

## INFLUENCE OF STRESS AND FLUOXETINE ON IMMOBILITY PERIOD OF MICE IN TAIL SUSPENSION TEST AND FORCED SWIM TEST

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### ABSTRACT

**Objective:** To investigate the influence of immobilization stress of 2 hrs and FLX on the immobility period of unstressed and stressed mice and to determine how stress and FLX modulates the effect of each other.

**Methods:** Mice were stressed by immobilization for 2 hrs and the immobility period of both unstressed and stressed mice were determined by using tail suspension test (TST) and forced swim test (FST).

**Results:** Immobilization stress of 2 hrs increased the immobility period of mice in both TST and FST. FLX reduced the immobility period of both unstressed and stressed mice in both TST and FST. Both immobilization stress of 2 hrs and FLX modulated the effect of each other. Immobility period of mice in which FLX was administered before the immobilization stress of 2 hrs had no significant difference from the immobility period of mice in which FLX was administered after the immobilization stress of 2 hrs.

**Conclusion:** It has been concluded that the immobility period of mice in which FLX was administered before the immobilization stress of 2 hrs had no significant difference from the immobility period of mice in which FLX was administered after the immobilization stress of 2 hrs.

**Keywords:** Stress, Depression, Fluoxetine, Immobility.

### INTRODUCTION

Depression is a heterogeneous disorder with symptoms characterized at psychological, behavioral, and physiological levels [1]. Various symptoms of depression are depressed mood, anhedonia, loss of energy, and low self-esteem [2]. Psychological stress plays an important role in the development of affective disorders in humans [3]. It has been suggested that the stress induces a behavioral state analogous to depression [4]. However, the duration of exposure, variability, and unpredictability of stressors are critical factors in the development of depression-like behavior [5,6]. In the animal experimental models, depression-like behavioral alterations is caused by the stressors such as immobilization stress [7]. Chronic immobilization stress in animals and psychological stress in human has been implicated in the pathophysiology of mood disorders [8]. Acute immobilization stress has also been reported to influence depression-like behaviors in the animals [9]. Thus, the depression-like alterations in the laboratory animals can be induced by the exposure of the laboratory animals to immobilization stress. In rodents, stress-induced behavioral depression can be assessed using tail suspension test (TST) and forced swim test (FST) [10]. In these tests, animal initially shows some escape-oriented behavior but after some escape attempts develop immobility [11]. The immobility of the animals is the behavioral despair which is similar to human depression and represents the psychomotor retardation in depressed patients [12-16].

Serotonin (5-HT) is neurotransmitter which is responsible for the regulation of the mood and emotions; therefore, 5-HT dysfunction contributes to depression or increases the vulnerability to depression [17,18]. 5-HT dysfunctioning contributes to depression is supported by the fact that the acute depletion of tryptophan (responsible for 5-HT synthesis) contributes to depression [19]. Therefore, the deficiencies of 5-HT or its reduced transmission contribute to the development of depression [20]. Therefore, drugs which correct the 5-HT neurotransmission could be used in the treatment of depression. Selective serotonin reuptake inhibitors

(SSRIs) enhance serotonergic neurotransmission, by blocking the 5-HT-binding site on the SERT, thus preventing 5-HT uptake into the neuron [21]. SSRIs mediated the inhibition of 5-HT reuptake results in the increase in synaptic levels of 5-HT that further activates inhibitory 5-HT<sub>1A</sub> autoreceptors, and therefore, reduces the neuronal firing, with consequent reduction of 5-HT release [22,23]. SSRIs are clinically proven antidepressants, increase the serotonergic transmission, and reduce the immobility period of the animal in TST and FST [12,13]. Fluoxetine (FLX), a standard antidepressant drug belonging to the category of SSRIs [24] reduces the immobility period of mice in TST and FST [25].

### METHODS

#### Animals

Swiss albino mice were used in the present study. All the mice were kept under controlled conditions of light and environmental and had free access to food and water. The testing was carried out between 9:00 and 16:00 hrs. The study protocols were approved by the Institutional Animal Ethics Committee, and care of the animals was carried out in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

#### Drugs

FLX was purchased from the Cadila Pharmaceuticals, Ahmedabad, India, and dose was used on the basis of literature [26].

#### Immobilization stress

Mice were stressed by immobilization for 2 hrs [27] by taping, all its four limbs and trunk against a wooden board [28].

#### TST

TST is the most commonly use test for the assessment of depression-like behavioral alterations in animals. In TST, each mouse was individually suspended at a height of 30 cm by adhesive tape from the tip of the tail, and the immobility was recorded over a period of 6 minutes [14].

## FST

FST is another most commonly use test for the assessment of depression-like behavioral alterations in animals. In FST, each mouse was individually forced to swim in the glass chamber containing water at a height of 15 cm maintained at  $26 \pm 1^\circ\text{C}$ . Each mouse shows vigorous movements during the initial 2 minutes period of the test. The immobility period was recorded during over a period of 4 minutes of the total 6 minutes testing period [15,16].

## Experimental groups

Group 1: Mice were administered with the vehicle and after 30 minutes immobility period was determined by TST followed by FST.

Group 2: Mice were immobilized for 2 hrs and after 2 hrs of immobilization, immobility period determined by TST followed by FST.

Group 3: Mice were administered with FLX (20 mg/kg, i.p.) and after 30 minutes immobility period determined by TST followed by FST.

Group 4: Mice were administered with FLX (20 mg/kg, i.p.) and after 2 hrs, immobility period was determined by TST followed by FST.

Group 5: Mice were administered with FLX (20 mg/kg, i.p.) and immediately after administration mice were subjected to immobilization for 2 hrs and after 2 hrs, immobility period was determined.

Group 6: Mice were immobilized for 2 hrs and after 2 hrs, FLX 20 mg/kg was administered by i.p. route and after 30 minutes of administration the immobility period was determined.

## Statistical analysis

Data were analyzed by one-way analysis of variance followed by Tukey's test. Values were expressed as mean  $\pm$  standard error of mean and  $p < 0.05$  was considered as statistically significant.

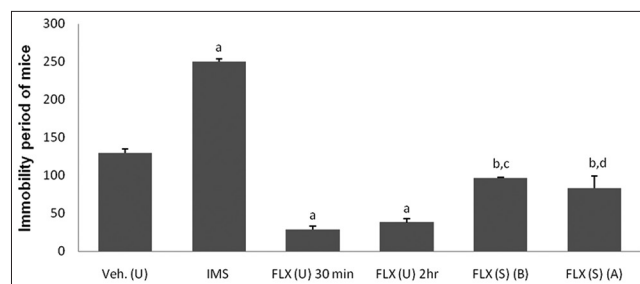
## RESULTS

### Effect of different treatments on the immobility period of mice in TST and FST

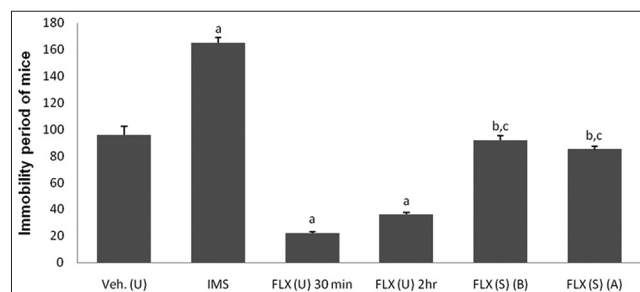
Immobilization stress of 2 hrs significantly increases the immobility period of mice in TST ( $p < 0.001$ ) and FST as compared to the vehicle-treated unstressed mice in the present study. Administration of FLX (20 mg/kg, i.p.) to the unstressed mice significantly reduced the immobility period after 30 minutes and even after the 2 hrs of the administration in TST ( $p < 0.001$ ) and FST ( $p < 0.001$ ) as compared to the vehicle-treated unstressed mice. Therefore, the antidepressant effect of the FLX (20 mg/kg, i.p.) persisted even after 2 hrs of the administration. Administration of FLX (20 mg/kg, i.p.) immediately before the immobilization of 2 hrs significantly reduced the immobility period in both TST ( $p < 0.001$ ) and FST ( $p < 0.001$ ) as compared to vehicle treated stressed mice. Furthermore, the administration of FLX (20 mg/kg, i.p.) immediately after the immobilization stress of 2 hrs; significantly reduced the immobility period in both TST ( $p < 0.001$ ) and FST ( $p < 0.001$ ) as compared to vehicle treated stressed mice ( $p < 0.0001$ ). The immobility period of FLX treated unstressed mice after 2 hrs was significantly lesser than the immobility period of mice in which the FLX was administered before the immobilization stress of 2 hrs in TST ( $p < 0.01$ ) and FST ( $p < 0.001$ ). The immobility period of FLX treated unstressed mice after 2 hrs was significantly lesser than the immobility period of mice in which the FLX was administered after the immobilization stress of 2 hrs in TST ( $p < 0.05$ ) and FST ( $p < 0.001$ ) (Figs. 1 and 2).

## DISCUSSION

Depression is heterogeneous and one of the most prevalent neuropsychiatric disorders that affect 20% of the world's population [29,30]. The exact mechanism that contributes to the depression is not known, but alterations in monoaminergic systems contribute to the pathogenesis of depression and, therefore, the drugs



**Fig. 1: Effect of different treatments on immobility period of mice in tail suspension test. Values were expressed as mean  $\pm$  standard error of mean  $F(5, 24) = 74.776$ . a:  $p < 0.001$  significant difference from the vehicle-treated unstressed mice; b:  $p < 0.001$  significant difference from the stressed mice; c:  $p < 0.01$  significant difference from fluoxetine (FLX) (U) 2 hrs; d:  $p < 0.05$  significant difference from FLX (U) 2 hrs. Veh. (U): Vehicle treated unstressed mice; IMS: Immobilization stressed mice; FLX (U) 30 minutes: FLX was administered 30 minutes before the behavioral testing in unstressed mice; FLX (U) 2 hrs: FLX was administered 2 hrs prior to the behavioral testing in unstressed mice FLX (S) (B): FLX was administered before subjecting the animals to immobilization. FLX (S) (A): FLX was administered after the animals were freed from the immobilization of 2 hrs. Doses are mentioned in mg/kg**



**Fig. 2. Effect of different treatments on immobility period of mice in forced swim test. Values were expressed as mean  $\pm$  standard error of mean  $F(5, 24) = 167.45$ . a:  $p < 0.001$  significant difference from the vehicle-treated unstressed mice; b:  $p < 0.001$  significant difference from the stressed mice; c:  $p < 0.001$  significant difference from fluoxetine (FLX) (U) 2 hrs. Veh. (U): Vehicle treated unstressed mice; IMS: Immobilization stressed mice; FLX (U) 30 minutes: FLX was administered 30 minutes before the behavioral testing in unstressed mice; FLX (U) 2 hrs: FLX was administered 2 hrs before the behavioral testing in unstressed mice FLX (S) (B): FLX was administered before subjecting the animals to immobilization. FLX (S) (A): FLX was administered after the animals were freed from the immobilization of 2 hrs. Doses are mentioned in mg/kg**

that influence the monoaminergic system influences depression-like behavioral alterations [31]. Stress is a stimulus that disturbs the homeostasis of the body and induces depressive disorders through the activation of the neuroendocrine system, neurotransmitter changes, and pro-inflammatory cytokines [32]. The depression-like behavioral alterations is caused by various stressors such as immobilization stress [7,9,27] in the present study the immobilization stress of 2 hrs significantly enhances the immobility period of the mice in both TST and FST. Poleszak *et al.* have also showed that the 2 hrs immobilization increases the immobility period of mice [27]. Thus, the acute immobilization for 2 hrs significantly enhances the depression in mice subjected to the immobilization.

TST and FST are the most commonly used test for the assessment of depression-like behavioral alteration in mice [14-16]. In both TST

and FST, the mice initially show some escape-oriented behaviors but develop the immobility after some time. This immobility developed by the mice reflects the behavioral despair, and it is related to the stress-induced depression in the humans [12-14]. Therefore, the drugs or the agents which reduce the immobility period of the mice in the TST and FST exert antidepressant effect [33-35]. Both TST and FST are highly sensitive to clinically used antidepressants such as SSRIs [36]. SSRIs are clinically proven antidepressants, increases the serotonergic transmission, and reduces the immobility period of the animal in TST and FST [12,13]. In the present study, the administration of FLX (20 mg/kg, i.p.) 30 minutes and 2 hrs before the testing in unstressed mice significantly reduced the immobility period of mice in both TST and FST as compared to the vehicle-treated unstressed mice. Therefore, our results showed the antidepressant effect of the FLX (20 mg/kg, i.p.) persisted even after the 2 hrs.

Administration of FLX (20 mg/kg, i.p.) immediately before the immobilization of 2 hrs significantly reduced the immobility period of mice in both TST and FST significantly as compared to vehicle treated stressed mice. So, it has been suggested that the FLX (20 mg/kg, i.p.) significantly exerted the antidepressant effect in the stressed mice also and significantly counteracts the effect of 2 hrs immobilization. Thus, FLX can be used in the treatment of the stress induced depression as suggested by the results of the present study. However, the mechanism by which FLX counteracts the effect of immobilization stress of 2 hrs could not be determined by the present study. However, the administration of FLX (20 mg/kg, i.p.) immediately after the immobilization stress of 2 hrs; also, significantly reduced the immobility period of mice in both TST and FST significantly as compared to vehicle treated stressed mice, but the immobilization stress of 2 hrs also significantly reduces the antidepressant effect of FLX (20 mg/kg, i.p.) in stressed mice, because the immobility period of FLX (20 mg/kg, i.p.) treated stressed was significantly higher than the FLX (20 mg/kg, i.p.) unstressed mice. Therefore, it has been demonstrated that the immobilization stress of 2 hrs also influences the antidepressant effect of the FLX in stressed mice. However, the mechanism by which immobilization stress of 2 hrs influence the antidepressant activity of the FLX in stressed mice could not be explained by the present study. However, there was no significant difference in the immobility period of mice in both TST and FST in which FLX (20 mg/kg, i.p.) was administered before the immobilization stress of 2 hrs from the mice in which FLX (20 mg/kg, i.p.) was administered after the immobilization stress of 2 hrs. Thus, both FLX and stress influence the effect of each other, if the FLX counteracts the effect of the immobilization stress, then immobilization stress also influences the effect of FLX in the stressed condition. However, the mechanism how they influence the effect of each other required further work.

## CONCLUSION

It has been concluded from the present study that both FLX and immobilization stress influence the effect of each other. FLX reduces the depressive symptoms enhances by the immobilization stress of 2 hrs. Furthermore, the immobilization stress of 2 hrs reduces the antidepressant effect of FLX (20 mg/kg, i.p.) in stressed mice. The immobility period of mice in which the FLX administered before the immobilization stress of 2 hrs has no significant difference the immobility period of mice in which the FLX was administered after the exposure to the immobilization stress of 2 hrs.

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