Full Length Research Paper

Neuroprotective potential of *Ocimum sanctum* (Linn) leaf extract in monosodium glutamate induced excitotoxicity

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The aim of this study was to investigate the potent neuroprotective property of ethanol extract of *Ocimum sanctum* (EEOS) leaf (Holy basil, Family: Labiataea) against excitotoxicity induced neurodegeneration by using monosodium-L-glutamate (MSG) in Sprague-Dawley rats. The animals received EEOS (50, 100 and 200 mg/kg) and memantine (MMT, 20 mg/kg) daily for 7 days. On all the 7 days, MSG (2g/kg, i.p.) was administered one hour before drug treatment. The animals were observed for neurobehavioral performance on 1st, 3rd, 5th and 7th day. Oxidative damage and histopathological analysis were also assessed. EEOS (100 and 200 mg/kg, p.o.) and MMT (20 mg/kg, i.p.) administration significantly improved body weight and attenuated locomotor activity, rotarod performance and foot-fault test as compared with MSG treated group. In addition, EEOS was found to restore reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT), super oxide dismutase (SOD) and Na^+-K^+ ATPase. Conversely, the elevated level of lipid peroxidation and nitrite concentration in MSG treated group was attenuated significantly in EEOS group in comparison to MSG treated group. Histopathological evaluation showed that treatment with EEOS and MMT significantly attenuated neuronal death and increased the density of neurons after MSG treatment. Thus, these findings suggest that EEOS contains rosmarinic acid and ursolic acid in addition to other bioactive principles may have utility in the preventing and/or treating the neurodegenerative diseases and its protective effects may be due to the amelioration of excitotoxicity, oxidative stress, neurological and behavioral alterations. However, further studies are necessary to clearly define mechanism responsible.

Key words: *Ocimum sanctum*, F Holy basil, sodium glutamate, neurological, neurodegeneration. rosmarinic acid

INTRODUCTION

Glutamate is present in very high concentrations in the brain and is believed to be a major excitatory neurotransmitter in the mammalian central nervous system (CNS) (Fonnum, 1984). L-monosodium glutamate (MSG), however, has been shown to be toxic to neurons *in vivo* (Mcbean and Roberts, 1984; Kubo et al., 1993) and *in*

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vitro (Zhou et al., 2009; Michaels and Rothman, 1990). This neurotoxicity of L-glutamate has been implicated both in the acute degenerative changes that occur after status epilepticus, hypoglycemia, ischemia, and trauma, and in such chronic neurodegenerative disorders as Huntington's disease, olivopontocerebellar atrophy, Alzheimer's dementia, Parkinsonism, and amyotrophic lateral sclerosis (ALS) (Hynd et al., 2004; Coyle and Puttfarcken, 1993). Although the detailed mechanisms are still not fully clarified, growing evidence points to a key role of receptor-mediated intracellular Ca^{2+} overload and increased reactive oxygen species (ROS) production in governing glutamate-mediated neurotoxicity. This type of over excitation-induced oxidative stress and intracellular Ca^{2+} metabolism disorders have been identified to execute cell death via distinct downstream signaling cascades, including activation of potentially lethal second messengers and enzymes, disturbance of mitochondrial function, and inhibition of anti-apoptotic pathways (Greenwood and Connolly, 2007; Montal, 1998). Thus, neuroprotection against glutamate-induced neurotoxicity has been therapeutic strategy for preventing and/or treating both acute and chronic forms of neurodegeneration (Meldrum, 2002).

In spite of its ubiquitous role as a neurotransmitter, glutamate is highly toxic to neurons, a phenomenon dubbed '*excitotoxicity*' (Choi, 1988). Studies in tissue culture indicate that glutamate receptor mediated neuronal degeneration can be separated into two distinct forms: acute and delayed form of neuronal degeneration. Studies have demonstrated that synaptic glutamate release and uptake are energy (ATP)-dependent, and any impairment or breakdown may lead to generation of ROS and inactivation of glutamate reuptake mechanism leading to excessive glutamate accumulation. If the circumstance continues unabated, there is excessive influx of Na^+, Cl^- and Ca^{2+}, via post-synaptic ion channels producing swelling and destruction of post synaptic elements not only in the immediate vicinity but also the entire neuron as well. Upon destruction of neurons by this mechanism, additional glutamate may be released further increasing the level of extracellular glutamate and thereby propagating the excitotoxicity and death of additional glutamate-sensitive neurons in the region of involvement (Nicholis and Attwell, 1990; Novelli et al., 1988).

Published reports indicates that several medicinal plants have been found to possess *in vitro* neuroprotection against glutamate excitotoxicity (Li et al., 2007; Nobre et al., 2008), may open a promising approach for treating glutamate-associated neurodegenerative disease. *Ocimum sanctum* (*O. sanctum* Linn. Family: Labiatae) is a well-known, widely distributed, and highly esteemed sacred medicinal herb especially for Hindus in the Indian subcontinent. Traditionally, it has been used as nerve tonic to alleviate the problems related to the nervous system. In Ayurveda, *O. sanctum* is described as rasayana (plants having adaptogen like properties). Ayurvedic rasayanas are those drugs, which prevent ageing, increase longevity, impart immunity, improve mental functions and add vigor and vitality to the body (Bhargava and Singh, 1981). Recently, it has been demonstrated that *O. sanctum* exhibited significant neuroprotective effect in models of cerebral reperfusion injury and long-term hypoperfusion (Yanpallewar et al., 2004), anticonvulsant (Jaggi et al., 2003), antistresss (Samson et al., 2006), anti-inflammatory (Godhwani et al., 1987), antioxidant (Kelm, 2000), and antidepressant (Sudhakar et al., 2010) properties.

Major phytochemical compounds in *O. sanctum* leaf include eugenol (volatile oil), palmitic , stearic, oleic, linoleic, linolenic acids (fixed oil), luteolin, orientin, vicenin (flavonoids), rosmarinic acid (RA, phenylpropnoid) and ursolic acid (UA, triterepene) (Kelm, 2000). Therefore, the present study was planned to standardize the ethanol extract of *O. sanctum* (EEOS) leaf for RA and UA and evaluate its possible effects on glutamate-induced general behavioral and biochemical alterations for the first time, in an attempt to assess and validate the central neuroprotective effects of *O. sanctum* leaf.

MATERIALS AND METHODS

Plant material

The aerial parts of the plant *O. sanctum* was collected from Bhavani, Erode district, Tamil Nadu, India. It was taxonomically identified by Survey of Medicinal Plants and Collection Unit, Ooty, Tamilnadu, India, and an herbarium of the plant is preserved (OS/234) in the Department of Pharmacognosy, J.S.S. College of Pharmacy, Ooty, Tamil Nadu, India. The plant was identified and authenticated by Dr. S. Rajan and Dr. D. Suresh Baburaj, Survey of Medicinal Plants and Collection Unit, Ootacamund, Tamilnadu, India.

Dried leaves of *O. sanctum* were coarsely powdered (1.9 kg ± 0.5 dry basis) and subjected to extraction by cold maceration with 90% ethanol at room temperature with continuous stirring (300 rpm) for 7 days, after defatting with petroleum ether (60-80°C). The solvents were evaporated with rotary vacuum evaporator until a solid residue was formed and was stored in desiccator. The solid residue was then made into a fine suspension using 0.5% Tween 80. The extract yield of the *O. sanctum* was found to be 17.38 % w/w. (Joshi and Parle, 2006).

Estimation of rosmarinic acid (RA) and ursolic acid (UA) in EEOS by LC-MS

The liquid chromatography (LC) separation was carried out on a Phenomenex C_{18} (150 x 4 mm i.d., 5µ) with single quadrupole MS analyzer. The mobile phase consisting of 0.5% formic acid-acetonitrile (75:25%) and ammonium acetate-acetonitrile (70:30%) were used for RA and UA respectively, and delivered at a flow rate of 0.5 ml/min. The column temperature was maintained at 30°C. The optimum operating parameters of the APCI interface in negative mode were: Heat block temperature 400°C, CDL temperature 450°C, nebulizing gas (N_2) 1.5 L/min, drying gas (N_2) 10 L/min, and detector voltage 1.3 kV. Quantification was achieved using selected ion-monitoring (SIM) mode of ion at m/z 359 for RA and 455 for UA.

Animals

Healthy Sprague-Dawley rats (age in the range of 4-6 weeks) with weight in range between 120-160 g, were used. Animals were obtained from the Central Animal House of the J.S.S. College of Pharmacy, housed in colony cages at an ambient temperature of 25 ± 2°C and 45-55% relative humidity with 12 h light / dark cycles with lights on at 20:00. Pelleted chow (Brook Bond, Lipton, India) and tap water were freely available in the home cages. A protocol for the use of animals study was approved by the Institutional Animal Ethical Committee, under the regulation of CPCSEA, New Delhi (JSSCP/IAEC/PhD/Ph.Cology/01/2005-06).

MSG administration

MSG (2 g/kg body weight) dissolved in physiological saline solution or sodium chloride solution at an equimolar concentration (control group) was injected intraperitonealy (i.p.) for 7 consecutive days, according to a previous protocol used to determine the MSG effects on neurodegeneration (Ramanathan et al., 2007).

Drug and treatment schedule

Memantine (MMT, Sigma Chemicals, St. Louis, USA) and EEOS was prepared as fine suspension in 0.5% Tween 80 and administered depending upon the body weight (5 ml/kg) of the rats. Experimental study included various treatment groups, consisting of 6 animals in each group. Group-1: Control (vehicle administered); Group-2: MSG (2 g/kg, i.p.); Group-3: MSG (2g/kg, i.p.) + EEOS (50 mg/kg, p.o.); Group-4: MSG (2g/kg) + EEOS (100 mg/kg, p.o.); Group-5: MSG (2 g/kg) + EEOS (200 mg/kg, p.o.); Group-6: MSG (2 g/kg, i.p.) + MMT (20 mg/kg, i.p.); Group-7: EEOS (200 mg/kg, p.o.) per sec (only) treatment. All the groups were subjected daily to drug treatment at 0900 h, for 7 days, starting from day 1 after one hour of MSG treatment (0800 h).

Examination of general behavior

Body weight

The body weight of the animals was recorded immediately before administration of MSG or drug treatment, from the day of first injection (day 1) and continued for 7 days thereafter, by weighing on a top loading balance with accuracy to ± 0.1 g. Changes in body weight were calculated by subtracting the weight of the animal obtained on every day from that of the animal weight immediately before the first MSG injection and expressed as g% change (changes in body weight per 100 g).

Locomotor activity

The locomotor activity of each animal was assessed using a photo-electric actimeter, 1 h after administration of MSG or drug treatment, on 1st, 3rd, 5th and 7th day. The apparatus consists of a stainless steel box containing transparent cages (270 × 220 × 110 mm) in which the animals' horizontal activity is measured by two light beams connected to a photoelectric cell. The total number of beam crossings is recorded over a period of 5 min (Ramanathan et al., 2007).

Rotarod activity

Animals were tested for muscular coordination and balance using the rotarod on 1st, 3rd, 5th and 7th day, 1 h after MSG or drug treat-

ment. The animals were given a prior training session before actual recording on rotarod apparatus (Inco, India). The animals were placed individually on the horizontal rotating bar (diameter 2.5 cm, 20 r.p.m.) of the rotarod apparatus and the time the animal could stay on the rotarod was measured (max. 2 min). Total time spent on the rotating bar was registered using a stopwatch, and the number of falls during the session was also recorded (Dunham and Miya, 1957).

Foot-fault test

The animals were acclimatized for 2 min on an elevated stainless steel grid floor before MSG administration for one week. The foot-fault test was performed 1 h after administration of MSG or drug treatment on 1st, 3rd, 5th and 7th day, according to a published method of Barth and Stanfield (1990). Rats were placed on an elevated stainless steel grid floor 50×40 cm, 1 m above the floor with 3 cm^2 holes and a wire diameter of 0.4 cm. Each animal was placed on the grid and observed for 2 min. An animal is said to have satisfactorily performed a foot-fault when it misplaced a fore or hind limb and the paw falls through between the grid bars. The excess of left (ipsilateral foot-faults) to right (contralateral foot-faults) was recorded. Only the side difference of foot-faults was used for the statistical evaluation to eliminate the influence of the extent of activity in different rats.

Biochemical estimations

Dissection and homogenization

On day 8, following behavioral testing, animals were sacrificed by decapitation under ether anesthesia and the brains were removed immediately and dissected. The hippocampus and striatum, of all the animals were isolated, rinsed in ice-cold isotonic saline and packed with ice and stored at -70°C. A 25% homogenate was prepared in potassium phosphate buffer (100 mM, pH 7.5) containing 0.15 M KCl. The homogenate was then centrifuged at 10000 x g at 4°C for 20 min and the resultant supernatant was separated and used for biochemical estimations.

Total glutathione estimation (GSHt)

GSHt was measured by the enzymatic recycling procedure in which reduced glutathione (GSH) is sequentially oxidized by 5, 5-dithiobis-(2- nitrobenzoic acid) (DTNB) to oxidized glutathione (GSSG) which is then reduced by NADPH in the presence of glutathione reductase (GR) back to GSH (Griffith, 1980) . GSH was used as an external standard, and the level of GSHt in the samples was expressed as nanomoles of GSH/mg protein.

Glutathione peroxidase estimation (GPx)

The reaction mixture consisted of phosphate buffer (0.05 M, pH 7.0), 1 mM ethylenediaminetetraacetic acid (EDTA), sodium azide (1 mM), GR (1 EU/ml), GSH (1 mM), NADPH (0.2 mM), hydrogen peroxide (0.25 mM), and 0.1 ml of supernatant in a final volume of 2 ml. The disappearance of NADPH at 340 nm was recorded at room temperature. The enzyme activity was calculated as nanomoles of NADPH oxidized per minute per milligram of protein using a molar extinction coefficient $6.22×10^{-3}$ M^{-1} cm^{-1} (Mohandas et al., 1984).

Glutathione reductase estimation (GR)

The assay mixture consisted of phosphate buffer (0.1 M, pH 7.6),

NADPH (0.1 mM), EDTA (0.5 mM), GSSG (1 mM), and 0.05 ml of supernatant in a total volume of 1 ml. The enzyme activity was quantified at room temperature by measuring the disappearance of NADPH at 340 nm and was calculated as nanomoles of NADPH oxidized per minute per milligram of protein using molar extinction coefficient of 6.22×10^{-3} M^{-1} cm^{-1} (Mohandas et al., 1984).

Superoxide dismutase estimation (SOD)

Brain SOD activity was determined colorimetrically according to the method of Kakkar et al. (1984). This assay relies on the ability of the enzyme to inhibit the phenazine methosulfate-mediated reduction of nitroblue tetrazolium dye which can be measured at 560 nm.

Catalase estimation (CAT)

In brief, potassium phosphate buffer (65 mM, pH 7.8, 2.25 ml) and 100 µl supernatant or sucrose (0.32 M) were incubated at 25°C for 30 min. Hydrogen peroxide (7.5 mM; 650 µl) was added to initiate reaction. Decomposition of hydrogen peroxide in the presence of CAT was followed at 240 nm. The results are expressed as units (U) of CAT activity/mg of protein (Beers and Sizer, 1952).

Lipid peroxidase estimation (LPO)

LPO in terms of thiobarbituric acid reactive substances (TBARS) was measured using the method of Okawara et al. (1979). Lipid peroxide content was expressed as nanomoles of malondialdehyde (MDA) / mg of protein. The calibration curve was prepared by using 1,1,3,3-tetra ethoxypropane as standard.

Nitrite estimation

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined with a colorimetric assay with Greiss reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by Green et al (1982). Equal volumes of supernatant and Greiss reagent were mixed, and then the mixture was incubated at 25°C for 10 min in the dark. The concentration of nitrite was assayed at 540 nm and calculated with reference to the absorbance of the sodium nitrite standard curve.

Na$^+$- K$^+$ ATPase estimation

Na$^+$-K$^+$ ATPase was assayed by taking 250 µl of Tris HCl (184 mM; pH 7.5) buffer followed by the addition of 50 µl of 600 mM NaCl, 50 µl of 50 mM KCl, along with 50 µl of 1 mM Na-EDTA and 50 µl of 80 mM ATP. The reaction mixture was pre-incubated at 37°C for 10 min. Then 25 µl of 10% homogenate was added to the test alone and further incubated at 37°C for 1 h. The reaction was immediately arrested by the addition of 10% trichloroacetic acid (TCA). Control samples were carried out under the same conditions with the addition of 0.1 mM ouabain. The precipitate was removed by centrifugation at 3500 rpm for 10 min. The supernatant (0.5 ml) was used for the estimation of inorganic phosphorous according to method of Sovoboda and Mossinger (1981). The specific activity of the enzyme corresponds to the difference between the total ATPase activity and the activity measured in the presence of 1 mM ouabain (ouabain-resistant activity).

Protein estimation

Protein was assayed according to Lowry et al. (1951) using the Folin-Ciocalteus phenol reagent with bovine serum albumin as standard.

Histological examination

On the 8th day, the animals were anesthetized with diethyl ether and underwent transcardialperfusion with 0.9% saline followed by 4% para-formaldehyde in 0.1 M phosphate buffer (pH7.3). The hippocampus, harvested from the rat brain, was post-fixed over night in parformaldehyde, processed and embedded in paraffin. The fixed brains were cut into 5 µm sections on a microtome. Levels of sections and CA1 area in the hippocampus were found with the help of the stereotaxic atlas (Paxinos and Watson, 2007), and were stained with cresyl-violet. The hippocampal damage was determined by counting the number of intact neurons in the stratum pyramidale within the CA1 subfield at a magnification of 400 and the counts of neurons were determined per square millimeter by using a standardized ocular grid. The neurons counted in random high-power fields using a light microscope (Olympus BH-2, Japan) incorporating a square graticule in the eyepiece (eyepiece x10, objective x40,a total side length of 0.225mm). Neurons density was assessed by counting the number of cells in 200 high power field in hippocampal tissue preparations of each group. The neurons density in each site was calculated and recorded as number of NCs/mm^2. The tissue compartments were used to record the neurons distribution in hippocampal tissue. Six sections from each animal were used for counting.

### Histological examination

The brain tissue was fixed in 10% formalin for one week, washed in running tap water for 24 h and dehydrated in ascending series of ethanol (50–90 %), followed by absolute alcohol. The samples were cleared in xylene and immersed in a mixture of xylene and paraffin at 60ºC. The tissue was then transferred to pure paraffin wax of the melting point 58°C and then mounted in blocks and left at 4°C. The paraffin blocks were sectioned on a microtome at thickness of 5 µm and mounted on clean glass slides and left in the oven at 40°C to dryness. The slides were deparafinized in xylene and then immersed in descending series of ethanol (90–50%). The ordinary haematoxylin and eosin stain was used to stain the slides (Paxinos and Watson, 2007).

Liquid chromatography-mass spectrophotometry (LC-MS) method validation

The assay was shown to be linear over the range of 100-1000 ng/ml (r^2 = 0.9997 and 0.9999 for RA and UA respectively). The limit of detection (LOD) and limit of quantification (LOQ) for RA and UA were found to be 1 and 3 ng/ml, and 2 and 6 ng/ml, respectively. The method was shown to be reproducible and reliable with intraday precision below 0.14 and 0.11% and interday precision below 0.71 and 0.46% and mean recovery excess of 94.38 and 92.92% for RA and UA, respectively.

Statistical analysis

All data are presented as mean ± SEM. The behavioral and biochemical data was analyzed using two ways ANOVA and one way analysis of variance (ANOVA) followed by Tukey-Kramer post hoc analysis was used to analyze differences between the groups. A probability level (p) of value of less than 0.05 was considered to be statistically significant. The statistical analysis was performed using GraphPad Prism for Windows (Graph Pad Prism Software (version 4.03), San Diego, California, USA).
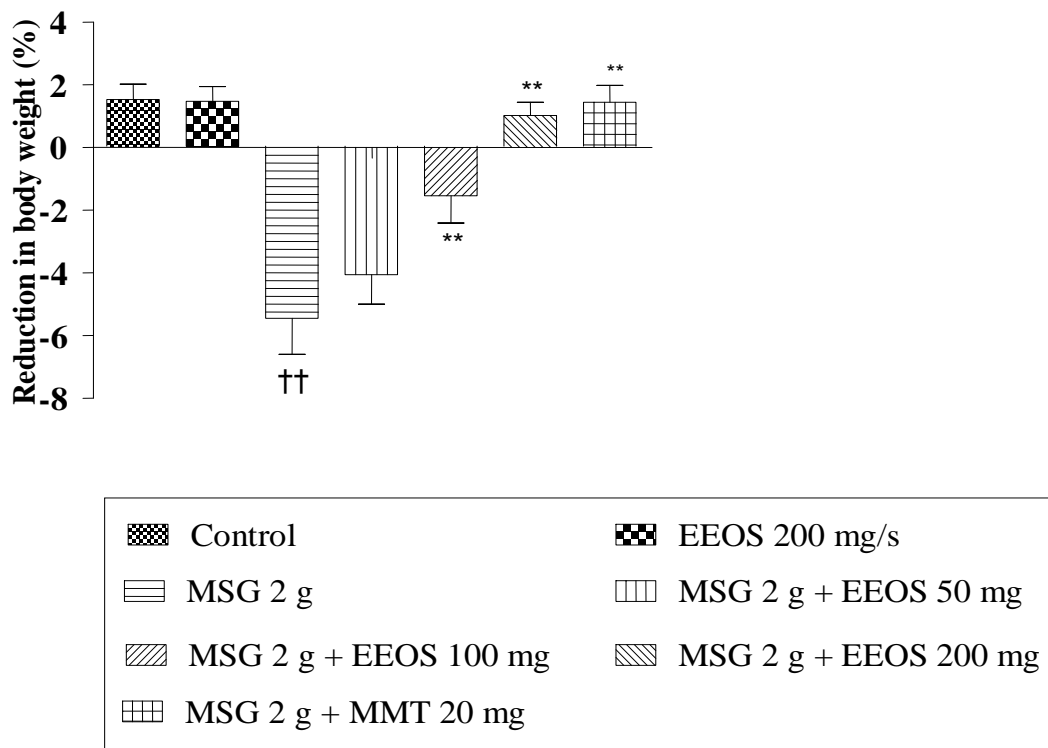
Figure 1. Effect of EEOS and memantine on MSG induced change in rat body weight. Values are expressed in mean±SEM; significance with Tukey's test following one way ANOVA is indicated as [†]p<0.05 and [††]p<0.01 compared to control or *p<0.05 or **p<0.01 compared to MSG.

RESULTS

LC-MS estimation of RA and UA

LC-MS assay achieved higher sensitivity and better specificity for analysis of RA and UA. The method offers sensitivity, with a LOQ of 3 and 6 ng/ml for RA and UA, respectively, without interference from other phytoconstituents present in the herb. Using the optimized conditions, the quantity of RA and UA in EEOS was found to be 0.27 and 0.40% w/w, respectively. The method is suggested to be ideally suited for rapid routine analysis of RA and UA.

### Effect of EEOS and MMT on MSG induced change in body weight

The body weights of the experimental animals are shown in Figure 1. Administration of MSG displayed significantly greater decrease in body weights (–11.45) of the rats as compared to control group. EEOS (100 and 200 mg/kg) and MMT (20 mg/kg) co-treatment significantly improved the body weight (P<0.001) as compared to MSG treated group. EEOS (200 mg/kg) per se treatment demonstrated had no statistically significant effect on the body weight as compared to control group.

Effect of EEOS and MMT on MSG induced alterations in locomotor activity and rotarod performance

Co-administration of MSG for 7 days significantly reduced locomotor activity and rotarod performance as compared to the control group. EEOS (100 and 200 mg/kg) and MMT (20 mg/kg) treatment significantly improved the locomotor activity (Figure 2) (P<0.001) and fall off time (rotarod performance) (Figure 3) as compared to MSG treated animals (P<0.001). EEOS (200 mg/kg) per se treatment exhibited no significant effect on locomotor activity and fall off time (rotarod performance) as compared to control group.

Effect of EEOS and MMT on MSG induced alterations in foot-fault test

Figure 4 shows the number of foot-faults in each group. The number of foot-fault was significantly greater in the MSG treated group than that in the control group. The number of foot-fault was significantly (P<0.001) attenuated in EEOS (100 and 200 mg/kg) and MMT (20mg/kg) co-treated group than that in the MSG treated group. EEOS (200 mg/kg) per se treatment demonstrated no statistically significant effect on foot-fault test as compared to control group.
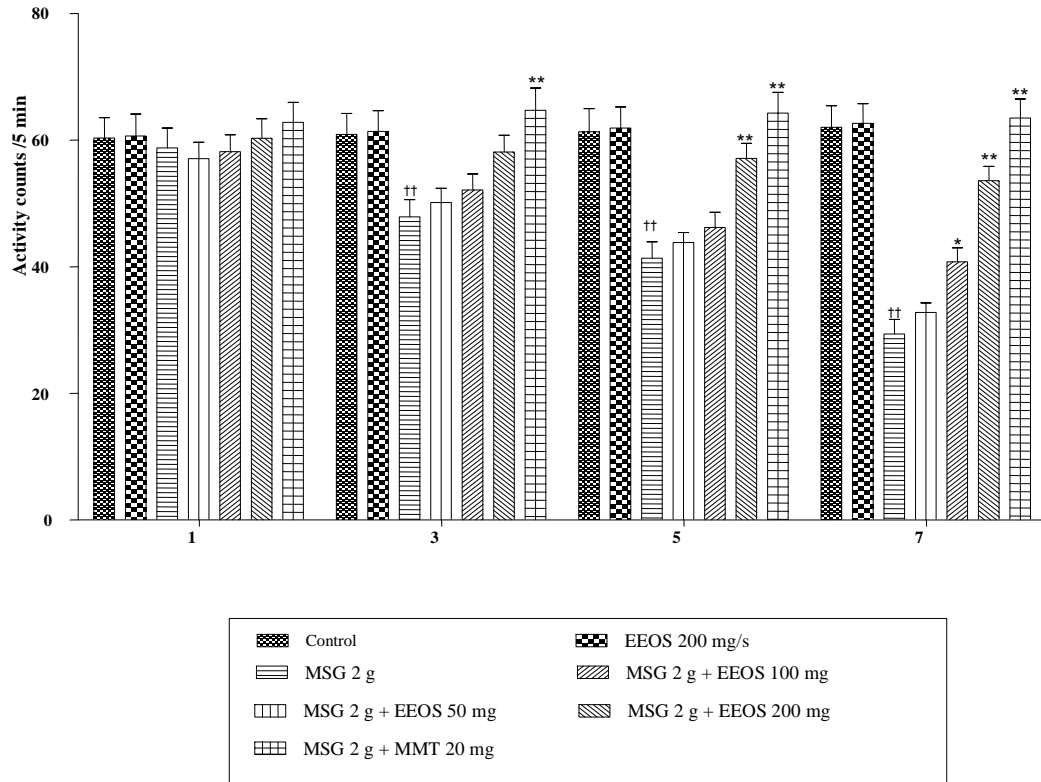
Figure 2. Effect of EEOS and memantine on locomotor activity in MSG treated rats. Values are expressed in mean±SEM; significance with Tukey's test following one way ANOVA is indicated as [†]p<0.05 and [††]p<0.01 compared to control or *p<0.05 or **p<0.01 compared to MSG.
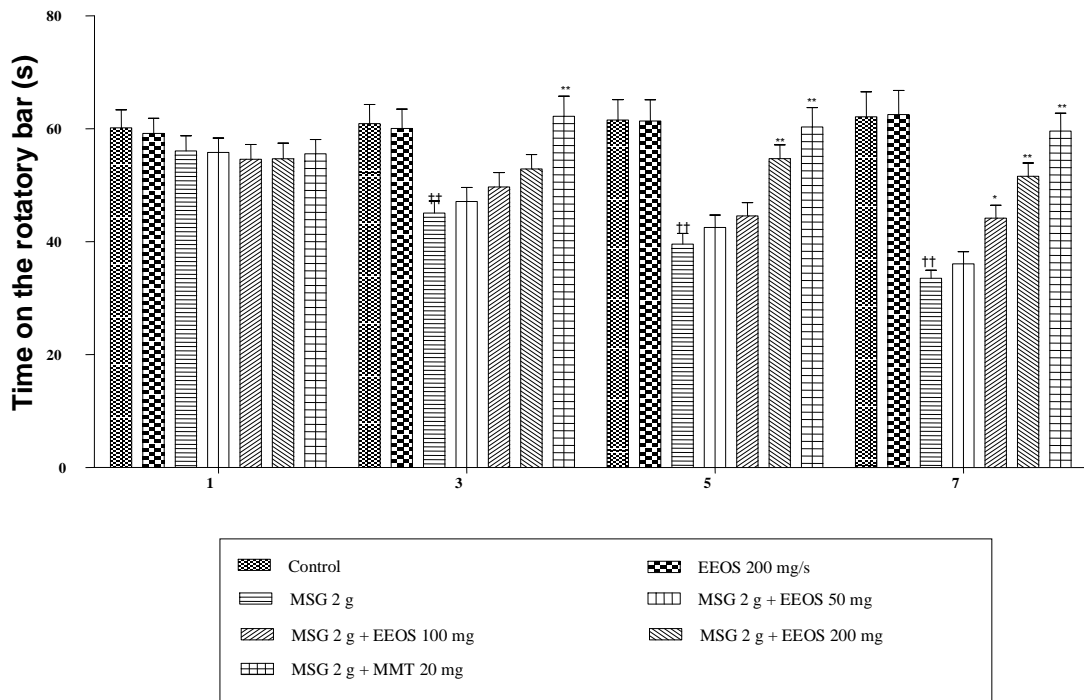
Figure 3. Effect of EEOS and memantine on performance of rota-rod test in MSG treated rats. Values are expressed in mean±SEM; significance with Tukey's test following one way ANOVA is indicated as [†]p<0.05 and [††]p<0.01 compared to control or *p<0.05 or **p <0.01 compared to MSG.
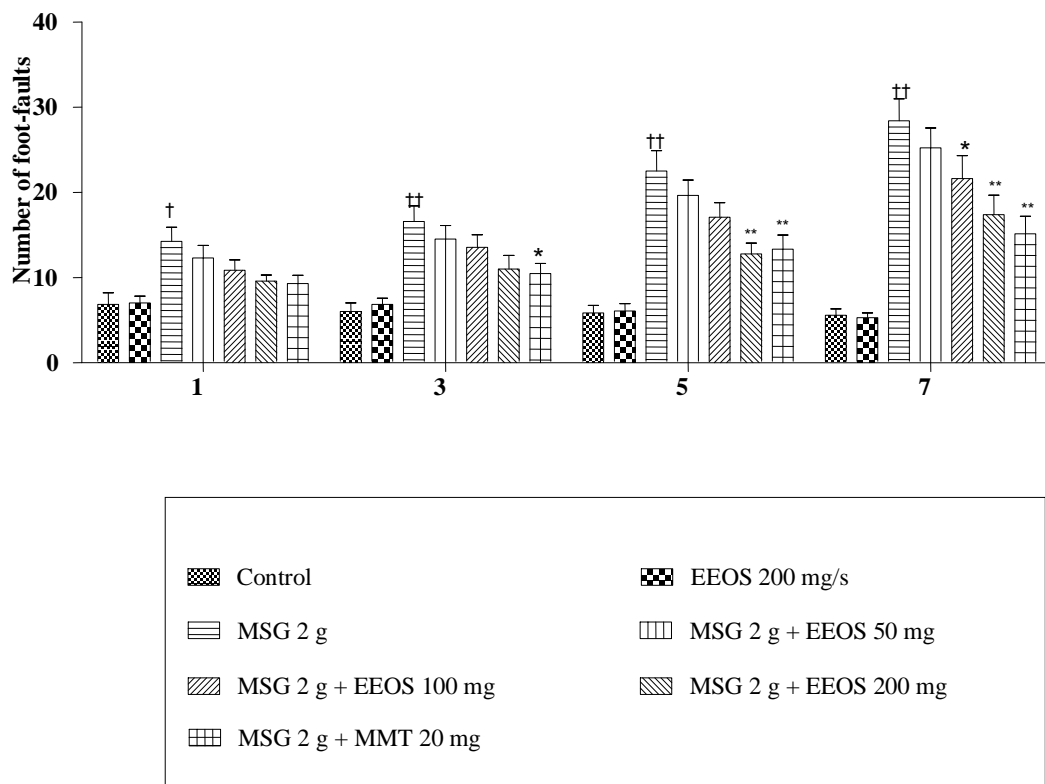
Figure 4. Effect of EEOS and memantine on the performance of foot-fault test in MSG treated rats. Values are expressed in mean±SEM; significance with Tukey's test following one way ANOVA is indicated as [†]p<0.05 and [††]p<0.01 compared to control or *p<0.05 or **p<0.01 compared to MSG.

Effect of EEOS and MMT on MSG induced GST, GPx, and GR enzymes level

A significant decrease in the GSHt, GPx and GR level was observed in hippocampus and striatum of the MSG treated groups as compared to the control group (Table 1). On the other hand, EEOS (100 and 200 mg/kg) and MMT (20 mg/kg) treatment significantly stimulated GSHt, GPx and GR levels both in hippocampus (P<0.001) and striatum (P<0.001) in comparison to MSG treated groups, whereas EEOS (200 mg/kg) per se treatment demonstrated no significant change in GSHt, GPx and GR levels, as compared to control group.

Effect of EEOS and MMT on MSG induced SOD and CAT enzyme level

The data in Table 2 indicates that SOD and CAT enzyme levels in hippocampus and striatum were significantly decreased (P<0.001) in MSG treated animals with respect to control group and significantly increased in EEOS (100 and 200 mg/kg) and MMT (20 mg/kg) treated groups (P<0.001) with respect to MSG treated group both in the hippocampus (P<0.001) and striatum (P<0.001), whereas EEOS (200 mg/kg) per se treated animals

demonstrated no significant change in SOD and CAT enzymes level as compared to control group.

Effect of EEOS and MMT on MSG induced lipid peroxidation (MDA) and nitrite content

A significant increase in MDA and nitrite content in hippocampus and striatum was observed in MSG treated group when compared to control group. EEOS (100 and 200 mg/kg) and MMT (20 mg/kg) demonstrated significant attenuation in lipid peroxidation and nitrite concentration both in hippocampus (P<0.001) and striatum (P<0.001) comparison to MSG treated group (Table 3). EEOS (200 mg/kg) per se treatment demonstrated no significant effect on MDA and nitrite content as compared to control group.

Effect of EEOS and MMT on Na^+- K^+ ATPase activity

Figure 6 shows that MSG administration significantly decreased Na^+-K^+ ATPase activity in hippocampus and striatum as compared to the control group. However, EEOS (100 and 200 mg/kg) and MMT (20mg/kg) treatment enhanced Na^+-K^+ ATPase activity significantly,

Table 1. Effect of EEOS and memantine on MSG induced change in GSH, GPx and GR levels of brain hippocampus and striatum.

Treatment	nmol GSH/mg protein		GPx (nmol NADPH oxidized/min/mg protein)		GR (nmol NADPH oxidized/min/mg protein)	
	Hippocampus	Striatum	Hippocampus	Striatum	Hippocampus	Striatum
Control	3.54±0.52	2.86±0.47	42.56±2.17	37.89±2.25	50.14±2.65	54.14±3.13
EEOS 200 mg per sec	3.82±0.60	3.25±0.62	44.24±2.08	40.24±2.40	54.62±3.30	58.55±3.50
MSG 2g	1.04±0.12††	0.92±0.07††	18.70±1.22††	16.60±1.06††	28.73±1.72††	28.58±1.59††
MSG 2 g + EEOS 50 mg	1.14±0.15	1.03±0.09	21.42±1.28	18.40±1.30	31.13±2.14	30.84±2.35
MSG 2 g + EEOS 100 mg	2.69±0.20*	2.57±0.16*	30.05±1.45*	26.19±1.53*	43.65±2.54*	43.51±2.28*
MSG 2 g + EEOS 200 mg	3.26±0.24*	2.86±0.28*	38.30±1.72**	33.58±1.84**	55.82±2.70**	50.77±2.94**
MSG 2 g + MMT 20 mg	3.70±0.35**	3.24±0.35**	42.53±2.29**	40.13±2.42**	58.45±3.14**	55.45±2.66**

Values are expressed in mean±SEM; significance with Tukey's test following one way ANOVA and indicated as †p<0.05 and ††p<0.01 compared to control or *p<0.05 or **p<0.01 compared to MSG.

Table 2. Effect of EEOS and memantine on MSG induced change in SOD and catalase levels of brain hippocampus and striatum.

Treatment	SOD levels (U of SOD/mg protein)		Catalase levels (µmol of catalase/mg protein)	
	Hippocampus	Striatum	Hippocampus	Striatum
Control	1.71±0.17	1.25±0.12	36.8±2.85	29.2±2.50
EEOS 200 mg/s	1.83±0.27	1.30±0.15	37.1±2.60	32.5±2.72
MSG 2 g	0.84±0.06††	0.64±0.05††	15.3±1.32††	13.5±1.16††
MSG 2 g + EEOS 50 mg	1.07±0.08	0.86±0.07	17.4±1.63	15.1±1.24
MSG 2 g + EEOS 100 mg	1.63±0.13*	1.34±0.12*	27.2±2.40*	26.2±2.49*
MSG 2 g + EEOS 200 mg	1.86±0.20*	1.52±0.18*	33.8±2.84**	32.5±3.18**
MSG 2 g + MMT 20 mg	2.25±0.21**	1.80±0.21**	39.4±3.25**	38.6±3.51**

Values are expressed in mean±SEM; significance with Tukey's test following one way ANOVA is indicated as †p<0.05 and ††p<0.01 compared to control or *p<0.05 or **p<0.01 compared to MSG.

Table 3. Effect of EEOS and memantine on MSG induced change in MDA and nitrite levels of brain hippocampus and striatum.

Treatment	MDA levels (µmol of MDA/mg protein)		Nitrite levels (µmol of nitrite/mg protein)	
	Hippocampus	Striatum	Hippocampus	Striatum
Control	0.97±0.05	0.76 ± 0.04	098.20 ± 4.12	094.53±4.12
EEOS 200 mg/s	0.87±0.04	0.68 ±0.06	092.45 ± 4.65	089.08±3.95
MSG 2 g	1.86±0.14††	1.47 ± 0.15††	224.93 ± 9.71††	195.73±8.94††
MSG 2 g + EEOS 50 mg	1.73±0.10	1.26± 0.12	208.52±9 .39	173.26±8.44
MSG 2 g + EEOS 100 mg	1.31±0.08*	1.05± 0.09*	171.58± 6.73*	146.44±7.09*
MSG 2 g + EEOS 200 mg	1.18±0 .06**	0.95 ± 0.07**	129.96 ±5.94**	118.16±5.60**
MSG 2 g + MMT 20 mg	1.05±0.04**	0.83 ± 0.05**	112.23±5.23**	105.35±4.38**

Values are expressed in mean±SEM; significance with Tukey's test following one way ANOVA is indicated as †p<0.05 and ††p<0.01 compared to control or *p<0.05 or **p<0.01 compared to MSG.

both in hippocampus (P<0.001) and in striatum (P<0.001) when compared with MSG treated groups (Table 4).

Further, there was no significant change found in EEOS (200 mg/kg) per se treated group of rats as compared to

Table 4. Effect of EEOS and memantine on MSG induced change in Na$^+$/K$^+$-ATPase activity levels of brain hippocampus and striatum.

Treatment	Na$^+$/K$^+$-ATPase activity (nmol of inorganic phosphorus liberated/min/mg protein)	
	Hippocampus	**Striatum**
Control	360.53±22.25	301.53±17.20
EEOS 200 mg/s	388.49±23.54	321.15±19.13
MSG 2 g	138.71±08.78[††]	127.16±07.44[††]
MSG 2 g + EEOS 50 mg	186.20±10.30	161.24±09.60
MSG 2 g + EEOS 100 mg	278.67±13.25**	240.22±12.48**
MSG 2 g + EEOS 200 mg	347.02±18.18**	293.43±17.30**
MSG 2 g + MMT 20 mg	356.82±21.40**	298.17±18.58**

Values are expressed in mean±SEM; significance with Tukey's test following one way ANOVA and indicated as [†]$p<0.05$ and [††]$p<0.01$ compared to control or *$p<0.05$ or **$p<0.01$ compared to MSG.

control group.

Histological studies

The histological results are shown in Figure 5a-g. In the control group, the morphology of neurons CA1 region of the hippocampal tissues were normal, borders distinct and cell membrane integrity was preserved. Nucleus borders were regular and nucleolus was distinctively observed EEOS (200 mg/kg, Figure 5a) per se treatment demonstrated no significant effect on the morphology of neurons in CA1 region of the hippocampal tissues as compared to control group (Figure 5b). The observations of cresyl-violet stained Figure 5c showed unequivocal signs of neuron death with extensively dark pyknotic and shrunken nuclei located in the CA1 pyramidal cell layer of rats following 7 days of MSG treatment, in comparison to control group animals. In contrast to this, the cellular structures of neurons in EEOS (100 and 200 mg/kg) and MMT (20 mg/kg) treated group were found to be normal and the nuclei and the nucleoli were observed to be more distinct in hippocampal CA1 region in comparison with the control group (Figure 5e,f and g). On the other hand, animals treated with EEOS (50 mg/kg,) exhibited very little neuroprotection as evidenced from the extensive loss of hippocampal CA1 cell bodies and decreased neuronal density in MSG treated animals in comparison with control group animals (Figure 5d). The effect of EEOS and MMT treatment on neuron density was examined by quantitative histopathological analysis. In MSG treated group, hippocampal neuron density was significantly decreased (Figure 6) in comparison with the control group (p <0.001). However, treatment with EEOS (100 and 200 mg/kg) and MMT (20 mg/kg) significantly preserved the neurons of hippocampal regions (P<0.001) as compared to MSG treated group. Further, treatment with EEOS (50 mg/kg) did not show any significant inhibition against the decreased neuronal cell density due to MSG treatment. EEOS (200 mg/kg) per se treatment

exhibited no significant effect on hippocampal neuron density as compared to control group.

DISCUSSION

The present study highlights the neuroprotective effect of EEOS against MSG-induced neurotoxicity. The results of present study indicate that the treatment with EEOS significantly improved body weight and motor deficits, marked reduction in oxidative stress, restored antioxidant defense mechanisms and reduction in histological changes characterized by MSG-induced neurodegeneration study. Administration of MSG for seven days exhibited significantly reduced body weight, locomotor activity, muscle grip strength test and foot fault test in rats. These finding are consistent with earlier reports including those from our laboratory, which showed a variety of neurobehavioral abnormalities and motor deficits in rats following MSG administration (Ramanathan et al., 2007). In the present study, EEOS treatment significantly attenuated changes in body weight and motor function.

Glutamate toxicity is a major contributor to pathological cell death within the nervous system and appears to be mediated by ROS (Coyle at al., 1981). There are two forms of glutamate toxicity: excitotoxicity pathway relies on the hyper-activation of glutamate receptors and non-receptor mediated oxidative glutamate toxicity (Murphy et al., 1989). Reports indicated that there may be a close relation between activation of glutamate receptor and oxidative stress. Hyperactivation of glutamate receptor has been implicated in inhibition of cystine transport, GSH depletion, and lipid peroxidation (Bondy and Lee, 1993). Oxidative glutamate toxicity is initiated by high concentrations of extracellular glutamate that prevent cystine uptake into the cells via the cystine/glutamate antiporter system, resulting in depletion of intracellular cysteine and glutathione. Glutathione (GSH) depletion induces cellular accumulation of ROS, leading to cell
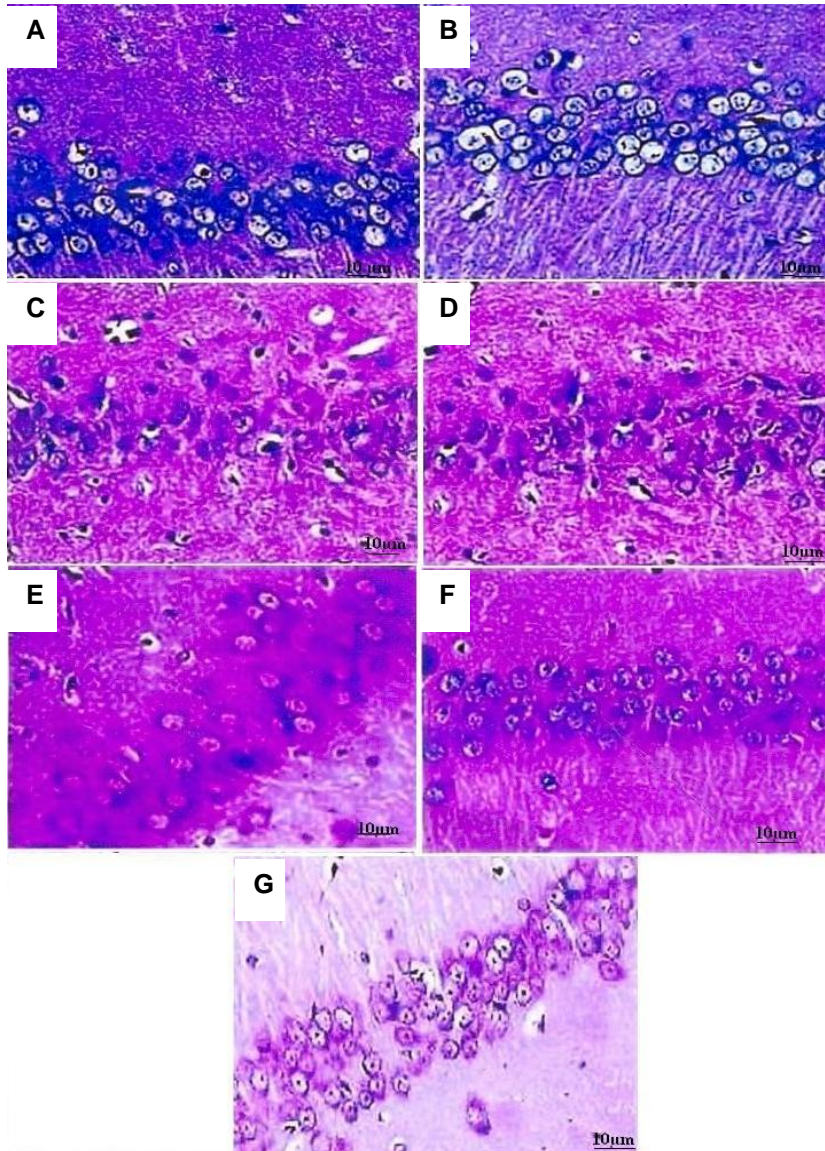
Figure 5. (Panel a–g) The effect of EEOS and memantine on the density of neurons in hippocampal CA1 region of MSG and drug treated rat brains. Photomicrographs of cresyl-violet stained brain hippocampus CA1 sections. (Panel a) Vehicle-treated control group showing healthy neurons; (Panel b) EEOS (200 mg/kg) *per se* treatment showing intact neurons; (Panel c) MSG treated group showing signs of neuron death with extensively dark pyknotic and shrunken nuclei located in the CA1 pyramidal cell; (Panel d) EEOS (50 mg/kg) little/no protective effect on MSG induced neurodegeneration; (Panel e) EEOS (100 mg/kg.) showing neuroprotection against MSG induced neurotoxicity; (Panel f) EEOS (200 mg/kg) showing more neuroprotection against MSG induced neurotoxicity; (Panel g) memantine (20 mg/kg) showing more protection against MSG induced neurodegeneration (Scale bars represent 10µm, magnification:40×).

injury. GSH is one of the most important tripeptide and plays an important role in maintaining cellular oxidant homeostasis by detoxifying ROS with antioxidative enzymes such as SOD, GPx, CAT and GR (Dringen, 2000). The Na^+-K^+ ATPase activity was reduced or insufficient to maintain ionic balances during and immediately after episodes of ischemia, hypoglycemia, epilepsy, and after administration of glutamate agonists (LEES) (Lees, 1991). In the present study, intra-peritoneal administration MSG caused oxidative damage as evidenced by decreased levels of GSH, GPx, GR, SOD, CAT, Na^+-K^+ ATPase activities and elevated melondialdehyde and
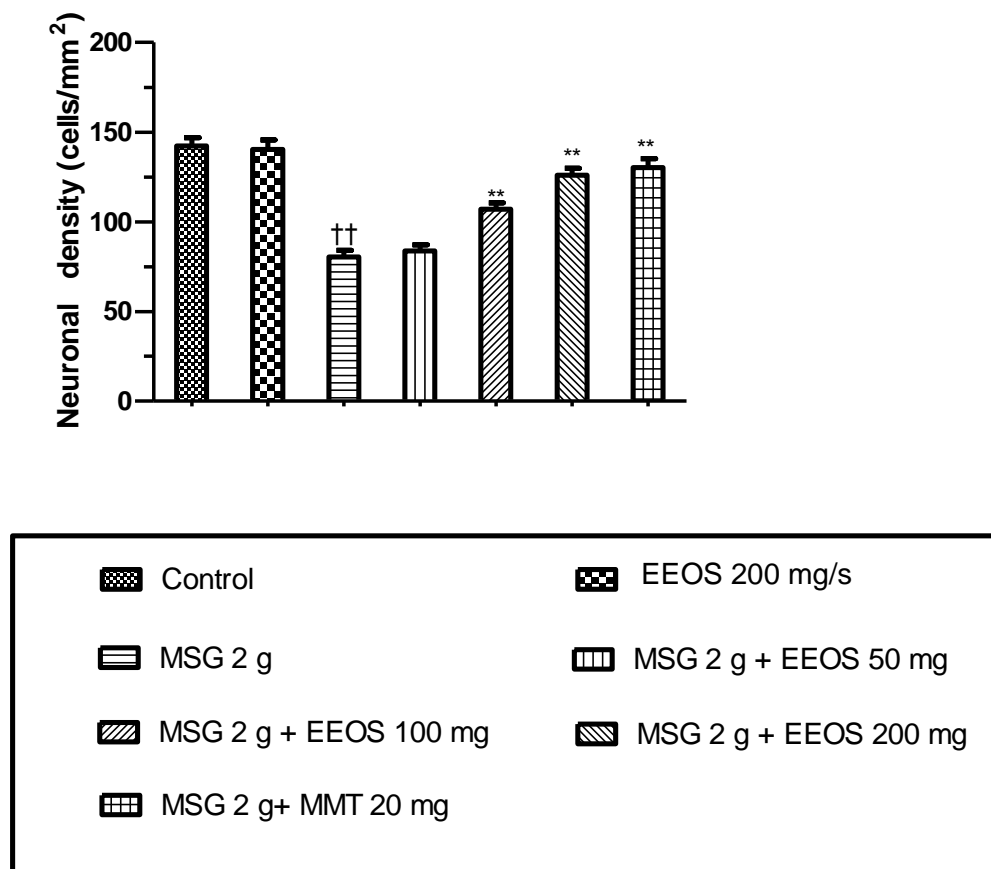
Figure 6. Effect of EEOS and memantine on neuronal density in the cresyl-violet stained hippocampal CA1 section of MSG treated rats. Values are expressed in mean±SEM; significance with Tukey's test following one way ANOVA is indicated as $^{†}p<0.05$ and $^{††}p<0.01$ compared to control or $^{*}p<0.05$ or $^{**}p<0.01$ compared to MSG.

nitrite content in the brain, as compared with the vehicle control rats, these results are consistent with earlier report (Ramanathan et al., 2007; Lees, 1991). Previous studies demonstrated that *O. sanctum* has a potent antioxidant activity by scavenging free radicals (Kelm, 2000). *O. sanctum* extracts and their fractions showed strong inhibitors of *in vitro* lipid peroxidation of erythrocytes membranes and also exhibited anti-lipidperoxidative effects *in vivo* both in normal and in hypercholesterolemia-induced stress conditions (Geetha et al., 2004). Yanpallewar et al. (2004) reported that pretreatment of *O. sanctum* prevented the oxidative stress caused by cerebral reperfusion injury as well as attenuated the behavior deficits and histopathological alterations secondary to long-term hypoperfusion. Strikingly, EEOS treatment significantly increased the activities of all these enzymes and decreased the level of LPO and nitrite in the brain of MSG treated rats. Our results strongly suggest that EEOS can strengthen anti-oxidative defense against free radicals induced by MSG *in vivo*.

It is well reported that MSG administration produces morphological and histological changes in rat brain that further results in learning and memory impair-ment (Coyle et al.,1981; Ali et al, 2000). Furthermore, neonatal administration of MSG has been reported to destroy the hippocampal CA1 structure while other neurons such as CA3, the dentate gyrus and the frontal cortex are much less vulnerable in rodents and impair the acquisition of discrimination learning (Kubo et al., 1993). In the studies carried out with MSG treated rat, unequivocal signs of neuron death with extensively dark pyknotic and shrunken nuclei located in the CA1 pyramidal cell layer have been determined. Oral administration of EEOS remarkably attenuated MSG induced neuronal loss and also decreased pyknotic cell density in a dose dependent manner. The cell counts showed that the number of cells in the hippo-campus sections were significantly lower in the MSG treated groups than in the control group. On the other hand, the number of cells in the treatment groups was closer to the values of the control group compared to the MSG treated group. This result suggests that EEOS has neuroprotective effects against MSG induced neuronal

death.

Previous phytochemical studies of *O. sanctum* have led to the isolation of terpenoids, fatty acids, triglycerides esters and phenolic derivatives (Norr and Wagner, 1992; Sukari et al., 1995). The content of RA and UA in the leaves of EEOS was quantified by LC-MS and was found to be 0.27 and 0.40% w/w, respectively. Therefore it can be assumed that EEOS attenuates behavioral and biochemical alterations against MSG induced excitotoxicity depending on rosmarinic acid, ursolic acid and other active principles present in EEOS leaves.

The effect of EEOS was comparable and equipotent to that of MMT, a known N-methyl-D-aspartate receptor (NMDAR) antagonists. MMT (20 mg/kg) administration significantly improved body weight and attenuated the altered neurofunctional paradigms (locomotor activity, rotarod performance and foot-fault test) significantly and prevented the neurodegeneration on MSG treatment. In addition, MMT significantly increased the activities of antioxidant enzymes and decreased the level of lipid peroxidation and nitrite in the brain of MSG treated rats. These effects could be attributed to the antagonizing activity at NMDAR, leading to controlling the glutamate excitotoxicity resulting in the improved body weight, motor functions and oxidative defense. These findings are in line with previous reports regarding modulatory effect of MMT on neurological deficits, anxiogenic behavior, histological changes, lipid peroxidation, Na$^+$-K$^+$ ATPase activity and antioxidant enzymes following injuries such as hypoxia/ischemia and CNS injuries (Ozsuer et al., 2005; Liu et al., 2009).

The effect of EEOS was comparable and equipotent, to that of known NMDAR-antagonists memantine. The most acceptable explanation for the neuroprotective action of memantine is to block preferentially the opening of the NMDA channel due to prolonged exposure to extracellular glutamate while still allowing for physiological activation of the NMDAR. It was also found that memantine decreased the levels of SOD, CAT and GSH with a corresponding increase in TBAR levels in all the regions studied. These effects could be attributed to the antagonizing activity at NMDAR, leading to controlling the glutamate excitotoxicity resulting in the preservation of brain antioxidant system.

The present study has provided experimental evidence for neuroprotective effects of EEOS against MSG induced excitotoxicity might caused at least in part by the increase in the activity of antioxidant enzymes with reduction in lipid peroxidation and nitrite concentration and improvement in behavioral activities.

Thus, these findings suggest that EEOS may have utility in the preventing and/or treating the neurodegenerative diseases and its protective effects may be due to the amelioration of excitotoxicity, oxidative stress, neurological and behavioral alterations. Further study is required to understand, more fully, the mechanisms of neuropharmacological effects of *O. sanctum*.

ABBREVIATIONS

CNS, Central nervous system; **MSG,** monosodium glutamate; **ALS,** amyotrophic lateral sclerosis; **ROS,** reactive oxygen species; **EEOS,** ethanol extract of *Ocimum sanctum*; **RA,** rosmarinic acid; **UA,** ursolic acid; **SIM,** selected ion-monitoring; **MMT,** memantine; **GSH,** reduced glutathione; **DTNB,** 5, 5-dithiobis-(2-nitrobenzoic acid; **GSSG,** oxidized glutathione; **GR,** glutathione reductase; **GPx,** glutathione peroxidase; **SOD,** superoxide dismutase; *CAT,* catalase; **LPO,** lipid peroxidase; **LOD,** limit of detection; **LOQ,** limit of quantification; **LC-MS,** liquid chromatography-mass spectrophotometry; **NMDAR,** N-methyl-D-aspartate receptor.

REFERENCES

Ali MM, Bawari UK, Babu MGN (2000). Locomotor and learning deficits in adult rats exposed to monosodium-l-glutamate during early life. Neurosci. Lett. 284:57-60.

Barth TM, Stanfield BB (1990). The recovery of forelimb-placing behavior in rats with neonatal unilateral cortical damage involves the remaining hemisphere. J. Neurosci. 10:3449-3459.

Beers RF, Sizer IW (1952). A Spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 195:133-140.

Bhargava KP, Singh N (1981). Anti-stress activity of *Ocimum sanctum* Linn. Indian J. Med. Res. 73:443-451.

Bondy SC, Lee CP (1993). The relationship between excitotoxicity and oxidative stress in the central nervous system. Free. Rad. Biol. Med. 14:633-642

Choi DW (1988). Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. Trends Neurosci. 11:465–467.

Coyle JT, Bird SJ, Evans RH, Gulley RL, Nadler JV, Nicklas WJ, Olney JW (1981). Excitatory amino acid neurotoxins: selectivity, specificity, and mechanisms of action. Neurosci. Res. Prog. Bull. 19:421-427.

Coyle JT, Puttfarcken P (1993). Oxidative stress, glutamate, and neurodegenerative disorders. Science 262:689-695.

Dringen R (2000). Metabolism and functions of glutathione in brain. Prog. Neurobiol. 62:649-671.

Dunham NW, Miya TS (1957). A note on a simple apparatus for detecting neurological deficit in rats and mice. J. Am. Pharm. Assoc. 46:208-209.

Fonnum F (1984). Glutamate: a neurotransmitter in mammalian brain. J. Neurochem. 42:1-11.

Geetha M, Kedlaya R, Vasudevan DM (2004). Inhibition of lipid peroxidation by botanical extracts of *Ocimum sanctum*: *In vivo* and *in vitro* studies. Life Sci. 76:21-28.

Godhwani S, Godhwani JL, Vyas DS (1987). *Ocimum sanctum*: An experimental study evaluating its anti-inflammatory, analgesic and antipyretic activity in animals. J. Ethnopharmacol. 21:153-163.

Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannebaum SR (1982). Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal. Biochem. 126:131-138.

Greenwood SM, Connolly CN (2007). Dendritic and mitochondrial changes during glutamate excitotoxicity. Neuropharmacology 53:891-898.

Griffith OW (1980). Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal. Biochem. 106:207-212.

Hynd MR, Scott HL, Dodd PR (2004). Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. Neurochem. Int. 45:583-595.

Jaggi RK, Madaan R, Singh B (2003). Anticonvulsant potential of holy basil, *Ocimum sanctum* Linn., and its cultures. Indian. J. Exp. Biol.

41:1329-33.

Joshi H, Parle M (2006). Evaluation of nootropic potential of *Ocimum sanctum* Linn. in mice. Indian J. Exp. Biol. 44:133-13.

Kakkar P, Das B, Viswanathan PNA (1984). Modified spectro-photometric assay of superoxide dismutase. Indian. J. Biochem. Biophys. 21:130-132.

Kelm MA, Nair MG, Strasburg GM, De WDL (2000). Antioxidant and COX inhibitory phenolic compounds from *Ocimum sanctum* Linn. Phytomedicine 7:7-13.

Kubo T, Kohira R, Okano T, Ishikawa K (1993). Neonatal glutamate can destroy the hippocampal CA1 structure and impair discrimination learning in rats. Brain Res. 616:311–314.

Lees GJ (1991). Inhibition of sodium-potassium-ATPase: a potentially ubiquitous mechanism contributing to central nervous system neuropathology. Brain Res. Rev. 16:283-300.

Li N, Liu B, Dluzen DE, Jin Y (2007). Protective effects of ginsenoside Rg2 against glutamate-induced neurotoxicity in PC12 cells. J. Ethnopharmacol. 111:458-463.

Liu C, Lin N, Wu B, Qiu Y (2009). Neuroprotective effect of memantine combined with topiramate in hypoxic–ischemic brain injury. Brain Res. 1282:173-182.

Lowry OH, Rosenborough NJ, Farr AL, Randal RJ (1951). Protein measurement with Folin- phenol reagent. J. Biol. Chem. 193:265-275.

Mcbean GJ, Roberts PJ (1984). Chronic infusion of L- glutamate causes neurotoxicity in rat striatum. Brain. Res. (1984) 290:372-375.

Meldrum BS (2002). Implications for neuroprotective treatments. Prog. Brain. Res. 135:487-495.

Michaels R, Rothman SM (1990). Glutamate neurotoxicity in vitro: antagonist pharmacology and intracellular calcium concentrations. J. Neurosci. 10:283-292.

Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller D (1984). Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney possible implications in analgesic nephropathy. Biochem. Pharmacol. 33:1801-1807.

Montal M (1998). Mitochondria, glutamate neurotoxicity and the death cascade. Biochem. Biophys. Acta. 1366:113-126.

Murphy TH, Miyamoto M, Sastre A, Schnaar RL, Coyle JT (1989). Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. Neuronsci. 2:1547-1558.

Nicholis D, Attwell D (1990). The release and uptake of excitatory amino acids. Trends Pharmacol. Sci. 11:462–468.

Nobre Jr HV, Maia FD, De Oliveira RA (2008). Neuroprotective actions of tannins from *Myracrodruon urundeuva* on 6-hydroxydopamine-induced neuronal cell death. J. Herbs Spices Med. Plants 13:41-57.

Norr H, Wagner H (1992). New constituents from *Ocimum sanctum.* Planta Med. 58:574.

Novelli A, Reilly JA, Lysko PG, Henneberry RC (1988). Glutamate becomesneurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. Brain Res. 451:205–212.

Okawara M, Katsuki H, Kurimoto E, Shibata H, Kume T, Akaike A (1979). Resveratrol protects dopaminergic neurons in midbrain slice culture from multiple insults. Biochem. Pharmacol. 73:550-560.

Ozsuer H, Gorgulu A, Kiris T, Cobanoglu S (2005). The effects of memantine on lipid peroxidation following closed-head trauma in rats. Neurosurg. Rev. 28:143-147

Paxinos G, Watson C (2007). The Rat Brain: In Stereotaxic Coordinates. Elsevier Science, London.

Ramanathan M, Sivakumar S, Anandvijaykumar PR, Saravanababu C, Pandian R (2007). Neuroprotective evaluation of standardized extract of *Centella asciatica* in monosodium glutamate treated rats. Indian J. Exp. Biol. 45:425-431.

Samson J, Sheela Devi R, Ravindran R, Senthilvelan M (2006). Biogenic amine changes in brain regions and attenuating action of *Ocimum sanctum* in noise exposure. Pharmacol. Biochem. Behav. 83:67-75.

Sovoboda P, Mossinger B (1981). Catecholamines and brain microsomal Na^+-K^+ ATPase protection against lipoperoxidative damage. Biochem. Pharmacol. 30:427-432.

Sudhakar P, Gopalakrishna HN, Akshaya A (2010). Antidepressant activity of ethanolic extract of leaves of *Ocimum sanctum* in mice. J. Pharm. Res. 3:624-626.

Sukari MA, Rahmani M, Lee GB, Takahashi S (1995). Constituents of stem barks of *Ocimum sanctum*. Fitoterapia 66:552-553.

Yanpallewar SU, Rai S, Kumar M, Acharya SB (2004). Evaluation of antioxidant and neuroprotective effect of *Ocimum sanctum* on transient cerebral ischemia and long-term cerebral hypoperfusion. Pharmacol. Biochem. Behav. 79:155-164.

Zhou S, Chena X, Gu X, Dinga F (2009). *Achyranthes bidentata* Blume extract protects cultured hippocampal neurons against glutamate-induced neurotoxicity. J. Ethnopharmacol. 122:547-554.