Original Article

Effect of cilnidipine on depression-like behaviour in male Swiss mice: A study using the tail suspension test

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ABSTRACT

Objective: The objective is to evaluate cilnidipine and compare it with fluoxetine on the depression model in male Swiss mice, utilising the tail suspension test and locomotor activity test.

Materials and Methods: The animals were categorised into four groups, each consisting of six individuals ($n = 6$ per group). The subjects were given the test drug doses of cilnidipine at 5 mg/kg and 10 mg/kg, as well as fluoxetine at 10 mg/kg, for a duration of 21 days through an intraperitoneal route. On days 1, 14 and 21, the locomotor activity was assessed using the actophotometer, while the antidepressant activity was assessed using the tail suspension test (TST). The duration of immobility was assessed using the total sleep time method for a 5-minute period, while the number of counts was monitored for 10 min using an actophotometer.

Results: Cilnidipine at a dosage of 10 mg/kg demonstrates a reduction in symptoms of depression when compared to the standard control. Neither cilnidipine 5 mg/kg nor 10 mg/kg resulted in a noteworthy decrease in locomotor activity.

Conclusion: The present study demonstrated a substantial antidepressant effect of cilnidipine 10 mg/kg dosage. More research is needed to validate the results reported.

Keywords: Depression, Hypertension, Dihydropyridines, Tail suspension test, Neurotransmission, Corticosterone

INTRODUCTION

Depression is a highly significant and widespread psychiatric disorder characterised by persistent feelings of hopelessness, diminished interest or enjoyment, low energy levels, inadequate diet and sleep and even thoughts of suicide. It significantly affects daily activities and psychosocial functioning.[1] Since 2008, the World Health Organisation has recognised depression as the third most significant source of illness burden. It will likely be ranked first by 2030.[1,2] Common mental disorders, such as depression, have a significant association with both the causes and consequences of various non-communicable diseases, including hypertension, myocardial infarction, stroke, dementia, epilepsy and endocrine disorders, including diabetes and hypothyroidism.[3] Recently, it has been found that the chronic nature of these medical conditions is an additional cause of depression symptoms in the patient. Treatment of such comorbidities often necessitates the concurrent use of multiple drugs, which leads to a pharmaceutical interaction between drugs or between a medicine and a condition which can improve or worsen the primary disorder

or comorbidity in the latter case, thus contributing to a significantly higher healthcare burden.[4]

The current therapy options for depression, such as selective serotonin reuptake inhibitor (SSRIs), selective noradrenaline (NA) receptor blockers and other older classes of drugs, are insufficient in achieving full recovery for most patients. In addition, many patients do not respond well to these medications. Only 30–40% of patients achieve remission with a single treatment, and most of these patients are switched to another medication or augmented with another drug.[5] As a result, there is a significant demand for additional treatment choices for depressive illnesses.

Calcium channel blockers (CCBs) have been observed in various studies that affect the release of central neurotransmitters such as NA and gamma-aminobutyric acid.[6,7] Research has shown that CCBs may have an antidepressant effect by increasing intracellular calcium release through GABA-A receptors and NA, both of which are involved in the development of depression.[7] Furthermore, CCBs that may cross the blood-brain barrier have been linked to a reduced occurrence of neuropsychiatric disorders, such as depression.[8]

A comprehensive analysis and synthesis of existing studies found that around 21.3% of persons with hypertension also experience depression.[9] Another study reported that depression was a risk factor for uncontrolled blood pressure in hypertension patients.[10] These findings emphasise the significance of treating patient's mental health issues with hypertension, as well as the possible effects of depression on controlling blood pressure. One of the main concerns is the cohabitation of depression and hypertension, and better therapies for depression in hypertensive individuals are required.[9,11] Dihydropyridines (DHPs) are lipophilic and the most effective CCBs; among them, cilnidipine has a pleiotropic effect with cerebroselective action. The current research aims to investigate the effects of cilnidipine on a mouse model.

MATERIALS AND METHODS

We purchased male Swiss albino mice from the central animal house, weighing between 20 and 30 grammes. The mice were kept in hygienic polypropylene cages furnished with sterile rice husk bedding. They were housed in typical laboratory settings with free reign over food and drink. The Institutional Animal Ethical Committee granted permission for the use of the mice in this investigation, and their registration number (CPCSEARegNo.627/PO/Re/S/02/CPCSEA). Before the testing day, the animals were habituated to a 12:12 h light-dark cycle for 7 days. The research was carried out in compliance with the guidelines set out by the New Delhi-based Committee for Control and Supervision of Experiments with Animals.

The current investigation spanned a duration of 1.5 years, commencing in September 2022 and concluding in March 2024. The animals were divided into four groups, with six mice in each category [Tables 1 and 2]: Group A: Control (0.2 mL of distilled water), Group B: Fluoxetine (Standard) at a dosage of 10 mg per kilogramme, Group C: Cilnidipine at a dosage of 5 mg per kilogramme and Group D: Cilnidipine at a dosage of 10 mg per kilogramme. Fluoxetine and cilnidipine solution were prepared freshly directly before injection by dissolving them in 2 mL physiological saline and injected intraperitoneally. Twenty-four hours after the last treatment, animals were exposed to antidepressants. The tail suspension test was used to assess the antidepressant effect. On the 1st, 14th and 21st of the experiment, the effects of cilnidipine on the effectiveness of antidepressant activity were investigated using the tail suspension test (TST).

Tail suspension test

The test was devised by Steru *et al.* in 1987. On the 1st day, 30 min after a single dose of test/vehicle,[16] a mouse is attached to a hook using adhesive tape, positioned 1 cm from the end of its tail, for a duration of 5 min. The mouse should be 15 cm away from the nearest object. When mice hang motionless and still for at least a minute, they are considered immobile. Immobility time in seconds is recorded. The mice were removed and returned to their cages and provided with food and water.

On the 14th and 21st day, 30 min after the drug was given, mice underwent the tail suspension test, and the length of time they remained motionless was measured for a period of 5 min. The mean immobility of all groups was calculated for the 1st, 14th and 21st day for analysis.[12-16]

Actophotometer

It was devised by Dews in 1953. After administering a single dosage of test/vehicle, mice from various groups were individually placed in the actophotometer on the 1st day. The number of movements made by the mice was then observed and recorded for a duration of 10 min before returning them to their cages. The same experiments were repeated on the 14th and 21st day, half an hour after drug administration.

The mean of all groups was calculated for the 1st, 14th and 21st day for analysis.[17,18]

Table 1: Grouping of animals.

Group	Treatment	Dose
A	Normal saline	0.2 mL
B	Fluoxetine	10 mg/kg (0.2 mL)
C	Cilnidipine	5 mg/kg (0.1 mL)
D	Cilnidipine	10 mg/kg (0.2 mL)

Table 2: Effect of various drugs on duration of immobility in Tail suspension test.

Day of study	Duration of immobility in seconds			
	Control	Fluoxetine 10mg/kg	Cilnidipine 5mg/kg	Cilnidipine 10mg/kg
1	184.4±4.1	176±3.3*	180.2±1.8	183.7±7.6
14	183.4±4.1	175.7±1.6**	179.8±3	177.3±2.3*#
21	182.1±4	171.1±1.9***	177.3±1.6*#	173.02±1.5**##

Values are expressed as mean ±SD. One-way ANOVA followed by Bonferroni's multiple comparison test. *$p<0.05$, **$p<0.001$ in comparison to control group after same duration of treatment; #$p<0.05$, ##$p<0.01$ in comparison to individual dug groups after same duration of treatment. SD: Standard deviation.

Table 3: Effect of various treatment on locomotor activity in actophotometer.

Day of study	Duration of immobility in seconds			
	Control	Fluoxetine 10mg/kg	Cilnidipine 5mg/kg	Cilnidipine 10mg/kg
1	21.83±2.9	19.66±2.4	22±2.8	21.6±4.6
14	22.6±3.1	20±3.7	23.8±3.7	20±3.7
21	20±3.3	18.8±3.4*	19.8±4.2	20±5.8

Values are expressed as mean±SD. One-way ANOVA followed by Bonferroni's multiple comparison test. *$p<0.05$ in comparison to control group after same duration of treatment

Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the data, and the experimental findings were reported as the mean value plus or minus the standard deviation (ANOVA). To compare with the control group, a *post hoc* analysis was carried out using GraphPad Prism software 8.0.1 and Bonferroni's multiple comparison tests. $P < 0.05$ was found to be statistically significant.

RESULTS

Effects of fluoxetine and cilnidipine in TST on day 1

The duration of immobility, measured in seconds, was recorded for various groups throughout a 6-min period. For the control group, the average duration of immobility was 182.4 ± 4.1, while for the fluoxetine group, it was 176 ± 3.3. For the cilnidipine 5 mg/kg dose, the duration was 180.2 ± 1.8, and for the cilnidipine 10 mg/kg dose, it was 183.7 ± 7.6. The duration of immobility was significantly shorter in the fluoxetine group as compared to the control group ($P < 0.05$). When compared to the control group, the administration of cilnidipine at dosages of 5 mg and 10 mg did not provide statistically significant results [Table 3 and Graph 1].

Effects of fluoxetine and cilnidipine in TST on day 14

The duration of immobility, measured in seconds, was recorded for various groups throughout a 6-min period. In the fluoxetine group, the average duration of immobility was 175.7 ± 1.6, whereas in the control group, it was 183.4 ± 4.1.

For the cilnidipine 5 mg/kg dose, the duration was 179.8 ± 3, and for the cilnidipine 10mg/kg dose, it was 177.3 ± 2.3. The immobility duration was much shorter in the fluoxetine ($P < 0.01$) and cilnidipine (10 mg) groups as compared to the control group. Furthermore, there was a statistically significant drop in the cilnidipine 10 mg group ($P < 0.05$) as compared to the fluoxetine group. When compared to the control group, the administration of cilnidipine 5 mg did not provide statistically significant results [Table 3 and Graph 1].

Effects of fluoxetine and cilnidipine in TST on day 21

The duration of immobility, measured in seconds, was recorded for various groups throughout a 6-min period. In the fluoxetine group, the average length of immobility was 171.1 ± 1.9 min, whereas in the control group, it was 184.1 ± 4. For the cilnidipine 5 mg/kg dose, the duration was 177.3 ± 1.6, and for the cilnidipine 10 mg/kg dose, it was 173.02 ± 1.5. The duration of immobility was notably reduced in the fluoxetine ($P < 0.001$), cilnidipine (5 mg) and cilnidipine (10 mg) groups compared to the control group ($P < 0.05$). A statistically significant decrease was seen in both the cilnidipine 5 mg and cilnidipine 10 mg groups when compared to the fluoxetine group ($P < 0.05$ and $P < 0.01$, respectively). In the fluoxetine group, the length of immobility was significantly shorter on days 1, 7 and 21. In the cilnidipine 5 mg/kg group, there was a significant decrease in immobility only on the 21st day compared to the control and fluoxetine groups. On the 14th day and onward, cilnidipine at a dosage of 10 mg/kg demonstrates a notable decrease. On the 21st day, in comparison to the standard
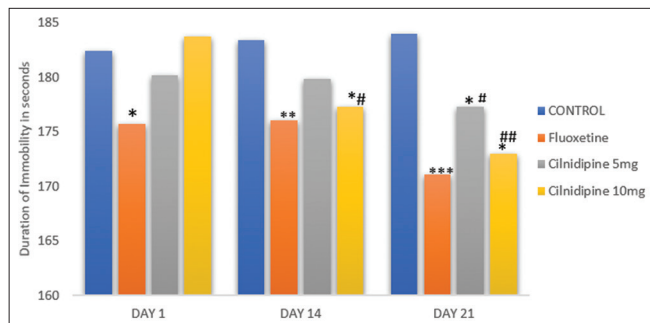
Graph 1: Effect of various drugs on duration of immobility in tail suspension test. Values are expressed as mean±SD. One-way ANOVA followed by Bonferroni's multiple comparison test. *$p<0.05$, **$p<0.001$ in comparison to control group after same duration of treatment; #$p<0.05$, ##$p<0.01$ in comparison to individual dug groups after same duration of treatment.
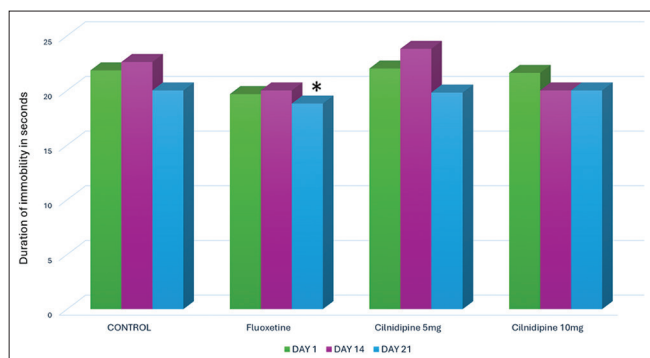
Graph 2: Effect of various treatment on locomotor activity in actophotometer. Values are expressed as mean±SD. One-way ANOVA followed by Bonferroni's multiple comparison test. *$p<0.05$ in comparison to control group after same duration of treatment.

drug fluoxetine, cilnidipine 10 mg/kg group showed similar results [Table 3 and Graph 1].

Actophotometer in male Swiss albino mice

The locomotor activity was assessed for different groups over 10 min. The locomotor activity were assessed in terms of number of counts. On day 1, in the control group was 21.83 ± 2.9 while in the fluoxetine group was 19.66 ± 2.4, cilnidipine 5 mg/kg dose: 22 ± 2.8 and cilnidipine 10 mg/kg dose: 21.6 ± 4.6. On day 14, counts in the control group were 22.6 ± 3.1 while in the fluoxetine group was 20 ± 3.7, cilnidipine 5 mg/kg dose: 23.8 ± 3.7 and cilnidipine 10 mg/kg dose: 20 ± 3.7. Results for day 21 were control group – 20 ± 3.3 while in the fluoxetine group was 18.8 ± 3.4, cilnidipine 5 mg/kg dose: 19.8 ± 4.2 and cilnidipine 10 mg/kg dose: 20 ± 5.8.

On day 21, the average mobility of the fluoxetine group was substantially different from that of the control group. On days 1, 14 and 21, there was no discernible statistical difference in the average movement activity between the treatment group and the control and standard groups.

DISCUSSION

This research aimed to evaluate the antidepressant effects of cilnidipine in male Swiss albino mice using the tail suspension test (TST), an experimental model of depression. While existing data supports the use of CCBs such as verapamil, nifedipine and nimodipine as antidepressants, there has been no prior investigation into the potential antidepressant properties of cilnidipine, an L/N-type CCB. The main goal of this study was to address this gap. Based on the findings, cilnidipine demonstrates an antidepressant effect and may serve as an adjunctive treatment for patients with depression and hypertension.

Cilnidipine was administered at doses of 5 mg and 10 mg for 21 days. The results revealed that the 10 mg dose significantly reduced immobility time in the TST, with effects becoming more pronounced on days 7 and 21 ($P < 0.05$, <0.01, respectively). *Post hoc* analysis indicated that the 10 mg dose produced the most substantial antidepressant response by the 21st day. These dose-dependent effects are consistent with findings from recent studies.[18]

Fluoxetine, a standard antidepressant, was used as a control in this study. Fluoxetine functions primarily as a selective serotonin reuptake inhibitor (SSRI), preventing the reabsorption of serotonin in presynaptic neurons. However, research by Traboulsie *et al.*[20] indicates that fluoxetine also inhibits various neuronal ion channels, including the T-type calcium channels (CaV3.1, CaV3.2 and CaV3.3), with norfluoxetine showing greater inhibitory effects on CaV3.3. These findings suggest that fluoxetine's ability to block T channels might enhance its antidepressant effects.[19]

In a study by Yamawaki *et al.* (1998),[21] rapid administration of antidepressants inhibited calcium signalling in both neuronal and glioma cells.[20] Additional research by Cohen *et al.* (1997) and Biała (1998)[23] demonstrated that DHP CCBs exhibited antidepressant-like effects in rats.

There is further evidence indicating that tricyclic antidepressants interact with calcium channels, suggesting that calcium channels may play a role in the mechanisms of antidepressant drugs.[23] Brain-penetrant CCBs have been associated with a reduced risk of mental and neurological disorders compared to other CCBs, such as amlodipine or diltiazem. These findings highlight the potential advantages of using CCBs that can cross the blood-brain barrier in reducing the risk of neuropsychiatric conditions.[24]

In older adults with hypertension, studies have shown that combining CCBs with SSRIs improves cognitive function and alleviates depressive symptoms. This suggests that adding

CCBs to SSRI therapy may enhance mood and cognitive abilities in elderly patients.[25]

However, a meta-analysis revealed an association between CCB use and an increased risk of depression. Specifically, these blockers were linked to higher rates of depression compared to other antihypertensive drugs, such as diuretics, beta-blockers and angiotensin antagonists, indicating that CCBs may carry some risk for depression. Hence, caution should be exercised due to the reported association between CCBs and an increased risk of depression, warranting further investigation into their long-term effects on mental health.

Limitation and further recommendations

This study has certain limitations:
1. Significant antidepressant effects were observed with cilnidipine at clinically utilised dosages. However, the precise mechanism of action of cilnidipine cannot be explained just by changes in behavioural research. To further comprehend the occurrences, more research is necessary
2. Further investigation is required to ascertain the antidepressant properties and molecular mechanisms of cilnidipine before recommending its use for treating depression in animal models
3. To verify the existence of these effects and investigate the underlying processes, more research utilising long-term therapy and other animal models of depression is required.

We anticipate that this data can shed light on the impact of cilnidipine on depression and has instructional implications for future pathology research on depression.

CONCLUSION

The study's findings indicate that cilnidipine, a DHP CCB that is frequently employed as an antihypertensive, also possesses antidepressant properties. This is evidenced by a reduction in the amount of time male Swiss albino mice spend immobile on the Forced swim test (FST) and TST. The drugs showed results like that of the standard drug Fluoxetine. However, lower doses of the test drugs (Cilnidipine 5 mg) did not provide significant effects. Cilnidipine's antidepressant effect is dosage and treatment duration dependent.

According to our findings, cilnidipine may be a newer target for antidepressant activity, and more research is needed to generalise this impact to patients. It can be used to improve the quality of life of hypertensive individuals suffering from depression when two medications are required.

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