

## Hepatoprotective activity of *Eria Mysorensis* Lindl. (orchidaceae) pseudobulb against carbontetrachloride induced toxicity

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### Abstract

The ethanolic extract of pseudobulb of *Eria mysorensis* at the dose of 100mg/kg body weight (oral) was studied for the hepatoprotective effect using Carbontetrachloride induced liver damage in wistar albino rats. Ethanolic extract showed significant ( $p < 0.05$ ) hepatoprotective effect by lowering the serum levels of various biochemical parameters such as serum Glutamic Oxaloacetate Transaminase (SGOT), Serum Glutamic Pyruvates Transaminase (SGPT), Alkaline Phosphatase (ALP), Total Bilirubin (TBL), Total Cholesterol (CHL) and by increasing the levels of Total Protein (TPTN) and Albumin (ALB) in the selected model. These biochemical observations were in turn confirmed by histopathological examinations of liver sections and are comparable with the standard hepatoprotective drug Silymarin (100mg/kg body weight) which served as a positive control. The overall experimental results suggested that the biologically active phytoconstituents such as flavonoids, terpenoids, sterols, phenols, saponins and alkaloids present in the ethanolic extract of plant *Eria mysorensis*, may be responsible for the significant hepatoprotective activity and the results justify the use of *Eria mysorensis* as a hepatoprotective agent.

**Keywords:** ALP, *Eria mysorensis* (pseudobulb), hepatoprotective, SGOT, SGPT, silymarin

### INTRODUCTION

India has one of the largest tribal population in the world. The forest plays a vital role in the economy as well as daily needs of the tribals. In times of scarcity when the staple food is in short supply tribals collect many types of wild roots and tubers to supplement their meagre food available at home (Vidarthi, 1987). Although orchids are being cultivated and valued mainly for ornamental purposes, some of them are used from time immemorial in traditional practices to treat various medical conditions. More than 13 species of orchids of traditional importance are reported in Kerala forests (Muktesh Kumar, 1987). Orchids, commonly the members of Orchidaceae, normally grow as epiphytes or often as lithophytes or sometimes as saprophytes. It is one of the most diversified plant families of angiosperms comprising of 18,500 species under 788 genera (Mabberley, 1997), mainly distributed in tropical, subtropical to subtemperate regions of the globe. In India, Orchidaceae is the second most diversified family comprising of 184 genera and 1229 species. *Eria* is a large genus of orchids with more than 500 species distributed in tropical Asia, Malaysia, Australia, Polynesia and other Pacific islands. It is used for its antidiabetic activity, hepatoprotective activity and aphrodisiac activity. Most of the species have a typical odour of rotting carcasses which attracts the flies to help in their pollination process.

Liver a chief site for intense metabolism and excretion has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved

with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction (Ward and Daly 1999). The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamins. Thus, to maintain a healthy liver is a crucial factor for overall health and well being. But it is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol and prescribed and over-the-counter drug which can eventually lead to various liver ailments like hepatitis cirrhosis and alcoholic liver disease (Sharma et al., 1991 and Subramaniam & Pushpangadan 1999). Thus liver diseases are some of the fatal diseases in the world today. Therefore, many folk remedies from plant origin are tested for their hepatoprotective activity in experimental animal model. Carbon tetrachloride ( $\text{CCl}_4$ ) induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts (Rubinstein, 1962 and Suja et al., 2002).

### MATERIALS AND METHODS:

Plant material *E. mysorensis* was collected from Velliangiri hills Coimbatore District and is the major hills range of Western Ghats, Tamilnadu, India. Collected medicinal plants were identified with the help of field floras (Gamble, 1957; Mathew, 1983; Chandrabose and Nair 1988). Identification was confirmed at the Botanical survey of India, Southern circle, Coimbatore, Tamilnadu.

#### Preparation of the extract:

Plant material pseudobulb was washed with clean tap water to remove dirt and dried under shade to constant

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weight for 20 days. The pseudobulbs were then cut in to pieces and later grinded to powder using an electrical mill. The powdered material were exhaustively extracted with ethanol for 8hrs using Soxhlet apparatus. The obtained extract was evaporated to dryness at 38°C.

#### Animals:

Wistar Albino rats weighing 180-240g were used in this evaluation. These rats aged between 2.5 and 3 months were procured from animal house of the laboratory of Agricultural University, Trissur, Kerala. They were housed in well ventilated stainless steel cages at room temperature (24±2°C) in hygienic conditions under natural light and dark schedule and were fed on standard laboratory diet. Food and water were given *Adlibitum*.

#### Experimental design for hepatoprotective activity

The rats were divided into 4 groups of 5 rats each. The hepatoprotective activity of the plant extracts was tested using CCl<sub>4</sub> model. Group I (normal control) received neither the plant extract nor CCl<sub>4</sub> for 72 hrs ie., they receive food and water only.

#### Carbontetrachloride induced hepatotoxicity:

Rats were divided into four groups of 5 animals each. The rats of control group (I) received three doses of 5% gumacaciamucilage (1ml/kg, per oral.) at 12 hour intervals (0 hour, 12 hour and 24 hour). The rats of Carbontetrachloride group (II) received three doses of vehicle at 12 hour intervals and a single dose of Carbontetrachloride (1.25ml/kg i.p.) diluted in liquid paraffin (1:1) 30 minutes after the administration of 1st dose of vehicle. The rats of standard group (III) received three doses of Silymarin (100mg/kg) at 0 hour, 12 hour

and 24 hour. Carbontetrachloride was administered (1.25ml/kg i.p.) 30 minutes after the first dose of

silymarin. While the rats of test group (IV) received three doses of test extract at the dose of 200mg/kg body weight per oral at 0 hour, 12 hour and 24 hour. Carbontetrachloride was administered (1.25ml/kg i.p.) 30 minutes after the first dose of silymarin test extract (Rao and Mishra, 1997) After 36 hour of administration of Carbontetrachloride, blood was collected and serum was separated and used for determination biochemical parameters.

#### Assessment of Hepatoprotective Activity:

In the present study the hepatoprotective activity was evaluated biochemically and histopathologically. After 72 hours of drug treatment, the animals were dissected under ether anesthesia. Blood from each rat was withdrawn from carotid artery at the neck and collected in previously labeled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 3000 rpm for 15 minutes. The separated serum were used for the estimation of biochemical parameters viz., Alanine aminotransferase (ALT/SGPT), Aspartate aminotransferase (AST/SGOT), cholesterol, bilirubin and glucose.

#### RESULTS AND DISCUSSION:

The effects of ethanol extracts of *E. mysorensis* on initial weight and final weight in normal and liver injured rats were observed. The initial and final body weight, mean weight gain or loss and differences between groups II, III and IV were represented in the table 1. A significant weight loss was observed in the liver injured control group. The body weights were increased in *E. mysorensis* extract treated groups (100, 200, 500 mg/kg b.wt.), where as a significant improvement was observed

**Tab.1:** Effect of *E. mysorensis* on the body weight of the rats before and after treatment in the normal, liver damaged and drug treated rats

Treatment	Dose	Initial Body Weight(gm)	Final Body Weight(gm)	Mean Weight Gain(G↑)/ Loss(L↓) (gm)	Percentage Difference
Normal control	0.9% saline	173.24±5.34	179.78±4.88	6.54↑	3.77
Liver damaged control	0.9% saline	184.45±6.67	168.58±5.23	15.87↓	8.60
Liver damaged Animal + <i>E. mysorensis</i> extract	100(mg/kg)	182.14±6.62	191.57±5.28	9.43↑	5.17
	200(mg/kg)	178.34±4.85	189.42±6.58	11.08↑	6.21
	500(mg/kg)	169.88±4.74	174.38±5.63	4.50↑	2.64
Standard Drug (Silymarin)	100(mg/kg)	186.96±4.48	196.33±5.78	9.37↑	5.01

in the group III treated with the standard drug, Silymarin .

The liver injured rats showed a significant elevation in the total bilirubin, conjugated bilirubin and unconjugated bilirubin when compared to the control group. The administration of ethanol extracts of *E. mysorensis* treated groups at the concentrations of 100,200,500mg/kg b.wt showed significant decreases in the levels of total bilirubin, conjugated bilirubin and unconjugated bilirubin when compared to the liver damaged groups. *E. mysorensis* extracts treated groups were significantly comparable to the liver injured rats, received silymarin orally at the dose of 100mg/kg b.wt. (Table- 2 )This result is compared to the liver injured rats received silymarin orally at the dose of 100mg/kg b.wt. for 14 days. The liver injured group when treated with ethanol extracts of *E. mysorensis* showed elevated levels of all enzymes in group III and IV as compared to the control group.

Estimating the activities of serum marker enzymes, like SGPT, SGOT, ALP can make the assessment of liver function when liver cell plasma membrane is damaged. A variety of enzyme normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepato cellular damage (Mitra, *et al.*, 1998). The tendency of these enzymes to return to near normal level in extract administered group is a clear manifestation of antihepatotoxic effects of the extract.

In accordance with these results, it may be confirmed that due to the presence of phytoconstituents such as flavonoids, terpenoids, sterols, phenols, saponins and alkaloids which are present in the ethanolic extract could be considered as responsible for the significant hepatoprotective activity.

#### CONCULSION:

In summary, the current study demonstrated that ethanol extract showed a potent protective effect against CCl<sub>4</sub>-induced liver injury. Ethanol extract pretreatment significantly inhibited the increase of the serum aminotransferase activities, attenuated oxidative stress-induced mitochondrial dysfunction, and decreased the pathological changes. Thus *E. mysorensis* can be utilized as a therapeutic against the liver diseases.

**Note:**Kongu Nadu Arts and science college has ethical clearance no is(659/02/a/CPCSEA)

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**Tab.2:** Effect of *E. mysorensis* extracts on the bilirubin levels (Conjugated and unconjugated) of normal, liver damaged and drug treated rats

Groups	Parameters			
	Dose	Total bilirubin (mg/dl)	Conjugated (mg/dl)	Unconjugated (mg/dl)
Normal	0.9% saline	0.70±0.036	0.20±0.04	0.50±0.03
Liver damaged control	0.9% saline	2.73±0.11	1.46±0.06	1.10±0.05
Liver damaged Animal + <i>E. mysorensis</i> extract	100(mg/kg)	1.22±0.03	0.26±0.02	0.96±0.03
	200(mg/kg)	1.02±0.04	0.21±0.04	0.81±0.04
	500(mg/kg)	0.89±0.03	0.19±0.02	0.70±0.06
Standard Drug (Silymarin)	100(mg/kg)	0.79±0.06	0.18±0.05	0.61±0.05

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