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NARDOSTACHYS GRANDIFLORA EXTRACT ATTENUATES GLUTAMIC ACID INDUCED LIPID PEROXIDATION IN HIPPOCAMPUS AND CEREBRAL CORTEX OF MICE

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ABSTRACT:

Oxidative stress participates in the etiology of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. The brain with its high lipid content, low level of free radical eliminating enzymes may be prime target of free radical damage. Plant flavonoids have significant effect on protection of brain against free radicals. *Nardostachys grandiflora* (Jatamansi) has a profile of activity that is consonant with putative anti-stress and antioxidant activity. In the present work, we examined the neuroprotective effects of *Nardostachys grandiflora* against lipid peroxidation (LPO) in parallel with the level of reduced glutathione (GSH) in hippocampus and cerebral cortex regions of mouse brain. The results of the present study showed that the extract of *Nardostachys grandiflora* has significant antioxidant property which may prevent the progression of neuronal cell injury.

KEY WORDS: Nardostachys grandiflora, Glutamic Acid, Hippocampus, Cerebral Cortex, Mice.

INTRODUCTION:

Neurodegeneration involves degeneration of circumscribed group of neurons that may be functionally of neuroanatomically connected (Beal 1997). Neurons degenerated are not replaced resulting in cognitive loss, dementia, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease and cerebellar degeneration. During neurodegeneration there is a deterioration of neurological tissue. Neurodegenerative diseases represent a large group of neurological disorders with heterogeneous clinical and pathological expressions affecting specific subsets of neurons in specific functional and anatomical systems. Neurodegenerative disorders are major cause of mortality and disability and as a result of increasing life spans represent one of the key medical research challenges.

Oxygen is a dangerous friend. Evidence indicates that oxidative stress can lead to cell and tissue injury. However, the same free radicals that are generated during oxidative stress are produced during normal metabolism and may account for gross cellular damage and the degenerative process of biological aging (Cross 1987; Harman 1956,1981). Oxidative stress

is a shift towards the pro-oxidant in the pro-oxidant/antioxidant balance that can occur as a result of increase in oxidative metabolism. Its increase at the cellular level can come as a consequence of several factors, including exposure to alcohol, cold, medications, trauma, infections, toxins, radiation, poor diet etc. (Singh et al, 2004). Oxidative stress in a cellular system occurs when the production of free radical moieties exceeds the antioxidant capacity of that system. If cellular antioxidant do not remove free radicals, radicals attack and damage proteins, lipids and nucleic acids. The oxidized or nitrosylated products of free radical attack have decreased biological activity, leading to loss of energy metabolism cell signaling, transport and other functions (Vincent et al., 2004). Evidence suggests that free radical damage may be a major factor in the degeneration of nerve cells in Parkinson'd disease (PD) (Grimes et al. 1988). Alzheimer's disease (AD) may involve oxidative stress and the accumulation of free radicals leading to excessive lipid peroxidation and degeneration of brain neurons (Sano et al. 1997). Evidence for increased oxidative damage in AD comes from reports of increased lipid peroxidation in AD cerebral cortex (Subbarao et al 1990.; Palmer and Burns 1994). Oxygen free radical induced lipid peroxidation has convincingly recognized as a significant factor in the pathophysiology of acute CNS injury (Braughler and Hall 1989; Siesjo et al.1989).

Excitotoxicity refers to neuronal cell death caused by activation of excitatory amino acid receptors. Glutamate is the primary excitatory neurotransmitter in the central nervous system. Increased amount of glutamate in synaptic cleft leads to neurotoxicity (Choi et al 1987). Interaction of glutamate with specific membrane receptors is responsible for many neurologic functions including cognition, memory and sensation. In various neurodegenerative diseases, excessive activation of glutamate receptors may mediate neuronal injury or death (Lipton and Rosenberg 1994).

When ROS are generated in living system a wide variety of antioxidants come into play (Auroma 1993, Diplock 1993, Halliwell 1990). Glutathione is an important cellular antioxidant and plays a major role in protecting cells against oxidative stress (Tirmenstein et al 2000). Studies have shown that a prolonged depletion of reduced glutathione (GSH) in the brain is associated with oxidative neuronal death (Regan and Guo 2001). Glutathione provides critical defense system for the protection of cells from oxidative and other forms of stress (Dickson and Forman 2002, Hayes and McLellan 1999). Glutathione provides cells with multiple defenses against both ROS and their toxic by products.

Recent researches have shown that phyotchemicals having antioxidant properties might reduce the symptoms of neurodegeneration. Some studies have indicated that phenolic

substances such as flavonoids are considerably potent antioxidants (Cao et al., 1997). Flavonoids have the property to scavenge free radical and prevent lipid peroxidation (Morel et al., 1993)

MATERIALS AND METHODS:

1) Plant extract preparation : Powdered plant materials were extracted in acetone at room temperature to obtain a brown solid extract. The extract was further fractioned by chloroform and organic layer was collected and evaporated. The extract was fractionated by 90% methanol. The extract was vacuum dried and dissolved in water for diet preparation.
2) Dose preparation : The dose of *Nardostachys grandiflora* was decided by measuring the total antioxidant power of plant extracts and comparing those activities with standard L-ascorbic acid solution of known concentration. The amount of 250mg/kg b.w. extracts of *Nardostachys grandiflora* were given to experimental mice.
3) Animal grouping : To observe the response of plant extract in prevention of neurodegeneration in glutamate induced excitotoxicity, animals were divided into different groups. These animals were treated with different concentrations of glutamic acid and extract of *Nardostachys grandiflora*.
4) Tissue preparation : Control and experimental swiss albino mice were decapitated at the end of the experiment and the brain regions such as cerebral cortex and hippocampus were dissected out and homogenized for the assays of lipid peroxidation, protein carbonyl content and reduced glutathione assay.
5) Lipid peroxidation assay : Lipid peroxidation was measured by thiobarbituric acid reactive substance (TBARS) (Ohkawa et al., 1979). Tissue was homogenized in phosphate buffer (pH 7.4). Homogenized samples were taken and mixed with 0.2ml sodium lauryl sulfate, 0.8% aqueous solution of thiobarbituric acid. To the mixture distilled water and mixture of n-butanol and pyridine was added. The mixture was centrifuged for 15 minutes. The amount of Maloondialdehyde (MDA) formed was measued by the absorbance of organic layer at 532nm in a Perkin Elmer spectrophotometer. Lipid peroxidation was calculated in terms of MDA equivalents.
6) Reduced Glutathione (GSH) Assay : The GSH assay was quantified by the method of Jollow et al 1974. Briefly, homogenate was centrifuged at 16,000 x g for 15 minutes. at 4oC. 0.5ml supernatant waas taken and mixed with 2.5 ml of 0.1mol/l disodium hydrogen phosphate buffer (pH 8.0) and 1ml of 0.1mmol/l Ellamn's reagent. The absorbance was measured at 412 nm in a Perkin Elmer spectrophotometer. A calibration curve was prepared using GSH as a standard.

7) Statistical analysis : All data are expressed as mean + S.E.M. Statistical comparisons were made relative to the appropriate control group buy student's t-test and analysis of variance. The 0.05 level was selected as point of minimal statistical significance in every comparison.

RESULTS:

Lipid Peroxidation (LPO) : Increasing concentrations of glutamate (doses of 1,5,10 mg/kg b.w) resulted in an increased lipid peroxidation in hippocampus and cerebral cortex of brain (Fig1,2). This increase was dose dependent in both hippocampus and cerebral cortex regions of mice brain. When the animals were administered with extract of *Nardostachys grandiflora* the lipid peroxidation was declined significantly ($p<0.05$) in both the regions of mice brain. (Fig 1,2).

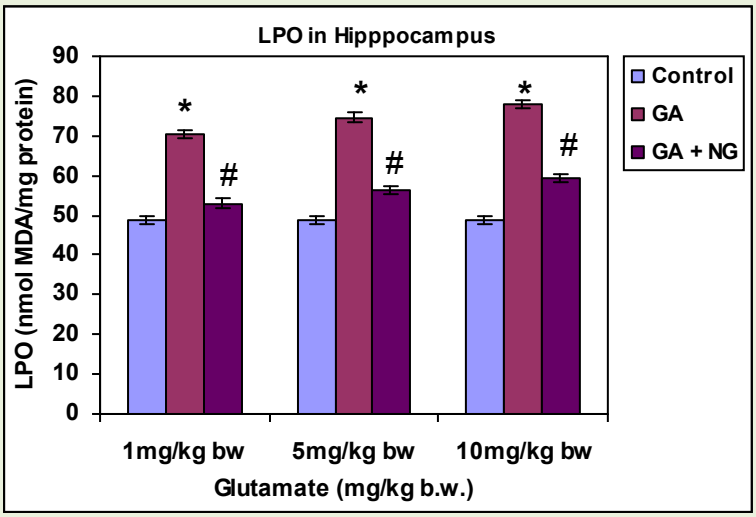

Fig 1: Lipid peroxidation (LPO; n moles MDA mg^{-1} protein) in hippocampus after the treatment with glutamate (GA) (1, 5, 10 mg/kg b.w.) and extract of *Nardostachys grandiflora* (NG).

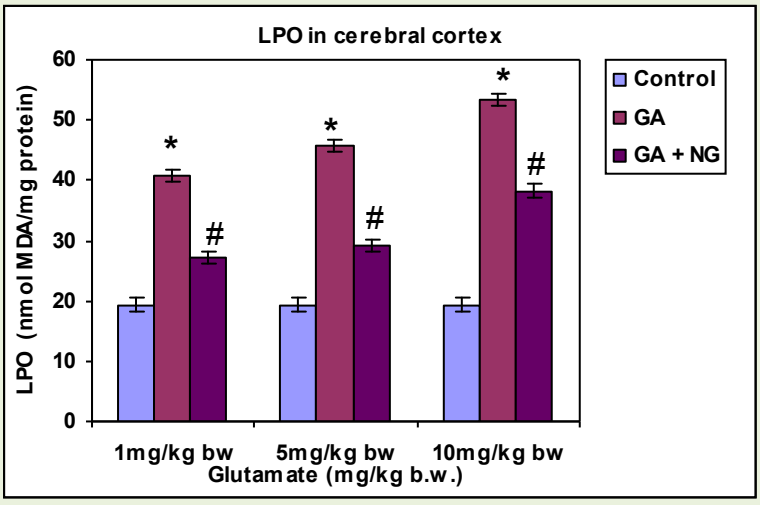

Fig 2: Lipid peroxidation (LPO; n moles MDA mg^{-1} protein) in cerebral cortex after the treatment with glutamate (GA) (1, 5, 10 mg/kg b.w.) and aqueous extract of *Nardostachys grandiflora* (NG).

Reduced glutathione (GSH) : Administration of three regimens of glutamic acid (1, 5, 10 mg/kg b.w.) resulted in a significant ($p<0.05$) decline in the level of GSH in both hippocampus and cerebral cortex regions of brain (fig 3,4). In view of increased oxidative burden in brain by three regimens of glutamic acid, depletion of GSH level in both hippocampus and cerebral cortex may enhance the neuronal death by increasing the vulnerability to oxidative stress. The administration of extract of *Nardostachys grandiflora* caused an elevation of GSH levels in both hippocampus and cerebral cortex regions of glutamic acid treated mice.

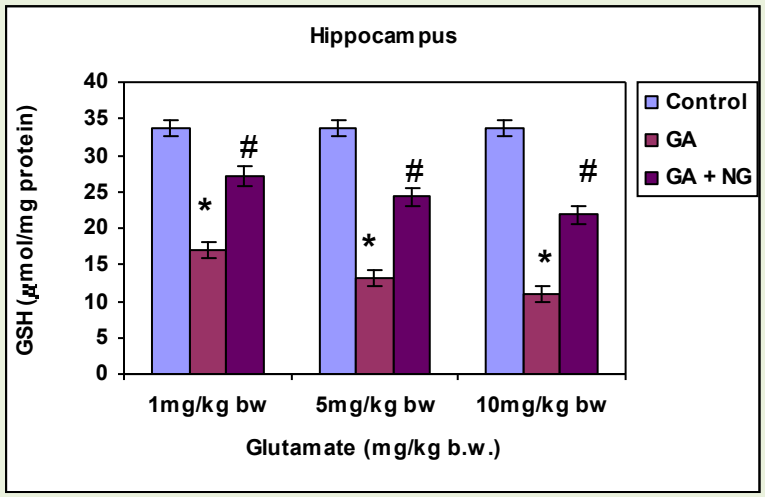

Fig 3: Reduced glutathione levels (GSH; □ moles mg^{-1} protein) in hippocampus after the treatment with glutamate (GA) (1, 5, 10 mg/kg b.w.) and extract of *Nardostachys grandiflora* (NG).

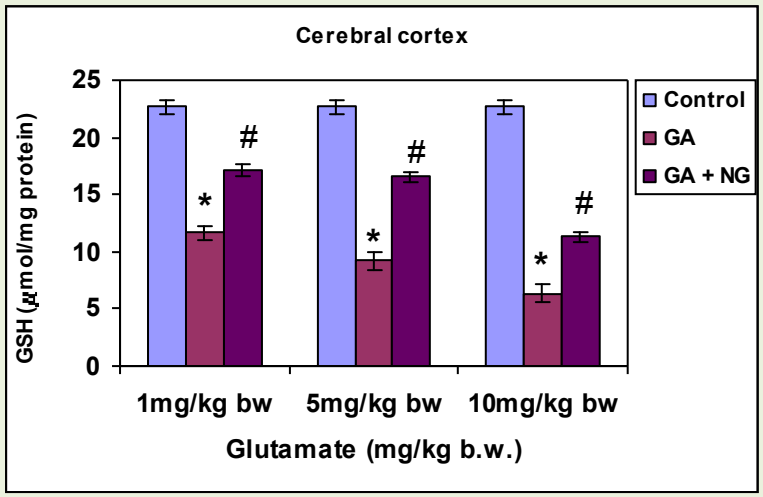

Fig 4: Reduced glutathione levels (GSH; □ moles mg^{-1} protein) in cerebral cortex after the treatment with glutamate (GA) (1, 5, 10 mg/kg b.w.) and aqueous extract of *Nardostachys grandiflora* (NG) .

DISCUSSION:

Apoptosis play a key role in cell death observed in neurodegenerative diseases marked by a progressive loss of neurons. High concentrations of excitatory neurotransmitter glutamate can

accumulate in the brain and are thought to be involved in the etiology of a number of neurodegenerative disorders. Studies have shown that at high concentration, glutamate is potent neurotoxin and is capable of destroying neurons. Evidence suggests that glutamate excitotoxicity involves oxidative stress and apoptosis.

Hydroxyl radical is most reactive free radical produced in biological systems and mainly responsible for the initiation of lipid peroxidation. A variety of indices have been developed to assess free radical mediated injury of free radical generation in vivo. Among substrates for attack by free radicals, lipids have been the most extensively studied. The present study was done to examine the excitotoxic effects of glutamic acid on hippocampus and cerebral cortex regions of mice brain. Result from the present study show an increased lipid peroxidation in hippocampus and cerebral cortex regions indicating increased production of reactive oxygen species in hippocampus and cerebral cortex regions of brain. Studies have shown that increased ROS causes high lipid peroxidation (Duarte et al 2000). The findings of the present study suggests an increased oxidative stress due to excessive glutamate concentrations in these brain regions. Increase in lipid peroxidation suggest the oxidative damage of lipids by glutamate excitotoxicity.

Oxidative stress is important in the pathophysiological mechanisms underlying acute central nervous system (CNS) injury. The discovery and development of potent antioxidant agents has been one of the most interesting and promising approaches in the search for treatment of CNS injury. Antioxidants of varying chemical structures have been investigated as therapeutic agents in the treatment of CNS injury. Nature has evolved several enzymes and antioxidants which act in a combined fashion to help protect biological system against oxidative damage. These antioxidants are substances, which provide protection against the toxic effects of ROS and their precursors in cell. Reduced glutathione and enzymes associated with its metabolism provide a major defense against ROS-induced cellular damage (Cardozo-Pelaez et al. 2000). Two enzymes that are essential components of the effect of GSH on ROS are GSH-Px (glutathione peroxidase) and GST (glutathione S-transferase). Results of the present study revealed considerable decline in hippocampus and cerebral cortex GSH in glutamate treated mice. This may be due to its utilization to challenge the prevailing oxidative stress under the influence of $O_2^{\cdot-}$. Significant improvement in antioxidant status was observed as marked by increase in GSH levels in both hippocampus and cerebral cortex regions of *Nardostachys grandiflora* extract treated mice. It was also observed that the level of lipid peroxidation was declined significantly on treatment of mice with extract of *Nardostachys grandiflora*. This suggests that the extract of Nardostachys grandiflora

supplementation effectively protected hippocampus and cerebral cortex region of brain against glutamate induced excitotoxicity.

CONCLUSION:

The present study revealed that glutamate causes oxidative damage to lipids leading to increased lipid peroxidation and reduced GSH content in both hippocampus and cerebral cortex regions of mice brain. In conclusion, the present study clearly shows that extract of *Nardostachys grandiflora* protected brain tissue from free radical derived oxidation and increased protection of neuronal degeneration. Those effects are also due to increasing antioxidant redox status of cell by increasing reduced glutathione levels.

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