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ORIGINAL ARTICLE

Alteration in Memory and Electroencephalogram Waves with Sub-acute Noise Stress in Albino Rats and Safeguarded by *Scoparia dulcis*

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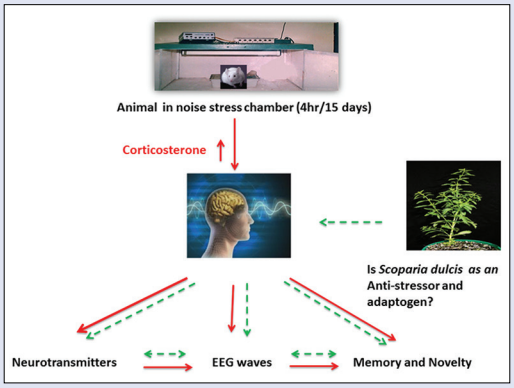
ABSTRACT

Background: Noise stress has different effects on memory and novelty and the link between them with an electroencephalogram (EEG) has not yet been reported. **Objective:** To find the effect of sub-acute noise stress on the memory and novelty along with EEG and neurotransmitter changes. **Materials and Methods:** Eight-arm maze (EAM) and Y-maze to analyze the memory and novelty by novel object test. Four groups of rats were used: Control, control treated with *Scoparia dulcis* extract, noise exposed, and noise exposed which received *Scoparia* extract. **Results:** The results showed no marked difference observed between control and control treated with *Scoparia* extract on EAM, Y-maze, novel object test, and EEG in both prefrontal and occipital region, however, noise stress exposed rats showed significant increase in the reference memory and working memory error in EAM and latency delay, triad errors in Y-maze, and prefrontal and occipital EEG frequency rate with the corresponding increase in plasma corticosterone and epinephrine, and significant reduction in the novelty test, and significant reduction in the novelty test, amplitude of prefrontal, occipital EEG, and acetylcholine. **Conclusion:** These noise stress induced changes in EAM, Y-maze, novel object test, and neurotransmitters were significantly prevented when treated with *Scoparia* extract and these changes may be due to the normalizing action of *Scoparia* extract on the brain, which altered due to noise stress.

Key words: Acetylcholine, corticosterone, electroencephalogram, epinephrine, noise stress, *Scoparia dulcis*

SUMMARY

- Noise stress exposure causes EEG, behavior, and neurotransmitter alteration in the frontoparietal and occipital regions mainly involved in planning and recognition memory
- Only the noise stress exposed animals showed the significant alteration in the EEG, behavior, and neurotransmitters
- However, these noise stress induced changes in EEG behavior and neurotransmitters were significantly prevented when treated with *Scoparia* extract
- These changes may be due to the normalizing action of *Scoparia dulcis* (adoptogen) on the brain which altered by noise stress.



Abbreviations used: EEG: Electroencephalogram, dB: Decibel, EPI: Epinephrine, ACH: Acetylcholine, EAM: Eight-arm maze.

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INTRODUCTION

Stress is one of the important factors that threatens homeostasis to all the age groups due to its unavoidable nature. Among the various stressors, noise stress is one among the stress that could not be avoided. Apart from noise effects on cardiovascular diseases, hypertension, ischemic heart disease, gastrointestinal, respiratory diseases, and alteration in the immune system has been reported earlier.[1] Noise stress causes alterations in neurotransmitter at the different regions of the brain,[2] with the action of stress hormones on the receptors present on the brain region such as hippocampus[3] was reported. According to Smith *et al.*[4] the unique effect of stress on the memory varies depends on the type and nature of stressor. Stress-induced neurotransmitters and hormones release might be responsible for a behavioral change which includes learning and memory.[5] McEwen,[6] reported that prefrontal cortex undergoes structural changes in spine and dendrite and thus the neuronal plasticity occurs. The stress responses over the areas of brain may be due to the high-affi ty, rapidly-activated mineralocorticoid receptors whereas the low-affi ty glucocorticoid receptors are involved in the termination of stress reactions via the negative feedback of hypothalamic–pituitary–adrenal axis.[7] Other than these regions, occipital cortex (medial extra striate visual cortex) has also been involved in visual recognition memory.[8] The activity of these brain areas can be recorded cortically by using electroencephalogram (EEG).[9] Most of the studies have focused

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only on the effect of stress on the memory and not on the retrieval process and novelty, that is, after the storage of information, and further its association with EEG wave's alternations, especially on the prefrontal and occipital lobe involved in cognitive abilities and visual recognition memory remains unclear. Plants have been a valuable source of natural products for maintaining human health for many years. More recently, there has been a greater search for natural therapies. *Scoparia dulcis* is a folk-medicinal plant, well known for its antioxidant and antidiabetic property,[10] it also has other numerous properties such as analgesic, anti-inflammatory and[11] neurotrophic activities.[12] These unique effects may be due to the presence of phytochemicals, such as phenols, saponins, tannins, amino acids, flavonoids, terpenoids, and catecholamines,[13] involved in biological manipulations and activities. With the background knowledge of the various properties of *Scoparia*, the present study is to determine the protective role of aqueous extract of *S. dulcis* as against the noise stress (antistressor) and whether the sub-acute noise stress exposure at the level of retrieval process could affect the memory and novelty in rats by altering prefrontal and occipital parietal EEG along with the neurotransmitters changes.

MATERIALS AND METHODS

The experiments were carried out by using healthy adult male Wistar rats (180–200 g). The study was initiated with a proper approval by the Institute's Animal Ethical Committee (IAEC No.: 01/19/2013). Animals were divided into four groups (control, control treated with *Scoparia*, noise exposed, and noise stressed rats treated with *Scoparia*). All the rats used in this study were maintained in constant temperature with a 12 h light: 12 h dark cycle and allowed free access to food and water. All groups were handled similarly.

Noise stress procedure

Noise stress was produced in the laboratory rats by using loud speakers (15 W), installed 30 cm above the cage, driven by white noise generator set at 100 dB noise level or above uniformly throughout the cage, and monitored by using sound level meter. Each animal was exposed to noise stress for 4 h/day for 15 days. Control group rats were also kept in the above-described cage during the corresponding period of time, without noise stimulation to avoid the influence of handling stress on the evaluation of effects due to noise exposure.[14]

Preparation of aqueous extract of *Scoparia dulcis*: Preparation of *Scoparia dulcis* plant extract

S. dulcis was collected from the local area and confi med by the botanist and a sample was deposited in herbarium (Reg. No.: NIS/MB/63/2012). Hundred grams of air dried leaves' portions of the plant, *S. dulcis* was soaked in 1 L of distilled water overnight. Th s was subsequently filtered into a beaker using filter paper and funnel. The filtrate was concentrated at 40°C to constant weight using a Rotavapor apparatus. The residue was collected and stored at −4°C. The concentrate was then reconstituted into a stock solution of 200 mg/ml in distilled water. The required volume of this solution (calculated on the basis of animal weight) was administered daily by gavage.

Assay of corticosterone

The corticosterone assay[15] is based on the oxidation of corticosteroids with ferric iron (III) in acidic medium and subsequent complex with ferrous iron (II) and potassium hexacyanoferrate. 0.5 µl was mixed with appropriate volumes of the working solutions of corticosterone and were transferred into a series of 10 ml volumetric flasks. 2 ml of sulfuric acid and 2 ml of ferric chloride were added to 0.5 ml of potassium hexacyanoferrate (III) solution. Th s mixture was heated in a water bath maintained at 70°C ± 2°C for 30 min with occasional shaking and diluted to the 5 ml mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Eight-arm maze

Eight-arm radial maze is used to assess spatial learning and memory. [16] A group of animals was trained prior to the experiment and during training, food act as the reward to animals, and placed in their respected respective arms. Initially, animals were allowed to freely explore the maze with all arms baited with cereal. The adaptation occurs after a week, each rat was individually housed in a small cage. The adaptation and maze test were performed between 10 and 12 h. On 3rd day, for each rat, a piece of cereal in only four of the eight-arms was kept and were trained to locate the four food rewards. Each individual rat had its own set of four rewarded arms. The room contained several visual reference cues on the wall and only four arms (fi ed for each animal). Each trial began with the placement of the animal on the central platform facing toward arm number one and ended when the rat had visited the four baited arms and the time taken was noted. Otherwise, the rats were given a maximum of after a period of 10 min. Reference and working memory error and time taken to complete the task were analyzed in all the groups.

Y-maze

It is used for the assessment of spatial memory and working memory. Before the stress exposure, rats were trained in Y-maze.[17] Rats were chosen in random order and placed in an arm of the Y-maze (start arm) with one of the arms blocked off (novel arm) and allowed to explore the start arm and remaining arm (alternate arm) for 5 min. After a 1 min delay, rats were placed back in the start arm and allowed to explore all arms freely for 5 min.The time spent in each arm of the maze, latency period, and triad arm errors were recorded manually. Only after the completion of stress exposure for 15 days, the animals were allowed to repeat the same procedure described before the stress exposure and the latency period and triad arm errors were recorded and compared before and after the stress exposure. The maze was placed in a sound attenuated room under dim illumination. Numerous visual cues were placed on the walls of the testing room and kept constant during the entire behavioral testing. To avoid manual error and bias, a double scoring was done with the help of a fellow researcher.

Novel object recognition test

The novel object test[18] consists of three phases. (1) Habituation, (2) familiarization, and (3) test. Habituation in the arena for 10 min/2 sessions with an interval of 4 h for a single day. The next day (i.e., after 24 h) familiarization session was carried out for 10 min using two identical marble pots with a minimum distance of 90 cm apart. After familiarization session, the interval of 1 h was given before the start of the test session. The test session was conducted by replacing one of the marble pot with novel object (pot of different shape and color and the time spent by each rat with the novel object when compared to different groups were noted and expressed in graph). The amount of time taken to explore the new object provides an index of recognition memory.

Electrode implantation surgery procedure

Animals were anesthetized with ketamine/xylazine (90/15 mg/kg, i.p.). Toe pinch method was used to check the responsiveness after the administration of anesthetize agent once every 5 min then fi ed the animal in stereotaxic frame, and a recording electrode was fi ed on the surface of the scalp after the small incision was made in the skin, after the cleaning procedures aseptically. Electrode was placed on the scalp and glued with dental acrylic resin on the scalp surface of animal's head and the

incision was closed and sutured by exposing the electrode. Postoperative care was taken as per ethical guidelines with the administration of saline and antibiotic to prevent fluid loss during incision and sepsis. After the recovery, the electrodes were connected to the EEG equipment (RMS EEG-24 Brain View Plus system - Recorders and Medicare Systems (p) Ltd, Chandigarh, India). The EEG activity was recorded for 10 min approximately in anesthetized rats. Signals were filtered between 1 and 70 Hz. Recordings were analyzed by RMS/EEG-24 Super spec version 1.1 and expressed in amplitude (μv) and frequency (Hz).

All the experiments were performed at the same time of day (10–12:00 am) to avoid the circadian rhythms. The behavioral studies (eight-arm maze [EAM], Y-maze, and novel object recognition test) was done for each of the animals (4 groups/6 animals) three times and the graphs were made on the average sum of three readings for each rat.

Neurotransmitter analysis: Acetylcholine and epinephrine

The brain tissue of frontal region was well homogenized using ice cold PBS (0.02 mol/L, pH 7.0–7.42) after homogenizing centrifuge for 15 min at 5000 rpm and the supernatant were used for the assay immediately using the protocol mentioned in the BG (EPI- Epinephrine) ELISA kit (Blue Gene Biotech,China) Catalogue no: E02A0007, (ACH- Acetylcholine) Catalogue No: E02A0711 and using ELISA micro plate, the yellow color developed was read immediately at 450 nm OD, after the stop solution was added.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). All the data were analyzed with the SPSS for windows statistical package (version 20.0, SPSS Institute Inc., Cary, North Carolina). The statistical signifi ance among the four different groups was analyzed by using one-way ANOVA test followed by Tukey's multiple comparison tests and the signifi ance level was fi ed at $P < 0.05$.

RESULTS

Effect of sub-acute noise stress on corticosterone levels

The data were summarized in Figure 1 with mean ± SD. The plasma corticosterone level was measured in all the four different groups. There were no signifi ant changes observed in the corticosterone level when compared among control, control treated with *Scoparia* extract, and noise exposed rats which received *Scoparia* extract. However, only noise exposed rats showed a signifi ant increase in the corticosterone level compared to the rest of the groups. Th s indicates noise exposure acts as a stressor and *Scoparia* extract was found to be benefic al.

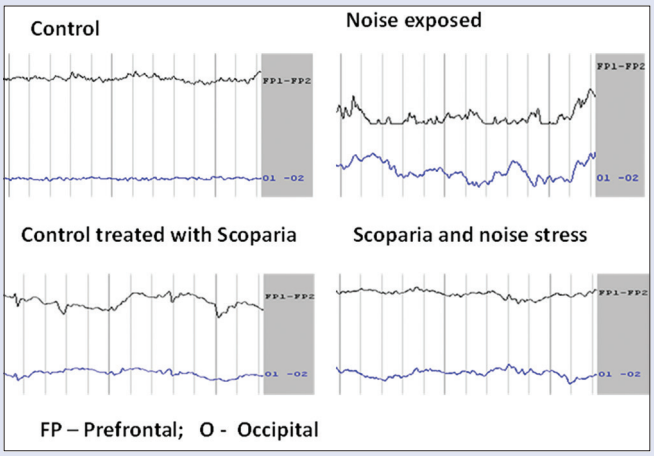

Figure 1: Effect of sub - acute noise stress on rat EEG waves (FP: Prefrontal; O: Occipital)

Eight-arm radial maze

Working and reference and memory error; time taken to complete the task

The data from various groups are given in Figures 2-4 and were expressed with mean ± SD. There were no signifi ant changes in working and reference memory errors among the control, control treated with *Scoparia* extract, and noise exposed rats which received *Scoparia* extract. However, there was a signifi ant difference in both working and reference memory error only in noise exposed rats caused by stress.

Y-maze

Triad errors and latency period

The data were summarized in Figures 5 and 6 with mean ± SD. Triad error is given by the wrong sequence and re-entry in the same arm whereas the latency period indicates the time taken for the onset of movement. Only stress exposed animals shows a signifi ant change in triad error and latency period and there was no signifi ant variation was observed among the other groups studied.

Time spent in arm A, B, and C (novel arm)

The data were summarized in Figure 7 with mean ± SD. There were no signifi ant changes in the time spent in arm A, B, and C (novel arm) in the entire groups studied, including the stress exposed animals.

Effect of sub-acute noise stress on novel object recognition test

The data were summarized in Figure 8 with mean ± SD. A signifi ant difference in novel object recognition test was observed only in noise exposed rats when compared with control and control treated with *Scoparia* extract. However, when noise animals received *Scoparia* extract. It showed a normal response.

Effect of sub-acute stress on electroencephalogram waves (A, B, C, and D)

Van Lier *et al.*,[19] reported the presence of alpha and beta waves in rats based on the frequency as alpha 1 (9–10 Hz), alpha 2 (11–12 Hz), beta

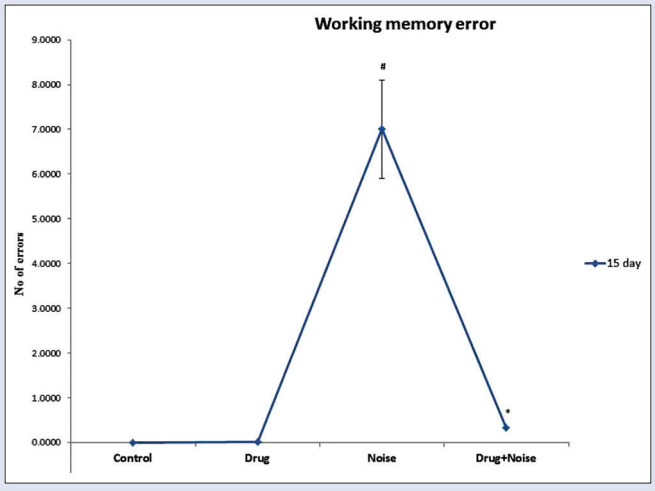

Figure 2: Effect of sub - acute noise stress on Eight arm radial maze (*Compare to control, drug control and drug + noise treated, #Compare to noise stress treated alone)

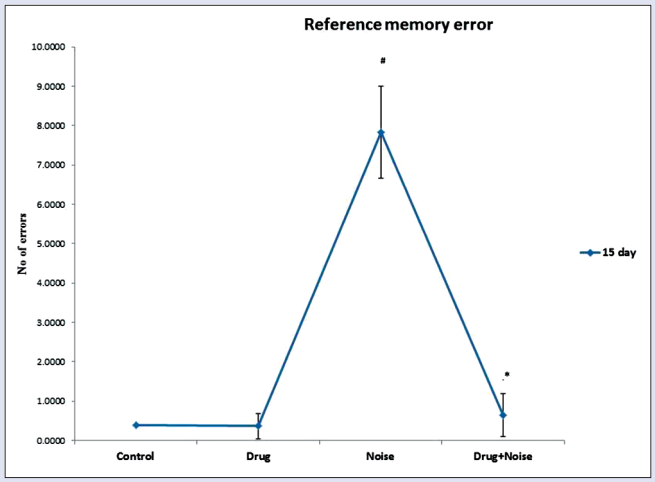

Figure 3: Effect of sub - acute noise stress on Eight arm radial maze (*Compare to control, drug control and drug + noise treated, #Compare to noise stress treated alone)

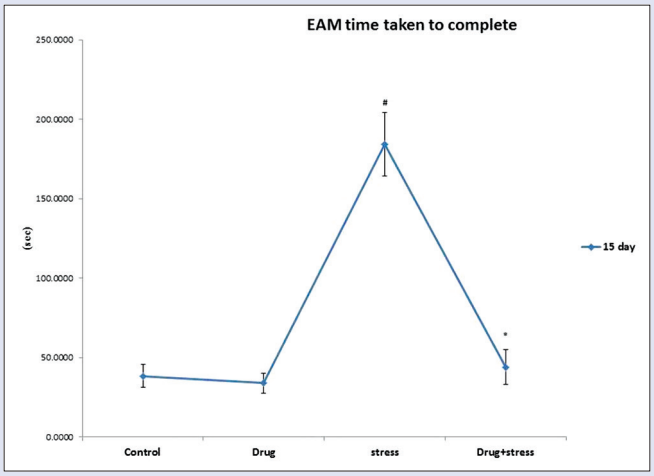

Figure 4: Effect of sub - acute noise stress on Eight arm radial maze (*Compare to control, drug control and drug + noise treated, #Compare to noise stress treated alone)

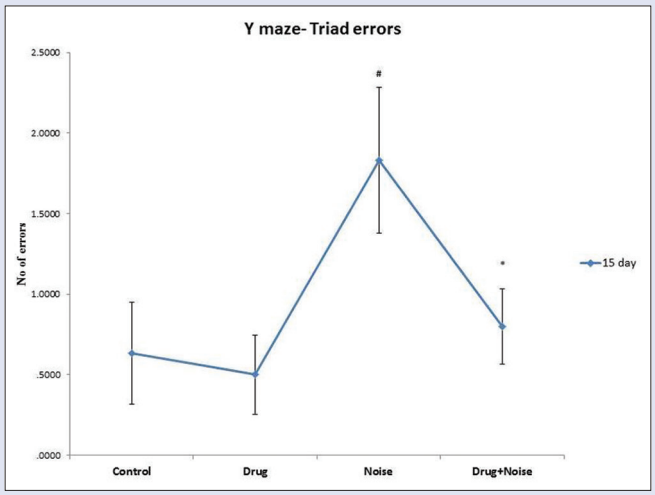

Figure 5: Effect of sub - acute noise stress on Y maze (*Compare to control, drug control and drug + noise treated, #Compare to noise stress treated alone)

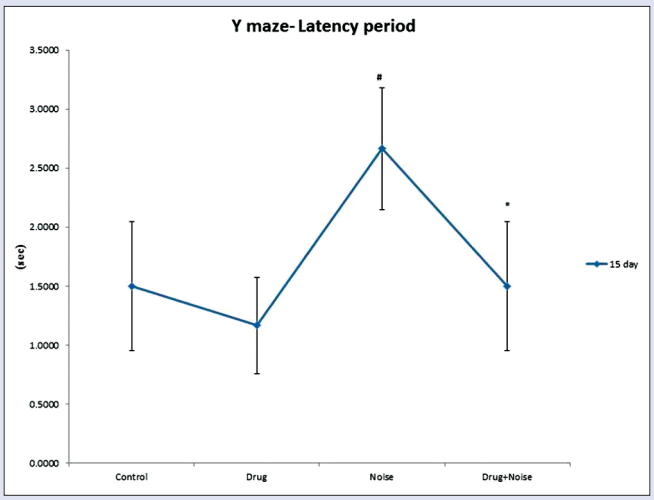

Figure 6: Effect of sub - acute noise stress on Y maze (*Compare to control, drug control and drug + noise treated, #Compare to noise stress treated alone)

1 (13–17 Hz), beta 2 (18–20 Hz), beta 3 (21–30 Hz), beta 4 (31–100 Hz), theta (6–8 Hz), and delta (1–5 Hz). Based on this, the frequency was analyzed. The amplitude was measured using software from RMS EEG – 24 brain new-plus.

Prefrontal and occipital regions (amplitude and frequency) were summarized in Figures 9 and 10 with mean ± SD. The control and control treated with *Scoparia* extract demonstrates no signifi ant changes on both amplitudes as well as in the frequency of prefrontal (beta 1) and occipital region (alpha 2) EEG waves. Only the noise exposed animals showed the signifi ant increase in the prefrontal (beta 2) and occipital regions (beta 1). EEG frequency and decrease in amplitude were compared with control and control treated with *Scoparia* extract. However, these noise stress induced changes in EEG were prevented completely in the animals receiving *Scoparia* extract.

Effect of sub-acute stress on neurotransmitter

The data for the brain ACH and norepinephrine were summarized in Figure 11. Only the stress exposed animals showed a signifi ant increase in the EPI and a decrease in the ACH when compared to control and control treated *Scoparia* extract and stressed rat with *Scoparia* extract. Moreover, these groups did not differ among themselves. However, in noise stress, animals received *Scoparia* extract and the neurotransmitter levels were similar to controls.

DISCUSSION

The noise stress affects the memory and brain EEG waves that were associated with the neurotransmitter changes. Normal thinking and decision making with higher cognitive abilities are mainly associated with the prefrontal cortex during nonstress conditions,[19] and it involves the activation and transmission of signals in the neurons of the prefrontal cortex. It also has connections with other regions of the brain to regulate cognitive, emotion, and memory functions. Other than prefrontal cortex, parietal and occipital cortex also plays a role by transferring the highly processed visuospatial information, oculomotor attention guidance[20] to the dorsolateral prefrontal cortex.[21] Further, occipital cortex other than visual functions, also has nonvisual

functions such as spatial recognition memory and tactile.[22] Especially medial extra striate visual cortex in mediating memory for visual object information[23] and transfer the information to parietal and temporal cortex via "what pathway" through the ventral stream.[24] Hence, all the information and signals were carried out to the frontal cortex for further processing and analyzing by different pathways and connections. The results in the present study describes that only noise stress exposed rats shows a signifi ant increase in the reference and working memory errors as well as the time taken to complete the task in EAM. Th s could be the effect of stress on prefrontal cortex and its connections involved in memory tasks because of the increase in corticosterone and EPI caused by stress[25] and its action on the receptors of prefrontal cortex that alters neuronal response pattern which was well noticed by the marked increase in prefrontal EEG frequency and the frontal EPI after being exposed to stress compared to control. These marked alterations were observed not only on the EEG frequency but also with the novel object recognition behavior. Normally, rats have more tendency to explore the new environment and novel object[26] and even the present results shows control rats spent more time with the novel object when compared to the stress exposed rats, where it shows the marked decrease in the time spent with novel object compared with the familiar object. Usually, the information about the new and old visual object and its recognition were identifi d by the medial extra striate visual cortex.[24] Once gets identifi d, the signals were further processed and recognized with the hippocampus[27] and frontal cortex.[28] The impairment in the exploratory behavior of rats, spatial recognition, and working memory were further assessed by using Y-maze[29] and the results showed that only stress exposed animals, showed a signifi ant variation in triad error, latency period, and prefrontal and occipital EEG frequency compared with control and control received *Scoparia* extract. One of the possible reason for all these behavioral and EEG alterations after subjected to stress is mainly due to the increase in corticosterone and catecholamines and its action on neurons that cause glutamate release which augment or alters the frequency of miniature excitatory postsynaptic potentials in hippocampal pyramidal neurons.[30] However, the EEG recordings also demonstrate the decrease in prefrontal and occipital EEG amplitude

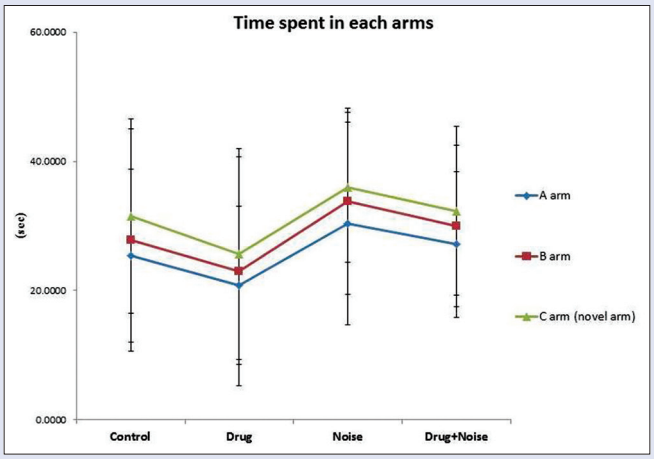

Figure 7: Effect of sub - acute noise stress on Y maze (*Compare to control, drug control and drug + noise treated, #Compare to noise stress treated alone)

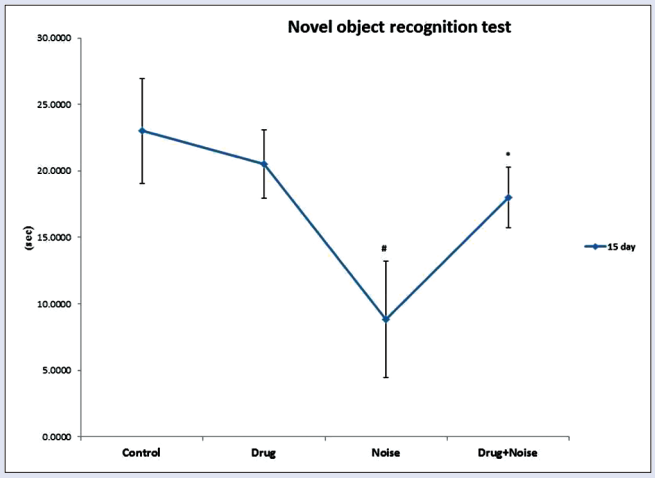

Figure 8: Effect of sub - acute noise stress on Novel object recognition test (*Compare to control, drug control and drug + noise treated, #Compare to noise stress treated alone)

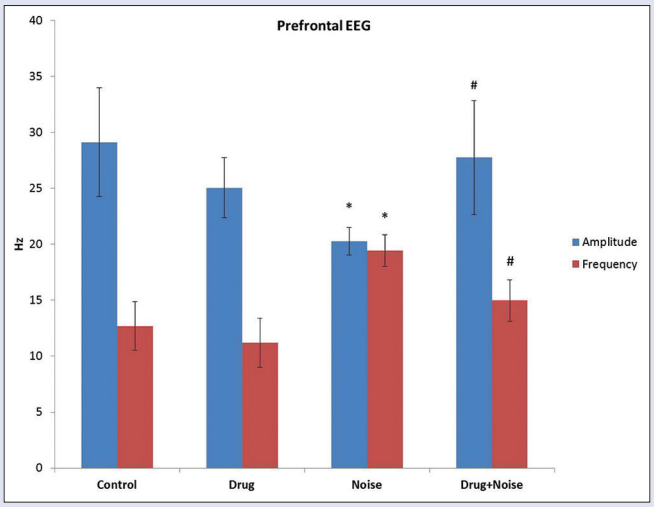

Figure 9: Effect of sub - acute noise stress on rat pre frontal EEG (*Compare to control, drug control and drug + noise treated, #Compare to noise stress treated alone)

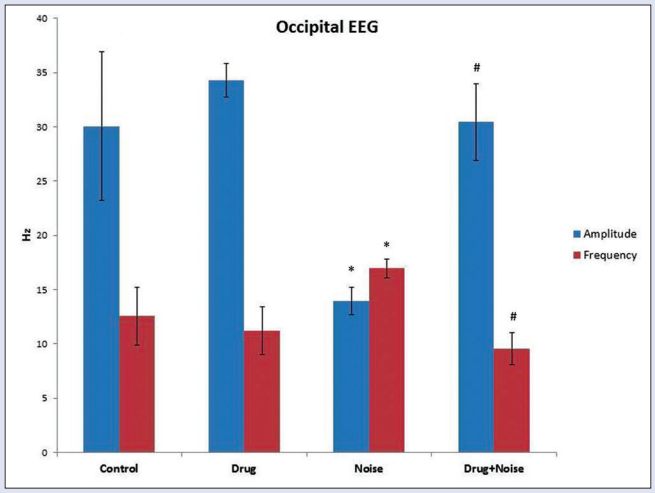

Figure 10: Effect of sub - acute noise stress on rat occipital EEG (*Compare to control, drug control and drug + noise treated, #Compare to noise stress treated alone)

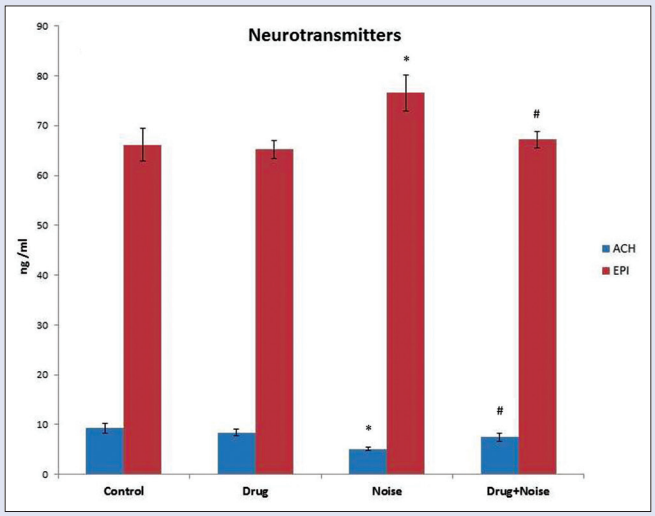

Figure 11: Effect of sub - acute noise stress on Neurotransmitters. (*-compare to control, drug control and drug + noise treated, #- compare to noise stress treated alone)

and with the corresponding increase in frequency. Hence, when the rats were exposed to noise stress, it may affect hippocampus and amygdala in the temporal lobe followed by hypothalamus in brainstem that it deranges the normal processing of signals between the frontal, parietal and occipital cortex essential for the normal spatial recognition and working memory. These memory changes is well associated with the alteration in ACH in frontal cortex region of stress exposed animals in this study was well in agreement with the earlier report of[31] whom were worked on environmental enrichment. The alteration of EEG frequency in prefrontal in noise stress exposed animals showed beta 2 compared to control and control treated with *Scoparia* extract (they showed beta 1). However, in the case of occipital frequency of noise exposed animals showed beta 1 compared to control and control treated with *Scoparia* extract, which shows alpha 2. All this alteration in the rhythm pattern of EEG waves may be due to the involvement of stress alteration on signaling pathways[20] and neurotransmitters levels[2] in the brain. However, all these noise stress induced changes were prevented and normalized compared to control after the treatment with aqueous extract of *S. dulcis* and even it brought back the EEG rhythm pattern to normal such as in control animals and suggesting the protective role of *S. dulcis*. The possible cause may be due to the potent antioxidant activity of *S. dulcis*[10] or the components/molecules in the extract. However, further studies required to be done to understand the mechanism of noise stress and its action on signaling pathways.

CONCLUSION

In spite of repeated exposure, noise stress is not adoptable and the elevated corticosteroid could not be ignored. Noise stress is defin tely activating the various brain regions and reported to increase the free radicals and could alter the brain neurotransmitters, these may be the cause behind the alteration observed in EEG as well as in behavior. Since noise stress could not be avoided the herbal remedy can be preferred. As a normal animal with *S. dulcis* did not show any alteration it action is such as an adoptogen and hence its usage can be recommended as a prophylactic.

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Conflicts of interest

There are no confli ts of interest.

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