Effect of methanolic extract of *Asparagus racemosus* Willd. on lipopolysaccharide induced-oxidative stress in rats

Mohammad Parwez Ahmad[1], Arshad Hussain*[1], Hefazat Hussain Siddiqui[1], Shadma Wahab[1] and Manoranjan Adak[2]

[1]Faculty of Pharmacy, Integral University, Lucknow, India
[2]Department of Biochemistry, Anna Medical College & Research Center, Mauritius

Abstract: Lipopolysaccharide (LPS) induced oxidative stress and impairment of normal physiological function generally categorized by increased anxiety and reduced mobility. Therefore, the present study was to find out the effect Methanolic extract of *Asparagus racemosus* (MEAR) in lipopolysaccharide (LPS)-induced oxidative stress in rats. LPS-induced oxidative stress in rats was measured by locomotor activity by photoactometer test, anxiety with elevated plus maze test and also studied the oxidative stress markers, nitric oxide and cytokines. The obtained data shows that LPS markedly exhausted ($p<0.001$) brain-reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) significantly increased ($p<0.001$) the level of malondialdehyde (MDA), nitric oxide and the activity of cytokines in the brain. MEAR supplementation resulted in normalization of brain GSH and CAT and SOD and decreases in the levels of MDA with reduction of nitric oxide and cytokines in the brain. The action of the extract at dose of 200 mg/kg was almost similar to the standard drug, quercetin (100mg/kg, p.o.). These present study conclude that MEAR administration significantly ($P<0.05$) reduced LPS- induced oxidative-stress and intensely suggest that *Asparagus racemosus* Willd. is a functionally newer type of cerebroprotective agent.

Keywords: Oxidative stress, *Asparagus racemosus*, lipopolysaccharide, cytokine, endotoxin.

INTRODUCTION

Lipopolysaccharide (LPS) is a gram-negative bacterial endotoxin and a key element that gives to organ failure, including brain injury (Kheir-Eldin *et al*., 2001). The degree of oxidative stress and the severity of resulting organ damage might depend on the imbalance between excess production of reactive oxygen species (ROS) and antioxidant defensive mechanism (Tanguy *et al*., 2003). LPS binding to immune cells initiates a cascade of happenings that up-regulate manifestation of the inflammatory cytokines including tissue necrosis factor -α (TNF-α), interleukin-6, (IL-6) and interleukin-1β (IL-1β). TNF-α and other cytokines stimulates the production of ROS and reactive nitrogen intermediates (RNIs) by activated macrophages causing brain due to the oxidative stress (Victor and Fuente, 2003). Additionally, LPS induces passage of stimulated polymorph nuclear leukocytes (PMNs) into the brain, which creates another source of free radicals. The oxidative stress thus generated, induces a rapid alteration in the antioxidant generation systems by depleting the cellular stores of endogenous antioxidants such as superoxide dismutase (SOD), catalase, glutathione (Richard *et al*., 1990; Wojewoda *et al*., 2010; Sangeeta *et al*., 2011). *Asparagus racemosus* Willd. is a main therapeutic plant of tropical and subtropical region of India and identified as rasayana and commonly called shatavari. In india its widely used in gastrointestinal disorder, as antitussive, as an immunostimulant and as antispasmodic (Bopana and Saxena, 2007). *Asparagus racemosus* has been used in Ayurveda as a galactagogue, anodyne, antispasmodic, diuretic, antiurolithiatic activity, hepatoprotective action and in diabetic nephropathy (Sharma *et al*., 2000; Jagannath *et al*., 2012; Acharya *et al*., 2012; Somania *et al*., 2012). The present study was aimed to evaluate the activity of methanolic extract of *Asparagus racemosus* on lipopolysaccharide induced oxidative stress in rats.

**Corresponding author:* e-mail: Integralpharmacy@gmail.com

MATERIALS AND METHODS

Plants materials

Roots of *Asparagus racemosus* Willd. was collected from Hamdard Dawakhana, Lucknow, India. The plant material was identified and authenticated by a taxonomist of National Botanical Research Institute (NBRI), Lucknow, India and the voucher specimen number NBRI-SOP-202 was deposited in the departmental herbarium.

Chemicals

All chemicals used were of analytical grade. Thiobarbituric acid (TBA); 2,6,-di-tert-butyl-4-hydroxy-toluene (BHT); trichloroacetic acid (TCA); Hydrogen peroxide (H_2O_2); EDTA; Tris buffer; Potassium dihydrogenortho phosphate; Disodium hydrogen ortho phosphate were obtained from CDH, Mumbai, NADPH; DTNB were obtained from Hi Media, Mumbai, cytokine ELISA kits obtained from eBioscience and Cayman Chemical USA, Lipopolysaccharide Serotype E. coli

0111: B4 were obtained from Sigma Chemicals, USA and standard drug quercetin were obtained from Total herb solution Mumbai.

Animals

Adult male (8 weeks) Sprague Dawley (SD) rats weighing 150-160g were used in this study. The animals were procured from the Division of Laboratory Animal, Central Drug Research Institute (CDRI), Lucknow, India. They were kept in polyacrylic cages in-group of seven and maintained under standard housing condition (room temperature 25±1°C and humidity 60-65%) with 12h light and dark cycle. The food and water were available adlibitum. Experiments were performed as per internationally followed ethical standards. This study was approved by Institutional Animal Ethics Committee (IAEC) of Integral University and CPCSEA No-IU/Pharm/Ph.D/CPCSEA/12/03.

Preparation of plant extract

The root of *Asparagus racemosus was* air-dried and dried materials were pulverized in a laboratory mill to a moderately fine powder. The powder was filled into soxhlet apparatus and exposed to hot continuous percolation using methanol (95% v/v) as solvent. The extract was concentrated under vacuum and dried in a vacuum desiccator (yield 10.4% w/w).

Acute toxicity studies

Acute toxicity: The acute toxicity of the extract of Root of *Asparagus racemosus* Willd. was evaluated in mice using the up and down procedure (OECD, 2001). Mice received methanolic extract at various doses (500-2000mg/kg) orally by gavage. The animals were observed for abnormal behaviour continuously for the first 4h after dosing. Finally, the number of survivors was noted after 24 h.

Experimental design

Animals were randomly divided into seven groups as follows: Group I (Control group) 1% Carboxy methyl cellulose (CMC) 5ml/kg p.o. once a day, and one dose of normal saline at day 21 from the start of the experiment (4 mg/kg i.p.). Group II (LPS group) 1% CMC 5ml/kg, p.o. once a day, and one dose of LPS Serotype E. coli 0111: B4 at day 21 from the start of the experiment (4 mg/kg, i.p.). Groups III (standard group) Quercetin 100 mg/kg, p.o. daily, and one dose of LPS at day 21 from the start of the experiment (4mg/kg, i.p.). Groups IV and V (Drugs treated groups) 100mg/kg and 200mg/kg, p.o. daily, and one dose of LPS at day 21 from the start of the experiment (4mg/kg i.p.) respectively. Groups VI 200 mg/kg of test drug and 100mg/kg standard drug p.o. and one dose of LPS at day 21 from the start of the experiment (4mg/kg, i.p.). VII (*perse* group) 200mg/kg, p.o. test drug daily for 21 days.

Anxiety was assessed in the elevated plus mazes 3 h after the LPS or saline administration on 21 day. Motor activity was assessed in the photoactometer test 4h after the LPS or saline administration on 21 day. Rats were transcardially perfused with cold saline followed by 4 % formalin in phosphate buffer-saline (o.1 M; p^H 7.4). The brains were removed and stored -80°C and later use for biochemical estimation.

Parameters investigated

(i) Behavioural tests

Elevated plus maze test (Corbett *et al.,* 1986). and Photoactometer test (Ahmad *et al.,* 2012) carried out by reported procedure.

(ii) Biochemical estimations

Estimation of Thio Barbituric Acid Reactive Substances (TBARS), (Ohkawa *et al*., 1979) glutathione(GSH) (Sedlak and Lindsay,1968), Catalase (CAT) (Clairbone, 1985), Super oxide dismutase (SOD) (Marklund and Marklund, 1974) and NO and cytokine (eBioscience and Cayman Chemical USA) carried out according to the manufacturer's instructions.

STATISTICAL ANALYSIS

The values were expressed as mean ±SEM. Statistical analysis was done by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. P values<0.05 were considered as significant.

RESULTS

Assessment of elevated maze test

LPS induced an anxiogenic effect in only LPS treated group and significantly (P<0.05) reduced the time spent in open arm in plus maze. Oral administration of MEAR for 21 days increased time spent in open arm and significantly reversed the effect of LPS (table 1).

Assessment of photoactometer response

LPS suppress Locomotor activity was increased by *Asparagus racemosus* root extract and significantly (P<0.05) increased (table 1).

Reduced glutathione (GSH) content in brain

In the present study, LPS administration (4mg/kg, i.p, once) resulted in significant depletion of brain GSH compared to the corresponding control value. Administration of 100mg/ kg and 200 mg/kg MEAR as an oral daily for 21 days as well as LPS (4mg/kg, i.p, once at the 21day) markedly increased brain (p<0.001) GSH content as it reached normal levels (table 2).

Malondialdehyde (MDA) content in brain

By evaluation of MDA (as indicator of lipid peroxides formation) in rat brain, the obtained data (table 2) explain that LPS administration (4mg/kg, i.p, once) into rats

Table 1: Assessment of Plus maze and Photoactometer

Group	Drugs treatment	Time duration in open arm in sec	No of entries in open arm	Locomotor activity
I	Control	204.6±5.115#	6.6±0.509#	178±10.677##
II	LPS	99.6±3.558*	2.00±0.136*	121±6.403*
III	QT	180.8±3.338#	5.2±0.374#	170±8.509###
IV	AR_1	140.4±3.265#	2.4±0.244 ns	123±5.380ns
V	AR_2	150±3.536#	4.00±0.362##	169.8±8.777###
VI	AR_2+QT	181.4±2.943#	5.6±0.244#	171±8.741###
VII	AR_2perse	198.8±3.513	7±0.316	176.4±10.815

Table 2: Assessment of oxidative stress markers

Drugs treatment	GSH (µg/mg protein)	CAT (nmol H_2O_2/mg protein)	SOD (Units/mg protein)	TBARS (nmoles MDA/mg protein)
Control	3.452±0.080#	22.2±0.860#	2.604±0.106#	3.658±0.076#
LPS	1.51±0.050*	9.00±0.707*	1.46±0.1281*	7.56±0.096*
QT	3.13±0.070#	19.4±0.500#	2.42±0.128#	3.79±0.051#
AR_1	1.894±0.110ns	11.4±0.678ns	1.76±0.114ns	7.06±0.092###
AR_2	1.972±0.037##	13.2±0.66###	2.02±0.073##	5.26±0.136#
AR_2+QT	3.35±0.065#	20.4±0.678#	2.46±0.0812#	3.704±0.011#
AR_2*perse*	3.516±0.036#	22.8±0.583	2.68±0.086	3.746±0.130

Table 3: Assessment of cytokines level and nitric oxide in brain

Group	Drugs treatment	TNF-α Pg/ml	IL-6 Pg/ml	IL-1β Pg/ml	NO (µmol nitrite/mg of wet tissue)
I	Control	33.00±0.707#	138.4±2.657#	38.4±1.364#	3.38±0.086#
II	LPS	52.8±1.71*	208±5.762*	79.4±1.503#*	8.42±0.0712*
III	QT	37.00±1.09#	144±2.429#	43.4±1.288#	5.866±0.1388#
IV	AR_1	42.8±1.715#	185.6±1.806#	69.8±1.772#	7.01±0.135#
V	AR_2	40.8±1.68#	182.8±5.809#	65.4±1.240#	6.322±0.066#
VI	AR_2+QT	35.4±0.927#	143.4±2.804#	43.6±1.631#	5.78±0.105#
VII	AR_2*perse*	34.4±1.50	138.6±2.315	38±0.707	3.57±0.129

LPS-Lipopolysacchride, AR_1- Lower dose of *Asparagus racemosus* extract, AR_2- Higher dose of *Asparagus 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 (Number of animal per group) # = p<0.001, ##= p<.01, ### = p<0.05, * = p<0.001, # Vs. GroupII, *Vs. GroupI

markedly increased the level of brain MDA compared to the corresponding control animals. However animals administered both MEAR (100mg/kg, p.o daily) in 21 successive days and injected with a single dose of LPS (4 mg/kg, i.p) at the 21day showed marked reduction (p<0.001) in brain MDA content in comparison with only LPS-administered animals.

Superoxide dismutase (SOD) content in brain

LPS administration decreases the level of SOD in to rats. Animal administered MEAR 100mg/kg and 200 mg/kg p.o. daily for 21 days and administration with single dose of LPS (4mg/kg i.p) at the 21_{th} day marked increase (p<0.001) in SOD in comparison with only LPS-administered animals.

Catalase (CAT) content in brain

LPS administration decreases the level of CAT in to rats. Animal administered MEAR 100mg/kg and 200mg/kg p.o. daily for 21_{th} days and injected with single dose of LPS (4mg/kg i.p) at the 21day marked increase (p<0.001) in CAT in comparison with only LPS-administered animals.

Nitric oxide content in brain

In the rats who were treated with *Asparagus racemosus* 100mg/kg and 200mg/kg, the levels of NO were significantly reduced as compared to disease control (table 3) shows the effect of the significant and dose dependent recovery on the LPS induced elevation of the NO levels in animals.

Cytokines content in brain

In the rats who were pretreated with *Asparagus racemosus*, the levels of cytokines were significantly reduced (p<001) as compared to disease control (table 3) shows the effect of the significant and dose dependent recovery on the LPS induced elevation of the cytokines levels in animals.

DISCUSSION

Medicinal plant and their products are accepted for their influential pharmacological activities in health and disease treatment. They are able to exert antioxidant, antiradical and hepatoprotective activities (Amor *et al*., 2009). In the present study, administration of LPS to rats resulted in development of oxidative stress which leads to suppressed locomotor and exploratory activity, induced an anxiogenic response and damage in brain tissue in rats. This effect was pointed out by an increase in the concentration of lipid peroxidation, cytokines, nitric oxide and decrease in the concentration of the GSH, SOD and Catalase. It is well accepted that LPS causes oxidative stress by intensification of proinflammatory cytokines production and by inducing the generation of ROS by different mechanisms (Victor *et al*., 2001; Zhao *et al*., 2008; Jaworek *et al*., 2007). Lipid peroxidation causes tissues injury by inactivation of membrane enzymes and receptors, depolymerization of polysaccharides as well as protein cross-linking and fragmentation (Luqman and Rizvi, 2006). This results in severe metabolic dysfunction and loss of cell integrity as well as genomic stability. Brain tissues have rich polyunsaturated fatty acids and are known for its high oxygen uptake. Therefore, it is more susceptible to oxidative stress than other tissues (Rozenberg *et al*., 2006). Both the doses of extract of *Asparagus racemosus* Willd. root (MEAR) significantly decreased brain cytokines levels after 6 h of LPS administration as compared to rats treated with disease control. In the present investigation increased the levels of GSH, SOD, catalase and decrease the level of lipid peroxidation cytokines and nitric oxide significantly in the LPS-challenged animals after the Root of *Asparagus racemosus* supplementation. Improvement of antioxidant status in root of *Asparagus racemosus* Willd. supplemented groups is in agreement with earlier findings (Gulec *et al*., 2006; Bansal *et al*., 2005). Increased TNF-α has been repeatedly shown to play a pivotal role in LPS -induced brain injury. TNF-α is a multifunctional cytokine secreted by activated macrophages, monocytes, neutrophils and Natural killer-cells. In accession to its direct cytotoxic effects, it is able to accelerate chemokines macrophage chemotactic protein-1 and vascular cell adhesion molecule-1, which is the key to hyper inflammation and ensuing brain damage. *Asparagus racemosus* Willd. is a medicinal plant with well-known antioxidant property (Hussain *et al*., 2011). Scientific evaluation of this claim using experimental model of LPS induced oxidative stress in rats was ascertained in this study. *Asparagus racemosus* Willd. contains steroidal saponins that are present in the root (Nandgopal *et al*., 2011). This constituent might be responsible for this effect. In conclusion, Oral administration of methanolic extract of *Asparagus racemosus* Willd. root (MEAR) protected rats from LPS induced brain injury. The protection may be due to the decrease of oxidative stress, which occurs by alteration in levels of antioxidant enzymes in oxidative stress rats. These observations propose 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 methanolic extract of *Asparagus racemosus* Willd. as a cerebroprotective agent.

REFERENCES

Acharya SR, Acharya NS, Bhangale JO, Shah SK and Pandya SS (2012). Anti-oxidant and hepatoprotective action of Asparagus racemosus Willd. root extracts. *Ind. J. Exp. Biol.,* **50**(11): 795-801.

Ahmad MP, Hussain A, Najmi AK, Manocha A, Akhtar MH and Wahab S (2012). Effect of the aqueous extract of *Mentha arvensis* on haloperidol induced catalepsy in albino mice. *J. Clin. Diagns. Res.,* **6**(2): 1-5.

Amor ILB, Skandrani I, Boubaker J, Sghaier MB, Neffati MA, Bhouri W, Bouhlel I, Chouchane N, Kilani S, Guedon E, Ghoul M, Ghedira K and Chekir-Ghedira L (2009). Investigation of biological activity of polar extracts isolated from Phlomiscrinita Cav ssp. *Drug. Chem. Toxicol.,* **32**: 38-46.

Auto-oxidation of pyrogallol and a convenient assay for super oxide dismutase. *Eur. J. Biochem.,* **47**: 469-474.

Bansal AK, Bansal M, Soni G and Bhatnagar D (2005). Protective role of vitamin E pre treatment on *N*-nitrosodiethylamine induced oxidative stress in rat liver. *Chem. Biol. Interact.,* **156**: 101-111.

Bopana N and Saxena S (2007). Asparagus racemosus-Ethnopharmacological evaluation and conservation needs. *J. Ethnopharmacol.,* **110**: 1-15.

Clairbone A (1985). Assay of Catalase. *In*: Greenwald RA, ed. Hand book of method of oxygen free radical research; Boca Raton Fla; CRC Press, pp.283-284.

Corbett R, Fielding ST, Cornfeldt M and Dunn RW (1991). GABA mimetic agents display anxi-olytic effects in the social interaction and elevated plus maze procedures. *Psychopharmacol.,* **104**: 312-316.

Gulec M. Gurel A and Armutcu F (2006). Vitamin E protects against oxidative damage caused by formaldehyde in the liver and plasma of rats. *Mol. Cell. Biochem.,* **290**: 61-67.

Hussain A, Ahmad MP, Wahab S, Hussain MS and Ali M (2011). A Review on Pharmacological and phytochemical Profile of *Asparagus racemosus* Willd. *Pharmacology Online,* **3**: 1353-1364.

Jagannath N, Chikkannasetty SS, Govindadas D and Devasankaraiah G (2012). Study of anti-urolithiatic activity of Asparagus racemosus on albino rats. *Ind. J. Pharmacol.,* **44**(5): 576-579.

Jaworek J, Konturek SJ and Macko M (2007). Endotoxemia in newborn rats attenuates acute pancreatitis at adult age. *J. Physiol. Pharmacol.,* **58**: 131-147.

Kheir-Eldin AA, Motawi TK, Gad MZ and Abd-El-Gawad HM (2001). Protective effect of vitamin E, β-carotene and N-acetylcysteine from the brain oxidative stress induced in rats by lipopolysaccharide. *Int. J. Biochem. Cell B.,* **33**: 475-482.

Luqman S and Rizvi SJ (2006). Protection of lipid peroxidation and carbonyl formation in proteins by capsaicin in human erythrocytes subjected to oxidative stress. *Phytother. Res.,* **20**: 303-306.

Marklund S and Marklund G (1974). Involvement of super oxide anion radical in the auto oxidation of pyrogallol and a convenient assay for super oxide dismutase. *Eur. J. Biochem*., **47**:469-474.

Nandgopal M, Murlidharan P and Thurumurugan G (2011). Cerebroprotective effect of root of *Asparagus racemosus* Willd. in global cerebral ischemia in rats. *J. Pharmacol. Toxicol.,* **6**(1): 49-61.

Ohkawa H, Ohishi N and Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.,* **95**: 359-64.

Reichenberg A, Kraus T, Haack M, Schuld A, Pollmacher T and Yirmiya R (2002). Endotoxin induced changes in food consumption in healthy volunteers are associated with TNF-alpha and IL-6 secretion. Psycho-neuroendocrinology, **27**: 945-956.

Richard C, Lemonnier F, Thibault M. Couturier M and Auzepy P (1990). Vitamin E deficiency and lipoperoxidation during adult respiratory distress syndrome. *Crit. Care. Med.,* **18**: 4-9.

Rozenberg S, Besse S and Brisson H (2006). Endotoxin-induced myocardial dysfunction in senescent rats. *Critical. Care.,* **10**: 124-132.

Sangeeta PS, Naveen T, Anurag K and Kanwaljit C (2011). Effect of quercetin on lipopolysaccharide induced-sickness behavior and oxidative stress in rats. *Ind. J. Pharmacol.,* **43**:192-196

Sedlak J and Lindsay RH (1968). Estimation of total, protein bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem.,* **25**: 192-205.

Somania R, Singhai AK, Shivgunde P and Jain D (2012). Source *Asparagus racemosus* Willd. (Liliaceae) ameliorates early diabetic nephropathy in STZ induced diabetic rats. *Indian. J. Exp. Biol.,* **50**(7): 469-475.

Tanguy S, De Leiris J, Besse S and Boucher F (2003). Ageing exacerbates the cardiotoxicity of hydrogen peroxide through the Fenton reaction in rats. *Mech. Ageing. Dev.,* **124**: 229-235.

The Organization of Economic Co-operation Development (OECD) (2001). The OECD Guideline for Testing of Chemical: 420 Acute Oral Toxicity. OECD, Paris, pp.1-14.

Victor VM and Fuente MD (2003). Changes in the superoxide production and other macrophage functions could be related to the mortality of mice with endotoxin-induced oxidative stress. *Physiol. Res.,* **52**: 101-110.

Victor VM, Guayerbas, N, Puerto M, Medino S and Fuente MDL (2000). Ascorbic acid modulates *in vitro* the function of macrophages from mice with different models of endotoxic shock. *Immunopharmacol.,* **46**: 89-101.

Zho L, Chen YH and Wang HJ (2008). Reactive oxygen species contribute to lipopolysaccharide-induced tetrageneis in mice. *Toxicol. Sci.,* **103**: 149-157.