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# Evaluation of Protective Role of *Ocimum sanctum* Leaf Extract in Excitotoxicity-induced Neurobehavioral Deficits Based on Specific Changes in the Structure of Feeding Behavior, Diuretic and Anxiety Paradigms in Female Rats

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The aim of this study was to investigate the potent neuroprotective property of ethanol extract of *Ocimum sanctum* leaf (EEOS, 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 $kg^{-1}$) and memantine (MMT, 20 mg $kg^{-1}$) daily for 7 days. On all the 7 days, MSG (2 g $kg^{-1}$, i.p.) was administered one hour before drug treatment. The animals were evaluated for the neurobehavioral performance studies, body weight, food intake, diuretic activity, natriuretic and chloruretic effect immediately after MSG administration from day 1 to day 7. On the day 7, the rats were sacrificed and the brain was removed for histopathological examination. Significant differences in behavioural performance between the control and MSG-treated rats were also found in all paradigms studied. Treatment with EEOS reversed the water-excretory-diuretic effect of MSG and caused a significant dose dependent enhancement in the volume of urine output. An interesting observation made during the study was an increased excretion of $Na^+$ and $Cl^-$ ions and reduced excretion of $K^+$, in the urine samples of EEOS-treated rats. Therefore, it may be concluded that the decrease in body weight and hypophagia, along with enhanced water intake and diuresis observed in MSG-treated rats were found to be attenuated by treatment with EEOS. Histopathological evaluation showed that treatment with EEOS and MMT significantly protected the neurodegeneration of the CA1 neuronal cells.

**Key words:** *Ocimum sanctum*, monosodium glutamate, diuretic, excitotoxicity, behavior, neurodegeneration

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## INTRODUCTION

The excitotoxicity of monosodium-α-glutamate (MSG) has been an area of considerable investigation in animals and humans (Olney, 1969). MSG administration in neonatal (Gonzalez-Burgos *et al*., 2001), perinatal (Ishikawa *et al*., 1997), post-natal (Macho *et al*., 1999) and in adult hood (Choudhary *et al*., 1996) is known to induce profound neuroanatomical disturbances in rodents. Glutamate is the predominant Excitatory Amino Acid (EAA) neurotransmitter in the Central Nervous System (CNS) where it modulates fast synaptic excitation at most synapses (Sakina *et al*., 1990). Ironically, excessive amounts of glutamate may be potentially neurotoxic in hypoxia, ischemia and geneses of various chronic neurodegenerative diseases in addition to its vital role as a neurotransmitter (Rothman and Olney, 1986). EAA-mediated neuronal injury models can provide important insights about the pathophysiology of a range of acute and chronic neurological disorders that are mediated, at least in part, by EAA receptor overactivation (Choi and Rothman, 1990).

Although glutamate-induced cell death is associated with both apoptic and necrotic changes (Ankarcrona *et al*., 1995), the mechanism of cell death remains to be established. Many studies have reported that acute form of glutamate excitotoxicity is cellular selective and different EAAs produce distinctive degenerative patterns in the presence of agonists; it is predominantly mediated by ionotropic glutamate receptors (iGluR) (Olney, 1990). The two deleterious processes can be distinguished by differences in time-dependence and ionic characteristics (Choi, 1992).

MSG produces necrosis within the preoptic and arcuate nuclei of the hypothalamus and of scattered neurons within the median eminence (nucleus tuberalis) (Kubo *et al*., 1993). Specific damage of neurons was observed in the CA1 region of the hippocampus, while neurons of the hippocampal CA3 and dentate gyrus were less damaged (Kubo *et al*., 1993). This results in a syndrome characterized by stunted growth, neuroendocrine disturbances and an increase in fat carcass content that is not accompanied by hyperphagia (Olney, 1969). Behavioral abnormalities such as increased irritability, hypoactivity and deficits in spontaneous alternation, open field activity, maze running, as well as pattern of discrimination (Hlinak *et al*., 2005), locomotor and learning deficits were also observed in adult rats injected with MSG in early (Ali *et al*., 2000) and adult life and attributed to the destruction of hippocampal CA1 cytoarchitecture (Kubo *et al*., 1993). A positive consequence of this complexity is that there are many possible strategies for intervention. These include anti-inflammatory treatments, blockers of $Ca^{2+}$ and $Na^{+}$ channels, glutamate receptors (GluR) antagonists and release inhibitors, free radical scavengers, neurotrophic factors, inhibitors of apoptosis (Michel *et al*., 1999).

*Ocimum sanctum* (*O. sanctum* Linn). Family:Labiatae) is a well known, widely distributed and highly esteemed and a sacred medicinal herb especially for Hindus in the Indian subcontinent. Several recent investigations of *O. sanctum* indicating, neuroprotective, antidepressive, antianxiety, antistress, antiulcer, adaptogenic, analgesic, antipyretic, anti-inflammatory, immunomodulatory, cardioprotective, hyolipedemic, hypoglycemic, hepatoprotective, diuretic, radioprotective, anticarcinogenic, bronchitis, rheumatism, pyrexia and antioxidant properties have been reported (Sundaram *et al*., 2012a; Devi, 2001; Singh *et al*., 2002).

There are, indeed, a multitude of paradigms assessing various aspects of the behavioral performance and cognitive abilities. Till now, some of the paradigms were not used at all in the evaluation of *O. sanctum* leaf extract against behavioral consequences of adult female rats in MSG-induced excitotoxicity. Moreover, EEOS has been reported to be neuroprotective in models of cerebral reperfusion injury and long-term hypoperfusion (Yanpallewar *et al*., 2004), anticonvulsant in electroshock-induced model (Sakina *et al*., 1990) and as antistress in preventing the aberrations in the turnover of brain neurotransmitters (NA, DA, 5-HT and 5-HT) in swimming and gravitational stress (Singh *et al*., 2002) and in various periods of noise exposure (Samson *et al*., 2006), respectively.

Although, *O. sanctum* shares all the medicinal properties with other rasayans in the group, its neuroprotective effect in different experimental neurodegenerative models are still debatable. Actually, little is known about the feeding pattern and feeding rhythms that underlie the hypophagia of the MSG treated rats. The general behavior, locomotor activity and histopathological examination of the CA1 region of hippocampus of rat brain, pattern of food and water intake and the perturbation in the diuretic paradigms and the measurement of urinary ions were used for the first time, to assess the central protective effect of *O. sanctum* against MSG-induced neuronal injury. We anticipated that behavioral tests used in the present study could contribute to the evaluation of potential drugs effective in

the prevention of functional deficits induced by neurotoxic agent and may shed an insight into the mechanism of action. Their investigation might help us to understand the eating behavior disturbances induced by arcuate nucleus lesions.

## MATERIALS AND METHODS

**Animals:** Adult 3-4 months old female Sprague Dawley rats weighing between 120-160 g were used. Animals were procured from the Central Animal House of the institute, housed in colony cages at an ambient temperature of 25±2°C and 45-55% relative humidity with 12 h light/dark cycles. They had free access to pellet chow (Brook Bond, Lipton, India) and water *ad libitum*. Animals were exposed only once to the experiments on any day of the study, performed between 09:00 to 17:00 h to avoid the influences of circadian rhythm. 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/PhCology/01/2005-06).

**Plant material and extraction:** 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 a herbarium of the plant is preserved in Department of Pharmacognosy, J.S.S. College of Pharmacy, Ooty. The whole plant was washed and leaves were separated from other aerial parts, freed from earthy material and shade dried with occasional sifting at room temperature. Dried leaves were coarsely powdered (1.9 kg±0.5 dry basis) and subjected to extraction by cold maceration with 90% ethanol (17.38% yield) at room temperature with continuous stirring (300 rpm) for 7 days, after defatting with pet ether (60-80°C). The solvents were evaporated with rotary vacuum and stored in a desiccator and then made in to a fine suspension using 0.5% Tween 80. The principle chemical constituents (rosmarinic acid and ursolic acid) of the extract were identified and quantified using LC-MS technique (Sundaram *et al*., 2012b).

### Experimental procedure

**Drug treatment:** The animals (36) were divided into 6 groups of six animals each. Except vehicle treated group, all other groups (non-fasted) received MSG (2 g $kg^{-1}$) intraperitonealy for 7 days to induce excitotoxicity (Ramanathan *et al*., 2007). The drugs EEOS (50, 100 and 200 mg $kg^{-1}$; p.o.), memantine (20 mg $kg^{-1}$; i.p.), a known NMDA receptor (NMDAR) antagonist, were administered after one hour of MSG treatment. The doses of the extract were determined in view of the results of a pilot study and our preliminary experiments that assessed the ability of *O. sanctum* to attenuate the MSG-induced neurobehavioral changes.

The animals were subjected to the following behavioural studies immediately after MSG administration from day 1 to day 7. On the day 7, the rats were sacrificed and the brain was removed for histopathological examination.

### Behavioral tests

**General behaviour:** The animals were observed for 45 min immediately after the administration of MSG from day 1 to day 7. During the entire period of study, the animals were observed for any changes in behavior and scored 5, when they exhibited any of the following behavior and scored 0 for normal behaviour (Zuccarello and Anderson, 1993). The characteristic essential behavioral features include: (1) Restlessness, (2) Increased motor activity, (3) Increased exploratory behavior, (4) Aggressiveness, (5) Increased grooming and rearing behavior, (6) Increased sensitivity to touch and sound, (7) Enhanced irritation, (8) Anticipatory autonomic reactivity, (9) sleep and (8) Fecal counts.

**Ambulatory behaviour:** The actimeter test was performed independently as a test to record the effects of the drugs on the spontaneous locomotor activity of rats using a photo-electric actimeter. This apparatus consists of a stainless steel box containing transparent cages (270×220×110 mm) in which the animal's 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. In order to reduce any neophobic response to the test conditions, the cages had been previously dirtied by rats other than those used for the test and there was no cleaning between trials.

**Body weight:** The body weight of the animals was monitored daily by weighing on a top loading balance with accuracy to ±0.1 g. All measurements were made every day between 08:30 and 09:15 h, immediately before administration of MSG or drug treatment, starting from the day of injection (day 1) and continued for 7 days thereafter. 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% changes (% changes in body weight per 100 g of rat).

**Food intake:** The measurement of food intake was studied by presenting pre-weighed food to the animals in all the groups immediately following MSG and drug treatment. The amount of food consumed by the animals (food intake g $g^{-1}$ body weight of rat) was evaluated by weighing the remaining amount of food, 24 h after food presentation with accuracy to ±0.1 g for 7 days. Spillage of food pellets was rare but any obvious spillage was noted and those data excluded from the analysis. The food pellets were placed at a height accessible to the experimental animals (5 cm from the floor of the cage), so they did not need to rear up to reach water and food.

**Water intake:** The animals in all the groups had free access to water during the entire duration of the study. Water intake was studied by measuring the volume of water (water intake mL $g^{-1}$ body weight of rat) consumed over a 24 h period for 7 days, following injection of MSG, in all the groups. Clean water was provided in graduated burettes with drinking spouts allowing direct volumetric measurements of intake to the nearest 0.1 mL. The drinking spouts were placed at a height accessible to the experimental animals (5 cm from the floor of the cage), so that they did not need to rear up to reach water.

**Diuretic activity:** Immediately after the injection of MSG, on all 7 days, the rats were placed in metabolic cages specially designed to separate urine and faecal matter and kept at an ambient temperature of 25±2°C and 45-55% RH with 12 h day light/dark cycles. The urine was collected in measuring cylinders up to 5 h, following MSG and drug treatment, in all the groups. The animals were not deprived of water and food during the 5 h period on all the days. The cumulative volume of urine collected over a period of 5 h, immediately following MSG and drug treatment on all the 7 days, was measured with accuracy to ±0.01 mL and expressed, as mL of urine output in 5 h (Lipschitz *et al*., 1943).

**Estimation of sodium, potassium and chloride ions in rat urine samples:** The urine samples collected for 5 h following MSG and drug treatments were immediately analyzed for $Na^+$ and $K^+$ ion concentration by flame photometry while $Cl^-$ ion concentration was estimated as sodium chloride, by titrating with silver nitrate solution (2.906 g $L^{-1}$) using 1 drop of 5% potassium chromate solution as indicator and the values are expressed in mEq $mL^{-1}$ of urine.

**Histopathological studies:** On 7th day, the rats were anesthetized with ether and sacrificed by cervical dislocation; the brains were quickly removed, weighed, rinsed in ice-cold isotonic saline and one half of the brain was processed as follows: the brain samples from different groups of rats was stored in FAM mixture (40% formaldehyde, acetic acid and methanol in the ration of 1:1:8) for histopathological analysis. The brains of all the animals were extracted and embedded in paraffin. A sagital section of 5 μm thickness of was carefully prepared using microtome and the tissues were stained using haematoxylin and eosin. The stained sections of brain tissues were then assessed for the extent of neuroprotective actions of drugs in CA1 region of hippocampus.

**Statistical analysis:** The data are expressed as Mean±SEM from six observations in each group. The behavioral data were subjected to One-way Analysis of Variance (ANOVA), followed by Dennett's multiple comparison post-tests. A probability level (p) of value of less than 0.05 was considered to be statistically significant. The statistical analysis was carried out using GraphPad Prism for Windows (GraphPad Prism Software (version 4.03), San Diego, California, USA).

## RESULTS

### Behavioural tests

**General behaviour:** Rats treated with MSG exhibited a prolonged aggressive behaviour in comparison to the control rats (2.8±0.91). Treatment with EEOS resulted in profound calmness. While the MSG-treated group scored 3.8±0.68, the scores of animals treated with EEOS were 3.5±0.51, 3.35±0.2 and 2.8±0.32 for 50, 100 and 200 mg $kg^{-1}$, respectively. Memantine treated animals exhibited little or no obvious change in the behaviour (2.4±0.36).

**Ambulatory behaviour:** Figure 1 summarizes the data on the cage locomotor activity on day 7. In comparison to the controls (48.50±2.33) the locomotion of MSG-treated rats (15.67±1.20, $p<0.01$) was significantly decreased. The effects of MSG were significantly attenuated in rats following treatment with EEOS in doses employed (50 mg $kg^{-1}$: 21.67±1.05; 100 mg $kg^{-1}$: 30.67±2.17 and 200 mg $kg^{-1}$: 43.00±1.21). Memantine (52.67±1.99) significantly increased the ambulatory behavior in rats.

**Body weight:** The percentage changes in body weight across a 7-day period for all experimental groups are shown in Fig 1. MSG-treated animals displayed significantly greater decrease in body weight on day 2 (-1.48 g%, $p<0.05$) and day 3 (-0.27 g%, $p<0.05$), in comparison to control group which gained +1.74% g and

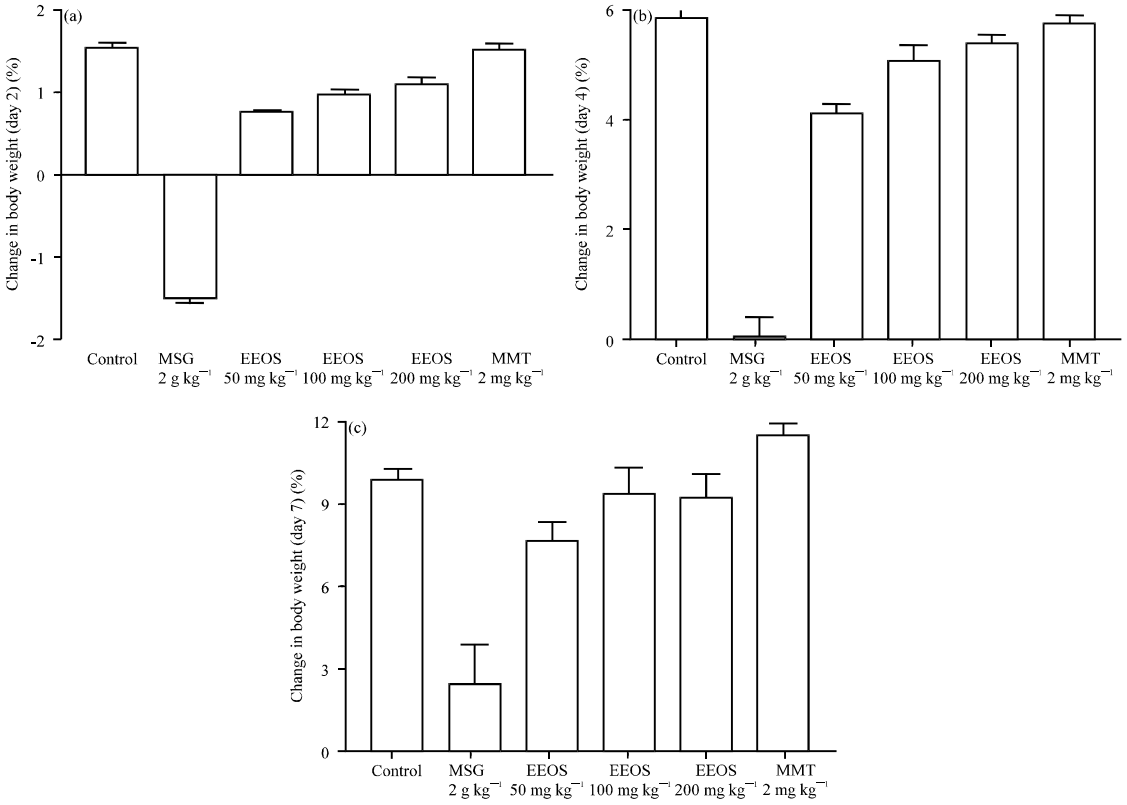


Fig. 1(a-c): Effect of EEOS and memantine on percentage changes in body weight (g) of rats treated with MSG, (a) Day 2, (b) Day 4 and (c) Day 7. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$-compared with control group. $^{x}p<0.05$, $^{y}p<0.01$, $^{z}p<0.001$-compared with MSG group

+3.36% g weight, on day 2 and 3, respectively. Treatment with EEOS for 7 days, prevented the suppression of rat body growth induced by MSG insult. Analysis of data revealed a reversal in the loss of body weight in animals following treatment with the different doses of the extract employed: Rats treated with EEOS 50 mg kg$^{-1}$ gained+0.76 g% and +1.30 g% on day 2 and day 3, respectively; EEOS 100 mg kg$^{-1}$ gained +0.98 g% g and +4.17 g% g on day 2 and day 3, respectively and EEOS 200 mg kg$^{-1}$ gained +1.10 g% and +4.81 g% on day 2 and day 3, respectively, in comparison to animals of the respective group on day 1. Memantine treated animals were found to be undisturbed in the rate of growth of body weight mass in comparison to control and MSG-treated animals. In contrast to the first 3 days of the study, the increase in body weight of rats was observed to be even in all groups except the MSG only treated.

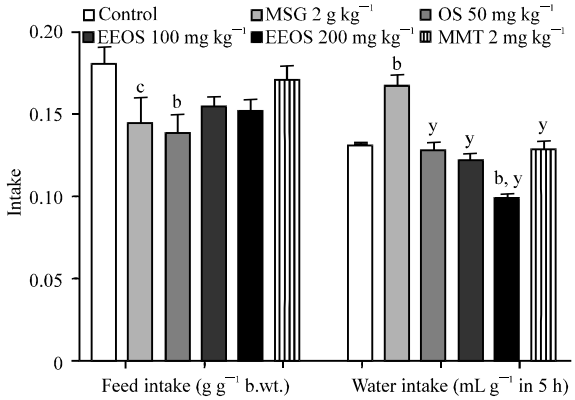


Fig. 2: Effect of EEOS and MMT on water and food intake in MSG-treated rats. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$: Compared with control group. $^{x}p<0.05$, $^{y}p<0.01$, $^{z}p<0.001$: Compared with MSG group

**Food intake:** The effect of MSG-treatment on the total amount of food consumed by rats in the different experimental groups is represented in Fig. 2. The suppression of food intake was found to be highest with the MSG-treated group (0.145 g g$^{-1}$ b.wt.), in comparison to control (0.181 g g$^{-1}$ b.wt.) and all other groups (Fig. 1). Hypophagia was associated with reduced time spent in eating and meal duration. The suppression of food intake

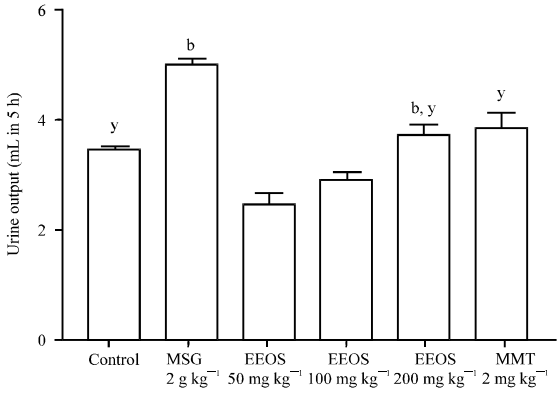


Fig. 3: Effect of EEOS and memantine on urine output in MSG-treated rats, $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$: Compared with control group, $^{x}p<0.05$, $^{y}p<0.01$, $^{z}p<0.001$: Compared with MSG group

in rats treated with EEOS (50 mg $kg^{-1}$: 0.138 g $g^{-1}$ b.wt.; 100 mg $kg^{-1}$: 0.155 g $g^{-1}$ b.wt.; 200 mg $kg^{-1}$: 0.152 g $g^{-1}$ b.wt.) at different dose level was found to be significantly attenuated following MSG insult. The hypophagia was found to be minimal with memantine treated rats.

**Water intake:** Analysis of cumulative water intake across 7 days of testing revealed that MSG-treated rats consumed significantly more water (0.168 mL $g^{-1}$ b.wt.) in comparison to control group (0.131 mL $g^{-1}$ b.wt.). The study revealed a significantly decreased water intake in doses employed with EEOS (50 mg $kg^{-1}$: 0.128 mL $g^{-1}$ b.wt.; 100 mg $kg^{-1}$: 0.122 mL $g^{-1}$ b.wt. and 200 mg $kg^{-1}$: 0.099 mL $g^{-1}$ b.wt.; $p<0.01$) in comparison to control and MSG-treated group (Fig. 2). Similarly, memantine treated rats had diminished levels of water intake (0.129 mL $g^{-1}$ b.wt.; $p<0.01$), in comparison to MSG-treated group. The results indicate that administration of MSG had increased the consumption of water and this effect was antagonized by treatment with EEOS and memantine.

**Diuretic activity (urine output):** MSG (5.0±0.11 mL, $p<0.01$) caused a significant increase in urine output in comparison to control group (3.45±0.08 mL). Treatment with EEOS reversed the water-excretory-diuretic effect of MSG and caused a significant dose dependent enhancement in the volume of urine output (Fig. 3). The excretion of urine, in memantine treated rats, during 5 h duration, was found to be 3.85±0.29 mL ($p<0.01$), in comparison to MSG-treated group. A dose-dependent decrease in urine excretion was observed in all the animals treated with different doses of EEOS. Animals treated with EEOS (50 mg $kg^{-1}$: 2.46±0.12; 100 mg $kg^{-1}$: 2.89±0.15; 200 mg $kg^{-1}$: 3.72±0.20), excreted significantly ($p<0.01$) less volume of urine, in comparison to MSG-treated animals during the test 5 h period.

**Measurement of $Na^+$, $K^+$ and $Cl^-$ ions in rat urine:** Though treatment of EEOS was found to display a dose-dependent enhancement in natriuretic and chloruretic effect, EEOS was found to significantly inhibit the excretion of $K^+$ (Fig. 4). MSG caused a significant ($p<0.01$) decrease in the excretion of $Na^+$ (36.7±4.30 mEq $L^{-1}$ in 5 h) and $Cl^-$ ions (28.81±1.76 mEq $L^{-1}$) in comparison to the respective control rats. Treatment with EEOS caused a significant ($p<0.01$) and gradual elevation in the excretion of $Na^+$ (EEOS 50 mg $kg^{-1}$: 68.59±3.55; 100 mg $kg^{-1}$: 94.63±3.23; 200 mg $kg^{-1}$: 121.73±2.28 mEq $L^{-1}$) and $Cl^-$ ions (50 mg $kg^{-1}$: 53.33±3.07 mEq $L^{-1}$; 100 mg $kg^{-1}$: 76.66±2.74 mEq $L^{-1}$; 200 mg $kg^{-1}$: 91.77±3.75 mEq $L^{-1}$). In sharp contrast and in comparison to the control rats (61.34±2.26 mEq $L^{-1}$), the excretion of $K^+$ was found to be reduced with the doses of EEOS employed (EEOS 50 mg $kg^{-1}$: 71.76±2.98; 100 mg $kg^{-1}$: 64.69±1.35; 200 mg $kg^{-1}$: 57.11±4.44 mEq $L^{-1}$). In comparison to control and MSG-treated animals, memantine significantly attenuated the MSG-induced inhibition of $Na^+$ and Cl-ion excretion.

**Histopathological studies:** Sagital sections of the brain samples stained with haematoxylin and eosin to study the neurodegenerative effect of EEOS in the CA1 hippocampus region of rat brain are shown in Fig. 5a-f. The CA1 regions of the control rats were found to be intact with no neuronal loss. The MSG-treated rats showed loss of CA1 neuronal anatomy and integrity and demonstrated distinct degeneration of hippocampal neuronal cells indicating excitotoxicity to MSG. Treatment with EEOS and memantine significantly protected the neurodegeneration of the CA1 neuronal cells. However, in comparison to standard drug (memantine), the effects of EEOS were found to be less.

## DISCUSSION

This study supports and extends earlier findings, that MSG-induced excitotoxicity produced behavioural changes in adult female rats (Ramanathan *et al*., 2007). Significant differences in behavioural performance between the control and MSG-treated rats were also found in all paradigms studied. Taken together, the

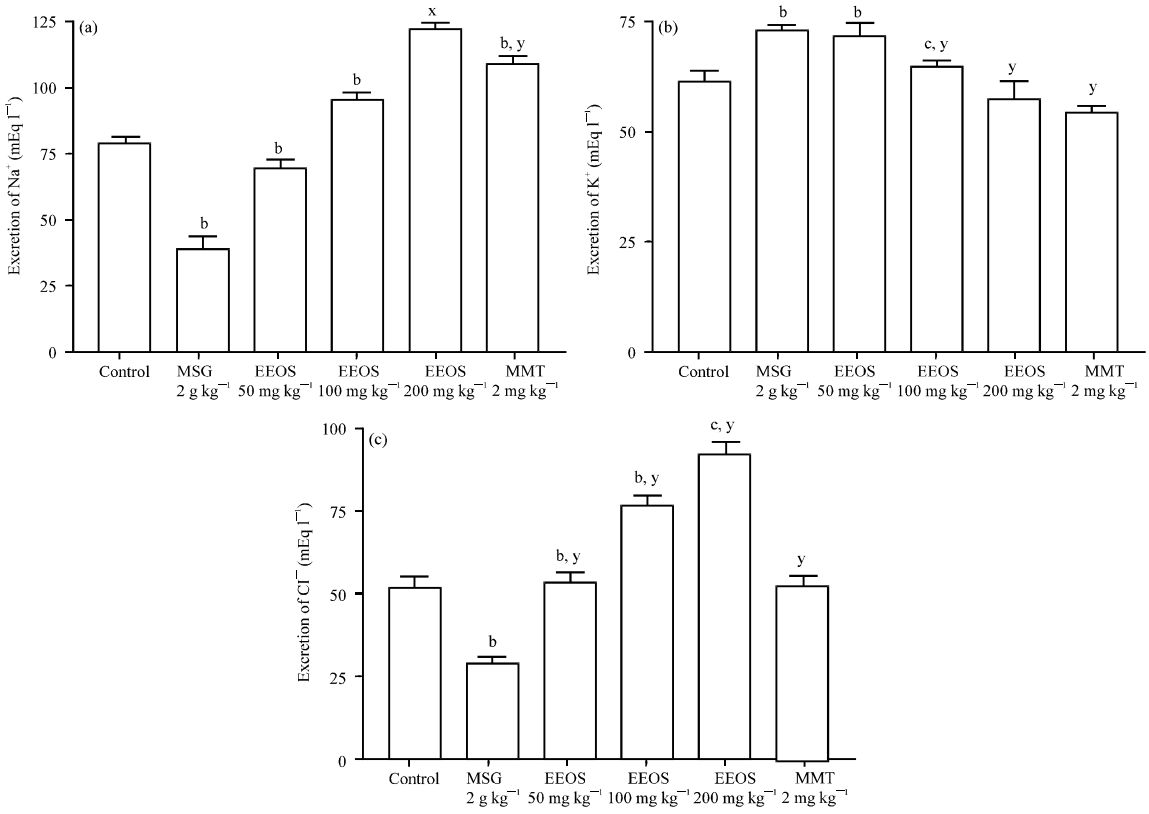


Fig. 4(a-c): Effect of EEOS and MMT on excretion of urinary ions in MSG-treated rats, (a) Sodium, (b) Potassium and (c) Chloride, $^{a}p<0.05$, b,$p<0.01$, $^{c}p<0.001$: Compared with control group, $^{x}p<0.05$, $^{y}p<0.01$, $^{z}p<0.001$: Compared with MSG group

results demonstrate an augmentation relationship between (a) Increased excretion of $Na^+$ and $Cl^-$ ions (b) The behaviour paradigms observed and (c) Excitotoxicity.

Glutamate is released by an estimated 40% of all synapses in the CNS (Coyle and Puttfarcken, 1993) and its interaction with specific membrane receptors are responsible for many neurological functions, including cognition, memory, behaviour, movement and sensation development (Headley and Grillner, 1990). In addition, excitatory neurotransmitters are important in influencing the developmental plasticity of synaptic connections in the nervous system. However, in a variety of pathologic conditions, including stroke and various neurodegenerative disorders, excessive activation of glutamate receptors may mediate neuronal injury of death. This form of injury appears to be predominantly mediated by excessive influx of $Ca^{2+}$ into neurons through ionic channels, triggered by activation of glutamate receptors (Choi, 1985). While it was first observed in neonatal animals with relatively low systemic doses of glutamate (Olney, 1969), it was subsequently found to occur in adult animals following higher systemic doses of glutamate (Ramanathan *et al*., 2007). The studies on the general behavioural parameters indicated that MSG-treated animals had significantly lower body weight than the control. The significant suppression of body weight in MSG-treated rats coincides with a number of previous reports (Pradhan and Lynch, 1972; Squibb *et al*., 1981). Considering that MSG-treated animals develop obesity at later age (Takasaki *et al*., 1979), the growth curves, must be followed for several months to observe this effect. Unfortunately, this was not the essence of the present study. A significant suppression in food consumption and increased water intake was noted with MSG treated rats and these effects were attenuated by treatment with EEOS.

Further the study showed that MSG-treated rats were characterized by disturbances in feeding pattern and feeding rhythms and extends the analyses of its behavioural syndrome as due to consumption of less food and more water than normal rats, which may mainly be by decreased meal size and eating rate. This agrees with

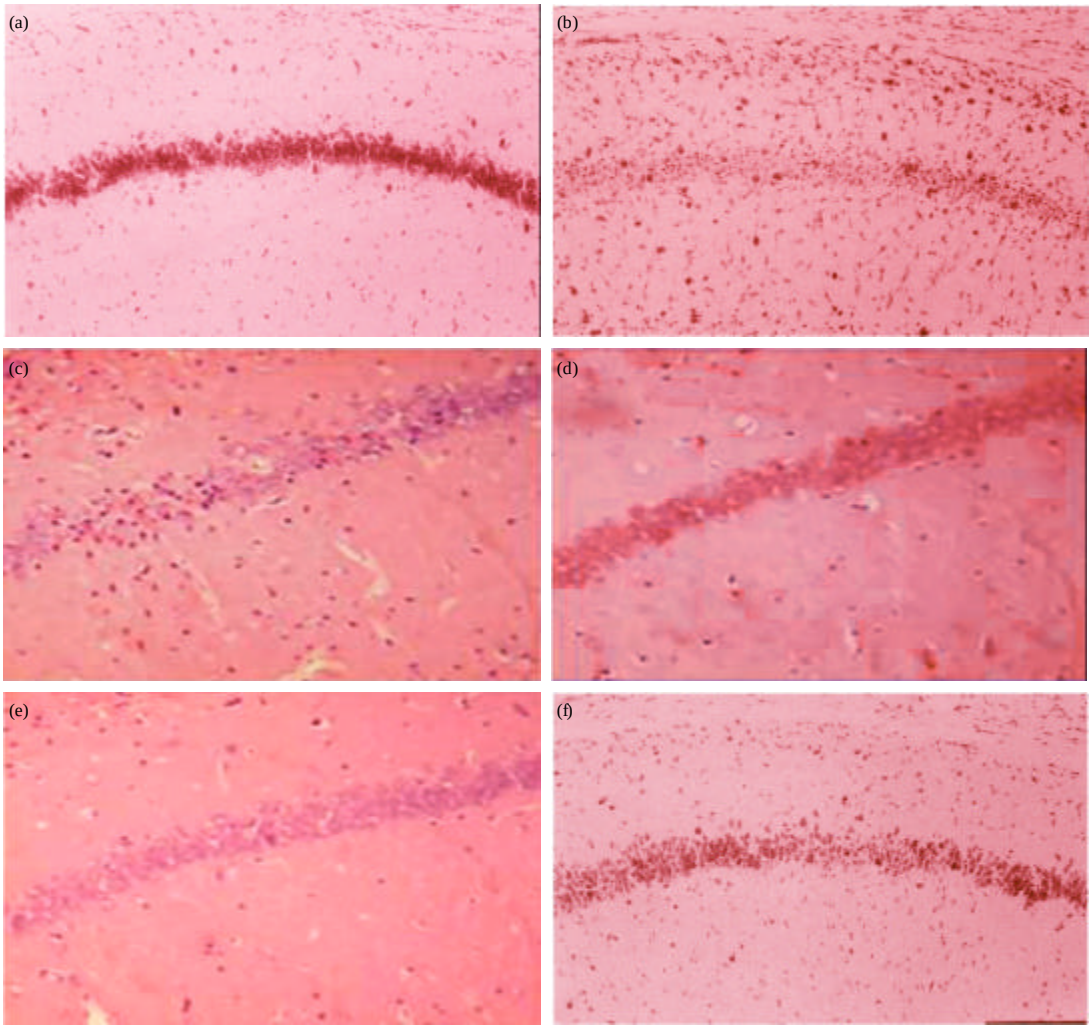


Fig. 5(a-f): Haematoxylin eosin-stained section of CA1 region of (a) Control rats, (b) MSG-treated rats, (c) EEOS (50 mg $kg^{-1}$) treated rats, (d) EEOS (100 mg $kg^{-1}$) treated rats, (e) EEOS (200 mg $kg^{-1}$) treated rats and (f) Memantine-treated rats

previous experiments recording gross food intake only (Beck *et al*., 1993). Among the brain transmitter systems that play a role in food intake and might support the behaviour syndrome of the MSG-treated rats, is intra-hypothalamic arcuate-paraventricular neuropeptide Y (NPY) network. When continuously infused in cerebral ventricles, NPY induces a disruption of dark/light rhythms of food intake independently of its orexigenic properties (Beck *et al*., 1997). Furthermore, several studies have reported that the hypothalamic NPY is depleted in MSG-treated rats (Stricker-Krongrad *et al*., 1994). These reported findings may very well describe the profound disturbances in food pattern and rhythm behavior and the loss in body weight, to MSG-induced lesions of the arcuate nucleus.

Contrary to the observed effect of EEOS on other behavioural parameters mentioned here in this study, EEOS did not antagonize the diuretic activity of MSG. This phenomenon may be explained on the basis that, in a compromised physiological state following exposure to severe stress and/or neurotoxic insult like MSG, EEOS may not antagonize the MSG-induced changes on physiological and urinary parameters. It could be argued, that *O. sanctum* may act as a diuretic agent and under

compromised physiological conditions like severe stress, as in this study, it may actually act to antagonize the effect of stressors or neurotoxins responsible for changes in urinary parameters, by enhancing the rate of clearance of MSG from the body of animals exposed to neurotoxins. It is possible, that EEOS may have exerted its diuretic activity by down regulating the tubular reabsorption of water and $Na^+$ and where the role or the influence of polar compounds, as the flavonoids and terepenoids present in the plant cannot be neglected.

This study suggests another underlying mechanism: high intracellular levels of $Na^+$ and $Cl^-$ ions augment glutamate-induced excitotoxicity. Even though MSG-treated groups excreted more urine, an analysis of the urine composition revealed a significant diminishment in the levels of excretion of $Na^+$ and $Cl^-$ ions, with reduced $K^+$ ions. It may be suggested that the diuresis produced by MSG may be due to aquaretic mechanism and may contribute to the neurodegenerative property of MSG. From the findings in our study, it was observed that EEOS-treated groups displayed a dose-related increase in natriuretic and chloruretic effect. An interesting observation made during the study was an increased excretion of $Na^+$ and $Cl^-$ ions in the urine samples of EEOS-treated rats, in contrast to reduced excretion of $K^+$, which could suite the argument that an increased intracellular concentration of $Na^+$ and $Cl^-$ ions had occurred following MSG insult. Also a dose dependent parallelism exists between the aqueous and ionic excretion were observed with rat urine samples of MSG-treated group. Therefore, it seems reasonable to think, that diuresis produced by EEOS, is more related to saluretic mechanisms than aquaretic mechanisms observed in MSG-treated group. It is well known, that in a condition of $Na^+$ over load and, if the renal tubules are not capable of absorbing it, $Na^+$ appears in the urine with extra water (Loew *et al*., 1991). In this study, EEOS has been shown to enhance the excretion of $Na^+$ and $Cl^-$ ions, substantiating the previously reported claims that the acute post-synaptic swelling induced by glutamate and GluR can be prevented by removal of extracellular $Na^+$ and $Cl^-$ (Rothman, 1985; Rothman and Olney, 1986). It was also reported that removal of $Na^+$ and $Cl^-$ ions from extracellular fluid, attenuated the glutamate-induced biochemical changes in cell cultures (Rothman *et al*., 1987). Depleting $Na^+$ and $Cl^-$ ions could lead to inhibition of post synaptic activation of GluR and reduced i[$Ca^{2+}$] accumulation and i[$Ca^{2+}$]-mediated neurodegeneration with obvious changes in the behavioural, biochemical and morphological observations.

It is possible to consider that chronic application of MSG might retard the development of glutamate synapses. It has been demonstrated that MSG treatment impairs an optimal development of both the glutamatergic and cholinergic neurotransmission mechanisms (Hatt, 1999; Natarajan and Wilkinson, 1997). An increased exploratory performance with a subsequent decrease in the habituation rate has been described; the males were more sensitive to neonatal MSG exposure than the females (Dubovicky *et al*., 1999). Specific damage of neurons observed in the CA1 region of rat hippocampus with MSG is consistent with previous findings (Kubo *et al*., 1993). The neuroanatomical disintegrity observed in this study was found to be mitigated by with treatment with EEOS (Fig. 3) and it is possible to attribute the behavioural changes to the damage caused to hypothalamus of the rat brain.

The present study on the behaviour of rats revealed that the prolonged aggressive behaviour exhibited by the MSG-treated rats was attenuated by treatment with EEOS, causing a profound calmness, consistent with a previous finding in our laboratory (Ramanathan *et al*., 2007). Therefore, it may be summarized that the decrease in body weight and hypophagia, along with enhanced water intake and diuresis observed in MSG-treated rats were found to be attenuated by treatment with EEOS. These findings are also consistent with a previously reported role of *O. sanctum* on behavioral studies in cerebral reperfusion-induced neurodegenerative model in rats (Yanpallewar *et al*., 2004). It is a well under stood phenomenon, that levels of neurotransmitters in the CNS play a crucial role in the behavioral homeostasis of animals. Any undue disturbances in these levels could lead to behavioral disturbances like anxiety, depression, cognitive and memory disorders, disturbances in feeding patterns, body temperature and urinary output. Therefore, we may envisage that EEOS may have antagonized the MSG-induced changes, especially in the hypothalamus, as observed from the histopathological studies and may have contributed to the positive behavioral parameters by relieving anxiety, depression and enhanced cognition and memory.

The effect of EEOS was comparable and equipotent to that of memantine, a known NMDAR-antagonists. 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. 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. Following a series of experiments with $Ca^{2+}$, NMDAR and AMPA antagonists, many researchers

had wondered (Krieglstein, 1997; Lippert *et al*., 1994) whether it is sufficient to block one $Ca^{2+}$ channel to diminish the increase in (i[$Ca^{2+}$]), when $Ca^{2+}$ may enter through various gates into the neuron and concluded that the idea may be to prevent most of the pathological $Ca^{2+}$ influx by blocking at least the main gates for $Ca^{2+}$. Thus, mechanisms other than NMDAR activation could also cause excitotoxicity and hence they have to be assessed in further studies to exactly pinpoint the mechanism of action of *O. sanctum*. Furthermore, the attenuation of MSG-induced changes in behavior (general and ambulatory) following EEOS treatment may be attributed predominantly to its central iGluR antagonistic and, possibly to, inhibition of post synaptic activity at the GluR.

## CONCLUSION

A dose-dependent decrease in urine excretion was observed in all the animals treated with different doses of EEOS. An interesting observation made during the study was an increased excretion of $Na^+$ and $Cl^-$ ions and reduced excretion of $K^+$, in the urine samples of EEOS-treated rats. Therefore, it may be summarized that the decrease in body weight and hypophagia, along with enhanced water intake and diuresis observed in MSG-treated rats were found to be attenuated by treatment with EEOS.

We hypothesized that behavioral and histological tests used in the present study could contribute to the evaluation of potential drugs effective in the prevention of functional deficits induced by neurotoxic agent and may shed an insight into the mechanism of action and neuroprotective effects of *O. sanctum*.

The present study has provided experimental evidence for *O. sanctum* as a neuroprotective agent and emphasizes the need to understand, more fully, the mechanisms of neuropharmacological effects of *O. sanctum*.

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