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Research Article

MICHELIA CHAMPACA MITIGATES NOISE STRESS-INDUCED OXIDATIVE AND BEHAVIORAL ALTERATIONS IN RAT BRAIN REGIONS

MALATHI S[1], VIDYASHREE HM[2], RAVINDRAN RAJAN[3]*

[1]Department of Physiology, Vels Medical College and Hospital, Chennai, Tamil Nadu, India. [2]Department of Physiology, Dr Chandramma Dayananda Sagar Institute of Medical Education and Research, Kanakapura, Karnataka, India. [3]Department of Physiology, Dr. ALM PG Institute of Basic Medical Sciences, University of Madras, Chennai, Tamil Nadu, India.
***Corresponding author: Ravindran Rajan; Email: ravindran89@gmail.com**

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ABSTRACT

Objectives: Noise is a globally prevalent environmental stressor known to contribute to a range of psychophysiological issues, including auditory defects and non-auditory disorders. The brain plays a central role in recognizing, interpreting, and responding to such stressors. The objective of the present study was to evaluate the anxiolytic, neuroprotective, and antioxidant effects of *Michelia champaca* in rats with noise-induced stress.

Methods: Animals were exposed to noise stress (100 dB for 4 h/day) for 1 day and 30 days, after which endogenous free radical levels and antioxidant activities were assessed in discrete brain regions.

Results: It demonstrated that noise exposure significantly ($p<0.05$) elevated free radical production and disrupted antioxidant status across various brain regions. Noise exposure also affected membrane bound enzymes. A marked decrease ($p<0.05$) was observed in the activities of Ca^{2+}-ATPase, Mg^{2+}-ATPase, and Na^+/K^+-ATPase. Behavioral assessments showed that noise exposed rats exhibited increased anxiety-like behaviors compared to controls. However, treatment with *M. champaca* (400 mg/kg body weight) effectively ($p<0.05$) mitigated these effects. The plant extract reduced free radical levels, restored antioxidant enzyme activities, and normalized the functions of membrane-bound enzymes, bringing them closer to control levels.

Conclusion: The findings suggest that *M. champaca* possesses potent free radical scavenging and antioxidant properties, likely due to its phytochemical constituents. These properties contribute to its therapeutic potential in alleviating biochemical and behavioral abnormalities induced by noise stress.

Keywords: *Michelia champaca*, Membrane-bound enzymes, Anxiolytic, Free radical scavenging activity, Noise stress.

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INTRODUCTION

In modern life, humans are subjected to various forms of stress, among which noise is one of the most prevalent. Sources of environmental noise include urban traffic, aircraft, industrial machinery, and household appliances, all of which contribute significantly to daily stress exposure [1]. Prolonged exposure to environmental noise has been associated with a range of physiological disturbances. Both acute and chronic noise exposure can lead to the overproduction of free radicals such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH•), contributing to oxidative stress overwhelms the body's antioxidant defenses [2,3].

Oxidative stress arises when the generation of reactive oxygen species (ROS) surpasses the neutralizing capacity of the body's endogenous antioxidants, including enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). These oxygen-derived free radicals can damage lipids, proteins, and nucleic acids, thereby compromising cellular function and integrity [4,5]. The brain is particularly susceptible to oxidative damage due to its high oxygen demand, abundant lipid content, and comparatively low antioxidant defense, making neural tissue a primary target for oxidative injury [6].

Membrane-bound ATPases, such as Na^+/K^+-ATPase, Ca^{2+}-ATPase, and Mg^{2+}-ATPase, are lipid-dependent enzymes essential for ion transport and the maintenance of electrochemical gradients critical for neuronal excitability and function [7]. Among these, Na^+/K^+-ATPase is especially important, as it consumes up to 40–50% of the brain's total ATP to maintain sodium and potassium gradients, particularly at nerve endings [8]. Alterations in the activity of this enzyme have been observed in numerous neurodegenerative and neuropsychiatric disorders, suggesting a link between Na^+/K^+-ATPase dysfunction and impaired neurotransmission [9,10]. Despite its significance, the impact of noise-induced oxidative stress on membrane ATPase activity in the central nervous system remains underexplored.

Over the past three decades, antioxidant-based treatments have gained considerable attention for their role in managing complex disorders such as atherosclerosis, diabetes, Alzheimer's disease, stroke, and cancer. In this context, natural antioxidants derived from medicinal plants have shown promising therapeutic potential [11]. One such plant is *Michelia champaca* L. (Magnoliaceae), commonly known as Yellow *Champaca*. Conventionally used in Indian medicine, *M. champaca* has been employed to treat a wide array of ailments, including fever, inflammation, eye disorders, rheumatism, colic, and abdominal tumors [12,13]. Pharmacological studies have highlighted its wound healing [14], antimicrobial [15], antidiabetic [16], antitumor, anti-inflammatory [17], antioxidant [18], and anti-infective [19] properties. However, there is a notable lack of research evaluating the neuroprotective and antioxidant efficacy of its methanolic extract in animal models of noise-induced stress.

Therefore, the present study was undertaken to investigate the neuroprotective effects of *M. champaca* on noise stress-induced redox imbalance, anxiety-like behaviors, and alterations in membrane-bound ATPase activity in discrete brain regions of rats.

METHODS

Chemicals

All the chemicals used in these studies were of analytical grade and obtained from Sisco Research Laboratory, Mumbai, India.

Identification and extraction of flower extract

Flowers of *M. champaca* were collected from Chennai and were 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/94/2013. The collected flowers were cleaned, dried under shade at room temperature. Then, the flower was ground into a coarse powder with the help of a suitable grinder. A total of 10 g of *M. champaca* dry flowers were soaked in 100 mL (twice, i.e., 2×100 mL) of methanol for 8–10 days at room temperature in dark conditions, stirring every 18 h using the sterile rod. The final extracts were filtered using a Whatman No 1 filter paper. The filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator and stored at 4°C for further use. An oral dose of 400 mg/kg/b. weight was administered orally in this study.

Experimental design

The experiments were carried out using healthy adult male Wistar rats (200–220 g). The study was initiated with a proper approval by the Institute's Animal Ethical Committee (IAEC No: 01/23/2015). Animals were divided into six groups consisting of six animals each one set of animals were employed for behaviors, DNA fragmentation and another set of animals were used for antioxidants (control, acute noise, acute noise treated with M. champaca, *chronic noise stress,* and *chronic noise stress treated with* M. champaca and M. champaca treated control 400 mg/kg given orally). All the rats used in this study were maintained at a constant temperature with a 12 h light: 12 h dark cycle and allowed free access to food and water. All groups were handled similarly.

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.

Noise stress induction

Noise stress induction Broadband white noise at 100-dBA intensity was produced by a white noise generator, amplified by an amplifier connected to a loudspeaker fixed 30 cm above the animal cage. A sound level meter was used to measure the intensity of the noise [20]. Noise stress group animals and treated animal with noise exposure was exposed for 4 h/day for 1 day (acute stress) and 30 days (chronic stress). Control rats were kept in the above-described cage during the consistent period, without noise stimulation to evade the impact of handling-stress on an appraisal of effects due to noise exposure.

Sample collection

The experimental procedure was done between 8 and 10 a.m., to avoid circadian rhythm-induced changes. After the experimental procedure, animals were deeply anesthetized with ketamine/xylazine (90/50 mg/kg.b.w.) and blood was collected from an internal jugular vein, serum was separated by centrifugation at 3000 rpm at 4°C for 15 min. The brain was immediately removed and washed with ice-cold phosphate buffered saline (PBS). The homogenates (10% w/v) of individual brain regions and liver were prepared in a Teflon-glass tissue homogenizer, using ice-cold Tris HCl (100 mM, pH 7.4) buffer and ice-cold PBS.

Brain dissection and biochemical determinations

The brain was immediately removed and washed with ice-cold 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 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 <2 min. 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 [21]. 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 SOD) and ice-cold PBS, centrifuged separately in the refrigerated centrifuge at 3,000 g for 15 min.

Stress markers like lipid peroxidation (LPO) were determined as described by Ohkawa *et al*. [22] and protein thiol was estimated as per Sedlack and Lindsay [23]. Protein carbonyl (PC) was determined as per Levine *et al*. [24]. 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*. [25] and hydrogen peroxide (H_2O_2) levels were determined as per Pick and Keisari [26]. Biochemical determination of enzymatic antioxidants like SOD was estimated according to Marklund and Marklund [27] and CAT according to the method of Sinha [28]. The activity of GPx was estimated as described by Rotruck *et al*. [29]. Glutathione reductase (GR) that utilizes nicotinamide adenine dinucleotide phosphate (NADPH) to convert metabolized glutathione (GSSG) to the reduced form was assayed by the method of Horn and Burns [30]. Biochemical estimation of non-enzymatic antioxidants like reduced GSH was estimated by the method of Moron *et al*. [31]. Vitamin C and Vitamin E contents were determined according to Omaye *et al*. [32] and Desai [33].

Determination of membrane bound enzymes

Na+ K+ ATPase by the method of Bloj *et al*. [34], Ca2+ ATPase by Hjerten and Pan [35], and Mg2+ ATPase by the method of Ohnishi *et al*. [36] were determined.

DNA fragmentation assay

Separation of DNA molecules from the extracted samples was performed by Borriello *et al*. [37].

Behavioral analysis

Place preference task/light and dark test

The light/dark box was used to assess the anxiety level of rodents. The box was divided into two compartments, 18×15×15 inches (long, wide, and high) light compartment with open at the top and 12×15×15 (long, wide, and high) the dark compartment that was fully enclosed. The divider between the two compartments and contained 3×4 inches (wide and high) opening at floor level. This allows the animal entries between compartments. At the beginning of testing, each animal was placed in the center of the light compartment. Behavior subsequently was videotaped for 5 min. Behaviors were scored by an observer who was blind to the treatment conditions. The measures scored were (1) initial latency to enter the dark compartment, (2) time spent in the brighter area, and (3) time spent in the dark compartment [38].

Hole-board (HB) test

HB box 60×60×45 with matt painted metallic walls and a plastic covered wooden floor which bears four equally spaced holes (3.8 cm in diameter) is divided into 36 square (10×10 cm) the HB provokes neophobia leading to exploratory and escape behavior and allows the estimation of motor activity. Parameters recorded: (i) Frequency of rearing (no of times the rat stood on its hindlimbs). (ii) The frequency of external ambulation (no of line crossings in peripheral areas close to the wall). (iii) Frequency of internal ambulation (no of line crossings in the central area). (iv) Frequency and duration of head dipping which has been validated as a measure of exploratory activity and anxiety. The number of head-dips is assumed to be inversely proportional to the anxiety state [39] and frequency and duration of stereotypic behavior (grooming and scratching).

Statistical analysis

Data were expressed as the mean ± standard deviation (SD). All the data were analyzed with the Statistical Package for the Social Sciences

(SPSS) for Windows statistical package (version 20.0, SPSS Institute Inc., Cary, North Carolina). The statistical significance among the four different groups was analyzed using one-way analysis of variance test followed by Tukey's multiple comparison tests and the significance level was fixed at $p<0.05$.

RESULTS

M. champaca attenuates noise-induced upregulation of free radicals and NO level

Increased levels of hydrogen peroxide (H_2O_2) (Fig. 1) and NO (Fig. 2) were observed in discrete brain regions – namely, the prefrontal cortex, cerebellum, striatum, hippocampus, and hypothalamus – in both acute and chronic noise stress groups. Notably, the chronic stress group exhibited a significantly higher concentration of H_2O_2 and NO ($p<0.05$) compared to the control group. However, treatment with *M. champaca* in the respective stress-exposed groups resulted in a significant reduction in these oxidative markers, particularly in the chronic stress condition. No significant changes were observed between the control group and the acute noise group treated with *M. champaca*, nor between the control and the group treated with *M. champaca* alone. These results indicate that the plant possesses notable free radical scavenging activity, as evidenced by its capacity to attenuate oxidative stress in the majority of the examined brain regions.

M. champaca on noise-induced elevated stress markers

Increased LPO was observed in discrete brain regions (prefrontal cortex, cerebellum, striatum, hippocampus, and hypothalamus) under acute stress, and more significantly in the chronic noise stress group, when compared to the control and *M. champaca*-treated control groups. This indicates increased LPO irrespective of brain region. However, the chronic stress groups treated with *M. champaca* showed a significant ($p<0.05$) decrease in LPO levels Fig. 3.

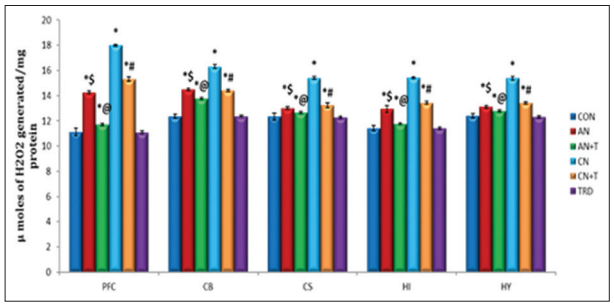

Fig. 1: Effect of *Michelia champaca* on H_2O_2 level in rat brain discrete regions after noise exposure (100 dB/4 h/day). The symbols represent statistical significance: *@#$ <p=0.05.*- Compared with saline control, @-compared with acute noise, #-compared with chronic noise. $- Acute noise compared with chronic noise

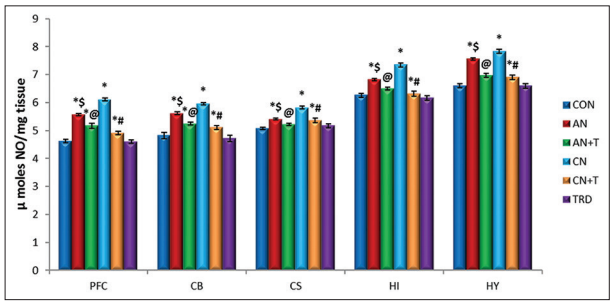

Fig. 2: Effect of *Michelia champaca* on nitric oxide level in rat brain discrete regions after noise exposure (100 dB/4 h/day). The symbols represent statistical significance: *@#$ <p=0.05.*- Compared with saline control, @-compared with acute noise, #-compared with chronic noise. $- Acute noise compared with chronic noise

A significant decrease ($p<0.05$) in Fig. 4 protein thiol levels was also observed in both acute and chronic noise stress groups, with a more pronounced reduction in the chronic stress group compared to the control and *M. champaca*-treated control groups. In contrast, stress groups treated with *M. champaca* showed a significant increase in protein thiol levels.

A significant increase ($p<0.05$) in Fig. 5 PC content was observed in all brain regions, with the chronic stress group showing a more marked increase than the acute stress group. Treatment with *M. champaca* significantly reduced PC levels in both acute and chronic stress groups, although the values did not return to control levels – except in the prefrontal cortex, hippocampus, and hypothalamus of the acute stress group treated with *M. champaca*.

Importantly, the drug-treated control group did not show any deviation from the untreated control group in any of the brain regions studied.

M. champaca on noise-induced changes in enzymatic anti-oxidants status

Increased levels of the enzymatic antioxidants SOD and GPx were observed in the discrete brain regions of the acute noise-stressed group, compared to the control and *M. champaca*-treated control groups. However, the chronic stress group showed a more significant increase in SOD and GPx levels, indicating an imbalance between oxidants and antioxidants in the brain.

In contrast, noise-exposed animals treated with *M. champaca* showed normalized SOD levels in the prefrontal cortex, cerebellum, striatum, hypothalamus, and hippocampus. GPx levels in the cerebellum and hypothalamus also returned to normal, comparable to both the *M champaca*-treated control and untreated control groups. No variation was observed between the control and *M. champaca*-treated control groups in any of the regions studied.

A significant decrease in CAT and GR levels was observed in the discrete brain regions of the noise-stressed group, when compared to the control and *M. champaca*-treated groups. However, the acute stress group did not show any alteration in CAT activity. Notably, noise-stressed animals treated with *M. champaca* showed a significant increase in GR levels in the prefrontal cortex, striatum, and hypothalamus, while levels in the cerebellum and hippocampus remained similar to those in both *M. champaca*-treated controls and the untreated control group.

Again, no variation was observed between the control and *M. champaca*-treated control groups across any of the regions studied Table 1.

M. champaca on noise-induced changes in non-enzymatic anti-oxidants status

A significant decrease in non-enzymatic antioxidants such as GSH, Vitamin E (Vit-E), and Vitamin C (Vit-C) was observed in the noise-stressed group compared to the control and *M. champaca*-treated control groups (Table 1). The chronic stress group exhibited a more pronounced decrease than the acute stress group.

However, noise-exposed animals treated with *M. champaca* showed a significant increase in GSH levels in the cerebral cortex, hypothalamus, striatum, and hippocampus, while levels in the cerebellum remained comparable to those of the control and *M. champaca*-treated control groups.

In contrast, animals exposed to chronic noise and treated with *M. champaca* still showed a significant decrease in Vit-E levels across all brain regions. Notably, treatment with *M. champaca* during acute noise exposure was able to maintain normal Vit-E levels in regions such as the prefrontal cortex and hypothalamus, comparable to both the control and *M. champaca*-treated control groups.

Table 1: Effect of *M. champaca* on noise stress on stress markers in rat brain discrete region after noise exposure (100 dB/4 h/day)

Parameter	Regions	Control	Acute noise	Acute noise treated with *M. champaca*	Chronic noise	Chronic noise treated with *M. champaca*	*M. champaca* treated control
Superoxide dismutase	Prefrontal Cortex	0.61±0.027	0.86±0.010 *$	0.63±0.014@	0.33±0.018*	0.58±0.030#	0.63±0.014
	Cerebellum	0.76±0.026	0.81±0.008*$	0.76±0.010@	0.55±0.018*	0.72±0.028*#	0.76±0.018
	Striatum	0.65±0.027	0.73±0.012*$	0.67±0.014@	0.47±0.021*	0.63±0.014#	0.65±0.018
	Hippocampus	1.52±0.056	1.71±0.010*$	1.49±0.071@	1.03±0.029*	1.46±0.032#	1.52±0.052
	Hypothalamus	1.23±0.018	1.30±0.014*$	1.24±0.011@	1.05±0.022*	1.28±0.030*#	1.23±0.014
Catalase (mm H_2O_2 utilized/ mg protein)	Prefrontal Cortex	5.13±0.062	5.10±0.133$	5.08±0.084	4.34±0.174*	4.83±0.084*#	5.07±0.085
	Cerebellum	6.22±0.064	6.25±0.047$	6.24±0.078	5.36±0.208*	5.84±0.056*#	6.19±0.057
	Striatum	5.12±0.066	5.12±0.106$	5.11±0.081	4.13±0.166*	4.82±0.044*#	5.12±0.066
	Hippocampus	6.59±0.148	6.52±0.192$	6.56±0.278	4.81±0.162*	5.74±0.059*#	6.38±0.060
	Hypothalamus	4.53±0.076	4.48±0.100$	4.48±0.147	3.75±0.151*	4.22±0.055*#	4.53±0.083
Glutathione peroxidase (mg GSH utilized/mg protein)	Prefrontal Cortex	4.82±0.088	7.74±0.074*$	5.61±0.064*@	9.73±0.061*	6.81±0.110*#	4.90±0.060
	Cerebellum	4.14±0.063	7.31±0.095*$	5.39±0.044*@	9.45±0.103*	6.58±0.081*#	4.15±0.051
	Striatum	4.81±0.047	7.21±0.047*$	5.12±0.047*@	9.29±0.064*	6.34±0.048*#	4.83±0.058
	Hippocampus	4.31±0.122	7.52±0.056*$	5.19±0.054*@	9.67±0.072*	5.58±0.062*#	4.32±0.010
	Hypothalamus	3.93±0.063	5.76±0.062*$	4.96±0.046*@	7.73±0.059*	5.01±0.66*#	3.97±0.063
Glutathione reductase (nmol/L of NADPH oxidized/minute/mg of protein).	Prefrontal Cortex	0.087±0.003	0.073±0.002*$	0.080±0.001*@	0.058±0.001*	0.081±0.002*#	0.087±0.003
	Cerebellum	0.066±0.001	0.054±0.003*$	0.062±0.001*@	0.041±0.002*	0.067±0.002#	0.067±0.001
	Striatum	0.069±0.001	0.058±0.003*$	0.065±0.001@	0.046±0.002*	0.064±0.004*#	0.069±0.003
	Hippocampus	0.070±0.003	0.061±0.002*$	0.067±0.003@	0.035±0.003*	0.069±0.002#	0.070±0.004
	Hypothalamus	0.091±0.002	0.082±0.002*$	0.088±0.001	0.047±0.005*	0.083±0.003*#	0.090±0.002
Reduced glutathione (mg of GSH/mg of protein	Prefrontal Cortex	0.42±0.003	0.32±0.002*$	0.39±0.003*@	0.28±0.002*	0.37±0.004*#	0.41±0.005
	Cerebellum	0.41±0.003	0.37±0.004*$	0.40±0.006@	0.31±0.003*	0.40±0.004#	0.40±0.004
	Striatum	0.40±0.005	0.38±0.002*$	0.39±0.001*@	0.32±0.003*	0.39±0.002*#	0.41±0.002
	Hippocampus	0.31±0.002	0.28±0.002*$	0.31±0.002@	0.27±0.001*	0.30±0.002*#	0.31±0.003
	Hypothalamus	0.26±0.003	0.23±0.002*$	0.25±0.002@	0.21±0.002*	0.24±0.003*#	0.26±0.003
Vitamin-C (mg/mg of protein).	Prefrontal Cortex	1.75±0.024	1.53±0.045*$	1.62±0.021*@	1.20±0.020*	1.67±0.031*#	1.75±0.018
	Cerebellum	1.53±0.052	1.37±0.019*$	1.45±0.025*@	1.27±0.017*	1.40±0.038*#	1.51±0.051
	Striatum	1.74±0.023	1.55±0.035*$	1.60±0.042*@	1.28±0.023*	1.48±0.023*#	1.73±0.017
	Hippocampus	1.49±0.038	1.21±0.042*$	1.38±0.028*@	1.13±0.025*	1.40±0.019*#	1.48±0.036
	Hypothalamus	1.58±0.031	1.40±0.043*$	1.46±0.036*@	1.32±0.024*	1.46±0.030*#	1.56±0.037
Vitamin-E (mg/mg of protein).	Prefrontal Cortex	4.206±0.034	3.843±0.041*$	4.136±0.041@	2.871±0.066*	3.333±0.036*#	4.211±0.052
	Cerebellum	4.320±0.024	3.168±0.041*$	3.420±0.038*	2.406±0.061*	3.888±0.066*#	4.303±0.033
	Striatum	4.321±0.026	3.248±0.045*$	3.565±0.035*@	2.825±0.034*	3.775±0.036*#	4.211±0.037
	Hippocampus	4.386±0.033	4.138±0.042*$	4.215±0.035*	3.163±0.046*	3.863±0.043*#	4.338±0.037
	Hypothalamus	4.220±0.033	4.105±0.030*$	4.160±0.042	2.835±0.050*	3.660±0.055*#	4.205±0.026

Table 1 effect of *M. champaca* on noise stress on stress markers in rat brain discrete region after noise exposure. Values are expressed as Mean±SD, n=6. The symbols represent statistical significance: *@#$ < p=0.05. *- Compared with saline control, @- Compared with acute noise, #-compared with chronic noise. $- Acute noise compared with chronic noise. *M. champaca*: *Michelia champaca*, SD: Standard deviation

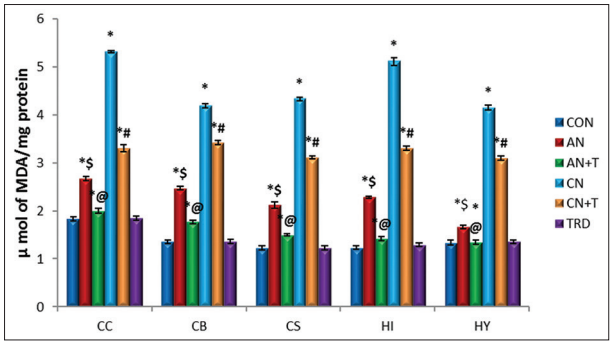

Fig. 3: Effect of *Michelia champaca* on lipid peroxidation level in rat brain discrete regions after noise exposure (100 dB/4 h/day). The symbols represent statistical significance: *@#$ <p=0.05.*- Compared with saline control, @-compared with acute noise, #-compared with chronic noise. $- Acute noise compared with chronic noise

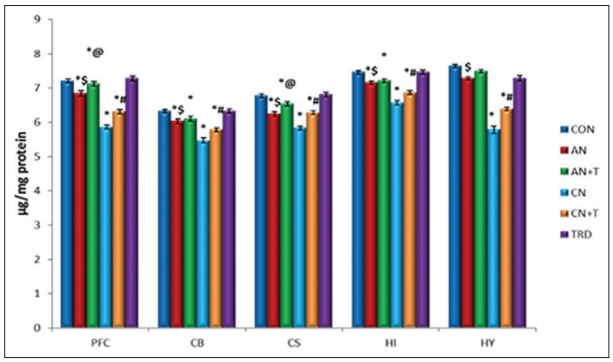

Fig. 4: Effect of *Michelia champaca* on protein thiol level in rat brain discrete regions after noise exposure (100 dB/4 h/day). The symbols represent statistical significance: *@#$ <p=0.05. *- Compared with saline control, @-compared with acute noise, #-compared with chronic noise. $- Acute noise compared with chronic noise

Furthermore, a significant increase in Vit-C levels was observed in all brain regions of the noise-exposed animals treated with *M. champaca*, compared to the stressed groups. The *M. champaca*-treated control group did not show any deviation from the untreated control group.

Effect of noise stress on membrane bound enzymes

The Na^+/K^+-ATPase activity levels across the experimental groups are summarized and presented in Fig. 6. Animals exposed to both acute and chronic noise stress showed a significant decrease in Na^+/K^+-ATPase

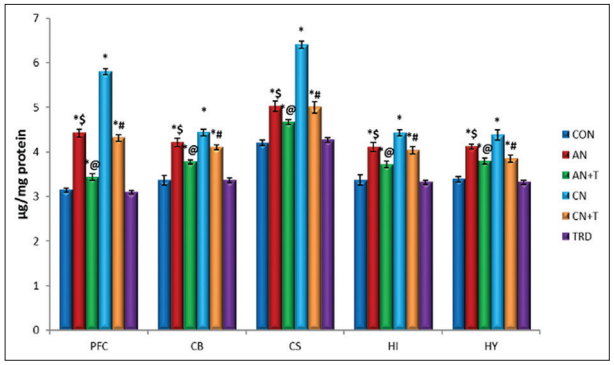

Fig. 5: Effect of *Michelia champaca* on protein carbonyl level in rat brain discrete regions after noise exposure (100 dB/4 h/day). The symbols represent statistical significance: *@#$ <p=0.05. *- Compared with saline control, @-compared with acute noise, #-compared with chronic noise. $- Acute noise compared with chronic noise

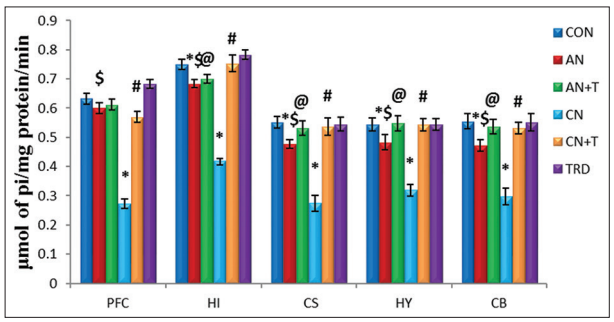

Fig. 6: Effect of noise stress on Na+K+ATPase level. Values are expressed as Mean±SD, n=6. The symbols represent statistical significance: *@#$ <p=0.05. *- Compared with saline control, @-compared with acute noise, #-compared with chronic noise. $-Acute noise compared with chronic noise. SD: Standard deviation

activity compared to control animals, with the chronic stress group exhibiting a more pronounced reduction than the acute stress group. In contrast, animals treated with *M. champaca* demonstrated a significant increase in Na^+/K^+-ATPase activity, indicating a protective effect of the treatment.

The Mg^{2+}-ATPase activity levels across the experimental groups are summarized and presented in Fig. 7. Animals exposed to both acute and chronic noise stress showed a significant decrease in Mg^{2+}-ATPase activity compared to control animals, with the chronic noise group showing a more pronounced reduction than the acute stress group. However, noise-exposed animals treated with *M. champaca* exhibited a significant increase in Mg^{2+}-ATPase activity compared to the untreated stressed groups. Notably, *M. champaca* treatment was able to restore Mg^{2+}-ATPase activity to levels comparable to the control group, suggesting its potential as a therapeutic agent against noise-induced stress.

The Ca^{2+}-ATPase activity levels in the experimental groups are summarized and presented in Fig. 8. Animals exposed to both acute and chronic noise stress showed a significant decrease in Ca^{2+}-ATPase activity compared to control animals. However, the stress groups treated with *M. champaca* demonstrated a significant increase in Ca^{2+}-ATPase activity relative to the noise-exposed groups. *M. champaca* treatment was able to restore the enzyme activity to near-normal levels comparable to those of the control group across all brain regions studied. Notably, in the acute noise group treated with *M. champaca*, no alterations were observed in any of the membrane-bound enzymes

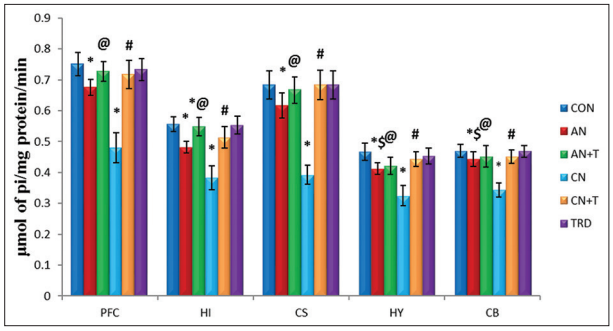

Fig. 7: Effect of noise stress on Mg2+ATPase level. Values are expressed as Mean±SD, n=6. The symbols represent statistical significance: *@#$ <p=0.05. *-Compared with saline control, @-compared with acute noise, #-compared with chronic noise. $-Acute noise compared with chronic noise. SD: Standard deviation

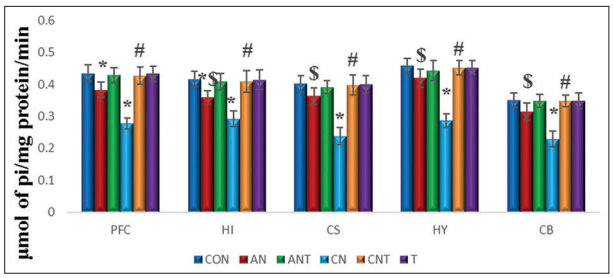

Fig. 8: Effect of noise stress on Ca2+ATPase level. Values are expressed as Mean±SD, n=6. The symbols represent statistical significance: *@#$ <p=0.05. *-Compared with saline control, @-compared with acute noise, #-compared with chronic noise. $-Acute noise compared with chronic noise. SD: Standard deviation

evaluated, indicating the effectiveness of the treatment in preserving enzymatic function under stress.

Effect of *M. Champaca* on histopathology of brain discrete regions of Wistar Albino rats on exposure to noise stress

Histology of prefrontal cortex

Histological examination of the prefrontal cortex in the noise-stressed group revealed a reduction in neuronal cell size and disruption of the normal architecture of the cortical layers. In addition, pyknotic and darkly stained nuclei were observed, indicating neuronal damage, compared to the control and *M. champaca*-treated control groups. However, treatment with *M. champaca* during noise exposure effectively prevented these alterations. The neuronal structure and cortical organization in this group remained comparable to those of the control and *M. champaca*-treated alone groups. No morphological changes were observed in the neuronal structures of either the control or *M. champaca*-treated alone groups.

Histology of hippocampus

Histological analysis of the hippocampus in the noise-exposed group revealed mature granule cells with rounded vesicular nuclei, along with the presence of pyknotic nuclei in the dentate gyrus, indicating neuronal damage, when compared to the control and *M. champaca*-treated control groups. In contrast, animals exposed to noise but treated with *M. champaca* showed no such abnormalities; no cells with dark, pyknotic nuclei were observed, and the hippocampal architecture closely resembled that of the control and *M. champaca*-treated alone groups. No morphological changes were observed in the dentate gyrus of either the control or *M. champaca*-treated control groups.

Effect of noise stress on DNA fragmentation in brain

Electrophoresis of DNA isolated from the whole brain revealed a marked increase in DNA fragmentation in the chronic noise-exposed group, indicating significant genomic damage. In contrast, treatment with *M. champaca* during chronic noise exposure reduced the smear pattern, suggesting a protective effect against stress-induced DNA damage. No DNA fragmentation was observed in the *M. champaca*-treated control group or the untreated control group. These results indicate that chronic noise stress has the potential to induce DNA damage across the whole brain, whereas acute noise stress did not result in detectable DNA fragmentation.

Effect of noise stress on HB test

The data are summarized in Figs. 11 and 12 were expressed with Mean±SD. The following parameters were assessed in the HB test head dip, fecal bolus, grooming, rearing, scratching, external ambulation, internal ambulation, and immobilization.

Number of Head Dips

The number of head dips was lower in the acute noise-stressed group compared to the control group, although the difference was not statistically significant. In contrast, the chronic noise-stressed group showed a significant increase ($p<0.05$) in head dips compared to controls. However, treatment with *M. champaca* in the chronic noise-stressed group reduced the number of head dips to levels similar to those observed in the control group.

Number of fecal pellets

A significant increase ($p<0.05$) in the number of fecal pellets was observed in the chronic noise-stressed group compared to both the control and acute stress groups. This increase was significantly reduced in the *M. champaca*-treated stress groups when compared to their respective untreated stress groups.

Grooming, rearing, and scratching behavior

Chronic stress led to a reduction in grooming, rearing, and scratching behaviors when compared to the control animals, with statistically significant decreases ($p<0.05$). The acute noise group also showed a decrease in these behaviors, though not significantly. Treatment with *M. champaca* in the chronic stress group improved all three behaviors, suggesting potential anti-anxiety effects of the extract.

External and internal ambulation

A significant reduction ($p<0.05$) in external ambulation was observed in the chronic stress group compared to controls. Both internal and external ambulation activities were significantly lower in the chronic group compared to the acute group. Treatment with *M. champaca* significantly ($p<0.05$) increased both internal and external ambulation in the chronic noise-stressed animals, bringing them to levels comparable with those of the control group.

Immobilization

The chronic stress group exhibited a significant increase ($p<0.05$) in immobilization time compared to the control group. There were also significant differences between the acute and chronic noise-stressed groups. Treatment with *M. champaca* restored immobilization behavior to normal levels, comparable to those of the control group.

Effect of noise stress on place preference test

Time spent in brighter areas

Animals exposed to chronic noise stress spent significantly less time in the brighter area compared to control animals. When compared to the acute noise-stressed group, the chronic stress group also showed a significant reduction ($p<0.05$) in time spent in the bright area. However, animals in the chronic noise-stressed group treated with *M. champaca* showed a significant improvement, spending more time in the bright area, similar to control levels.

Time spent in darker areas

Chronic noise-stressed animals spent significantly more time in the dark area compared to control animals. A notable difference was also observed between the acute and chronic noise-stressed groups. Treatment with *M. champaca* in the chronic stress group significantly ($p<0.05$) reduced the time spent in the dark area. Although animals exposed to acute stress spent more time in the dark area compared to controls, the difference was not statistically significant.

DISCUSSION

Stress arises in response to situations perceived as threatening to an organism and activates two primary physiological systems: the sympathetic nervous system and the hypothalamus–pituitary–adrenal (HPA) axis. Activation of the HPA axis results in the release of corticosteroids from the adrenal cortex.

Studies in rats have shown that auditory stimulation causes widespread brain activation through the induction of c-fos mRNA. These noise-induced excitations are transmitted subcortically through the amygdala, a key component of the auditory processing system, and subsequently activate the HPA axis. Noise exposure may lead to behavioral suppression and cause time-dependent alterations in plasma levels of catecholamines, ACTH, and cortisol.

An elevation in corticosterone levels accelerates the generation of free radicals [40]. The generation of free radicals is a fundamental feature of normal cellular functions. However, excessive generation and inadequate removal of free radicals result in destructive and irreversible cellular damage [41].

ROS are essential to normal cellular processes, including mitochondrial respiration, phagocytosis, arachidonic acid metabolism, ovulation, and fertilization. Among ROS, hydroxyl and alkoxy radicals are particularly reactive and rapidly interact with macromolecules within cells, potentially causing damage. These free radicals damage vital cellular components such as DNA, proteins, carbohydrates, and lipids, leading to cellular dysfunction and disruption of homeostasis. Proteins damaged by ROS or reactive nitrogen species (RNS) undergo structural changes and loss of enzymatic activity.

Cell membranes, rich in polyunsaturated fatty acids, are especially vulnerable to oxidative degradation, leading to the formation of products such as malondialdehyde, which disrupt normal cellular function and integrity. Noise exposure increases levels of ROS such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide. In response, the activity of antioxidants and associated enzymes is upregulated to counteract and eliminate the excess ROS [42].

The neuronal biochemical environment is particularly vulnerable to ROS due to its high concentration of unsaturated lipids, which are prone to peroxidation and oxidative modification. Free radicals target the double bonds of these fatty acids, triggering a cascade of reactions that further damage adjacent unsaturated fatty acids [43]. The brain consumes about 20% of the body's total oxygen, making it especially susceptible to peroxidation. Furthermore, it has relatively low antioxidant defenses – only about 10% of those found in the liver.

Various forms of stress have been associated with enhanced free radical generation, resulting in oxidative damage. Oxidative stress is characterized by reduced levels of antioxidant defense components – such as CAT, GSH, and total thiols – or by elevated levels of ROS and RNS in the body [44].

Noise is among the most pervasive sources of environmental stress. It is well known that an intense stress response results in the generation of ROS such as hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and superoxide anion radicals (O_2•−), which cause LPO – especially in membranes – and play a key role in tissue injury. The levels of

ROS in tissues are determined by the balance between LPO and the effectiveness of the antioxidant defense system [45].

Several studies have shown that exposure to noise frequently leads to the generation of free radicals and induces oxidative and nitrosative stress in neurons. NO, a highly reactive signaling molecule, plays a significant role in the central nervous system. It is a unique messenger that serves diverse physiological functions throughout the body. A significant increase in both H_2O_2 and NO levels has been observed in discrete brain regions. Demirel *et al*. ($p<0.05$) reported increased NO generation following 20 days of noise exposure [46]. Elevated NO levels are associated with increased nitrosative stress, leading to cellular degeneration and necrosis.

In our findings, elevated NO levels were particularly noticeable in the hippocampus and hypothalamus, (Fig. 2) suggesting that these areas are more affected by chronic noise exposure than other brain regions and by acute stress. This aligns with the findings of Lidija *et al*., who reported that increased NO production in functionally connected brain regions indicates these areas which are more susceptible to noise-induced injury [47].

The elevated H_2O_2 levels observed in the noise-exposed group (Fig. 1) indicate that $O_2\bullet-$, when dismutated by SOD, is converted into harmful ROS such as H_2O_2 and hydroxyl radicals. These findings suggest that NO and H_2O_2 levels must be tightly regulated to prevent oxidative and nitrosative damage in specific brain regions.

However, in the noise-exposed group treated with *M. champaca*, there was a significant ($p<0.05$) decrease in both NO and H_2O_2 levels, likely due to the downregulation of their activity. This protective effect is attributed to the plant's phytochemicals [48], which possess strong antioxidant properties.

A significant increase in LPO in specific brain regions further underscores the damaging effects of noise exposure. This is consistent with the findings of Samson *et al*., [20] who observed that elevated LPO products can adversely affect cellular functions, as they may be mutagenic and carcinogenic.

A significant inverse correlation was observed between malondialdehyde (a marker of LPO) and the levels of Vitamins C and E in the noise-exposed groups (Table 1), compared to the control and *M. champaca*-treated groups. Vitamins C and E work synergistically, with Vitamin E acting as a crucial lipid-soluble antioxidant that disrupts the chain reactions of LPO. Vitamin C aids in regenerating Vitamin E by reducing the α-tocopheroxyl radical back to α-tocopherol, thus preventing pro-oxidant effects.

In noise-exposed groups, levels of both Vitamins C and E were reduced, correlating with increased LPO. Treatment with *M. champaca* extract significantly lowered LPO levels, (Fig. 3) indicating its strong anti-LPO potential. This effect may be due to the bioactive compounds in the plant that enhance antioxidant defense mechanisms and disrupt LPO chains.

The GSH-dependent antioxidant pathway is vital for maintaining cell survival. GSH facilitates the regeneration of α-tocopherol (active Vitamin E) either directly or indirectly by reducing dehydroascorbate to ascorbate [49]. Low total thiol concentrations are typically indicative of accelerated turnover, a consequence of increased oxidative and nitrosative stress. This is consistent with our finding of decreased protein thiol levels in the noise-exposed group, which points to the depletion of essential thiol groups and a reduction in GSH levels."

Significant increases in SOD and GPx activities, along with decreased GR activity, were observed in specific brain regions in the noise-stressed group (Table 1). This suggests increased production of superoxide radicals. The coordinated action of enzymatic and non-enzymatic antioxidants is essential to maintain redox balance. The increase in SOD activity in the noise-exposed group supports the notion of heightened free radical production, particularly H_2O_2.

GPx and CAT detoxify peroxides with GSH serving as an electron donor. GR then reduces oxidized glutathione (GSSG) back to its active form, GSH, using NADPH as a cofactor. The reduction in GR activity in the noise-exposed group further explains the diminished GSH levels (Table 1). Literature also supports this, describing GSH as a vital thiol-containing tripeptide critical for cellular survival, [50] with its dysregulation contributing to the onset and progression of various neurodegenerative diseases.

In addition, GSH aids in detoxifying electrophilic compounds and peroxides through enzymes such as glutathione-S-transferase (GST) and GPx. These findings suggest that elevated SOD, GPx, CAT, and GST activities act as part of a compensatory response to oxidative stress, showing that the brain is attempting to maintain homeostasis.

Nevertheless, the detrimental effects of noise exposure are evident in the depletion of GSH, a critical component of the antioxidant defense system. Numerous studies have shown that alterations in antioxidant enzyme activities under stress conditions are associated with decreased GSH levels and increased LPO, ultimately leading to oxidative cell death [51].

Thus, excessive free radical production and disrupted antioxidant balance may form a link between environmental/occupational noise exposure and the development of multifactorial diseases. However, *M. champaca* extract showed neuroprotective effects by restoring antioxidant levels and potentially facilitating the reduction of GSSG back to GSH. The observed increase in GSH content may help reduce reactive oxygen and nitrogen species (RONS) generation.

The enhanced activity of antioxidant enzymes in response to acute noise exposure might represent an adaptive protective mechanism. The therapeutic efficacy of *M. champaca* was evident in the noise-exposed group treated with the extract, as it restored antioxidant levels and reduced free radicals to near-control levels in specific brain regions.

The Na^+/K^+-ATPase catalyzes the hydrolysis of ATP and couples this process to the active transport of Na^+ and K^+ across the cell membrane, thereby establishing and maintaining the transmembrane Na^+/K^+ gradient. Similarly, Ca^{2+}-ATPase facilitates the active transport of Ca^{2+}, helping to maintain low intracellular calcium concentrations essential for cellular homeostasis. Mg^{2+}-ATPases are cell surface enzymes that hydrolyze intracellular ATP [52].

Na^+/K^+-ATPase is a critical enzyme involved in neural excitability, metabolic energy production, and the uptake and release of catecholamines and serotonin [53,54]. Impairment of Na^+/K^+-ATPase activity is known to result in neural dysfunction. Several studies have shown that the structural properties and lipid composition of the synaptosomal membrane are essential for the optimal function of this enzyme, and even low concentrations of free radicals can inhibit Na^+/K^+-ATPase activity in the brain [55].

Increasing evidence suggests that excessive production of free radicals in the brain, and the resulting imbalance between oxidative species and antioxidant defenses are linked to the pathogenesis of neurodegenerative diseases [56]. One of the primary targets of ROS is membrane lipids, which undergo peroxidation. LPO not only alters the membrane lipid environment and compromises structural integrity but also impairs the activity of membrane-bound enzymes such as acetylcholinesterase and various ATPases. Inhibition of ATPase activity results in neuronal dysfunction due to disrupted cation transport and altered neurotransmitter uptake and release.

Excessive LPO can damage the phospholipid bilayer and associated proteins, making the membrane leaky. This results in an influx of Na^+

and water, accompanied by an efflux of K^+. [57] Previous studies have reported that oxidative stress [58] inhibits the activity of Na^+/K^+ and Mg^{2+}-ATPases. The oxidative damage to membrane lipids and proteins, particularly the oxidation of thiol groups, [59] may be responsible for the inactivation of Na^+/K^+-ATPase, which is rich in these groups.

In the present study, chronically stressed rats exhibited elevated levels of LPO (Fig. 3), increased formation of lipid hydroperoxides and PCs, and reduced GSH levels in the brain. These changes indicate oxidative modification of proteins likely due to excessive free radical production. In contrast, rats treated with *M. champaca* under stress conditions showed restoration of membrane enzyme activity.

GSH, the most abundant non-protein thiol, plays a key role in maintaining cellular redox status and serves as a first-line defense against oxidative stress in the brain [60]. The reduced levels of GSH observed in the chronically stressed group (Table 1) could be due to its increased utilization in response to elevated free radical levels and LPO. Administration of *M. champaca* significantly (Fig. 6-8) restored the activities of membrane-bound ATPases, suggesting that it helps preserve membrane integrity and normal brain function.

The free radical scavenging properties of *M. champaca*, along with its role in maintaining mitochondrial function, may contribute to the enhanced activity of ATPases in chronically stressed rats. This effect is likely attributable to antioxidant-rich phytochemicals such as Kaempferol is one of the key compounds present in the Michelia champaca- has been shown to enhance antioxidant activity and reduce lipid peroxidation in cell membranes as also observed by lee *et al*. Ashafaq *et al*. [62] further emphasized the growing relevance of antioxidants in oxidative stress-related disorders and their potential as therapeutic agents.

Khalid *et al*. [63] reported that administration of kaempferol significantly enhanced membrane-bound ATPase activities in the liver, heart, kidney, and erythrocytes of diabetic rats. In our study, decreased Na^+/K^+, Mg^{2+}, and Ca^{2+}-ATPase activities in the noise-stressed group (Fig. 6-8) suggest altered ionic fluxes that may disturb normal neuronal function. However, *M. champaca* treatment significantly restored these enzyme activities, demonstrating protective effects against noise-induced oxidative stress by inhibiting LPO and protein oxidation, and by enhancing endogenous antioxidant defenses in both plasma and brain tissues.

These results strongly suggest that the antioxidant and anti-lipid peroxidative properties of *M. champaca* contribute to the improved activity of ATPases and the maintenance of ionic equilibrium (Na^+/K^+, Ca^{2+}, and Mg^{2+}) in noise-induced oxidative stress. By exerting a stabilizing effect, *M. champaca* appears to protect the brain by preserving membrane structure and function.

Effect of noise stress on histological changes in discrete brain region

The present histopathological study demonstrates that noise stress induces significant cellular changes in the prefrontal cortex and hippocampus (Figs. 9 and 10). Hematoxylin and eosin-stained sections of these brain regions showed normal histoarchitecture in both the control group and the group treated with *M. champaca* alone.

In contrast, the brains of noise-stressed rats revealed varying degrees of structural alteration and less distinct tissue organization. Notably, numerous neurons appeared shrunken, pyknotic, and darkly stained, with small, irregular nuclei. Degenerated pyramidal cells exhibited darkened, shrunken cell bodies, and nuclear abnormalities. These changes were more pronounced in the chronic noise stress (Figs. 9 and 10) group compared to the acute stress group.

However, treatment with *M. champaca* extract appeared to prevent these histopathological alterations in the prefrontal cortex (Fig. 9) and hippocampus (Fig. 10), suggesting a neuroprotective effect against noise-induced damage.

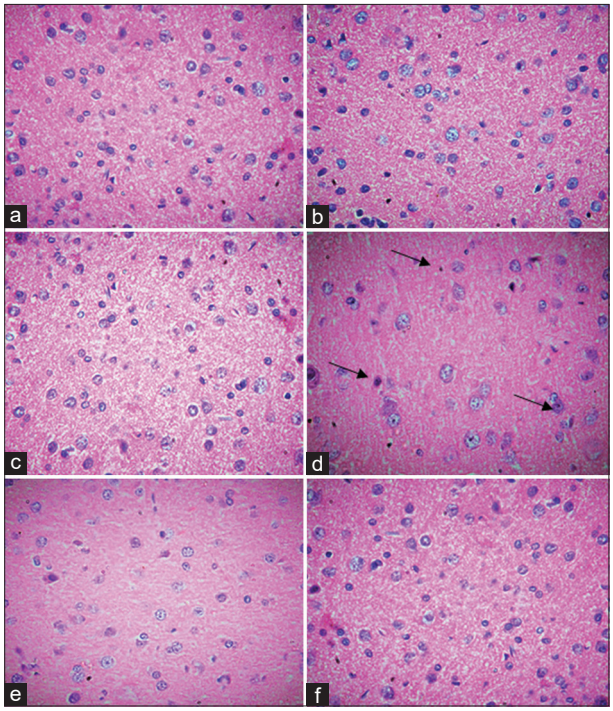

Fig. 9: Illustrates H&E-stained neuronal cells of rat's prefrontal cortex in different experimental groups at ×40 magnification. (a) Control, (b) acute noise stress alone, (c) acute noise stress + *Michelia champaca*, (d) chronic noise stress, (e) chronic noise stress+ *M. champaca*, and (f) *M. champaca* treated alone group

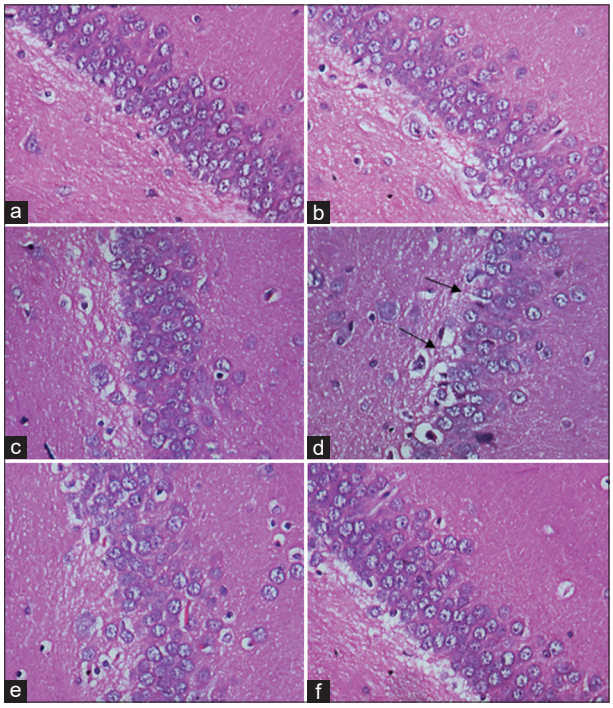

Fig. 10: Illustrates H&E-stained neuronal cells of rats Hippocampus in different experimental groups at ×40 magnification. (a) Control, (b) acute noise stress alone, (c) acute noise stress + *Michelia champaca*, (d) chronic noise stress, (e) chronic noise stress+ *M. champaca*, and (f) *M. champaca* treated alone group

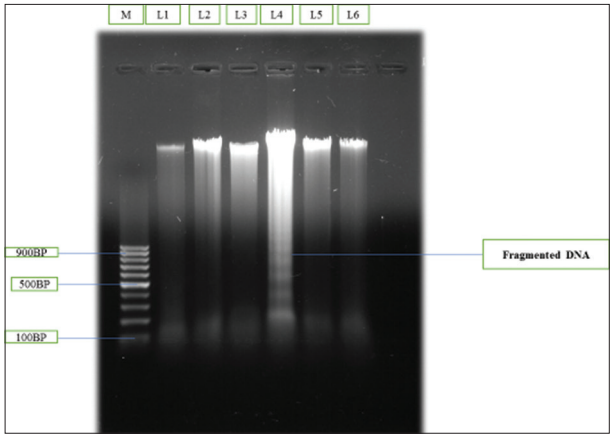

Fig. 11: Effect of *Michelia champaca* on DNA fragmentation in whole brain of noise-exposed adult Wister rats. L1-1KBLadder, L2-Control, L3-Acute noise, L4-Acute noise treated with *M. champaca* L5-Chronic noise, L6-Chronic noise treated with *M. champaca, and* L7- *M. champaca* alone

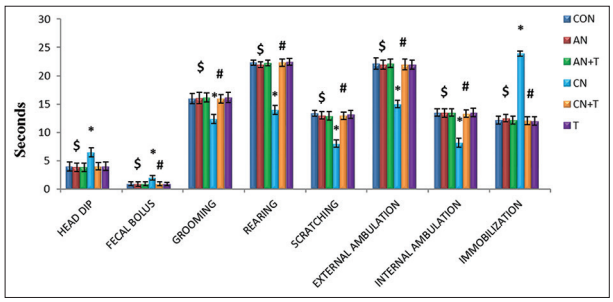

Fig. 12: Effect of noise stress on hole board test. Values are expressed as Mean±SD, n=6. The symbols represent statistical significance: *@#$ <p=0.05. *-Compared with saline control, @-compared with acute noise, #-compared with chronic noise. $-Acute noise compared with chronic noise. SD: Standard deviation

Our findings are consistent with those reported by Laijun *et al*. [64], who reported that high-frequency steady noise leads to various degrees of pathological damage in multiple organs, including the brain, liver, spleen, and heart. Similarly, Cook and Wellman [65] noted that chronic stress causes cognitive deficits along with neurochemical and morphological changes in the cortex. This may be attributed to the medial prefrontal cortex being a primary target for glucocorticoids released during the stress response.

Alaa Abousetta *et al*. [66] also observed degenerated pyramidal neurons with darkened, shrunken cell bodies, nuclear irregularities, and disrupted mitochondrial cristae in noise-exposed animals. These degenerative changes may ultimately result in neuronal death, reflected by a reduced cell population and the presence of loosely packed neurons. Neuronal degeneration and loss following noise exposure are likely due to the physiological stress imposed by excessive noise.

Noise levels exceeding 90 dB are recognized as stressors, which activates HPA axis [67]. Which in turn elevate cortisol levels that bind to glucocorticoid receptors – abundant in the hippocampus – ultimately resulting in hippocampal atrophy [68]. In addition, noise stress induces excessive production of ROS, contributing to oxidative DNA damage and the activation of apoptotic pathways [69].

The observed reduction in cell density and the presence of loosely packed neurons following noise exposure may also be due to the inhibition of adult neurogenesis. This could result from abnormal acoustic input to the brain, either due to overactivity during exposure or altered neuronal activity as a consequence of hearing loss. Furthermore, steroid release triggered by noise stress may suppress the generation of new neurons in the dentate gyrus of the hippocampus [70].

DNA fragmentation

Electrophoresis of DNA isolated from the whole brain revealed a smear pattern (Fig. 11), these findings indicate that chronic noise exposure has the potential to induce DNA damage. ROS, generated as a result of oxidative stress, are known to impair chromatin structure, causing both single- and double-strand DNA fragmentation, which ultimately leads to cell death through necrosis or apoptosis. ROS-mediated DNA fragmentation is often triggered and amplified by hydroperoxides or polyunsaturated fatty acids through LPO.

A reduction in intracellular GSH levels – caused by inhibited cysteine transport or suppressed GSH biosynthesis – can also lead to excessive ROS accumulation and subsequent cell death in certain mammalian cells [71]. This finding aligns with our study, in which oxidative stress and increased LPO were observed following noise exposure.

Neurons are particularly susceptible to oxidative stress due to their high metabolic demand to support electrical and synaptic activity. Therefore, the integrity and efficiency of DNA repair systems are critical for neuronal survival and function, especially under conditions of increased oxidative stress [72]. The link between elevated ROS levels and DNA toxicity is well-established [61]. ROS-induced DNA damage includes single-strand breaks and inter-/intra-strand crosslinks [73].

Malondialdehyde (MDA), a byproduct of LPO, is believed to contribute to cellular toxicity by forming cross-links between proteins and nucleic acids, particularly in the hippocampus following noise exposure [74]. Van Campen *et al*. [75] reported increased levels of 8-hydroxy-2'-deoxyguanosine (8OHdG) – a biomarker of oxidative DNA damage – in the brain and liver (in addition to the cochlea) of rats exposed to loud noise (120 dB), supporting the hypothesis that ROS play a key role in DNA damage in both central and peripheral organs.

These findings suggest that the link between noise exposure, oxidative stress, and DNA damage warrants further investigation, given the potential for long-term mutagenic effects [76]. The DNA fragmentation observed in the chronically stressed group in our study is consistent with findings by Gerardo Barroso *et al*. [77], who also reported that oxidative DNA damage is associated with elevated LPO, reduced antioxidant enzyme activity (GPx, GST, and GR), and decreased GSH levels.

In contrast, rats exposed to noise but treated with *M. champaca* showed reduced DNA damage, comparable to the control and *M. champaca*-alone groups. This suggests that *M. champaca* supplementation can mitigate noise-induced genotoxic effects. Previous studies have reported loss of DNA integrity in discrete brain regions of rats following exposure to 100 dBA noise for 12 h [78]. Similar ROS-induced DNA damage has been documented in the myocardium [79] and adrenal glands [80] following loud noise exposure.

Yu-Ling *et al*. [81] demonstrated that plant-derived phenolic compounds – such as phenolic acids, flavonoids, and catechins – are effective in scavenging ROS and preventing oxidative DNA damage. Recently studies have confirmed that phenolic compounds possess significant free radical scavenging capabilities. Groups have the ability to protect against free radicals [82] Antioxidants are also recognized for their protective effects against degenerative diseases, including cancer, by reducing DNA damage [83]. Pool-Zobel *et al*. [84] reported that an antioxidant-rich diet significantly lowered endogenous DNA strand breaks in lymphocytes [85]. Multiple observational and interventional studies support the role of antioxidant vitamins in decreasing DNA damage and cancer risk [86].

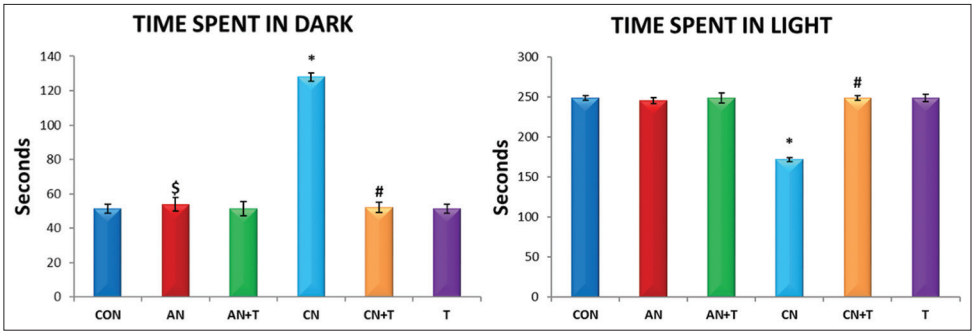

Fig. 13: (a and b) Effect of noise stress on place preference test. Values are expressed as Mean±SD, n=6. The symbols represent statistical significance: *@#$ <p=0.05. *-Compared with saline control, @-compared with acute noise, and #-compared with chronic noise. $-Acute noise compared with chronic noise. SD: Standard deviation

Hofer *et al*. [87] highlighted the protective potential of curcumin – a non-toxic, antioxidant-rich compound – alongside plant extracts from lotus, onion, *Trapa*, and sunflower, all of which showed strong protective effects against DNA damage.

Flavonoids, in particular, have demonstrated the ability to protect DNA from oxidative damage. These compounds enhance the activity of antioxidant enzymes such as CAT, GSH, SOD, GSH, and GPX and while simultaneously downregulating the expression of apoptotic proteins [88,89] Abdallah M. Kanakis *et al*. [90,91] proposed that flavonoids such as kaempferol, delphinidin, and quercetin form complexes with DNA, shielding it from ROS-induced damage. These interactions have been studied using FTIR and UV-visible spectroscopy. Other flavonoids such as hesperidin and naringenin have shown stabilizing effects on DNA in various *in vitro* models [92,93].

Tawani and Kumar [94] reported that quercetin can stabilize G-quadruplex structures in telomerase sequences, offering potential anticancer benefits. Seufi *et al*. [95] demonstrated the chemoprotective effects of quercetin in preventing DNA mutations in a hepatocellular carcinoma model. Additional studies showed quercetin protects against H_2O_2-induced DNA damage in Caco-2 and Hep G2 cells [96], as well as against pulmonary and benzo[a]pyrene-induced DNA damage in mice by inhibiting cytochrome P4501A1 [97]. In PC12 neuronal cells, quercetin inhibited both ROS production and DNA strand breaks [98]. Srivastava *et al*. [99] also noted quercetin's role in tumor suppression through cell cycle arrest and mitochondrial pathway-mediated apoptosis.

Flavonoids may also modulate DNA repair mechanisms, enhancing their protective role against various DNA-damaging agents. In some instances, DNA fragmentation may be a reversible process if cellular viability is maintained and repair pathways are activated [100].

In our study, treatment with *M. champaca* in noise-exposed rats mitigated DNA fragmentation. This protective effect is likely due to its bioactive compounds – particularly phenols and flavonoids such as quercetin and kaempferol – which appear to play a significant role in counteracting noise stress-induced DNA damage.

Effect of noise stress on anxiety level

The Light and Dark (L&D) test is a widely used animal model for screening anxiolytic or anxiogenic drugs [101], leveraging the animal's natural preference for dark environments. This paradigm is based on the innate aversion of rats to brightly lit spaces. Anxiolytic compounds reduce this aversion, [102] resulting in increased time spent in the light compartment and a greater number of transitions between the two compartments, thereby indicating the anxiolytic potential of a tested substance [103].

In the present study, rats subjected to chronic noise stress exhibited increased time spent in the dark compartment compared to the brightly lit one (Fig. 13a and b), suggesting that noise exposure induces anxiety-like behavior. However, animals exposed to noise but treated with *M. champaca* extract showed a restoration of this behavioral alteration, with a significant increase in time spent in the light compartment, similar to that of the control group.

Kuloglu *et al*. [104] emphasized that patients with anxiety disorders exhibit higher activity of antioxidant enzymes such as SOD and GPx, along with elevated LPO, suggesting that oxidative metabolism may play a role in the regulation of anxiety. Similarly, Souza *et al*. [105] reported that increased protein oxidation in the frontal cortex induces anxiety-like behavior in place preference tests, without affecting locomotor activity. Desrumaux *et al*. [106] also demonstrated that Vitamin E deficiency in the brain leads to oxidative stress and anxiogenic behavior, without impairing locomotion in mice.

Our findings are consistent with these reports. Chronic noise-exposed animals showed a significant increase in SOD, GPx, and LPO levels, accompanied by a reduction in Vitamin E and Vitamin C levels across all brain regions studied. These animals also exhibited decreased overall mobility and ambulation, indicative of heightened anxiety. Treatment with *M. champaca* effectively reversed these changes, supporting the anxiolytic potential of the extract.

The anxiolytic-like effects of *M. champaca* were further validated using the HB test. This test has recently gained popularity as a reliable model of anxiety and offers a simple method to assess the behavioral response of rodents to a novel environment [107]. Head-dipping behavior in the HB test is particularly sensitive to changes in the animal's emotional state [108]. In the present study, *M. champaca* treatment significantly (Fig. 12) improved behavioral parameters such as fecal bolus count, immobilization time, rearing, grooming, ambulation, and head dips. These improvements further reflect the anxiolytic efficacy of the extract, in agreement with previous findings [109].

CONCLUSION

The results indicate that chronic noise stress induces oxidative damage in the rat brain. However, treatment with the methanolic extract of *M. champaca* effectively restored the activity and levels of antioxidant defense mechanisms in the brain under noise stress. Notably, the *M. champaca*-treated group showed protection against cortical neuronal alterations typically observed under noise exposure. Furthermore, rats in the *M. champaca*-treated group did not exhibit anxiety-like behaviors despite being subjected to noise stress.

This protective effect is likely attributable to the presence of bioactive compounds in *M. champaca* with anxiolytic properties and a strong ability to enhance antioxidant status in stressed animals. These findings suggest that the adaptogenic effects of *M. champaca* are primarily mediated through its antioxidant activity. Based on these findings, the present study suggests that supplementation with *M. champaca* may be beneficial in managing oxidative stress, anxiety disorders, and

neurodegeneration associated with environmental stressors such as noise. It can thus be considered a potential neuroprotective agent for preventing noise-induced cognitive impairments in everyday life.

AUTHORS' CONTRIBUTIONS

First author – Dr. Malathi. S – experiments and manuscript preparation and formulation of experimental design were done. Second author – Dr. Vidyashree H.M – Manuscript edition and formulation of experimental design were done corresponding and third author – Dr. Ravindran Rajan – formulation of experimental design and approved.

CONFLICTS OF INTEREST

None.

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REFERENCES

1. Ravindran R, Rathinasamy SD, Samson J, Senthilvelan M. Noise-stress-induced brain neurotransmitter changes and the effect of *Ocimum sanctum* (Linn) treatment in albino rat's. J Pharmacol Sci. 2005;98(4):354-60. doi: 10.1254/jphs.fp0050127, PMID 16113498
2. Endo T, Nakagawa T, Iguchi F, Kita T, Okano T, Sha SH, *et al*. Elevation of superoxide dismutase increases acoustic trauma from noise exposure. Free Radic Biol Med. 2005;38(4):492-8. doi: 10.1016/j.freeradbiomed.2004.11.008, PMID 15649651
3. Manikandan S, Devi RS. Antioxidant property of alphfa-asarone against noise-stress-induced changes in different regions of rat brain. Pharmacol Res. 2005;52(6):467-74. doi: 10.1016/j.phrs.2005.07.007, PMID 16140022
4. Elsayed NM, Gorbunov NV. Interplay between high energy impulse noise (blast) and antioxidants in the lung. Toxicology. 2003;189(1-2):63-74. doi: 10.1016/s0300-483x(03)00153-7, PMID 12821283
5. Mercan U. Importance of free radicals in toxicology. YYUVet Fak Derg. 2004;15(1-2):91-6.
6. Contestabile A. Oxidative stress in neurodegeneration: Mechanism and therapeutic perspectives. Curr Top Med Chem. 2001;1(6):553-68. doi: 10.2174/1568026013394723, PMID 11895131
7. Adhirai M, Selvam R. Effect of cyclosporin A on tissue lipid peroxidation and membrane bound phosphatases in hyperoxaluric rat and the protection by vitamin E pretreatment. Jpn J Med Sci Biol. 1997;50(1):9-17. doi: 10.7883/yoken1952.50.9, PMID 9354965
8. Jorgensen PL, Hakansson KO, Karlish SJ. Structure and mechanism of Na,K-ATPase: Functional sites and their interactions. Annu Rev Physiol. 2003;65:817-49. doi: 10.1146/annurev.physiol.65.092101.142558, PMID 12524462
9. Gamaro GD, Streck EL, Matté C, Prediger ME, Wyse AT, Dalmaz C. Reduction of hippocampal Na+, K+-ATPase activity in rats subjected to an experimental model of depression. Neurochem Res. 2003;28(9):1339-44. doi: 10.1023/a:1024988113978, PMID 12938855
10. Hernández-R J, Na. Na+/K(+)-ATPase regulation by neurotransmitters. Neurochem Int. 1992;20(1):1-10. doi: 10.1016/0197-0186(92)90119-c, PMID 1363908
11. Devasagayam TP, Tilak JC. Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidants in human health: Current status and future prospects. J Assoc Physicians India. 2004;52:794-804.
12. Kirtikar KR, Basu BD. Indian Medicinal Plants. Uttaranchal. 2nd ed., Vol. 8. Oriental Enterprises; 2001. p. 2604.
13. Sobhagini N, Soumit KB, Malaya KM. Ethano-medicobotanical survey of Kalahandi district of Orissa. Indian J Trad Knowl. 2004;3:72-9.
14. Dwajani S, Shanbhag TV. *Michelia champaca*: Wound healing activity in immuno suppressed Rats J Altern Med. 2009;7:6.
15. Khan MR, Kihara M, Omoloso AD. Antimicrobial activity of *Michelia champaca*. Fitoterapia. 2002;73(7-8):744-8. doi: 10.1016/s0367-326x(02)00248-4, PMID 12490248
16. Jarald EE, Joshi SB, Jain DC. Antidiabetic activity of flower buds of *Michelia champaca* Linn. Indian J Pharmacol. 2008;40(6):256-60. doi: 10.4103/0253-7613.45151, PMID 21279181
17. Vimala R, Nagarajan S, Alam M, Susan T, Joy S. Anti-inflammatory and antipyretic activity of *Michelia champaca* Linn., (White variety), Ixora brachiate Roxb. and *Rhynchosia cana* (Wild.) D.C. flower extract. Indian J Exp Biol. 1997;131(12):1310-4.
18. Hasan SM, Hossain MM, Akter R. Jamila M, Mazumder ME, Rahman S. DPPH free radical scavenging activity of some Bangladeshi medicinal plants. J Med Plants Res. 2009;3:875-9.
19. Rangasamy O, Raoelison G, Rakotoniriana FE, Cheuk K, Urverg-Ratsimamanga S, Quetin-Leclercq J, *et al*. Screening for anti-infective properties of several medicinal plants of the *Mauritians flora*. J Ethnopharmacol. 2007;109(2):331-7. doi: 10.1016/j.jep.2006.08.002
20. Samson J, Sheela Devi R, Ravindran R, Senthilvelan M. Effect of noise stress on free radical scavenging enzymes in brain. Environ Toxicol Pharmacol. 2005;20(1):142-8. doi: 10.1016/j.etap.2004.12.059, PMID 21783581
21. Glowinski J, Iversen LL. Regional studies of catecholamines in the rat brain. I. The disposition of [3H]norepinephrine, [3H]dopamine and [3H] dopa in various regions of the brain. J Neurochem. 1966;13(8):655-69. doi: 10.1111/j.1471-4159.1966.tb09873.x, PMID 5950056
22. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8. doi: 10.1016/0003-2697(79)90738-3, PMID 36810
23. Sedlack J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;25:192-205.
24. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, *et al*. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 1990;186:464-78. doi: 10.1016/0076-6879(90)86141-h, PMID 1978225
25. Moshage H, Kok B, Huizenga JR, Jansen PL. Nitrite and nitrate determinations in plasma: A critical evaluation. Clin Chem. 1995;41(6 Pt 1):892-6. doi: 10.1093/clinchem/41.6.892, PMID 7768008
26. Pick E, Keisari Y. A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. J Immunol Methods. 1980;38(1-2):161-70. doi: 10.1016/0022-1759(80)90340-3, PMID 6778929
27. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. J Biochem. 1974;47:469-74.
28. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972;47(2):389-94. doi: 10.1016/0003-2697(72)90132-7, PMID 4556490
29. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science. 1973;179(4073):588-90. doi: 10.1126/science.179.4073.588, PMID 4686466
30. Horn HD, Burns FH. Assay of glutathione reductase activity. In: Bergemeyer HV, editor. Methods of Enzymatic Analysis. New York: Academic Press; 1978. p. 142-6.
31. Moron MS, Difieree JW, Mannervik KB. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta. 1979;582:67-8.
32. Omaye ST, Turnbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Methods Enzymol. 1979;62:3-11.
33. Desai ID. Vitamin E analysis methods for animal tissues. Methods Enzymol. 1984;105:138-47. doi: 10.1016/s0076-6879(84)05019-9, PMID 6727662
34. Bloj B, Morero RD, Farias RN, Trucco RE. Membrane lipid fatty acids and regulation of membrane-bound enzymes. Allosteric behaviour of erythrocyte Mg 2+ -ATPase, (Na + +K +)-ATPase and acetylcholinesterase from rats fed different fat-supplemented diets. Biochim Biophys Acta. 1973;311:67-79.
35. Hjerten S, Pan H. Purification and characterization of two forms of a low-affinity Ca2+-ATPase from erythrocyte membranes. Biochim Biophys Acta. 1983;728:281-8.
36. Ohnishi T, Suzuki T, Suzuki Y, Ozawa K. A comparative study of plasma membrane Mg2+ -ATPase activities in normal, regenerating and malignant cells. Biochim Biophys Acta. 1982;684:67-74.
37. Borriello A, Roberto R, Della Ragione F, Iolascon A. Proliferate and survive: Cell division cycle and apoptosis in human neuroblastoma. Haematologica. 2002;87(2):196-214. PMID 11836171
38. Salim S, Sarraj N, Taneja M, Saha K, Tejada-Simon MV, Chugh G. Moderate treadmill exercise prevents oxidative stress-induced anxiety-like behavior in rats. Behav Brain Res. 2010;208:545-52.
39. Bilkei-Gorzó A, Gyertyán I. Some doubts about the basic concept of hole-board test. Neurobiology (Bp). 1996;4(4):405-15. PMID 9200132
40. McIntosh LJ, Sapolsky RM. Glucocorticoids increase the accumulation of reactive oxygen species and enhance adriamycin-induced toxicity in neuronal culture. Exp Neurol. 1996;141(2):201-6. doi: 10.1006/exnr.1996.0154, PMID 8812153
41. Lopaczynski W, Zeisel SH. Antioxidants, programmed cell death,

and cancer. Nutr Res. 2001;21(1-2):295-307. doi: 10.1016/S0271-5317(00)00288-8
42. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition. 2002;18(10):872-9. doi: 10.1016/s0899-9007(02)00916-4, PMID 12361782
43. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol. 2009;7(1):65-74. doi: 10.2174/157015909787602823, PMID 19721819
44. Hacioglu G, Senturk A, Ince I, Alver A. Assessment of oxidative stress parameters of brain-derived neurotrophic factor heterozygous mice in acute stress model. Iran J Basic Med Sci. 2016;19(4):388-93. PMID 27279982
45. Anbuselvam C, Vijayavel K, Balasubramanian MP. Protective effect of Operculina turpethum against 7,12-dimethyl benz(a)anthracene induced oxidative stress with reference to breast cancer in experimental rats. Chem Biol Interact. 2007;168(3):229-36. doi: 10.1016/j.cbi.2007.04.007, PMID 17531963
46. Demirel R, Mollaoğlu H, Yeşilyurt H, Üçok K, Ayçiçek A, Akkaya M, *et al*. Noise induces oxidative stress in rat. Eur J Gen Med. 2009;6(1):20-4. doi: 10.29333/ejgm/82631
47. Radenovic L, Selakovic V, Janac B, Todorovic D. Effect of glutamate antagonists on nitric oxide production in rat brain following intra hippocampal injection. Arch Biol Sci (Beogr). 2007;59(1):29-36. doi: 10.2298/abs0701029r
48. Wei LS, Wee W, Siong JY, Syamsumir DF. Characterization of antimicrobial, antioxidant, anticancer property and chemical composition of *Michelia champaca* seed and flower extracts. J Pharm Sci. 2011;4:19-24.
49. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44-84. doi: 10.1016/j.biocel.2006.07.001, PMID 16978905
50. Allen EM, Mieyal JJ. Protein-thiol oxidation and cell death: Regulatory role of glutaredoxins. Antioxid Redox Signal. 2012;17(12):1748-63. doi: 10.1089/ars.2012.4644, PMID 22530666
51. Ahmad A, Rasheed N, Chand K, Maurya R, Banu N, Palit G. Restraint stress-induced central monoaminergic and oxidative changes in rats and their prevention by novel *Ocimum sanctum* compounds. Indian J Med Res. 2012;135(4):548-54. PMID 22664506
52. Sabbadini RA, Dahms AS. Biochemical properties of isolated transverse tubular membranes. J Bioenerg Biomembr. 1989;21(2):163-213. doi: 10.1007/BF00812068, PMID 2473982
53. Mata M, Hieber V, Beaty M, Clevenger M, Fink DJ. Activity-dependent regulation of Na+,K+-ATPase an isoform mRNA expression *in vivo*. J Neurochem. 1992;59(2):622-6. doi: 10.1111/j.1471-4159.1992.tb09415.x, PMID 1321232
54. Hernandez R, Na B. Brain Na+,K+-ATPase activity possibly regulated by a specific serotonin receptor. Brain Res. 1989;408:399-402.
55. Lees GJ. Inhibition of sodium-potassium-ATPase: A potentially ubiquitous mechanism contributing to central nervous system neuropathology. Brain Res Brain Res Rev. 1991;16(3):283-300. doi: 10.1016/0165-0173(91)90011-v, PMID 1665097
56. Halliwell B. Oxidative stress and neurodegeneration: Where are we now? J Neurochem. 2006;97(6):1634-58. doi: 10.1111/j.1471-4159.2006.03907.x, PMID 16805774
57. Smejtek P, Paulis-Illangasekare M. Modification of ion transport in lipid bilayer membranes in the presence of 2,4-dichlorophenoxyacetic acid. I. Enhancement of cationic conductance and changes of the kinetics of nonactin-mediated transport of potassium. Biophys J. 1979;26(3):441-66. doi: 10.1016/S0006-3495(79)85264-9, PMID 263687
58. Hazarika A, Sarkar SN, Kataria M. Subacute toxicity of anilofos, a new organophosphorus herbicide in male rats: Effect on lipid peroxidation and ATPase activity. Indian J Exp Biol. 2001;39(11):1113-7. PMID 11906103
59. Unlüçerçi Y, Koçak H, Seferoğlu G, BekpInar S. The effect of aminoguanidine on diabetes-induced inactivation of kidney Na(+),K(+)- ATPase in rats. Pharmacol Res. 2001;44(2):95-8. doi: 10.1006/phrs.2001.0842, PMID 11516257
60. Dringen R, Gutterer JM, Hirrlinger J. Glutathione metabolism in brain metabolic interaction between astrocytes and neurons in the defense against reactive oxygen species. Eur J Biochem. 2000;267(16):4912-6. doi: 10.1046/j.1432-1327.2000.01597.x, PMID 10931173
61. Lee YJ, Suh KS, Choi MC, Chon S, Oh S, Woo JT, *et al*. Kaempferol protects HIT-T15 pancreatic beta cells from 2-deoxy-D-ribose-induced oxidative damage. Phytother Res. 2010;24(3):419-23. doi: 10.1002/ptr.2983, PMID 19827031
62. Ashafaq M, Varshney L, Khan MH, Salman M, Naseem M, Wajid S, *et al*. Neuromodulatory effects of hesperidin in mitigating oxidative stress in streptozotocin induced diabetes. BioMed Res Int. 2014;2014:249031. doi: 10.1155/2014/249031, PMID 25050332
63. Al-Numair KS, Veeramani C, Alsaif MA, Chandramohan G. Influence of kaempferol, a flavonoid compound, on membrane-bound ATPases in streptozotocin-induced diabetic rats. Pharm Biol. 2015;53(9):1372-8. doi: 10.3109/13880209.2014.982301, PMID 25853957
64. Xue L, Zhang D, Yibulayin X, Wang T, Shou X. Effects of high frequency noise on female rat's multi-organ histology. Noise Health. 2014;16(71):213-7. doi: 10.4103/1463-1741.137048, PMID 25033787
65. Cook SC, Wellman CL. Chronic stress alters dendritic morphology in rat medial prefrontal cortex. J Neurobiol. 2004;60(2):236-48. doi: 10.1002/neu.20025, PMID 15266654
66. Abousetta A, Makhlouf NA, El-Beshbishy RA. The effects of concomitant Ginkgo intake on noise induced hippocampus injury. Possible auditory clinical correlate. Egypt J Ear Nose Throat Allied Sci. 2014;15(3):231-9. doi: 10.1016/j.ejenta.2014.05.003
67. Jáuregui-Huerta F, Garcıa-Estrada J, Ruvalcaba-Delgadillo Y, Trujillo X, Huerta M, Feria-Velasco A, *et al*. Chronic exposure of juvenile rats to environmental noise impairs hippocampal cell proliferation in adulthood. Noise Health. 2011;13(53):286-91. doi: 10.4103/1463-1741.82961, PMID 21768732
68. García-Segura LM. Hormones and Brain Plasticity. Vol. 17. Oxford: Oxford University Press; 2009. p. 170-1. doi: 10.1093/acprof: oso/9780195326611.001.0001
69. Dharini S. Effect of *Acorus calamus* Linn and its active principle on noise stress-induced changes on immune system. Int J Biomed Adv Res. 2012;3:494-501.
70. Kraus KS, Mitra S, Jimenez Z, Hinduja S, Ding D, Jiang H, *et al*. Noise trauma impairs neurogenesis in the rat hippocampus. Neuroscience. 2010;167(4):1216-26. doi: 10.1016/j.neuroscience.2010.02.071, PMID 20206235
71. Higuchi Y. Chromosomal DNA fragmentation in apoptosis and necrosis induced by oxidative stress. Biochem Pharmacol. 2003;66(8):1527-35. doi: 10.1016/s0006-2952(03)00508-2, PMID 14555231
72. Liu D, Croteau DL, Souza-Pinto N, Pitta M, Tian J, Wu C, *et al*. Evidence that OGG1 glycosylase protects neurons against oxidative DNA damage and cell death under ischemic conditions. J Cereb Blood Flow Metab. 2011;31(2):680-92. doi: 10.1038/jcbfm.2010.147, PMID 20736962
73. Caraceni P, De Maria N, Ryu HS, Colantoni A, Roberts L, Maidt ML, *et al*. Proteins but not nucleic acids are molecular targets for the free radical attack during reoxygenation of rat hepatocytes. Free Radic Biol Med. 1997;23(2):339-44. doi: 10.1016/s0891-5849(96)00571-0, PMID 9199897
74. Cheng L, Wang SH, Chen QC, Liao XM. Moderate noise induced cognition impairment of mice and its underlying mechanisms. Physiol Behav. 2011;104(5):981-8. doi: 10.1016/j.physbeh.2011.06.018, PMID 21726571
75. Van Campen LE, Murphy WJ, Franks JR, Mathias PI, Toraason MA. Oxidative DNA damage is associated with intense noise exposure in the rat. Hear Res. 2002;164(1-2):29-38. doi: 10.1016/s0378-5955(01)00391-4, PMID 11950522
76. Emerit I. Reactive oxygen species, chromosome mutation, and cancer: Possible role of clastogenic factors in carcinogenesis. Free Radic Biol Med. 1994;16(1):99-109. doi: 10.1016/0891-5849(94)90246-1, PMID 8300000
77. Barroso G, Morshedi M, Oehninger S. Analysis of DNA fragmentation, plasma membrane translocation of phosphatidylserine and oxidative stress in human spermatozoa. Hum Reprod. 2000;15(6):1338-44. doi: 10.1093/humrep/15.6.1338, PMID 10831565
78. Frenzilli G, Ryskalin L, Ferrucci M, Cantafora E, Chelazzi S, Filippo S, *et al*. Loud noise exposure produces DNA, neurotransmitter and morphological damage within specific brain areas. Front Neuroanat. 2017;11(49):1-16.
79. Lenzi P, Frenzilli G, Gesi M, Ferrucci M, Lazzeri G, Fornai F, *et al*. DNA damage associated with ultrastructural alterations in rat myocardium after loud noise exposure. Environ Health Perspect. 2003;111(4):467-71. doi: 10.1289/ehp.5847, PMID 12676600
80. Frenzilli G, Lenzi P, Scarcelli V, Fornai F, Pellegrini A, Soldani P, *et al*. Effects of loud noise exposure on DNA integrity in rat adrenal gland. Environ Health Perspect. 2004;112(17):1671-2. doi: 10.1289/ehp.7249, PMID 15579411
81. Lee YL, Yang JH, Mau JL. Antioxidant properties of water extracts from Monascus fermented soybeans. Food Chem. 2008;106(3):1128-37. doi: 10.1016/j.foodchem.2007.07.047

82. Tiwari A. Comparative analysis of phytochemicals in acacia catechu bark extracts from Guna district, Madhya Pradesh. Int J Curr Pharm Res. 2024;16(3):76-81. doi: 10.22159/ijcpr.2024v16i3.4074
83. Bub A, Watzl B, Blockhaus M, Briviba K, Liegibel U, Müller H, *et al*. Fruit juice consumption modulates antioxidative status, immune status, and DNA damage. J Nutr Biochem. 2003;14(2):90-8. doi: 10.1016/s0955-2863(02)00255-3, PMID 12667600
84. Pool-Zobel BL, Bub A, Müller H, Wollowski I, Rechkemmer G. Consumption of vegetables reduces genetic damage in humans: First results of a human intervention trial with carotenoid-rich foods. Carcinogenesis. 1997;18(9):1847-50. doi: 10.1093/carcin/18.9.1847, PMID 9328185
85. Pool-Zobel BL, Bub A, Liegibel UM, Treptow-van Lishaut S, Rech kemmer G. Mechanisms by which vegetable consumption reduces genetic damage in humans. Cancer Epidemiol Biomarkers Prev. 1998;7: 891–9.
86. Brennan LA, Morris GM, Wasson GR, Hannigan BM, Barnett YA. The effect of vitamin C or vitamin E supplementation on basal and H2O2-induced DNA damage in human lymphocytes. Br J Nutr. 2000;84:195-202.
87. Hofer T, Karlsson HL, Möller L. DNA oxidative damage and strand breaks in young healthy individuals: A gender difference and the role of life style factors. Free Radic Res. 2006;40(7):707-14. doi: 10.1080/10715760500525807, PMID 16983997
88. Abdallah M, Neseem DI, Elgazayerly ON, Abdelbary AA. Topical delivery of quercetin loaded transfersomes for wound treatment: *In vitro* and *in vivo* evaluation. Int J App Pharm. 2021;13(5):189-97. doi: 10.22159/ijap.2021v13i5.41345
89. Idacahyati K, Nurdianti L, Husni SS, Gustaman F, Wulandari WT. Nephroprotective activity of ethanol extract of kirinyuh (*Chromolaena odorata* L.) in gentamicin induced nephrotoxicity in Wistar rats. Int J App Pharm. 2021;2021(13 Special Issue 3):53-6. doi: 10.22159/ijap.2021.v13s3.11
90. Kanakis CD, Tarantilis PA, Polissiou MG, Tajmir-Riahi HA. Interaction of antioxidant flavonoids with tRNA: Intercalation or external binding and comparison with flavonoid-D1A adducts. DNA Cell Biol. 2006;25(2):116-23. doi: 10.1089/dna.2006.25.116, PMID 16460235
91. Kanakis CD, Tarantilis PA, Polissiou MG, Diamantoglou S, Tajmir-Riahi HA. DNA interaction with naturally occurring antioxidant flavonoids quercetin, kaempferol, and delphinidin. J Biomol Struct Dyn. 2005;22(6):719-24. doi: 10.1080/07391102.2005.10507038, PMID 15842176
92. Bi S, Qiao C, Song D, Tian Y, Gao D, Sun Y, *et al*. Study of interactions of flavonoids with DNA using acridine orange as a fluorescence probe. Sens Actuators B. 2006;119(1):199-208. doi: 10.1016/j.snb.2005.12.014
93. Hegde AH, Prashanth SN, Seetharamappa J. Interaction of antioxidant flavonoids with calf thymus DNA analyzed by spectroscopic and electrochemical methods. J Pharm Biomed Anal. 2012;63:40-6. doi: 10.1016/j.jpba.2012.01.034, PMID 22349882
94. Tawani A, Kumar A. Structural Insight into the interaction of Flavonoids with human telomeric sequence. Sci Rep. 2015;5:17574. doi: 10.1038/srep17574, PMID 26627543
95. Seufi AM, Ibrahim SS, Elmaghraby TK, Hafez EE. Preventive effect of the flavonoid, quercetin, on hepatic cancer in rats via oxidant/antioxidant activity: Molecular and histological evidences. J Exp Clin Cancer Res. 2009;28(1):80. doi: 10.1186/1756-9966-28-80, PMID 19519916
96. Aherne SA, O'Brien NM. Protection by the flavonoids myricetin, quercetin, and rutin against hydrogen peroxide-induced DNA damage in Caco-2 and Hep G2 cells. Nutr Cancer. 1999;34(2):160-6. doi: 10.1207/S15327914NC3402_6, PMID 10578483
97. Jin NZ, Zhu YP, Zhou JW, Mao L, Zhao RC, Fang TH, *et al*. Preventive effects of quercetin against benzo[a]pyrene-induced DNA damages and pulmonary precancerous pathologic changes in mice. Basic Clin Pharmacol Toxicol. 2006;98(6):593-8. doi: 10.1111/j.1742-7843.2006.pto_382.x, PMID 16700823
98. Silva JP, Gomes AC, Coutinho OP. Oxidative DNA damage protection and repair by polyphenolic compounds in PC12 cells. Eur J Pharmacol. 2008;601(1-3):50-60. doi: 10.1016/j.ejphar.2008.10.046, PMID 18996367
99. Srivastava S, Somasagara RR, Hegde M, Nishana M, Tadi SK, Srivastava M, *et al*. Quercetin, a natural flavonoid interacts with DNA, arrests cell cycle and causes tumor regression by activating mitochondrial pathway of apoptosis. Sci Rep. 2016;6:24049. doi: 10.1038/srep24049, PMID 27068577
100. Sancar A. DNA repair in humans. Annu Rev Genet. 1995;29(1):69-105. doi: 10.1146/annurev.ge.29.120195.000441
101. Mi XJ, Chen SW, Wang WJ, Wang R, Zhang YJ, Li WJ, *et al*. Anxiolytic-like effect of paeonol in mice. Pharmacol Biochem Behav. 2005;81(3):683-7. doi: 10.1016/j.pbb.2005.04.016, PMID 15970315
102. Keerthi Priya CS, Malathi S, Rajan R. Effect of sleep deprivation on reminiscence and apprehension behavior in Wistar albino rats. Asian J Pharm Clin Res. 2020;13(1):196-201.
103. Yadav AV, Kawale LA, Nade VS. Effect of *Morus alba* L. (mulberry) leaves on anxiety in mice. Indian J Pharmacol. 2008;40(1):32-6. doi: 10.4103/0253-7613.40487, PMID 21264159
104. Kuloglu M, Atmaca M, Tezcan E, Gecici O, Tunckol H, Ustundag B. Antioxidant enzyme activities and malondialdehyde levels in patients with obsessive-compulsive disorder. Neuropsychobiology. 2002a;46(1):27-32. doi: 10.1159/000063573, PMID 12207144
105. Souza CG, Moreira JD, Siqueira IR, Pereira AG, Rieger DK, Souza DO, *et al*. Highly palatable diet consumption increases protein oxidation in rat frontal cortex and anxiety-like behavior. Life Sci. 2007;81(3):198-203. doi: 10.1016/j.lfs.2007.05.001, PMID 17574275
106. Desrumaux C, Risold PY, Schroeder H, Deckert V, Masson D, Athias A, *et al*. Phospholipid transfer protein (PLTP) deficiency reduces brain vitamin E content and increases anxiety in mice. FASEB J. 2005;19(2):296-7. doi: 10.1096/fj.04-2400fje, PMID 15576481
107. Takeda H, Tsuji M, Matsumiya T. Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. Eur J Pharmacol. 1998;350(1):21-9. doi: 10.1016/S0014-2999(98)00223-4
108. Nolan NA, Parkes MW. The effects of benzodiazepines on the behaviour of mice on a hole-board. Psychopharmacologia. 1973;29(3):277-86. doi: 10.1007/BF00414043, PMID 4702279
109. Hranilovic D, Cicin-Sain L, Bordukalo-Niksic T, Jernej B. Rats with constitutionally upregulated/downregulated platelet 5HT transporter: Differences in anxiety-related behavior. Behav Brain Res. 2005;165(2):271-7. doi: 10.1016/j.bbr.2005.07.008, PMID 16139900