ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

## ORIGINAL RESEARCH

# Neuro psychopharmaco logical effect of felodipine with reference to senile dementia of alzheimer's type model in mice

**[1]Rohit Dwivedi,[2]Suyog Sindhu, [3]RishiPal, [4]Arpita Singh,[5]Rajendra Nath, [6]Rakesh K Dixit, [7]Amod K Sachan**

[1]3rd year Junior Resident,[2]Assistant Professor, [3]Prof.(Junior Grade), [5,6]Professor, [7]Professor & Head, Department of Pharmacology,KGMU,Lucknow, Uttar Pradesh, India
[4]Prof.(Junior Grade),Department of Pharmacology, RML,Lucknow, Uttar Pradesh, India

**Correspondence:**
Dr. Rohit Dwivedi
3rd year Junior Resident,Department of Pharmacology,KGMU,Lucknow, Uttar Pradesh, India
**Email:** rohithims@gmail.com

**Abstract**
**Background:** Little is known about the therapeutic potential of calcium channel blockers in Alzheimer's disease, a neurodegenerative disorder in elderly. We, therefore, tried to identify the probable neuroprotective effect of FELODIPINE, a calcium channel blocker (CCB) in Alzheimer's disease.
**Methods:** The work was conducted in the Department of Pharmacology & Therapeutics, King George's Medical University, Lucknow. A total number of 72 female swiss albino mice were used in the study. Mice were irregularly divided into 12 groups each carrying 6 mice. Alzheimers model was made by administering scopolamine [3 mg/kg i.p.] and was tested for cognition via cooks pole climbing apparatus by recording the conditioned avoidance response time, learning and memory by elevated plus maze by recording transfer latency, and anti-depression activity by forced swim test by recording period of immobility.
**Results:** In terms of cognition enhancing effect, felodipine treated group showed cognition enhancing effect, but it was found to be comparable than the standard drug donepezil. In terms of antidepressant effect, the test drug felodipine lowered the depression. However the effect was found to be less than the standard drug imipramine. In terms of learning the test drug felodipine showed effect which were lower than the standard drug donepezil.
**Conclusion:** The present study supports the probable neuroprotective effect of felodipine in senile dementia of Alzheimer's model in mice.
**Keywords:** Neuroprotective, felodipine, alzheimers disease

## Introduction

Alzheimer's disease (AD) is a progressive, age-related neurodegenerative disorder characterized by progressive loss of neurons from specific areas of the brain mainly the hippocampus and cerebral cortex leading to impairment of memory and cognition. It is the most common form of dementia and is becoming more widespread and putting a greater strain on healthcare systems. Despite significant efforts over the last 35 years, the cognitive degeneration of Alzheimer's disease has remained frustratingly resistant to prospective disease-modifying treatments. [1]

According to an estimation by the Center for Disease Control, the number of people above age 65 will rise from 420 million to approximately 1 billion from 2000 to 2030.

At present there are above 36.5 million people worldwide who are affected by dementia and the majority of them are associated with Alzheimer's disease. 5-7 million new cases are

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

estimated to be recorded in the geriatric population annually.As projected by census data there will be 13.8 million people diagnosed with AD dementia by 2050. [2]
The proportion of the geriatric population in India is projected to be 19.1% approximately 316 million. Estimation of Global Burden of Disease Study states that 3.74 million people in South Asia had dementia, including 2.93 million people from India in 2016.
The WHO estimates that the global, number of the person with dementia will increase from the current 50 million to 82 million in 2030 and 152 million in 2050. South Asia, especially India, will be a major contributor to this increase due to its large population.[3]
The cause and progression of the disease are not well understood. Altered homeostasis in calcium movement in and out of the cell is important in multiple diseases of CNS. This explains the therapeutic reason behind blocking the various subtypes of voltage-activated calcium channels (VACCs) expressed in neurons. Alleviation of Ca2+ entry excess elicited by those blockers may restore the alteration in synaptic transmission, synaptic plasticity, and gene expression to normal parameters, ending the enhanced neuronal vulnerability.[4]
In mitochondria, Ca2+ levels are closely regulated. When high Ca2+ levels are attained within mitochondria, important mitochondrial functions are harmed, resulting in increased production of reactive oxygen species and activation of apoptosis, both of which are processes that occur in Alzheimer's disease.[5]There is strong evidence that dysregulation of intracellular calcium plays a key role in the pathogenesis of Alzheimer's disease, and specifically, that beta-amyloid may induce increases in intracellular calcium leading to neuronal cell dysfunction and death.[6]

**Material and methods**

The work was conducted in the Department of Pharmacology & Therapeutics, King George's Medical University, Lucknow, after getting approval from the Institutional Animal Ethics Committee (IAEC).
72 adult healthy female swiss albino mice of similar body constitution (in terms of age, body weight), weighing 20-30 gm had been used in the study. Mice were procured from the animal house of the Indian Institute of Toxicology Research [IITR, Lucknow]. The animals were allowed to access food water *ad libitum*and were kept in the institutional animal house of King George's Medical University (KGMU) under a temperature-controlled environment [25±2◦C], humidity (60% ± 10%) with 12 hours light / 12 hours dark cycle. All experiments were carried out between 0090 and 1700 hrs.The animals were housed for 2 weeks before the experiments to acclimatize to laboratory temperature.The care of animals was done as per CPCSEA guidelines**.**
The maintenance of the animals and the experimental procedures were in accordance with the 'Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH Publication no. 85-23 revised1996, Latest revision in 2011) and the guiding principles of IAEC which were strictly adhered to the guidelines of CPCSEA.
Cook's Pole Climbing Apparatus use to study cognitive function, mainly a response to conditioned stimuli during learning & its retention. Mice were trained to act in a certain way (climbing a pole) in response to a signal (buzzer) to avoid a noxious stimulus. Response to the signal is conditioned response while response to noxious stimulus is unconditioned response Mice were administered normal saline (control group), standard treatment and test drugs at 60 min before the test. Mice were trained to climb a pole within 30 sec when shock was given. The shock was then preceded by a buzzer for 15 sec. This was done for 2-3 times a day for 8 days till mice were trained to climb the pole at the sound of the buzzer. Trained mice were treated with the drugs and CR was noted.
We used Forced swim test as pharmacological model for assessing antidepressant activity. The device consisted of a transparent cylinder (14cm in diameter and 19cm in height) filled

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

to a depth of 12cm with water (25±2 °C) so that the animal's hind paws could not touch the bottom. The animal had an initial burst of activity in an attempt to flee, but gradually settled into a motionless posture, making only the movements required to keep its head above water. Mice were administered normal saline (control group), standard treatment and test drugs at 60 min before the test. Mice were gently dropped into a transparent cylinder for 6 minutes, one hour following medication treatment. The decrease in the duration of immobility is considered to have a good predictive value in the evaluation of potential antidepressant agents.

Elevated plus maze (EPM) was used to assess the retention of learning and memory. Transfer latency (TL) is defined as the time taken by the rodent to move into any one of the closed arms with all its four paws. The mice which did not enter into one of the arms within 90secs, was gently pushed into one of the covered arms and the TL was assigned as 90secs. Baseline TL (seconds) was recorded prior to start of experiment and was repeated after administering, test, control, and standard drugs. The mice were allowed to explore the maze for 10 seconds before returning back to their cages. The procedure was directed in dim light room and apparatus was cleaned before placing each mouse in apparatus. Transfer latency was recorded with the help of a stopwatch.

**Dosage forms, dosage, and sources of the drugs**

The test drug used was injectable Felodipine in dose 5 mg/kg BW,i.p.[7]The standard drugs used were Donepezil in dose of 3 mg/kg i.p[8] and Imipramine in dose of 20 mg/kg BW, i.p.[9] Drug for inducing amnesia was Scopolamine in dose of 3 mg/kg, i.p.[10]

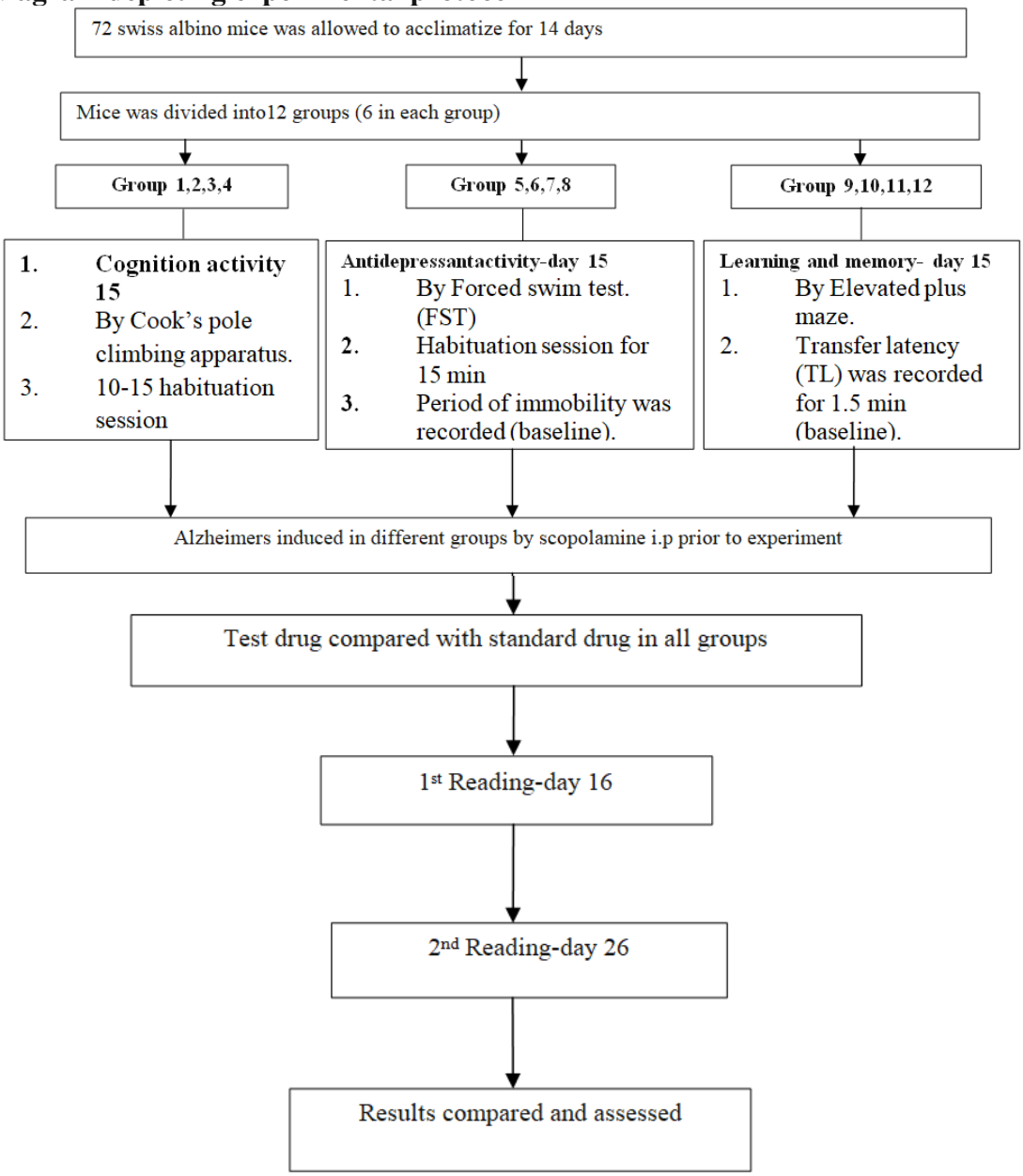
Each of the above-mentioned drugs was dissolved/diluted in normal saline (vehicle) just before administration. The strength of the solution was adjusted in such a way that 0.1ml of solution contained the desired dose that was to be administered in an individual mouse.

**Animal grouping**

| | | | |
|---|---|---|---|
| | COGNITION | Group 1 | Normal saline |
| | | Group 2 | Scopolamine [3 mg/kg i.p.] |
| | | Group 3 | Scopolamine [3 mg/kg i.p.] + felodipine[5mg/kg i.p] |
| | | Group 4 | Scopolamine [ 3 mg/kg i.p.]+ Standard drug Donepezil [ 3 mg/kgbw] |
| 2) | ANTIDEPRESSANT ACTIVITY | Group 5 | Normal saline |
| | | Group 6 | Scopolamine [3 mg/kg i.p.] |
| | | Group 7 | Scopolamine [3 mg/kg i.p.] + felodipine [5mg/kg] |
| | | Group 8 | Scopolamine [ 3 mg/kg i.p.] + Standard drug Inj imipramine 20 mg/kg BW |
| 3) | LEARNING AND MEMORY | Group 9 | Normal saline |
| | | Group10 | Scopolamine [3 mg/kg i.p.] |
| | | Group11 | Scopolamine [3 mg/kg i.p.] + felodipine [5mg/kg i.p] |
| | | Group12 | Scopolamine [ 3 mg/kg i.p.] + Standard drug Inj Donepezil 3 mg/kg W |

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

**Flow diagram depicting experimental protocol**




## Results

### I. Cognition Activity

**Table 1: Intergroup comparison of cognition time at different time intervals**

| Groups | Day 16 | | | Day 26 | | |
|---|---|---|---|---|---|---|
| | Mean | Standard deviation (SD) | Significance | Mean | Standard deviation (SD) | Significance |
| Group I (NS) | 3.167 | .7528 | F= 7.338 p-value=0.002 | 3.333 | .5164 | F= 53.185 p-value<0.001 |
| Group II (S) | 10.833 | 5.6006 | | 14.667 | 2.8752 | |
| Group III (S+F) | 7.667 | .8165 | | 9.833 | .7528 | |
| Group IV (S+I) | 8.500 | 1.0488 | | 11.833 | 1.1690 | |

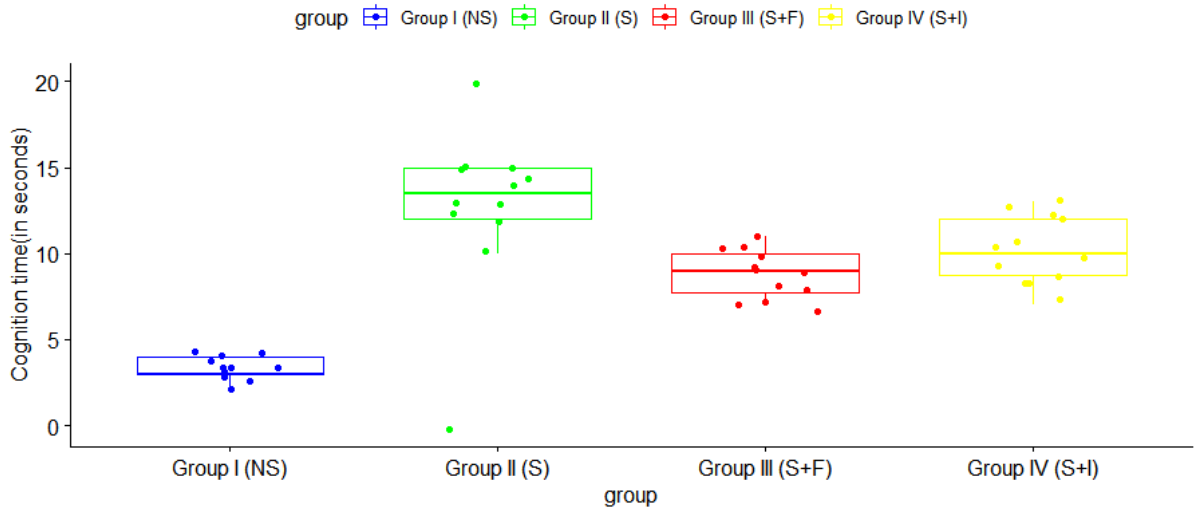
ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

This table shows the output of the ANOVA analysis and whether there is a statistically significant difference between the group means.

The significance value of cognition time for Day 16 is 0.002 (i.e., $p = .002$), which is below 0.05, and, therefore, there is a statistically significant difference in the mean cognition time between the different groups.

The significance value of cognition time for Day 26 is <0.001(i.e., $p = .001$), which is below 0.05, and, therefore, there is a statistically significant difference in the mean cognition time between the different groups.

**Fig 1: Distribution of cognition time by different group**




From the boxplots, we can see that the center of the distributions appears to be little different. The median cognition time for group II is slightly higher than the median cognition time of the group I, group III and group IV.

**Fig 2: Mean cognition time by groups. Cognition times are expressed as z scores. Error bars represent standard errors.**

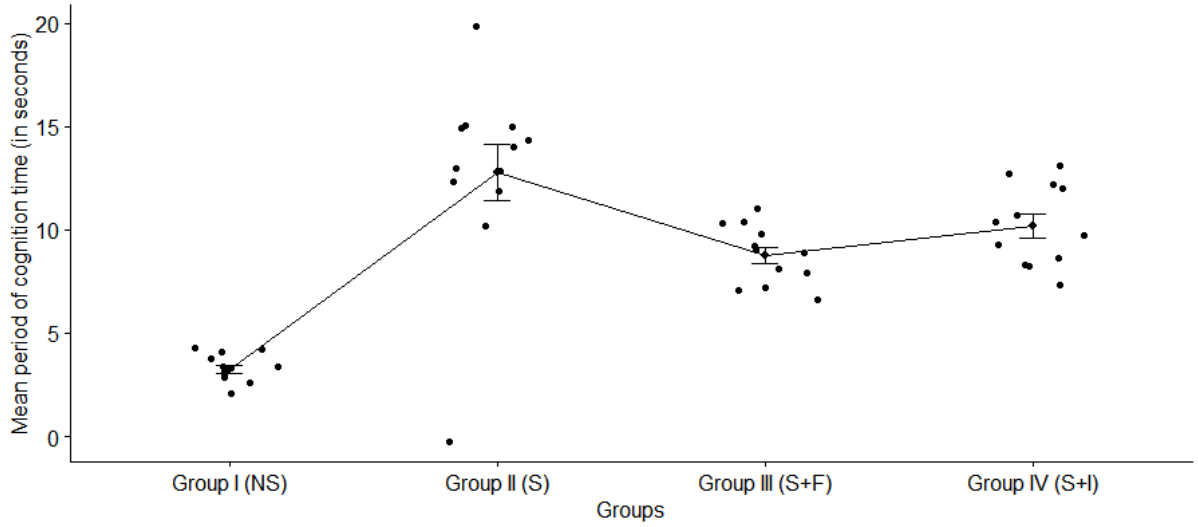



**Table 2: Between group difference in cognition time at different time intervals**

| **Multiple Comparisons** (Tukey HSD) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Groups | Groups | **Day 16** | | | **Day 26** | | |
| | | Mean Difference | Std. Error | p-value | Mean Difference (I-J) | Std. Error | p-value |

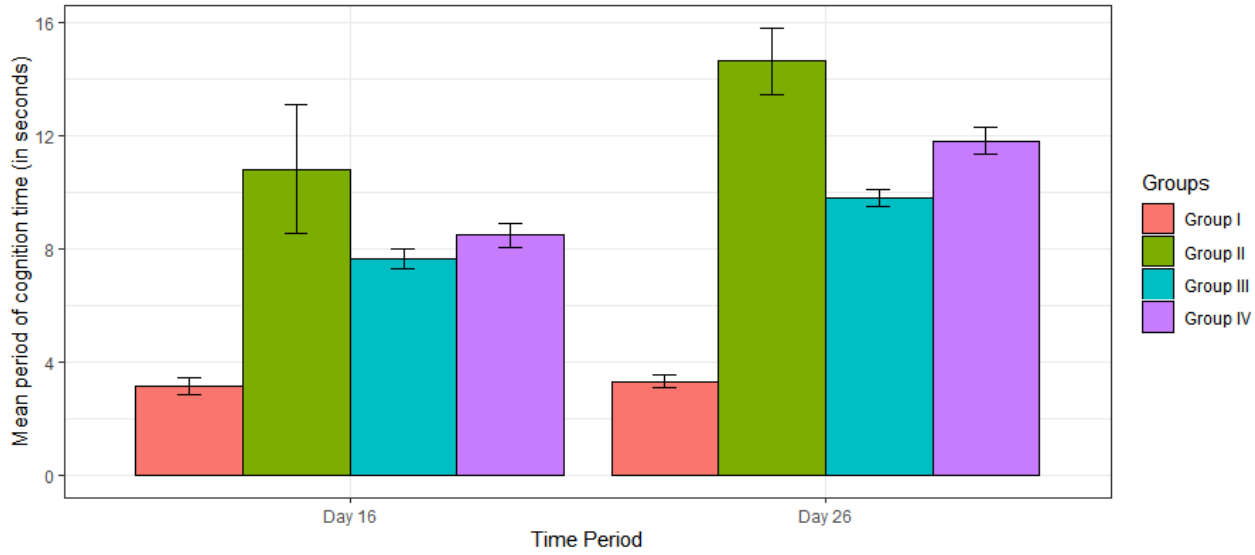
ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Group I (NS) | Group II (S) | -7.6667* | 1.6758 | .001 | -11.3333* | .9339 | .000 |
| | Group III (S+F) | -4.5000 | 1.6758 | .063 | -6.5000* | .9339 | .000 |
| | Group IV (S+I) | -5.3333* | 1.6758 | .022 | -8.5000* | .9339 | .000 |
| Group II (S) | Group I (NS) | 7.6667* | 1.6758 | .001 | 11.3333* | .9339 | .000 |
| | Group III (S+F) | 3.1667 | 1.6758 | .264 | 4.8333* | .9339 | .000 |
| | Group IV (S+I) | 2.3333 | 1.6758 | .518 | 2.8333* | .9339 | .031 |
| Group III (S+F) | Group I (NS) | 4.5000 | 1.6758 | .063 | 6.5000* | .9339 | .000 |
| | Group II (S) | -3.1667 | 1.6758 | .264 | -4.8333* | .9339 | .000 |
| | Group IV (S+I) | -.8333 | 1.6758 | .959 | -2.0000 | .9339 | .174 |
| Group IV (S+I) | Group I (NS) | 5.3333* | 1.6758 | .022 | 8.5000* | .9339 | .000 |
| | Group II (S) | -2.3333 | 1.6758 | .518 | -2.8333* | .9339 | .031 |
| | Group III (S+F) | .8333 | 1.6758 | .959 | 2.0000 | .9339 | .174 |
| *. The mean difference is significant at the 0.05 level. | | | | | | | |

The table 2, **Multiple Comparisons**, shows which groups differed from each other. The Tukey post hoc test was used for conducting post hoc tests on a one-way ANOVA. There is a statistically significant difference in Day 16 cognition time between the group I and group II ($p$ = 0.001), as well as between group I and group IV ($p$ = 0.022). However, there were no differences between the group I vs. group III ($p$ = 0.063); group II vs. group III ($p$ = 0.264); group II vs. group IV ($p$ = 0.518), and group III vs. group IV ($p$ = 0.959)

There is a statistically significant difference in Day 26 cognition time between the group I vs. group II ($p$<0.001); group I vs. group III ($p$ <0.001); group I vs. group IV ($p$ <0.001), as well as between group II and group III ($p$ <0.001); group II and group IV ($p$=.031). However, there were no differences between the group III and group IV ($p$ = .174).

**Fig 3: Intergroup comparison of cognition time**




3. Intragroup change in baseline period of cognition time (using paired t-test)

| Group | Time | Mean | Standard | Standard | T | Significance | 95% |
|---|---|---|---|---|---|---|---|

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

| | | | deviation (SD) | error mean | | (p-value) | confidence interval of the difference | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Group I (NS) | Day 16 | 3.167 | 0.753 | 0.3073 | -0.415 | 0.695 | -1.198 | 0.865 |
| | Day 26 | 3.333 | 0.516 | 0.211 | | | | |
| Group II (S) | Day 16 | 10.833 | 5.600 | 2.286 | -1.176 | 0.293 | -12.213 | 4.546 |
| | Day 26 | 14.667 | 2.875 | 1.173 | | | | |
| Group III (S+F) | Day 16 | 7.667 | 0.816 | 0.333 | -4.540 | 0.006 | -3.393 | -0.939 |
| | Day 26 | 9.833 | 0.752 | 0.307 | | | | |
| Group IV (S+I) | Day 16 | 8.500 | 1.048 | 0.428 | -5.00 | 0.004 | -5.047 | -1.619 |
| | Day 26 | 11.833 | 1.169 | 0.477 | | | | |

A paired t-test was used to determine whether there was a statistically significant mean difference between the cognition times observed on Day 16 to a cognition time observed on Day 26, for each of the group separately. The mean cognition time of group I and group II for Day 16 and Day 26 were not statistically significant.The mean cognition time of group III was higher for Day 26 (9.833 ± 0.752 in seconds) as opposed to Day 16 (7.667± 0.816 in

The mean cognition time of group IV was higher for Day 26 (11.833 ± 1.169 in seconds) as opposed to Day 16 (8.500± 1.048 in seconds).

A statistical significant increase of 2.166 seconds, $t$ = -4.540, $p$ = .006 was observed in group III for cognition time on Day 26.

A statistical significant increase of 3.33 seconds, $t$ = -5.00, $p$ = .004 was observed in group IV for cognition time on Day 26.

**II. Evaluation of anti-depressant effect**

**Table 4: Intergroup comparison of period of Immobility at different time intervals**

| Groups | Day 16 | | | Day 26 | | |
|---|---|---|---|---|---|---|
| | Mean | Standard deviation (SD) | Significance | Mean | Standard deviation (SD) | Significance |
| Group 5 | 149.500 | 1.8708 | F= 173.449 p-value<0.001 | 154.333 | 2.7325 | F= 194.578 p-value<0.001 |
| Group 6 | 189.333 | 3.8816 | | 194.833 | 3.6560 | |
| Group 7 | 179.667 | 3.1411 | | 184.167 | 3.0605 | |
| Group 8 | 171.333 | 3.3862 | | 179.833 | 2.4833 | |

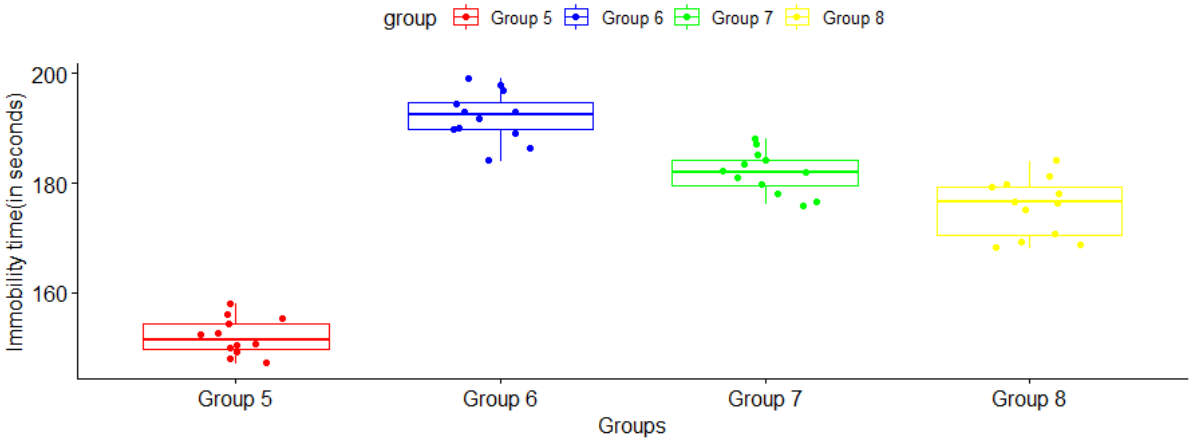
Table 4 shows the output of the ANOVA analysis and whether there is a statistically significant difference between the group means.

The significance value of immobility time for Day 16 is <0.001 (i.e., $p$ <0.001), which is below 0.05, and, therefore, there is a statistically significant difference in the mean immobility time between the different groups.

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

The significance value of immobility time for Day 26 is <0.001(i.e., $p$ <0.001), which is below 0.05, and, therefore, there is a statistically significant difference in the mean immobility time between the different groups.

**Fig 4: Distribution of immobility time by different group**




From the boxplots, we can see that the center of the distributions appears to be little different. The median immobility time for group 6 is slightly higher than the median immobility time of the group 5, group 7 and group 8.

**Fig 5: Mean immobility time by groups. Immobility times are expressed as z scores. Error bars represent standard errors.**

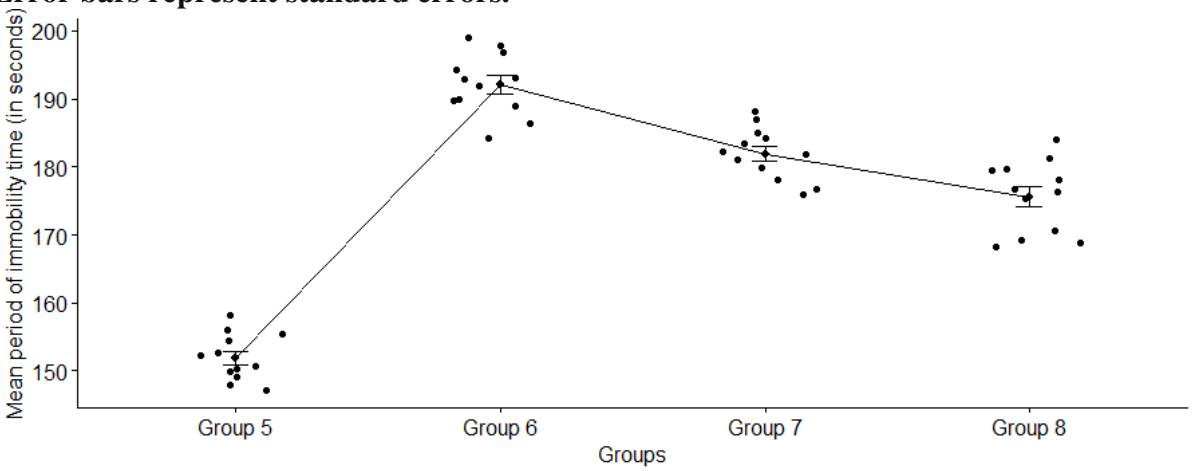



**Table 5: Between group difference in period of Immobility at different time intervals**

| **Multiple Comparisons** (Tukey HSD) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Groups | Groups | **Day 16** | | | **Day 26** | | |
| | | Mean Difference | Std. Error | p-value | Mean Difference (I-J) | Std. Error | p-value |
| Group 5 | Group 6 | -39.8333* | 1.8235 | .000 | -40.5000* | 1.7408 | .000 |
| | Group 7 | -30.1667* | 1.8235 | .000 | -29.8333* | 1.7408 | .000 |
| | Group 8 | -21.8333* | 1.8235 | .000 | -25.5000* | 1.7408 | .000 |
| Group 6 | Group 5 | 39.8333* | 1.8235 | .000 | 40.5000* | 1.7408 | .000 |
| | Group 7 | 9.6667* | 1.8235 | .000 | 10.6667* | 1.7408 | .000 |
| | Group 8 | 18.0000* | 1.8235 | .000 | 15.0000* | 1.7408 | .000 |
| Group 7 | Group 5 | 30.1667* | 1.8235 | .000 | 29.8333* | 1.7408 | .000 |
| | Group 6 | -9.6667* | 1.8235 | .000 | -10.6667* | 1.7408 | .000 |

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Group 8 | 8.3333* | 1.8235 | .001 | 4.3333 | 1.7408 | .092 |
| Group 8 | Group 5 | 21.8333* | 1.8235 | .000 | 25.5000* | 1.7408 | .000 |
| | Group 6 | -18.0000* | 1.8235 | .000 | -15.0000* | 1.7408 | .000 |
| | Group 7 | -8.3333* | 1.8235 | .001 | -4.3333 | 1.7408 | .092 |
| *. The mean difference is significant at the 0.05 level. | | | | | | | |

The table 5, **Multiple Comparisons**, shows which groups differed from each other. The Tukey post hoc test was used for conducting post hoc tests on a one-way ANOVA. There is a statistically significant difference in Day 16 immobility time between the group 5 vs. group 6 ($p$<0.001); group 5 vs. group 7 ($p$ <0.001); group 5 vs. group 8 ($p$ <0.001); group 6 vs. group 7 ($p$<0.001); group 6 vs. group 8 ($p$<0.001), and group 7 vs. group 8 ($p$<0.001).

There is a statistically significant difference in Day 26 immobility time between the group 5 vs. group 6 ($p$<0.001); group 5 vs. group 7 ($p$ <0.001); group 5 vs. group 8 ($p$ <0.001); group 6 vs. group 7 ($p$<0.001), and group 6 vs. group 8 ($p$<0.001). However, there were no differences between the group 7 and group 8 ($p$ = .092).

**Fig 6: Intergroup comparison of immobility time**

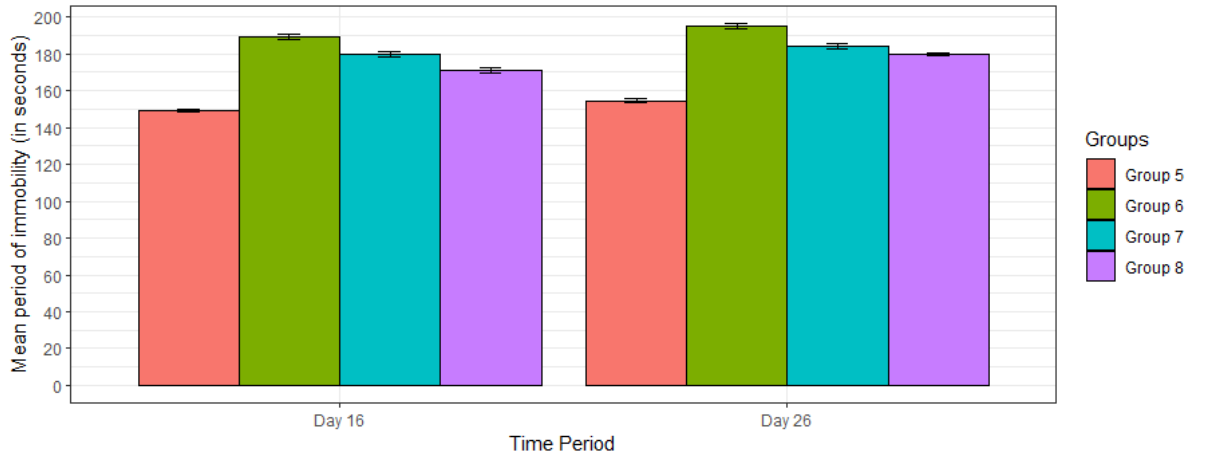



**Table 6:Intra-group change in baseline period of immobility (using paired t-test)**

| Group | Time | Mean | Standard deviation (SD) | Standard error mean | t | Significance (p-value) | 95% confidence interval of the difference | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Group 5 | Day 16 | 149.50 | 1.8708 | .7638 | -2.586 | .049 | -9.63 | -0.028 |
| | Day 26 | 154.33 | 2.7325 | 1.1155 | | | | |
| Group 6 | Day 16 | 189.333 | 3.8816 | 1.5846 | -2.133 | .086 | -12.12 | 1.12 |
| | Day 26 | 194.833 | 3.6560 | 1.4926 | | | | |
| Group 7 | Day 16 | 179.667 | 3.1411 | 1.2824 | -3.576 | 0.016 | -7.734 | -1.265 |
| | Day 26 | 184.167 | 3.0605 | 1.2494 | | | | |
| Group 8 | Day 16 | 171.333 | 3.3862 | 1.3824 | -8.295 | <0.001 | -11.13 | -5.86 |
| | Day 26 | 179.833 | 2.4833 | 1.0138 | | | | |

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

A paired t-test was used to determine whether there was a statistically significant mean difference between the immobility times observed on Day 16 to immobility times observed on Day 26, for each of the group separately. The mean immobility time of group 6 for Day 16 and Day 26 was not statistically significant.
The mean immobility times of group 5 was higher for Day 26 (154.33 ± 2.73 in seconds) as opposed to Day 16 (149.50± 1.87 in seconds).
The mean immobility times of group 7 was higher for Day 26 (184.16 ± 3.06 in seconds) as opposed to Day 16 (179.66± 3.14 in seconds).
The mean immobility times of group 8 was higher for Day 26 (179.83 ± 2.48 in seconds) as opposed to Day 16 (171.33± 3.38 in seconds).
A statistical significant increase of 4.83 seconds, $t$ = -2.586, $p$ = .049 was observed in group 5 for immobility times on Day 26.
A statistical significant increase of 4.5 seconds, $t$ = -3.576, $p$ = .016 was observed in group 7 for immobility times on Day 26.
A statistical significant increase of 8.5 seconds, $t$ = -8.295, $p$ = <0.001was observed in group 8 for immobility times on Day 26.

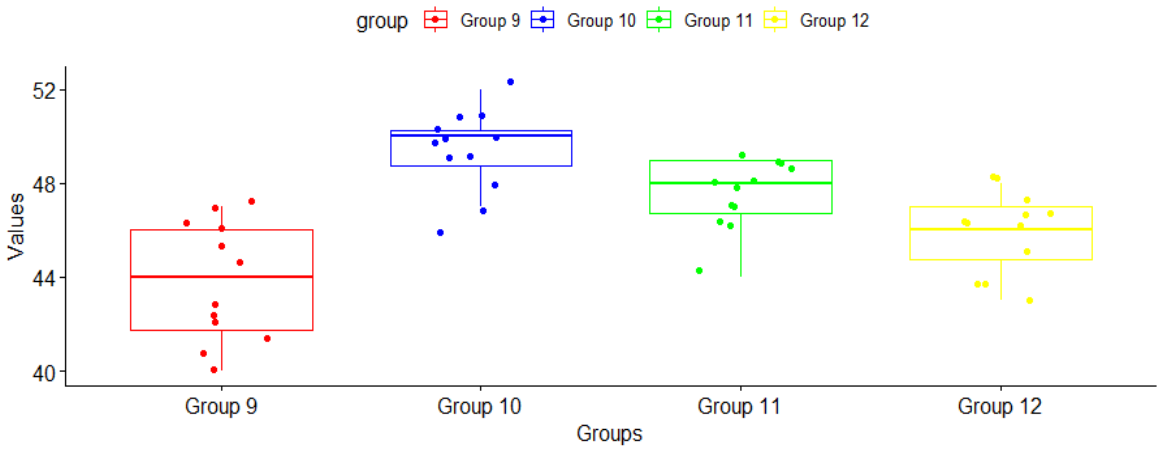
**III. Evaluation of elevated plus maze effect:**

**Table 7: Intergroup comparison of period of elevated plus maze effect at different time intervals**

| Groups | Day 16 | | | Day 26 | | |
|---|---|---|---|---|---|---|
| | Mean | Standard deviation (SD) | Significance | Mean | Standard deviation (SD) | Significance |
| Group 9 | 46.000 | .8944 | F= 19.935 p-value<0.001 | 41.500 | 1.0488 | F= 26.786 p-value<0.001 |
| Group 10 | 49.833 | 1.3292 | | 49.000 | 2.0976 | |
| Group 11 | 48.667 | .5164 | | 46.333 | 1.3663 | |
| Group 12 | 47.167 | .7528 | | 44.667 | 1.2111 | |

Table 7 shows the output of the ANOVA analysis and whether there is a statistically significant difference between the group means.
The significance value of learning and memory, transfer latency time for Day 16 is <0.001 (i.e., $p$ <0.001), which is below 0.05, and, therefore, there is a statistically significant difference in the mean transfer latency time between the different groups.
The significance value of transfer latency time for Day 26 is <0.001(i.e., $p$ <0.001), which is below 0.05, and, therefore, there is a statistically significant difference in the mean transfer latency time between the different groups.

**Fig 7: Distribution of transfer latency timetime by different group**



ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

From the boxplots, we can see that the center of the distributions appears to be little different. The median transfer latency time for group 10 is slightly higher than the median transfer latency time of the group 9, group 11 and group 12.

**Fig 8: Mean transfer latency time by groups. Transfer latency times are expressed as z scores. Error bars represent standard errors.**

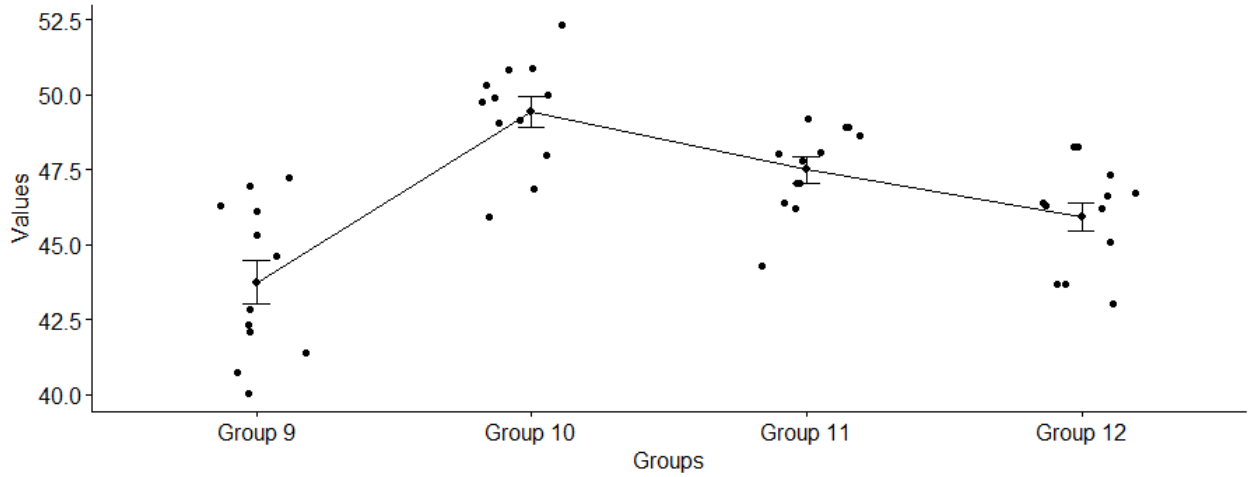



**Table 8: Between group difference in period of elevated plus maze effect at different time intervals**

| **Multiple Comparisons** (Tukey HSD) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Groups | Groups | **Day 16** | | | **Day 26** | | |
| | | Mean Difference | Std. Error | p-value | Mean Difference (I-J) | Std. Error | p-value |
| Group 9 | Group 10 | -3.8333* | .5323 | .000 | -7.5000* | .8580 | .000 |
| | Group 11 | -2.6667* | .5323 | .000 | -4.8333* | .8580 | .000 |
| | Group 12 | -1.1667 | .5323 | .160 | -3.1667* | .8580 | .007 |
| Group 10 | Group 9 | 3.8333* | .5323 | .000 | 7.5000* | .8580 | .000 |
| | Group 11 | 1.1667 | .5323 | .160 | 2.6667* | .8580 | .026 |
| | Group 12 | 2.6667* | .5323 | .000 | 4.3333* | .8580 | .000 |
| Group 11 | Group 9 | 2.6667* | .5323 | .000 | 4.8333* | .8580 | .000 |
| | Group 10 | -1.1667 | .5323 | .160 | -2.6667* | .8580 | .026 |
| | Group 12 | 1.5000* | .5323 | .048 | 1.6667 | .8580 | .243 |
| Group 12 | Group 9 | 1.1667 | .5323 | .160 | 3.1667* | .8580 | .007 |
| | Group 10 | -2.6667* | .5323 | .000 | -4.3333* | .8580 | .000 |
| | Group 11 | -1.5000* | .5323 | .048 | -1.6667 | .8580 | .243 |
| *. The mean difference is significant at the 0.05 level. | | | | | | | |

The table 8, **Multiple Comparisons**, shows which groups differed from each other. The Tukey post hoc test was used for conducting post hoc tests on a one-way ANOVA. There is a statistically significant difference in Day 16 transfer latency time between the group 9 vs. group 10 ($p$<0.001); group9 vs. group 11 ($p$ <0.001); group 10 vs. group 12 ($p$ <0.001), and group 11 vs. group 12 ($p$=0.048). However, there were no differences between the group 9 vs. group 12 ($p$ = .160), and group 10 vs. group 11 ($p$ = .160).

There is a statistically significant difference in Day 26 transfer latency time between the group 9 vs. group 10 ($p$<0.001); group9 vs. group 11 ($p$ <0.001); group 9 vs. group 12 ($p$ =0.007); group 10 vs. group 11($p$ =0.026), and group 10 vs. group 12 ($p$ <0.001). However, there were no differences between the group 11 and group 12 ($p$ = .243).

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

**Fig 9: Intergroup comparison of learning and memory time**

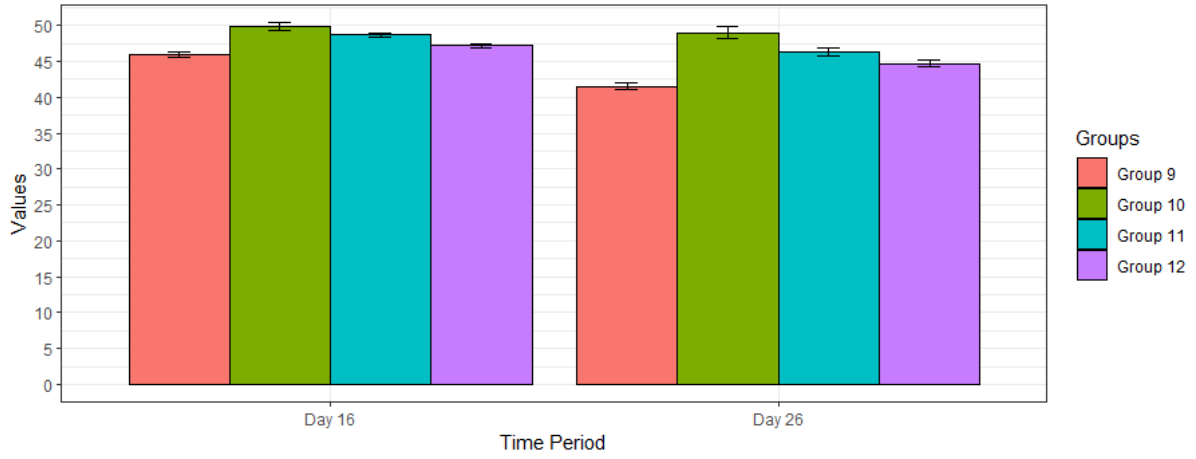



**Table 9: Intra-group change in baseline period of elevated plus maze value (using paired t-test)**

| Group | Time | Mean | Standard deviation (SD) | Standard error mean | t | Significance (p-value) | 95% confidence interval of the difference | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Group 9 | Day 16 | 46.000 | .8944 | .3651 | 7.268 | .001 | 2.9085 | 6.0915 |
| | Day 26 | 41.500 | 1.0488 | .4282 | | | | |
| Group 10 | Day 16 | 49.833 | 1.3292 | .5426 | .822 | 0.448 | -1.772 | 3.439 |
| | Day 26 | 49.000 | 2.0976 | .8563 | | | | |
| Group 11 | Day 16 | 48.667 | .5164 | .2108 | 4.719 | 0.005 | 1.0624 | 3.6043 |
| | Day 26 | 46.333 | 1.3663 | .5578 | | | | |
| Group 12 | Day 16 | 47.167 | .7528 | .3073 | 3.478 | .018 | .6523 | 4.3477 |
| | Day 26 | 44.667 | 1.2111 | .4944 | | | | |

A paired t-test was used to determine whether there was a statistically significant mean difference between the transfer latency time observed on Day 16 to a transfer latency time observed on Day 26, for each of the group separately. The mean transfer latency time of group10 for Day 16 and Day 26 were not statistically significant.

The transfer latency time of group 9 was higher for Day 16 (41.500± 1.0488 in seconds) as opposed to Day 26 (46.000± .8944in seconds).

The transfer latency time of group 11 was higher for Day 16 (48.667± .5164 in seconds) as opposed to Day 26 (46.333± 1.3663 seconds).

The transfer latency time of group 12 was higher for Day 16 (47.167± .7528 in seconds) as opposed to Day 26 (44.667± 1.2111 seconds).

A statistical significant increase of 4.5 seconds, $t = 7.268$, $p = .001$ was observed in group 9 for transfer latency time on Day 16

A statistical significant increase of 2.334 seconds, $t = 4.719$, $p = .005$ was observed in group 11 for transfer latency time on Day 16

A statistical significant increase of 2.5 seconds, $t = 3.478$, $p = .018$ was observed in group 12 for transfer latency time on Day 16

## Discussion

Alzheimer's disease is a neurologic ailment that causes the brain to shrink (atrophy) and the death of brain cells. Alzheimer's disease is the most frequent form of dementia, which is defined as a progressive loss of cognitive, behavioural, and social abilities that impairs a

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

person's capacity to operate independently. The present study was conducted to investigate the neuropsychopharmacological effect of antidepressant, cognitive enhancing properties of felodipine in mice.

In terms of cognition enhancing effect, Felodipine showed cognition enhancing effect, but it was found to be comparable than the standard drug donepezil. In Cooks pole apparatus, cognition activity was assessed by measuring conditioned avoidance response time and was found that group treated with Felodipine showed cognition enhancing effect (decrease in cognition time 9.833 ±.7528) than scopolamine treated group(14.667±2.8752) and was statistically significant but it was found to be comparable than the standard group treated with donepezil (11.833±1.1690)The mechanism underlying CCB's protective effect against scopolamine-induced dementia in our tests could be owing to its action on the slow L-type calcium channel, which reduces cellular calcium influxto have a good predictive value in the evaluation of potential antidepressant agents. It was investigated the feasibility of modifying Alzheimer's pathology with the L-type voltage-gated calcium channel blockers verapamil, diltiazem, isradipine and nimodipine.[6]The study showed that Aβ oligomers are strongly associated with increased intracellular Ca2+ and CCBs, especially isradipine, can prevent such an influx at nanomolar concentrations and protect MC65 cells. Results suggested the possibility that isradipine with ready bioavailability in brain tissue may have value in clinical trials of patients with or at risk for AD.

A study[11]was conducted to examine the cellular expression of all L Type Calcium Channel subunits around beta-amyloid plaques by in situ hybridization using 35S-labeled oligonucleotides. In cortical organotypic brain slices of adult Alzheimer mice, it was demonstrated that LTCC blockers increased angiogenesis, which was further potentiated by substance P and concluded that, brain vessels associated with beta-amyloid plaques express substance P and an LTCC and may play a role in angiogenesis.

Astudy[12] was conducted to investigate the effect of diltiazem in AlCl3 -induced dementia in mice. Morris water maze test and elevated plus maze were utilized to evaluate learning and memory. Various biochemical estimations including brain acetylcholinesterase activity (AChE), brain total protein, thiobarbituric acid-reactive species (TBARS) level, reduced glutathione (GSH) level, nitrate/nitrite, and superoxide dismutase (SOD) were measured. The results indicate that diltiazem significantly improves AlCl3-induced memory impairment and biochemical changes

Some scientists [13] investigated the role of flunarizine (a non-selective calcium channel blocker) on cerebral ischemic–reperfusion associated cognitive dysfunction in aged mice and concluded that, a non-selective calcium channel blocker can be useful in I/R associated cognitive dysfunction due to its antioxidant, anti-infarct and modulatory actions of neurotransmitters & calcium channels.

A study showed [7] that felodipine, an anti-hypertensive and L-type calcium channel blocker, activates autophagy and clears a variety of aggregate-prone, neurodegenerative disease-associated proteins. In mouse brains, felodipine can remove mutant -synuclein at plasma concentrations similar to those reported in patients taking the medicine. This is linked to neuroprotection in mice, implying that this chemical has potential for use in neurodegeneration.

In a study [14], it was stated that CCBs can be implicated for the treatment of AD patients with SARS-CoV-2 infection. In both cases, CCBs are useful to stabilize the normal Ca2+ neuronal physiological concentration in AD brain and are also able to inhibit the viral vulnerability on Ca2+ imbalance and inhibition at several stages of the virus life cycle.

Depression was assessed using the Forced Swim Model by measuring the period of immobility and was found to be decreased in Felodipine treated group (179.667±3.1411) than

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

scopolamine treated group (189.333 ± 3.8816) and was statistically significant.However, the immobility time was found to be lower than the standard group treated with imipramine.
Scientists [15] haveinvestigated the mechanisms underlying behavioral responses to various doses of scopolamine in mice to clarify the involvement of L-type voltage-dependent calcium channels in its modes of action.
The present study was conducted to investigate the neuropsychopharmacological effect of antidepressant, cognitive enhancing properties of felodipine in mice. Majority of geriatric population suffers from loss of memory and other cognitive function without any known etiology. Therefore age related complications particularly senile dementia, needs a long term therapeutic preventive strategies to promote quality of life and to reduce burden on family.
In a study[16], it was investigated the effects of long-term treatment with verapamil, a calcium channel blocker on the development of cognitive impairment in aged animals. Verapamil was studied at a low dose (1mg/kg/d) in a mouse model of sporadic Alzheimer's disease (sAD). Oral treatment with verapamil or vehicle was started, 24 h postintracerebroventricular (ICV) streptozotocin/(STZ), in 12-month-old animals and continued for 3 months. Cognitive function was assessed using established tests for spatial learning, short-term/working memory, and long-term/reference memory. Findings demonstrated that long-term low-dose verapamil effectively prevents development of ICV/STZinduced cognitive impairment. It mitigates the astrogliosis and synaptic toxicity otherwise induced by ICV/STZ in the hippocampus of aged animals. These findings indicate that long-term, low-dose verapamil may delay progression of AD in susceptible subjects of advanced age.
In terms of cognition enhancing effect, Felodipine showed cognition enhancing effect, but it was found to be comparable than the standard drug donepezil. The mechanism underlying CCB's protective effect against scopolamine-induced dementia in our tests could be owing to its action on the slow L-type calcium channel, which reduces cellular calcium influx. Calcium is involved in causing oxidative damage and excitotoxicity, both of which are important in scopolamine-induced dementia and related changes.
In terms of learning the test drug felodipine showed effect which were lower than the standard drug donepezil. Scientists [17] have studied the effects of lacidipine (L-type CCB) on learning and memory functions using the scopolamine mouse model of AD. Swiss albino mice (20–25 g) were administered lacidipine (1 and 3 mg/kg) for 14 days. Scopolamine, an anti-muscarinic drug, was given (1 mg/kg) from days 8 to 14. The mice were subjected to elevated plus maze (EPM) and passive-avoidance (PA) paradigms. Lacidipine prevented the amnesia against scopolamine and reduced the oxidative stress and AChE activity in the brain of mice. Lacidipinepretreatment was able to avert scopolamine induced memory impairment and oxido-nitrosative stress in mice
Astudy[18] was conducted earlier to find out the effect of calcium channel blockers on learning and memory using elevated plus maze and novel recognitionobject tests. Ten groups of animals were treated with CCBS and scopolamine.
Both prophylactic and curative studies were carried out. It was concluded that observed that verapamil was good in prophylactic studies and diltiazem in curative studies.

## Conclusion

The present study supports the probable neuroprotective effect of Felodipine in senile dementia of Alzheimer's model in mice andrequires further studies to promote felodipine as cognition enhancer drug.

## Reference

1. Lei, P., S. Ayton, and A.I. Bush, *The essential elements of Alzheimer's disease.* J Biol Chem, 2021. **296**: p. 100105.

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

2. Sengoku, R., *Aging and Alzheimer's disease pathology.* Neuropathology, 2019. **40**(1): p. 22-29.
3. Ravindranath, V. and J.S. Sundarakumar, *Changing demography and the challenge of dementia in India.* Nat Rev Neurol, 2021. **17**(12): p. 747-758.
4. Juan-Alberto Arranz-Tagarro, Cristóbal de los Ríos, Antonio G García & Juan-Fernando Padín (2014) Recent patents on calcium channel blockers: emphasis on CNS diseases, Expert Opinion on Therapeutic Patents, 24:9, 959-977, DOI: 10.1517/13543776.2014.940892
5. Calvo-Rodriguez, M. and B.J. Bacskai, *Mitochondria and Calcium in Alzheimer's Disease: From Cell Signaling to Neuronal Cell Death.* Trends Neurosci, 2021. **44**(2): p. 136-151
6. Anekonda, T.S., Quinn, J.F., Harris, C., Frahler, K., Wadsworth, T.L. and Woltjer, R.L., 2011. L-type voltage-gated calcium channel blockade with isradipine as a therapeutic strategy for Alzheimer's disease. Neurobiology of disease, 41(1), pp.62-70.
7. Siddiqi, F.H., Menzies, F.M., Lopez, A., Stamatakou, E., Karabiyik, C., Ureshino, R., Ricketts, T., Jimenez-Sanchez, M., Esteban, M.A., Lai, L. and Tortorella, M.D., 2019. Felodipine induces autophagy in mouse brains with pharmacokinetics amenable to repurposing. Nature communications, 10(1), pp.1-14.
8. Hashimoto T, Hatayama Y, Nakamichi K, Yoshida N. Procognitive effect of AC-3933 in aged mice, and synergistic effect of combination with donepezil in scopolamine-treated mice. Eur J Pharmacol. 2014 Dec 15;745:123-8.
9. Kitamura Y, Araki H, Nagatani T, Takao K, Shibata K, Gomita Y. Influence of imipramine on the duration of immobility in chronic forced-swim-stressed rats. Acta Med Okayama. 2004 Dec;58(6):271-4.
10. Biradar, S.M., H. Joshi, and T.K. Chheda, *Neuropharmacological effect of Mangiferin on brain cholinesterase and brain biogenic amines in the management of Alzheimer's disease.* Eur J Pharmacol, 2012. **683**(1-3): p. 140-7.
11. Daschil, N., Kniewallner, K.M., Obermair, G.J., Hutter-Paier, B., Windisch, M., Marksteiner, J. and Humpel, C., 2015. L-type calcium channel blockers and substance P induce angiogenesis of cortical vessels associated with beta-amyloid plaques in an Alzheimer mouse model. Neurobiology of aging, 36(3), pp.1333-1341.
12. Rani, A., Sodhi, R.K. and Kaur, A., 2015. Protective effect of a calcium channel blocker “diltiazem” on aluminum chloride-induced dementia in mice. Naunyn-Schmiedeberg's archives of pharmacology, 388(11), pp.1151-1161.
13. Gulati, P., Muthuraman, A. and Kaur, P., 2015. Investigation of the role of non-selective calcium channel blocker (flunarizine) on cerebral ischemic–reperfusion associated cognitive dysfunction in aged mice. Pharmacology Biochemistry and Behavior, 131, pp.26-32.
14. Danta, C.C., 2020. Calcium channel blockers: a possible potential therapeutic strategy for the treatment of Alzheimer’s dementia patients with SARS-CoV-2 infection. ACS Chemical Neuroscience, 11(15), pp.2145-2148.
15. Yu H, Li M, Shen X, Lv D, Sun X, Wang J, Gu X, Hu J, Wang C. The Requirement of L-Type Voltage-Dependent Calcium Channel (L-VDCC) in the Rapid-Acting Antidepressant-Like Effects of Scopolamine in Mice. Int J Neuropsychopharmacol. 2018 Feb 1;21(2):175-186.
16. Ahmed, H.A., Ismael, S., Mirzahosseini, G. and Ishrat, T., 2021. Verapamil prevents development of cognitive impairment in an aged mouse model of sporadic Alzheimer’s disease. Molecular Neurobiology, 58(7), pp.3374-3387.

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

17. Khurana, K., Kumar, M. and Bansal, N., 2021. Lacidipine Prevents Scopolamine-Induced Memory Impairment by Reducing Brain Oxido-nitrosative Stress in Mice. Neurotoxicity Research, 39(4), pp.1087-1102.
18. Sekhar, D.S., Shwetha, B.K., Haimavathi, B. and Vikram, P., 2016. The effect of calcium channel blockers against scopolamine induced cognitive impairment and oxidative stress. Int. J. Basic Clin. Pharmacol, 5(5), pp.2199-2211.