

**ANTIDEPRESSANT ACTIVITY OF CILOSTAZOL: AN  
EXPERIMENTAL STUDY.**

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**ABSTRACT**

**Objective:** Besides antiplatelet activity, cilostazol has been reported to posses antidepressant activity. Therefore it was thought worthwhile to compare antidepressant activity of cilostazol with that of fluoxetine, a clinically used antidepressant. **Methods:** In this study, the antidepressant activity of cilostazol was compared with that of fluoxetine in mice by forced swim test, tail suspension test and forced swim test with activity wheel. **Results:** Cilostazol (20 mg/kg) administered intraperitoneally showed antidepressant activity as it significantly decreased the immobility time when subjected to forced swim and tail suspension test and increased the number of rotations of wheel when subjected to forced swim test with activity wheel.

However the activity of cilostazol was found to be less than that of fluoxetine (20mg/kg).

**Conclusion:** The present study suggests that cilostazol possesses potential antidepressant activity which could be of clinical importance for the patients suffering from depressive disorders.

**KEYWORDS:** *Cilostazol, forced swim test, tail suspension test, water wheel test.*

## INTRODUCTION

Depression is an affective disorder characterized by change in mood, lack of interest in the surroundings, psychomotor retardation and melancholia<sup>[1]</sup>. In comparison to men, women are twice likely to suffer from depression as the life time risk of depression varies from 5 to 12% in men and 10 to 25% in women<sup>[2,3]</sup>. It is also the leading cause of disability which leads to suicidal tendency among patients of depression. Patients with cardiovascular diseases are at higher risk of developing depression and when it develops, there is two-fold increase in cardiac motility and morbidity for population suffering from various coronary heart diseases.<sup>[4-6]</sup>

Cilostazol(6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1H)-quinolinone), a 2-oxo-quinoline derivative is mainly used as an antiplatelet agent and in peripheral vascular diseases with intermittent claudication. It also has antithrombotic, vasodilator, antimutagenic and cardiogenic properties which have been observed in various preclinical studies.<sup>[7-9]</sup> It is a potent inhibitor of the enzyme phosphodiesterase 3A,<sup>[10]</sup> found in hippocampus, striatum and other sites in brain<sup>[11]</sup> due to which it has been reported as an antidepressant, antipsychotic, enhancer of memory and also as aid in improving cognitive functions in various preclinical studies.<sup>[12-15]</sup> The phosphodiesterase inhibitors present a potentially powerful means to manipulate second messengers such as cAMP and cGMP which play an important role in neuronal cell functions.<sup>[16]</sup>

## MATERIAL AND METHODS

Experiments were performed on Swiss albino mice three / four months of age (25 to 30 g) of either sex. They were procured from the central animal house of Nims University. The animals were housed in standard polypropylene cages and kept under controlled room temperature ( $24 \pm 2^\circ\text{C}$ ) in a 12 hour light dark cycle. They were given standard pellet diet and water ad libitum. They were acclimatized to the laboratory conditions at least one day prior to the behavioral experiments. Food was withdrawn 12 hour before the experiments. The animal handling was performed according to the Good Laboratory Practice (GLP) guidelines,<sup>[17]</sup> and efforts were made to minimize animal suffering. All the experiments were performed after the prior permission from the Institutional Animal Ethics Committee (IAEC), NIMS Medical College, Jaipur, India (Approval number: NIMS/MC/PO/2013/158).

Cilostazol (Cipla, India) and Fluoxetine (Cadila Pharmaceuticals, India) were used for the experimentation. Cilostazol was given at a dose of 20 mg/kg i.p for 15 days. Fluoxetine was

given at a dose of 20 mg/kg i.p for 15 days. Cilostazol was dissolved in dimethyl sulphoxide whereas fluoxetine was dissolved in distilled water.<sup>[18-20]</sup>

### Grouping of animals

GROUP	TREATMENTS
CONTROL(n=6)	Treated with distilled water (i.p.,15 days)
STANDARD(n=6)	Treated with Fluoxetine(20 mg/kg i.p., 15 days)
TEST (n=6)	Treated with Cilostazol (20mg/kg i.p., 15 days)

All the drugs were administered one hour before the test procedure.

### Parameters assessed

#### Forced Swim Test (FST)

Forced swim test in glass jar was performed as described by Porsolt et al. with few modifications.<sup>[17,21,22]</sup> This test consists of two parts, an initial training period of 15 min followed 24 h later by actual test for 5 min duration. Mice were individually forced to swim inside a vertical borosilicate glass cylinder of diameter 15 cm and height 40 cm containing fresh water to a height of 15 cm and maintained at  $25 \pm 1^\circ\text{C}$ . At this level of water, animals were not able to support themselves by touching the bottom or the side walls of the chamber with their hind paws or tail. Water in the chamber was changed after subjecting each animal to FST since used water alters the behavior. Mice placed in the cylinder for the first time were initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2 to 3 min, activity began to subside and was interspersed with phases of immobility or floating of increasing length. After 5 to 6 min, immobility reached a plateau where the mice remained immobile for approximately 80% of the time. After 15 min in the water, the mice were removed, wiped with dry cloth and allowed to dry before being returned to their home cages. The mice were again placed in the cylinder 24 h later and drugs were administered 1 hr prior to testing on last day of dosing. The duration of immobility was recorded for a period of 5 min swimming session with the help of video recorder and subsequently analyzed. Mice were considered immobile when they ceased struggling and remained floating motionless in water, making only those movements necessary to keep their head above water.

#### Tail Suspension Test (TST)

Tail suspension test was done as described by Steru et al.<sup>[23,24]</sup> Each animal was individually suspended on a string held by a metal stand, by an adhesive tape placed 1 cm from the tip of the tail. This string was 58 cm above the table top. The total period of immobility was

recorded through a video recorder for 6 min and subsequently analyzed. Mice were considered immobile when they hung passively and became completely motionless. The test was conducted in dim lighted room and during the experiment, each animal under test was both acoustically and visually isolated from other animals.

### **Forced swim test with activity wheel**

Forced swim test with activity wheel is a modified porsolt forced swim test with minor modifications.<sup>[25,26]</sup> Mice were forced to swim in an apparatus consisting of a water tank 19 x 10 x 13.5 cm with a water wheel in its centre. The tank was filled with water ( $25 \pm 1^\circ\text{C}$ ) to a height of 9 cm. When placed in the tank, mice tried to escape from the tank but ended up in rotating the wheel. The number of times the wheel was rotated by mice in a 5 min test period, as recorded in the digital counter of the instrument, was noted. The tank was cleaned after experiment with each animal.

### **Statistical Analysis**

All the results of tail suspension test, forced swim test with activity wheel and forced swim test were expressed as the mean  $\pm$  standard deviation. Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparisons test to determine statistical significance of difference in various groups. Statistical significance was set at  $p < 0.05$ .<sup>[27]</sup>

## **RESULTS AND DISCUSSION**

### **Forced swim test**

In this test [Table 1], mice treated with cilostazol (20 mg/kg) showed decrease in immobility time, which was significant ( $84.42 \pm 3.24$ ;  $P < 0.001$ ) when compared with control ( $116.23 \pm 2.68$ ). Mice treated with fluoxetine (20mg/kg) also showed a significant decrease in the immobility time ( $62.11 \pm 2.98$ ;  $P < 0.001$ ) as compared to control ( $116.23 \pm 2.68$ ). Similarly, mice treated with fluoxetine showed significant decrease in immobility time ( $62.11 \pm 2.98$ ) and thus fluoxetine was found to possess more effective antidepressant activity when compared with Cilostazol ( $84.42 \pm 3.248$ ;  $P < 0.001$ ).

**Table 1. Effect of cilostazol (20mg/kg) and fluoxetine (20mg/kg) on duration of immobility in the forced swim test. The reduction in immobility time (in seconds) in forced swim test by the two drugs fluoxetine (20mg/kg) and cilostazol (20mg/kg) is significant ( $P < 0.001$ ).**

Treatment	N	Mean [Immobility time]	Standard Deviation
Control	6	116.23	2.68
Fluoxetine (20mg/kg)	6	62.11	2.98
Cilostazol (20mg/kg)	6	84.42	3.24

### Tail Suspension Test

In this test [Table 2], mice treated with cilostazol (20 mg/kg) showed decrease in immobility time, which was significant ( $94.98 \pm 3.57$ ;  $P < 0.001$ ) when compared with control ( $132.53 \pm 2.79$ ). Mice treated with fluoxetine (20mg/kg), also showed a significant decrease in the immobility time ( $73.4 \pm 3.05$ ;  $P < 0.001$ ) as compared to control ( $132.53 \pm 2.79$ ). Similarly mice treated with fluoxetine showed significant decrease in immobility time ( $73.4 \pm 3.05$ ) and thus fluoxetine was found to possess more effective antidepressant activity when compared to cilostazol ( $94.98 \pm 3.57$ ;  $P < 0.001$ ).

**Table 2. Effect of cilostazol(20mg/kg) and fluoxetine (20mg/kg) on duration of immobility(in seconds) in the tail suspension test. The reduction in immobility time in tail suspension test by the two drugs fluoxetine (20 mg/kg) and cilostazol (20 mg/kg) is significant ( $P < 0.001$ ).**

Treatment	N	Mean [Immobility Time]	Standard Deviation
Control	6	132.53	2.79
Fluoxetine (20mg/kg)	6	73.4	3.05
Cilostazol (20mg/kg)	6	94.98	3.57

### Forced Swim Test with Activity Wheel

In this test [Table 3], mice treated with cilostazol (20 mg/kg) showed increase in number of rotations of water wheel, which was significant ( $14.5 \pm 2.74$ ;  $P < 0.001$ ) as compared with control ( $6.5 \pm 1.05$ ). Mice treated with fluoxetine (20mg/kg), also showed a significant increase in number of rotations of water wheel ( $26.33 \pm 2.42$ ;  $P < 0.001$ ) as compared to control ( $6.5 \pm 1.05$ ). Similarly mice treated with fluoxetine showed increase in number of rotations of water wheel ( $26.33 \pm 2.42212$ ) and thus fluoxetine was found to possess more effective antidepressant activity when compared with cilostazol ( $14.5 \pm 2.74$ ;  $P < 0.001$ ).

**Table 3. Effect of cilostazol (20mg/kg) and fluoxetine (20mg/kg) on number of rotations in forced swim test with activity wheel. The increase in number of rotations of wheel in forced swim test with activity wheel by the two drugs fluoxetine (20mg/kg) and cilostazol (20mg/kg) is significant (P<0.001).**

Treatment	N	Mean [No. of rotations of wheel]	Standard Deviation
Control	6	6.5	1.05
Fluoxetine (20mg/kg)	6	26.33	2.42
Cilostazol (20mg/kg)	6	14.5	2.74

The results of forced swim test and tail suspension test with cilostazol (20 mg/kg) in mice show that the immobility time was significantly decreased and in water wheel test, number of turns of wheel was significantly increased by the drug treatment. The drug cilostazol is thought to be a potent inhibitor of phosphodiesterase III enzyme, which increases cAMP in hippocampus by which the framework of pathophysiology and pharmacotherapy converge on cAMP-mediated signaling rather than on neurotransmitter system.<sup>[10,11,16]</sup> It has also been seen that cilostazol increases insulin like growth factor-I (IGF-I) in hypothalamus, which is responsible for improving cognitive functions in patients of depression leading to antidepressant like action.<sup>[28]</sup> Moreover, some clinical studies have shown that the drug cilostazol when administered to cardiovascular patients with depression who underwent angioplasty and on adjuvant dual antiplatelet therapy, is effective in treating patients with mild to moderate depression which was seen by a decrease in Montgomery-Asberg Depression Rating Scale (MADRS) scoring compared to baseline ratings.<sup>[29]</sup> Further studies are required to arrive at exact mechanism of action of cilostazol as antidepressant.

The standard drug fluoxetine reduced the immobility time in forced swim test and tail suspension test and increased the number of turns of wheel in Water Wheel Test. Fluoxetine, a selective serotonin reuptake inhibitor, acts by inhibiting the uptake of serotonin by the neurons in the brain and enhancing serotonin neurotransmission through action on various serotonin receptors.<sup>[30]</sup>

## CONCLUSION

It can be concluded that cilostazol can be a drug of future specially for cardiovascular patients with mild to moderate depression as it has multiple uses and is devoid of serious adverse effects which occur with the use of various established antidepressant drugs. Further studies are required to confirm and know the exact mechanism of action of cilostazol for its antidepressant effect.

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