

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

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ABSTRACT

The whole plant extract of *evolvulus alsinoides* was evaluated for its antioxidant activity in hypoxia induced neurodegeneration. The extract was given orally in to two different doses (200mg/kg and 400 mg/kg) for 7 days. Simantaneously rats were allowed to drink the water containing (2g/l) sodium nitrate. Parameters under study were TBAR, SOD, Nitrates and protein. The orally administrated extracts significantly decrease nitrates, TBAR levels and significantly increases the SOD in hypoxia induced rats. But the extract did not alter protein level in glutamate treated rats

KEYWORDS: *Evolvulus alsinoides*, monosodium glutamate. memantine, antioxidant activity

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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 etc¹. Inducement of synthesis of NO generation may precipitate hypoxia due to its more affinity to hemoglobin (Hb). Sodium nitrite oxidizes oxyhemoglobin to methemoglobin and also yielding nitrites (NO₂), and nitrates (NO₃). Nitric oxide binds slowly and reversibly to hemoglobin that eventually auto reduces by a first order reaction to HbFe²⁺NO. Further NO₂ oxidises oxyhemoglobin by an auto catalytic reaction and finally NO₂ bind reversibly to methemoglobin to yield a mixture of complexes. All this reaction put the biological system into oxidative stress and resulting apoptosis. This statement supports our findings with sodium nitrite administration in rats². *Evolvulus alsinoides* a traditional ayurvedic medicine have a better neuro protection and antioxidant properties³.

MATERIALS AND METHODS

Plant Material

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

Animals

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 a group of 4-5 in colony cages at ambient temperature of 25 ± 2°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 °C with 5 ml methanol.

Experimental design

Hypoxia was induced by allowing the rats to drink water containing (2 g/l) sodium nitrite⁴. The rats drinking about 20-25 ml NaNO₂ water per day were selected for study. *Evolvulus alsinoides* (200 & 400 mg/kg) were orally administered on nitrostatic animals and they were subjected to biochemical estimation.

Experimental groups

Healthy female Sprague Dawley rats (150-180kg) were selected for this study. Experimental animal were grouped into four, contains six animals each and treated as follows: Group-I received vehicle and serve as control. Group-II received sodium nitrite drinking water (2g/l) serve as negative control. Group-III& IV received sodium nitrite drinking water (2g/l), simultaneously rats were treated with ethanolic extract of *evolvulus alsinoides* (200&400mg/kg, p.o) respectively serve as a treatment groups. 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 table⁵:

Ingredients with Volume

Tissue Homogenate supernatant - 0.5 ml (brain)

8% sodium dodecyl sulphate (SDS) - 0.2 ml

20% acetic acid solution - 1.5 ml

(adjusted at pH 3.5 with 1N NaOH/ 0.1 N HCl)

0.9 aqueous solution of thiobarbituric acid - 1.5 ml

(Adjusted to pH 7.4 with 1N NaOH/ 0.1 N HCl solution)

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 Folin's 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/protein⁶.

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/ml⁷.

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 hypoxia induced rats

A significant increase in plasma nitrates was observed in hypoxia induced animals when compared with control group. Whereas, administration of *Evolvulus alsinoides* (200 & 400 mg/kg) decreased the nitrates level in comparison to sodium nitrite treated group. (Table 1)

Effects of whole plant extracts of *Evolvulus alsinoides* on super oxide dismutase level in normal and hypoxia induced rats

Sodium Nitrite significantly depleted the superoxide dismutase level in plasma. Administration of *Evolvulus alsinoides* (200 & 400 mg/kg) to the hypoxic rats significantly elevated the superoxide dismutase level. (Table 2)

Effects of whole plant extracts of *Evolvulus alsinoides* on TBAR level in normal and hypoxia induced rats

A significantly increase in TBAR levels in brain was observed with sodium nitrite treated animals in comparison to the control animals, administration of *Evolvulus alsinoides* 200 mg/kg and 400 mg/kg significantly decreased TBAR level in comparison to hypoxic group (Table 3).

Effects of whole plant extracts of *Evolvulus alsinoides* on protein level in normal and hypoxia induced rats

Sodium nitrite and *Evolvulus alsinoides* (200 & 400 mg/kg) did not alter the brain protein level. (Table 4)

DISCUSSION

Earlier reports revealed that animal treated with sodium nitrite in drinking water was resulted in hypoxic condition⁴. It is well documented that hypoxia/ischemia condition may lead to an intracellular Ca^{2+} over load which correlates to neuronal injury and degeneration⁸ and drugs which can prevent this influx of Ca^{2+} were shown to prevent the neuronal degeneration against hypoxic/ ischemic conditions⁹. Amnesia induced by sodium nitrite was correlated to the metabolic function involved in the biological system. It is also shown a close relationship between sodium nitrite induced oxidative metabolism and cholinergic function¹⁰⁻¹¹. Hypoxia and ischemia results over accumulation of glutamate and activate the post synaptic glutamate receptors, which initiate the detrimental biochemical cascade in the post synaptic neuron. These processes ultimately lead to DNA degradation, lipid peroxidation and neuronal cell death¹². Our present study revealed that the animals treated with *Evolvulus alsinoides* dose 200 & 400mg/kg/b.w. (p.o) was observed for significant antioxidant property and also it is proved by our present reports that sodium nitrite

treatment in the rat leads to elevation in the nitrates and TBAR levels along with decreased SOD level indicates oxidative stress in the sodium nitrite administration. *Evolvulus alsinoides* significantly altered these enzyme variations in plasma and brain. Our findings also supported by Bhattacharya, et al., 1994¹³

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 is 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* on plasma nitrite in hypoxic induced rats

Groups	Nitrates
Control	11.57 ± 1.26 ^{aa}
Hypoxia	29.625 ± 2.35 ^{acc}
Hypoxia + EA 200 mg/kg	24.95 ± 2.60 ^{aa}
Hypoxia + EA 400 mg/kg	22.8 ± 1.48 ^{ac}

Data are Expressed as Mean ± SEM (n=3)
^{aa}P<0.01, ^aP < 0.05 compared with control
^{cc}P<0.01, ^cP<0.05 compared with Hypoxia

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* on superoxide dismutase in hypoxic induced rats

Groups	SOD
Control	18.91 ± 1.530 ^{aa}
Hypoxia	13.5 ± 1.28 ^{ac}
Hypoxia + EA 200 mg/kg	17.06 ± 1.49
Hypoxia + EA 400 mg/kg	18.52 ± 1.98

Data are Expressed as Mean ± SEM (n=3)
^{aa}P<0.01, ^aP < 0.05 compared with control
^{cc}P<0.01, ^cP<0.05 compared with Hypoxia

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* on brain TBAR in hypoxic induced rats

Groups	TBAR activity	
	Hippocampus	Striatum
Control	0.123 ± 0.00	0.122 ± 0.02
Hypoxia	0.378 ± 0.02 ^{aa}	0.35 ± 0.03 ^{aa}
Hypoxia + EA 200 mg/kg	0.319 ± 0.01 ^{aa}	0.30 ± 0.03 ^{aa}
Hypoxia + EA 400 mg/kg	0.275 ± 0.03 ^{aa}	0.25 ± 0.04 ^a

Data are Expressed as Mean ± SEM (n=3)
^{aa}P<0.01, ^aP < 0.05 compared with control
^{cc}P<0.01, ^cP<0.05 compared with Hypoxia

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* on total proteins in hypoxia induced rats

Groups	Protein	
	Hippocampus	Striatum
Control	1.09 ± 0.10	1.03 ± 0.05
Hypoxia	1.07 ± 0.03	1.00 ± 0.05
Hypoxia + EA 200 mg/kg	1.05 ± 0.112	1.02 ± 0.04
Hypoxia + EA 400 mg/kg	1.00 ± 0.07	0.99 ± 0.09

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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