International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (1): 541-547
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Antioxidant Activity Effect of *Asparagus Racemosus* Whole Plant Extracts in Albino Rats

R. Panneerselvam[a] and S. Palani[b*]

[a] Research Centre, Manonmanium Sundaranar University, Tirunelveli, Tamil Nadu, India
[b] Arunai Engineering College. Thiruvannamalai, Tamil Nadu, India

Received: 10 Oct 2018 / Accepted: 8 Nov 2018 / Published online: 01 Jan 2019
Corresponding Author Email: spalanitvm@gmail.com

Abstract

*Asparagus racemosus (*AR) is one of the oldest medicinal herbs of India, is an ingredient of Indian Ayurvedic drug used for the treatment of liver disorders. In Indian traditional system of medicine, the fruits are also used in the treatment of hepatic disorders by tribal people. Acetaminophen (APAP) is used as an analgesic which produces liver and kidney necrosis in mammals at high doses. The aim of the present study was to investigate the hepatoprotective and antioxidant activities of the ethanol extract of *Asparagus racemosus* at two doses level of 250 mg/kg and 500 mg/kg B/W on acetaminophen- induced hepatotoxicity in rats. The results of study showed that APAP significantly increased serum levels of GOT and GPT, ALP and total bilirubin. In addition, the ethanol extract of TP significantly ($p<0.01$) elevated the decreased level of antioxidant enzymes such as superoxide dismutase (SOD) catalase (CAT), glutathione peroxidase (GPX), glutathione-s-transferase (GST) and reduced glutathione (GSH). Histological analysis of the liver of these rats revealed marked necro-inflammatory changes by APAP and ethanol extract of TP attenuated the necro-inflammatory changes in the liver. The activity of ethanol extract of *Asparagus racemosus* at 500 mg/kg B/W was comparable to the standard drug silymarin (25mg/kg B/W). This study reveals that ethanol extract of Ethanolic Extract of Asparagus Racemosus (whole plant) showed significant hepatoprotective and antioxidant properties from APAP induced liver damage and oxidative stress.

Keywords
Asparagus racemosus, SOD, APAP and GSH

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INTRODUCTION

Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. APAP is an antipyretic analgesic drug that is available over-the-counter, and an overuse of APAP

can cause overproduction of ROS during formation of N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P450 [1]. Many studies have demonstrated that overproduction of Reactive oxygen species (ROS) [such as super oxide anion, hydroxyl radical and hydrogen peroxide] can further aggravate the oxidative stress and the result is a unifying mechanism of injury that occurs in many developments of clinical disease processes, such as heart disease, diabetes, liver injury, cancer, aging, etc. [2-6]. Maintaining the balance between ROS and antioxidant enzymes (especially superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)) is therefore crucial and could serve as a major mechanism in preventing damage by oxidative stress. This balance has been suggested to play an important role in drug toxicity, such as from acetaminophen (APAP) [7]. This mechanism has been suggested to participate in the development of oxidative stress and injury in APAP-induced hepatotoxicity [8]. that arises from infectious diseases, oxidative damages, *etc*.

MATERIALS AND METHODS

Plant material

The whole plant of *Asparagus racemosus* collected from Nilgiri hills, Ooty, Tamilnadu region and authenticated through Government Arts College, Ooty. Voucher specimen (AECBT-01/2011-2012) has been retained in the Anna bioresearch foundation, Arunai Engineering college, Thiruvannamalai, Tamilnadu, India.

Extraction

The whole plant was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol (90% v/v) in soxhlet apparatus at 60°C [17]. The solvent was completely removed by rotary vacuum evaporator. The extract was freeze dried and stored in vacuum desiccators.

Animals

Studies were carried out using Wistar albino male rats (150-200g), obtained from Indian Veterinary Preventive Medicine (IVPM), Ranipet, Tamilnadu, India. The animals were grouped and housed in polyacrylic cages (38 x 23 x10 cm) and maintained under standard laboratory conditions (temperature 25 ± 2ºC) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by Poultry Research Station, Nandhanam, India and fresh water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the University Animal's Ethical Committee (NO.1011/C/06/CPCSEA).

Acute toxicity study

The safety study was carried out using OECD guide lines No. 423. Three rat of same age group and weight were taken for a single dose of ethanol extract of *Asparagus racemosus* up to the highest dose of 2000 mg/kg orally. The animals were observed for 1 hr continuously and then hourly up to 4 hr and finally after every 24 h up to 15 days for any mortality or gross behavioral changes [18]

Experimental treatments

Animals were divided into five groups of six animals each. Group I treated with vehicle (distilled water) was kept as normal. Group II treated with a single dose of acetaminophen (AAP) of 500mg/kg body weight was kept as toxin control. Group III and IV were treated with ethanol extract of Asparagus racemosus 250 mg/kg and 500 mg/kg body wt plus AAP and Group V were fed with standard drug silymarin 25 mg/kg daily for seven days. The extract was administered by oral gavages 1 h before AAP administration [19].

Preparation of serum from blood

After 24 h, animals were sacrificed by chloroform anesthesia. The blood samples of each animal were taken and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 600× g for 15 min and analyzed for various biochemical parameters including serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT) [20], alkaline phosphatase (ALP) [21], bilirubin [22] and total protein [23].

Preparation of liver homogenate

Hepatic tissues were homogenized in KCl [10 mM] phosphate buffer (1.15%) with ethylene-diamine tetra acetic acid (EDTA; pH 7.4) and centrifuged at 12,000×g for 60 min. The supernatant was used for assay of the marker enzymes (glutathione peroxidase, glutathione-s-transferase, superoxide dismutase and catalase), reduced glutathione, thiobarbituric acid reactive substances (TBARS) content, and protein estimation.

Biochemical estimation of markers of oxidative stress

SOD activity was determined according to previous report [25]. CAT activity was determined from the rate of decomposition of H_2O_2 by the reported method [26]. GPx activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3 [27]. Glutathione reductase activity was

assayed according to previous reports [28]. Protein content in the tissue was determined by earlier method reported [29-30], using bovine serum albumin (BSA) as the standard.

Histopathological study

On completion of closing regimen animals were sacrificed and the liver dissected out. Paraffin sections were prepared for histological examination and following standard procedure [31]. Hematoxylin-eosin stained sections were observed.

Statistical analysis

The obtained results were analyzed for statistical significance using one-way ANOVA followed by Dunnet test using the graph pad statistical software for comparison with control group and acetaminophen treated group. $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Acute toxicity study

Rat fed with ethanol extract of *Asparagus racemosus* up to 2000 mg/kg, exhibited no mortality or any sign of gross behavioral changes which were observed initially for 72 h and finally up to 15 days.

The effect of *Asparagus racemosus* on serum marker enzymes is presented in table 1 and fig 1, 2 & 4. The serum levels of GOT & GPT, ALP and total bilirubin were marked significantly ($p< 0.01$) elevated and that of protein levels significantly ($p< 0.01$) decreased in acetaminophen treated animals, indicating liver damage. Administration of ethanolic extract of *Asparagus racemosus* at the doses of 250 and 500 mg/kg remarkably ($p< 0.05$; $p< 0.01$) prevented hepatotoxicity.

Acetaminophen treatment caused a significant ($P<0.01$) decrease in the level of SOD, catalase, GPX and GST in liver tissue when compared with control group. The treatment of *Asparagus racemosus* at the doses of 250 and 500 mg/kg resulted in a significant ($P<0.05$; $P<0.01$) increase of SOD, catalase, GPX and GST when compared to Group II (Table 2;). The standard drug, silymarin treated animals also showed a significant ($P<0.01$) increase in antioxidant enzymes levels compared to Group II. Morphological observations showed an increased size and enlargement of the liver in acetaminophen treated groups. These changes were reversed by treatment with silymarin and also *Asparagus racemosus* with the two different groups, that administered two different doses.

Histopathological profile of the normal animal showed normal hepatocytes with well-preserved cytoplasm and there was no sign of inflammation, which has been illustrated in Fig 5 (a). The acetaminophen treated animals showed severe centrilobular necrosis and fatty infiltration (Fig 5 b). Treatment with different doses of ethanol extract of *Asparagus racemosus and* silymarin produced mild degenerative changes and absence of centrilobular necrosis when compared with control [Fig 5 (c), 5 (d) & 5 (e)]. All these results indicate a hepatoprotective potential by the ethanol extract of *Asparagus racemosus*

The safe evaluation study of ethanol extract of *Asparagus racemosus* showed that no mortality of rat occurred, at a limit dose of 2000 mg/kg body weight given. This is an indication that the extract has low acute toxicity. According to [32], substances with LD_{50} of 1000 mg/kg body weight/oral route are regarded as being safe or of low toxicity.

Acetaminophen a widely used antipyretic analgesic drug produces acute hepatic damage on accidental over dosage. The hepatic damage is established that, a fraction of acetaminophen is converted via the cytochrome P450 pathway to a highly toxic metabolite; N–acetyl–p–benzoquinamine (NAPQI) [1] which is normally conjugated with glutathione and is excreted in urine. In overdose situations, however, glutathione levels are exhausted, and NAPQI can directly modify susceptible protein residues in what is widely believed to be the first step in a cascade of biochemical events leading to hepatocyte death [33 -40].

In the present study, rats treated with AAP developed a **s**ignificant hepatic damage and oxidative stress, resulted in a marked increase in serum SGOT, SGPT, SALP and total bilirubin levels. This is indicative of cellular leakage and loss of functional integrity of cell membrane in liver [41]. However, the total protein level decreased. There was a significant ($P<0.01$) restoration of these enzyme levels on administration of the ethanol extract in a dose dependent manner and also by silymarin at a dose of 25 mg/kg.

The reversal of increased serum enzymes in acetaminophen induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [42-43]. Effective control of ALP, bilirubin and total protein levels points towards an early improvement in the secretary mechanism of the hepatic cells, as well as repair of hepatic tissue damage caused by APAP. This indicates the anti-lipid per oxidation

and/or adaptive nature of the systems as brought about by plant extract against the damaging effects of free radical produced by APAP.

Decrease in enzyme activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury [44]. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. *Asparagus racemosus* causes a significant increase in hepatic SOD activity and thus reduces reactive free radical induced oxidative damage to liver.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [45]. Therefore, reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. A higher dose (500 mg/kg) of *Asparagus racemosus* and silymarin increases the level of CAT.

Both reductions of GPX &GSH activity in AAP-treated rats as observed in this study indicate the damage to the hepatic cells. Administration of *Asparagus racemosus extract* promoted the reactivation of hepatic glutathione reductase enzyme in AAP-treated rats. The restoration of GSH level after the administration of plant extract to such AAP treated rats is due to the protective effect of the ethanol extract.

Urea, uric acid and creatinine were elevated and after treatment their level reduced to nearing normal [23].

Severe centrilobular necrosis and fatty infiltration in hepatocytes was produced by acetaminophen. Treatment with different doses of ethanolic extract of *Asparagus racemosus* produced only mild degenerative changes and absence of centrilobular necrosis, indicating *Asparagus racemosus* treatment significantly rescued these signs of inflammation and necrosis, suggesting that Asparagus *racemosus* treatment conferred hepatoprotectivity.

CONCLUSION

The ethanolic extract of *Asparagus racemosus* significantly protects against liver injuries as well as oxidative stress, resulting in improved serum biochemical parameters such as SGOT, SGPT and SALP. The reduced levels of parameters of SOD, CAT, GSH, GPX, and GST in acetaminophen-treated rats were significantly increased by treatment with ethanol extract of *Asparagus racemosus*. Urea, uric acid and creatinine were elevated and after treatment their level reduced to nearing normal. EEAR at 500mg/kg exhibits potent pharmacological activity and holds significant therapeutic potential. Further studies to characterize the active principles and to elucidate the mechanism are in progress.

Table 1. Effect of Ethanolic Extract of *Asparagus Racemosus* (EEAR) on serum enzymes (ALT, AST and ALP), total bilirubin and total protein on acetaminophen (APAP) induced hepatotoxicity in rats.

Groups and Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Total Protein (mg/dl)
Group 1 Control (NaCl 0.9% w/v)5ml/kg	44.50± 1.47	48.26± 2.73	14.03± 0.45	1.65± 0.05	7.47± 0.18
Group 2 Acetaminophen (750mg/kg)	97.25± 4.28 ^{a,**}	98.25±2.75^{a,**}	28.71± 1.23 ^{a,**}	6.44± 0.22 ^{a,**}	5.09± 0.19 ^{a,**}
Group 3 EEAR + Acetaminophen (250mg/kg+750mg/kg)	87.83± 2.00 ^{b,*}	88.91± 2.38 ^{b,*}	24.52± 0.55 ^{b,*}	5.55± 0.07 ^{b,*}	5.21± 0.15 ^{b,*}
Group 4 EEAR + Acetaminophen (500mg/kg+750mg/kg)	79.06± 1.66 ^{b,**}	71.65±2.47^{b,**}	18.36± 0.29 ^{b,**}	3.41± 0.31 ^{b,**}	5.43± 0.09 ^{b,**}
Group 5 Silymarin + acetaminophen (25mg/kg +750mg/kg)	60.23± 2.59 ^{b,**}	54.98±1.10^{b,**}	16.01± 0.43 ^{b,**}	1.90± 0.05 ^{b,**}	6.49± 0.13 ^{b,**}

Values are expressed mean ± S.D for six rats in each group. a as compared with control, b As compared with APAP, ** represents *P<0.001*, * represents *P<0.01*.

Table 2. Effect of Ethanolic Extract of Asparagus Racemosus (EEAR) on antioxidants levels (SOD, CAT, TBARS, GSH, GPx and GST) of liver homogenate in acetaminophen induced heapatotoxicity in rats

Groups and treatment	SOD (units of activity/mg protein)	CAT (micromoles of H_2O_2 decomposed/mg protein/min)	TBARS (nanomoles of MDA formed/mg protein/h)	GSH (µg/mg protein)	GPx (nmol of GSH oxidized/min/mg protein)	GST (Units/mg protein)
Group 1 Control (NaCl 0.9% w/v)5ml/kg	0.93± 1.47	23.03±4.66	120.83±9.41	7.14±0.95	6.82±0.54	0.37±0.05
Group 2 Acetaminophen (750mg/kg)	0.66± 0.19 [a, **]	6.49± 1.2 [a, **]	187.24± 11.2 [a, **]	1.55± 0.23 [a,**]	4.33± 0.22 [a, **]	0.16± 0.28 [a, **]
Group 3 EEAR + Acetaminophen (250mg/kg+750mg/kg)	0.74± 0.15 [b, *]	14.51± 2.00 [b, *]	134.04± 9.8 [b, *]	4.75± 0.55 [b, *]	5.06± 0.07 [b, *]	0.29± 0.05 [b, *]
Group 4 EEAR + Acetaminophen (500mg/kg+750mg/kg)	0.84± 0.09 [b, **]	20.83± 1.66 [b, **]	111.57± 9.47 [b, **]	5.26± 0.29 [b, **]	6.13± 0.31 [b, **]	0.34± 0.09 [b, **]
Group 5 Silymarin + acetaminophen (25mg/kg +750mg/kg)	1.02± 0.13 [b, **]	21.28± 1.23 [b, **]	112.82± 1.10 [b, **]	7.48± 0.43 [b, **]	8.04± 0.05 [b, **]	0.40± 0.03 [b, **]

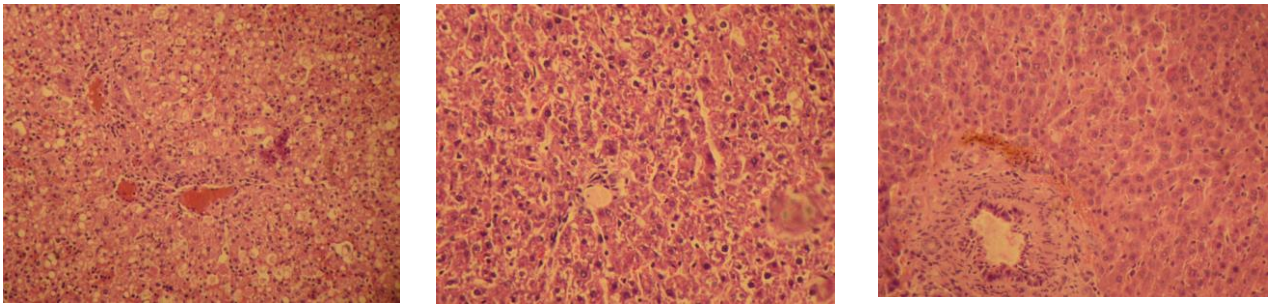
Values are expressed mean ± S.D for six rats in each group. a as compared with control, b as compared with APAP, ** represents *P<0.001*, * represents *P<0.01*.

Table 3. Effect of treatment with Ethanolic Extract of Asparagus Racemosus (EEAR) on the serum Urea (mg/dl), uric acid (mg/dl) and creatinine levels (mg/dl) in rats with APAP-induced hepatotoxicity

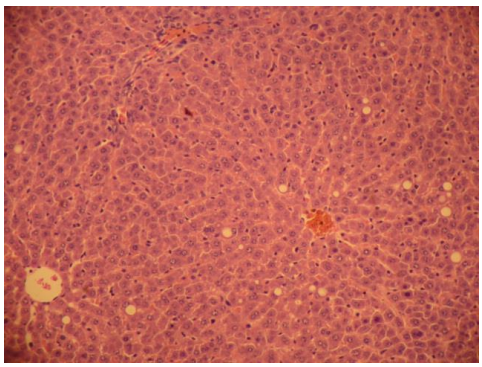
Groups and treatment	Group 1 Control (NaCl 0.9% w/v)	Group 2 APAP (750 mg/kg)	Group 3 EEAR (250mg/kg) + APAP (750mg/kg)	Group 4 EEAR (500mg/kg) + APAP + 750mg/kg)	Group 5 MV (500mg/kg) Silymarin+ acetaminophen
Urea(mg/dl)	26.88±0.24	60.42±0.52 [a,**]	39.18±0.42 [b, *]	32.44±0.32 [b, **]	25.13±0.24
Uric acid(mg/dl)	4.75±1.32	12.23±2.84 [a,**]	9.64±1.56 [b, *]	7.26±1.02 [b, **]	5.82±1.42
Creatinine(mg/dl)	0.8±0.32	1.48± 0.24 [a,**]	1.1±0.32[b, *]	0.93± 0.12 [b, **]	0.82±0.62

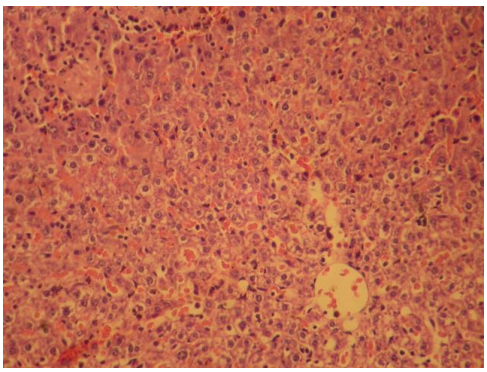
Values are expressed mean ± S.D for six rats in each group. a as compared with control, b As compared with APAP, ** represents *P<0.001*, * represents *P<0.01*.

A **B.** **C.**



D. **E**





Liver Histology of control and experimental rats (40X)
(A) Normal liver histology.
(B) APAP fed rat liver shows infiltration with inflammatory cells, micro and macro vesicular fatty changes.
(C) APAP +EEAR (250 mg/body weight) administrated rat hepatocytes shows reduced abnormalities such as vesicular fatty changes and inflammation.
(D) APAP + EEAR (500 mg/body weight) supplemented hepatocytes shows the normal appearance
(E) APAP + Silymarin supplemented hepatic cells shows the recovered changes.

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