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Research Article

Hepatoprotective Activity of "*Asparagus Racemosus* Root" On Liver Damage Caused By Paracetamol in Rats

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ABSTRACT

Aqueous extract of *Asparagus racemosus* root (AEAR) was evaluated for its hepatoprotective activities in rats. The plant extract (150 and 250 mg/kg, p.o.) showed a remarkable hepatoprotective and antioxidant activity against paracetamol-induced hepatotoxicity as judged from the wet liver weight, serum marker enzymes, antioxidant levels and histopathological studies on liver tissues. Paracetamol-induced a significant rise in wet liver weight, aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin with a reduction of superoxide dismutase (SOD) and catalase. Treatment of rats with different doses of plant extracts (150 and 250 mg/kg) significantly altered serum marker enzymes and antioxidant levels to near normal against paracetamol-treated rats. The activity of the extracts was comparable to the standard drug, silymarin (100 mg/kg, p.o.). Histopathological changes of liver sample were compared with respective control. Results indicate the hepatoprotective properties of AEAR against paracetamol-induced hepatotoxicity in rats.

INTRODUCTION

The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction [1]. The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals. Inspite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate [2]. And Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2,50,000 new cases each year.

In India, about 40 polyherbal commercial formulations reputed to have hepatoprotective action are being used. It has been reported that 160 phytoconstituents from 101 plants have hepatoprotective activity [3].

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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 [4].

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. Hence, people are looking at the traditional systems of medicine for remedies to hepatic disorders with significant hepatoprotective action.

Asparagus racemosus (Liliaceae) is a popular vegetable consumed in many parts of the world and grows naturally throughout India, Asia, Australia and Africa. It is commonly used for the treatment of diarrhoea, dysentery, rheumatism, nervous breakdown, and is thought to be an aphrodisiac [5]. The root of the plant has also been claimed by traditional healers to possess antidiabetic properties.

Studies on the extracts of *A. racemosus* have revealed a wide range of biological activities. These include antimutagenic, antitumor, antifungal [6,7], diuretic [8] Antiulcer activity [9] and immunostimulatory effects [10]. *A. racemosus* has been considered to be a lactogogue in lactational inadequacy [11] and useful to decrease post-operative adhesions and it also have Anticandidal activity [12]. The protective effects of *A. racemosus* against the myelosuppression with single and multiple doses of cyclo-phosphamide have also been demonstrated [10]. Asparagus roots inhibited the growth of human leukaemia HL-60 cells [13].

From the traditional knowledge it is very clear that the plant *Asparagus racemosus* have the hepato protective activity [14]. 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. About 1000 gm of the powdered material was subjected to cold maceration for 7 days with continuous stirring. The extract was filtered and the filtrate was concentrated at reduced pressure by Rotary Vaccum Evaporator. The aqueous 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 [15]. 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 100mg/kg, p.o)
Group D - Aqueous extract of *Asparagus racemosus* root (150mg/kg, p.o)
Group E - Aqueous 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.

Table 1: Effect of aqueous extract of Asparagus racemosus root on Paracetamol-induced hepatotoxicity

Parameters	Normal control	Toxicant control	Standard	AEAR 150 mg/kg	AEAR 250 mg/ kg
WET LIVER WEIGHT	1.98 ± 0.180	4.48 ± 0.231	2.36±0.103***	3.18±0.172**	2.71 ±0.147***
SGPT	28.42±0.167	115.39±1.480	48.91±0.082***	87.6±0.3016**	74.056±0.240**
SGOT	35.97±0.419	162.67 ±0.546	78.87±0.717**	124.72±0.723*	111.93±0.62**
ALP	29.48±0.438	178.37±0.452	32.76±0.305***	84.048±0.4852*	52.32±0.725**
TOTEL BILIRUBIN	0.35±0.009	1.58±0.012	0.612±0.05***	1.42±0.026*	1.12±0.012**
CAT	92.38±0.446	27.05±0.664	79.81±4.79***	34.46±0.251**	45.98±0.159**
SOD	11.03±0.581	3.47±0.578	8.53±0.157***	5.73±0.078*	7.12±0.081**

Values are expressed as mean ± 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

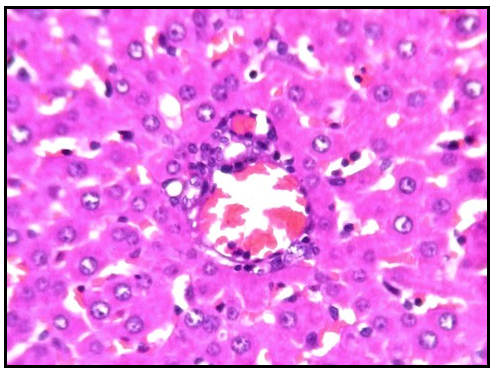

Figure 1: Normal control group (Group A)

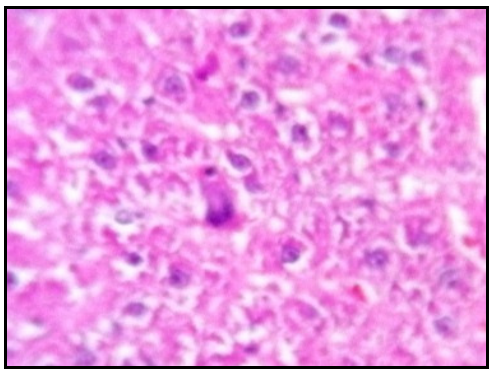

Figure 2: Paracetamol treated group (Group B)

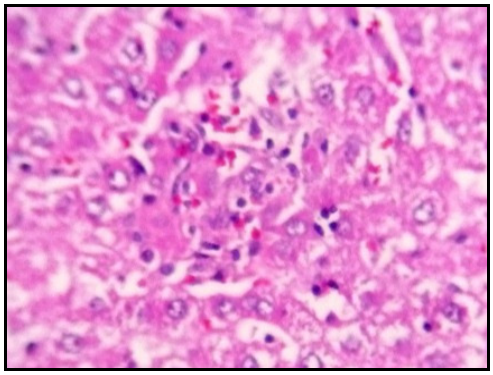

Figure 3: Sylimarine treated group (Group C)

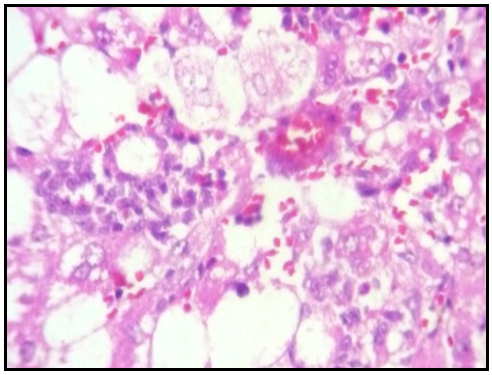

Figure 4: AEAR 150 mg/kg treated group (Group D)

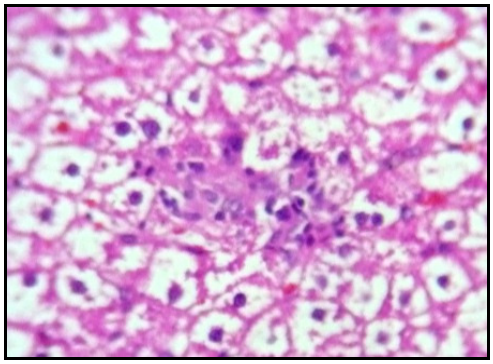

Figure 5: EEAR 250 mg/kg treated group (Group E)

Statistical analysis:
The values were expressed as mean ± 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

The results of hepatoprotective effect of aqueous extracts on paracetamol-intoxicated rats are shown in Table 1. It was observed in the paracetamol intoxicated group total bilirubin, Alanine aminotransferase (ALT/SGPT), Aspartate aminotransferase (AST/SGOT) and Serum Alkaline Phosphatase (SALP) activities were significantly increased as compared to control group. The elevated activities of serum AST and ALT were significantly reduced in the animal groups treated with aqueous extracts. The activities of SOD and CAT were significantly reduced in the paracetamol -intoxicated group, while they were significantly elevated in the groups pretreated with aqueous extract.

Histopathological examination of the liver section of the rats treated with toxicant showed intense Effaced architecture, Apoptotic hepatocytes and Congested central veins (fig.2) compare to normal control group (fig.1). 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.

DISCUSSION

In case of toxic liver, Wet liver weight was increased. Toxicants induced hepatotoxicity produce fatty changes and also it is observed that there is a fall in serum lipids in another series of experiments. In chronic alcoholics, ethanol produces hepatomegaly. In this case water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass [16]. It is reported that liver mass is important parameters in ascertaining the hepatoprotective effect of the drugs. Treatment with aqueous extracts of the roots of *Asparagus racemosus* significantly reduced the wet liver weight of animals and hence it possesses statistically significant hepatoprotective activity.

Serum AST and ALT activities were used as a marker of liver damage. Paracetamol produces an experimental damage to the liver cells. 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 [17] which is one of the most important natural antioxidants of the hepatocytes, renders the cell remarkably susceptible to oxidative stress [18]. The reduction of AST and ALT activities by the extracts is an indication of repair of hepatic tissue damage induced by paracetamol. The aqueous extract induced suppression of increased ALT and AST activities. Thus, administration of AEAR revealed hepatoprotective activity against the toxic effect of parcetamol, which is also supported by histological studies.

In case of toxic liver, bilirubin levels are elevated. Hyperbilirubinemia can result from impaired hepatic uptake of unconjugated bilirubin. Such a situation can occur in generalized liver cell injury. Certain drugs (e.g., rifampin and probenecid) interfere with the net uptake of bilirubin by the liver cell and may produce a mild unconjugated hyperbilirubinemia [19]. Bilirubin level rises in diseases of hepatocytes, obstruction to biliary excretion into duodenum, in haemolysis and defects of hepatic uptake and conjugation of bilirubin pigment such as in Gilbert's disease [20]. In the current study treatment of animals with aqueous extract of *Asparagus racemosus* root significantly decrease the levels of in serum which is an indication of hepatoprotective activity.

Oxidative stress induced due to the generation of free radicals and/or decreased antioxidant level in the target cells and tissues has been suggested to play an important role in carcinogenesis [21]. During cell membrane damage, various enzymes leak down to the circulatory fluid and their assessment in serum serves as markers in clinical studies. SOD is the first antioxidant enzyme to deal with oxyradicals by accelerating the dismutation of superoxide to hydrogen peroxide, while CAT is a peroxisomal hem protein that catalyses the removal of hydrogen peroxide formed during the reaction catalyzed by SOD. Thus, SOD and CAT acts mutually supprtive anti oxidative enzymes, which provide protective defense against reactive oxygen species [22].

The present study revealed that SOD and CAT activities decreased in parcetamol injected animal, which may be due to altered antioxidant status. This is in accordance with results that indicated a decreased CAT in paracetamol injected animals may be due to the utilization of antioxidant enzymes in the removal of released H_20_2 released [23]. SOD and CAT activities increased significantly in the treated group versus the untreated animals.

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 AEAR 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 [24]. 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 aqueous 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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