



HEPATOPROTECTIVE ACTIVITY OF AN ETHANOLIC EXTRACT OF STEMS OF ANISOCHILUS CARNOSUS AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

An ethanolic extract of stems of *Anisochilus Carnosus* (EEAC) was studied for hepatoprotective activity against carbon tetrachloride (CCl₄) induced hepatotoxicity in rats. Hepatotoxicity was induced in Albino wistar rats of either sex by intraperitoneal injection of CCl₄ [CCl₄ in olive oil 1:1]. Ethanolic extract of *Anisochilus Carnosus* was administered to the experimental rats at two dose levels 200 and 400mg/kg body weight. The hepatoprotective effect of the extract was evaluated by the assay of liver function biochemical parameters like Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP), Total Bilirubin and Total Protein. In ethanolic extract treated animals, the toxic effect of CCl₄ was controlled significantly as compared to the normal and the standard drug silymarin treated group.

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

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 effects¹. Treatment of many liver diseases is symptomatic and often disappointing, since much is still obscure about their etiology². 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 disorders³. 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 disorder⁴. 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 carnosus*, *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 branched⁵. The plant has been traditionally used for the treatment of gastrointestinal disorders, cough, cold, fever and ulcer^{6, 7}. Anti-ulcer activity of *Anisochilus Carnosus* leaf extract in pylorus ligated rats has been reported⁸. Literature reviews indicated that the hepatoprotective activity of stems of *Anisochilus Carnosus* has not been clinically evaluated so far. In the present study, the hepatoprotective activity of ethanolic extract of stems of *Anisochilus Carnosus* against carbon tetrachloride induced hepatotoxicity in rats is reported.

MATERIALS AND METHODS

Collection of plant material

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

Extraction of plant material

The plant material was dried under shade at room temperature, reduced to moderately coarse powder and extracted successively

with 95% ethanol using soxhlet apparatus. The ethanolic extract was dried under vacuum. The defatted ethanolic extract of *Anisochilus 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 viz. alkaloids, flavonoids and glycosides^{9, 10}.

Animals and acute toxicity studies

Wistar albino rats of both sexes (weighing 130-160gm) were used in the present study. They were housed in clean polypropylene cages (38×23×10cm) with not more than three animals per cage and maintained under standard laboratory condition (temperature 25±2°C) 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 method¹¹. 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 CCl₄ (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 200mg/kg in a day for 10 days and **Group V** received EEAC 400mg/kg in a day for 10 days. The dose administered was recorded^{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. The level of significance was found out by Dunnett's test wherein all the groups are compared against toxicant group.

RESULTS

In CCl₄ induced hepatotoxicity, the administration of the toxicant CCl₄ 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 CCl₄ induced hepatotoxicity in rats (Biochemical Parameters)

| Group No. | SGPT (IU/L) | SGOT (IU/L) | ALP (IU/L) | Total Bilirubin (mg/dl) | Total Protein (gm/dl) |
|---|-------------|-------------|-------------|-------------------------|-----------------------|
| I (Control) | 23.21±0.27 | 34.65±0.24 | 86.7±0.49 | 0.62±0.02 | 6.9±0.20 |
| II (Toxicant) ^a | 44.99±0.47* | 54.32±0.30* | 143.4±0.53* | 0.76±0.03** | 6.52±0.21*** |
| III (Standard) ^b | 29.05±0.43* | 37.92±0.16* | 102.3±0.31* | 0.68±0.02*** | 16.12±0.44* |
| IV (Treatment group EEAC-200mg/kg) ^b | 36.06±0.29* | 46.76±0.24* | 99.56±0.29* | 0.56±0.03* | 7.08±0.26*** |
| V (Treatment group EEAC-400mg/kg) ^b | 29.45±0.20* | 38.09±0.21* | 103±0.39* | 0.66±0.04*** | 13.54±0.26* |

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 Dunnett's test. Significant *at p<0.01, **at p<0.05, ***at p>0.05.

Figure 1 & 2 shows the potentiating effect of EEAC activity in CCl₄ induced hepatotoxicity which was found to be dose dependant with other groups showing significant reduction in the enzyme levels. These data suggests that the toxic effect of CCl₄ was controlled in the

animals treated with the ethanolic extract by way of restoration of the levels of the liver function biochemistry similar to that of the standard drug Liv-52.

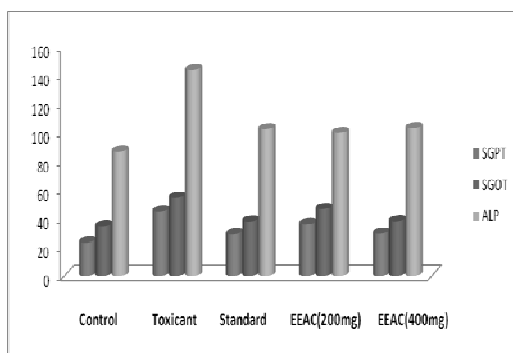


Fig. 1: Effect of EEAC on biochemical estimation of SGPT, SGOT and ALP of CCl₄ induced toxicity in rats

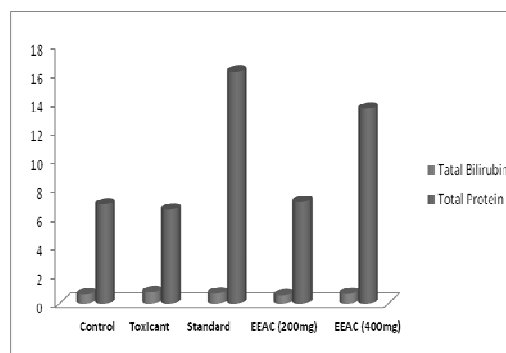


Fig. 2: Effect of EEAC on biochemical estimation of total bilirubin and total protein of CCl₄ induced toxicity in rat

DISCUSSION

CCl₄ 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 CCl₄. The increase in the levels of serum bilirubin reflected the depth of jaundice and the increase in transaminases and alkaline phosphate was the clear indication of the cellular leakage and loss of functional integrity of the cell membrane¹⁶.

Since CCl₄ 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 CCl₄ 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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