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THE ANTIANXIETY ACTION OF VERAPAMIL IN SWISS ALBINO MOUSE IN COMPARISON TO FLUOXETINE

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ABSTRACT: Introduction: Anxiety disorders are due to reduced serotonin or noradrenaline or GABA in the hippocampus. This study aims at investigating the anti-anxiety actions of verapamil. **Materials and Methods:** Swiss Albino mice of either sex were used. Group I – control recieved normal saline, Group II fluoxetine 20mg/Kg,Group III Verapamil (10mg/Kg), Group IV Verapamil (20mg/Kg) and Group V- Verapamil (10mg/Kg) and Fluoxetine. Each group had 5 mice. On day 9 of treatment mice were subjected to the marble burying test, light-dark exploration test, actophotometer , and social interaction study. **Results:** Mean (sd) Locomotor activity in the control group was 267.6 (±61.77), 462.6 (± 58.2) with Verapamil 10mg, 561.8 (±33.33) with Verapamil 20mg. Verapamil significantly increased locomotion (P<0.001). The number of entries into light area was 57.20±11.78 in the control group, 85±11.26 with Verapamil (10mg/Kg) and 102.6±7.93 with Verapamil 20 mg/kg. Verapamil significantly increased the number of entries towards light as compared to control mice (P <0.001). The number of entries into dark area was 262.4±16.65 in the control group, 30.6±8.88 with Verapamil 10mg/kg and 14.6±4.4 with Verapamil 20 mg/kg. Verapamil significantly decreased the number of entries towards dark as compared to control mice (P <0.001). Verapamil significantly increased number of social interactions as compared to control mice (P <0.001). **Conclusion:** Mice treated with Verapamil had reduced anxiety. This effect was dose dependant and comparable to fluoxetine.

INTRODUCTION: Anxiety is a state of fear, apprehension, tension, panic or restlessness Anxiety is perceived by patient as worry, social fear, performance fears, panic attacks, or avoidance behaviors. Patient experience palpitations, giddiness, and shortness of breath [1]. If these symptoms are found to affect the quality of life it is called anxiety disorders [2].

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The prevalence of anxiety disorders in India as per a multi centric study done across 12 states was 2.57% more in female of 40–59 age group, and urban metro dwellers [3]. Anxiety disorders are a result of reduced serotonin [4] noradrenaline or GABA in the central nervous system, especially the hippocampus.

The different voltage gated calcium channels have been exhibiting different roles in the etiology of anxiety. It has been shown that N-type voltage gated calcium channels control almost 50% of GABA released in the baso lateral amygdale which is one of the main sites of the brain related to anxiety [5]. Whereas the T type calcium channels

have a dual or rather opposite action in normal or basal state and when activated. The basal activity attenuates anxiety, while activation of these channels produces anxiety. In a study done on fear-conditioned rats, there was upregulation in expression of CaV1.2 and CaV1.3. These rodents did not have a startle response due to use of Nimodipine [7]. In contrast CaV1.2 haplo insufficiency or its deletion in the forebrain showed to induce an anxiety in mice [8].

Selective serotonin reuptake inhibitors (SSRI, eg, Fluoxetine) and serotonin-norepinephrine reuptake inhibitors (SNRI) remain first-line pharmacotherapy [1]. In patients with hypertension, it has been observed that 32% have been suffering from Anxiety [9]. If the calcium channel blockers are proven to reduce anxiety, they can be the drug of choice for anxiety associated with hypertension. The anti-anxiety action of verapamil is controversial [10, 11]. This study aims at investigating the anti-anxiety actions of verapamil.

MATERIALS AND METHODS: Study protocol was approved by the Institutional Animal Ethics Committee, approval number 2/2016. Animals and housing conditions-Swiss Albino mice of either sex of weight 19 to 31g, obtained from the animal house of Govt. Vellore Medical College, India were used in this study. Mice were housed in polypropylene plastic cages with paddy – husk bedding in hygienic conditions. There were 5 animals in each cage. The cages were kept in animal houses under controlled temperature (25±1°C) at a relative humidity of (60±2%). The mice were exposed to a 12-hour light and 12-hour dark cycle. There was no restriction to food or water. Experiments were conducted in the laboratory of the Pharmacology department during the day.

Drugs: Verapamil (obtained from Nicholas Piramal India Limited, Madhya Pradesh) was injected intraperitoneally. Fluoxetine (obtained from Mano Pharma, Orchid chemicals and Pharmaceuticals Ltd. Swiss Garnier Life Sciences, Himachal Pradesh). It was injected intraperitoneally (I.P.) after dilution with saline. Normal Saline (obtained from Fresenius Kabi India Pvt. Limited, Pune). All the drugs were administered in the morning session i.e. 9:30 am to 10:30 am on each day.

Experiment: Group I was the control groupbeing treated with intraperitoneal normal saline at a dose of 0.1ml/Kg mice. Group II was fluoxetine group where 20mg/Kg at concentration of 0.001/ml, was injected intraperitoneally (I.P.) for 9 days to 5 mice. Group III was Verapamil group where (10mg/Kg) at concentration of 0.001/ml, was injected intraperitoneally for 9 days to 5 mice. Group IV- Verapamil (20mg/Kg) was injected intraperitoneally (I.P.) to 5 swiss albino female mice respectively for 9 days. Group V- Verapamil (10mg/Kg) and Fluoxetine was injected intraperitoneally to 5 swiss albino mice for 9 days. On the day 9^{th} of treatment, the tests were performed after 45 minutes of drug administration and the parameters are estimated using Rota rod apparatus, Actophotometer, Tail suspension test, Forced swim test, Marble burying test, Light-dark exploration test and Social interaction study.

Digital Photoactometer: The locomotor activity (horizontal activity) was measured using a photoactometer [12, 13] (INCO, Ambala, India) which operates on photoelectric cells which are connected in circuit with a counter. Each mouse of the control and drug-treated group is in the activity cage for 5 minutes and then the counts are recorded. Statistically, centrally acting depressants have shown to inhibit the locomotor activity in rodents.

Light-dark Exploration Test: The apparatus consisted of a thermacolbox (44 x 21 x 21 cm) separated into two unequal compartments (dark and light) by a partition which had a small opening (8 x 5 cm) at floor level to enable the mouse to move between the lighted and dark areas [14]. The light compartment of the box was covered on top with a transparent plastic sheet and illuminated by a desk lamp (100 W). The dark compartment of the box was covered with black paper on all sides including the top. Mice were individually placed in the center of the light compartment, facing away from the partition, and allowed to freely explore the apparatus for 10 minutes. The number of light-dark transitions between the two compartments and the time spent in the bright and dark compartments were recorded.

Marble Burying Test: The marble-burying behavior test is based on the method of Uday Gaikwad *et al*, [15] Drugs were dosed intraperitoneally (i.p) 40 min before the test. The drug-treated mice were placed individually in cages (21 x 38 x 14 cm) containing 15 clean glass marbles of different colours (10 mm in diameter) evenly spaced on 5 cm deep paddy husk. The results of marble-burying behavior were expressed as the number of marbles at least two-thirds buried in the paddy husk after 30 minutes stay of the mouse in that cage.

Social Interaction Test: Social interaction test is used to determine the anxiety state of rodents.[16] The parameters like exploration, ambulation, sniffing, rearing, grooming, following, social contacts, sexual behavior, attack, fighting, biting defensive posture and immobility can be measured in this study. Two drug-treated mice of either sex are placed in a cage and their social interaction is studied for about 10 minutes. The presence of an unfamiliar social partner is the source of anxiety for the mice.

RESULTS:

Locomotor Activity: Mean (sd) Locomotor activity in the control group was 267.6(±61.77), whereas it was 590.8 (±25.4) in the group pre-treated with Fluoxetine. The increase in the locomotor activity of group pre-treated with Fluoxetine as compared to control was highly significant (*P*< 0.001). In the group pre-treated with Verapamil 10mg the locomotor activity was 462.6 (± 58.2). In the group pre-treated with Verapamil 20mg locomotor activity was 561.8 (±33.33). Verapamil at these two doses significantly increased endurance period as compared to control mice (*P*<0.001).

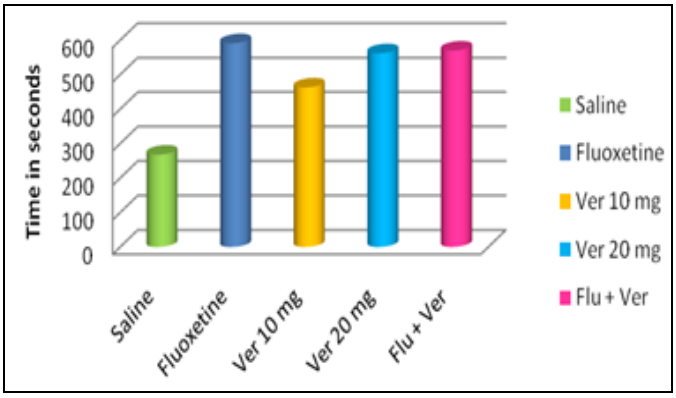

FIG. 1: LOCOMOTOR ACTIVITY

Locomotor activity of the groups pre-treated with Verapamil 10mg was not significantly different as compared to Fluoxetine pre-treated mice. In the groups pre-treated with both Verapamil (10mg/Kg) and Fluoxetine the locomotor activity was 569.6±39.90. Co-administration of Verapamil and Fluoxetine significantly increased Locomotor activity as compared to control mice (*P*<0.001).

Marble Burying Test: Mean number of marbles buried in the control group was 27.6±6.1, whereas it was 7.6±1.14 in the group pre-treated with Fluoxetine. The decrease in the number of marbles buried in the pre-treated with Fluoxetine group as compared to control was highly significant (*P*< 0.001). In the groups pre-treated with Verapamil 10mg, the number of marbles buried was 14 ±3.16 and in group which received Verapamil 20mg it was 9.2±0.83. Verapamil at these two doses significantly decreased the number of marbles buried as compared to control mice (*P* <0.001). Decrease in number of marbles buried of the groups pre-treated with Verapamil 10mg was not found to be significant as compared to Fluoxetine pre-treated mice. In the groups pre-treated with both Verapamil (10mg/Kg) and Fluoxetine the number of marbles buried was 9.2±1.1. Co-administration of Verapamil and Fluoxetine significantly decreased in the number of marbles buried as compared to control mice (*P* <0.001) and it was significantly lesser as compared to Fluoxetine pre-treated mice andsame as Verapamil 20mg.

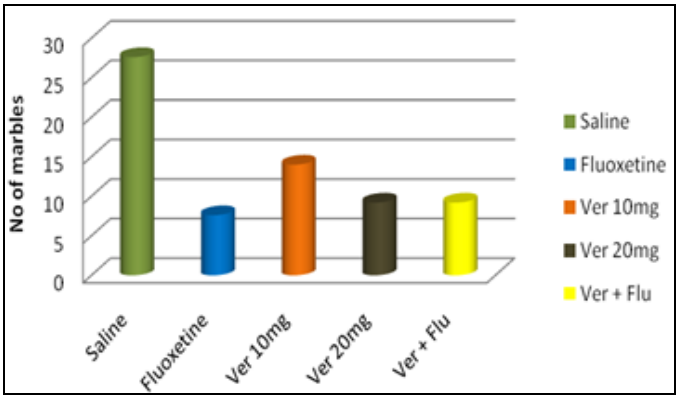

FIG. 2: MARBLE BURYING TEST

Lightand Dark Exploration Test: Fluoxetine treated mice increased the number of entries into light area and its mean was 123±13.93, whereas it was 57.20±11.78 in the control group Saline. The increase in the number of entries towards light in

the group pre-treated with Fluoxetine as compared to control was highly significant (*P* <0.001). In the groups pretreated with Verapamil 10 mg the number of entries towards light was 85±11.26. In the groups pretreated with Verapamil 20 mg, the number of entries towards light was 102.6±7.93. Verapamil at these two doses significantly increased the number of entries towards light as compared to control mice (*P* <0.001). In the groups pre-treated with both Verapamil (10mg/Kg) and Fluoxetine, the number of entries towards light was 103.6±12.26. Co-administration of Verapamil and Fluoxetine significantly increased in the number of entries towards light as compared to control mice (*P* <0.001) and it was significantly lesser as compared to Fluoxetine pre-treated mice and more or less same as Verapamil 20mg. In case of dark area, Fluoxetine treated mice had decreased number of entries into dark area and its mean was 13.4±3.98, whereas it was 262.4±16.65 in the control group Saline. The decrease in the number of entries towards dark in the group pre-treated with Fluoxetine as compared to control was highly significant (*P* <0.001). In the groups pretreated with two different doses of Verapamil for 9 days, the number of entries towards dark was 30.6±8.88 for group 3 (Verapamil 10mg) 14.6±4.4 for group 4 (Verapamil 20 mg). Verapamil at these two doses significantly decreased the number of entries towards dark as compared to control mice (*P* <0.001). Increase in the number of entries towards dark of the group pre-treated Verapamil 10mg was not found to be significant as compared to Fluoxetin pre-treated mice (group 2), but anxiolytic activity in animals administered with Verapamil 20mg (group4) was significantly more as compared to Fluoxetine pre-treated mice. In the groups pre-treated with both Verapamil (10mg/Kg) and Fluoxetine (10mg/Kg) for 9 days, the number of entries towards dark was 14±2.74 (group5). Co-administration of Verapamil and Fluoxetine significantly decreased in the number of entries towards dark as compared to control mice (*P* <0.001) and it was Significantly greater as compared to Fluoxetine pre-treated mice and more or less same as Verapamil 20mg.

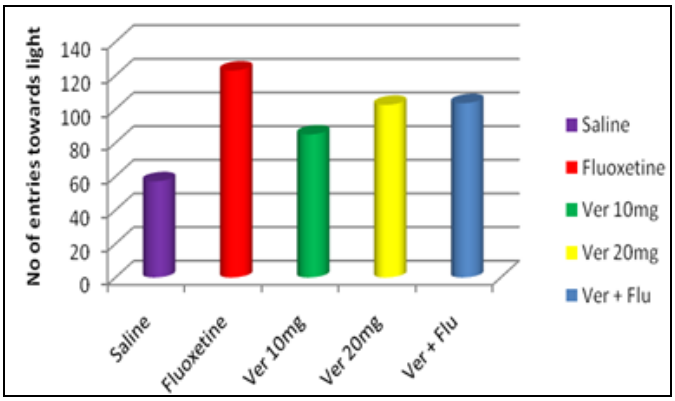

FIG. 3: LIGHT AND DARK-LIGHT AREA

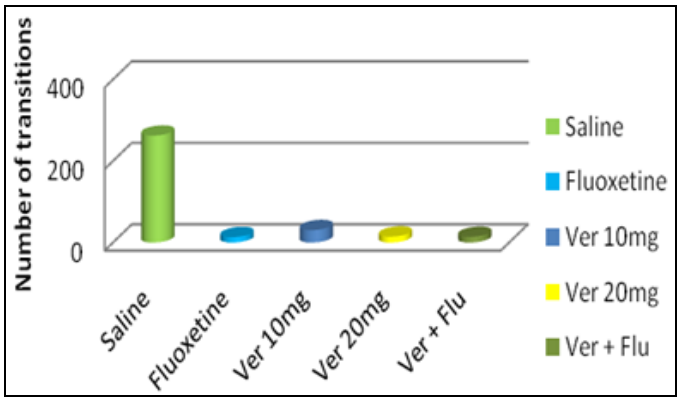

FIG. 4: LIGHT AND DARK-DARK AREA

Social Interaction: Fluoxetine treated mice increased in the number of social interaction and its mean was 24.8±2.59, whereas it was 11.2±4.66 in the control group Saline. The increase in the number of social interactions in the group pre-treated with Fluoxetine as compared to control was highly significant (*P* <0.001). In the groups pretreated with two different doses of Verapamil for 9 days, the number social interaction was 17.8±1.92 for group 3 (Verapamil 10 mg) 19.6±1.14 for group 4 (Verapamil 20mg). Verapamil at these two doses significantly increased in the number of social interactions as compared to control mice (*P* <0.001). Decrease in the number of social interactions of the groups pre-treated with Verapamil 10mg and 20mg was not found to be significant as compared to Fluoxetine pre-treated mice (group 2). In the groups pre-treated with both Verapamil (10mg/Kg) and Fluoxetine (10mg/Kg) for 9 days, the number of social interactions was 20.4±0.89 (group5). Co-administration of Verapamil and Fluoxetine significantly increased in the number of social interactions as compared to control mice (*P* <0.001) and it was significantly lesser as compared to Fluoxetine pre-treated mice but greater than Verapamil 10 and 20mg. In case of aggression, Fluoxetine treated mice decreased in the number of

attacks and its mean was 2.4±1.14, whereas it was 13.4±4.39 in the control group Saline. The decrease in the number of attacks in the group pre-treated with Fluoxetine as compared to control was highly significant (*P* <0.001). In the groups pretreated with two different doses of Verapamil, the number of attacks was 8.4±2.07 for group 3 (Verapamil 10mg) 7±1.23 for group 4 (Verapamil 20 mg). Verapamil at these two doses significantly decreased the number of attacks as compared to control mice (*P* <0.001). The increase in the attack of the group pre-treated Verapamil 10mg and 20mg was not found to be significant as compared to Fluoxetine pre-treated mice (group 2). In the groups pre-treated with both Verapamil (10mg/Kg) and Fluoxetine (10mg/Kg) for 9 days, the number of attacks was 6.4±1.52 (group5). Co-administration of Verapamil and Fluoxetine significantly decreased in the number of attacks as compared to control mice (*P* <0.001) and it was significantly greater as compared to Fluoxetine pre-treated mice but lesser than Verapamil 10 and 20mg.

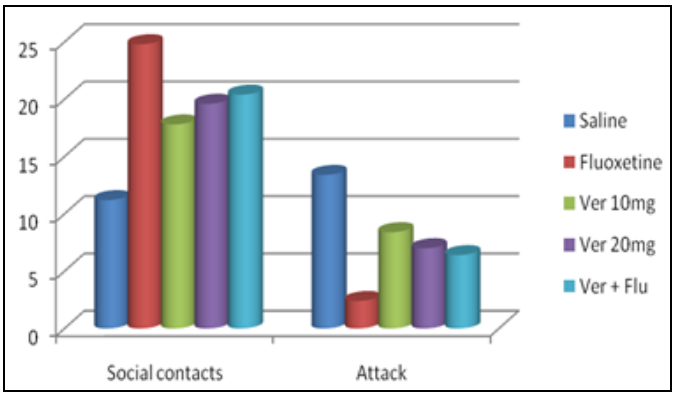

FIG. 5: SOCIAL INTERACTION

DISCUSSION: The combination of various methods to test anti anxietic effects of drugs is shown to better than adopting a single method [23]. In this study four experimental methods were adopted for testing the anti-anxiety effect of verapamil in Swiss albino mice. There was dose dependant increase in the locomotor activity of the mice with the use of Verapamil. There is clearly a benefit observed when compared to placebo as well as the positive control Fluoxetine. There was a decrease in locomotion when verapamil and Fluoxetine were given when compared to Fluoxetine group, but this was not significantly different. There were more marbles buried by the verapamil group as compared to placebo and also the effect of verapamil on marble burying was more than that of the fluoxetine group. The findings in the light and dark exploration test in this study were similar to that observed by Krishna *et. al* [10]. There was a decrease in the number of entries into the dark compartment and more entries into the dark compartment with both doses of verapamil. When the mice were allowed to interact with each other there were decreased number of attacks on each other and an increased social contact.

CONCLUSION: There was anti-anxiety effect observed with administration of verapamil when tested by with digital photoactometer, light and dark exploration test, social interaction and marble burying test. This effect was also seen to be dose dependant and comparable to fluoxetine.

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CONFLICTS OF INTEREST: Nil

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