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Original Article

Antioxidant mediated response of *Scoparia dulcis* in noise-induced redox imbalance and immunohistochemical changes in rat brain

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Abstract

Noise has been regarded as an environmental/occupational stressor that causes damages to both auditory and non-auditory organs. Prolonged exposure to these mediators of stress has often resulted in detrimental effect, where oxidative/nitrosative stress plays a major role. Hence, it would be appropriate to examine the possible role of free radicals in brain discrete regions and the "antioxidants" mediated response of *S. dulcis*. Animals were subjected to noise stress for 15 days (100 dB/4 hours/day) and estimation of endogenous free radical and antioxidant activity were carried out on brain discrete regions (the cerebral cortex, cerebellum, brainstem, striatum, hippocampus and hypothalamus). The result showed that exposure to noise could alleviate endogenous free radical generation and altered antioxidant status in brain discrete regions when compared to that of the control groups. This alleviated free radical generation (H_2O_2 and NO) is well supported by an upregulated protein expression on immunohistochemistry of both iNOS and nNOS in the cerebral cortex on exposure to noise stress. These findings suggest that increased free radical generation and altered anti-oxidative status can cause redox imbalance in the brain discrete regions. However, free radical scavenging activity of the plant was evident as the noise exposed group treated with *S. dulcis*[200 mg/(kg ·b·w)] displayed a therapeutic effect by decreasing the free radical level and regulate the anti-oxidative status to that of control animals. Hence, it can be concluded that the efficacy of *S. dulcis* could be attributed to its free radical scavenging activity and anti-oxidative property.

Keywords: free radicals, oxidative stress, *S. dulcis*, nitric oxide synthase, nitric oxide, hydrogen peroxide

Introduction

With rapid industrialization and urbanization in the modern society, noise has become one of the most widespread sources of environmental stressors in the living environments. Though the detrimental effect of noise is evident, the deleterious effect of noise is not as catastrophic and sudden as any other environmental pollution. Hence, it has often been overlooked as a potential threat. Exposure to noise exceeding 100 dB is regarded as a stressor[1]. In addition to the auditory loss, noise exposure may lead to detrimental effects that can interfere with body functioning and activity[2]. Though it is appreciable that the brain manages to overcome the

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stress situation, the physiologic stress response has often resulted in the release of hormonal mediators where they serve as the brain's alarm signals by showing adaptive plasticity as well as structural and functional changes[3]. However, prolonged exposure to these mediators of stress has often resulted in detrimental effect, where oxidative stress plays a major role[4].

Literature survey has revealed that noise can alter neurotransmitter levels[5], cause metabolic and anatomical changes in neurons, and reduce dendritic count, impaired memory and cognition[6]. Excessive corticosterone level and altered antioxidant enzyme status have often been regarded as the reason behind redox imbalance in the cell. Deficiencies in the antioxidant defense system impair protection against reactive oxygen/nitrogen species (ROS/RNS) leading to damage in vulnerable targets such as unsaturated fatty acyl chains in the membrane, proteins and DNA[7]. Of all the organs, neural tissue takes more than its share of oxidative abuse owing to the fact that neurons utilize 20% of the oxygen consumed by the body but constitute only 2% of the body weight, indicating its potential in generating ROS during oxidative phosphorylation. Furthermore, iron is particularly abundant in the brain; although iron is required for oxidative metabolism, it can also be toxic, due to its ability to catalyze ROS generation. In addition, neural tissues are rich in unsaturated fatty acids, making them vulnerable to lipid peroxidation and also contributing to the chain reaction of generating highly reactive cytotoxic molecules. Neural tissues also have low antioxidant enzyme levels and are not able to synthesize glutathione, a fundamental component of antioxidant machinery. Lastly, loss of neurons in the adult brain cannot be generally compensated by generation of new neurons[8]. However, the effects of stress on the brain do not necessarily constitute permanent "damage" per se and are amenable to recovery through preventative strategies and interventions that include pharmaceutical agents. Antioxidants are vital to combat oxidative/nitrosative damage as they can interfere with the oxidation process by chelating free catalytic metals and also acting as free radical scavengers. With growing interest in indigenous herbal plants for their therapeutic efficacy, various efforts are now concentrated in documenting herbal plants as a potential source of modern medicine.

S. dulcis belongs to the family *Scrophulariaceae,* which is known as a folk-medicinal plant, commonly known as sweet broom weed, and is fast becoming a medicinal plant of growing global interest. A number of speculated medicinal properties of *S. dulcis* has been validated by scientific research. Traditionally, the extracts of the plant have been shown to have anti-hyperglycemic, anti-inflammatory, and antioxidant capacity in vivo[9-10]. Its impact on lipid peroxidation[11] has also been reported. Phytochemical investigations on *S. dulcis* have shown that it contains steroids, diterpenoids, triterpenoids, flavonoids, benzenoids[12-13] and acetylated flavonoid glycosides[14]. It is believed that the pharmacological actions of *S. dulcis* are due to the presence of these phytochemicals in the plant.

Materials and methods

Chemicals

Primary antibodies were purchased from Sigma-Aldrich, USA and secondary antibodies were purchased from Merck Millipore, Bengaluru, India. DAB system was purchased from Pierce, USA. All other chemicals used were of analytical grade and obtained from Sisco Research Laboratory, Mumbai, India.

Identification and extraction of plant extract

The plant *S. dulcis* was purchased from IMPCOPS, Chennai and 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. A total of 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 stored at 4°C by freeze-dried for further investigation.

Animals

Healthy adult male albino rats of the Wistar strain weighing 180-220 g were maintained under standard laboratory conditions 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 studies were carried out in accordance with the established institutional guidelines regarding animal care and use. Animal welfare and the experimental procedures were carried out strictly in accordance with the Guide for Care and Use of Laboratory Animals (National Research Council of USA, 1996).

Noise stress induction procedure

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). Each rat was exposed for 4 hours/day for 15 days. To avoid the influence of handling-stress on evaluating the effects of noise exposure, the control rats were also kept in the above described cage during the corresponding period of time, without noise stimulation

Experimental groups

The rats were randomly divided into 4 groups and each group consisted of 6 rats. Aqueous extract of *Scoparia dulcis* (*S. dulcis*) was used for this study. Group Ⅰ rats were administered with saline (0.9%) orally for 48 days. Group Ⅱ rats were subjected to noise stress for 15 days (100 dB/4 hours/day) and noise stress induced changes were observed in this group. Group Ⅲ rats were administered with aqueous extract of *S. dulcis* o*rally* [200 mg/(kg·b·w)] according to Latha *et al.*[15] for 48 days. Group Ⅳ rats 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 day 33 onwards along with *S. dulcis* treatment.

To avoid variations in the results due to circadian rhythm, all the experiments were conducted between 8:00 and 10:00 am. After the experimental procedure, rats were anesthetized with ketamine/xylazine [90/50 mg/(kg·b·w)] and tissue samples were collected.

Brain dissection and biochemical determinations

The brain was immediately removed and washed with ice-cold phosphate-buffered saline (PBS). To expose the brain, the tip of curved scissors was inserted into the foramen magnum and a single lateral cut was made into the skull, extending forward on the left and right side. With a bone cutter, the dorsal portion of the cranium was peeled off and using a blunt forceps, the brain was dropped onto the ice-cold glass plate, leaving the olfactory bulbs behind. The whole process of removing the brain took less than 2 minutes. After removing the brain, it was blotted and chilled. Further dissection was made on the ice-cold glass plate. The discrete regions of the brain (the cerebral cortex, cerebellum, brainstem, striatum, hippocampus and hypothalamus) were dissected according to the method by Glowinski and Iverson[16]. The homogenates (10% w/v) of individual regions were prepared in a Teflon-glass tissue homogenizer, using ice-cold Tris-HCl (100 mmol/L, pH 7.4) buffer [only for superoxide dismutase (SOD)] and ice-cold PBS, centrifuged separately in the refrigerated centrifuge at 3,000 *g* for 15 minutes.

Stress markers like lipid peroxidation were determined as described by Ohkawa *et al.*[17] and protein thiol was estimated as per Sedlack and Lindsay[18]. Free radicals like nitric oxide (NO) levels were measured as total nitrite + nitrate levels with the use of the Griess reagent by the method of Moshage *et al.*[19] and hydrogen peroxide (H_2O_2) levels were determined as per Pick andKeisari[20]. Biochemical determination of enzymatic antioxidants like superoxide dismutase (SOD) was estimated according to Marklund and Marklund[21] and catalase (CAT) according to the method of Sinha[22]. The activity of glutathione peroxidase (GPx) was estimated as described by Rotruck *et al.*[23], whereas glutathione-S-transferase (GST) was estimated by the method of Habig *et al.*[24]. Glutathione reductase (GR) that utilizes NADPH to convert metabolized glutathione (GSSG) to the reduced form was assayed by the method of Horn and Burns[25]. Biochemical estimation of non-enzymatic antioxidants like reduced glutathione (GSH) was estimated by the method of Moron *et al.*[26]. Vitamin C and vitamin E

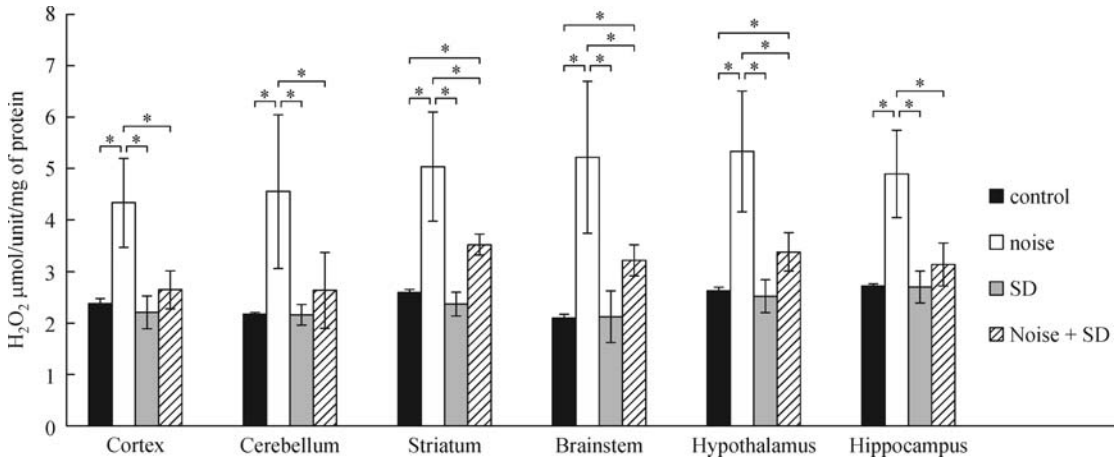

Fig. 1 **Effect of *S. dulcis* on H_2O_2 level in rat brain discrete regions after 15 days of noise (100 dB /4 hours/day).** Values are expressed as mean±SD, N = 6 and $P \leqslant 0.05$, SD: *S. dulcis*.

contents were determined according to Omaye *et al.*[27] and Desai[28].

Histology

Rats were anesthetized with ketamine/xylazine [90/50 mg/(kg·b·w)] and then perfused transcardially with PBS, followed by buffered 10% formalin. The brain was then removed and preserved in formalin until processed for histology. On processing, the tissues were kept on running water to remove formalin pigments and dehydrated with gradient alcohol. After impregnation with paraffin wax, the paraffin blocks were made. Sections were cut with 7-10 μm in thickness using Spencer Lens, rotatory microtome (No. 820, Newyork, USA) and then stained with hematoxylin and eosin.

Immunohistochemical analysis

Immunohistochemical analysis was carried out as per Bancroft and Cook[29] using the DAB universal staining kit. The sections were deparaffinized in xylene and dehydrated in ethanol. After washing with PBS, slides were incubated with 3% H_2O_2 in at room temperature for 15 minutes to quench endogenous peroxidase activity. After antigen retrieval (15 minutes at 90°C in 10 mmol/L citrate buffer, pH 6.0), the slides were incubated with blocking solution (10% normal goat serum) for 5 minutes at room temperature. Then, the sections were incubated overnight with primary antibody. Subsequently, the sections were incubated with HRP-conjugated secondary antibody for 30 minutes at room temperature, washed with PBS, followed by treatment with secondary antibody for 30 minutes at 37°C and washed. Then, the sections were treated with DAB chromogen for 15 minutes. Finally, the sections were washed with deionised water, counterstained with hematoxylin and mounted. Photographs were taken using Nikon microscope (Japan).

Statistical analysis

Statistical analysis was carried out using the SPSS statistical package version 17.0 (SPSS Inc., Chicago, IL, USA). The results were expressed as mean±SD and the data were analyzed by one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison tests when there was a significant "F" test ratio. The level of significance was set at $P \leqslant 0.05$.

RESULTS

S. *dulcis* attenuates noise-induced upregulation of free radicals and nitric oxide synthase (NOS)

Increased levels of H_2O_2 (***Fig. 1***) and NO (***Fig. 2***) were found in brain discrete regions (cerebral cortex, cerebellum, striatum, brainstem, hippocampus and hypothalamus) in Group Ⅱ when compared to Group Ⅰ and Group Ⅲ animals. Group Ⅱ animals showed a significant higher level of H_2O_2 and NO in brain discrete regions when compared to Group Ⅰ and Group Ⅲ. However, the brainstem, striatum and hypothalamus showed a significant decrease in H_2O_2 level in Group Ⅳ but significantly elevated from the Group Ⅰ and Group Ⅲ. No significant changes in the level of free radicals and NOS expression were observed in Group Ⅰ and Group Ⅲ. The plant free radical scavenging activity was appreciable as it was able to scavenge the radical species in a majority of regions. Immunohistochemical studies in the brain showed increased positive cell expression in nNOS (***Fig. 3***) and iNOS (***Fig. 4***) indicating upregu-

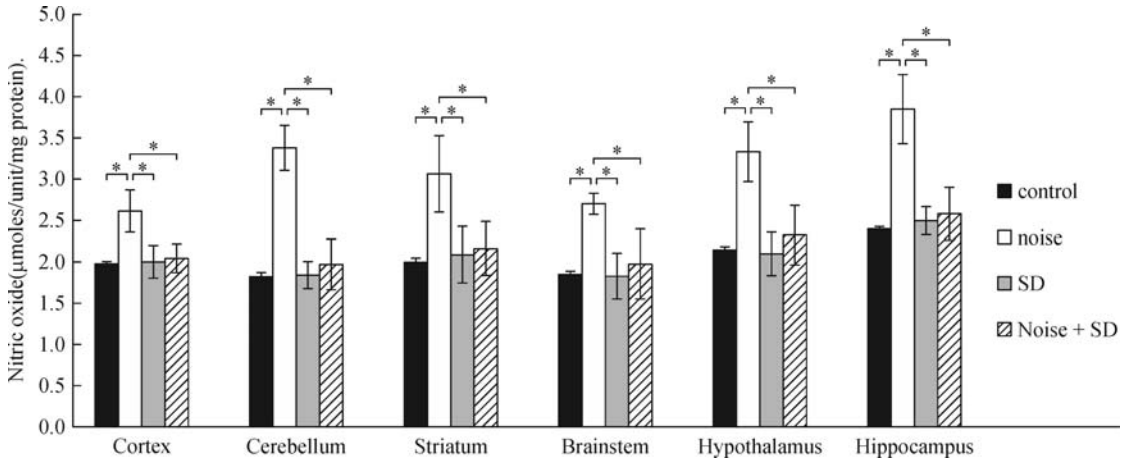

Fig. 2 **Effect of *S. dulcis* on nitric oxide level in rat brain discrete regions after 15 days of noise (100 dB/4 hours/day).** Values are expressed as mean±standard deviation, $n = 6$. The symbols represent statistical significance: $P < 0.05$.

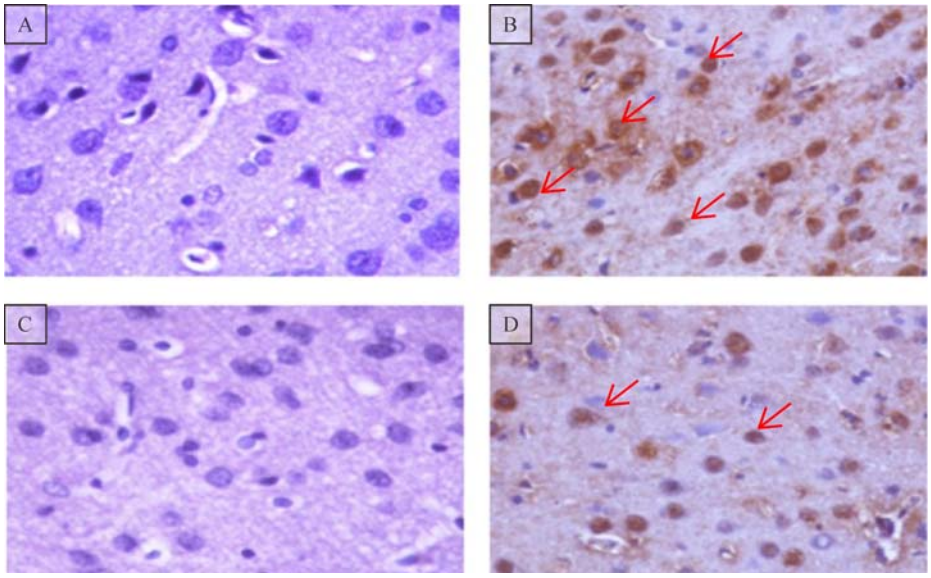

Fig. 3 **Effect of *S. dulcis* on nNOS level in rat brain after 15 days of noise (100 dB/4 hours/day).** A: control; B: noise; C: *S. dulcis* control; D: noise + *S.dulcis*. Red arrow indicates positive expression in neuronal cells.

lated expression for both nNOS and iNOS in Group Ⅱ. However, Group Ⅳ animals showed decreased expression when compared to Group Ⅱ. No changes in NOS protein expression were observed Group Ⅰ and Group Ⅲ.

S. *dulcis* on noise-induced elevated stress markers

Increased lipid peroxidation (LPO) was found in discrete brain regions (cerebral cortex, cerebellum, brain stem, striatum, hippocampus and hypothalamus) in Group Ⅱ, when compared with Group Ⅰ and Group Ⅲ indicating neurodegenerative effect of noise (***Table 1***). However, LPO level was found to be significantly decreased in Group Ⅳ compared with Group Ⅱ. Decreased protein thiol level (***Table 1***) in group Ⅱ animals was observed when compared to Group Ⅰ and Group Ⅲ. However, a significant increase in thiol level was observed in Group Ⅳ. The thiol level in Group Ⅲ did not differ from control animals.

S. *dulcis* on noise-induced changes in anti-oxidants status

Increased enzymatic antioxidant levels of SOD, CAT, GPx, and GST and decreased GR levels were observed

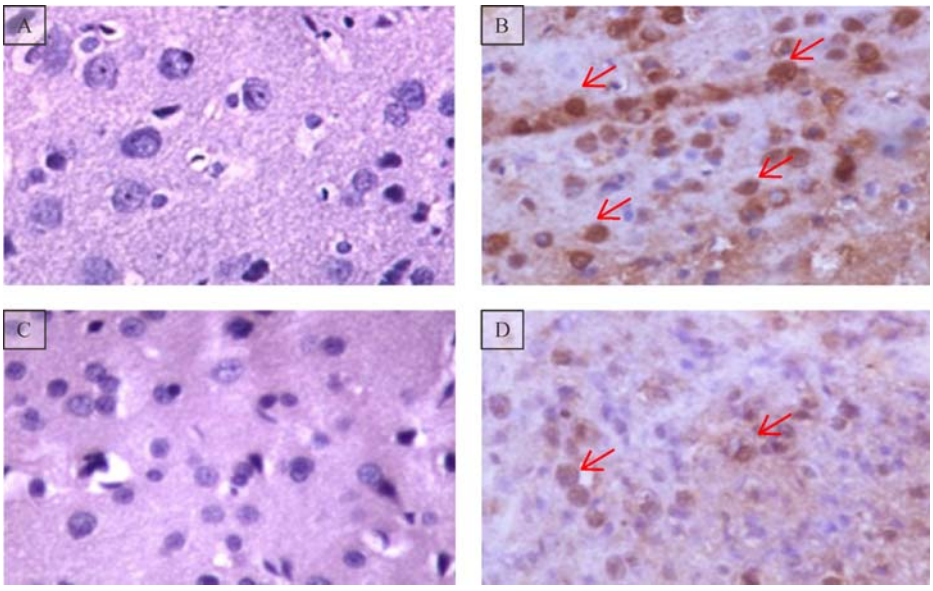

Fig. 4 **Effect of *S. dulcis* on iNOS level in rat brain after 15 days of noise (100 dB/4 hours/day).** A: control; B: noise; C: *S. dulcis* control; D: noise + *S.dulcis*. Red arrow indicates positive expression in neuronal cells.

Table 1 **Effect of *S. dulcis* on noise stress on stress markers in rat brain discrete region after 15 days of noise (100 dB/4 hour/day).**

		CONTROL	NOISE	S. DULCIS	NOISE + S.D
Lipid peroxidation (nm MDA/mg protein)	Cortex	1.91±0.215	3.24±0.502*	1.88±0.045#	2.21±0.198#
	Cerebellum	1.80±0.202	3.15±0.207*	1.72±0.046#	2.03±0.169#
	Striatum	1.63±0.248	2.89±0.671*	1.65±0.048#	1.87±0.088#
	Brainstem	1.41±0.212	2.66±0.205*	1.34±0.046#	2.02±0.120*#
	Hypothalamus	1.71±0.251	2.86±0.432*	1.60±0.031#	1.75±0.109#
	Hippocampus	1.66±0.175	3.21±0.390*	1.56±0.034#	2.35±0.259*#
Protein thiols (μg/mg protein)	Cortex	5.13±0.38	2.21±0.61*	4.77±0.39#	4.98±0.67#
	Cerebellum	4.97±0.30	2.03±0.78*	4.86±0.29#	4.01±0.69#
	Striatum	5.6±0.44	1.87±0.90*	4.74±0.52#	3.78±0.35*#
	Brainstem	4.78±0.67	2.02±0.98*	4.36±0.70#	3.89±0.56*#
	Hypothalamus	6.01±0.88	1.75±1.23*	5.36±0.32#	4.54±0.67#
	Hippocampus	5.86±0.51	2.35±0.78*	5.42±0.49#	3.96±0.43*#

Note: Values are expressed as mean±SD, $n = 6$ and significance at $P < 0.05$. * Compared with saline control and # Compared with noise stress.

Table 2 **Effect of *S. dulcis* on noise stress on enzymatic antioxidant in rat brain discrete region after 15 days of noise (100 dB/4 hour/day).**

		CONTROL	NOISE	S. DULCIS	NOISE + S.D
Superoxide dismutase (SOD) (units/mg protein)	Cortex	0.57±0.037	0.70±0.092*	0.53±0.068#	0.55±0.080#
	Cerebellum	0.74±0.046	0.89±0.095*	0.70±0.078#	0.68±0.09#
	Striatum	0.66±0.052	0.90±0.059*	0.62±0.079#	0.70±0.091#
	Brainstem	0.59±0.095	0.76±0.104*	0.56±0.048#	0.63±0.078
	Hypothalamus	1.93±0.113	2.86±0.527*	1.86±0.118#	2.33±0.417
	Hippocampus	1.34±0.117	2.66±0.547*	1.20±0.078#	1.90±0.116*#
Catalase (μm H_2O_2 utilized/mg protein)	Cortex	4.89±0.383	7.84±0.61*	4.77±0.396#	6.04±0.674*#
	Cerebellum	4.93±0.307	7.95±1.31*	4.86±0.291#	5.87±0.697*#
	Striatum	5.01±0.445	7.05±0.90*	4.74±0.528#	5.05±0.357#
	Brainstem	4.93±0.675	9.41±2.28*	4.36±0.703#	5.69±0.785#
	Hypothalamus	6.04±0.887	10.15±2.23*	5.36±0.321#	6.88±0.943#
	Hippocampus	5.76±0.514	10.47±1.92*	5.42±0.498#	7.87±0.790*#
Glutathione peroxidase (μg GSH utilized/ mg protein)	Cortex	5.31±0.386	7.42±0.81*	5.04±0.473#	6.98±0.394#
	Cerebellum	4.30±0.535	7.72±1.03*	4.35±0.304#	6.74±0.822#
	Striatum	4.91±0.527	7.67±0.94*	4.63±0.631#	6.21±0.537#
	Brainstem	4.47±0.363	5.79±0.82*	4.51±0.482#	5.24±0.864#
	Hypothalamus	4.45±0.638	8.40±1.66*	3.89±0.318#	5.29±0.659#
	Hippocampus	4.53±0.646	9.47±1.66*	4.23±0.420#	6.29±0.794*#
Glutathione-S-Transfrease (μmoles of CDNB utilized/minute/mg of protein).	Cortex	1.22±0.371	2.64±0.41*	1.19±0.170#	2.12±0.127*#
	Cerebellum	1.27±0.344	2.75±0.36*	1.33±0.291#	2.03±0.279*#
	Striatum	0.94±0.510	2.16±0.14*	1.19±0.151#	1.70±0.164*#
	Brainstem	1.33±0.242	2.76±0.80*	1.35±0.129#	2.27±0.203#
	Hypothalamus	2.03±0.241	3.25±0.62*	1.66±0.153#	2.08±0.468#
	Hippocampus	1.73±0.309	3.14±0.66*	1.78±0.245#	2.34±0.283#
Gutathionereductase (nmol/L of NADPH oxidised/ minute/mg of protein).	Cortex	0.071±0.004	0.04±0.004*	0.070±0.004#	0.05±0.006*#
	Cerebellum	0.055±0.004	0.03±0.003*	0.059±0.001#	0.04±0.004*#
	Striatum	0.061±0.005	0.04±0.004*	0.059±0.006#	0.04±0.003*#
	Brainstem	0.056±0.003	0.03±0.006*	0.058±0.006#	0.043±0.006*
	Hypothalamus	0.069±0.008	0.03±0.007*	0.071±0.003#	0.04±0.005*#
	Hippocampus	0.066±0.004	0.02±0.005*	0.064±0.003#	0.05±0.002*#

Note: Values are expressed as mean±SD, $n = 6$ and significance at $P < 0.05$. * Compared with saline control and # Compared with noise stress.

Table 3 **Effect of *S. dulcis* on non-enzymatic antioxidant in rat brain discrete region after 15 days of noise (100 dB/4 hours/day).**

		CONTROL	NOISE	S. DULCIS	NOISE + S.D
Reduced glutathione (μg of GSH/mg of protein	Cortex	3.02±0.21	1.79±0.18*	3.18±0.28#	2.47±0.38#
	Cerebellum	2.84±0.24	0.99±0.17*	2.73±0.08#	2.30±0.20*#
	Striatum	3.46±0.18	1.84±0.43*	3.34±0.42#	2.84±0.31*#
	Brainstem	3.15±0.51	1.69±0.47*	3.02±0.40#	2.74±0.23#
	Hypothalamus	4.97±0.76	2.24±0.63*	4.75±0.20#	3.78±0.47#
	Hippocampus	4.04±0.50	2.36±0.57*	4.30±0.32#	3.54±0.41#
Vitamin-E (μg /mg of protein).	Cortex	0.44±0.016	0.27±0.031*	0.43±0.023#	0.35±0.041*#
	Cerebellum	0.42±0.019	0.25±0.031*	0.40±0.015#	0.34±0.034*#
	Striatum	0.40±0.024	0.28±0.032*	0.41±0.063#	0.36±0.023#
	Brainstem	0.45±0.050	0.25±0.054*	0.44±0.039#	0.37±0.013*#
	Hypothalamus	0.49±0.054	0.24±0.042*	0.48±0.025#	0.35±0.027*#
	Hippocampus	0.48±0.034	0.35±0.017*	0.48±0.040#	0.44±0.056#
Vitamin-C (μg /mg of protein).	Cortex	0.51±0.05	0.28±0.03*	0.47±0.03#	0.37±0.07#
	Cerebellum	0.46±0.03	0.27±0.03*	0.44±0.02#	0.35±0.03*#
	Striatum	0.50±0.02	0.30±0.03*	0.48±0.06#	0.38±0.02*#
	Brainstem	0.48±0.06	0.28±0.08*	0.45±0.04#	0.42±0.07
	Hypothalamus	0.55±0.03	0.31±0.06*	0.51±0.04#	0.43±0.08
	Hippocampus	0.57±0.03	0.38±0.05*	0.53±0.04#	0.46±0.05#

Note: Values are expressed as mean±SD, $n = 6$ and significance at $P < 0.05$. * Compared with saline control and # Compared with noise stress.

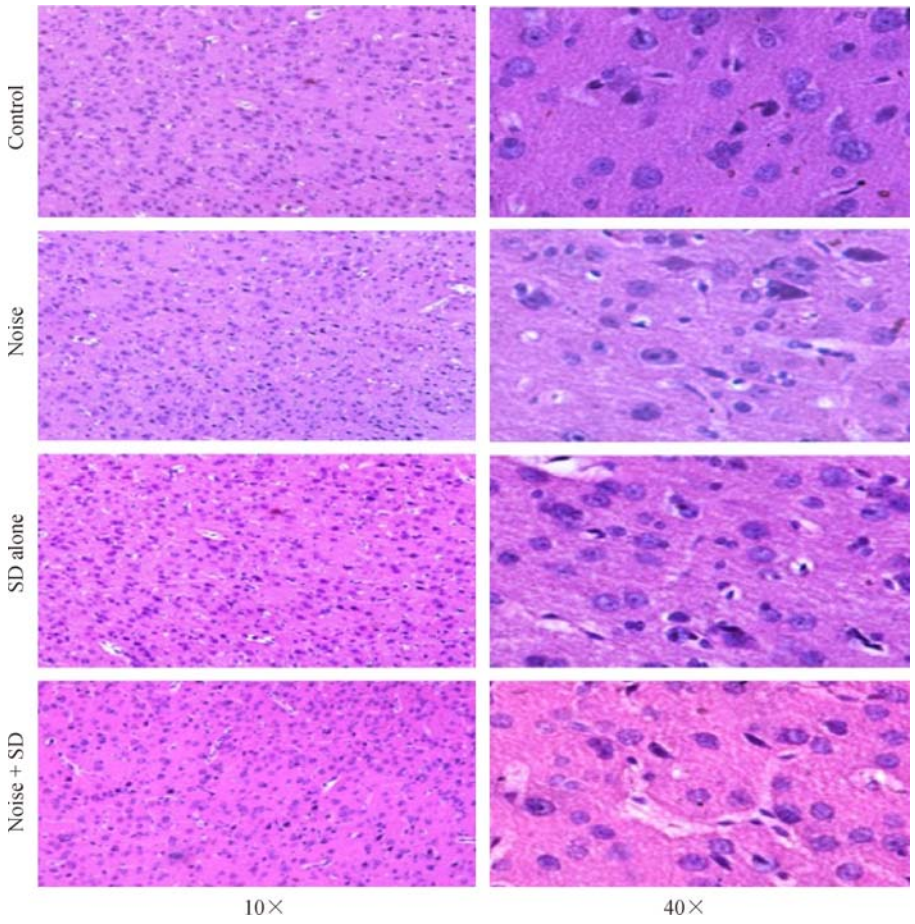

Fig. 5 **H&E staining of neuronal cells.** Effect of *S. dulcis* on cerebral cortex morphology in different experimental groups after 15 days of noise exposure (100 dB/hour/day).

in discrete brain regions (cerebral cortex, cerebellum, brain stem, striatum, hippocampus and hypothalamus) in Group Ⅱ when compared to Group Ⅰ and Group Ⅲ (***Table 2***). However, a significant decrease in SOD, CAT, GPx and GST levels were found, in contrast to significant increase in GR levels in Group Ⅳ. No significant changes in the level of free radicals were observed in Group Ⅲ when compared to the control animals. Conversely, we also observed a significant decrease in non-enzymatic antioxidants like GSH, vitamin E and vitamin C (***Table 3***) in Group Ⅱ when compared to Group Ⅰ and Group Ⅲ. However, a significant increase in GSH, vitamin C and vitamin E levels in *S. dulcis* treated Group Ⅳ in discrete brain regions. No significant changes in the level of free radicals were observed in Group Ⅰ and Group Ⅲ in GSH, vitamin C and vitamin E levels.

Histology

The cerebral cortex in Group Ⅰ and Group Ⅲ showed normal neuron architecture (***Fig. 5***). Cytoarchitecture of the cerebral cortex was not extensively disturbed as evident from the photomicrograph in Group Ⅱ noise (100 dB, 4 hours/day) exposed rats for 15 days. However, neurons were pyknotic and had darkly stained nuclei. Treatment with *S. dulcis* extract along with noise exposure for 15 days (4 hours/day) in Group Ⅳ prevented the alteration in the cerebral cortex which was observed in Group Ⅱ.

Discussion

Free radicals arising from either normal metabolism or induced by environmental or occupational sources interact continuously with the endogenous biologic antioxidant systems to attain an equilibrated redox state. Literature survey revealed that exposure to noise reportedly often resulted in free radical generation and neuronal oxidative/nitrosative stress. Du *et al.*[30] demonstrated that acute incubation of cortical neurons with corticosterone increased mitochondrial oxidation, membrane potential and calcium-holding capacity in a dose and time-dependent manner. Significant increase in H_2O_2 and NO levels was noticed in discrete brain regions. Demirel *et al.*[31] also reported increased NO generation when exposed to noise for 20 days. To substantiate our finding, immunohistochemical data showed positive cell expression for both neuronal nitric oxide synthase (nNOS) and inducible nitricoxide synthase (iNOS) in the noise-exposed Group Ⅱ animals. This upregulation of NOS possibly accounted for increased NO production. To support the neurotoxic effect of NO, Samadani *et al.*[32] reported that activation of nNOS in focal ischemia model promoted neurotoxicity and iNOS expression produced high levels of NO continuously (up to 1 μmol/L) from microglia or astrocytes, which induced neuronal death by causing inhibition of mitochondrial cytochrome oxidase in neurons[33-34].

Although NO production is the most widely studied reaction catalyzed by NOS, both iNOS and nNOS are also characteristically associated with the production of superoxide ($O_2^{\bullet-}$) when levels of *L*-arginine and tetrahydrobiopterin decrease[35]. The increased H_2O_2 level in the noise exposed Group Ⅱ animals is also justified because $O_2^{\bullet-}$, when dismutated with SOD, it is converted to harmful ROS such as H_2O_2 and hydroxyl radical ($^{\bullet}OH$). From these findings, it is obvious that NO and H_2O_2 levels should be regulated to prevent oxidative/nitrosative damage in brain discrete regions. However, the normalized regulated action of nitric oxide synthase activity and NO levels in Group Ⅳ animals when treated with *S. dulcis* could be credited to the therapeutic action of plant. However, the mechanisms contributing to the nitric oxide synthase expression in noise stress are not well understood.

Remarkable increased SOD, CAT, GPx, GST, LPO and decreased GR, GSH, Vit-C, Vit-E, protein thiols in discrete brain regions were observed in Group Ⅱ animals on exposure to noise-stress for 15 days (100 dB/4 hours/day). The synchronized action of both enzymatic and non-enzymatic antioxidant enzymes is needed to attain a redox balance of the cell. $O_2^{\bullet-}$ undergoes reaction with SOD as the first line of defense and is converted into H_2O_2 and singlet oxygen ($^1O_2$). This increased SOD activity in the noise exposed Group Ⅱ animals justifies the presence of upsurge in free radical production, especially H_2O_2 in brain discrete regions. GPx along with CAT detoxifies peroxides with GSH acting as an electron donor in the reduction reaction, producing oxidized glutathione (GSSG) as an end product. Glutathione reductase (GR) then catalyzes the reduction of GSSG to the sulfhydryl form glutathione requiring NADPH as a cofactor (GSH), which is a critical molecule in resisting oxidative stress and maintaining the reducing environment of the cell[36].

The decrease in GR level in noise exposed Group Ⅱ is also justified, because with increased free radical being the reason behind decreased GSH activity in noise exposed Group Ⅱ. A decrease in GR activity can be well correlated in this particular finding, as regeneration of GSH is limited with decreased GR activity. Literature survey also supports our finding where it is reported that, GSH is a ubiquitous thiol-containing tripeptide via the GSH dependent antioxidant pathway that plays a role in cell survival[37] and its dysregulation could

contribute to the initiation and progression of several neurodegenerative diseases. In addition, GSH also plays a role in the detoxification of a variety of electrophilic compounds and peroxides via catalyzing GST and GPx. This present finding reports a significant increase in GST activity in the discrete brain regions. This persistent decrease in GSH content in the noise exposed group is likely to be reasonable due to the concomitant increase in GST activity, as GSH acts as a substrate of the enzymatic reaction of GST[38].

From these findings, it is evident that increased SOD, GPx, CAT and GST may act as a mediator to stress response, indicating that the brain homeostatic mechanism is attempting to adapt to this stressor. However, the detrimental effect of noise was evident as we could observe a decrease in major thiol group (GSH), a fundamental antioxidant machinery. Several studies have shown that alteration of antioxidant enzyme activities in different kinds of stress were associated with a depletion of GSH and increased lipid peroxidation, all of which leading to oxidative cell death[39]. Thus, excessive free radical generation and altered antioxidant status may assume to serve as a linkage between the environmental/occupational noise and the manifestation of multifactorial diseases. However, *S. dulcis* extract showed neuroprotective effect as it could normalize the antioxidant status and above all, it could have also attributed to catalyze the reduction reaction of GSSG to GSH. This significant increase in GSH content may have helped attenuate the generation of reactive oxygen-nitrogen species (RONS).

A significant increase in LPO in discrete brain regions, supports the deteriorating effect on exposure to noise. High levels of lipid peroxidation products can adversely affects cellular functions as the end products of lipid peroxidation may be mutagenic and carcinogenic. In this study, a significant inverse correlation was recorded between malondialdehyde levels with regard to vitamin C and vitamin E levels in noise exposed Group Ⅱ when compared to Group Ⅰ and Ⅲ. Vitamin C and E work in close relation with each other because vitamin E is a major lipid-soluble antioxidant and the most effective chain-breaking antioxidant within the cell membrane protects membrane fatty acids from lipid peroxidation. On the other hand, vitamin C prevents the pro-oxidant activity of vitamin E by decreasing α-tocopheroxyl radical activity to α-tocopherol. The decreased vitamin C and E contents in noise exposed Group Ⅱ justify the increased LPO level when compared to Group Ⅰ and Ⅲ. Yet, the anti-lipid peroxidation activity of the plant is obvious as we observed significantly decreased LPO level in Group Ⅳ treated with *S.dulcis* extract when compared to noise exposed Group Ⅱ. This anti-lipid peroxidation activity could be attributed to the phytocompounds present in the plant and ultimately resulting in effective chain-breaking activity. However, the importance of GSH in maintaining the redox status cannot be ignored, as already discussed earlier with regard to contribution of GSH-dependent antioxidant pathway playing a major a role in cell survival; another possible way is the ability of GSH to regenerate α-tocopherol to its active form via reducing the tocopherol radical of vitamin E directly or indirectly, via reduction of semi-dehydroascorbate to ascorbate[40]. Low concentrations of total thiols are probably the result of an accelerated turn over linked to an elevated oxidative/nitrostative stress. This is in agreement with our finding where we observed decreased protein thiol level in noise exposed Group Ⅱ, indicating depletion in major thiol group which can be well corroborated with decreased GSH content.

The therapeutic efficacy of *S. dulcis* was evident in Group Ⅳ treated with *S. dulcis* as it was able to restore the antioxidant status and free radicals to that of the control groups in brain discrete regions. The aqueous extract at 200 mg/kg·b·w has also been reported to exhibit antihyperglycemic, antioxidant, antiapoptotic and cytoprotective action[15]. In addition, Wankhar *et al.*[41] identified polyphenols through High performance thin layer chromatography (HPTLC) which are known to possess antioxidant properties and they could have been the possible reason for the plant showing a protective role against lipid peroxidation and free radicals induced brain damages. These polyphenol hydroxyls which are very reactive in neutralizing free radicals (-R●) by donating a hydrogen atom (-RH) or an electron (-R●) chelating metal ions in aqueous solutions. In-depth studies are still needed to verify the mode of action of this plant as a potential therapy.

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