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Original Article

Effect of *Ocimum sanctum* on Noise Stress Induced Changes in the Membrane Bound Enzymes of Albino Rats in Brain

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ABSTRACT

Ocimum sanctum [OS] was selected for this study to understand its efficacy on the membrane bound enzymes of brain during noise exposure. In this study, wistar strain albino rats were divided into the four groups, control, OS, noise stress [100 dB/4h/ d] and noise stress+OS and treated. After 15 days of incubation to noise stress, estimation of Sodium [Na^+] Potassium [K^+], Magnesium [Mg^{2+}] and Calcium [Ca^{2+}] Adenosine Tri Phosphatases [ATPases] were carried out to determine the changes in the membrane bound enzymes [MBEs] of brain. Administration of the ethanolic extract of OS had a normalizing action on membranes of the cell and controlled the alteration of MBE due to noise stress in noise stress+OS group. In conclusion treatment with OS prevented the decrease in the levels of MBEs

Key words: *Ocimum sanctum,* Noise Stress, Sodium Potassium, Magnesium and Calcium ATPases.

INTRODUCTION

Stress is a condition in an organism that results from the action of one or more stresses that can be either external or internal origin [1]. Sound waves are produced when the air is mechanically disturbed. Sound propagates from a source in all directions. The rate at which the vibrations occur (the frequency) is expressed in cycles per second or Hertz (Hz). Sound is of great value for the living being as hearing has evolved for our need to alert, to warn and to communicate. Though sound is useful, when the sound level exceeds, it becomes a noise. Noise is the most widespread sources of environmental stress in living environment [2]. Noise may act as a non-specific stressor, inducing stress reactions which are in line with the general stress model [3]. Annoyance can be defined as "the expression of negative feelings resulting from interference with activities, as well as disruption of one's peace of mind and the enjoyment of one's environment" [4]. According to Babisch [5], noise activates the pituitary-adrenal-cortical axis and the sympathetic-adrenal-medullary axis. The general pattern of endocrine responses to noise is consistent with noise as a stressor, stimulating short-term physiological responses [6]. Nervous system is relatively more susceptible to free radical damage [7]. It was reported that neurotransmitters in discrete brain regions were found to be increased during noise stress even after 15 d of exposure [8]. In addition to generating free radical species, it also leads to increase in radical induced lipid peroxidation end products such as malondialdehyde [MDA] which is an indicator of lipid peroxidation processes [9].

The Physiological response to stress owing to activation of Hypothalamo pituitary adrenal axis is the subsequent release of corticosterone from the adrenal cortex into blood. The elevation of endogenous corticosterone because of stress response has been reported to accelerate the generation of free radicals. Lipid peroxidation is regarded as one of the basic mechanisms involved in tissue damage caused by free radicals. Peroxidation of membrane is accompanied by alteration of the structural and functional characteristics of membranes. Lipid peroxidation changes the activities of various enzymes. ATPases are very sensitive to peroxidation reactions and abnormal lipid peroxides affect ATPase activities. ATPases are intimately associated with the plasma membrane and participate in the energy requiring

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translocation of sodium, potassium, calcium and magnesium ions.

It has been reported that in real life even moderate environmental noise exposure can increase the acute release of stress hormones [10]. OS, a plant has anti-arthritic activity [11], antipyretic effect [12], anti-inflammatory effect [13] and analgesic activity [14]. There have been no studies on the ethanolic effect of OS on noise stress induced changes in MBEs of brain. Antioxidants play a protective role against reactive oxygen species [ROS]. Hence, the search for natural antioxidants is essential. Therefore this study to analyze the potential of OS proving the Pharmacological value of their efficiency as an antidote to stresses was needed.

MATERIALS AND METHODS

Animals: Wistar strain male albino rats, used for the study were reared in the animal house of the institute and all the rats were acclimatized to the standard conditions of constant ambient temperature [24-26 ºC] with 12 hrs dark photo period [lights on from 6.00 to 18.00 hrs]. The rats were provided *ad libitum* access to food [Rat feed, Hindustan Lever Ltd.] and water. Animal experiments were carried out after getting proper IACE and well as CPCSEA permission (IAEC no. 8/014/08).

Chemicals: All the biochemical used in this experiment were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Experimental Protocol: Animals were divided into 4 groups with 6 animals each: Group 1 consisted of Control rats to understand the normal levels, Group 2 consisted of OS, Group 3 consisted of 15 d noise stress exposed animals [100 dB for 4hrs/d] and Group 4 consisted of 15 d noise stress and also OS pretreated animals [100 mg/kg bw *ip*] to understand the effect of OS during stress exposure. After sub-acute [15d] noise exposure, the animals were sacrificed by cervical dislocation and the brains were removed quickly on ice cold plate. Whole brain was homogenized and centrifuged [12000 rpm, 4 ºC] for estimation of enzymes.

Noise Stress Induction Procedure: Noise become a stressor when the exposure limit exceeds 90 dBs Therefore the animals [15] were exposed to noise by two loudspeakers [15W], which was driven by a white noise generator [0-26kHz], and installed 30-cm above the cage and The noise level was set at 100 dB, the noise level was monitored by a sound level meter D2023 [S.NO-F02199; Cygnet systems, Gurgaon, Haryana, India]. Each treated animal were exposed for 4h/d for 15 ds. The control rats were kept in the corresponding period of time, without noise stimulation to evaluate the effect of stress.

Preparation of OS Extract: Fresh OS plants were identified and collected at IMPCOPS farms, Chennai and dried under shade, then the leaves were powdered and the ethanolic extract was prepared according to Bhargava and Singh. The method involves percolation at room temperature using 70% ethanol. 500 g of OS powder was mixed with 5l of ethanol and the container was kept at room temperature for 7 ds, and shaken 5-6 times daily. After 7 ds, the supernatant is decanted, filtered and stored as a residue. To the residue 2l of ethanol was added and percolated. This confirmed till the final end product in the form of residue was obtained.

MBE: Na^+-K^+ATPase: The activity of Na^+-K^+ATPase [ATP: Phosphohydrolase - EC. 3.6.1.3.] in the tissue was estimated by the method suggested by Bernabe *et al*., [16]. 0.1 ml of homogenated brain tissue was taken in centrifuge tubes and was incubated in a medium containing 1.5 ml Tris-Hydrochloride [HCl] buffer, 0.1 ml each Sodium chloride [NaCl], Potassium chloride [KCl], Magnesium Sulphate [$MgSO_4$], Ethylene Diamine Tetra Acetate [EDTA] and 0.1 ml Adenosine Tri Phosphate [ATP] for 30 min at 37 ºC. The reaction was arrested by the addition of 1.0 ml of 10% Tricyclic Acid [TCA]. The precipitate formed on addition of TCA in both the test and tissue control tubes was removed by centrifugation and the supernatant was transferred to fresh tubes. The reagent blank contained 1.8 ml of Tris-HCl buffer. The standard tubes taken at a concentration range of 2 to10 µg were placed in distilled water and were made upto1.8ml with Tris-HCl buffer. To all the above tubes, 0.5 ml of ammonium molybdate and 0.2 ml of 1-amino-2-napthol-4-sulphonic acid [ANSA] was added and left for 20 min for the development of blue color, which was read at 620 nm against the reagent blank using spectrophotometer. The activity of Na^+-K^+ATPase in the tissue was expressed as µmoles of phosphorous liberated/min/mg protein.

Ca^{2+}ATPase: The activity of Ca^{2+}ATPase [ATP: Phosphohydrolase - EC. 3.6.1.3.] in the tissues was estimated as described by the method suggested by Hjerten and Pan [17]. 0.1 ml of homogenated brain tissue was taken in centrifuge tubes and was incubated in a medium containing 1.5 ml Tris-HCl buffer, 0.1 ml Calcium chloride [$CaCl_2$] and 0.1 ml ATP for 30 min at 37 ºC. The reaction was arrested by the addition of 1.0 ml of 20% TCA. To the

tissue control containing the buffer, $CaCl_2$ and ATP, the 0.1 ml of homogenate was added only after addition of 1.0 ml of TCA. The precipitate formed after the addition of TCA in both the test and tissue control tubes was removed by centrifugation and the supernatant was transferred to fresh tubes. The reagent blank contained 1.8 ml of Tris-HCl buffer. The standard tubes taken at a concentration range of 2 to10 µg was placed in distilled water and was made up to 1.8 ml with Tris-HCl buffer. To all the above tubes, 0.5 ml of ammonium molybdate and 0.2 ml of 1-amino-2-napthol-4-sulphonic acid [ANSA] was added and left for 20 min for the development of blue color, which was read at 620 nm against the reagent blank using spectrophotometer. The activity of Ca^{2+}ATPase in the tissue is expressed as µmoles of phosphorous liberated/min/mg protein.

Mg^{2+}ATPase: The activity of Mg^{2+}ATPase [ATP: Phosphohydrolase - EC. 3.6.1.3.] in the tissues was estimated by the method suggested by Ohnishi *et al*. [18]. Tissue homogenate of volume 0.1 ml in a tube was incubated in a medium containing 1.5 ml Tris-HCl buffer, 0.1 ml Magnesium chloride [$MgCl_2$] and 0.1 ml ATP for 30 min at 37 ^{0}C. The reaction was arrested by the addition of 1.0 ml of 30% TCA. To the tissue control containing the buffer, $MgCl_2$ and ATP, the 0.1 ml of homogenate was added only after the addition of 1.0 ml of TCA. The precipitate formed after addition of TCA in both the test and tissue control tubes was removed by centrifugation and the supernatant was transferred to fresh tubes. The reagent blank contained 1.8 ml of Tris -HCl buffer. The standard tubes taken at a various concentration range of 2 to 10 µg was placed in distilled water and was made up to 1.8 ml with Tris-HCl buffer. To all the above tubes 0.5 ml of ammonium molybdate and 0.2 ml of 1-amino-2-napthol-4-sulphonic acid [ANSA] was added and left for 20 min for the development of blue color, which was read at 620 nm against the reagent blank using spectrophotometer. The activity of Mg^{2+}ATPase in the tissue was expressed as µmoles of phosphorous liberated/min/mg protein.

RESULTS

Ca^{2+}ATPase levels in the groups were studied summarized and given in figure: 1. Animals exposed to sub-acute noise stress for 15 d showed a significant decrease in the activity of Ca^{2+}ATPase, compared to control animals. Animals exposed to noise stress after pretreatment with OS showed a significant increase in the activity of Ca^{2+}ATPase, compared to noise exposed group of animals. Na^+-K^+ATPase levels in the groups were studied summarized and given in figure: 2. Animals exposed to sub-acute noise stress for 15 d showed a significant decrease in the activity of Na^+-K^+ATPase, compared to control animals. Animals exposed to noise stress after pretreatment with OS too showed a significant increase in the activity of Na^+-K^+ATPase, compared to noise exposed group of animals.

Mg^{2+}ATPase levels in the groups were studied summarized and given in figure: 3. Animals exposed to sub-acute noise stress for 15 d showed a significant decrease in the levels of Mg^{2+}ATPase activity compared to control animals. Similarly, animals exposed to noise stress after pretreatment with OS showed a significant increase in the activity of Mg^{2+}ATPase, compared to noise exposed group of animals.

It is essential to point out that OS could bring the activity to normal activity as that of the control in the stressed group indicating it can be an antidote for noise stress. The possible cause behind will be discussed.

DISCUSSION

In this study, there is marked alteration in the MBEs with regard to noise exposure. Because of the brain recognizes the sound levels and discriminates the stress level, it acts as a key organ and involves in interpreting and responding to potential stresses. It is the target for different stresses as indicated by the induced degenerative conditions. Researchers reported that the auditory system has the fastest metabolic rate in the brain. Noise stimulates the brain's reticular activating system to induce wake. Neural impulses spread from the reticular system to the higher cortex and throughout the central nervous system [4]. In response to stress, the brain activates several neuro-peptide-secreting enzymes. Noise exposure firstly increase the levels of ROS such as super oxide radicals, hydroxyl radicals and hydrogen peroxide. Secondly, the activity of antioxidants and related enzymes were increased in order to eliminate the overproduced ROS due to noise [19].

ROS and free radicals can break down cell membranes through lipid peroxidation, leading to cell death [20]. MDA is an indicator of lipid peroxidation processes which involves the formation of free radical species. ATPases are very sensitive to peroxidation reactions and abnormal lipid peroxides affects the ATPase activities. Na^+-K^+ATPase and Mg^{2+}ATPase activities were decreased. ATPases are intimately associated with the plasma membrane and participates in the energy requiring translocation of Na^+-K^+ ,Ca^{2+} and Mg^{2+} [19]. The MBEs such Na^+-K^+ ATPase,

Mg^{2+}ATPase and Ca^{2+}ATPase are responsible for the transport of Na^+, K^+, Mg^{2+} and Ca^{2+} ions across the cell membrane at the expense of ATP by hydrolysis [21].

Na^+-K^+ATPase is an enzyme concentrated at nerve ending membranes [22] where Na^+ exit and K^+ entry occur during neurotransmission. A close relationship between Na^+-K^+ATPase activity and neurotransmitter release has been demonstrated, suggesting that this enzyme could play a role in the mechanism of neurotransmission modulation [23].

It has been demonstrated that neuronal death associated with a decrease in Na^+-K^+ATPase activity is mediated via intracellular depletion of K^+ and accumulation of Ca^{2+} and Na^+ [24]. Since maintenance of Na^+-K^+ATPase is critical for normal brain function and reduction of this activity is related to selective neuron damage in the rat brain [25, 26, 27]. It is also conceivable that some substances can directly bind to the Na^+-K^+ATPase due to their specific chemical structures modulating its activity or alter the synaptic membrane where the enzyme is embedded, as it occurs in other substances which inhibit Na^+-K^+ATPase [28].

The calcium pump or Ca^{2+}ATPase in plasma membrane cause high Ca^{2+} affinity. The structure is responsible for the maintenance of Ca^{2+} concentration at the sub micro molar level [29, 30]. This could be the reason for membrane damage in cancer. Excessive Ca^{2+} influx is a phenomenon leading cellular death and Ca^{2+}ATPase activity decreased in cancer bearing animals. [20].

In this study there is a marked decrease in all the MBEs such as Na^+-K^+ATPase, Ca^{2+}ATPase and also Mg^{2+}ATPase. Na^+-K^+ATPase inhibition, in the presence of a non-lethal insult, activates the apoptotic cascade and neuronal injury probably by amplifying the disruption on K^+ homeostasis [31].
It was reported that catecholamines, Nor-Epinephrine [NE] and dopamine modify neuronal Na^+-K^+ATPase activity, respectively stimulate or inhibit the enzyme [31].

CONCLUSION

OS could prevent the changes by scavenging the free radical generated. There are reports that support the favorable actions during stress. It is also interesting to note that OS can bring the monoamine oxidase [MAO] level back to the normal level, which decreased during the swimming stress. Similarly, the changes in whole brain acetylcholine level after the exposure to noise stress was blocked by the OS extract and also reported about that the normal action of OS which helps the body to cope with stress in a better way. Therefore Treatment with OS effectively prevented the decrease in levels of Na^+-K^+ ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase caused by repeated noise stress exposure.

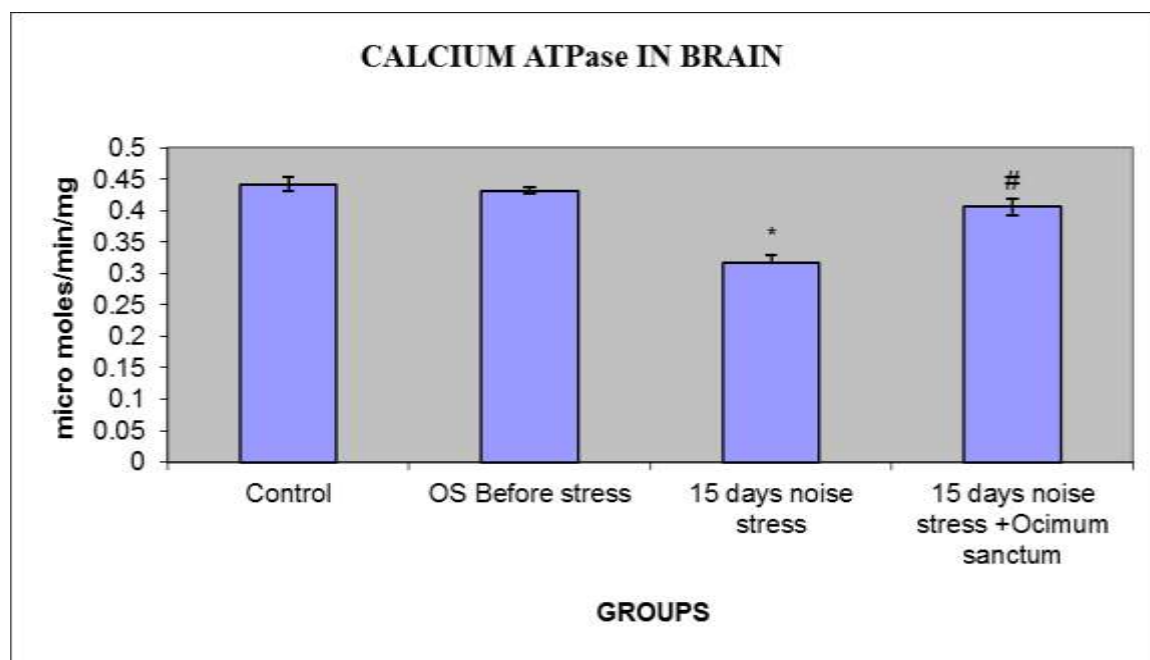

Fig: 1. Effect of OS on Ca^{2+}ATPase in Male Albino Rats Exposed to Noise-stress. Each column represents the mean ± S.D. of six animals. * compared with control; # compared with noise-stress. The symbols, *, # represent statistical significance at $p < 0.05$.

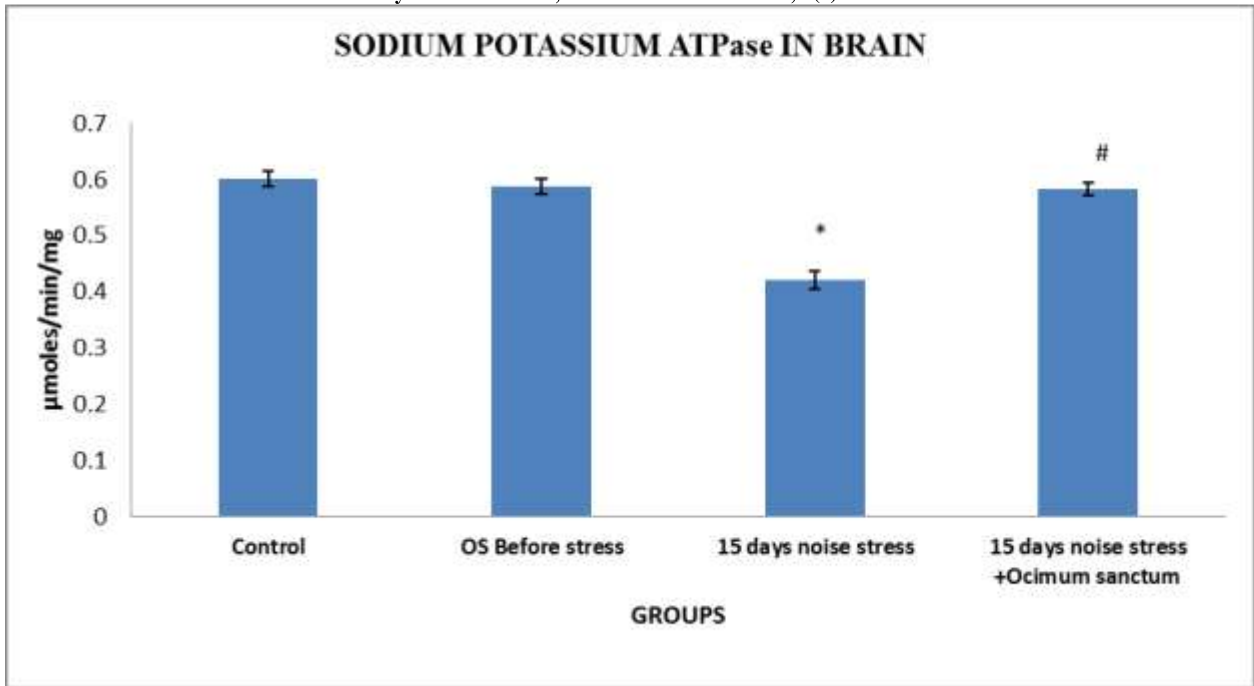

Fig: 2. Effect of OS on Na^{+}-K^{+}ATPase in Male Albino Rats Exposed to Noise-stress.
Each column represents the mean ± S.D. of six animals. * compared with control; # compared with noise-stress. The symbols, *, # represent statistical significance at $p < 0.05$.

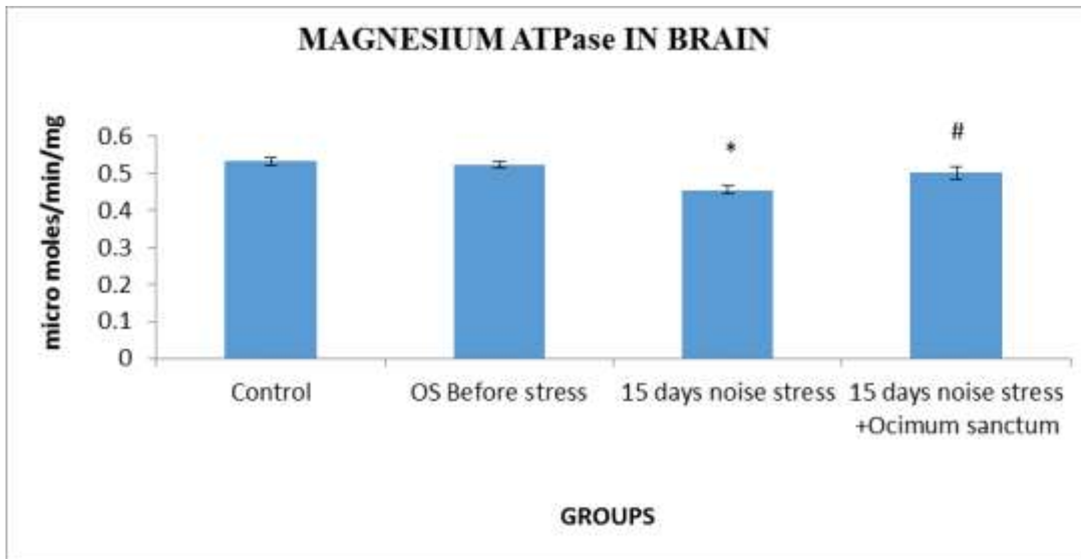

Fig: 3. Effect of OS on Mg^{2+}ATPase in Male Albino Rats Exposed to Noise-stress.
Each column represents the mean ± S.D. of six animals. * compared with control; # compared with noise-stress. The symbols, *, # represent statistical significance at $p < 0.05$.

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