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Effect of Eugenol on the Behaviour of Wistar Albino Rats Subjected to Noise Induced Stress

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Abstract: ***Introduction: The Eugenol is the major constituent of Ocimum sanctum Linn. The vast majority of work in Ocimum sanctum Linn has been completed so far but the effects of Eugenol on stress parameters were indistinct. The acute noise stress can modulate the anxiety related behavior such as Rota rod tests in Wistar albino rats. Aim & Objective: To assess the effects of stress on motor coordination and performance in rats after the administration of active principle of Ocimum sanctum Linn. Materials & Methods: All the animals were randomly divided into three groups with six animals in each. Group I: This group of rats served as control. Group II: Rats of this group were subjected to acute noise stress 4 hours /day and after that 6 consecutive days roto rod test were made. Group III: This group Eugenol is administered (50 mg/kg/day, IP for 15 consecutive days) after acute noise stress. Noise was produced by two loudspeakers (15 W), driven by a white-noise generator (0–26 kHz), and installed 30 cm above the cage. Control rats were kept in the above-described cage during the corresponding period of time, without noise stimulation. Rota rod is intended for testing coordination and impairment of locomotor ability of rats using well-established principle of a rotating rod on which an animal tries to maintain its balance. Results : The motor coordination of the noise exposed rats is compared with the control which can balance on the rota-rod for 300sec.There are no overlaps at df = 2,p = 0.002(fig:1) which shows that the time taken by the noise stressed and Eugenol treated rats balanced on the rota-rod varies significantly from that of the control. Conclusion: Our study indicated that acute noise stress significantly induced the alterations in motor coordination. Administration of Eugenol is an ideal anti stressor substance for the prevention of stress induced changes in motor coordination.***

Keywords: Wistar albino rat, Rota rod, Eugenol, Noise stress

1. Introduction

Eugenol (4-allyl-2-methoxyphenol), the principal chemical constituent of clove oil and tulsi has been primarily derived from a variety of plant sources, including *Eugenia caryophyllus*, *Oscimum sanctum* Linn and *Myristica fragrans*. For years Eugenol has been used in dental practice to relieve pain arising from a variety of sources, including pulpitis and dentinal hypersensitivity. In the recent past, a wealth of literature has been generated on Eugenol's antidepressant, antistress, anticonvulsant, and analgesic activities. Eugenol is also reported to possess anti-inflammatory, antioxidant, anaesthetic and muscle relaxant properties. A Rota rod tread mill device was used for the evaluation of motor coordination. Rats were placed on a horizontal rotating (16 RPM) rod. These rats had been selected for their ability to remain on the revolving bar for a period of time. After the administration of Eugenol (50mg/kg, I.P.) each mouse was placed on the rotating rod. The endurance time for each rat on the rota-rod was noted [1]

The purpose of this study was to assess the effects of stress on motor coordination and performance in rats after the administration of active principle of *Ocimum sanctum* Linn. The present study has been designed to investigate the protective role of Eugenol after acute noise induced stress in albino rats. The Eugenol is the major constituent of Ocimum sanctum Linn. The vast majority of work in Ocimum sanctum Linn. has been completed so far but the effects of Eugenol on stress parameters were indistinct, so present study will evaluate the changes in motor coordination effects after the rats exposed to acute noise stress and the protective role of Eugenol administration.

2. Aims & Objectives:

To assess the effects of stress on motor coordination and performance in rats after the administration of active principle of Ocimum sanctum Linn.

3. Materials and Methods

The study was approved by the Institute's Animal Ethical Committee (IAEC No 01/034/2010) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Healthy adult male Wistar rats weighing about 180-200 g have been used for this study.

4. Experimental Groups

All the animals were randomly divided into three groups with six animals in each.

Group I: This group of rats served as control.

Group II: Rats of this group were subjected to acute noise stress 4h/day and after that 6 consecutive days roto rod test were made.

Group III: This group Eugenol is administered (50 mg/kg/day, IP for 15 consecutive days) after acute noise stress

When noise exposure of any kind exceeds 90 dB, noise becomes a stressor [1]. Noise was produced by two loudspeakers (15 W), driven by a white-noise generator (0–

26 kHz), and installed 30 cm above the cage. The noise level was set at 100 dB uniformly throughout the cage and monitored by a sound level meter (Quest Electronics 215). This noise level was selected based on the intensity occurring in discotheques. Each treated animal was exposed for 4 hr/day for 15 days. To avoid the influence of handling-stress on evaluation of the effects due to noise exposure, control rats were kept in the above-described cage during the corresponding period of time, without noise stimulation.

ROTA ROD

Rota rod is intended for testing coordination and impairment of locomotor ability of rats using well-established principle of a rotating rod on which an animal tries to maintain its balance. The length of time the animal is able to maintain itself on the rotating rod describes an animal's alertness. The functional status and progressive modification of locomotor behavior of experimental animals in these runways were graded or scored during the noise stress period and after with Eugenol administration and compared with those of the control.

Equipment

Commercially available Rota Rod apparatus (Rota-Rod, INCO, India) modified as described: Dimensions of the apparatus: Rotating rod diameter is ca. 5 cm made of hard plastic material covered by grey rubber foam (cut from insulation material to cover water pipes); lanes width is about 5 cm. The apparatus must allow an accelerating speed from 4rpm to 40rpm in 300 sec.

5. Procedures

Minimum n=6 rat per experimental group. Rats to be housed three per cage at least one week before the test. On the day before testing rat should be individually marked to be easily identified on the test day. One suggestion is to mark their tails as 1, 2, 3 in each cage. Use a non-toxic, no odor black marker to make 1 or 2 or 3 stripes at the base of the tail.

On the day of testing, rat should be kept in their home cages and acclimate to the testing room for at least 15 min. Turn on the Rota rod apparatus. **Training phase**: Training trial 1 (t1): place three rats from the same cage/strain on lanes 1, 3 and 5 (i.e. leaving an empty lane between two rats).Try to have the rat on the rod walking forward to keep their balance. The rod is initially rotating at 4rpm constant speed to allow positioning of all the rat in their respective lanes. Keep their balance at 4 rpm constant speed for 60 sec. Clean apparatus with 70% Ethyl alcohol, detergent and wipe it dry. It consists of three trials separated by 10 min inter-trial intervals (ITI).

Training trial 2 (t2): repeat procedure as t1. Training trial 3 (t3): repeat procedure as t1.

Repeat trial 3 once if a mouse falls off the rod before 60 sec cut off (not more than 4 trials at maximum). All rats have to stay on rod at 4 rpm for 60 sec before moving on to the test. Leave at least a 30 min break between training and test phases. **Test phase:** It consists of three trials separated by 15 min inter trial intervals (ITI). It is possible to run a total of nine rats (three cages, 3 rats per cage) consecutively in one trial before moving to the next. Set apparatus to accelerating mode from 4 to 40rpm in 300 sec. The apparatus will indicate "acceleration waiting" of 4rpm constant speed until the start button is pressed. Test trial 1 (T1): place three rats from the same cage/strain on lanes 1, 3 and 5.

Once all the rats are "ready" push the start button and the rod will be accelerating from 4rpm to 40rpm in 300 sec. Record the latency and the rpm at which each rat falls off the rod.

If a rat is clinging on the rod and completes a full passive rotation, stop the timer for that rat by pushing down the lever and record the latency. Remove the rat and place it back in its home cage. Be very careful not to disturb the other rat that are still running in the adjacent lanes. Also take note of passive rotation on the data sheet. Clean apparatus with 70% ethyl alcohol, detergent and wipe it dry. Test the next two sets of three rats, repeating for both sets the procedure for trial 1. Leave a 15 min inter trial interval (ITI) between the beginnings of consecutive trials (e.g. T1-ITI-T2-ITI-T3). Test trial 2 (T2): repeat procedure as trial 1 (T1). Test trial 3 (T3): repeat procedure as trial 1 (T1). Also note on the data sheet, any observations during the test, including occurrence of jumping, passive rotations etc.

Eugenol

Eugenol, allyl chain-substituted guaiacol (2-methoxyphenol), is a clear to pale yellow oily liquid extracted from certain essential oils especially from clove oil and cinnamon.

6. Statistical Analysis

The statistical software package SPSS 10.0 for windows was used to analyze the data. Statistical analysis was undertaken by using ANOVA Tukey's multiple comparison tests. $P<0.5$ was considered statistically significant.
From this we can understand, control is significant with stressed and treated animals.

7. Results

The motor coordination of the noise exposed rats is compared with the control which can balance on the rota-rod for 300sec.There are no overlaps at df = 2,p = 0.002(fig:4) which shows that the time taken by the noise stressed and Eugenol treated rats balanced on the rota-rod varies significantly from that of the control.

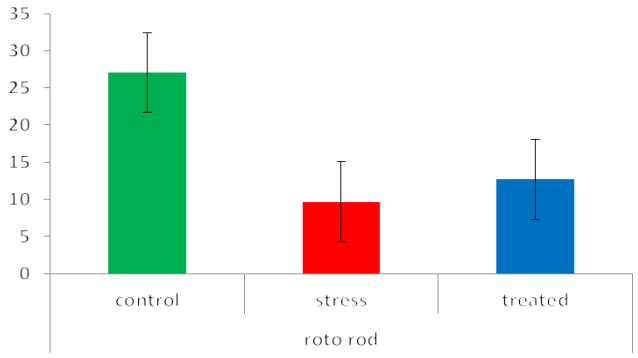

Figure 1

8. Discussion

Compounds present in Oscimum sanctum are Eugenol (a volatile oil), ursolic acid, rosmarinic acid, oleanolic acid, carvacrol, linalool, ß – caryophyllene and germacrene. Eugenol (1 – hydroxyl-2-methoxy-4-allyl benzene), has been found to be largely responsible for the therapeutic potentials of tulsi. Eugenol is reported to substantially modulate brain functions by regulating voltage-gated cation channels and release of neurotransmitters. It is interesting that the seven-day pretreatment with eugenol showed no significant genotoxicity. Similar nontoxic effects of eugenol were also reported earlier. Some doses of eugenol used in those studies were even higher than the ones used in the present work. [2,3] .It may also be mentioned that the doses of eugenol used in the present work were relatively quite low compared to its LD 50 dose in rat i.e. 2000 mg/kg body weight. Moreover the duration of treatment was also short. The mechanism underlying stress-induced tissue damages are not yet fully understood, however, accumulating evidence has implied that the production of free radicals plays a critical role in these processes [4,5,6]. Previous studies have indicated that stress stimulated numerous pathways leading to increased levels of free radicals [4, 6]. Oxygen radicals can attack proteins, nucleic acids and lipid membranes, thereby disrupting cellular functions and integrity. Brain is the target for different stressors because of its high sensitivity to stress induced degenerative conditions. Recent findings indicate that rapid and transient changes in polyamine metabolism, termed the polyamine-stress-response, may occur repeatedly in the brain after chronic intermittent stress. The ethanolic extract of OS leaves was found to prevent noise induced oxidative stress in discrete regions of the brain [7]. The anti-stressor effect of essential oil from leaves and seeds of OS in rats exposed to restrained stress has been reported. It is evident now that OS prevents the stress-induced changes in the central cholinergic system, cardiac system [8, 9]. The Rota rod Performance test measures parameters such as riding time (seconds) or endurance. Some of the functions of the Rota rod Performance test include evaluating balance and coordination. Rodents naturally try to stay on the rotating cylinder, or rota rod, and avoid falling to the ground.

The length of time that a given animal stays on this rotating rod is a measure of their balance, coordination, physical condition, and motor-planning. The speed of the rota rod is mechanically driven, and may either be held constant, or accelerated [10]. Animals have been shown to use compensatory behaviors in a variety of situations including the beam walk, staircase test, and the rota rod. [11, 12, 13] After a period of acute noise stress, the animals were trained in the rota rod showed depression in their activity. But after the administration of Eugenol, the depression was eliminated gradually but it did not reach the control level. This shows the anti-stressor effect of Eugenol. Since Eugenol has an antistressor effect, they can able to balance in the rota rod when compared to the stressed animals. From the above observations we can conclude strongly that Eugenol has an anti-stressor effect.

9. Conclusion

Our study indicated that acute noise stress significantly induced the alterations in motor coordination. Administration of Eugenol is an ideal anti- stressor substance for the prevention of stress induced changes in motor coordination.

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