

**EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *ASPARAGUS RACEMOSUS* ROOT AGAINST PARACETAMOL – INDUCED ACUTE LIVER INJURY IN RATS**

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**Summary**

Hepatoprotective activity of ethanol extract of *Asparagus racemosus* against paracetamol induced hepatic damage in albino rats was observed. In the present study the effect of ethanol extract of *Asparagus racemosus* on wet liver weight, bio chemical parameters such as SGPT, SGOT, ALP and Serum total bilirubin, anti oxidant studies such as SOD, CAT and Histopathological studies have been studied to find out the possible mechanism of hepatoprotection. It was observed that extracts of *Asparagus racemosus* has reversal effects on the levels of above mentioned parameters in paracetamol hepatotoxicity. The extract of *Asparagus racemosus* functions as a hepatoprotective agent and this hepatoprotective activity of *Asparagus racemosus* may be due normalization of impaired membrane function activity.

**Keywords:** *Asparagus racemosus*, glutathione, hepatoprotection, lipid peroxidation, paracetamol, serum marker enzymes.

**Abbreviations:** Ethanol extracts of *Asparagus racemosus* (EEAR), Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), Alkaline phosphatase (ALP), Catalase (CAT), Super oxide dismutase (SOD)

**Introduction**

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it 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 [1]. 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 & over-the-counter drug which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease [2][3]. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders.

It has been reported that 160 phytoconstituents from 101 plants have hepatoprotective activity [4]. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthines. Plant extracts of many crude drugs are also used for the treatment of liver disorders. Extracts of different plants of about 25 plants have been reported to cure liver disorders [5].

In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cell [6]. There are however, members of drugs employed in traditional system of medicine for liver affections [7]. Many formulations containing herbal extracts are sold in the Indian market for liver disorders. But management of liver disorders 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. Some of these plants have already been reported to possess strong antioxidant activity [8-10].

*Asparagus racemosus* Wild (Lilliaceae), commonly known as Shatavari is a perennial shrub, with a tuberous root-stock, stems covered with recurved spines, linear leaves arranged in a tuft, white flowers and sweet-scented appears in October. The plant occurs throughout India up to 1500 meters elevation. *Asparagus racemosus* is recommended in Ayurvedic texts for prevention and treatment of gastric ulcers as galatogogue and nervine tonic. The decoction of root has been used in blood diseases, diarrhoea, dysentery, cough, bronchitis and general debility [11-13]. Reports indicate that the pharmacological activities of root extracts include antiulcer [14], anti-tussive [15], antioxidant [16] and antibacterial activities [17].

From the traditional knowledge it is very clear that the plant *Asparagus racemosus* have the hepato protective activity [18]. But still no scientific and methodical investigation has so far been reported in literature regarding its action on liver. Therefore, the present investigation has been designed to study the possible mechanism of ethanolic extract of *Asparagus racemosus* root on the different parameter against paracetamol induced hepatic damage in albino rats.

### Materials and methods

**Plant material:** The roots of *Asparagus racemosus* used for the present studies were collected from poonoor in Calicut district of Kerala in India. The plant was identified, confirmed and authenticated by comparing with voucher specimen available at Calicut university herbarium, Department of botany, university of Calicut, Emerald by Botanist Dr. Pradeep AK. A voucher herbarium specimen was stored.

**Preparation of extracts:** Fresh roots of *Asparagus racemosus* were washed, shade dried, powdered, passed through a #60 mesh sieve and were extracted with alcohol (95% v/v) in a soxhlet apparatus by continuous heat extraction. The extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50°C. The alcohol extract was prepared in distilled water containing 2% v/v Tween 80 (as a suspending agent) for experimental purpose.

**Experimental animals:** Albino rats of either sex (150-200 gm) were used for the study. The animals were procured and housed in the animal house maintained under standard hygienic conditions, at 25 ± 1o C, humidity (60 ± 10%) with 12 hour day and night cycle, with food and water *ad libitum*. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC) of Bharathi College of pharmacy, Bharathinagara, Mandya. Studies were performed in accordance with the CPCSEA guidelines.

**Hepatoprotective activity:** The LD50 is >1g/kg. No toxic effects or mortality were observed with doses ranging from 50mg/kg to 1g/kg for four weeks. Acute and sub acute (15-30 days administration) toxicity studies did not detect any changes in vital organ function tests [19]. Hence hepatoprotective activity of alcohol extracts of *Asparagus racemosus* was studied by following methods.

#### **1. Paracetamol induced-hepatotoxicity:**

- Group A - Normal control
- Group B - Toxicant (paracetamol 500mg/kg, p.o.)
- Group C - Served as Standard (Silymarin 100 mg/kg, p.o)
- Group D - Ethanol extract of *Asparagus racemosus* root (150mg/kg, p.o)
- Group E - Ethanol extract of *Asparagus racemosus* root (250mg/kg, p.o)

**Experimental procedure:** Wistar rats of either sex weighing between 150-200 g were divided into five groups of six rats each. Group A was maintained as normal control, which was given distilled water only. Group B received paracetamol 500 mg/kg body wt by p.o. at every 72 h for 10 Days. Group C animals were treated with Silymarin (100 mg/kg p.o.) which served as standard. Groups D and E animals were treated with two different doses of alcohol extract of *Asparagus racemosus* (medium, high) respectively Group C, D and E were in toxicated with paracetamol (500 gm/kg) 1 h before the administration of Silymarin or extract for 10 days. The animals were then anesthetized using anesthetic ether, and blood collected by retro orbital puncture and biochemical parameters like ALT, AST, ALP, Total Bilirubin, were estimated. The animals were sacrificed by overdose of ether and autopsied. Livers from all animals were removed, washed with ice-cold saline, weighed. Small piece of liver tissue was collected and preserved in 10% formalin solution for histopathological studies. Livers of some animals were homogenized with ice-chilled 10% KCl solution and centrifuged at 2000 rpm for 10 min. Then the supernatant liquid was collected and the antioxidant parameters like Catalase and Super oxide Dismutase were estimated.

**Statistical analysis:** The values were expressed as mean  $\pm$  SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values  $< 0.05$  were considered as significant.

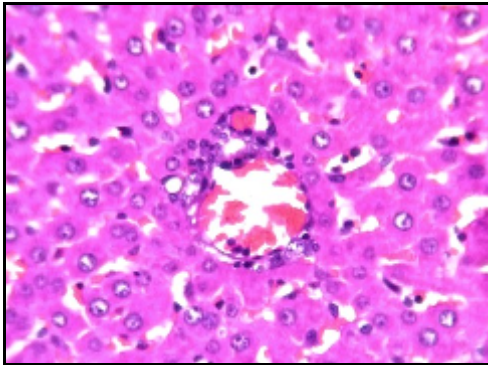
### Results

It was observed that the weight of the liver was increased in paracetamol-intoxicated rats but it was normal in drug-treated groups. And also the administration of paracetamol to the animals resulted in a marked increase in total bilirubin, Alanine aminotransferase (ALT/SGPT), Aspartate aminotransferase (AST/SGOT), Serum Alkaline Phosphatase (SALP) activities. The toxic effect of paracetamol 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 silymarin (Table 1). And also show a marked decrease in CAT and SOD levels. The toxic effect of paracetamol was controlled in the animals treated with the ethanolic extract by way of restoration of the levels of the liver anti oxidant parameters similar to that of the standard drug silymarin

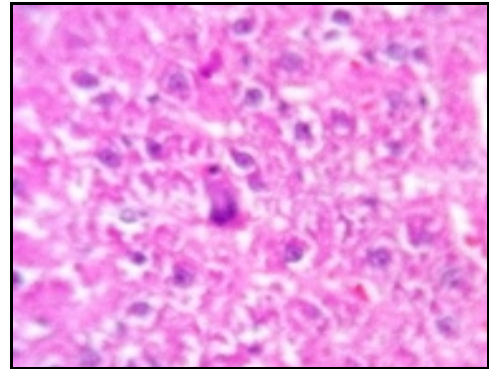
Parameters	Normal control	Toxicant control	Standard	EEAR 150 mg/kg	EEAR 250 mg/kg
WET LIVER WEIGHT	1.98 $\pm$ 0.180	4.48 $\pm$ 0.231	2.36 $\pm$ 0.103***	2.75 $\pm$ 0.104***	2.61 $\pm$ 0.116***
SGPT	28.42 $\pm$ 0.167	115.39 $\pm$ 1.480	48.91 $\pm$ 0.082***	84.48 $\pm$ 0.241**	67.16 $\pm$ 0.293**
SGOT	35.97 $\pm$ 0.419	162.67 $\pm$ 0.546	78.87 $\pm$ 0.717**	115.53 $\pm$ 0.576*	105.23 $\pm$ 0.55**
ALP	29.48 $\pm$ 0.438	178.37 $\pm$ 0.452	32.76 $\pm$ 0.305***	66.59 $\pm$ 1.589**	41.85 $\pm$ 0.676***
TOTEL BILIRUBIN	0.35 $\pm$ 0.009	1.58 $\pm$ 0.012	0.612 $\pm$ 0.05***	1.12 $\pm$ 0.029**	0.83 $\pm$ 0.013***
CAT	92.38 $\pm$ 0.446	27.05 $\pm$ 0.664	79.81 $\pm$ 4.79***	35.68 $\pm$ 0.709**	54.04 $\pm$ 0.62***
SOD	11.03 $\pm$ 0.581	3.47 $\pm$ 0.578	8.53 $\pm$ 0.157***	6.59 $\pm$ 0.157**	7.59 $\pm$ 0.056***

Values are expressed as mean  $\pm$  SEM (n=6) using one way ANOVA followed by Tukey Kramer's test. Where, \* represents significant at  $p<0.05$ , \*\* represents highly significant at  $p<0.01$ , \*\*\* represents very significant at  $p<0.001$

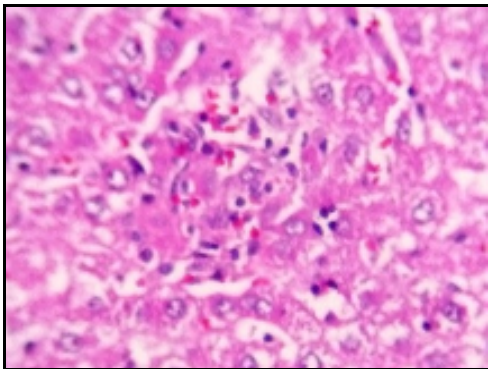
Histopathological examination of the liver section of the rats treated with toxicant showed intense Effaced architecture, Apoptotic hepatocytes and Congested central veins (fig.2). The rats treated with Silymarin (fig. 3) and extracts along with toxicant (fig.4 and fig.5) showed sign of protection against these toxicants to considerable extent as evident from formation of normal hepatic cards and absence of Effaced architecture, Apoptotic hepatocytes and Congested central veins.



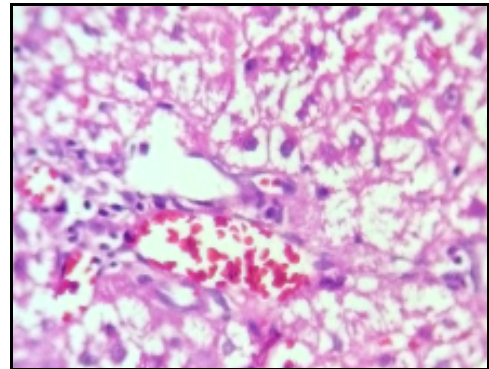
**Fig. 1** normal control treated group  
(Group A)



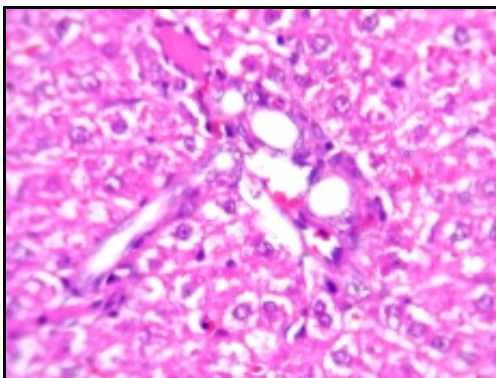
**Fig.2** paracetamol treated group  
(Group B)



**Fig.3** silymarine treated group  
(Group C)



**Fig..4** EEAR 150 mg/kg treated group  
(Group D)



**Fig..5** EEAR 250 mg/kg treated group  
(Group E)

### Discussion

Paracetamol is a common antipyretic agent, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses [20]. Protection against paracetamol-induced toxicity has been used as a test for potential hepatoprotective activity by several investigations [21]. The covalent binding of N-acetyl-pbenzoquinoneimine, an oxidation product of paracetamol, to sulfhydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity have been reported earlier [22] which is one of the most important natural antioxidants of the hepatocytes, renders the cell remarkably susceptible to oxidative stress [23].

In the assessment of liver damage by paracetamol, the determination of enzyme levels such as SGOT and SGPT is largely used. Necrosis or membrane damage releases the enzyme in to circulation; therefore, it can be measured in serum. A high level of SGOT indicates liver damage such as that due to viral hepatitis as well as cardiac infarction and muscle injury. SGPT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [24]. Serum ALP and bilirubin level on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary presence [25].

This present study evaluated the hepatoprotective activities of EEAR in paracetamol induced liver toxicity. Acute administration of paracetamol produced a marked elevation of the serum levels of SGOT, SGPT, ALP and total bilirubin in treated animals (Group B to E) when compared with that of control group (Group A). Treatment with EEAR at a dose of 150mg/kg and 250mg/kg significantly reduced the elevated levels of the enzymes. Treatment with EEAR decreased the SGOT, SGPT levels towards the respective normal value that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by paracetamol. The above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells. Effective control of alkaline phosphatase (ALP) and bilirubin levels points towards an early improvement in the secretory mechanism of the hepatic cell.

Catalase (CAT) and Super oxide dismutase (SOD) are the most imperative antioxidants in the human body. They play a chief role in scavenging oxygen free radicals, such as superoxide anion radicals ( $O_2^-$ ), hydroxyl radicals (OH), supplementary free radicals (FRs) as well as singlet oxygen ( $O_2$ ), hydrogen peroxide ( $H_2O_2$ ) and other reactive oxygen species (ROS) that are disproportionate in the human body, thereby shielding biological membranes of cells against oxidative and lipoperoxidative damages. In the present study, lower levels of CAT and SOD were observed in the paracetamol control group. Groups treated with EEAR showed the significant amplification in the concentration of CAT and sod as compared to paracetamol control group as like the standard silymarin treated group. Hence EEAR shows an antioxidant property which is also an evidence for its hepatoprotective activity.

Histological examination of the liver sections reveals that the normal liver architecture was disturbed by hepatotoxins intoxication which shows Apoptotic hepatocytes and Congested central veins. Treatment of rat with EEAR exhibit regenerative changes like normal appearance

of hepatic cells with nucleus, less vacuolization and fatty change supplements the protective effect of the extract. However the results strongly suggest an initiation of the process of liver regeneration, which is also evident from the various biochemical parameter results.

The preliminary phytochemical studies revealed the presence of flavonoids in ethanolic extract of *Asparagus Racemosus* willd. Various flavonoids have been reported for their hepatoprotective activity [26]. So the hepatoprotective effect of *Asparagus Racemosus* willd. may be due to its flavonoids content.

### **Conclusions**

In conclusion, the result of this study demonstrated that ethanolic extract of *Asparagus Racemosus* willd (150 mg/kg and 250 mg/kg) shows significant hepatoprotective activity against paracetamol induced liver damage rats. Hence the present study justified the traditional use of *Asparagus Racemosus* willd in the treatment of liver diseases.

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