Noise-Stress-Induced Brain Neurotransmitter Changes and the Effect of *Ocimum sanctum* (Linn) Treatment in Albino Rats

Rajan Ravindran¹, Rathinasamy Sheela Devi¹*, James Samson¹, and Manohar Senthilvelan¹

¹Department of Physiology, Dr. ALM. Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai – 600 113, India

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**Abstract.** In this modern world, stress and pollution are unavoidable phenomena affecting the body system at various levels. A large number of people are exposed to potentially hazardous noise levels in daily modern life, such as noise from work environments, urban traffic, and household appliances. A variety of studies have suggested an association between noise exposure and the occurrence of disorders involving extra-auditory organs such as disorders of the nervous, endocrine, and cardiovascular systems. In this study, Wistar strain albino rats were subjected to 100 dB broadband white noise, 4 h daily for 15 days. The high-pressure liquid chromatographic estimation of norepinephrine, epinephrine, dopamine, and serotonin in discrete regions of the rat brain indicates that noise stress can alter the brain biogenic amines after 15 days of stress exposure. *Ocimum sanctum* (OS), a medicinal herb that is widely claimed to posses antistressor activity and used extensively in the Indian system of medicine for a variety of disorders, was chosen for this study. Administration of the 70% ethanolic extract of OS had a normalizing action on discrete regions of brain and controlled the alteration in neurotransmitter levels due to noise stress, emphasizing the antistressor potential of this plant.

**Keywords:** noise stress, norepinephrine, dopamine, serotonin, *Ocimum sanctum*

**Introduction**

Stress has become an integral part of human life in this modern era. Among the innumerable stressors to which mankind is exposed, noise happens to be a commonly encountered stressor throughout the world. Even brief noise exposure is known to increase heart rate and peripheral vascular resistance, leading to a rise in blood pressure. On the other hand, experimental studies have demonstrated ultra structural modifications in rat cardiomyocytes mainly involving mitochondria. These subcellular alterations are related to an imbalance in calcium homeostasis, which is supposed to be sustained by increased catecholamine innervations (1).

Noise as a stressful stimulus is a widely accepted fact. However an effective agent to counter the noise-stress-induced biochemical alterations remains elusive. *Ocimum sanctum* (OS), a plant that is known as *Tulsi* in India and “Holy Basil” in English and known to have adaptogenic activity, was chosen for this study. Antipyretic effect (2), anti-arthritic activity, anti-inflammatory effect, and analgesic activity of this plant have been established. Even the antistressor activity of this plant has been studied (3 – 5). However there have been no studies on the effects of ethanolic extract of OS on noise-stress-induced catecholamine and indole amine changes in discrete brain regions like the cerebral cortex, cerebellum, hypothalamus, hippocampus, pons, and corpus striatum in rat. Therefore this study attempts to analyze the potential of OS as an effective antidote for noise stress.

**Materials and Methods**

Experimental animals were all healthy adult male albino rats of the Wistar strain, weighing 180 – 220 g. All the animals were maintained under standard laboratory conditions housed 3 per cage (29 cm × 22 cm × 14 cm) and were allowed free access to food and water. Appropriate ethical clearance was obtained for this work.
from the Institutional Animal Ethical Committee (IAEC no. 08/009/02 dated 27/12/02). All the animal experimentation involved in this work was done in accordance with national and institutional guidelines for the protection of animal welfare.

In order to avoid variations in the results due to circadian rhythm of biogenic amine levels and their metabolism, all the experiments involving the measurement of the catecholamine and indolamine levels were conducted between 8 a.m. and 10 a.m. After sub-acute noise exposure (15 days), the animals were sacrificed by cervical dislocation and the brains were removed quickly. Anesthesia was not used as it alters the brain amines. The experimental animals were divided into six groups and each group consists of six animals.

**Noise stress induction procedure**

When noise exposure of any kind exceeds 90 dB, noise becomes a stressor (6). Noise was produced by two loudspeakers (15 W), driven by a white-noise generator (0 – 26 kHz), and installed 30-cm above the cage. The noise level was set at 100 dB uniformly throughout the cage and monitored by a sound level meter D2023 (S.NO-F02199: Cygnet Systems, Gurgaon, Haryana, India). Each treated animal was exposed for 4 h/day for 15 days. To avoid the influence of handling-stress on evaluation of effects due to noise exposure, control rats were kept in the above-described cage during the corresponding period of time, without noise stimulation.

**OS pretreatment**

Fresh OS plants cultivated in the Center for Advanced Studies in Botany (CAS Botany) farm (University of Madras, Chennai, India) were collected. The plant was identified as *Ocimum sanctum* Linn, (Labiatae) and authenticated by Professor N. Anand, D.Sc., Director, CAS Botany (No. ARC/RR/2004/1091 September 2004). A herbarium of the specimen has also been deposited at the CAS Botany. The leaves were dried under shade, powdered, and the 70% ethanolic extract was prepared. The extract was concentrated under shade, powdered, and the 70% ethanolic extract deposited at the CAS Botany. The leaves were dried 2004). A herbarium of the specimen has also been authenticated by Professor N. Anand, D.Sc., Director, CAS Botany. The leaves were dried and powdered. The 70% ethanolic extract was prepared and concentrated under shade, powdered, and the 70% ethanolic extract deposited at the CAS Botany. The leaves were dried 2004). A herbarium of the specimen has also been authenticated by Professor N. Anand, D.Sc., Director, CAS Botany.

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The main components of OS are tannins (4.6%) and essential oil (up to 2%) eugenol (up to 42%), methylchavicol, linalool and 1,8-cineole as determined by gas liquid chromatography (7).

The animals were divided into six groups. Group 1 consisted of control animals, and this group was used for studying the base line values of the parameters studied. Group 2 consisted of noise-stressed animals. These animals were subjected to noise stress, 4 h/day for 15 days, and sacrificed on the 16th day. This group was used to study the effect of noise stress on the central neurotransmitter levels. Group 3 consisted of noise-stressed + OS-treated animals. These animals were pretreated with OS for 15 days and then exposed to noise stress for 15 days. During the noise stress period, they were also given OS extract by the i.p. route. The animals were sacrificed on the next day for neurotransmitters estimation. This group was used to study the effect of OS treatment in noise stress. Group 4 consisted of noise-stressed + PG-treated animals and this group was used to confirm that PG does not interfere with the OS activity. The remaining two groups consisted of control rats pretreated with OS for 15 days and control rats pretreated with PG for 15 days to rule out the action of OS and PG in the absence of noise stress.

**Determination of neurotransmitter concentrations**

The various brain biogenic amines in discrete regions of the rat brain were estimated by the method of Wagner et al. (8). The rats were sacrificed by cervical dislocation. After sacrifice, the brain was rapidly removed, and the cerebral cortex, cerebellum, hypothalamus, hippocampus, pons, and corpus striatum were dissected on an ice-cold plate (9). Concentrations of norepinephrine (NE), epinephrine (E), dopamine (DA), and 5-hydroxytryptamine (serotonin, 5-HT) were measured by high performance liquid chromatography (HPLC) coupled with electrochemical detection (ECD). All brain regions were homogenized with perchloric acid. The mobile phase contains citric acid, di sodium hydrogen orthophosphate, EDTA, octane-1-sulphonic acid sodium salt, and 14% methanol and was adjusted to the pH of 4.0 using di sodium hydrogen orthophosphate. Homogenates were centrifuged (12,000 rpm, 4°C) in a refrigerated centrifuge for 2 min, and then the internal standard dihydroxybenzylamine (DHBA) was added to the supernatant of brain homogenate and again centrifuged at 12,000 rpm for 20 min. The supernatant was filtered with 0.22-µm membrane filter, and 20 µl of sample was injected into the Rheodyne injector (Cotati, CA, USA) of the HPLC system, which is connected to an isocratic pump (Model 501; Waters Association, Millipore, MA, USA) and reverse phase column LiChroCART RP-18, for separation of indole amines and catecholamines. The reaction products were detected with an electrochemical detector (Model 460, Waters), which was coupled to the HPLC system and set at a potential of +0.60 V for the detection of monoamine.
neurotransmitters. The flow rate was maintained at 0.8 ml/min. Neurotransmitters were quantified using a C-R8A data processor (Shimadzu, Kyoto) and expressed as nanograms of neurotransmitter per gram of wet weight of brain tissue.

All the data were statistically analyzed by one-way analysis of variance (ANOVA). When the F test ratio was significant, the inter group differences were evaluated by Tukey’s multiple comparison test, and the significance level was fixed at $P < 0.05$. Baseline data were statistically analyzed by the unpaired Student’s t-test with vehicle control and OS-alone-treated groups.

**Results**

The NE levels in the groups studied are summarized in the Fig. 1. In all the brain regions, noise stress significantly increased the NE levels from the control ($df = 3, 23$). In all the brain regions, the OS-treated group showed significant decrease in NE levels compared to stress values ($*P < 0.05$). This indicates OS treatment attenuates the altered norepinephrine levels.

The E levels in the groups studied are summarized in Fig. 2. In all regions, sub-acute stress significantly increased the E levels from the control ($df = 3, 23$). In all regions, the treated group showed significant decrease in E levels compared to the stress values.

The DA levels in the groups studied are summarized in Fig. 3. In all the regions, sub-acute stress significantly increased the DA levels from control except pons ($df = 3, 23$). In all the regions, OS-treated group showed significant decrease in DA levels from stress values except pons and corpus striatum.

The 5-HT levels in the groups studied are summarized in the Fig. 4. In cerebellum, hypothalamus, pons and corpus striatum sub-acute stress significantly increased the 5-HT levels from control ($df = 3, 23$). In cerebellum, hippocampus and pons treated group showed significant decrease in 5-HT levels from stress values.

The results in the control and OS-treated animals are summarized in the Table 1. Vehicle (PG) and OS-alone-treated animals did not show any significant change from the control in NE, E, DA, and 5-HT levels in all the regions of the rat brain. At the same time, vehicle (PG) and control group also did not show any significant change from the control in all the regions of the rat brain.

**Discussion**

Stress is an unavoidable phenomenon that affects the body system at various levels. Any stress can induce three major changes: 1) exogenous stresses or endogenous stress initiating a disturbance in the body, 2) the physical and chemical disturbances induced by the stressor in the body, and 3) the body counteracts these responses. This third stage is quite variable from stressor to stressor and also from individual to individual, depending on whether they take stress as a positive one or negative one.
The effect of sub-acute noise stress on discrete brain regions of neurotransmitter level has not been reported earlier. In this study, since neurotransmitters in discrete brain regions were found to be increased during stress and the OS was found to prevent noise-stress-induced changes, it may be possible that the OS may have a normalizing effect on the levels of these neurotransmitters in discrete brain regions. Since the auditory input shows adaptation in a noisy environment, many people believe that it is not going to affect the body system like heat stress, cold exposure, etc. However, this study reveals that auditory stress persists within the body to induce changes. This study also confirms that noise stress can alter the brain biogenic amine levels even after
15 days of stress exposure. According to Pignatelli et al. (10), in rats under the influence of a noise stress, the adrenal function and the entire hypothalamic-pituitary-adrenal (HPA) axis were severely affected, resulting in the elevation of brain biogenic amines. Many workers have also reported the increased brain levels of E and NE in response to various stressors like isolation-rearing and immobilization stress (11).

Various workers have given different opinions about the changes in the levels of brain monoamines and indolamines in stress. However, the stressor-induced changes are reported with controversies. Various stressors like noise stress and immobilization stress have been reported to decrease (12) as well as to increase brain neurotransmitters levels. Some scientists have been reported no alteration in the biogenic amine levels (13). This may be due to the nature of the stressor, duration of the stress, and the animal used in their experiments.

The anti stressor activity of OS has been documented in different kinds of stress (3). The considerable increase in the neurotransmitter level in all the brain regions after noise exposure in this study is well consistent with other reports. The neurotransmitter concentration increases in certain brain regions during sub-acute noise stress exposure (14). Such an increase appears to be an active process. Particularly in the hypothalamus and striatum, the response of the neurotransmitter to sub-acute noise stress is more profound. Exposure of animals to noise stress for different durations markedly and rapidly increases the concentration of neurotransmitter in the hypothalamus and striatum (15).

Due to stress, consistent increases of DA are observed in the cerebral cortex, cerebellum, hypothalamus, hippocampus, and corpus striatum, except for the pons. According to Singh et al. (16), DA levels in the brain
elevate as a compensatory mechanism and as a precursor for synthesis of more E and NE to cope with the increased demand. The antistress drugs facilitate this compensatory mechanism. The higher 5-HT level in the hypothalamus than that in the pons under the control condition may be due to the handling of the control animals for the period of 15 days prior to the experiment, which might have altered the emotional response resulting in elevated 5-HT levels in the hypothalamus. The results of this study indicated that sub-acute exposure to noise induces an enhancement in striatal DA activity, as confirmed by the increased levels of DOPA in the stressed groups. Earlier reports also indicate that the noise stress decreases the striatal 3,4-dihydroxyphenylacetic acid (DOPAC) levels (a metabolite of dopamine), with the increase in DA levels in rats indicating a decrease in the catabolism of DOPA. The increase in the striatal DOPA accumulation after noise stress may be due to the elevated tyrosine hydroxylase activity resulting in an increase of 5-HT levels. The elevation of 5-HT in the hypothalamus during stress was consistent with the hypothesis that 5-HT is involved in HPA axis activation during noise stress (4). The 5-HT level of brain has also been reported to increase after swim stress (25). Another report (26) found that an acute or repetitive exposure to noise activates tryptophan hydroxylase in the cerebral cortex and midbrain. Since tryptophan hydroxylase is the enzyme that converts tryptophan to serotonin, it is likely that noise stress increases the activity of tryptophan hydroxylase, leading to increased 5-HT levels.

Delini-Stula et al. (17) administered haloperidol and sulpiride, D₂-receptor antagonists, along with OS extract and found that they blocked the effect of the OS, suggesting a possible D₂-receptor mechanism for OS extract. Bromocryptine, a potent D₂-receptor agonist, reduced the immobility duration, and when combined with OS, there was an apparent additive action. This indicates similar dopaminergic activation, and OS possesses an interesting profile of central nervous system activity, which may involve dopaminergic neurons.

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In this study, after OS extract pretreatment, the increase of 5-HT was prevented significantly. The altered neurotransmitter level did not return to the normal level in any of the regions, which indicates the need for different dosage testing.

It is clear that OS acts as an antistressor not only for a specific type of stressors like noise stress but also to many other stressors. However, the mechanism of action is very difficult to understand as its action seems to be nonspecific. However, we can reasonably hypothesize that OS may contain some substance that can block the afferent input so that the induction of alterations in the body is blocked. For a better understanding of this idea, it can be compared with the pain gate theory where the
endogenous endorphins block the sensory input. Probably, the analgesic substance in OS blocks the sensory input. These aspects require in depth studies to clarify them.

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