Protective Effect of Amaranthus Viridis Linn. Against Monosodium Glutamateinduced Oxidative Stress And Excitotoxic Brain Damage In Rat

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Abstract- *The aerial parts of Amaranthus viridis Linn was extracted with ethanol (80%) to obtain hydroalcoholic extract. The neuro protective activity of hydroalcoholic extraction of Amaranthus viridis Linn(HAEAV) was tested against monosodium glutamate (MSG) induced neurotoxicity in albino Wistar rats. In MSG model, the animals were pretreated with HAEAV (200 and 400 ml/kg p.o.) for 7 days. MSG (2 g/kg, i.p.) is administered up to 21 days conjugative days along glutamate with HAEAV. During pre-treatment physio pharmacological parameters were recorded. After 24hr, the assessment of loc omotor activity, beam walking test hanging wire test and the estimation of AchE, nitrates along with other antioxidant parameters (LPO, CAT, SOD, GSH, total thiols) were carried out. The HAEAV, Dextromethorphan (30 mg/kg, p.o) and normal saline were administered 1hr prior to the MSG treatment. On the 22ndday the animals were assessed for behavioral performance. On the 23rdday antioxidant profile from brain isolate were estimated and histopathology of brain was performed. Monosodium glutamate (MSG) significantly altered animal behavior, oxidative defense (raised levels of LPO, depletion of antioxidant levels), mitochondrial enzymes activity and loss of hippocampus neurons in the brain. So ,it was suggested that HAEAV showed protection against chemical(MSG) induced neurotoxicity in rats. The antioxidant, anti-inflammatory property of HAEAV may be responsible for its neuro protective action.The pretreatment of HAEAV significant ($p<0.001$) attenuated in physiological responses, ambulatory behavior, motor coordination, improved body balanced, grip strength, anxiogenic changes in both the MSG. The significant attenuation in the elevation of AchE, LPO and nitrite levels.Increase in GSH, SOD, CAT, TT was observed in groups treated with the HAEAV improves the behavioral and physiological parameters and reduced the histopathological changes induced by MSG The HAEAV pretreatment shows significant ($p<0.001$) reduction in the oxidative stress induced by MSGmodel. The present study suggests that the Amaranthus viridis linn possesses significant neuroprotective activity against MSG induced neurotoxicity in rats. The observed effect could be possibly attributed to its potential antioxidant activity and anti- inflammatory property due to the presence of phytoconstituents.*

I. INTRODUCTION

Neurodegenerative diseases, such as Parkinson's disease (PD), Alzheimer's disease (AD) and Huntington's diseases (HD), are progressive neurological disorders characterized by typical protein assemblies and cell death or apoptosis. Glutamate is the principal excitatory neurotransmitter in the nervous system and its concentration is strictly controlled in the brain. However, excessive release of glutamate into extracellular spaces leads to excitotoxic neuronal damage [1]. Monosodium glutamate (MSG) is a substance widely used as flavouring agent in the whole world. It is the sodium salts of glutamic acid. It is added to the food either as a purified monosodium salt or as a component of a mixture of amino acids and small peptides resulting from the acid or enzymatic hydrolysis of proteins. When it is added to food in relatively small quantities, the palatability of this food increases. There is substantial evidence that the sensory basis for this effect is that MSG stimulates the sense of taste [2]. The average daily intake of MSG per person in industrialized countries is 0.3–1.0 g but, in many countries, there are no limitations on the amount of it which is questioned due to its toxic effects. MSG is demonstrated as the Chinese restaurant syndrome that causes symptoms such as numbness, weakness, flushing, sweating, dizziness, and headache [3]. The Commercial production of MSG requires large vast of harmless bacteria to convert glutamate from sugars or starches into glutamic acid. This acid is then allowed to evaporate, and the remaining brownish white or white crystals are sold as pure MSG [4] MSG acts through the activation of both ionotropic and metabotropic glutamate receptor (iGluR and mGluR) found in the central nervous system (CNS). Hyper activation of these receptors has been reported to produce excitotoxicity and neuronal death [3] The main mechanisms of glutamate-induced neuronal toxicity are associated with excessive influx of calcium and formation of ROS, induction of apoptosis, mitochondrial dysfunction and translocation of

apoptosis-inducing factor (AIF) from the mitochondria to the cytosol and nucleus [5]. Once Exposure to MSG induced neuronal damage in hippocampal CA1 pyramidal cells connected with learning impairment, [6] Hyper- excitability and motor behavioural alterations [7] Moreover, it was proved that MSG could deplete the monoamine neurotransmitters of the hypothalamus region in treated rat. Glutamate serves a multitude of roles in mammalian brain; where it is an important mediator of sensory information, motor coordination, emotions, and cognition, including formation and retrieval of the memory an energy substrate; also, a potent neurotoxin [8]. And several studies were carried out to study the toxicity MSG is reported to produce neurotoxicity, cardio-toxicity, reproductive organ toxicity, obesity, nephrotoxicity, hepatotoxicity [9]. Hence, Plant extracts have been used as medicines, nutrition, and other industrial purpose. The natural products today symbolize safety in contrast to the synthetic drugs. A. viridis linn (Amaranthaceae) is spread throughout the world, growing under a wide range of climatic conditions. The plant is erect, having stems that are up to about 10–100 cm in length with branched glabrous leaves In Greek, Amaranthus is translated as "never fading flower" [10] They produce useful feed and food products. A. viridis L. (Amaranthaceae) has also been used in Indian traditional system. It is also a very good source of vitamins including vitamin A, B6, and C, riboflavin and foliates minerals like calcium, iron, zinc, magnesium. Inaddition, the whole plant possesses analgesic and anti-pyretic properties and is used for the treatment of pain and fever respectively in traditional systems of medicine [11]. Plant derived alkaloids are suggested to be natural antioxidants associated with prevention of various diseases and pathological conditions including malaria, diabetics, cancer, cardiac dysfunction etc. and also used as local anesthetics and pain relievers [12]. Many alkaloids are used as antiarrhythmic, anticholinergic, anti-tumor, vasodilating, antihypertensive, cough medicine, anesthetic, antiprotozoal, antidiabetic, antihyperlipidemic and antioxidant agents [13]. The present study reveals the protective "effect of evaluation of neuroprotective activity of Amaranthus viridis linn. Against chemically induced neurotoxicity in rats".

Materials and methods

Chemicals

Monosodium-L-glutamate monohydrate, Dextromethorphan, Dithiobisnitrobenzoate (DTNB), Vitamin E, Sodium chloride, Formalin, Hydrochloric acid (HCl), Hydrogen peroxide, Adrenaline, Dipotassium hydrogen phosphate, Sodium dihydrogen phosphate, Potassium dihydrogen phosphate, Sodium hydroxide, Thiobarbituric acid, Trichloroacetic acid, Ketamine, EDTA, Sodium carbonate, Sodium bicarbonate, Petroleum ether, Sodium thiosulphate, Ethyl acetate, Anesthetic ether.

Collection of plant material and extraction

The fresh plant of HAEAV was collected from Tumkuru and was authenticated by Department of Botany. The whole plant was shade dried and coarsely powdered. The coarse powder was subjected to Hydroalcoholic extraction of the leaves and stems of the plant were carried out separately, by suspending 100 g of the powder of each part in 2000 ml of 80% ethanol: water (80:20 v/v). The extraction was done by cold maceration for 3days at room temperature. The extracts were filtered through wattman filter paper. After filtration extract is subjected to evaporation. Finally, product is collected. However, the dose 200 and 400 mg/kg extract does will be prepared from concentrated extract which will be dissolved in normal saline [14].

Preliminary phytochemical screening

The methanol extract of HAEAV was screened for the presence of various phytoconstituents like flavonoids, tannins, proteins and amino acids, carbohydrates, Saponin, anthraquinone and alkaloids compounds [15].

Animals

Female Swiss Albino Wistar rats weighing 150–250 g was acclimatized to the experimental room at temperature 23±2 ◦C, controlled humidity conditions (50–55%) and 12 h light and dark cycle. They were caged with a maximum of two animals in polypropylene cage and were fed with standard food pellets and water ad libitum. All the studies conducted were approved by the institutional animal ethical committee of Sree Siddaganga College of Pharmacy (Reg. No. 551/2010-11), Tumakuru, Karnataka, according to prescribed guidelines of CPCSEA, Government of India.

Experimental design

The female rats were randomized into six groups comprising of eight animals in each group as given below. Solvent/HAEAV (200 and 400 mg/kg)/ Dextromethorphan) was administered orally and treated for 21 days as follows-
Group I- receives vehicle (10 ml/kg (p.o.) Group II- HAEAV (400mg/kg) Group III- receives MSG 2 g/kg (i.p.) Group IV- receives PEFIR 100 mg/kg (p.o.) + MSG 2 g/kg (i.p.) Group V- receives PEFIR 200 mg/kg (p.o.) + MSG 2 g/kg (i.p.) Group VI- Dextromethorphan 30 mg/kg (p.o.) +MSG 2 g/kg (i.p.) Group IV, V & VI administration of MSG solution was

done once a day orally after one hour of oral administration of HAEAV and Dextromethorphan extracts for 21 days. On 21th day, behavioural phenotypes were studied after one hour of MSG dosing and animals were sacrificed on 22th day for biochemical estimation and histopathological investigation.

Behavioural parameter

Locomotor activity

(Actophotometer test) Locomotor activity was evaluated by using actophotometer. Animals from all the groups in MSG induced neurotoxicity model were placed in actophotometer for 10 minutes and score was recorded. Difference in the change in locomotor activity in different groups of animals in the model was recorded [16].

Elevated plus maze

Elevated plus maze served as the exteroceptive model to evaluate short term memory in rat. To study the acquisition and retention of short-term memory, EPM was designed of two open arms (16x5cm) and two closed arms (16x5x12cm) extended from a central platform and the maze was raised to a height of 25cm from the floor. On the 7th day of dosing, after 30 min of scopolamine injection, each rat was placed at one end of the open arm facing away from the central platform, to measure the acquisition transfer latency time. Transfer latency was defined as the time taken by the animal to enter from open arm into the enclosed arm with all its four legs. The cut off time was 90sec if the animal didn't enter the enclosed arm within 90sec.The mouse was allowed to explore the maze for 1min and placed back into its cage [17]. Retention memory is examined after 24hr i.e., on 8th day of dosing. Significant reduction in transfer latency time is an index of the improved memory.

Beam Walking Test

Beam walking test was used to evaluate gross vestibulomotor function. The apparatus consisted of a rod 120 cm in length and with a diameter of 2.3 cm. A wooden box (20 cm 9 20 cm 9 10 cm) was set at one end of the rod as a nest for motivating the animal to cross the beam. The apparatus was suspended 50 cm above a cushion, which protected the animals against fall injury [18]. Rats were trained twice daily for 2 days for motor coordination. The time taken to traverse the beam was recorded. The cut-off time was taken as 120 sec.

Biochemical estimation

Preparation of Tissue Homogenates The animals were sacrificed under deep ether anaesthesia. The whole brain dissected out, blotted dry and immediately weighed. A 10 % brain homogenate was prepared with ice-cold phosphate buffered saline using Teflon-glass homogenizer [16]. The homogenate was centrifuged at 10,000 rpm at 4 C for 15 min and the pellet discarded. The supernatant obtained was used for biochemical estimations.

Acetyl cholinesterase (AchE)

The principle of the method is the measurement of the rate of production of thiocholine as acetyl thiocholine is hydrolysed. Briefly, 0.1ml of 0.01M DTNB was added to 2.6ml of 0.1M phosphate buffer (pH 8.0) 0.04ml of brain homogenate was added to the above mixture followed by incubation for 5min.Then 0.04ml of substrate (0.075Macetylthiocholine iodide) was added to the reaction mixture [19][20]. The readings were taken at 420nm continuously for 5min at 1min intervals. The results were expressed in μmol−1 min−1 mg protein−1using a molar extinction coefficient 1.36×104 M−1 cm−1.

Estimation of Reduced glutathione (GSH)

The assay is based on the principle of Ellman's reaction. The sulfhydryl group of glutathione reacts with DTNB (5, 5'-dithiobis-2-nitrobenzoic acid) and produces a yellow coloured 5-thio- 2-nitrobenzoic acid (TNB). Measurement of the absorbance of TNB at 412 nm provides an accurate estimation of glutathione in a sample. Briefly, 0.5 ml of homogenate is mixed with 0.1 ml of 25% TCA to precipitate proteins and centrifuged at 4000 rpm for 5 min. Then, 0.3 ml of the supernatant was mixed with 0.5 ml of 0.1M phosphate buffer (pH 7.4) and 0.2 ml of 10 mM DTNB [21]. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blanks. The glutathione content was calculated by using extension coefficient 13.6 x 103.

Estimation of superoxide dismutase (SOD)

SOD activity was measured based on the ability of SOD to inhibit spontaneous oxidation of epinephrine to adrenochrome at pH 10.2 we are measuring the concentration of adrenochrome at 295 nm Briefly, to 100 µl of brain homogenate add 0.8 ml of carbonate buffer (pH 10.2), incubate the above solution for 15 min initiate by adding 100 µl of adrenaline solution (1 mM). Record the change in absorbance at 295 nm for 5 min. One unit of SOD produces approximately 50% of auto-oxidation of adrenaline. Results were expressed as U/mg protein [22].

Lipid peroxidation (LPO)

The assay is based on TBARS and malondialdehyde (MDA) a break down product of lipid peroxidation that reacts give a chromogen (light pink colour) species measured at absorbance maximum at 535 nm. The Estimation of lipid peroxidation (LPO) is based on TBARS method by mix 1ml of the above supernatant with 2ml of TCATBA-HCL mix thoroughly and heated about 30min in boiling water bath then cooled in ice bath for 10min fallowed by centrifugation at6000rpm for 10min [23]. the absorbance of supernatant was measured at 532nm.the amount of LPO was determined by using 1.56*105 M-1cm-1as moles MDA/mg protein. Determined by using 1.56×105 M -1 cm -1 and the results were expressed as moles MDA/mg protein.

Estimation of Catalase (CAT)

The estimation is based on the property of Catalase to catalyses the decomposition of hydrogen peroxide to water. Briefly, to 0.95 ml of 10 mM H2O2 60 mM phosphate buffer (pH=7.0), 50 µl of the brain tissue supernatant was added and the rate of degradation of H2O2 was followed at 240 nm per min [24]. Catalase content in turns of U/mg of protein was estimated from the rate of decomposition of H2O2 using the formula k = 2.303/ t × log (A1/A2) s-1 (A unit of catalase is defined as the quantity which decomposes 1.0 mole H2O2 of per min at pH 7.0 and temperature 25ºC.

Histopathological studies

A section of the brain was fixed with 10% formalin and embedded in paraffin wax and cut into sections of 5 µm thickness. The sections were stained with haematoxylin and eosin dye for histopathological observations. Depending on the model, either hippocampal or striatal neurons were observed for morphological changes [24].

Statistical Significance

The data and other related values were expressed statistically as mean ± SEM. The Statistical difference in mean was analysed using one way ANOVA followed by Dennett's multiple comparison tests and the p< 0.05 value was considered as statistically significant.

Results

Behavioural assessment

Locomotor activity- Administration of MSG for 21 days in normal rats resulted significant (P<0.01) reduction in movement of animals compared to normal control animals. The HAEAV alone pre-treated group showed significantly (P<0.01) increase in locomotor count. Pre-treatment with HAEAV (200 and 400) mg/kg) and Dextromethorphan (30 mg/kg) in MSG induced rats significantly showed (p<0.05, p<0.01 and p<0.001) increased locomotor counts. Table1.

Elevated plus maze paradigm

In this study, mean initial transfer latency (ITL) on day 21th was relatively stable in all the animals within the group. MSG alone administered rats for 21 days showed significant (P<0.001) increase in retention transfer latency (RTL) compared to normal control animals. In contrast, MSG treated rats performed poor memory retention. This indicates there is cognitive dysfunction in MSG treatment. The HAEAV alone pre-treated significantly showed (P<0.001) when compared to normal group. Pre-treatment with HAEAV (200 and 400 mg/kg p.o.) and Dextromethorphan (30 mg/kg) in MSG treated rats showed significant (p<0.05, p<0.01 and p<0.001) improvement in memory performance when compared to MSG induced alone treated rats. Table-1.

Balance beam

Motor coordination was decreased significantly with the administrations of MSG, which leads to significant (P<0.001) increase in transfer latency time, in crossing the beam when compared with the control group animals. The HAEAV pre-treatment groups at low, high (200 and 400 mg/kg) and Dextromethorphan (30 mg/kg) doses, prevented an increase in transfer latency, in MSG administered rats significantly showed (p<0.05, p<0.01 and p<0.001) when compared with MSG alone treatment group by improving motor coordination and body balance. Table 1.

Table 1. Neuroprotective effect of HAEAV on behavioural parameters on MSG induced neurotoxicity in rats

Treatment	Locomotor activity (s)	Beam walking assay (s)	Elevated Plus Maze Test (% memory retention)
Normal control (normal saline 10 ml/kg)	162.2 ± 33.69	5.01±0.56	54.71 ±4.963
HAEAV (400 mg/kg)	141.4±8.69	5.23±0.46	37.6±2.56
MSG (2g/kg. i.p.)	47.40 ± 9.0321###	60.01±3.90###	23.40±0.9063###
MSG 2 g/kg + HAEAV 200 mg/kg	87.40 ± 8.764*	5.65±0.41*	34.13 ±1.078*
MSG 2 g/kg + HAEAV 400mg/kg	135.1 ± 20.56**	6.1±0.67**	49.78 ±6.470**
Dextromethorphan (Dextromethorphan 30 mg/kg +MSG 2 g/kg)	170.4 ±10.70***	5.15±0.40***	52.68 ± 6.511***

Table 1- Neuroprotective effect of HAEAV on MSG induced change in rat. Values are expressed in mean±SEM (n = 6/group). significance with Tukey's test following one way ANOVA is indicated as####p<0.001, ##p<0.01, # p<0.05 compared to control or ***p<0.001, **p<0.01, *p<0.05 significant difference from the control group.

Neuroprotective Effect of HAEAV **on AchE and LPO Activity in Rat's Brain**

The data in Figure's indicates that, the levels of AchE and LOP were measured in rat brain homogenates as a marker of central cholinergic status, which is compulsory in keeping typical cognitive functions. MSG treatment meaningfully increased the brain AchE and LPO level compared to control group. Administration of HAEAV (200 and 400 mg/kg) significantly ($P < 0.01$, $P < 0.05$, $P < 0.001$) decreases the AchE, LPO activity in the brain tissue of rats as compared to disease control and control group. Standard drug, Dextromethorphan treatment significantly ($P < 0.01$) inhibited the brain AChE and LPO level compared to their corresponding disease control and control group presented in Table 2.

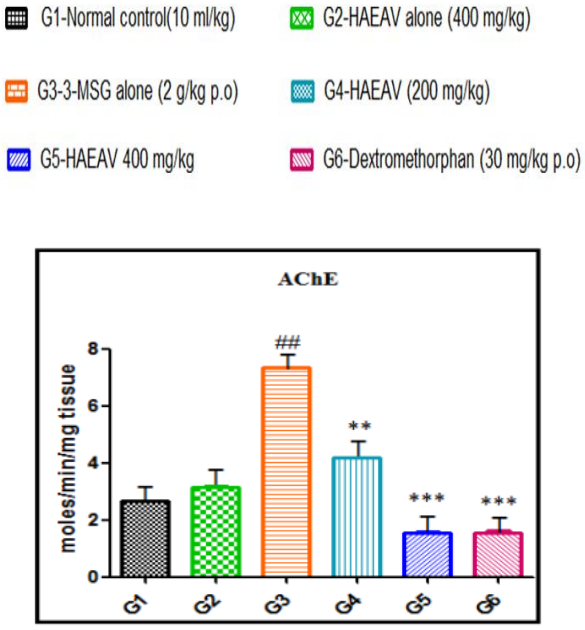

Figure 1. Neuroprotective effect of HAEAV on MSG of rat for Acetyl cholinesterase (AChE), Values are expressed in mean±SEM (n = 6/group). Significance with Tukey's test following one way ANOVA is indicated as ####p<0.001, ##p<0.01, # p<0.05 control or ***p<0.001, **p<0.01, *p<0.05 significant difference from the control group.

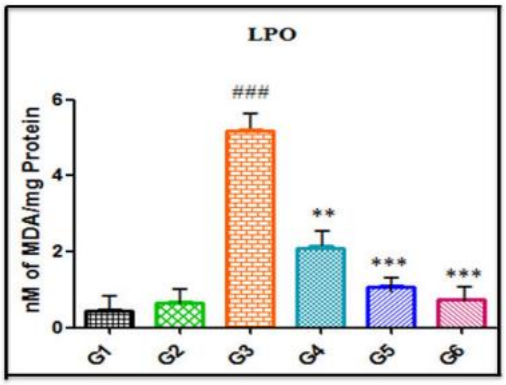

Figure 2. Neuroprotective effect of HAEAV on MSG of rat for Lipid peroxidation (LPO). Values are expressed in mean±SEM (n = 6/group). Significance with Tukey's test following one way ANOVA is indicated as ####p<0.001, ##p<0.01, # p<0.05 control or ***p<0.001, **p<0.01, *p<0.05 significant difference from the control group.

Neuroprotective Effect of HAEAV on GSH, CAT, SOD Activity in Rat's Brain.

The data in Figure's indicates that GSH, CAT and SOD 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 HAEAV (200 and 400 mg/kg) and dextromethorphan (30 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 HAEAV (200 mg/kg) per se treated animals demonstrated no significant change in GSH, CAT and SOD enzymes level as compared to control group.

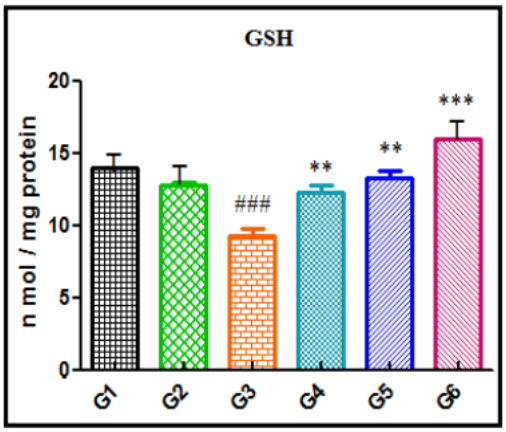

Figure 3. Neuroprotective effect of HAEAV on MSG of rat for GSH. Values are expressed in mean±SEM (n = 6/group). Significance with Tukey's test following one way ANOVA is indicated as ####p<0.001, ##p<0.01, # p<0.05

control or ***p<0.001, **p<0.01, *p<0.05 significant difference from the control group.

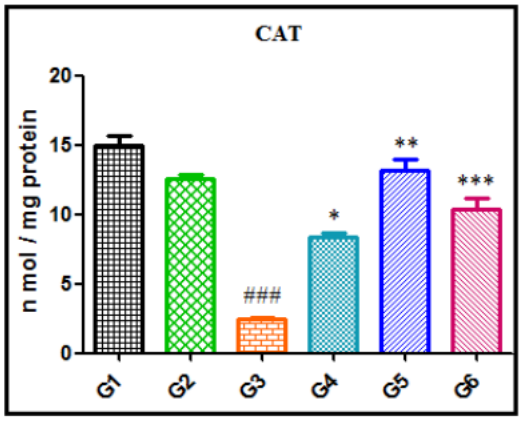

Figure 4. Neuroprotective effect of HAEAV on MSG of rat for CAT. Values are expressed in mean±SEM (n = 6/group). Significance with Tukey's test following one way ANOVA is indicated as ####p<0.001, ##p<0.01, # p<0.05 control or ***p<0.001, **p<0.01, *p<0.05 significant difference from the control group.

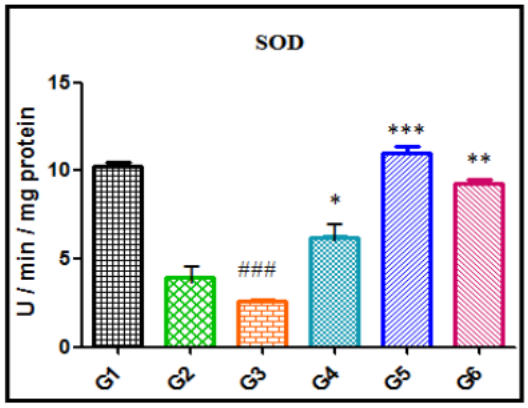

Figure 5. Neuroprotective effect of HAEAV on MSG of rat for GSH. Values are expressed in mean±SEM (n = 6/group). Significance with Tukey's test following one way ANOVA is indicated as ####p<0.001, ##p<0.01, # p<0.05 control or ***p<0.001, **p<0.01, *p<0.05 significant difference from the control group.

Table 2. Neuroprotective Effect of MEPA on biochemical parameters of rat brain antioxidant defence system.

Treatment	Tissue AchE(µmoles/min/mg tissue)	GSH (nmol/mg of tissue)	LPO (nmol/mg of tissue)	CAT (U/mg of tissue)	SOD (U/mg of tissue)
Normal control (normal saline 10 ml/kg)	2.701±0.16	14.51 ±1.287	0.5855±0.1235	17.18 ±2.123	14.65 ±1.212
HAEAV	3.24±0.21	13.2±0.64	0.761±0.251	13.5±0.04	3.36±0.31
MSG (2g/kg,i.p.)	7.36±0.63# #	10.81 ±0.1754###	5.271±1.128###	2.779 ± 0.8480###	2.853 ±0.7262###
MSG 2 g/kg + HAEAV 200mg/kg	4.18±0.83**	13.679 ±0.2294**	2.277±0.733**	10.70 ± 1.728*	6.428 ± 1.421*
MSG 2 g/kg + HAEAV 400mg/kg	1.57±74***	14.506 ± 0.2401**	1.123±0.151***	17.21 ± 2.327***	12.03 ± 1.835***
Dextromethorphan (Dextromethorphan 30mg/kg +MSG 2g/kg)	1.49±76***	16.4023 ±1.129***	0.8233±0.1417**	14.60 ± 1.245**	10.55 ± 1.322**

Table 2. The rats brain biochemical parameters are expressed as mean±SEM values (n = 6/group). Significance with Tukey's test following one way ANOVA is indicated as ####p<0.001, ##p<0.01, # p<0.05 control or ***p<0.001, **p<0.01, *p<0.05 significant difference from the MSG group.

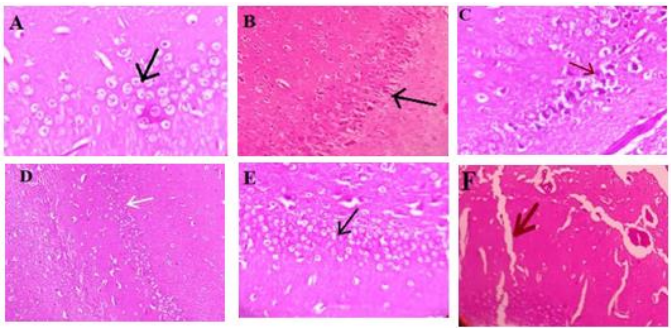

Figure 6. Effect of various treatments on CA1 region of hippocampus [(A) CA1 region of control vehicle treated

rats stained by eosin and haematoxylin staining and the region was found to be intact. (B) Administration of Amaranthus viridis linn alone with glutamate protected the CA1 region however in comparison to standard drugs effect Amaranthus viridis linn is significantly less. (C) CA1 region of HAEAV (200 mg/kg). (D) CA1 region of HAEAV (400 mg/kg). (E) Treatment of Dextromethorphan (30 mg/kg).

II. DISCUSSION

The present study highlights the protective role of Amaranthus viridis linn (HAEAV) on MSG-induced neurotoxicity by assessing its morphological, behavioural, biochemical, and histopathological parameters.Impairment of cellular calcium homeostasis, activation of nitric oxide synthesis, generation of free radicals, and programmed cell death which leads to progressive neurodegeneration [25] are common pathogenic mechanisms related to glutamatergic dysfunctions [26]. These mechanisms damage nucleic acids, protein, lipids, and potentially open mitochondrial permeability transition pore, which in turn can further stimulate ROS production, worsen energy failure and release proapoptotic factors such as cytochrome c into the cytoplasm [27]. Generation of high levels of ROS and down-regulation of antioxidant mechanisms results in neuronal death of neurodegeneration diseases [28][29]. It is believed that degenerative nerve diseases may decline many of our body's activities, including memory balance, movement, normal behavioural and heart function [25]. Neurotoxicity and disturbed function of glutamate neurotransmitter mainly due to excitotoxic neuronal damage and increases oxidative stress [29]. Phytochemicals from natural source found to have good antioxidant property and may be used in prevention and treatment of neurodegeneration diseases. So, proposed experiment aimed to study the effect of Amaranthus viridis linn on MSG-induced neurotoxicity in albino Wister rats.The result of present study indicates that the treatment with HAEAV significantly improved body weight and motor deficits. Administration of MSG for 21 days exhibited significantly reduced body weight, locomotor activity, elevated plus maze, beam walk test in rat. 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 [30]. Dextromethorphan is a synthetic opioid agonist functions additionally as an NMAD receptor antagonist [31]. The treatment of HAEAV showed excellent effect against the excitotoxic induced neurotoxicity by MSG.The result obtained in the present study exhibited that the treatment of HAEAV showed a protective effect against the MSG be restoring the behavioural change and bringing back the antioxidant levels to normal. In addition to its effect on behaviour changes, the active component of the HAEAV showed neuroprotective property and reserved free radical induced cell death in hippocampal region in a rat's brain and Significant changes in behavioural phenotypes in rat treated with MSG indicated abnormal or deficit neuronal function as compared to normal control animals. While treatment with HAEAV extract showed significant normalization of behavioural phenotypes in MSG-treated rats. Acetyl choline as a neurotransmitter has important role in the CNS and is implicated in behavioural as well as learning and memory and neurodegenerative diseases. Our results showed that administration of MSG in rats increased AChE activity in the hippocampus. Considering these findings, we speculate that increased AChE activity may lead to a reduction of cholinergic neurotransmission efficiency due to a decrease in acetyl choline level in the synaptic cleft, thus contributing to progressive cognitive impairment [32]. Glutathione (GSH) is highly abundant in all cell compartments and major soluble antioxidant. Reduced GSH/Oxidized GSH ratio is a major determinant of oxidative stress. There is significant evidence that the disturbance of glutathione homeostasis may either lead to or result from oxidative stress in neuronal disorders [33] [34]. Reduced glutathione was significantly decreased in brain tissue of MSG-treated rat. This reveals an imbalance between oxidants and defence mechanism. While treatment with HAEAV extracts showed significant increase in tissue glutathione in MSG-treated rat [35]. Increase in lipid peroxidation product has been observed in cases of oxidative stress and neuronal damage [36] [37]. A significant increase in lipid peroxidation in MSG-treated rat brain tissue indicated oxidative stress induced by MSG treatment. This indicated that MSG might be responsible for the production of reactive oxygen species (ROS). Products of lipid peroxidation, such as malondialdehyde (MDA) and unsaturated aldehydes are capable of inactivating many cellular proteins by forming protein cross-linkages [38]. Treatment with HAEAV significantly decreased the lipid peroxidation product in brain tissue of rat as Compared to MSG-treated. While, catalase is the enzyme responsible for the breakdown of hydrogen peroxide into water and oxygen. The activity of both enzymes is sufficient for removal of ROS in normal homeostasis. There must be a balance between oxidation and antioxidant level in the system for healthy biological integrity to be maintained [34]. Superoxide dismutase is considered to be one of the most active enzymes which dismutase superoxide anions produced during metabolism in cells. It converts superoxide radical into less toxic hydrogen peroxide [39]. Usually, the neuronal damage and oxidative stress are caused by many chemical agents i.e., metals, gases, pesticides and some inorganic chemicals. Which also shows a decrease in antioxidant defense enzyme activity [33] [37]. Treatment with HAEAV extract significantly increased the activity of the enzymes in

brain tissue in MSG-treated rat.It is well reported that MSG administration produces morphological and histological changes in rat brain that further results in learning and memory impairment [1]. Furthermore, the administration of MSG has been reported to destroy the hippocampal CA1 structure. In the studies carried out with MSG treated rat, unequivocal signs of neuron death with extensively dark pyknotic, intercellular space and shrunken nuclei located in the CA1 pyramidal cell layer have been determined. Oral administration of HAEAV remarkably attenuated MSG induced neuronal loss and also decreased pyknotic cell density in a dose dependent manner. The cell counts showed that the numbers of cells in the hippocampus sections were significantly lower in the MSG treated groups than in the control grouped. 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 HAEAV has neuroprotective effects against MSG induced neuronal death. Previous phytochemical studies of HAEAV have led to the isolation of flavonoids, terpenoids, fatty acids, steroids, alkaloids, glycolsides, and phenolic compounds. [40]. Therefore, it can be assumed that HAEAV attenuates behavioural and biochemical alterations against MSG induced excitotoxicity depending on antioxidant, anti-inflammatory activity and other active principles present in HAEAV leaves [41].

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Conflict of interest

No conflict of interest.

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