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Research Article

FREE RADICAL SCAVENGING ACTIVITY OF *EVOLVULUS ALSINOIDES* ON GLUTAMATE INDUCED NEURODEGENERATION

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ABSTRACT
The whole plant extract of evolvulus alsinoides was evaluated for its antioxidant activity in glutamate induced neurodegeneration. The extract was given orally in to two different doses (200mg/kg and 400 mg/kg) for 7 days .Simultaneously rats were treated with monosodium glutamate (5mg/kg; i.p) and for comparison purpose NMDA antagonist memantine (5mg/kg) was used as a standard drug. Parameters under study were TBAR, SOD, Nitrates and protein. The orally administrated extracts and memantine (5mg/kg; i.p) significantly decrease nitrates, TBAR levels and significantly increase the SOD levels in glutamate treated groups. But the extract and memantine did not alter protein level in glutamate treated rats

KEYWORDS: Evolvulus alsinoides, Monosodium glutamate. Memantine, Antioxidant activity.

INTRODUCTION
Oxidative Stress is implicated as one of the primary factors that contribute to the development of neurodegenerative disease like Alzheimer's, Parkinsonism and neurological conditions like epileptic seizures, stroke, brain damage, neurotrauma etc [1]. The broad range of neuro degenerative disease are now believed to be caused by the excito toxic action of Glutamate/aspartate.Excess of glutaminergic stimulation cause on over activation NMDA receptors. This leads to calcium overflow in to the neurons, activating the catabolic enzymes .Increased activity of this enzyme promote the cell death as a result of hypoxia and ischemia eventually cause the memory loss, Confusion, Anxiety and etc[2] .

Evolvulus alsinoides a traditional ayurvedic medicine have a better neuro protection and antioxidant properties[3]

MATERIALS AND METHODS
The plant was collected in the surroundings of Coimbatore and authenticated by Botanical Survey of India (BSI), Coimbatore, India.

Female Sprague Dawley Rats (150-180 g) were used for the study. Animals were procured from the central animal house of our institute. Animals were housed in groups of 4-5 in colony cages at ambient temperature of 25 $\pm$ 2^{o}C and 45-55% relative humidity with 12 hours light/ dark cycle. They had free access to pellet chow *(*Brook bond, Lipton, India*)* and water *ad libitum,* animals were exposed only once to every experiment.

Extraction of *Evolvulus alsinoides* By Cold Maceration
The extracts of the plant *Evolvulus alsinoides* were prepared by cold maceration technique by treating with 50% ethanol for seven days. The extract was filtered and concentrated under vacuum. Finally the

crude extract of *Evolvulus alsinoides* was separately treated with 1 ml of 10% ammonia solution and then extracted by shaking for 15 min at 60 ^{o}C with 5 ml methanol.

Experimental Design

Glutamate (2mg/kg,ip) was administered continuously for seven days. Simultaneously rats were administered with *Evolvulus alsinoides* (200 & 400 mg/kg, P.O) and NMDA antagonist memantine (5mg/kg, ip).

Experimental Groups

Healthy female Sprague Dawley rats (150-180kg) were selected for this study. Experimental animal were grouped into five, contains six animals each and treated as follows: Group-I received vehicle (1% Tween80, 5ml/kg, ip) and serve as control. Group-II received monosodium glutamate (5mg/kg,ip) serve as negative control. Group-III received monosodium glutamate (5mg/kg, ip) simultaneously rats were administered with NMDA antagonist memantine (5mg/kg, ip) serve as a positive control. Group-IV& IV received monosodium glutamate and Ethanolic extract of *Evolvulus alsinoides* (200&400mg/kg, p.o) respectively serve as a treatment group. The injections were administered above manner for 7 days.

Blood Collection and Preparation of Brain Tissues Homogenate

On 14th day the Rats were euthanised by thiopental sodium (45 mg/kg i.p.), blood was collected for about 20 sec in a Eppendroff tubes, containing anti-coagulant solution (50 µl). The anti-coagulant treated blood was used for nitrate and SOD estimation. On 15th day Brain was collected from euthanised rats and hippocampus and striatum were removed and weighed immediately. The weighed samples were homogenized in chilled 10% KCl solution (10 ml/gm tissue). Homogenized samples were centrifuged at 2000 rpm for 10 min. Finally the clear supernatant was separated to measure TBAR, Protein levels.

Bio Chemical Estimation

The estimation of TBAR, SOD, Nitrates, Protein were determined by standard analytical kit

Estimation of TBAR

The method was followed to estimate total amount of lipid peroxidation product (Thiobarbituric acid reacting substances) in the homogenate. The incubation mixture was prepared as shown in table[4]:

Ingredients	**Volume**
Tissue Homogenate (supernatant	0.5 ml (brain)
8% sodium dodecyl sulphate (SDS)	0.2 ml
20% acetic acid solution (adjusted at pH 3.5 with 1N NaOH/ 0.1 N HCl)	1.5 ml
0.9 aqueous solution of thiobarbituric acid (adjusted to pH 7.4 with 1N NaOH/ 0.1 N HCl solution)	1.5 ml

The incubation mixture was made upto 5.0 ml with double distilled water and then heated in boiling water bath for 30 min. After cooling, the red chromogen was extracted into 5 ml of the mixture of n-butanol and pyridine (15:1v/v) centrifuged at 4000 rpm for 10 min, the organic layer was taken and its absorbance at 532 nm was measured. 1, 1, 3, 3 tetra ethoxypropane (TEP) was used as an external standard and the level of lipid peroxides was expressed as nmole of MDA/100g protein. The calibration curve for TEP was prepared by the above procedure taking TEP as standard. Linearity was obtained over the range of 80-240 nmoles of TEP.

Estimation of Total Protein

Protein was estimated following the method. 50 µl of brain homogenate was incubated at room temperature for 20 min along with 45 0µl of distilled water and 5 µl of copper reagent. After 20 min 5 µl of 1N Folins phenol reagent was added and samples were vortexed. After 15 min the colour intensity was read at 640 nm. Protein calibration curve was prepared by taking bovine serum albumin (100 mg/ml) as standard. It is expressed as mg/protein[5].

Estimation of Nitrates

Nitrites concentration in the plasma/brain was determined as nitrates by using Griss reagent. 400 µl of plasma was mixed with equal volume of griss reagent, and the optical density was determined at 540 nm. A calibration curve was generated using 0.1 sodium nitrite as a standard. The nitrates level in the plasma expressed as µg/ml[6].

Estimation of SOD

The activity of SOD was estimated following the method. It involves the measurement of the inhibition of the formation of the blue coloured formozan dye from nitro blue tetrazolium (NBT) in the presence of phenazine methosulphate (PMS) and reduced nicotinamide adenine dinucleotide (NADH). The whole brain was dissected out following the procedure mentioned earlier. One unit of activity of SOD was defined as the amount of enzyme that inhibits the rate reaction by 50% under special conditions. The incubation mixture consisted of sodium pyrophosphate buffer (pH 8.3, 0.052 M, 1.2 ml), phenazine methosulphate (186 μM), nitroblue tetrazolium (300 μM) and NADH (780 μM, 0.2 ml). The reaction was initiated by the addition of NADH; following incubation was done for 90 seconds at 37°C the reaction was terminated by the addition of glacial acetic acid (1 ml). N-butanol (4 ml) was added, shaken vigorously, centrifuged at 4000 rpm for 1 min. and the upper butanol layer was read at λ- 560 nm against butanol blank.

Statistical Analysis

Biochemical data were subjected to one way ANOVA followed by Newman Keuls multiple comparison post hoc test, using Grad Pad Prism version 3.00 for windows (GradPad Software, San Diego, California, USA). A value of less than 0.05 has been taken as significant.

RESULTS

Effects of whole plant extracts of *Evolvulus alsinoides* on nitrates level in normal and monosodium glutamate treated rats

A significant increase in brain nitrates was observed with glutamate administration in comparison to the control rats reflects more nitrates level in plasma. Administration of *Evolvulus alsinoides* (200 & 400 mg/kg) decreased the nitrates level in comparison to glutamate treated group. (Table no.1)

Effects of whole plant extracts of *Evolvulus alsinoides* on super oxide dismutase level in normal and monosodium glutamate treated rats

A significantly depleted the superoxide dismutase level in the brain was observed with glutamate administrated groups when compared to control group. Administration of *Evolvulus alsinoides* (200 & 400 mg/kg) to the glutamate treated rats significantly elevated the superoxide dismutase level (Table no.2)

Effects of whole plant extracts of *Evolvulus alsinoides* on TBAR level in normal and monosodium glutamate treated rats

A significantly increase in TBAR levels in brain was observed with glutamate treated animals in comparison to the control animals, administration of *Evolvulus alsinoides* 200 mg/kg and 400 mg/kg significantly decreased the TBAR level in comparison to the glutamate treated group (Table no.3)

Effects of whole plant extracts of *Evolvulus alsinoides* on protein level in normal and monosodium glutamate treated rats

Glutamate and *Evolvulus alsinoides* (200 & 400 mg/kg) treated groups did not alter the brain protein level (Table no.4)

DISCUSSION

A number of pharmacological data indicate the involvement of glutamate in pathophysiology of anxiety and depression[7]. Several neurotransmitters mediate the different components of anxiety, including excitatory amino acids such as glutamate, inhibitory amino acids such as gamma-amino butyric acid (GABA), and monoaminergic neurotransmitters such as catecholamines and indoleamines. Different aspects of the anxiety response are mediated by various neurotransmitters in anatomically distinct areas. Thus, imprinting of emotionally traumatic memories is mediated, in part, by norepinephrine's action through the beta-adrenergic receptors in the amygdala. The development of conditioned fear is mediated by dopamine-1 receptors in the amygdala, leading to facilitation of declarative memory associations through the hippocampus[8] Blocking the basal glutamate excitation generated by ionotropic receptor could elicite significant anxiolytic effect. Indeed, the administration of antagonists of the NMDA and Non-NMDA type receptors into the baselaterel amgdala has been shown to reduced anxiety in animal mode [9,10].

The present findings of our study showed a significant antioxidant activity of *Evolvulus alsinoides* 200 & 400mg/kg b.wt. on glutamate induced rats which is also strongly supported with our present antioxidant findings. Our present findings also supported by Lafon-Cazal, et al., 1993[11] and Hammer, et al., 1993 [12].

CONCLUSION

The role of oxidative stress in the genesis of neurodegeneration diseases has been widely studied. The high oxygen consumption rate coupled with low antioxidant potential of the brain are the main triggering factor for the enhanced release of free radicals. Oxidative stress is implicated in the pathogenesis of the number of neurodegenerative diseases like Alzheimer's, Parkinsonism, Stroke, etc.

The present study concludes that *Evolvulus alsinoides* may be effective in therapy of various neurodegenerative diseases, which may be due to an effective free radical scavenging property of the plant, will be one of the reason.

In future, the study may be recommended to find out the active phytoconstituents which are responsible for antioxidant property. Further in anxiety there is a variety of neurotransmitters are said to be involved. A systematic investigation in effects of the *Evolvulus alsinoides* on major neurotransmitters involved may be investigated. These investigations are now in progress.

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Table 1: Effect Of *Evolvulus alsinoides, Memantine* On Brain Nitrates In Monosodium Glutamate Induced Anxiety Rats

Data are Expressed as Mean ± SEM (n=3)

Groups	Nitrates	
	Hippocampus	Striatum
Control	0.310 ± 0.06	0.25 ± 0.02
MSG	2.194 ± 0.14 ^{aabb}	1.82 ± 0.03 ^{aabb}
MSG + Memantine 5 mg/kg	1.65 ± 0.03 ^{aab}	1.38 ± 0.02 ^{aab}
MSG + EA 200 mg/kg	2.07 ± 0.04 ^{aa}	1.71 ± 0.03 ^{aab}
MSG + EA 400 mg/kg	1.74 ± 0.03 ^{aabb}	1.44 ± 0.01 ^{aab}

^{aa}P<0.01, ^{a}P < 0.05 compared with control
^{bb}P<0.01, ^{b}P<0.05 compared with MSG
EA – *Evolvulus alsinoides*
Biochemical data were subjected to one way ANOVA followed by Newman Keuls multiple comparison post hoc test

Table 2: Effect Of *Evolvulus alsinoides, Memantine* On Brain Superoxide Dismutase In Monosodium Glutamate Induced Anxiety Rats

Data are Expressed as Mean ± SEM (n=3)

Groups	SOD	
	Hippocampus	Striatum
Control	0.65 ± 0.03	0.68 ± 0.02
MSG	0.28 ± 0.02 ^{aabb}	0.28 ± 0.06 ^{aabb}
MSG + Memantine 5 mg/kg	0.49 ± 0.01 ^{aab}	0.48 ± 0.02 ^{aabb}
MSG + EA 200 mg/kg	0.031 ± 0.3 ^{aa}	0.33 ± 0.02 ^{aa}
MSG + EA 400 mg/kg	0.44 ± 0.02 ^{aab}	0.48 ± 0.04 ^{aab}

^{aa}P<0.01, ^{a}P < 0.05 compared with control
^{bb}P<0.01, ^{b}P<0.05 compared with MSG
EA – *Evolvulus alsinoides*
Biochemical data were subjected to one way ANOVA followed by Newman Keuls multiple comparison post hoc test

Table 3: Effect Of *Evolvulus alsinoides, Memantine* On Brain TBAR In Monosodium Glutamate Induced Anxiety Rats

Data are Expressed as Mean ± SEM (n=3)

Groups	TBARS	
	Hippocampus	Striatum
Control	0.123 ± 0.00	0.122 ± 0.02
MSG	0.485 ± 0.02 ^{aabb}	0.465 ± 0.02 ^{aabb}
MSG + Memantine 5 mg/kg	0.279 ± 0.00 ^{aab}	0.24 ± 0.04^{bb}
MSG + EA 200 mg/kg	0.357 ± 0.03^{aabb}	0.34 ± 0.03 ^{aab}
MSG + EA 400 mg/kg	0.299 ± 0.04^{aab}	0.28 ± 0.04^{ab}

^{aa}P<0.01, ^{a}P < 0.05 compared with control
^{bb}P<0.01, ^{b}P<0.05 compared with MSG
EA – *Evolvulus alsinoides*
Biochemical data were subjected to one way ANOVA followed by Newman Keuls multiple comparison post hoc test

Table 4: Effect of *Evolvulus alsinoides*, *Memantine* On Total Proteins In Monosodium Glutamate Treated Rats

Groups	Protein	
	Hippocampus	Striatum
Control(2ml/kg)	0.45.026	0.44±0.02
MSG(2gm/Kg)	0.42±0.032	0.42±0.04
EA (200 mg/kg)	0.43±0.027	0.45±0.01
EA (400 mg/kg)	0.43±0.019	0.46±0.00
Memantine(5 mg/kg)	0.44±0.020	0.42±0.00

Data are Expressed as Means± SEM (n=3)
Biochemical data were subjected to one way ANOVA followed by Newman Keuls multiple comparison post hoc test

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