

HEPATOPROTECTIVE ACTIVITY OF *ERIA MYSORENSIS* LINDL. (ORCHIDACEAE) PSEDOBULP 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 inturn 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 suggests 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: Hepatoprotective activity, *Eria mysorensis*, pseudobulb.

1. 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 of supply tribals collect many types of wild roots and tubers to supplement their meagre food available at home (Vidyarthi, 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 called for 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 of 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 vitamin. 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; Subramaniam and Pushpangadan, 1999). Thus liver diseases are some of the fatal disease in the world today. Therefore, many folk remedies from plant origin are tested for its hepatoprotective liver damage in experimental animal model. Carbon tetrachloride (CCl₄) induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts (Rubinstein, 1962 and Suja *et al.*, 2002).

2. 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.

2.1. Preparation of the extract

Plant material pseudobulb was washed with clean tap water to remove dirt and dried under shade to constant weight for 20 days. The pseudobulb 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.

2.2. 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 condition under natural light and dark schedule and were fed on standard laboratory diet. Food and water were given ad-libitum.

2.3. 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 CCl₄ model. Group I (normal control) received neither the plant extract nor CCl₄ for 72 hrs that is they receive food and water only.

2.4. 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.

2.5. 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 some biochemical parameters like Alanine aminotransferase (ALT/SGPT), Aspartate aminotransferase (AST/SGOT), cholesterol, bilirubin and glucose.

3. RESULTS AND DISCUSSION

The effect 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 in the group III treated with the standard drug, Silymarin (Fig. 1).

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 and Fig. 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.

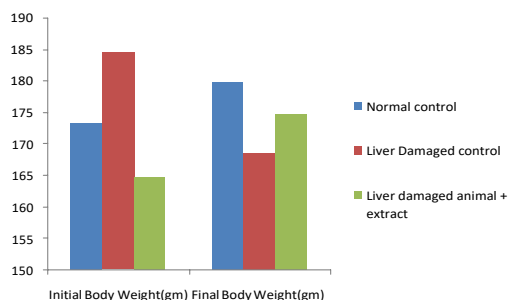
Estimating the activities of serum marker enzymes, like SGPT, SGOT, ALP can make the assessment of liver function when liver cell plasma membrane is damaged, a variety of enzyme normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful

quantitative marker of the extent and type of hepatocellular damage (Mitra *et al.*, 1998). The tendency of these enzymes to return to near normally in extract administered group is a clear manifestation of antihepatotoxic effects of the extract.

Table 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

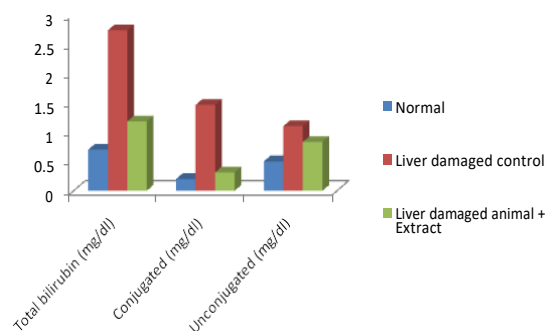
| Treatment | Dose | Initial Body Weight(gm) | Final Body Weight(gm) | Mean Weight Gain(G)/ Loss(L) (gm) | Percentage Difference |
|------------------------------|------------|-------------------------|-----------------------|-----------------------------------|-----------------------|
| Normal control | 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 + | 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 |
| <i>E. mysorensis</i> extract | 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 |

Fig. 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.



responsible for the significant hepatoprotective activity.

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



In accordance with these results, it may be confirmed 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,

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

| Groups | Dose | Parameters | | |
|------------------------------|-------------|-------------------------|--------------------|----------------------|
| | | 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 + | 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 |
| <i>E. mysorensis</i> extract | 500(mg/kg) | 0.89±0.03 | 0.19±0.02 | 0.70±0.06 |
| | 100(mg/kg) | 0.79±0.06 | 0.18±0.05 | 0.61±0.05 |

4. CONCLUSION

In summary, the current study demonstrated that ethanol extract showed a potent protective effect against CCl₄-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 therapeutics against the liver diseases.

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