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ALTERATIONS IN COCHLEAR ANTIOXIDANT LEVELS & HAIR CELL PROTEINS OF SPRAGUE DAWLEY (SD) RATS EXPOSED TO CHRONIC NOISE EXPOSURE

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ABSTRACT
The sound that is beyond the normal range is considered noise. Noise is supposed to be an alarming environmental pollutant. It also affects various bodily functions. Exposure to noise over a long period leads to permanent hearing impairment and cochlear dysfunction known as Noise-Induced Hearing Loss (NIHL). The current aim of the study is to observe the cochlear proteins, biochemical changes in the inner ear after chronic noise exposure and the role of a synthetic steroid (Prednisolone) when given at a low dose as treatment. Sprague Dawley (SD) rats weighing about 250-300g were grouped into 4 groups: Group I- Control, Group II- Chronic Noise Exposure (100dBA 4 hours/day for 30 days), Group III -Treatment (Prednisolone,1mg/kg b.w. administered orally, dissolved in 0.9% Saline given for 7 days after the noise exposure), Group IV- Drug only. After 30 days of noise exposure, anxiety behaviour, such as the Open Field Test (OFT) and Elevated Plus Maze (EPM), has been observed, and the results are interpreted using the software ANYMAZE. The animals are then euthanised, then the inner ear is harvested for antioxidant assays, Plasma Corticosterone estimation and protein estimations using the ELISA kits. Our study demonstrates the behavioural changes and protein estimations which was altered in the noise-exposed groups that haven't shown effectiveness against the treatment with a low dose of prednisolone, while the biochemical assay observed in the inner ear shows reduced oxidative stress with the treatment of prednisolone. From the results obtained, it has been concluded that a low dose of prednisolone can be used to lower the oxidative stress alone, but furthermore, more studies should be carried out to check the negative effect on behaviour and protein estimations.

KEYWORDS: Hearing loss, Cochlear Hair cells, Prednisolone, inner ear antioxidants. Chronic noise exposure.

INTRODUCTION
The ultimate purpose of the sound is to alert and warn about the surroundings. The major organ involved in the hearing process is the inner ear; its direct connection to the "fight or flight" neural mechanism is involved in the evocation of emotions via the autonomic nervous system (ANS). Because of this protective machinery, sound waves are registered even in sleep.[1] The receptor organ for hearing is the hydromechanical frequency analyser, named the cochlea, situated in the inner ear, which performs the real-time spectral decomposition of the acoustic signals.[2] The sensory cells for audition are the hair cells present in the inner ear that convert sound waves into receptor potential. The organ of Corti contains two types of specialised hair cells, outer hair cells (OHC) and inner hair cells (IHC). The outer hair cells serve the function of motor protein, and the inner hair cells act as primary receptor cells for audition.[3] The mammalian inner ear hair cell has lost its spontaneous regenerating capacity.[4] When compared to other sensory organs, the cochlea has a smaller number of hair cells, 17000 hair cells, since the absence of surplusage regeneration, even a lack of a few thousand hair cells, causes irreversible damage to the cochlea and the hearing mechanism.[5] There are many proteins present in the cochlear hair cells for the transduction of sound waves from the inner ear to the auditory nerves. One among them is the Otoferlin, a C_2-domain transmembrane

protein responsible for the synaptic exocytosis in the inner hair cells, deficiency of this Otoferlin profoundly found to deaf.[6] This protein is encoded by the OTOF gene, where any mutation in this gene causes nonsyndromic deafness (DFNB9).[7] Otoferlin interrelates with Calcium, SNAP-25 and syntaxin-1 to facilitate the exocytotic discharge of neurotransmitter glutamate. Without the presence of otoferlin, even though the conversion of sound waves into an electrical signal is achieved, this signal does not reach the brain.[8] The next protein target of this study is the motor molecules that mediate the cochlear amplification[9] and electromobility of the OHC, termed Prestin, encoded by the gene family SLC26A. It is a new type of motor protein, and its function is entirely different from the other motor proteins. The function relies directly on voltage-displacement conversion, where it operates at microsecond rates, whereas other motor protein has enzymatic functions.[10] If there are any homeostatic alterations to the membrane potential, OHCs act by rapidly changing the length axially at the acoustic frequencies up to 80 kHz. This prestin is responsible for the fast eM (deemed electromotility) of the OHCs.[11]

Sound beyond the safety limit is considered noise. Environmental noise has unavoidably become a major threat to many physiological and psychological illnesses.[12] This noise, being a stressor, affects both the auditory and non-auditory functions, yet its effect is not sudden and calamitous; but it is considered a promising threat.[13] Overexposure to noise causes the inflammation of the cochlea, leading to cochlear dysfunction Nosie Noise-Induced Hearing Loss (NIHL).[14] According to Samson *et al.*, 2007[15], when the cochlea was exposed to different kinds of noise, alterations were observed in the plasma corticosterone, Heat Shock Proteins (Hsp70) expressions and levels of brain Norepinephrine (NE). While thinking of treatment for NIHL and Cochlear dysfunction, the treatment is still under development, and no such strong medications have been formulated. Hence, this research focuses on the low dosage of synthetic corticosteroids named Prednisone/Prednisolone used in various treatments of disease and disorder. Low doses of prednisolone along with NSAIDS have been used to treat rheumatoid arthritis,[16] pain, asthma, and Sjögren's. Some studies have concluded that steroids are an effective drug for the treatment of NIHL.[17] Though keeping in mind the side effects of high doses of steroids, this study has been conceived to run low doses of steroids for NIHL. Thus, the objective of the current research is to observe i)cochlear protein (Prestin & Otoferlin) changes, ii) Biochemical levels, iii) behavioural assessments, iv) Plasma corticosterone estimation after noise overexposure, and to study the effects of low steroidal on noise overexposure.

MATERIALS AND METHODS

Animals

16 Healthy Adult Sprague Dawley (SD) rats weighing about 250 – 300 g were used for this current study. Animals were housed and maintained in standard conditions of 12h light/dark at 25°C with sufficient feed and water *ad libitum* Housed 3 animals per cage (29cm×22cm×14cm) were kept at the Central Animal House Facilities at our institute. The study was conducted after the approval of the institutional Animal Ethical Committee (02/03/2022).

Experimental design

This study consists of 6 animals in four groups

I. Control
II. Chronic Noise Exposure (100dBA noise for 4 hours/per day for 30 days)
III. Treatment (Prednisolone, 1mg/kg b.w. administered orally, dissolved in 0.9% Saline given for 7 days after the noise exposure)
IV. Drug-only.

After 30 days of noise exposure, animals were euthanised, and the excised organs were examined for the following observations. Once the animals had been sacrificed inner ear was taken for further analysis.

Noise Stress Induction

The sound that is above 100 dBA is considered noise. The Stress and treatment group animals were exposed to 100 dBA of white noise generated by two speakers (120W) installed 30cm above the cage in a soundproof chamber. The animals were exposed to white noise for over 30 days, 4 hours/day. The intensity of the sound is measured by the sound level meter D2023 (S.NO-F02199: Cygnet Systems, Gurgaon, Haryana, India).

ANIMAL ANXIETY BEHAVIOR

Open Field Test (OFT)

After 30 days of stress, an open field test has been recorded to assess the anxiety and social behaviour of the animals. The apparatus consists of a large rectangular wooden box (100cm×100 cm) with of 40 cm. The floor contains a clean, dark plastic sheet with a grid marked in paint, dividing 25 equal squares.[18] Before introducing the animals into the open field, 70% Ethanol was used for cleaning purposes. The animal was placed in the centre of the apparatus, and the video was recorded for 5 minutes.[19]

Elevated Plus Maze (EPM)

The Elevated plus Maze is also one of the anxiety behaviours. The apparatus includes a wooden setup with two perpendicular arms, viz., two open arms (25×5×0.5cm) and two closed arms (25×5×16cm).[20] For behaviour recording, the animal is placed in the central zone, and the animal's behaviour is recorded for 5 minutes.[21]

BIOCHEMICAL ASSESSMENTS

Lipid Peroxidation (LPO) (Ohkawa et al., 1979)

Adding 0.2ml of homogenate in 0.2ml of SDS, 1.5ml Of Acetic acid (AA) and 1.5ml of TBA, followed by heating at 95°C for about 1hr. After cooling, add 1ml of water

and 5ml of the pyridine mixture, then centrifuge for 10 mins at 4000rpm. With the supernatant, read absorbance at 532nm.[22]

Catalase (CAT) (Sinha, 1972)
Add a mixture of 4ml of H2O2, 5ml of Phosphate Buffer, to 1ml of Homogenate. From this mixture, 1ml was added to 2ml of dichromatic-acetic acid at 60s intervals and heated for 10 mins. OD was observed at 570nm.[23]

Glutathione peroxidase (GPX)
By adding 0.2 ml of sample, 0.4 ml of phosphate buffer, 0.1 ml of sodium azide, 0.2 ml of GSH, 0.1 ml of H2O2 in 1 ml of DH_20. 1 ml of 10%TCA was added before centrifugation at 2000 rpm. 0.2ml of Supernatant was collected and diluted with 1.8ml of DH_20 and 1ml of 5%TCA kept at 37°C at 20 minutes. Lately, 0.5ml of supernatant, 4ml of disodium hydrogen phosphate and 0.5 ml of DTNB. Absorbance is measured at 420mn.[24]

Reduced Glutathione Assay (GSH)
Here, 1ml of homogenate and 1ml of TCA were added and centrifuged. 0.5ml of supernatant is taken and added to 2ml of DTNB, and the absorbance is seen at 412nm.[25]

Vitamin C Assay
The absorbance was seen at 520nm by adding 0.5ml of plasma, and 1ml of TCA in 0.5 ml of DH_2O and centrifuging. After centrifugation, 0.5 ml supernatant was collected and added to 0.2 ml of DTC and incubated for 3 hrs. After incubation, 1.5 ml of H_2SO_4 was added, and the OD was observed.[26]

PLASMA CORTICOSTERONE
The Plasma were collected from the euthanised animal's blood. The collected blood is centrifuged at 4ºC, 5000rpm for 10 mins. The supernatant is collected, followed by adding ferric chloride, potassium hexacyanoferrate were added and the total mixture is heated for 70ºC for 30 minutes, and the OD is observed at 780nm using the spectrophotometry.[27]

PROTEIN ESTIMATION
Prestin Estimation
The prestin estimation was measured by Rat Prestin (Slc26a5) ELISA Kit purchased from MyBioSource (Cat No. MBS7612463). The Sensitivity of the kit is 18.75pg/ml. After serial dilution of the standards given in the kit, add a Biotin-labelled antibody, followed by HRP – Streptavidin Conjugate working solution and TMB substrate in the dark the absorbance is measured at 459nm.

Otoferlin Estimation
The Otoferlin estimation was measured by the OTOF ELISA kit purchased from MyBiosource (Cat No. Ms9330975). The detection range of the kit is 3.12ng/ml-100ng/ml.

RESULTS
Noise-induced hearing Loss (NIHL) is the major reason for temporary/permanent hearing loss prevailing because of environmental and inheritance factors. In a standard homeostatic condition, there is a balance between the oxidant and antioxidant levels; any disturbance or increase in free radicals in the body has a direct impact on many systems, and those disturbances also lead to cochlear dysfunction.[28] Chronic noise exposure leads to neurobehaviour alterations in rats.[29]

ANIMAL BEHAVIOUR
Open Field Test (OFT)
In **Figure 1**, **Rearing** the stress group shows a significant decrease in rearing when compared to the control and the treatment group shows a significant increase when compared to the stress group, **Grooming**, **Time Freezing**, and **Time in Peripheral Zone** there were no significant changes amongst the groups and in **Time in Central Zone** the stress group shows a no significant changes when compared to control. The statistical data were obtained from the Graphpad Prism Software. The Open Field Test (OFT) has been observed. One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. The data are expressed as Mean ± SD. p-value ≤ 0.05 (*); ≤ 0.01 (**); ≤ 0.001(***) was considered statistically significant. p-value ≥ 0.05 was considered not significant (ns).

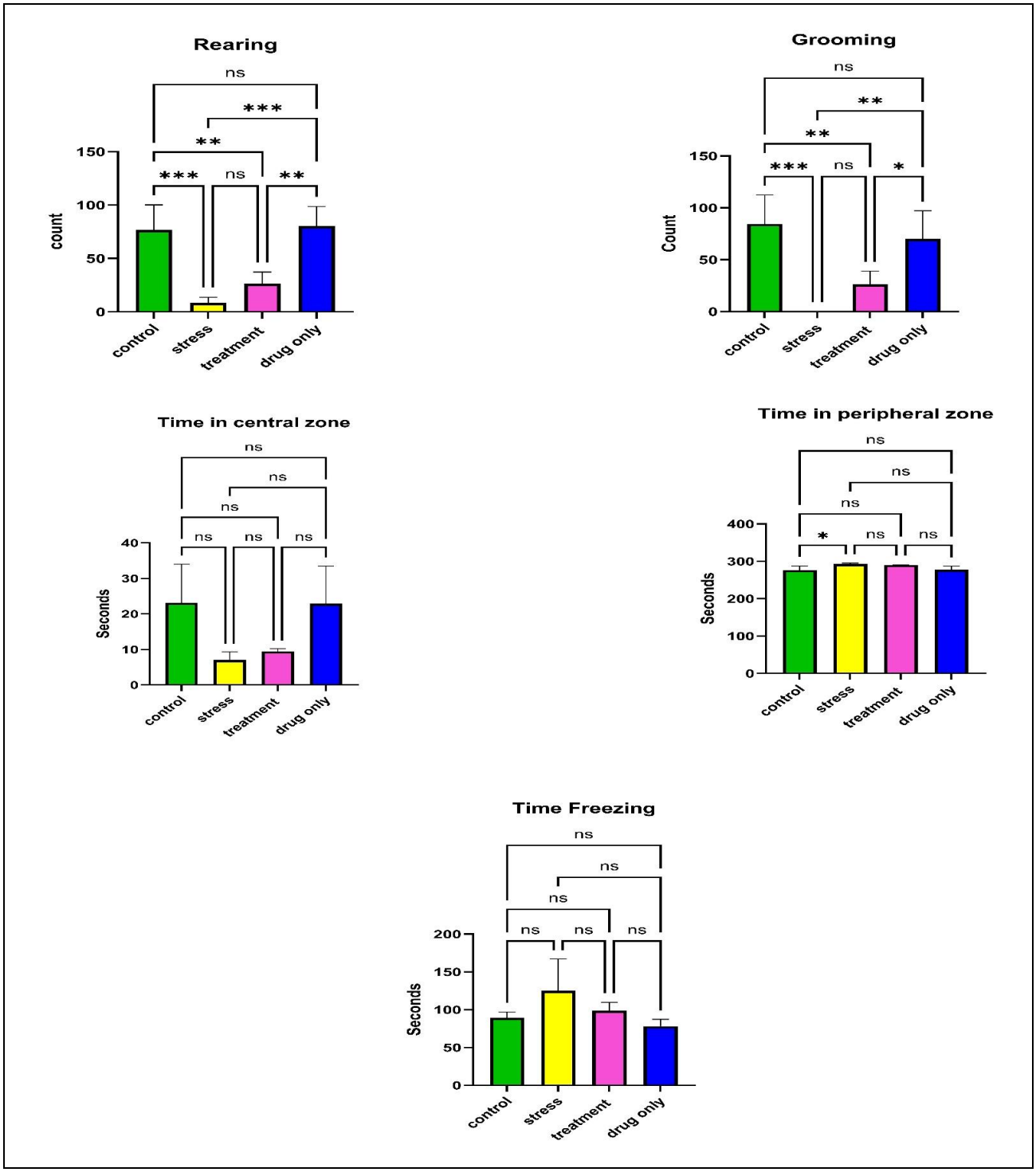

Figure 1: Open Field Test. p-value ≤ 0.05 (*); ≤ 0.01 (**); ≤ 0.001(***)

Heat map

Figure 2 shows the heat map of the animals, which were analysed using the ANYMAZE software. The colour intensity starts from blue to red, indicating the blue as the less explored area, whereas the red colour is the more explored area. In the figure below **Control** shows the control group where the animals explores both the central and the peripheral zone, **Stress group** shows the heat map of the stressed animals that are exposed to the chronic noise explore the peripheral zone alone, **treatment group** shows the treatment group where the animals explore the peripheral zone with varying mobility, **drug only** shows the heat map of the drug only group animals.

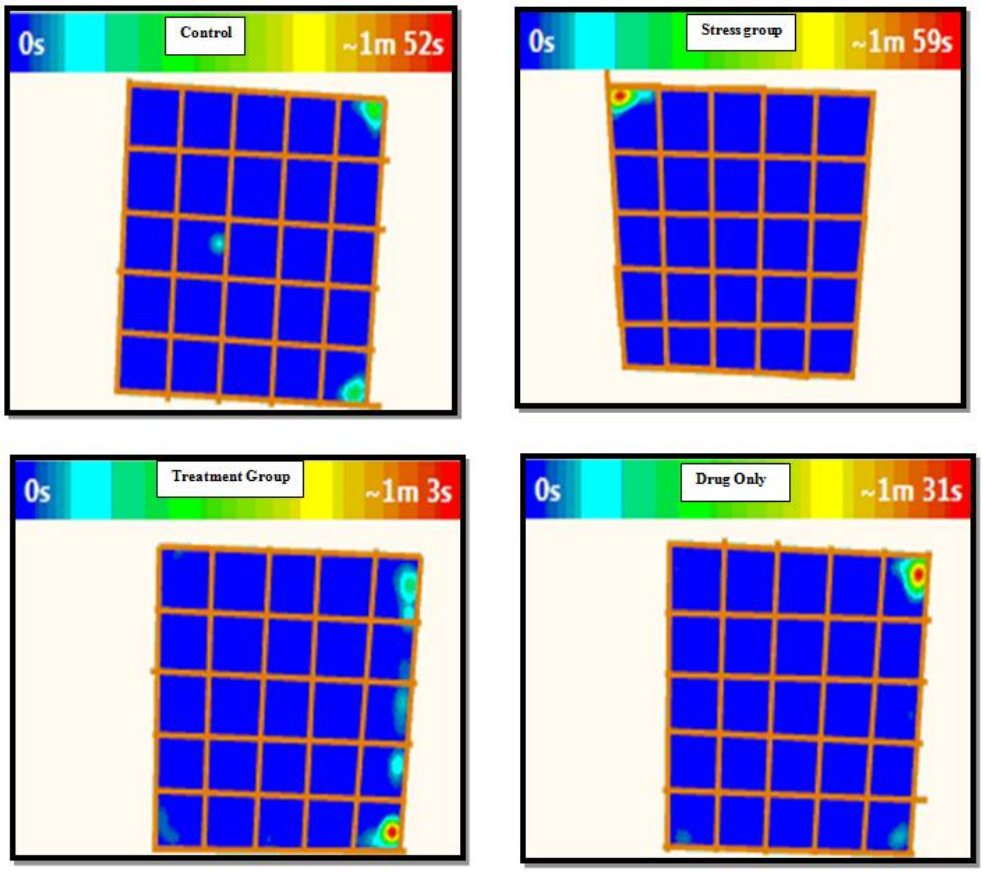

Figure 2: Heat map of Open field test. Analyzed using the ANYMAZE software.

Track Plot

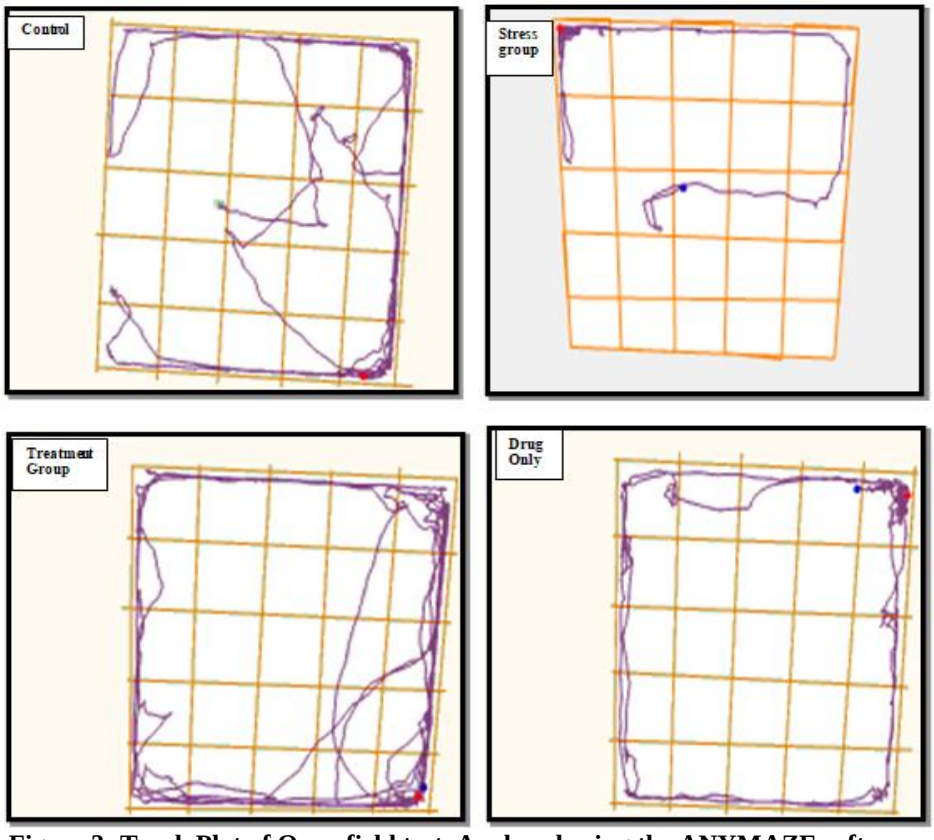

Figure 3: Track Plot of Open field test. Analyzed using the ANYMAZE software.

Figure 3 shows the track plot of the animals exploring the open field. **Control** shows the mobility of the control animal around the open field, **stress group** shows the mobility of the stress group animals, **treatment group** shows the track of the treatment group animals, and **drug only** shows the mobility of the drug only group animals.

Elevated Pluz Maze (EPM)

In Figure 2, there were no significant changes observed in any of the four groups. One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. The data are expressed as Mean ± SD. p-value ≤ 0.05 (*); ≤ 0.01 (**); ≤ 0.001(***) was considered statistically significant. p-value ≥ 0.05 was considered not significant (ns).

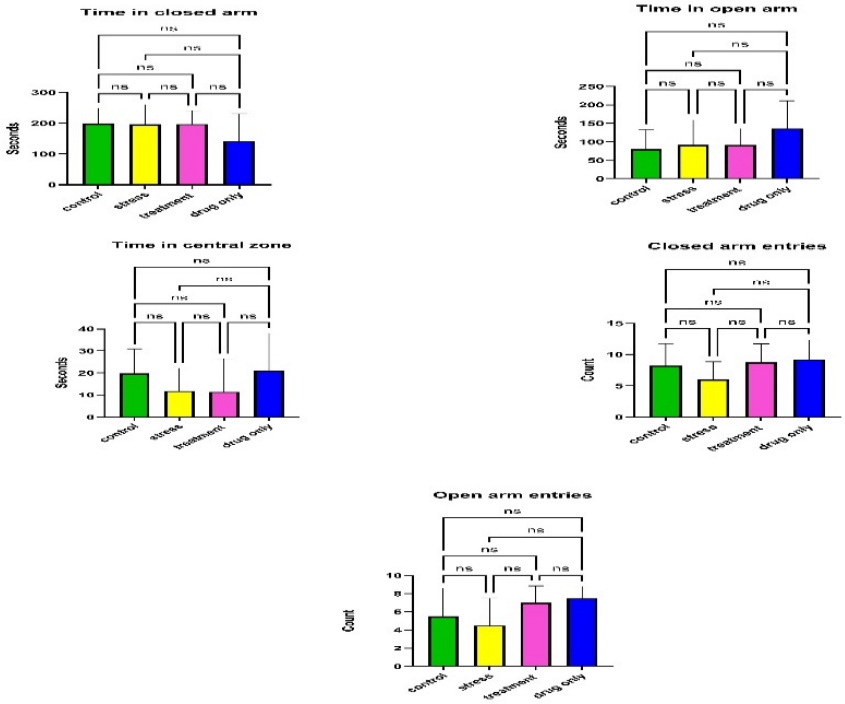

Figure 4: Elevated Plus Maze. p-value ≤ 0.05 (*); ≤ 0.01 (**); ≤ 0.001(***)

Heat map

Figure 5 shows the heat map of the animals in the elevated plus maze. **Control** shows the heat map of the control group animal, **Stress group** shows the heat map of the stress group animals, **Treatment group** shows the heat map of the treatment group, **Drug only** shows the heat map of the drug-only group animals.

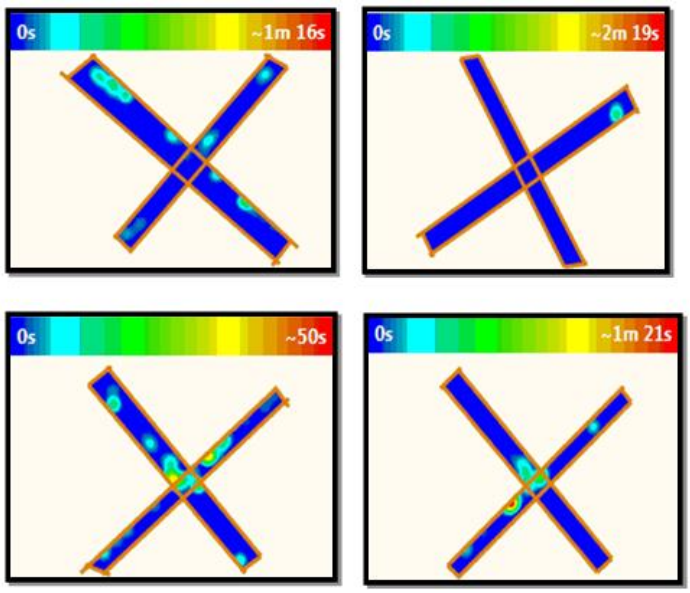

Figure 5: Heat map of Elevated Plus maze. Analysed using ANYMAZE software.

Track Plot

Figure 6 shows the heat map of the animals in the elevated plus maze. **Control** shows the track plot of the control group animal, **stress group** shows the mobility of the stress group animals, **treatment group** shows the track of the treatment group, and **drug only** shows the track of the drug-only group animals.

ANTIOXIDANTS

In antioxidant assessments, Glutathione Peroxidase (GPX), Catalase (CAT), Lipid peroxidase (LPO), Glutathione reductase (GSH), and Vitamin C assays were done to check the oxidative stress of the inner ear from the noise exposure. These assays have been observed in the inner ear. The oxidative stress has been significantly increased in LPO, CAT, GSH and GPX in the inner ear of the stress group when compared to the control. The treatment has responded, showing significant changes when compared to the control. One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. The data are expressed as Mean ± SD. p-value ≤ 0.05 (*); ≤ 0.01 (**); ≤ 0.001(***) was considered statistically significant. p-value ≥ 0.05 was considered not significant (ns).

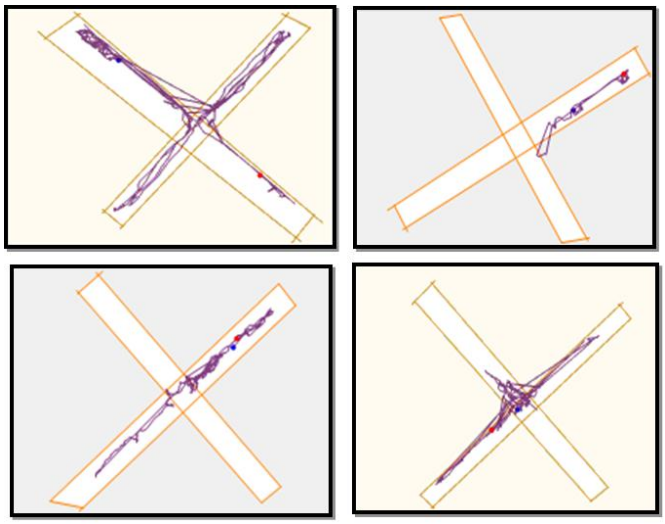

Figure 6: Track Plot of Elevated Plus Maze.Analyzed using the ANYMAZE software.

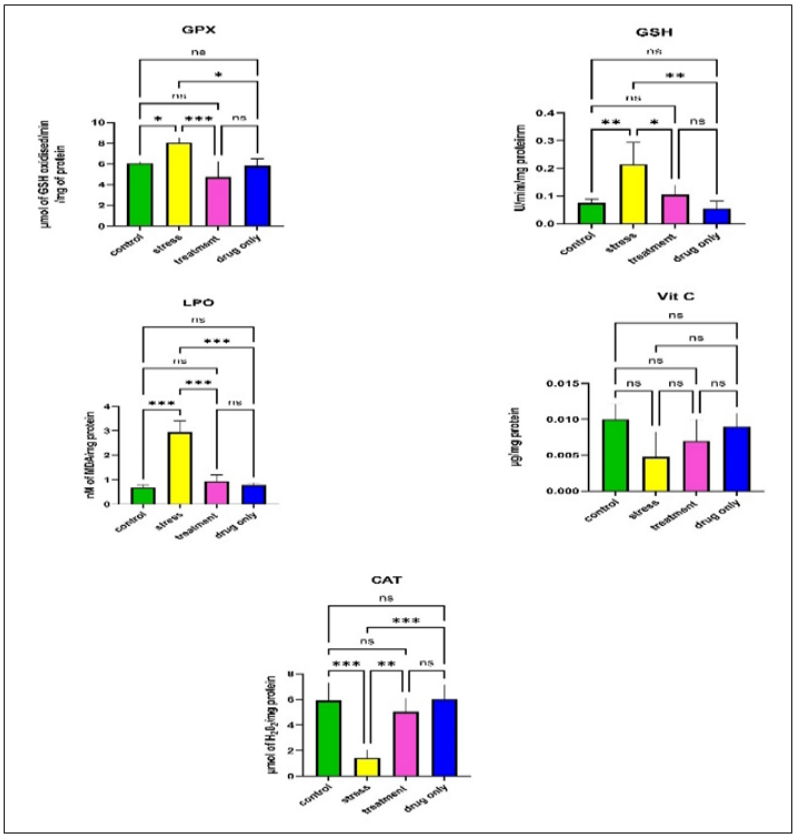

Figure 7: Antioxidants. p-value ≤ 0.05 (*); ≤ 0.01 (**); ≤ 0.001(***).

PLASMA CORTICOSTERONE

In this estimation, the stress hormone is found to be significantly increased in the stress group when compared to all the other groups. One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. The data are expressed as Mean ± SD. p-value ≤ 0.05 (*); ≤ 0.01 (**); ≤ 0.001(***) was considered statistically significant. p-value ≥ 0.05 was considered not significant (ns).

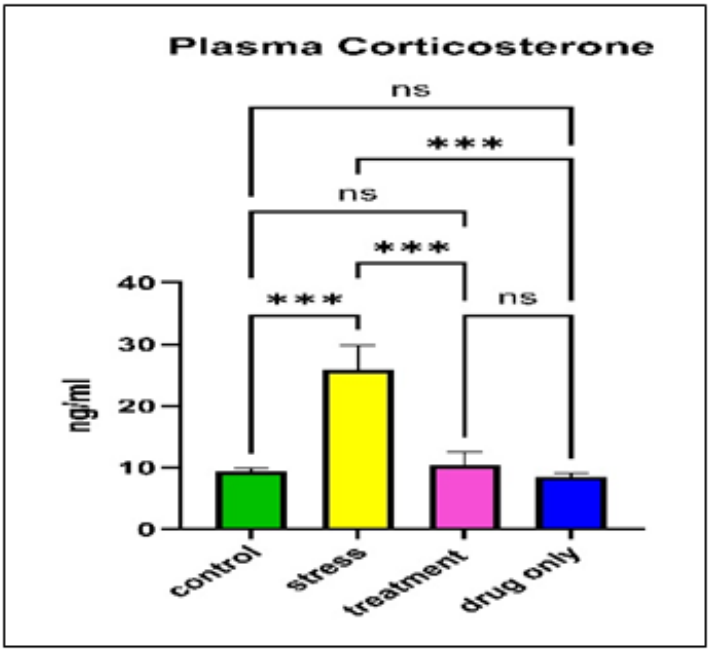

Figure 8: Plasma Corticosterone. p-value ≤ 0.05 (*); ≤ 0.01 (**); ≤ 0.001(***)

PROTEIN ESTIMATION

Two cochlear proteins were estimated using the ELISA KIT purchased from MYBIOSOURCE. The proteins estimated were the Prestin present in the outer hair cells and otoferlin present in the inner hair cells. The prestin present in the inner ear of the cochlea shows a significant increase in the stress group when compared to all other groups, and the otoferlin present in the inner ear has been significantly decreased in the stress group when compared to the control. One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. The data are expressed as Mean ± SD. p-value ≤ 0.05 (*); ≤ 0.01 (**); ≤ 0.001(***) was considered statistically significant. p-value ≥ 0.05 was considered not significant (ns).

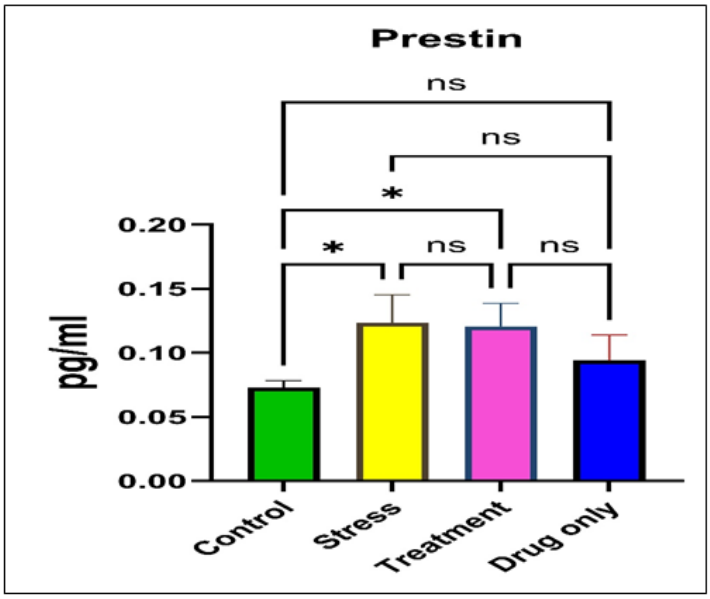

Figure 9: Prestin estimation. p-value ≤ 0.05 (*); ≤ 0.01 (**); ≤ 0.001(***).

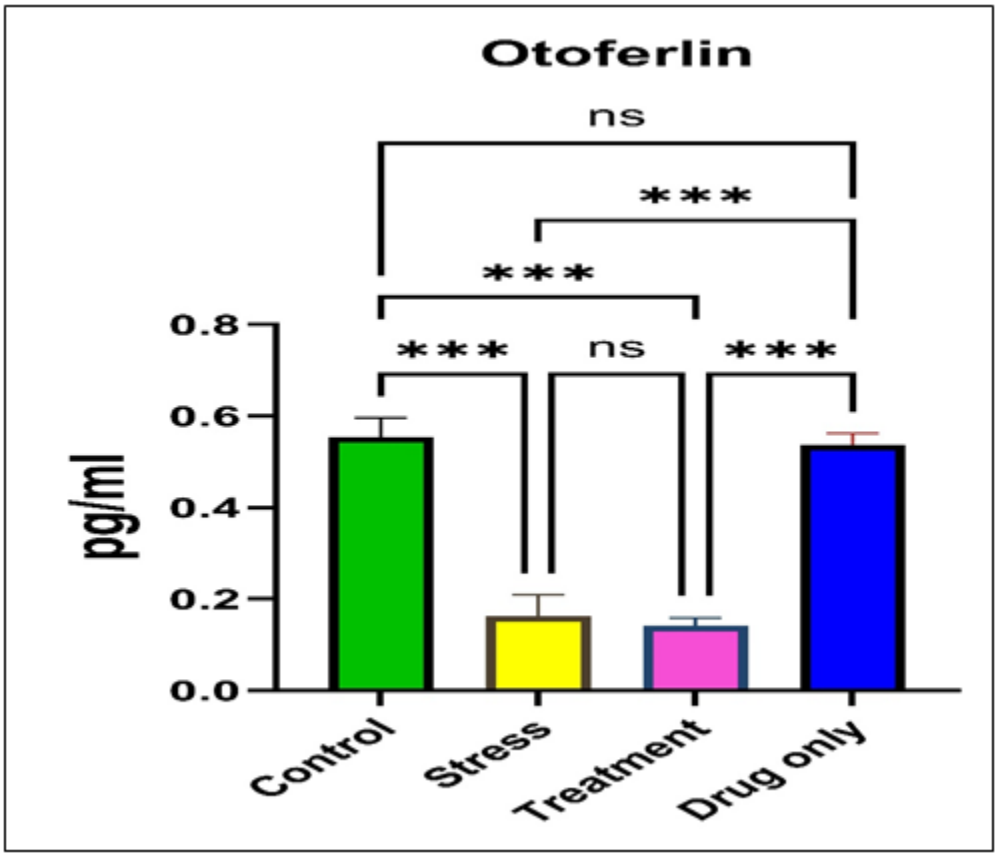

Figure 10: Otoferlin estimation. p-value ≤ 0.05 (*); ≤ 0.01 (**); ≤ 0.001(***)

DISCUSSION

ANIMAL BEHAVIOUR

Open Field Test (OFT)

Calvin Hall (1934), the originator of this OFT, has claimed that defecation in the field is considered to be pusillanimous, and over 30 or more dependent variables for OFT have been measured.[30] This OFT has become the major parameter for observing the anxiety and social and exploratory status of the animals. Freezing episodes, rearing, grooming, time spent in the central zone and time spent in the peripheral zone are the variables assessed along with the track plot and heat map of the animals. Here, the stressed animals show less rearing, absence of grooming and decreased other variables, which indicates the 'refractory loss of interest'. Over-noise exposure can lead to the sensitisation of the amygdala, causing the inhibition of K+ channels and thus facilitating the lateral amygdala neuronal excitability, which results in the development of anxiety-like behaviour[31] that results in the absence of all variables in stressed animals. The treatment of prednisolone doesn't seem to have significant changes in the stress group. The heat map of the OFT describes the visual representation of the animals in the open field area, where it shows the concentration gradient of the animals in the OFT, and the track plot shows the path of the animal taken during the recording of the behaviour. The control animal tends to explore the entire zone with active rearing, the stress group shows lesser exploration of all the zones with increasing freezing duration, the treatment group and the drug-only group show greater exploration.

Elevated Plus Maze (EPM)

The animals of all groups tend to show no significant changes in all the parameters observed may be because the animals might have been exposed to the apparatus other than the EPM earlier. Also, a reduction in exploratory activities indicates a high level of anxiety.[32] The heat map and the track plot of the animals in the EPM show that the control group explores both the closed and open arms, the stress group explores the closed arm more with the increasing freezing duration, the treatment group and the drug only group explore as well as the control.

The heat map and the track plot observation in the OFT and EPM animals exposed to chronic noise have not been reported. Thus, the heatmap and track plot show the presence and path of the animal travelled during the behaviour recording, but keeping in mind the parameters to be observed both the behaviour hasn't responded to the treatment.

ANTIOXIDANTS

Noise exposure can cause deleterious effects on the cellular level; it has been verified that noise can generate reactive oxygen (ROS) and reactive nitrogen species (RNS) inside the cell.[33] Lipid Peroxidation (LPO) is important biomolecule produced when the cell undergoes

oxidative stress[34], and LPO has been reported to increase 4 folds immediately after noise exposure and thus shows an increase in the inner ear of the noise-exposed animals, indicating oxidative stress.[35] Thus, our study has found that the treatment of prednisolone retains the LPO levels in the normal range. The Catalase (CAT) vary their concentration in various regions, having elevated function in muscle with reduced function in the liver.[36] Our study has found that the CAT activity in the inner ear has decreased when compared to control, and the treatment with prednisolone has shown effectiveness. Reduced Glutathione (GSH) is the primary metabolite of the Glutathione Peroxidase (GPX); previous studies show that the GPX level is reduced after noise exposure.[37] Our study indicates that the GPX and GSH levels increase in the stressed animal group; this is due to the upregulation of these antioxidant enzymes, which are carried by the activation of the Nrf2 pathway.[38] This upregulation is due to the increased oxidative stress inside the cochlea, and our treatment with prednisolone has reduced the oxidative stress, thus bringing the GPX and GSH levels under control.

PROTEIN ESTIMATIONS

The motor molecule prestin present in the OHC mediates the electromobility of the OHC. The noise-exposed animal groups show an increase in prestin level compared to the control. Certain studies state that the increase is due to the upregulation of prestin mRNA and prestin molecules post noise exposure, returning to baseline after 4 weeks.[39] Since this prestin is assessed immediately after the noise, the treatment isn't effective. The cochlear protein Otoferlin, present in the IHC of the cochlea, is involved in the synaptic-vesicle membrane fusion and performs exocytosis.[40] The protein otoferlin has been decreased in noise noise-induced group, but the treatment hasn't worked here.

Studies have shown that treatment with a low dose of prednisolone is a better cure. Administration of prednisolone to transient ischemic cochlear injury has reduced the extent of cochlear damage, but this mechanism remains unclear.[41] Thus, our study aims to use this prednisolone in NIHL, but the results have not been achieved in all cases. Animal behavioural alterations due to noise overexposure haven't been reversed with the treatment of prednisolone; the antioxidation alteration in the inner ear due to noise overexposure has induced oxidative stress, and thus the treatment has been effective in reducing the ROS. Regarding the cochlear proteins, a low dose of prednisolone administration has shown a null effect.

PLASMA CORTICOSTERONE

The stress hormone corticosterone has been significantly increased in the noise-exposed group. The increase in the stress hormone when exposed to noise for above 30 days shows the defence mechanism of the body and the involvement of the hypothalamic-pituitary-adrenal-adrenal- adrenal axis, which induces the accelerated secretion of CRH, ACTH, and POMC gene expressions.[42] Chronic exposure causes the sensitisation of the amygdala, increasing cortisol release.[43]

CONCLUSION

To conclude, our study expected prednisolone to be a perfect drug of choice for NIHL; only the oxidative damage in the inner ear has responded to prednisolone, but the behaviour assessment and cochlear protein have been altered after the noise exposure, and the treatment hasn't worked in NIHL. To our finding, the mode of administration of the drug is intraperitoneal, so it will not cross the blood-labyrinth barrier (BLB), and cannot bind to the treatment for the cochlear proteins.

REFERENCES

1. Westman JC, and Walters JR. Noise and stress: a comprehensive approach. Environmental Health Perspectives, 1981; 41.
2. Peter Dallos. The active cochlea. The Journal of Neuroscience, 1992; 12: 4575-4585.
3. Renato Nobili; Fabio Mammano; Jonathan Ashmore. How well do we understand the cochlea?. TINS, 1998; 21(4): 0–167.
4. Xu Shan, Yang NinG. Research Progress on the Mechanism of Cochlear Hair Cell Regeneratin. Frontiers in Cellulat Neurosciences, 2021; 15.
5. Edge AS & Chen Z. Hair cell regeneration. Current Opinion in Neurobiology, 2008; *18*(4): 377-382.
6. Isabelle Roux, Saaid SafieddinE, Régis Nouvian, M'hamed Grati, Marie-Christine Simmler, Amel Bahloul, Isabelle Perfettini, Morgane Le Gall, Philippe Rostaing, Ghislaine Hamard, Antoine Triller, Paul Avan, Tobias Moser, Christine Petit. Otoferlin, Defective in a Human Deafness Form, Is Essential for Exocytosis at the Auditory Ribbon Synapse. Cell, 2006; 127(2): 0–289.
7. Jain RK, Pingle SK, Tumane RG, Thakkar LR, Jawade AA, Barapatre A, Trivedi M. Cochlear Proteins Associated with Noise-induced Hearing Loss: An Update. Indian J Occup Environ Med, 2018; 22(2): 60-73.
8. Hussain SAS, Ali MH, Imtiaz MH, Al Hasibuzzaman M. Breaking the silence: gene therapy offers hope for OTOF-mediated hearing loss, editorial. Ann Med Surg (Lond), 2024; 86(9): 4950-4951.
9. Dehghani-Ghahnaviyeh S, Zhao Z, Tajkhorshid E. Lipid-mediated prestin organization in outer hair cell membranes and its implications in sound amplification. Nat Commun, 2022; 13(1): 6877.
10. Dallos P, Fakler B. Prestin, a new type of motor protein. Nat Rev Mol Cell Biol, 2002; 3: 104–111.
11. Ge J, Elferich J, Dehghani-Ghahnaviyeh S, Zhao Z, Meadows M, von Gersdorff H, Tajkhorshid E, Gouaux E. Molecular mechanism of prestin electromotive signal amplification. Cell, 2021; 184(18): 4669-4679.
12. Zaman M, Muslim M, Jehangir A. Environmental noise-induced cardiovascular, metabolic and mental

health disorders: a brief review. Environ Sci Pollut Res Int, 2022; 29(51): 76485-76500.
13. Wankhar W, Srinivasan S, Rajan R, Sheeladevi R. Antioxidant mediated response of Scoparia dulcis in noise-induced redox imbalance and immunohistochemical changes in rat brain. J Biomed Res, 2017; 31(2): 143-153.
14. Winston JT, Peter R, Srdjan M, Tan WJ, Thorne PR, Vlajkovic SM. Noise-induced cochlear inflammation. World J Otorhinolaryngol, 2013; 3(3): 89-99.
15. Samson J, Sheeladevi R, Ravindran R, Senthilvelan M. Stress response in rat brain after different durations of noise exposure. Neurosci Res., 2007; 57(1): 143-7.
16. Conn DL. Resolved: Low-dose prednisone is indicated as a standard treatment in patients with rheumatoid arthritis. Arthritis Rheum, 2001; 45(5): 462-7.
17. Mutlu A, Ocal FCA, Erbek S, Ozluoglu L. The protective effect of adrenocorticotropic hormone treatment against noise-induced hearing loss. Auris Nasus Larynx, 2018; 45(5): 929-935.
18. Tatem KS, Quinn JL, Phadke A, Yu Q, Gordish-Dressman H, Nagaraju K (2014). Behavioral and locomotor measurements using an open field activity monitoring system for skeletal muscle diseases. J Vis Exp, 2014; (91): 51785.
19. Kraeuter AK, Guest PC, Sarnyai Z. The Open Field Test for Measuring Locomotor Activity and Anxiety-Like Behavior. Methods Mol Biol., 2019; 1916: 99-103.
20. Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc., 2007; 2(2): 322-8.
21. Komada M, Takao K, Miyakawa T (2008). Elevated plus maze for mice. J Vis Exp, 2008; (22): 1088.
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.
23. Sinha AK. Colorimetric assay of catalase. Anal Biochem, 1972; 47(2): 389-94.
24. 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.
25. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta, 1979; 582(1): 67-78.
26. 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.
27. Verma S and Singh S. Current and Future Status of Herbal Medicines. Veterinary World, 2008; 1: 347-350.
28. Plontke S, Zenner HP. Current aspects of hearing loss from occupational and leisure noise. Curr Top Otorhinolaryngol Head Neck Surg, 2004; 3: 6.
29. Arjunan A and Rajan R. "Impact of chronic noise on hippocampal morphology and its functions in wistar albino rats". Asian journal of pharmaceutical and clinical research, 2021; 14(7): 137-40.
30. Walsh RN, Cummins RA. The open field test: a critical review. Psychol Bull, 1976; 83: 482–504.
31. Peng X, Mao Y, Tai Y, Luo B, Dai Q, Wang X, Wang H, Liang Y, Guan R, Liu C, Guo Y, Chen L, Zhang Z, Wang H. Characterization of Anxiety-Like Behaviors and Neural Circuitry following Chronic Moderate Noise Exposure in Mice. Environ Health Perspect, 2023; 131(10): 107004.
32. Shoji H, Miyakawa T. Effects of test experience, closed-arm wall color, and illumination level on behavior and plasma corticosterone response in an elevated plus maze in male C57BL/6J mice: a challenge against conventional interpretation of the test. Mol Brain, 2021; 14(1): 34.
33. Manikandan S, Devi RS. Antioxidant property of alpha-asarone against noise-stress-induced changes in different regions of rat brain. Pharmacol Res, 2005 Dec; 52(6): 467-74.
34. Gaschler MM, Stockwell BR. Lipid peroxidation in cell death. Biochem Biophys Res Commun, 2017; 482(3): 419-425.
35. Chen L, Yu N, Lu Y, Wu L, Chen D, Guo W, Zhao L, Liu M, Yang S, Sun X, Zhai S. Hydrogen-saturated saline protects intensive narrow band noise-induced hearing loss in guinea pigs through an antioxidant effect. PLoS One, 2014; 9(6): e100774.
36. Coling D, Chen S, Chi LH, Jamesdaniel S, Henderson D. Age-related changes in antioxidant enzymes related to hydrogen peroxide metabolism in rat inner ear. Neurosci Lett, 2009; 464(1): 22-5.
37. Kil J, Pierce C, Tran H, Gu R, Lynch ED. Ebselen treatment reduces noise induced hearing loss via the mimicry and induction of glutathione peroxidase. Hear Res., 2007; 226(1-2): 44-51.
38. Ma Q. Role of nrf2 in oxidative stress and toxicity. Annu Rev Pharmacol Toxicol., 2013; 53: 401-26.
39. Chen GD. Prestin gene expression in the rat cochlea following intense noise exposure. Hear Res, 2006; 222(1-2): 54-61.
40. Jain RK, Pingle SK, Tumane RG, Thakkar LR, Jawade AA, Barapatre A, Trivedi M. Cochlear Proteins Associated with Noise-induced Hearing Loss: An Update. Indian J Occup Environ Med, 2018; 22(2): 60-73.
41. Maetani T, Hyodo J, Takeda S, Hakuba N, Kiyofumi G. Prednisolone prevents transient ischemia-induced cochlear damage in gerbils. Acta Otolaryngol Suppl, 2009; 562: 24-7.
42. Chandralekha G, Jeganathan R, Viswanathan, Charan JC. Serum leptin and corticosterone levels after exposure to noise stress in rats. Malays J Med Sci, 2005; 12(1): 51-6.
43. Ressler KJ. Amygdala activity, fear, and anxiety: modulation by stress. Biol Psychiatry, 2010; 67(12): 1117-9.