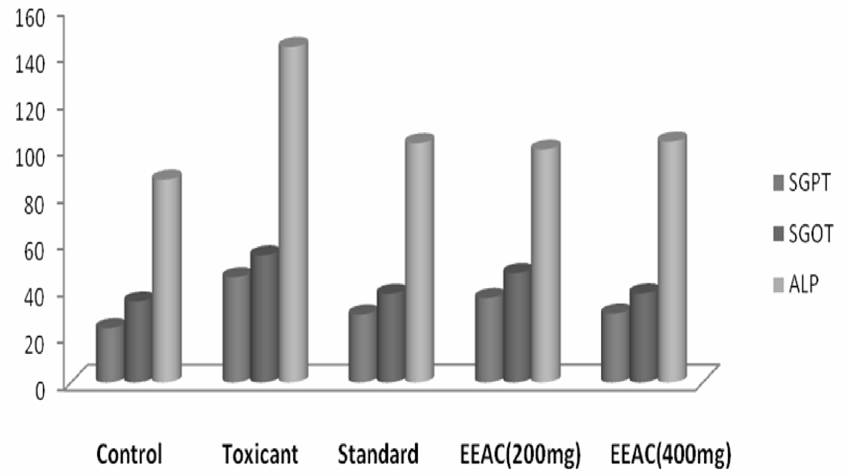

Figure 1: Effect of EEAC on biochemical estimation of SGPT, SGOT and ALP of CCl_4 induced toxicity in rats

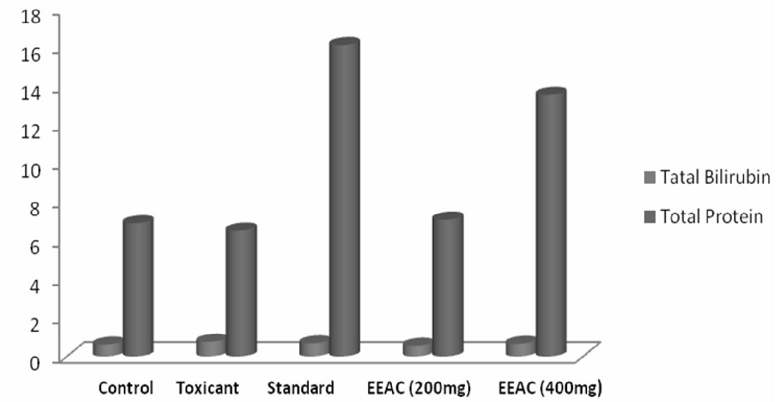

Figure 2: Effect of EEAC on biochemical estimation of total bilirubin and total protein of CCl_4 induced toxicity in rat

Discussion

CCl_4 is one of the most commonly used hepatotoxin in the experimental study of liver diseases. The lipid peroxidative degeneration of biomembranes is one of the major causes of hepatotoxicity of CCl_4. The increase in the levels of serum bilirubin reflected the depth of jaundice ad the increase in transaminases and alkaline phosphate was the clear indication of the cellular leakage and loss of functional integrity of the cell membrane [16].

Since CCl_4 involve activation by Cyt P-450, subsequent damage to the hepatocellular membrane by the toxic intermediate and increase in lipid peroxidation, the possible hepatoprotective mechanisms of *Anisochilus Carnosus* would be inhibition of the lipid peroxidation, stabilization of the hepatocellular membrane and enhancement of protein synthesis.

Conclusion

In the present pharmacological evaluation the whole stem extract (ethanolic) of *Anisochilus Carnosus* plant was extensively investigated for its hepatoprotective potential against CCl_4 induced hepatopathy. At the end of this study, a strong conclusion can be drawn that, the ethanolic extract of stems of Anisochilus Carnosus possess hepatoprotective activities more or less depending on the dose levels.

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by authors. Mr N Senthilkumar, collected a plant material and received an authentification report. He did the preliminary extraction processes and phytochemical screening. Assessment of hepatoprotective activity was carried out by Mr P Venkatesh and Mr A Dinakar. Finally the data's analoyzed by all the three authors and the manuscript was prepared by Mr P Venkatesh.

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