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RESEARCH ARTICLE

HEPATOPROTECTIVE ACTIVITY OF *ASPARAGUS RACEMOSUS* ROOT EXTRACT ON LIPOPOLYSACCHARIDE INDUCED OXIDATIVE STRESS IN RATS

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Abstract

Objective: Lipopolysaccharide (endotoxin) produces an inflammatory condition leading to multiple organ failure. LPS most potent bacterial products are used for induction of host oxidative stress responses and liver injury.
Methods: Present study was undertaken to investigate the effect of *Asparagus racemosus* Willd. root extract in lipopolysaccharide (LPS) induced oxidative stress in rats by measuring oxidative stress markers, nitric oxide, liver function test and cytokines.
Results: The obtained data showed that LPS administration significantly reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT), total cholesterol (TC) and albumin (ALB). There was significant increase in malondialdehyde (MDA), cytokines activity, serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphate (ALP), total bilirubin (TB) and nitric oxide (NO).
Conclusion: The methanolic extract of *Asparagus racemosus* (MEAR) administration significantly ($p<0.05$) reduced LPS-induced oxidative stress by normalizing liver GSH, SOD, CAT, MDA, NO, cytokines and liver function markers. MEAR significantly increased ALB and TC level. Results suggest that MEAR protects the liver against liver toxicity induced by LPS.
Keywords: *Asparagus racemosus*, cytokine, hepatoprotective, lipopolysaccharide, oxidative stress, quercetin**.**

INTRODUCTION

Lipopolysaccharide (LPS), gram-negative bacterial endotoxin induced hepatic failure has lead to high mortality. The severity of subsequent organ damage might depend on the difference between excess production of ROS and antioxidant defenses[1-3]. LPS binds to liver proteins, producing oxygen free radicals and proinflammatory cytokines[4]. Release of these toxic mediators is the contributing factor to most of LPS toxicity in the liver and in the systemic circulation[5-6].
LPS after binding to immune cells initiate a cascade of events that up-regulate expression of the inflammatory cytokines including TNF-α, IL-6 and IL-1β. TNF-α and other cytokines stimulate the production of reactive oxygen species (ROS) and reactive nitrogen intermediates (RNIs) by activated macrophages causing liver damage[7]. The oxidative stress generated induces a rapid alteration in the antioxidant systems by depleting the cellular stores of endogenous antioxidants such as GSH, SOD and CAT[8]. The liver plays very important role in the defense against LPS induced toxicity. *Asparagus racemosus* has been scientifically investigated in experimental animals for their anti-amnesic, diuretic, hypoglycaemic and antioxidant activity[9]. Earlier researchers have reported several phytoconstituents in plants viz. steroidal saponins, flavonoids, terpenoids, alkaloids, phenolics, carbo-hydrates, etc[10].
The aim of the present study was to assess the liver damage in LPS administered rats and the hepatoprotective effect of root of *A. racemosus*.

MATERIALS AND METHODS

Plant material and extract MEAR preparation

Root of *A. racemosus* Willd. was collected from Hamdard dawakhana, Lucknow in 2011. The plant material was identified and authenticated by a taxonomist of National Botanical Research Institute (NBRI) Lucknow and the voucher specimen number NBRI-SOP-202 was deposited in the departmental herbarium. The plant *A. racemosus was* air-dried and pulverized. The powdered material (500 gm) was packed in muslin cloth and subjected to soxhlet extractor with methanol for continuous hot extraction for 72 hrs separately. There after methanolic extract of plant was filtered through Whatman paper no. 42 and the resultant filtrates was concentrated under reduced pressure and finally vacuum dried[11]. The yield of the methanolic extract was 10.4%.

Animals

Adult male Sprague Dawley (SD) rats weighing 150–180 g were used in this study. They were kept in polyacrylic cages in group of 7 and maintained under standard housing condition (room temperature 25±1°C and humidity (60–65%) with 12 h light and dark cycle. The food and water were available ad libitum. The animals were procured from the Division of Laboratory Animal, Central Drug Research Institute, Lucknow, India. Experiments were performed as per internationally ethical standards, after obtaining clearance from animal ethics committee of the Integral University under Committee for the Purpose of Control and Supervision of Experiments on Animals, CPCSEA No-IU/Pharm/Ph.D/CPCSEA/12/03. Each group (control as well as treated) consists of 6 rats.

Drugs and Chemicals

Thiobarbituric acid (TBA), 2,6,-di-tert-butyl-4-hydroxy -toluene (BHT), trichloroacetic acid (TCA), Hydrogen peroxide (H_2O_2), EDTA, Tris buffer, Potassium dihydrogen orthophosphate, Disodium hydrogen ortho phosphate were obtained from CDH, Mumbai. NADPH; DTNB were obtained from Hi Media, Mumbai. Cytokine ELISA kits were obtained from e Bioscience and Cayman Chemical USA. Lipopolysaccharide Serotype E. coli 0111:B4 were obtained from Sigma Chemicals, USA. Quercetin was obtained from Total herb solution Mumbai, India.

Toxicity studies

An acute toxicity study was performed for *A. racemosus* according to the Organisation for Economic Co-operation and Development guidelines by acute toxic classic method[10]. Three female (Sprague dawley) SD rats were used for each step in this study. The animals were fasted for overnight with only water available, after which the extracts were administered intragastrically at different doses of 50 and 300 mg/kg. Food and water was withheld for a further 1-2 h after drug administration. Rats were closely observed for the initial 4 h after administration, and then once daily for 14 days to observe mortality. If mortality occurred in two of the three animals at any dose, then this dose was assigned as a toxic dose. If mortality occurred in one animal then the same dose was repeated to confirm the toxic dose. If mortality did not occur, the procedure was repeated for further higher doses, i.e. 2000 mg/kg.

Treatments

Animals were randomly divided into seven groups as follows:-

Group I (control group): 1% Carboxy methyl cellulose (CMC) 5 ml/kg p.o. once a day then challenged with normal saline i.p. on 21th day.

Group II (LPS group): CMC 5 ml/kg p.o. once a day and then challenged with LPS Serotype E. coli 0111:B4, (4 mg/kg i.p.) on 21th day[12].

Group III: (standard group) Quercetin 100 mg/kg p.o. daily and then challenged with LPS (4 mg/kg i.p.) on 21th day[13].

Group IV and V (Drugs treated groups): 100 mg/ kg and 200 mg/kg p.o. daily respectively and then challenged with LPS (4 mg/kg i.p.) on 21th day.

Group VI: 200 mg/kg of test drug and 100 mg/kg standard drug p.o. daily then challenged with LPS (4mg/kg i.p.) on 21th day.

Group VII (Perse group): 200 mg/kg. p.o. daily for 21th day. After six hours of LPS or saline injection blood was collected from tail vein for liver function test and all the animals were sacrificed, liver was removed, stored and homogenates was used for biochemical estimation.

Determination of liver MDA content

Suspension medium (1 ml) was taken from the 10% tissue homogenate. TCA (0.5 ml of 30%) was added to it, followed by TBA reagent (0.5 ml of 0.8%). The tubes were then covered with aluminium foil and kept in shaking water bath for 30 minutes at 80ºC. After half an hour tubes were taken out and kept in ice-cold water for further thirty minutes and ccentrifuged at 3000 rpm for 15 minutes[15]. The absorbance of the supernatant was read at 540 nm at room temperature against appropriate blank.

Determination of liver glutathione

Liver tissue (300 mg) was homogenized in EDTA (5 -8 ml of 0.02 M) and then cold distilled water (4 ml) was added to it. After mixing it well, TCA (1 ml of 50%) was added and shaken intermittently for 10 minutes using a vortex mixer. After 10 minutes the contents were centrifuged at 6000 rpm for 15 minutes. Following centrifugation, supernatant (2 ml) was mixed with Tris buffer (4 ml of 0.4 M). The whole solution was mixed well and DTNB (0.1 ml of 0.01M) was added to it. The absorbance was read within 5 min of the addition of DTNB at 412 nm against a reagent[16].

Determination of liver catalase

Liver tissue was homogenized in 50 mM/L potassium phosphate buffer with a ratio of 1:10 w/v. The homogenate was centrifuged at 10,000 rpm at 4ºC in a cooling centrifuge for 20 minutes. Catalase activity was measured in supernatant obtained after centrifugation. Supernatant (50 µl) was added to cuvette containing 2.95 ml of 19 mM/L solution of H_2O_2 prepared in potassium phosphate buffer. The change in absorbance was monitored at 240 nm wavelength at 1 minute interval for 3 minutes. Presence of catalase decomposes H_2O_2 leading to decrease in absorbance[17].

Determination of Superoxide dismutase

The supernatant was assayed for SOD activity by inhibiting pyrogallol autoxidation. Cytosolic

supernatant (100 µl) was added to Tris HCl buffer (pH 8.5). The final volume of 3 ml was adjusted with the same buffer. Pyrogallol (25 µl) of was added and changes in absorbance at 420 nm were recorded at 1 minute interval for 3 minutes. The increase in absorbance at 420 nm after the addition of pyrogallol was inhibited by the presence of SOD[18].

Markers of liver function

The activity of biochemical parameters such as AST and ALT were estimated by Reitman and Frankel method[19], ALP and TB were estimated by King and Dangerfield method[20,21] ALB and TC level were estimated by the methods of Webster and Zlatkis, respectively[22,23].

Determination of Nitric oxide (NO): Griess Reaction

After the experiment, animals were sacrificed and the tissues were washed with PBS (pH 7.4) and placed on ice as described earlier. Sample (50 µl) was added with Griess reagent (100 µl) and reaction mixture was incubated for about 5-10 minutes at room temperature. The optical density was measured at 540 nm in microplate reader according to the reagent manufacturer's protocol. Calculations were done after generating a standard curve from sodium nitrite in the same buffer as used for preparation of homogenate[24].

Enzyme-linked immunosorbent assay

Cytokines were measured from tissue samples using commercially available ELISAs for rat TNF-α, IL-1β and IL-6. The ELISAs were operated according to the manufacturer's instructions. The intensity of the color measured is in proportion to the amount of rat cytokine bound in the initial steps. The sample values were then read off from the standard curve[25].

Statistical Analysis

All results are expressed as mean ± SEM. Groups of data was compared with analysis of variance (ANOVA) followed by Tukey-kramer multiple comparison test, $p<0.05$ was considered statstically significant.

RESULTS AND DISCUSSION

Acute toxicity

The extract from roots of *A. racemosus* administered orally to rats up to dose of 2000 mg/kg showed no toxicity and animal death during the evaluated period thus suggesting low toxicity of the extract. One-tenth and one-twenty of the maximum tolerated dose of the extract tested (2000 mg/kg) for acute toxicity did not indicate mortality and were selected for evaluation of the effect of *A. racemosus* i.e. 100 and 200 mg/kg.

Table 1: Effect of lipopolysaccharide, Quercetin and *A. racemosus* extract alone and in combination on oxidative stress markers in liver.

Group	Drug treatment	TBARS(nmols MDA/mg protein)	GSH(µg/mg protein)	CAT (nmolH_2O_2/mg protein)	SOD (Units/mg protein)
I	Control	3.502±0.0596	3.292±0.043	19.625±0.939	2.64±0.086
II	LPS	6.33±0.0743***	1.666±0.035***	9.912±0.919***	1.424±0.067***
III	QT	3.974±0.0812$^{###}$	2.876±0.073$^{###}$	15.716±0.285$^{###}$	2.20±0.087$^{###}$
IV	AR_1	5.614±0.1062$^{##}$	1.794±0.022^{ns}	12.200±0.504^{ns}	1.584±0.058^{ns}
V	AR_2	5.386±0.117$^{###}$	1.942±0.054$^{#}$	13.788±0.359$^{#}$	1.784±0.0508$^{#}$
VI	AR_2+QT	3.802±0.049$^{###}$	3.204±0.0423$^{###}$	17.70±0.475$^{###}$	2.324±0.0305$^{###}$
VII	AR_2 perse	3.598±0.113	3.516±0.036	19.292±1.066	2.616±0.099

LPS-Lipopolysaccharide, AR_1- Lower dose of *A. racemosus* extract, AR_2- Higher dose of *A. racemosus* extract, QT-Quercetin
Results are expressed as mean ± SEM. The results were analyzed by Analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. n=6, $^{#}$ = $p<0.05$, $^{##}$= $p<.01$, $^{###}$ = $p<0.001$, *** = $p<0.001$, $^{#}$ Vs. Group II, * Vs. Group I

Oxidative stress markers

The level of GSH, SOD and CAT activity decreased and the level of lipid peroxidation (TBARS) in LPS only treated group increased. MEAR (100 mg/kg and 200 mg/kg p.o.) supplementation was given for 21 days daily and on 21st day single dose of LPS (4 mg/kg i.p) was injected which restored depleted level of antioxidant enzyme i.e. GSH, SOD and CAT in the meanwhile decreased the level of TBARS (Table 1).

Liver function tests

Liver function markers AST, ALT, ALP, TC, ALB and TB were assessed. Administration of LPS (4 mg/kg, i.p, once) resulted in marked increase in serum AST, ALT, ALP and TB where as decrease in TC and ALB compared to corresponding control group (Table 2).

Table 2: Effect of lipopolysaccharide, Quercetin and *A. racemosus* extract alone and in combination on liver injury markers.

Drug treatment	SGOT U/l	SGPT U/l	ALKP U/l	TB mg/dl	ALB g/dl	CHL mg/dl
Control	138.6±8.599	113.24±7.314	317.4±10.773	0.924±0.058	3.542±0.0737	83.69±4.344
LPS	269.6±12.902***	196.2±8.009***	397.6±8.447***	1.504±0.048***	1.736±0.030***	21.908±4.432***
QT	172.4±8.078$^{###}$	128.8±7.940$^{#}$	322.6±12.428$^{###}$	1.142±0.0731$^{###}$	2.774±0.052$^{###}$	75.626±3.018$^{###}$
AR_1	256.3±9.257^{ns}	179.4±6.337^{ns}	388.6±4.79^{ns}	1.292±0.033^{ns}	1.85±0.0761^{ns}	34.126±4.194^{ns}
AR_2	209.2±10.012$^{##}$	163.2±5.113$^{#}$	331.2±8.691$^{###}$	1.2±0.024$^{##}$	2.066±0.049$^{##}$	42.86±6.344$^{#}$
AR_2+QT	169.2±7.612$^{###}$	124.8±7.453$^{###}$	320.2±8.628$^{###}$	1.046±0.0710$^{###}$	2.924±0.021$^{###}$	77.482±3.292$^{###}$
AR_2 perse	142.61±10.559	105±4.980	320.8±6.946	0.966±0.0403	3.582±0.0582	80.976±3.277

Results are expressed as mean ± SEM. The results were analyzed by Analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. n=6, $^{#}$ = $p<0.05$, $^{##}$= $p<0.01$, $^{###}$ = $p<0.001$, *** = $p<0.001$, $^{#}$ Vs. Group II, * Vs. Group I

Table 3: Effect of lipopolysaccharide, Quercetin and *A. racemosus* extract alone and in combination on cytokines and nitric oxide level.

Group	Drug treatment	IL-1β Pg/ml	IL-6 Pg/ml	TNF-α Pg/ml	NO (µ mol nitrite/mg of wet tissue)
I	Control	37.4±2.542	136.4±4.377	32.6±1.364	3.24±0.108
II	LPS	73.6±5.115***	196.4±6.964***	51.6±3.027***	7.446±0.198***
III	QT	52.8±3.555$^{\#\#}$	149.31±6.189$^{\#\#\#}$	37.12±2.345$^{\#\#}$	5.488±0.140$^{\#\#\#}$
IV	AR_1	69.6±3.140^{ns}	168.21±5.391$^{\#}$	45.4±4.707^{ns}	6.718±0.093$^{\#}$
V	AR_2	56.8±2.289$^{\#\#\#}$	163.6±6.787$^{\#\#\#}$	38.34±1.732$^{\#}$	6.632±0.087$^{\#\#}$
VI	AR_2+QT	50.8±2.672$^{\#\#\#}$	140.4±5.636$^{\#\#\#}$	36.6±3.763$^{\#\#}$	4.854±0.145$^{\#\#\#}$
VII	AR_2 perse	37±2.074	137.81±5.187	37.2±0.734	3.106±0.174

Results are expressed as mean ± SEM. The results were analyzed by Analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. n=6, $^{\#}$ = p<0.05, $^{\#\#}$= p<.01, $^{\#\#\#}$ = p<0.001, *** = p<0.001, $^{\#}$ Vs. Group II, * Vs. Group I

MEAR supplementation (21 day) along with LPS (4 mg/kg i.p on 21th day) resulted in significant reduction in AST, ALT, ALP and TB whereas the level of TC and ALB increased compared with only LPS-treated group (Table 2).

Nitric oxide

In the rats pretreated with *A. racemosus*, the levels of NO significantly reduced compared to disease control (Table 3). Dose of 200 mg /kg was more effective than that of 100 mg/ kg.

Cytokine Activity

In the rats pretreated with *A. racemosus*, the levels of cytokines significantly reduced ($p<0.001$) as compared to disease control (Table 3). There was dose dependent recovery on the LPS induced elevation of the cytokines level in rats.

In the present study, administration of LPS to rats resulted in development of oxidative stress which led to damage in liver tissue in rats. This effect was indicated by an increase in the concentration of lipid peroxidation (TBARS), cytokines, nitric oxide and decrease in the concentration of the GSH, SOD and CAT (catalase). MEAR supplementation (21 day) along with LPS (4 mg/kg i.p on 21th day) resulted in reduction in AST, ALT, ALP and TB whereas the level of TC and ALB increased compared with only LPS-treated group. LPS causes oxidative stress by intensification of proinflammatory cytokines production and by inducing the generation of ROS by different mechanisms[26]. Lipid peroxidation causes tissues injury by inactivation of membrane enzymes and receptors, depolymerization of polysaccharides as well as protein cross-linking and fragmentation[27]. Liver tissues are rich in polyunsaturated fatty acids and are known for its high oxygen uptake. Therefore, it is more susceptible to oxidative stress than other tissues[28].

In current study *A. racemosus* root (MAER) significantly decreased liver cytokines level after 6 hr of LPS administration as compared to rats treated with disease control. Root of *A. racemosus* supplementation increased the levels of GSH, SOD, CAT and decreased the level of TBARS, cytokines and nitric oxide significantly in the LPS-challenged animals. In a study done by Palanisamy and Manian showed that *A. racemosus* extract has hepatopotective activity by inhibiting production of free radical via inhibition of hepatic CYP2E1, increasing removal of free radical by induction of antioxidant enzyme and improving non-enzymatic thiol antioxidant GSH[28].

Thus *A. racemosus* acts as a free radical scavenger[29]. In addition to its direct cytotoxic effects, it is able to induce chemokines macrophage chemotactic protein-1 and vascular cell adhesion molecule-1, which is the key to hyper inflammation and consequent liver damage[30]. *A. racemosus* is a medicinal plant with well-known antioxidant property. Scientific evaluation of this claim using experimental model of LPS induced oxidative stress in rats was ascertained in this study.

CONCLUSIONS

Oral administration of methanolic extract of *A. racemosus* root (MEAR) protected rats from LPS induced liver injury. The protection may be due to the reduction of oxidative stress which occurs by alteration in levels of antioxidant enzymes in rats. These observations suggest that MEAR may be clinically viable protection against variety of conditions where cellular damage is a consequence of oxidative stress. In conclusion, the present study provides experimental evidence for MEAR as a hepato-protective agent.

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AUTHOR'S CONTRIBUTION

Ahmad MP: writing, review, and editing. **Perween T:** writing, review, project administration. **Singh S:** methodology, investigation, formal analysis. **Sinha R:** conceptualization, methodology. **Hussain A:** investigation, data curation. **Wahab S:** writing, review, and editing. **Jha AK:** writing, review. All the authors approved the finished version of the manuscript.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

None to declare.

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