Biomedicine & Pharmacotherapy 86 (2017) 475–481

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Original article

# Role of *Scoparia dulcis* linn on noise-induced nitric oxide synthase (NOS) expression and neurotransmitter assessment on motor function in Wistar albino rats

Wankupar Wankhar, Sakthivel Srinivasan, Loganathan Sundareswaran, Dapkupar Wankhar, Ravindran Rajan, Rathinasamy Sheeladevi*

*Department of Physiology, Dr. ALM PG Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 600 113, India*

ARTICLE INFO

*Article history:*
Received 28 October 2016
Received in revised form 9 December 2016
Accepted 14 December 2016

*Keywords:*
Noise
Rota rod
Narrow beam
Motor co-ordinations
Monoamine
Purkinje cells

ABSTRACT

Noise pollution is one of the most widespread and fast growing environmental and occupational menaces in the modern era. Exposure to noise above 100 dB is not adaptable through the brain homeostatic mechanism. Yet, the detrimental effects of noise have often been ignored. Developing reliable animal models to understand the neurobiology of noise stress and advance our research in the field of medicine to impede this growing stressor is needed. In this study experimental animals were divided into four groups, (i) Control and (ii) *S. dulcis* extract (200 mg/kg b w) treated control group. (iii) To mimic the influence of noise, animals in this group were exposed to noise stress (100 dB/4 h/day) for 15 days and finally, (iv) Noise exposed treated with *S. dulcis* extract (200 mg/kg b w) group. Rota-rod and narrow beam performance results showed impaired motor co-ordination in noise exposed group on both 1st and 15th day when compared to controls. This impaired motor function on exposure to noise could be attributed to the altered norepinephrine, dopamine and serotonin levels in both the striatum and cerebellum. Moreover, the motor impaired associated changes could also be attributed to upregulated nNOS and iNOS protein expression in the cerebellum resulting in increased nitric oxide radical production. This increased reactive free radicals species can initiate lipid peroxidation mediated changes in the cerebellar Purkinje cells, which is responsible for initiating inhibitory motor response and ultimately leading to impaired motor co-ordination.

Treatment with *S. dulcis* extract (200 mg/kg b w) could control motor impairment and regulate neurotransmitter level as that of control groups when compared to noise exposed group. One key aspect of therapeutic efficacy of the plant could have resulted due to attenuated lipid peroxidation mediated damages on the cerebellar Purkinje cells thereby regulating motor impairment. Thus, targeting the antioxidant and free radicals scavenging properties of the plant could serve as a potential therapeutic to combat this environmental stressor.



## 1. Introduction

The sound that is generally loud or unpleasant, unexpected or undesired is often termed as noise. Noise is gradually becoming the most pervasive environmental and occupational stress pollutant in a modern era. It is known that brain recognize and discriminates the level of stress by showing adaptive plasticity through possible mechanisms of hypothalamic-pituitary-adrenal and sympathoadrenal medullary (HPA/SAM) axis where neurotransmitters and systemic hormones interact to produce structural as well as functional changes [1]. However, in-spite of these adaptive homeostatic system, exposure to noise above 100 dB is not adaptable through the brain physiological homeostatic mechanism [2]. Sakthivel et al. [3] reported the influence of free radical and altered antioxidant activity on noise-induced lipid peroxidation in the brain. Neurons are especially vulnerable to free radical mediated damage caused by lipid peroxidation, with the subsequent formation of end products like 4-hydroxynonenal [4] and not very efficient antioxidant defense system [5]. Recent findings by Wankhar et al., [6] have reported that altered cytokine secretion especially increase inflammatory cytokine on exposure

* Corresponding author at: Department of Physiology, Dr. ALM.PG. Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 600 113, Tamilnadu, India.
*E-mail address:* drsheeladeviibms@gmail.com (R. Sheeladevi).

http://dx.doi.org/10.1016/j.biopha.2016.12.054
0753-3322/

to noise could have resulted in neuroinflammation with reactive oxygen/nitrogen species (RONS) playing a major role in eliciting neuronal DNA smear pattern in the brain. The detrimental effect of noise is evident, at least in general terms, this would readily account for the maladaptive effects of noise stress and enhance the susceptibility to RONS-induced neurobehavioral and neurodegenerative disease. Repeated noise stress exposure has also been reported to alter stress hormones [7], produces metabolic and anatomical changes in neurons, reduced dendritic count, impaired memory, cognition [8,9]. Psychiatric disorders such as depression, anxiety, annoyance, locomotory behaviour etc [10,11] and the onset of developing Alzheimer disease [12] has also been reported. In line with these findings, evidence also suggests that stress adversely affects the motor function in both humans [13] and rodents [14]. Nonetheless, reports on whether cerebellum and striatum centres responsible for performing motor co-ordination can succumb to the adverse effects of noise stress are yet to be studied.

In-spite of advance research in the field of medicine a remedy for this fast growing environmental/occupational stressor is limited. A number of data have revealed that antioxidant-mediated therapy on oxidative/nitrosative stress-induced damages has provided a commendable result. Plant based herbal therapy has been practised in Indian traditional system of herbal medicine for years. The plant of our interest *Scoparia dulcis*, traditionally has been used as one of the remedies for stomach troubles, hypertension, diabetes, inflammation, bronchitis, hemorrhoids, hepatosis, analgesic, antipyretic, antiviral, emmenagogue, anti-inflammatory, cytotoxic, anti-septic, emollient, febrifuge, anti-diarrhoeal, anti-spasmodic, hepatoprotective, [15–18] neuroprotective and anticholinergic [19]. In addition, literature survey also supports the therapeutic effects of *S. dulcis* extract against innumerable oxidative stress associated disease [20,21]. HPTLC analysis established a class of compounds that include terpenoids, flavonoids, stilbenes, phenolic compounds and proanthocyanidins [22] which are known to possess potent antioxidant activity and free radical scavenging activity. Thus, the present study was aimed at investigating the possible involvement of noise-induced oxidative/nitrosative stress on motor functioning and also the search of the herbal remedy to combat this environmental stressor.

## 2. Materials and method

### 2.1. Chemicals

Primary antibodies were purchased from Sigma Aldrich, USA and the secondary antibodies were purchased from (Merck, India). DAB system was purchased from Pierce, USA. The antibody was purchased from Pierce, USA. Norepinephrine (NE), dopamine (DA), 5-hydroxytryptamine (serotonin 5-HT) and dihydroxybenezylamine (DHBA) standards were purchased from Sigma-Aldrich. All other chemicals were of analytical grade obtained from Sisco research Laboratory, Bombay, India.

### 2.2. Extraction of plant extract

The plant *Scoparia dulcis* purchased from Indian Medical Practitioners Co-operative Pharmacy and Stores (IMPCOPS), Chennai and was authenticated by Dr D. Aravind, Department of Medicinal Botany. Voucher specimens have been deposited at the herbarium of National Institute of Siddha, Reg no NIS/MB/62/2012. 500 g of *S. dulcis* leaves were extracted with 1.5 l of sterile distilled water using the Soxhlet apparatus at 60 °C. The extract was then filtered through Whatman No 1 filter paper and then freeze dried stored at 4 °C for further investigation.

### 2.3. Animals

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. 01/20/2013 dated 20/02/2013). All the animal experimentation involved in this work was done in accordance with national and institutional guidelines for the protection of animal welfare.

### 2.4. Experimental groups

Animals were randomly divided into 4 groups and each group consisted of 6 animals. Group I animals were administrated with saline (0.9%) orally for 48 days and Group II animals were subjected to noise stress for 15 days (100 dB/4 h/day) and noise stress induced changes were observed in this group. Group III animals were administrated with aqueous extract of *S. dulcis* alone (200 mg/kg b w) according to Latha et al. [23] for 48 days. Group IV animals were treated with aqueous extract of *S. dulcis* (200 mg/kg.b.w) for 32 days and were further subjected to noise stress for 15 days from 33rd day onwards along with *S. dulcis* treatment. In order to avoid variations in the results due to circadian rhythm and their metabolism, all the experiments were conducted between 8 am to 10 am.

### 2.5. Noise stress induction procedure

When noise exposure exceeds 100 dBA, noise becomes a stressor [14]. Noise was produced by two loudspeakers (15W), 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). Animal were then exposed for 4 h/day for 15 days. To avoid the influence of handling stress on evaluation the effects of noise exposure in control rats, animals were kept in the above-described cage during the corresponding period of time, without noise stimulation.

### 2.6. Assessment of motor co-ordination

Motor co-ordination was assessed using the conventional rota rod test according to Dunham and Miya [24], and narrow beam-walk was performed according to Kolb and Whishaw [25].

### 2.7. 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. [26]. The rats were sacrificed by cervical dislocation. After sacrifice, the brain was rapidly removed and the two regions responsible for motor co-ordination, the striatum and cerebellum were dissected on an ice-cold plate [27]. Concentrations of norepinephrine (NE), dopamine (DA), and 5-hydroxytryptamine (serotonin, 5-HT) were measured using high performance liquid chromatography (HPLC) coupled with electrochemical detection (ECD). Brain regions were homogenized with Perchloric acid. The mobile phase contains citric acid, disodium hydrogen orthophosphate, EDTA, octane-1-sulphonic acid sodium salt, 14% methanol and was adjusted to the pH of 4.0 using disodium hydrogen orthophosphate. Homogenates were centrifuged (12,000 rpm, 4 °C) in a refrigerated centrifuge for 2 min, and then the internal standard dihydroxybenezylamine (DHBA) was added to the supernatant of brain homogenate and again centrifuged at 12,000 rpm for 20 min. The supernatant was