Cerebroprotective Effect of Root Extract of *Asparagus racemosus* Willd. in Global Cerebral Ischemia in Rats

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ABSTRACT

The present study investigated the role of methanolic root extract of *Asparagus racemosus* Willd. (MEAR) in the levels of antioxidants, metabolic enzymes, neurotransmitters and histopathology studies in a model of global cerebral ischemia in rats. Global cerebral ischemia was induced by temporary bilateral carotid artery occlusion for 15 min followed by reperfusion in Wistar rats and the animals were pretreated with MEAR (200 and 400 mg kg^{-1}) for 7 days before induction. After induction of ischemia by BCAO animals were again treated with MEAR for 7 days and the animals were sacrificed. Homogenized content of brain were estimated in control, sham and treatment groups. MEAR showed the significance of $p < 0.01$ done by ANOVA and Dunetts in the levels of antioxidants such as superoxide dismutase, glutathione peroxidase, glutathione reductase, lipid peroxidation, catalase, protein levels, metabolic enzyme acetylcholine esterase and neurotransmitters glutamate, dopamine and serotonin levels. At the 7 days after ischemia a maximum of 85% protection of neurons in CA1region showed in treatment group at the dose 400 mg kg^{-1}. The results showed cerebroprotective nature of MEAR and protection may be due to reduction of oxidative stress which occurs by alteration in levels of antioxidants, neurotransmitters and MEAR had the potential to use in treatment of ischemia.

Key words: *Asparagus racemosus*, bilateral carotid artery occlusion, antioxidants, neurotransmitters, CA1

INTRODUCTION

Owing to the global trend towards improved quality of life, there is considerable evidence of an increase in demand for medicinal plant. Use of plants for treating various ailments of both man and animal is as old practice as man himself. India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society whether directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine (Valery and Fan, 2007). In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems (Ayurveda, Siddha and Unani; Patwardhan *et al.*, 2005).

A stroke or brain attack occurs when a blood clot blocks the blood flow in a vessel or artery or when a blood vessel breaks, interrupting blood flow to an area of the brain. When either of these

things happens, brain cells begin to die (Wu *et al.*, 2004). In recent years, several reviews have been published on the effect (Gong and Sucher, 2002) and potential benefits of traditional Eastern medicine in stroke (Kim, 2005). It has been suggested that some herbal medicines (Wu *et al.*, 2004), or their products, may improve microcirculation in the brain and protect against ischemic reperfusion injury possess neuroprotective properties (Lee *et al.*, 2005) and inhibit apoptosis (Bei *et al.*, 2004), thus justifying their use in ischemic stroke patients. *Asparagus racemosus* is mainly known for its phytoestrogenic properties. With an increasing realization that hormone replacement therapy with synthetic oestrogens is neither as safe nor as effective as previously envisaged; the interest in plant-derived oestrogens has increased tremendously making *Asparagus racemosus* particularly important. The plant has been shown to aid in the treatment of neurodegenerative disorders and in alcohol abstinence-induced withdrawal symptoms. In Ayurveda, *Asparagus racemosus* has been described as a *rasayana* herb and has been used extensively as an adaptogen to increase the non-specific resistance of organisms against a variety of stresses (Vijay *et al.*, 2009). Besides use in the treatment of diarrhoea and dysentery, the plant also has potent antioxidant, immunostimulant, anti-dyspepsia and antitussive effects (Bopana and Saxena, 2007).

Asparagus racemosus has been used in Ayurveda as a galactagogue, aphrodisiac, anodyne, diuretic, antispasmodic and nervine tonic since time immemorial (Sharma *et al.*, 2000). The plant finds use in about 64 ayurvedic formulations which include traditional formulations such as Shatavari kalpa, Phalaghrita, Vishnu taila, etc. (Unnikrishnan, 1998). Abana® (containing 10 mg Satavari root extract per tablet), Diabecon® (containing 20 mg Satavari root extract per tablet), EveCare® (containing 32 mg Satavari root extract per 5 mL syrup), Geriforte®(containing 20 mg Satavari root powder per tablet), Himplasia® (containing 80 mg Satavari root powder per tablet), Lukol® (containing 40 mg Satavari root extract per tablet) and Menosan® (containing 110 mg Satavari root extract per tablet) are some formulations containing *Asparagus racemosus* developed by Himalaya Herbal Healthcare, India. Since *Asparagus racemosus* is said to be a potent antioxidant, based on this claim an attempt has been made in this study in the treatment of cerebral ischemia induced by bilateral carotid artery occlusion in rats.

MATERIALS AND METHODS

Animals: The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals). IAEC Reference number IAEC/XIII/01/CLBMCP/2008-2009 Dated on 04/09/2008. Colony inbred strains of male Wistar albino strain rats of male sex weighing 250-300 g were used for the pharmacological studies. The animals were kept under standard conditions (day/night rhythm) 8.00 a.m. to 8.00 p.m., 22±1°C room temperature, in polypropylene cages. The animals were feed on standard pelleted diet and water *ad libitum*. The animals were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory conditions. It was randomly distributed into six different groups with six animals in each group under identical conditions throughout the experiments. Food was withdrawn 18 h prior to surgery, however. Principles of Laboratory Animals Care and Use (NIH Publication No. 85-23, revised 1985) guidelines were followed throughout.

Chemicals and reagents: Fine chemicals such as acetyicholine thio-iodide, glutamate dehydrogenase, doamine, serotonin has been obtained from Sigma (USA). All other chemicals and

reagents were of the highest analytical grades available locally. Estimation of protein was done using the ready to use kit (Zydus Pathline, India).

Plant material and standardisation of extract: The roots of *Asparagus racemosus* Willd. were collected from Central Research Institute, Chennai. The plant material was identified during July 2008 and authenticated by Dr. Sasikala Ethirajulu, Research Officer (Pharmacognosy) of Central Research for Siddha, Arumbakkam, Chennai-600106 and Dr. G.V.S. Murthy Joint Director of Botanical Survey of India, Southern circle, Coimbatore-641003. (Authentication No. BSI/SC/5/23/08-09/Tech-893 dated 09/09/08, Botanical survey of India). Freshly collected roots of *Asparagus racemosus* Willd. were dried in shade and pulverized to get a coarse powder. A weighed quantity of the powder (980 g) was passed through sieve number 40 and subjected to hot solvent extraction in a soxhlet apparatus using methanol at a temperature range of 60-80°C, respectively. Before and after every extraction the powder bed was completely dried and weighed. The filtrate was evaporated to dryness at 40°C under reduced pressure in a rotary vaccum evaporator. A brownish black waxy residue was obtained. The percentage yield of methanolic extract was 17.5% w/w. The extract (MEAR) was subjected to preliminary phytochemical screening for the presence or absence of phytoconstituents and the methanolic root extract showed the presence of alkaloids, carbohydrates, steroids, proteins, phenols, flavanoids, gums and mucilage, glycosides, saponins, terpenes, triterpinoids, sterols and showed the absence of tannins. The animals received this extract orally in dosages of 200 and 400 mg kg^{-1} day^{-1} dissolved in distilled water. The particular dose was selected on the basis of our preliminary and acute oral toxicity studies that assessed the ability of MEAR to attenuate the excitotoxity provoked oxidative damage. These plant extraction and phytochemical screening were carried out in C.L.Baid Metha College of Pharmacy, Chennai, India during Dec.-Feb. 2008.

Experimental procedure
Induction of global cerebral ischemia: Global cerebral ischemia was induced by temporary bilateral carotid artery occlusion followed by reperfusion (Colbourne *et al.*, 1998). Anaesthetized rat with thiopentone sodium at a dose of 45 mg kg^{-1} b.wt. Rats were transfered to the surgery table. Checked anesthesia level intermittently (e.g., toe pinch) and level adjusted accordingly. Ventral neck region was shaved. Area was washed with 70% ethanol to cleanse. All loose fur were removed and treated with betadine solution. Temperature measurement was carried out. Started recording temperature and controlled with help of following method (Iwasaki *et al.*, 1989). Body temperature was easily controlled (37.0°C) with a heating blanket. An infrared lamp was also effective in maintaining temperature, but it must not be too close to cause burns. Animal was draped. A small midline skin incision was made in neck (~ 2 cm long). The thyroid gland was gently separated with non-traumatic forceps. Both common carotid arteries were isolated. Care was taken to avoid damaging the vagal nerves and separated with the help of curved forceps. Silk suture was looped under each artery for each access to vessels. Vessels were made free enough to allow easy and rapid placement of clamps. Non-traumatic vessel clamps was applied to each artery for a defined period (10-15 min) and after that allowed for reperfusion. Area was infiltrated with warm saline to prevent drying out of tissue during occlusion. After the end of defined period clamps were removed and checked arteries for good reflow. The order of clamp application and removal should be the same and within 10 sec of each other minimize asymmetrical injury. Silk suture was gently removed around each vessel. Incision was infiltrated with a few drops of lignocaine. Incision was

sutured and post surgical care was given to the animal and they were retuned to their home cages. Sham-operated control animals underwent all the surgical procedure except occlusion of BCAO.

Steady state experiment: For the studies of global cerebral ischemia the male Wistar strain rats were randomized into 6 different groups (n = 6 per group) Group 1: Animals (POSITIVE CONTROL) with sham operation (without occlusion) and treated with control vechicle (normal saline) only (p.o.). Group 2: Animals with sham operation (without occlusion) and treated with 200 mg kg^{-1} of MEAR dissolved in distilled water (p.o.). Group 3: Animals with sham operation (without occlusion) and treated with 400 mg kg^{-1} of MEAR (p.o.). Group 4: Animals (NEGATIVE CONTROL) with BCAO and treated with control vechicle only (p.o). Group 5: Animals with BCAO and treated with 200 mg kg^{-1} of MEAR (p.o.). Group 6: Animals with BCAO and treated with 400 mg kg^{-1} of MEAR (p.o). In our study the animals were pretreated with MEAR for a period of 1 week (200 and 400 mg kg^{-1}) p.o. The animals were anaesthetized with thiopentone sodium (45 mg kg^{-1}) i.p. and stroke was induced by occlusion of bilateral carotid artery (BCAO) for defined period (10 min) with aneurism clamps placed on both arteries and later clamps were removed to allow reperfusion and animals were then returned to their cages. The treatment was continued for another week after surgery with root extract and the animals were sacrificed with overdose of thiopentone sodium and the brain was removed and homogenized. The homogenized content was used for the estimation of anti-oxidant, metabolic enzymes and various neurotransmitter levels. Histopathology of hippocampal CA1 region was carried out.

Biochemical analysis: Assessment of oxidant-antioxidant status of the rat forebrains subjected to global cerebral ischemia was done by measuring the levels of superoxide dismutase (SOD) (Marklund and Marklund, 1974), glutathione peroxidase (GSH-Px) (Lawrence *et al.*, 1976), glutathione reductase (GSH-Rd) (Dubler and Anderson, 1981), lipid peroxidation (Bergmeyer *et al.*, 1965; Ohkawa *et al.*, 1979), catalase (Bergmeyer *et al.*, 1965). Estimation of lipid peroxidation was done by measuring the levels of malondialdehyde (MDA), a by-product of lipid peroxidation. 7 days after ischemia, rats from each group were decapitated; the brains were quickly removed and homogenized in ice-cold sodium pyrophosphate buffer (pH 8.3) in a ratio of 50 mg mL^{-1}.

Estimation of protein: The protein content of brain tissue was estimated by following the method of (Lowry *et al.*, 1951) using bovine serum albumin as standard.

Metabolic enzyme and neurotransmitters estimation: Assessment of various metabolic enzyme and neurotransmitters were carried out in rats subjected to global cerebral ischemia.

Estimation of metabolic enzyme acetylcholine esterase: Twenty milligrams of brain tissue per mL of phosphate buffer (pH 8, 0.1 M) was homogenized in a potter-Elvehjem homogenizer (Ellman *et al.*, 1961). A 0.4 mL aliquot of brain homogenate was added to a cuvette containing 2.6 mL of 0.1 M phosphate buffer (pH.8). One hundred microliter of the DTNB reagent was added to the photocell, the absorbance was measured at 412 nm. Twenty microliter of the acetylthiocholine iodide was added. Changes in absorbance were recorded and the change in absorbance per minute was calculated. The enzymatic activity is expressed as μmoles/min/g tissue.

Estimation of glutamate: In this study, animals were decapitated 3 h after the last behavioral session. The brains were immediately excised and cerebellum discarded (Bernt and Bergmeyer, 1965). The cortex, striatum and the sub cortical parts were separated and weighed. The sub cortical region of the brain comprised all the remaining parts of the forebrain after dissection of the cerebral cortex and striatum, including the hippocampus, thalamus, hypothalamus, amygdale and other sub thalamic structures.

Deproteinization: Weighed quantity of brain portion was homogenized with 2 parts by weight of perchloric acid and centrifuge for 10 min at 3000 rpm. Adjust 3.0 mL supernatant fluid to pH 9 with 1.0 mL phosphate solution. Allow to stand 10 min in an ice bath and then filter through a small, fluted filter paper. Allow to warm to room temperature, dilute 1:10 and take 1.0 mL for the assay.

Assay system: Wavelength : 340 nm, light path : 1 cm, final volume : 3.35 mL, room temperature. For each series of measurements prepare reagent blank containing water instead of sample. Pipette successively into cuvettes, Glycine-hydrazine buffer (2 mL), sample (1 mL), ADP solution (0.1 mL). Mix, read extinction E1 at 340 nm then add GIDH solution (0.05 mL), mix. Allowed sample and blank to stand for 45 min and measured extinction E_2 at 340 nm. Calculated the difference between E_1 and E_2 for sample and blank:

$$\Delta E_{sample} - \Delta E_{blank} = \Delta E_{glutamate} \text{ is used for the calculations}$$

Estimation of dopamine: On the day of experiment rats were sacrificed, whole brain was dissected out (Kepe *et al.*, 2006) and separated the subcortical region (including the striatum). Weighed a specific quantity of tissue and was homogenized in 3 mL HCl Butanol in a cool environment. The sample was then centrifuged for 10 min at 2000 rpm. The 0.8 mL of supernatant phase was removed and added to an Eppendorf reagent tube containing 2 mL of heptane and 0.25 mL 0.1 M HCl. After 10 min, shake the tube and centrifuge under same conditions to separate two phases. Upper organic phase was discarded and the aqueous phase was used for dopamine assay.

Dopamine assay: To 0.02 mL of the aqueous phase, 0.005 mL 0.4 mL HCl and 0.01 mL EDTA/Sodium Acetate buffer (pH 6.9) were added, followed by 0.01 mL iodine solution for oxidation. The reaction was stopped after 2 min by the addition of 0.1 mL sodium thiosulphate in 5 M Sodium hydroxide. 10 M Acetic acid was added 1.5 min later. The solution was then heated to 100°C for 6 min. When the sample again reaches room temperature excitation and emission spectra were read (330 to 375 nm) in a spectrofluorimeter. Compared the tissue values (fluorescence of tissue extract-fluorescence of tissue blank) with an internal reagent standard (fluorescence of internal reagent standard-fluorescence of internal reagent blank).Tissue blanks for the assay were prepared by adding the reagents of the oxidation step in reversed order (sodium thiosulphate before iodine). Internal reagent standards were obtained by adding 0.005 mL distilled water and 0.1 mL HCl Butanol to 20 ng of dopamine standard.

Estimation of serotonin: Three milligrams of brain was homogenized in Hcl-n-butanol for 1 min in glass homogenizer. The sample was centrifuged for 10 min at 2000 rpm (Kepe *et al.*, 2006). The supernant phase was removed and added to eppendorf reagent tubes containing heptane and HCl.

After 10 min of vigorous shaking the tube was centrifuged under same conditions as above in order to separate 2 phases. The aqueous phase was taken and O-Phthaldialdehyde was added. The flurophore was developed by heating to 100°C for 10 min. After the sample reach the equilibrium the intensity readings was measured at 360-470 nm. The total biochemical estimation was done in C.L. Baid Metha College of Pharmacy, Chennai, India during April-Aug. 2009.

Histopathological examination: Seven days after ischemia, rats from each group were anesthetized (Al-Majed *et al.*, 2006) with sodium Thiopentone (100 mg kg^{-1}). Rats were then transcardially perfused with cold saline followed by 4% formalin in phosphate- buffered saline (0.1 M; pH 7.4). The brains were removed from the skull and fixed in the same fixative for 24 h. Thereafter, the brains were embedded in paraffin and 5 μm thick sections were coronally cut at the level of the dorsal hippocampus by a rotator microtome. The segments of the hippocampal CA1 region per 1000 μm lengths from bregma -3.3, -3.8 and -4.3 were counted for viable cells. Tissue sections were stained with hematoxylin and eosin. The hippocampal damage was determined by counting the number of intact neurons in the stratum pyramidal within the CA1 subfield at a higher magnification. Only neurons with normal visible nuclei were counted. The mean number of CA1 neurons per millimeter linear length for both hemispheres in sections of dorsal hippocampus was calculated for each group of animals. An observer who was unaware of the drug treatment for each rat made all the assessments of the histological section 2.10. Histopathological studies and Statistical analysis were conducted in Research Lab, GIET School of pharmacy, Rajahmundry, India during Sep.-Dec. 2009.

Statistical analysis: The statistical analysis was carried out using Graph pad prism 4.0 software. All values were expressed as Mean±SEM. Data analysis was done by one-way ANOVA followed by Dunetts Multiple Comparison Tests. Difference level at $p < 0.05$ was considered as statistically significant condition.

RESULTS AND DISCUSSION

MEAR treatment blocks global cerebral ischemia induced oxidative stress: Table 1 shows the effect of MEAR treatment (200 mg and 400 mg kg^{-1} day^{-1} for 14 days) on oxidant-antioxidant status of rat measured after ischemia-reperfusion, there was a decrease in the superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd), catalase and increase in the lipid peroxidation levels in Stroke induced (negative control) group when compared with the control group and negative control group which showed significance of $p < 0.01$ when compared with control group. The group treated with 200 and 400 mg kg^{-1} MEAR showed significant ($p < 0.01$) increase in the superoxide dismutase), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd), catalase and decrease in the lipid peroxidation level when compared with negative control group. The group treated with 200 and 400 mg kg^{-1} MEAR showed the significance of $p < 0.01$ as shown in Table 1.

MEAR treatment attenuates global cerebral ischemia altered protein level: There was a decrease in the protein level in stroke induced (negative control) group when compared with the control group and negative control group which showed significance of $p < 0.01$ when compared with control group. The group treated with 200 and 400 mg kg^{-1} MEAR showed significant $p < 0.01$ increase in the protein level when compared with negative control group. The group treated with 200 and 400 mg kg^{-1} MEAR showed the significance of $p < 0.01$ as shown in Table 2.

Table 1: Effect of MEAR in the levels of antioxidants

Groups	SOD (units/mg protein)	GSH-Px (units/min/ mgprotein)	GSH-Rd (units/min/ mgprotein)	MDA (nM/mg protein)	Catalase (μmol/mg protein/min)
Sham (saline)	9.00±0.274	37.20±0.255	33.30±0.255	2.16±0.068	2.54±0.025
Sham (200 mg kg^{-1})	8.85±0.224	37.00±0.224	33.40±0.332	2.22±0.037	2.50±0.056
Sham (400 mg kg^{-1})	9.00±0.387	37.20±0.300	33.60±0.187	2.24±0.510	2.52±0.037
Ischemia (saline)	6.60±1.187a**	28.20±0.300a**	25.40±0.187a**	4.54±0.0509a**	1.72±0.025a**
Ischemia+MEAR (200 mg kg^{-1})	8.00±0.224b**	32.40±0.187b**	29.10±0.185b**	3.26±0.075b**	2.08±0.037b**
Ischemia+MEAR (400 mg kg^{-1})	9.30±0.123b**	37.20±0.300b**	33.50±0.274b**	2.24±0.0509b**	2.49±0.033b**

Values are expressed as Mean±SEM of 6 animals; Comparisons were made between a. Sham control vs. ischemia control and b. ischemia vs. Treatment groups; **Represents the statistical significance of $p<0.01$ done by ANOVA, followed by Dunnets Multiple Comparison Test

Table 2: Effect of MEAR in the levels of proteins

Groups	1	2	3	4	5	6
Gram dL^{-1}	1.83±0.026	1.87±0.034	1.84±0.019	1.12±0.034a**	1.52±0.026b**	1.78± 0.020b**

Values are expressed as Mean±SEM of 6 animals; Comparisons were made between a. Sham control vs. ischemia control and b. ischemia vs. Treatment groups; **Represents the statistical significance of $p<0.01$ done by ANOVA, followed by Dunnets Multiple Comparison Test

Table 3: Effect of MEAR in the levels of neurotransmitters

Groups	Acetylcholine esrase (μM/min/g protein)	Glutamate (μM g^{-1})	Dopamine (pgm mg^{-1} tissue)	Serotonin (pgm mg^{-1} tissue)
Sham (saline)	17.70±0.095	76.00±0.224	659.0±1.225	823.6±2.731
Sham (200 mg kg^{-1})	17.64±0.098	75.80±0.255	658.0±1.265	824.4±2.249
Sham (400 mg kg^{-1})	17.60±0.071	75.90±0.367	657.8±1.625	823.4±2.337
Ischemia (saline)	20.60±0.187a**	86.40±0.187a**	538.0±1.924a**	730.0±2.702a**
Ischemia+ MEAR (200 mg kg^{-1})	18.58±0.049b**	80.90±0.187b**	598.2±1.158b**	787.2±1.844b**
Ischemia+ MEAR (400 mg kg^{-1})	17.62±0.058b**	76.30±0.255b**	646.4±1.749b**	813.8±2.035b**

Values are expressed as Mean±SEM of 6 animals. Comparisons were made between a. Sham control vs. ischemia control and b. ischemia vs. Treatment groups; **Represents the statistical significance of $p<0.01$ done by ANOVA, followed by Dunnets Multiple Comparison Test

MEAR treatment attenuates global cerebral ischemia induced neurotransmitter change: There was an increase in the acetylcholine esterase and glutamate levels and decrease in dopamine and serotonin levels in Stroke induced (negative control) group when compared with the control group and negative control group which showed significance of $p<0.01$ when compared with control group. The group treated with 200 and 400 mg kg^{-1} MEAR showed significant ($p<0.01$) decrease in the acetylcholine esterase and glutamate levels and increase in dopamine and serotonin levels when compared with negative control group. The group treated with 200 and 400 mg kg^{-1} MEAR showed the significance of $p<0.01$ as shown in Table 3.

MEAR treatment shows protection in CA1 region: There was a decrease in the number of neurons in the hippocampal region in stroke induced (negative control) group when compared with the control group and negative control group which showed significance of $p<0.01$ when compared
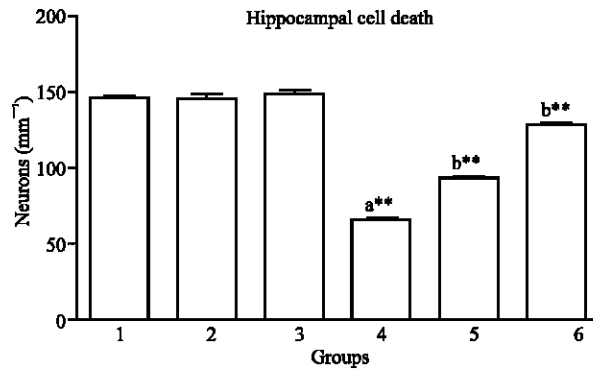
Fig. 1: Effect of MEAR on Neuron cell count in CA1 region in rat brain after ischemia. Results are expressed as Mean±SEM of 6 rats and data were analyzed by one-way ANOVA followed by Dunnets Multiple Comparisons Test. **Indicates significance between (aSham control vs. ischemia control and bIschemia vs. treatment groups)

with control group. The group treated with 200 and 400 mg kg^{-1} MEAR showed significant (p<0.01) increase in number of neurons in the hippocampal region when compared with negative control group which was confirmed from photomicrographs of histopathology study. The group treated with 200 and 400 mg kg^{-1} MEAR showed the significance of p<0.01 as shown in Fig. 1 and 2a-f photomicrographs.

Asparagus racemosus is a medicinal plant with well known antioxidant property and claimed to have anticoagulant property (Bopana and Saxena, 2007) as per folklore. Scientific evaluation of these claims using experimental model of bilateral carotid artery occlusion (BCAO) in rats induced cerebral ischemia was ascertained in this study. This was supported in our study by various behavioral, biochemical findings and histopathology studies. The present study revealed the cerebroprotective effect of the plant *Asparagus racemosus* in Cerebral ischemia induced by bilateral carotid artery occlusion in rats.

Bilateral carotid artery occlusion is the basic experimental inducing model of global cerebral ischemia in animals and common carotid arteries is the main arteries supplying blood to the brain from heart. The occlusion of these arteries for a period of 10 min leads to reduction in blood supply to the brain and the pathophysiological events starts and continues followed by reperfusion.

The primary elements of the pathophysiologic cascade following concussive brain injury include abrupt neuronal depolarization (Giza and Hovda, 2001), release of excitatory neurotransmitters, ionic shifts, changes in glucose metabolism, altered cerebral blood flow and impaired axonal function. These alterations can be correlated with periods of post concussion vulnerability and with neurobehavioral abnormalities. While the time course of these changes is well understood in experimental animal models, it is only beginning to be characterized following human concussion.

In the pathophysiology of stroke the neurotoxic pathway (Ohtaki *et al.*, 2005) which takes place alters the levels of neurotransmitters such as glutamate, acetycholine, dopamine, serotonin and norepinephrine levels and the levels of antioxidant enzymes such as superoxide dismutase, malondialdehyde, glutathione peroxidase, glutathione reductase, catalase and protein and neurons levels in animals. Hence estimation of various neurotransmitters and antioxidant enzyme levels and neurons cell count in hippocampal region was carried out in order to asses these levels in stroke induced animals and treatment groups.
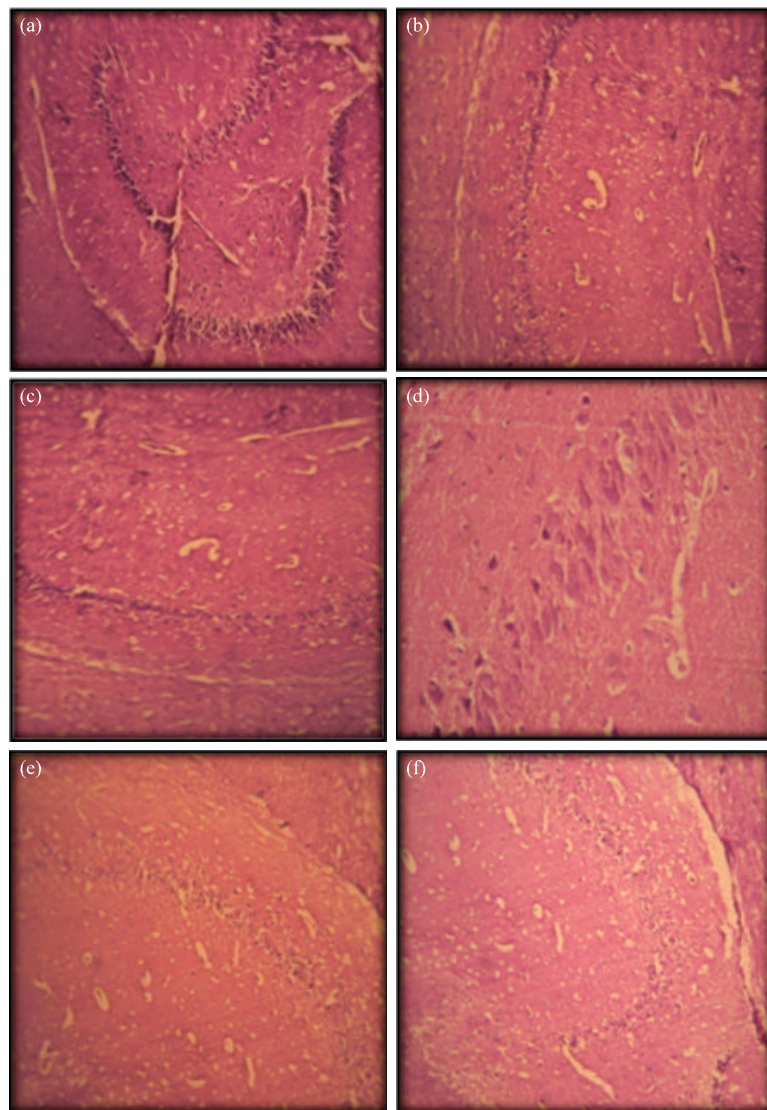
Fig. 2: Protective effect of MEAR against ischemia-mediated cell loss in the CA1 hippocampal area 7 days after ischemia in rat. Figure 2 illustrates neurons within the CA1 region of the hippocampus stained with hematoxylin and eosin at a magnification of 40× after transient cerebral ischemia. (a) Coronal sections showing intact neurons in the hippocampal CA1 region of the Sham control rats; (b) coronal sections showing intact neurons in the hippocampal CA1 region of the sham-treatment rats; (c) coronal sections showing intact neurons in the hippocampal CA1 region of the sham-treatment rats; administration of MEAR (200 and 400 mg kg^{-1} day^{-1} p.o.) for 14 successive days showed no effect on the number of the intact neurons in the hippocampal CA1 region; (d) most pyramidal cell died in the CA1 subfield 7 days following reperfusion in rats subjected to 10 min ischemia: (e and f) In contrast, administration of MEAR (200 and 400 mg kg^{-1} day^{-1} p.o.) 7 days before ischemia and continued for 7 successive days conferred cerebroprotection by markedly reduced number of damaged pyramidal cells in the CA1 subfield

Asparagus racemosus contains steroidal saponins (Shatavarins I-IV) that are present in the roots. Shatavarin IV is a glycoside of sarsasapogenin having two molecules of rhamnose and one molecule of glucose. Other active compounds such as quercetin, rutin (2.5% dry basis) and hyperoside are found in the flowers and fruits; while diosgenin and quercetin-3 glucuronide are present in the leaves (Anonymous, 1987). The presence of sarsasapogenin (Asmari *et al.*, 2004) in natural plants of *Asparagus racemosus* as well as in *in -vitro* cultures. DPPH (α,α-diphenyl-β-picrylhydrazyl) autography-directed separation resulted in the identification of a new antioxidant compound from *Asparagus racemosus* named racemofuran (Wiboonpun *et al.*, 2004).

The potential of methanolic extract of *Asparagus racemosus* roots against kainic acid (Parihar and Hemnani, 2004) (KA)-induced hippocampal and striatal neuronal damage in mice. Intra-hippocampal and intra-striatal injections of KA to anesthetized mice resulted in the production of excitotoxic lesions in the brain. After KA injection, impairment of hippocampus and striatal regions of brain was observed accompanied by increased lipid peroxidation, increased protein carbonyl content, decreased glutathione peroxidase (GPx) activity and reduced glutathione (GSH) content. GSH is an important antioxidant which acts as a nucleophilic scavenger of toxic compounds and as a substrate in the GPx-mediated destruction of hydroperoxides which would otherwise accumulate to toxic levels in brain tissues. The mice treated with *Asparagus racemosus* extract showed an enhancement in GPx activity and GSH content and reduction in membranal lipid peroxidation and protein carbonyl. They concluded that the plant extract plays the role of an antioxidant by attenuating free radical induced oxidative damage.

A polyherbal formulation containing the standardized extracts of *Withania somnifera, Ocimum sanctum, Asparagus racemosus* and *Emblica officinalis* was evaluated for its anti-stress activity in rats (Bhattacharya *et al.*, 2002). Neurological and psychiatric disorders together account for more chronic suffering than all other disorders combined (Cowan and Kandel, 2001). Treating these problems however, remains a challenging field in medical science. Keeping in mind the encouraging leads and the limited data regarding the use of *Asparagus racemosus* in treating neurological disorders; more studies need to be conducted to fully exploit the potential of Satavari in this area.

ROS including hydrogen peroxide (H_2O_2) and superoxide radical (O_2^-) are produced by a number of cellular oxidative metabolic processes (Adibhatla and Hatcher, 2006) involving xanthine oxidase, NAD(P)H oxidases, metabolism of arachidonic acid by cyclooxygenases and lipoxygenases, monoamine oxidases and the mitochondrial respiratory chain.

The sequential univalent reduction of oxygen generates superoxide, hydrogen peroxide and hydroxyl radical (Kontos, 2001). The generation of hydroxyl radical is dependent on catalysis by ferrous iron. In addition, superoxide and nitric oxide produce peroxynitrite, which spontaneously generates hydroxyl radical independently of iron-mediated catalysis. Several enzymes, including superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase, are endogenous antioxidants that process specific free radical scavenging properties. There will be decrease and increase in the levels of these antioxidant enzymes in the stroke induced and treatment groups (Chan, 1996).

There was a decrease in the superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase, proteins and increase in lipid peroxidation level in stroke induced (negative control) group when compared with the control group and negative control group which showed significance of $p < 0.01$ when compared with control group. The group treated with 200 and 400 mg kg^{-1} MEAR showed significant ($p < 0.01$) increase in the superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase, proteins and decrease in lipid peroxidation level when compared with negative control group.

Neurotransmitter release during cerebral ischemia has been extensively studied and is thought to play a key role in excitotoxic neuronal death. The changes in neurotransmitter release and its metabolism may reflect changes in cellular metabolism during ischemia (Kondoh *et al.*, 1994). The purpose is to assess the alterations in extracellular dopamine and serotonin and their metabolites under varied degrees of ischemia in rat striatum to elucidate the pathophysiology of cerebral ischemia. The metabolism of dopamine and serotonin is affected not only under conditions of severe ischemia but also under moderate ischemia. However, under severe ischemia, both dopaminergic and serotonergic systems are markedly affected, whereas under moderate ischemia, the dopaminergic system appears to be more vulnerable. The striatum, a region highly vulnerable to ischemia, is richly innervated both by the corticostriatal glutamatergic pathway and by nigrostriatal dopaminergic projections (Globus *et al.*, 1988), which have been shown to interact with each other.

There was an increase in the acetylcholine esterase, glutamate and decrease in the dopamine, serotonin level in stroke induced (negative control) group when compared with the control group and negative control group which showed significance of $p<0.01$ when compared with control group. The group treated with 200 and 400 mg kg^{-1} MEAR showed significant ($p<0.01$) decrease in the acetylcholine esterase, glutamate and increase in the dopamine, serotonin level when compared with negative control group.

During ischemic stroke, neurons at risk are exposed to pathologically high levels of intracellular calcium (Ca^{++}), initiating a fatal biochemical cascade (Gribkoffi *et al.*, 2001). To protect these neurons, we have developed openers of large-conductance, Ca^{++}-activated (maxi-K or BK) potassium channels, thereby augmenting an endogenous mechanism for regulating Ca^{++} entry and membrane potential. It is well documented that transient forebrain ischemia results in death of the neurons in the CA1 subregion of the hippocampus. Our results indicated that 10 min of forebrain ischemia induced selective neuronal damage in animals.

There was an increase in death of neurons in the CA1 subregion of the hippocampus stroke induced (negative control) group when compared with the control group and negative control group which showed significance of $p<0.01$ when compared with control group. The group treated with 200 and 400 mg kg^{-1} MEAR showed significant ($p<0.01$) protection against neuronal damage when compared with negative control group.

Treatment with MEAR offered protection against hippocampal CA1neuronal damage induced by 10 min of forebrain ischemia as evidenced by the fact that MEAR rescued most of CA1pyramidal neurons from ischemic death which was evident from photomicrograph: I of histopathology study.

The results of this study confirmed that MEAR protects rats from ischemia induced brain injury. This protection was evident from, the significant decrease in the elevated levels of malondialdehyde (lipid peroxidation) and reversal of the decreased GSH contents, catalase and SOD activities and protein levels and decrease in elevated levels of acetycholine esterase, glutamate and reversal of decreased dopamine and serotonin levels and the significant reduction in neuronal cell death in the hippocampal CA1 region to nearly normal levels after forebrain ischemia.

Though the results showed the same significance of $p<0.01$ in 200 and 400 mg kg^{-1} treatment groups. The datas showed in the results of 400 mg kg^{-1} treatment group showed higher cerebroprotective effect when compared to 200 mg kg^{-1} treatment group and MEAR showed dose-dependent protection.

CONCLUSION

In summary, oral administration of MEAR protected rats from ischemia-induced brain injury. The protection may be due to the reduction of oxidative stress which occurs by alteration in levels

of antioxidant enzymes and neurotransmitter levels in stroke induced animals. These observations suggest that MEAR may be a clinically viable protective against a variety of conditions where cellular damage is a consequence of oxidative stress. In addition, MEAR may have the potential to be used in the prevention of neurodegenerative diseases such as cerebral ischemia. In conclusion, the present study provides experimental evidence for MEAR as a cerebroprotective agent and emphasizes the need to understand more fully the neuropharmacological effects of *Asparagus racemosus*.

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