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ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 6, Issue 04, pp. 1326-1330, April, 2015

RESEARCH ARTICLE

TO STUDY THE CHANGES IN NOISE STRESS INDUCED MEMBRANE BOUND ENZYMES AND EFFECT OF *TRIPHALA* IN BRAIN OF ALBINO RATS

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ARTICLE INFO

Article History:
Received 04th January, 2015
Received in revised form
07th February, 2015
Accepted 19th March, 2015
Published online 30th April, 2015

Key words:

Triphala,
Noise Stress,
Sodium Potassium,
Magnesium,
Calcium ATPases.

ABSTRACT

Introduction: A large number of people frequently exposed to unavoidable noise in urban areas, industries, household appliances in this modern world in our daily life. Noise is one of the globally faced environment stressor, which was proved affecting the psycho physiological problems, auditory defect, non-auditory disorder.

Objective: *Triphala*was selected for this study to understand its efficacy on the membrane bound enzymes of brain during noise exposure.

Materials and methods: In this study, wistar strain albino rats were subjected to the four groups, Control, *Triphala* (which is a combination of three fruits by name (*Terminalia chebula, Terminalia bellerica, Emblica officinalis*), Noise stress (100dB/4h/ 15 d) and noise stress+*Triphla* and incubated for 15 days. After 15 days of incubation to noise stress, estimation of Sodium Potassium, Magnesium and Calcium ATPases were evaluated to determine the changes in the membrane bound enzymes of brain.

Result and discussion: Administration of the *Triphala* had a normalizing action on membranes of the cell and controlled the alteration of membrane bound enzymes due to noise stress in noise stress+*Triphala* group.

Conclusion: Therefore treatment with *Triphala* prevented the decrease in the levels of membrane bound enzymes.

INTRODUCTION

Stress is defined as a condition in an organism that results from the action of one or more stressors that may be either external or internal origin (Beckman and Ames, 1998). Noise is one of the most widespread sources of environmental stress in living environment (Halliwell and Gutteridge, 1999). Noise can act as a non-specific stressor, inducing stress reactions which are in line with the general stress model (Owens et al., 1990). Noise pollution can be defined as intrusive noise that disrupts, distracts, or detracts from regular functioning. It also increases aggression and reduce the processing of social cues seen as irrelevant to task performance as well as leading to coronary heart disease, hypertension, higher blood pressure, increased mortality risk, serious psychological effects, headache, anxiety, and nausea (Abbate *et al.,* 2005). Formation of reactive oxygen species is associated with the development of many animal and plant pathological conditions as well as natural aging (Beckman and Ames, 1998; Halliwell and Gutteridge, 1999).

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Free radicals are terminated or neutralized, by nutrient antioxidants, enzymatic mechanism, or by recombining with each other. Endogenous sources of free radicals include those that are generated intra-cellular, acting within the cell, but are released into the surrounding area. Exogenous sources of free radicals introduced into the body may be due to irradiation, chemicals, air pollutants, stress and some medications. Activation of Hypothalamo-pituitary adrenal axis is the subsequent release of corticosterone from the adrenal cortex into blood for Physiological response to stress (Owens *et al*., 1990). Stress response has been reported to accelerate the generation of free radicals for the elevation of endogenous corticosterone (McIntosh and Sapolsky, 1996). *Triphala* has been reported to treat anemia, jaundice, constipation, cough, asthma, fever, eye diseases, chronic ulcers, leucorrhoea, pyorrhea (Nadkarni, 1976) and also assists in the weight loss. Anti-cancer (Tokura and Kagawa, 1995), anti-mutagenic (Rani, et al., 1994) anti-aging properties and improves the mental faculties (Nadkarni, 1976), antioxidant (Vaidya *et al.,* 1998). Antioxidants play a protective role against reactive oxygen species. Promote the immune functions and intellect and render longevity to life (Sharma and Dash, 1998). Hence, the search for natural antioxidants is essential. Therefore this

study to analyze the potential of *Triphala* proving the Pharmacological value of their efficiency as an antidote to stressor is 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 (24-26ºc) with 12 hour dark photo period (lights on from 6.00 to 18.00 hours).the rats were provided *ad libitum* access to food (Rat feed, Hindustan Lever Ltd.) and water. Proper IACE and well as CPCSEA approval was obtained before the commencement of the experiments.

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 *Triphala* (1g/kg body weight), Group 3 consisted of 15 days noise stress exposed animals (100dB for 4hrs/day) and Group 4 consisted of 15 days noise stress and also *Triphala* pretreated animals (1g/kg b.w. orally) to understand the effect of *Triphala* during stress exposure. After sub-acute (15days) 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, 4ºC) for estimation of enzymes.

Noise stress induction procedure

Noise become a stressor When the exposure limit exceeds 90 dB (Ramsey, 1982). Therefore the animals 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/day for 15 days. The control rats were kept in the corresponding period of time, without noise stimulation to evaluate the effect of stress.

Preparation of *Triphala*

Triphla fruits were purchased from Indian Medical Practitioners Co-operative Pharmacy & Stores Ltd, Chennai, India and authenticated by the Director of Centre for Advanced Studies in Botany, University of Madras, India (No.ARC/DO-NA/AUTHENTICATION/2004/1830). *riphala* consist of equal proportion of weighed powder from *Terminalia chebula, Terminalia bellerica, Emblica officinalis. Triphala* (1g/kg body weight) was dissolved in saline (2ml) and administered for rats.

Membrane bound enzymes

Na^+-K^+ATPase

The activity of Na^+-K^+ATPase [ATP: Phosphohydrolase - EC. 3.6.1.3.] in the tissue was estimated by Bernabe *et al*. (1973). 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 each NaCl, KCl, $MgSO_4$, EDTA and 0.1 ml ATP for 30 min at 37^{o}C. The reaction was arrested by the addition of 1.0 ml of 10% 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 µ moles 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 Hjerten and Pan (1983). 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 $CaCl_2$ and 0.1 ml ATP for 30 min at 37^{0}C. The reaction was arrested by the addition of 1.0 ml of 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 methods of Ohnishi *et al*. (1982). 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 $MgCl_2$ 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 µ moles of phosphorous liberated/min/mg protein.

RESULTS

to noise stress after pretreatment with *Triphala* showed a significant increase in the activity of Ca $^{2+}$ATPase, compared to noise exposed group of animals. Na^{+}-K^{+}ATPase values in the groups were studied summarized andgiven in figure: 2. Animals exposed to sub-acute noise stress for 15 days showed

Table 1.Effect of *Triphala* on Membrane bound enzymes Na^{+}-K^{+}ATPase, Ca^{2+}ATPase and Mg^{+}ATPases in Male Albino Rats Exposed to Noise –Stress.

Parameters	Control	*Triphala*	Sub-acute Noisestress	*Triphala*+Sub-acute Noise stress
Na^{+}-K^{+}ATPase (µmoles/min/mg)	0.6±0.013	0.582±0.011	0.42±0.015	0.584±0.01
Ca^{2+}ATPase (µmoles/min/mg)	0.442±0.011	0.431±0.012	0.316±0.013	0.405±0.01
Mg^{2+}ATPase (µmoles/min/mg)	0.533±0.01	0.53±0.01	0.455±0.011	0.515±0.017

Values are expressed as mean ± S.D of six animals. *compared with control. #compared with noise-stress. The *, #symbols represent statistical significance at p< 0.05.

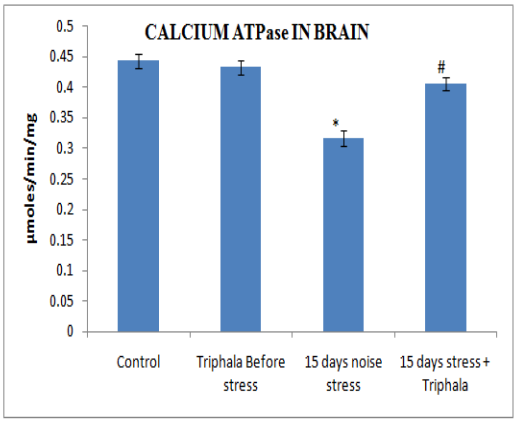

Fig 1. Effect of *Triphala* on Ca^{2+}ATPase in Male Albino Rats Exposed to Noise-stress.

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.

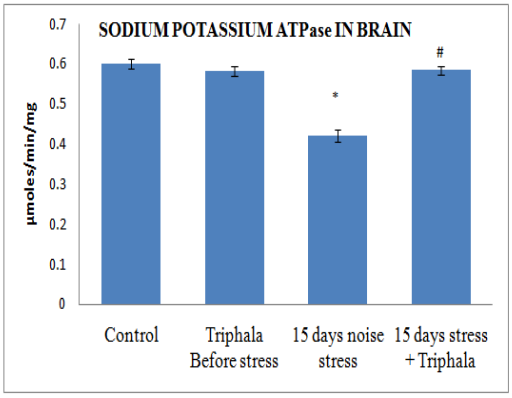

Fig 2. Effect of *Triphala* on Na^{+}-K^{+} ATPase in Male Albino Rats Exposed to Noise-stress.

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.

Ca^{2+}ATPasevalues in the groups were studied summarized and given in figure: 1. Animals exposed to sub- acute noise stress for 15 days showed a significant decrease in the activity of Ca^{2+}ATPase, compared to control animals. Animals exposed a significant decrease in the activity of Na^{+}-K^{+}ATPase, compared to control animals. Animals exposed to noise stress after pretreatment with *Triphala* showed a significant increase in the activity of Na^{+}-K^{+}ATPase, compared to noise exposed group of animals Mg^{2+}ATPase values in the groups were studied summarized and given in figure: 5. Animals exposed to sub-acute noise stress for 15 days 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 *Triphala* 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 *Triphala* 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.

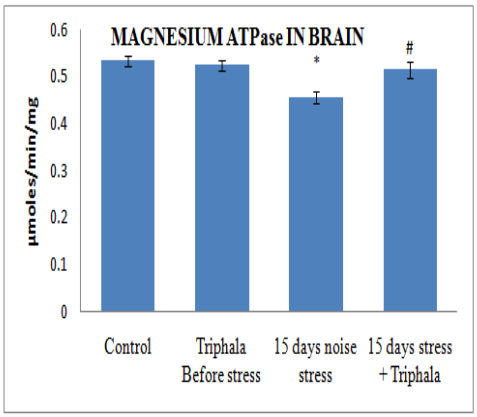

Fig 3. Effect of *Triphla* on Mg^{2+}ATPase in Male Albino Rats Exposed to Noise-stress.

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.

DISCUSSION

In this study, there are marked changes in the membrane bound enzymes with expose to noise. Brain acts as a key organ recognize and involve in interpreting and responding to potential stressors. Researchers suggested 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 (Suter, 1991). The brain activates several neuro-peptide-secreting systems response to stress. Chronic exposure to noise can cause hearing loss, annoyance, sleep disturbances and decreased performance in animals, including humans. (Passchier *et al.,* 2000; Berglund B *et al*., 1995). The brain regulates the entire process and instructing the rest of the body through release of hormones. Increase in the weight of adrenal glands in rats exposed to acute noise stress is reported by Jensen and Rasmussen, 1963. Adreno- corticotrophic hormone (ACTH) apart from increasing the secretory activity of adrenal cortex, could also cause hypertrophy and proliferation of adreno-cortical cells (Guyton, 1986). Acute as well as long-term exposure to noise can produce excessive free radicals such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) (Manikandan, *et al.,* 2005). Oxygen free radicals can attack protein, nucleic acids and lipid membranes thereby disrupting normal cellular functions and integrity (Endo *et al.,* 2005; Manikandan, *et al.,* 2005). Nervous system is relatively more susceptible to free radical damage (Scarfiotti, *et al.,* 1997). Ravindran et al. reported that neurotransmitters in discrete brain regions were found to be increased during noise stress even after 15 days of exposure (Ravindran *et al.,* 2005). In addition to generating free radical species, it also leads to increase in radical induced lipid per oxidation end products such as malondialdehyde (MDA) which is an indicator of lipid per oxidation processes (Derekoy, *et al.,* 2001). 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 (Paton et al., 1971). It was reported that catecholamines, Nor-Epinephrine (NE) and dopamine modify neuronal Na^+-K^+ATPase activity, respectively stimulate or inhibit the enzyme. There is evidence in the literature suggesting that inhibition of Na^+-K^+ATPase activity leads to neuronal death cause many pathological conditions in the central nervous system (Lees, 1993). Since this enzyme activity is highly susceptible to free radicals (Lees 1993), it may inhibit some metabolites via free radical generation. In this study there is a marked decrease in all the membrane bound enzymes 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 (Wang *et al*., 2003). Neuronal Nor-Epinephrine uptake arrests the activity of released Nor-Epinephrine, through a mechanism mediated by an active transport system dependent on Na^+-K^+ATPase activity (Paton, 1976). A free radical-induced decrease in Na^+-K^+ATPase activity has been documented in several studies (Rohn *et al*., 1996). The mechanism by which free radicals cause inhibition of the enzyme could be via per oxidation of the membrane. The dependence of Na^+- K^+ATPase activity on membrane fluidity is well known (Chong *et al*., 1985). Membrane lipid per oxidation result saltered in membrane fluidity (Richter 1987).

Conclusion

Triphala could prevent the changes by scavenging the free radical generated. There are reports that support the favorable actions during stress. *Triphala* could restore the antioxidant status and cell mediated immune response (Sri Kumar *et al.,* 2005). The noise-stress induced suppression in the neutrophil functions and increased corticosterone levels were significantly prevented by *Triphala* (Sri Kumar *et al*., 2005). Hence, it may be inferred that the observed changes after noise exposure is multi- factorial including free radical generation and alteration in the neurotransmitter level. Therefore Treatment with *Triphala* effectively prevented the decrease in levels of Na^+-K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase caused by repeated noise stress exposure.

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