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# Evaluating the Neuropsychopharmacological Profiling of a Calcium Channel Blocker Verapamil on Sleep, Depression, and Locomotion in Mice

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***ABSTRACT***

*This study aimed to evaluate the neuropsychopharmacological effects of Verapamil using three different behavioural tests in mice: Pentobarbitone-induced sleeping time, Forced Swim Test (FST), and Spontaneous Locomotor Activity (SMA) using an actophotometer. For Pentobarbitone-induced sleeping time, the control group treated with Pentobarbitone alone exhibited an average sleep duration of 84.85 ± 2.17 minutes. Verapamil at 5, 10, and 20 mg/kg increased sleep duration to 85.91 ± 2.21, 87.49 ± 2.17, and 92.64 ± 1.93 minutes, respectively, but these changes were not statistically significant. In the FST, Verapamil at 5 mg/kg slightly increased immobility time, while doses of 10 and 20 mg/kg significantly reduced immobility times to 117.20 ± 5.64 and 104.95 ± 5.26 seconds, respectively, indicating potential antidepressant-like effects at higher doses. The SMA test showed that while Verapamil at 5 and 10 mg/kg did not significantly alter locomotor activity, the 20 mg/kg dose significantly reduced photocell counts from 552.82 ± 15.67 to 462.50 ± 16.85, suggesting sedative properties at higher doses. These findings highlight Verapamil's potential effects on sleep, depression-like behaviour, and locomotor activity, warranting further investigation to elucidate the underlying*

*mechanisms.*

***Keywords:*** *Verapamil, Neuropsychopharmacology. Pentobarbitone-induced sleeping time, Forced* Swim Test (FST), Spontaneous Locomotor Activity (SMA), Calcium channel blocker, Antidepressant-like effects, Sedative

## INTRODUCTION

The one physiological characteristic that most clearly distinguishes humans from other animals is brain function. The principal problem of human civilization is brain function disorders, which can arise from primary or secondary malfunctions of other systems. Pharmacological intervention is a crucial intervention in this field. Without any biological proof, psychological inadequacy or excess refers to daily stressors including family issues, work obligations, and other pressures that cause anxiety, depression, insomnia, and other behavioural disorders. Extended periods of such behavioural aberrations can result in significant psychological problems as well as major organ dysfunctions, such as metabolic illnesses like diabetes and hypertension (Frackowiak, 2004, Tyler, 2012, Lassen et al., 1978).

The treatment of neurological and psychiatric disorders is connected to the boundaries of basic neuroscience through the field of neuropsychopharmacology. This scientific subject aims to comprehend how medications might specifically influence the central nervous system (CNS) in order to alleviate pain, enhance cognition, induce sleep, lower fever or hunger, inhibit movement disorders, or avoid seizures. Notably, this area of study aims to comprehend how medications can treat conditions like schizophrenia, mania, anxiety, or depression without affecting awareness (Hesdorffer, 2016, Tarazi and Schetz, 2005). The fundamental idea of neuropsychopharmacology is that medications that affect behaviour and help patients with neurological or psychiatric conditions function better do so by either amplifying or attenuating the efficacy of chemical transmission at the location of primary interneuronal communication, which is the specialised chemical junction known as synapses. In psychiatry, as in most of medicine, the diagnosis is primarily predicated on the recognition of identifiable patterns of subjective symptoms. Abnormalities in behaviour, mood, perceptions, thinking, and intellectual function are among these signs (Tarazi and Schetz, 2005, Longhena et al., 2021).

Calcium channel blockers (CCBs), primarily used for managing cardiovascular conditions such as hypertension and angina, have recently attracted attention for their potential neuropsychopharmacological effects. These drugs function by inhibiting the influx of calcium ions through L-type calcium channels, which are crucial not only for cardiac muscle contraction but also for numerous processes in the central nervous system (CNS) (Elliott and Ram, 2011, Russell, 1988). Understanding the broader impact of CCBs on the CNS has opened new avenues for research into their therapeutic potential beyond cardiovascular diseases. In the CNS, calcium channels play pivotal roles in regulating neurotransmitter release, neuronal excitability, and synaptic plasticity. These channels are involved in essential brain functions such as learning, memory, and mood regulation (McKeever and Hamilton, 2018). Dysregulation of calcium signalling has been implicated in various neuropsychiatric and neurodegenerative disorders, suggesting that modulating these pathways could have significant therapeutic benefits. Verapamil, for example, has demonstrated anxiolytic properties in some animal studies. By stabilizing calcium influx in neurons, Verapamil may reduce excessive neuronal firing associated with anxiety and hyperactivity disorders. This property makes CCBs a potential candidate for treating conditions such as generalized anxiety disorder (GAD) and attention-deficit/hyperactivity disorder (ADHD) (Lima et al., 2024, Liao et al., 2024, Li et al., 2024a, Lankford et al., 2024, Kutzsche et al., 2024). Moreover, calcium dysregulation is a known factor in the

pathophysiology of neurodegenerative diseases such as Alzheimer's and Parkinson's. Excessive intracellular calcium can lead to neuronal damage and death, contributing to the progression of these disorders. CCBs, by modulating calcium entry into neurons, may offer neuroprotective effects. For instance, stabilizing intracellular calcium levels can prevent the activation of calcium-dependent enzymes that lead to cell death, thereby potentially slowing disease progression and preserving neuronal function. This neuroprotective role of CCBs opens a promising therapeutic avenue for managing neurodegenerative diseases (Jæger et al., 2024, Iepsen et al., 2024, Hortua Triana et al., 2024, Ho et al., 2024, Hirakawa et al., 2024, Hernandez-Hernandez et al., 2024).

Clinical studies also support the potential neuropsychopharmacological benefits of CCBs. Some studies have reported improved cognitive function and reduced progression of cognitive decline in patients with Alzheimer's disease treated with CCBs. These effects are likely due to the combined impact of CCBs on calcium homeostasis, neurotransmitter release, and neuronal survival. Despite these promising findings, the precise mechanisms underlying the neuropsychopharmacological effects of CCBs remain to be fully elucidated. Further research is needed to explore the dose-response relationships, long-term effects, and potential side effects of CCBs in neuropsychiatric conditions. Additionally, understanding the interaction of CCBs with other neurotransmitter systems and signaling pathways in the brain will be crucial for optimizing their therapeutic use (Zhao et al., 2023, Wołek et al., 2023, Wickline et al., 2023, Welsh et al., 2023, Wei et al., 2023, Wardas et al., 2023). In conclusion, calcium channel blockers, particularly Verapamil, show significant promise beyond their traditional cardiovascular applications. Their ability to modulate calcium signaling in the CNS suggests potential benefits for treating a range of neuropsychiatric and neurodegenerative disorders. Continued research into the neuropsychopharmacological effects of CCBs could lead to new and effective treatments for conditions such as depression, anxiety, ADHD, and neurodegenerative diseases, ultimately improving patient outcomes and quality of life (Zhao et al., 2023, Wołek et al., 2023, Wickline et al., 2023, Welsh et al., 2023, Wei et al., 2023, Wardas et al., 2023).

Verapamil, a widely used calcium channel blocker primarily prescribed for cardiovascular conditions, has shown potential for neuropsychopharmacological effects, warranting comprehensive screening for its broader therapeutic applications. Its primary mechanism of action involves inhibiting L-type calcium channels, which are not only abundant in cardiac tissue but also present in various brain regions, influencing neurotransmitter release and neuronal excitability. This dual action suggests that Verapamil may exert significant effects on central nervous system (CNS) functions, presenting opportunities for treating neuropsychiatric disorders (Wu et al., 2024, Wang et al., 2024, Padmapriyadarsini et al., 2024).

Preliminary studies indicate that Verapamil can modulate neurotransmitter systems implicated in mood regulation, such as serotonin, dopamine, and norepinephrine pathways. For instance, its potential to reduce immobility time in the Forced Swim Test, a behavioral model for assessing antidepressant activity, suggests that Verapamil may possess antidepressant-like properties. Additionally, its impact on Spontaneous Locomotor Activity (SMA) further highlights its potential influence on behavioural and cognitive processes. High doses of Verapamil have demonstrated significant reductions in locomotor activity, similar to known sedatives like Chlorpromazine, indicating its potential utility in conditions characterized by hyperactivity or agitation. Furthermore, Verapamil's neuroprotective effects, mediated through calcium channel modulation, could be beneficial in managing neurodegenerative diseases. Calcium dysregulation is a known factor in the pathophysiology of disorders such as Alzheimer's and Parkinson's diseases, and Verapamil's ability to stabilize intracellular calcium levels could mitigate neuronal damage and slow disease progression (Liu et al., 2024, Li et al., 2024b, Kundu et al., 2024a, Kundu et al., 2024b, Johnston and Kennedy, 2024).

Given these preliminary findings, a thorough neuropsychopharmacological profiling of Verapamil is justified. This screening should encompass a range of behavioural, biochemical, and electrophysiological assays to elucidate its effects on mood, cognition, and neuroprotection. Such comprehensive profiling will determine the feasibility of repurposing Verapamil for neuropsychiatric conditions, potentially offering a novel therapeutic option for disorders with limited current treatments. Additionally, understanding its CNS effects could lead to optimized dosing regimens that maximize therapeutic benefits while minimizing adverse effects, enhancing the clinical utility of Verapamil beyond its cardiovascular applications (Levine, 2023, Komatsu et al., 2023, Kim et al., 2023, Kaisbain et al., 2023, Hsu et al., 2023, Forlenza et al., 2023, Dutta et al., 2023, Chen et al., 2023, Wu et al., 2022). Therefore, this present study was designed to investigate and evaluate screen the Neuropsychopharmacological profile of verapamil, a calcium channel blocker in various animal models of neuropsychopharmacology.

## MATERIAL AND METHODS

### Chemicals and Drugs

The study utilized a variety of drugs and chemicals sourced from reputable suppliers to ensure the accuracy and reliability of the results. Apomorphine Hydrochloride, obtained from Himedia in Mumbai, India, was employed for its dopaminergic effects, commonly used in neuropharmacological studies. Chlorpromazine Hydrochloride and Diazepam, both procured from Sigma Aldrich, Mumbai, India, served as standard antipsychotic and anxiolytic agents, respectively, in behavioral assays. Haloperidol, another antipsychotic, was supplied by Himedia, Mumbai, India, adding to the range of neuroleptic agents tested. Pentobarbitone Sodium, also from Sigma Aldrich, was used for its sedative properties, crucial for inducing sleep in animal models. Additionally, Pentylenetetrazol, sourced from Himedia, was utilized for its convulsant properties to study seizure susceptibility. Tween 80, an emulsifying agent, was acquired from LobaChem India and was likely used to ensure proper dispersion of the drugs in solution. These carefully selected drugs and chemicals were integral to the study, providing a comprehensive framework for examining the neuropsychopharmacological effects in the experimental models.

### Animals

For the investigation, albino mice (Swiss 22–25 gm, either female) were employed. The Agricultural University at Mannuthy, Thrissur, Kerala, provided all of the animals. Before the testing period began, they had unlimited access to food and drink. The mice were kept in groups of six to eight in polypropylene cages with a natural light-dark cycle before being employed. A distinct animal colony is developed and given time to adjust to its new surroundings before it is utilised in the research. Standard laboratory conditions were followed, and each animal was used just once. Every observation was conducted in a silent, evenly lit room with ambient temperature. Every observation was conducted in the experimental room from 9:00 to 17:00. Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), New Delhi, India, regulations were followed by the Institutional Animal Ethical Committee (IAEC) in approving all experimental procedures (Pereira et al., 2004).

### Pentobarbitone induced sleeping time

In the control group, pentobarbitone (PB) (45 mg/kg; i.p.) was given. The other three groups received three doses of verapamil (i.p., 5, 10, and 20 mg/kg) thirty minutes before receiving an injection of pentobarbitone (Siemens et al., 1974, Tsuji et al., 1996). Sleeping time was defined as the amount of time between losing and recovering the ability to fall asleep (the righting reflex). When the animal turns to resume its typical posture, note how long it takes it to wake up from sleep. determined when pentobarbitone and its combination with verapamil would start to act and how long it would last.

Table 1. Experimental design table for pentobarbitone induced sleeping time

| Group | Treatment Description | Dosage |
|---|---|---|
| Group – A | Control, received Pentobarbitone Sodium | 45 mg/kg; i.p. |
| Group – B | Test, received Verapamil + Pentobarbitone Sodium | Verapamil: 5 mg/kg; i.p. + Pentobarbitone Sodium: 45 mg/kg; i.p. |
| Group – C | Test, received Verapamil + Pentobarbitone Sodium | Verapamil: 10 mg/kg; i.p. + Pentobarbitone Sodium: 45 mg/kg; i.p. |
| Group – D | Test, received Verapamil + Pentobarbitone Sodium | Verapamil: 20 mg/kg; i.p. + Pentobarbitone Sodium: 45 mg/kg; i.p. |

**Forced swim test**

The forced swim test or behavioral despair swim test has been used as a test of depression like behavior. This test is sensitive to all major classes of antidepressant drugs (Can et al., 2012, Yankelevitch-Yahav et al., 2015). The procedures for the forced swim test (FST) were similar to those first described by Porsolt et al. (1977). Mice were placed individually in glass cylinder containing water. Animals (mice) were forced to swim individually for 15 minutes, in glass cylinder containing 15cm water at room temperature. Animals were individually trained in 15 minute sessions. This was "pre- test session". 24 hours later, the animals were treated with verapamil 5, 10, 20 mg/kg doses in 3 groups each with 6 mice. And the control group was treated with vehicle (1% aqueous solution of Tween 80) and each animal was again forced to swim in similar environment for 5 minutes in a "test-session" and duration of immobility time for each mouse was recorded. Each experimental group consisted of 6 animals (mice) and was chosen by means of completely randomized schedule.

Table 2. Experimental design table for the forced swim test

| Group | Treatment Description | Dosage |
|---|---|---|
| Group – A | Control, received 1% aqueous solution of Tween 80 | 10 ml/kg; i.p. |
| Group – B | Test, received Verapamil | 5 mg/kg; i.p. |
| Group – C | Test, received Verapamil | 10 mg/kg; i.p. |
| Group – D | Test, received Verapamil | 20 mg/kg; i.p. |

**Spontaneous Locomotor Activity (SMA) using Actophotomer**

Spontaneous locomotor activity was evaluated by using a photocell activity cage. Degree of depression was determined by this test (Gosavi et al., 2020b, Gosavi et al., 2020a). The actions of verapamil on spontaneous locomotor activity were measured automatically by using Actophotometer (Medicraft photo actometer, model No.600-40, S.No: P A-0149, India). The units of activity counts were arbitrary and based on the beam breaks by movement of the mice. The spontaneous locomotor activity of each mouse was recorded individually for 10 minutes using the Actophotometer. Verapamil was administered at 3 doses (5, 10, 20 mg/kg) to different groups of mice 30 minutes before the test and chlorpromazine (3 mg/kg, i.p.) as a standard drug was given 30 min before the test. The control group was treated with1% aqueous solution of Tween 80 intreperotoneally 30 min before the test. In each group 6 mice were kept.

Table 3. Experimental design table for spontaneous locomotor activity

| Group | Treatment Description | Dosage |
|---|---|---|
| Group – A | Standard, received Chlorpromazine | 3 mg/kg; i.p. |
| Group – B | Test, received Verapamil | 5 mg/kg; i.p. |
| Group – C | Test, received Verapamil | 10 mg/kg; i.p. |
| Group – D | Test, received Verapamil | 20 mg/kg; i.p. |

**Statistical analysis**

The statistical analysis in this study was meticulously designed to evaluate the effects of Verapamil on various neuropharmacological parameters. Data were expressed as mean ± standard error of the mean (SEM) for each group. Differences between groups were assessed using one-way analysis of variance (ANOVA), a robust statistical method for comparing multiple groups simultaneously to determine if there are any statistically significant differences among them. This was followed by Dunnett's test, a *post-hoc* analysis specifically used when comparing several treatment groups against a single control group. Dunnett's test is particularly advantageous as it controls for type I errors, thereby increasing the reliability of the results. The significance level was set at $p < 0.05$.

## RESULTS AND DISCUSSION

**Pentobarbitone - induced sleeping time**

The study aimed to evaluate the effect of Verapamil on Pentobarbitone-induced sleeping time in mice, using a control group and three different dosages of Verapamil. The control group, treated with Pentobarbitone (40 mg/kg, i.p) alone, exhibited an average sleeping duration of 84.85 ± 2.17 minutes. Mice administered with Verapamil at 5 mg/kg showed a slight increase in sleeping time to 85.91 ± 2.21 minutes, while those treated with 10 mg/kg and 20 mg/kg of Verapamil had increased durations of 87.49 ± 2.17 minutes and 92.64 ± 1.93 minutes, respectively. Despite these observed trends, the increases in sleeping time were not statistically significant, as indicated by the "ns" notation, meaning the results were not significant when analyzed using one-way ANOVA followed by Dunnett's test. The data suggest a dose-dependent trend where higher doses of Verapamil are associated with longer durations of Pentobarbitone-induced sleep. However, the lack of statistical significance implies that these increases could be attributed to natural variability rather than a definitive pharmacological effect of Verapamil. The standard error of the mean (SEM) values indicated consistency within each group, but the absence of significant differences highlights the need for further investigation. Future studies with larger sample sizes or alternative experimental designs might be necessary to clarify whether Verapamil truly affects Pentobarbitone-induced sleeping time. Additionally, exploring other parameters or mechanisms may provide more comprehensive insights into the interaction between Verapamil and Pentobarbitone. Overall, while the data hint at a possible interaction, the current study does not provide conclusive evidence of a significant effect of Verapamil on Pentobarbitone-induced sleep in mice.

Table 4. Effect of verapamil on Pentobarbitone - induced sleeping time in mice

| Treatment (mg/kg,i.p) | Duration of action (Min.) |
|---|---|
| Control PB (40 mg/kg, i.p) | 84.85 ± 2.17 |
| Verapamil (5) | 85.91 ± 2.21 $^{ns}$ |

| | |
|---|---|
| Verapamil (10) | 87.49 ± 2.17 ns |
| Verapamil (20) | 92.64 ± 1.93 ns |

Values are mean ± SEM of 6 animals in each group, ns (One way ANOVA followed by Dunnett's test as compared with Pentobarbitone treated group)

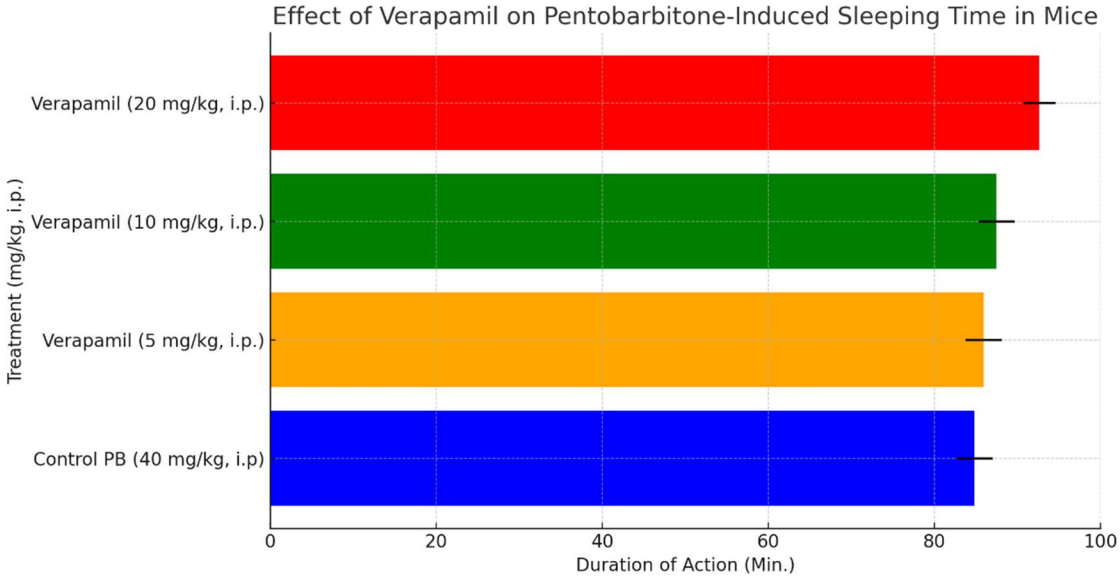


Figure 1. Effect of verapamil on Pentobarbitone - induced sleeping time in mice

**Forced swim test**

The study aimed to assess the impact of Verapamil on the duration of immobility in mice during the Force Swim Test, a common measure of depressive-like behavior. The control group, without Verapamil, showed an immobility duration of 160.73 ± 5.87 seconds, representing a baseline level of behavioral despair. When administered Verapamil at a dose of 5 mg/kg, the mice exhibited a slight increase in immobility time to 175.65 ± 5.88 seconds, suggesting no significant antidepressant effect at this dose. Interestingly, at higher doses of 10 mg/kg and 20 mg/kg, Verapamil significantly reduced immobility times to 117.20 ± 5.64 seconds and 104.95 ± 5.26 seconds, respectively. The reduction observed at 20 mg/kg was statistically significant (denoted by *), indicating a potential antidepressant-like effect at this dosage. This data suggests a dose-dependent response, where lower doses of Verapamil do not produce notable changes in behavior, but higher doses decrease immobility, potentially indicating reduced behavioral despair. The significant reduction at 20 mg/kg implies that Verapamil might have an antidepressant-like effect at sufficient doses, possibly through mechanisms involving calcium channel modulation, which could influence neurotransmitter release and mood regulation. However, the increase in immobility at the lowest dose indicates a complex relationship, possibly due to varying pharmacokinetics or receptor interactions at different concentrations. Overall, these findings highlight the potential of Verapamil, particularly at higher doses, to influence depressive-like behaviors in mice, warranting further research to elucidate its mechanisms and therapeutic potential in treating depression. The results also underscore the importance of dose optimization in pharmacological studies to uncover the full spectrum of a drug's effects

Table 5. Effect of verapamil on Force Swim Test in mice

| Treatment (mg/kg, i.p) | Duration of Immobility (seconds) |
|---|---|
| Control | 160.73 ± 5.87 |
| Verapamil (5) | 175.65 ± 5.88 |
| Verapamil (10) | 117.20 ± 5.64 |
| Verapamil (20) | 104.95 ± 5.26* |

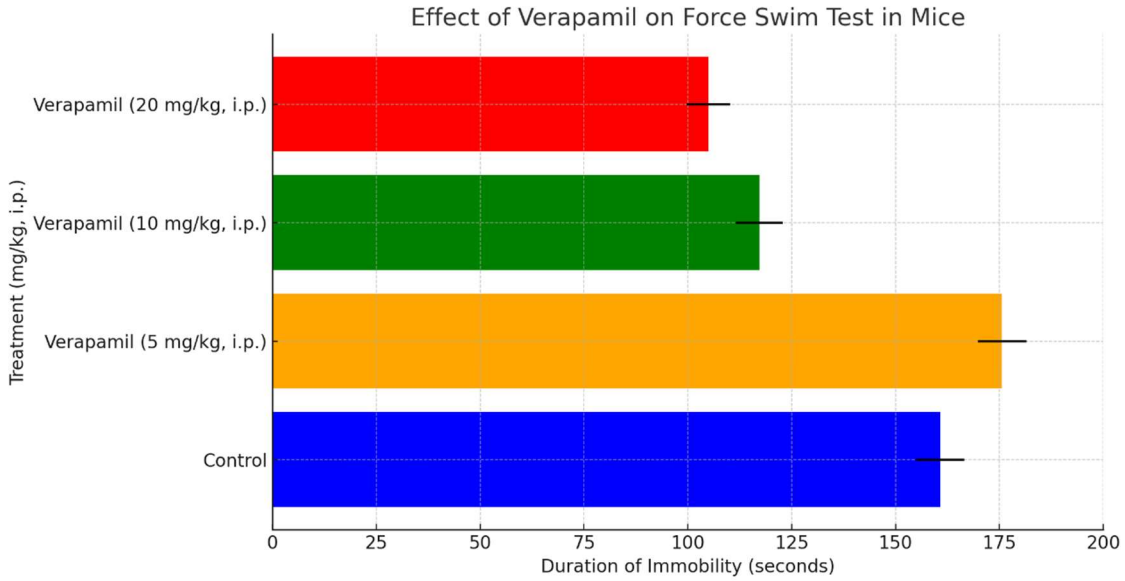


Figure 2. Effect of verapamil on Force Swim Test in mice

**Spontaneous Locomotor Activity (SMA) using Actophotometer**

The study investigated the effect of Verapamil on Spontaneous Locomotor Activity (SMA) in mice, using an actophotometer to measure photocell counts before and after administration. Chlorpromazine, a known sedative, served as the control at 3 mg/kg, significantly reducing photocell counts from 555.45 ± 15.75 to 436.83 ± 15.12 (*** indicates statistical significance). This marked reduction confirms Chlorpromazine's expected sedative effect, validating the experimental setup. Verapamil at 5 mg/kg resulted in a slight decrease in photocell counts from 554.91 ± 16.793 to 520.17 ± 16.98, but this change was not statistically significant, indicating minimal impact on SMA at this dose. Similarly, Verapamil at 10 mg/kg showed almost no change, with counts moving from 517.33 ± 15.77 to 514.17 ± 15.48, suggesting that this dose does not significantly alter locomotor activity. In contrast, Verapamil at 20 mg/kg produced a notable reduction in photocell counts from 552.82 ± 15.67 to 462.50 ± 16.85, which was statistically significant (***). This indicates a dose-dependent effect of Verapamil on locomotor activity, with higher doses exerting a sedative-like influence similar to that of Chlorpromazine. The findings suggest that while low to moderate doses of Verapamil do not

significantly affect SMA, higher doses (20 mg/kg) substantially decrease locomotor activity, indicating potential sedative properties. This dose-dependent reduction highlights Verapamil's influence on central nervous system activity, likely through calcium channel modulation affecting neurotransmitter release and neuronal excitability. The results warrant further investigation to better understand the therapeutic implications and mechanisms underlying Verapamil's impact on locomotor activity

Table 6. Effect of verapamil on Spontaneous Locomotor Activity (SMA) using Actophotometer

| Treatment (mg/kg,i.p) | Photocell count | |
|---|---|---|
| | Before administration | After administration |
| Chlorpromazine (3 mg/kg) | 555.45 ±15.75 | 436.83±15.12*** |
| Verapamil (5) | 554.91± 16.793 | 520.17±16.98 |
| Verapamil (10) | 517.33± 15.77 | 514.17±15.48 |
| Verapamil (20) | 552.82±15.67 | 462.50±16.85*** |

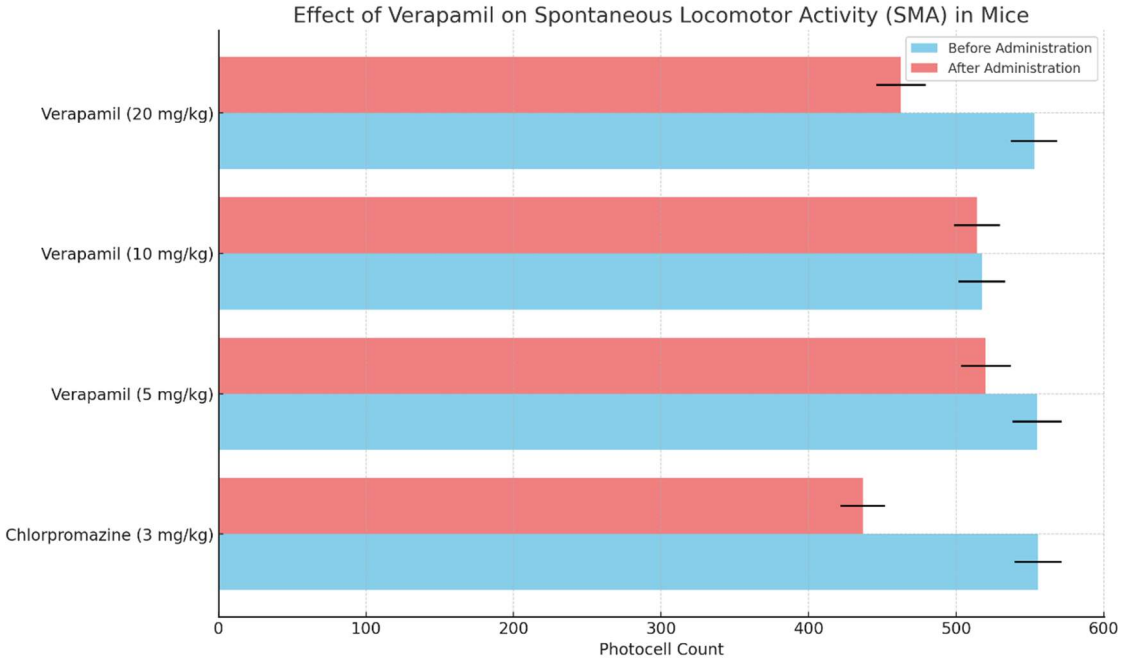


Figure 3. Effect of verapamil on Spontaneous Locomotor Activity (SMA)

## CONCLUSIONS

The study explored the neuropsychopharmacological effects of Verapamil in mice through various behavioural assays, revealing nuanced insights into its potential therapeutic applications. In the Pentobarbitone-induced sleeping time test, Verapamil demonstrated a dose-dependent increase in sleep duration, although these changes were not statistically significant. This suggests that while Verapamil may enhance Pentobarbitone-induced sleep, further studies with larger sample sizes and alternative methodologies are required to confirm this interaction. The Forced Swim Test provided compelling evidence for Verapamil's antidepressant-like effects at higher doses. Mice treated with 10 and 20 mg/kg of Verapamil exhibited significantly reduced immobility times, indicating a reduction in depressive-like behavior. This effect is likely mediated through Verapamil's modulation of calcium

channels, which influence neurotransmitter release and mood regulation. However, the slight increase in immobility at the lowest dose suggests a complex dose-response relationship that necessitates further investigation to fully understand the pharmacokinetics and receptor interactions involved. In the Spontaneous Locomotor Activity test, Verapamil showed significant sedative-like effects at the highest dose (20 mg/kg), markedly reducing locomotor activity similar to the known sedative Chlorpromazine. Lower doses of Verapamil did not significantly impact locomotor activity, highlighting a threshold effect where only higher doses influence CNS activity to a notable extent. This dose-dependent sedative property of Verapamil underscores its potential utility in conditions characterized by hyperactivity or agitation. Overall, the findings suggest that Verapamil exhibits diverse neuropsychopharmacological effects depending on the dosage. Its potential antidepressant and sedative effects at higher doses, coupled with minimal impact at lower doses, highlight the importance of dose optimization in therapeutic applications. Future research should focus on elucidating the precise mechanisms of Verapamil's action in the CNS, exploring its long-term effects, and investigating its potential in clinical settings for treating mood disorders, anxiety, and other neuropsychiatric conditions. These efforts will be crucial in determining the full therapeutic potential of Verapamil beyond its established cardiovascular applications.

## REFERENCES

1. CAN, A., DAO, D. T., ARAD, M., TERRILLION, C. E., PIANTADOSI, S. C. & GOULD, T. D. 2012. The mouse forced swim test. *JoVE (Journal of Visualized Experiments)*, e3638.
2. CHEN, Y., CAO, X., PAN, B., DU, H., LI, B., YANG, X., CHEN, X., WANG, X., ZHOU, T., QIN, A., ZHAO, C. & ZHAO, J. 2023. Verapamil attenuates intervertebral disc degeneration by suppressing ROS overproduction and pyroptosis via targeting the Nrf2/TXNIP/NLRP3 axis in four-week puncture-induced rat models both in vivo and in vitro. *Int Immunopharmacol,* 123**,** 110789.
3. DUTTA, D., NAGENDRA, L., RAIZADA, N., BHATTACHARYA, S. & SHARMA, M. 2023. Verapamil improves One-Year C-Peptide Levels in Recent Onset Type-1 Diabetes: A Meta-Analysis. *Indian J Endocrinol Metab,* 27**,** 192-200.
4. ELLIOTT, W. J. & RAM, C. V. S. 2011. Calcium channel blockers. *The Journal of Clinical Hypertension,* 13**,** 687.
5. FORLENZA, G. P., SCHAMBERGER, M. S. & BUCKINGHAM, B. A. 2023. Verapamil and Pancreatic Beta Cell Function in Pediatric Type 1 Diabetes-Reply. *Jama,* 330**,** 380.
6. FRACKOWIAK, R. S. J. 2004. *Human brain function*, Elsevier.
7. GOSAVI, D. D., KAMDI, A. S., KALAMBE, S. M. & BOHRA, P. N. 2020a. The spontaneous motor action of alcoholic excerpt of Withania coagulans fruits in Swiss albino mice by Actophotometer.
8. GOSAVI, D. D., KAMDI, A. S., KALAMBE, S. M. & BOHRA, P. N. 2020b. The spontaneous motor activity of aqueous extract of Withania coagulans fruits in Swiss albino mice by actophotometer. *International Journal of Basic & Clinical Pharmacology,* 9**,** 1440.
9. HERNANDEZ-HERNANDEZ, G., O'DWYER, S. C., YANG, P. C., MATSUMOTO, C., TIEU, M., FONG, Z., LEWIS, T. J., SANTANA, L. F. & CLANCY, C. E. 2024. A computational model predicts sex-specific responses to calcium channel blockers in mammalian mesenteric vascular smooth muscle. *Elife,* 12.
10. HESDORFFER, D. C. 2016. Comorbidity between neurological illness and psychiatric disorders. *CNS spectrums,* 21**,** 230-238.

11. HIRAKAWA, K., ASANO, R., UEDA, J., AOKI, T., TSUJI, A. & OGO, T. 2024. Calcium channel blockers in patients with pulmonary arterial hypertension receiving PAH-specific treatment. *Int J Cardiol,* 406**,** 132043.
12. HO, C., HA, N. T., YOUENS, D., ABHAYARATNA, W. P., BULSARA, M. K., HUGHES, J. D., MISHRA, G., PEARSON, S. A., PREEN, D. B., REID, C. M., RUITER, R., SAUNDERS, C. M., STRICKER, B. H., VAN ROOIJ, F. J. A., WRIGHT, C. & MOORIN, R. 2024. Association between long-term use of calcium channel blockers (CCB) and the risk of breast cancer: a retrospective longitudinal observational study protocol. *BMJ Open,* 14**,** e080982.
13. HORTUA TRIANA, M. A., MÁRQUEZ-NOGUERAS, K. M., FAZLI, M. S., QUINN, S. & MORENO, S. N. J. 2024. Regulation of calcium entry by cyclic GMP signaling in Toxoplasma gondii. *J Biol Chem,* 300**,** 105771.
14. HSU, N. C., TSAI, H. B. & HSU, C. H. 2023. Verapamil and Pancreatic Beta Cell Function in Pediatric Type 1 Diabetes. *Jama,* 330**,** 380.
15. IEPSEN, U. W., HJORTDAL, A. R., THUESEN, A. D., FINSEN, S. H., HANSEN, P. B. L. & MORTENSEN, S. P. 2024. The role of T-type calcium channels in elderly human vascular function: A pilot randomized controlled trial. *Exp Physiol,* 109**,** 779-790.
16. JÆGER, K. H., CHARWAT, V., WALL, S., HEALY, K. E. & TVEITO, A. 2024. Do calcium channel blockers applied to cardiomyocytes cause increased channel expression resulting in reduced efficacy? *NPJ Syst Biol Appl,* 10**,** 22.
17. JOHNSTON, C. U. & KENNEDY, C. J. 2024. Effects of the chemosensitizer verapamil on P-glycoprotein substrate efflux in rainbow trout hepatocytes. *Comp Biochem Physiol C Toxicol Pharmacol,* 275**,** 109763.
18. KAISBAIN, N., LIM, W. J. & KAISBAIN, V. 2023. Verapamil-Induced Hypotension in End-Stage Renal Disease: The Role of Calcium Gluconate. *Cureus,* 15**,** e33341.
19. KIM, D. K., HAN, D., BAE, J., KIM, H., LEE, S., KIM, J. S., JEONG, Y. G., SHIN, J. & PARK, H. W. 2023. Verapamil-loaded supramolecular hydrogel patch attenuates metabolic dysfunction-associated fatty liver disease via restoration of autophagic clearance of aggregated proteins and inhibition of NLRP3. *Biomater Res,* 27**,** 4.
20. KOMATSU, Y., YODOSHI, M., TAKEGAMI, M., YOKOYAMA, S. & HOSOMI, K. 2023. Association between hemorrhage and direct oral anticoagulants in combination with verapamil: Analysis of Japanese Adverse Drug Event Report database and electronic medical record data. *Int J Clin Pharmacol Ther,* 61**,** 148-158.
21. KUNDU, A., GHOSH, P. & BISHAYI, B. 2024a. Verapamil and tangeretin enhances the M1 macrophages to M2 type in lipopolysaccharide-treated mice and inhibits the P-glycoprotein expression by downregulating STAT1/STAT3 and upregulating SOCS3. *Int Immunopharmacol,* 133**,** 112153.
22. KUNDU, A., GHOSH, P. & BISHAYI, B. 2024b. Vitexin along with verapamil downregulates efflux pump P-glycoprotein in macrophages and potentiate M1 to M2 switching via TLR4-NF-κB-TNFR2 pathway in lipopolysaccharide treated mice. *Immunobiology,* 229**,** 152767.
23. KUTZSCHE, J., GUZMAN, G. A., WILLUWEIT, A., KLETKE, O., WOLLERT, E., GERING, I., JÜRGENS, D., BREITKREUTZ, J., STARK, H., BECK-SICKINGER, A. G., KLÖCKER, N., HIDALGO, P. & WILLBOLD, D. 2024. An orally available Ca(v)2.2 calcium channel inhibitor for the treatment of neuropathic pain. *Br J Pharmacol,* 181**,** 1734-1756.
24. LANKFORD, L., MADDALA, R., JABLONSKI, M. M. & RAO, P. V. 2024. Influence of the calcium voltage-gated channel auxiliary subunit (CACNA2D1) absence on intraocular pressure in mice. *Exp Eye Res,* 241**,** 109835.

25. LASSEN, N. A., INGVAR, D. H. & SKINHØJ, E. 1978. Brain function and blood flow. *Scientific American,* 239**,** 62-71.
26. LEVINE, L. A. 2023. A Primer on the History of Intralesional Verapamil Injection for Peyronie's Disease. *Urology,* 173**,** 5-7.
27. LI, X., FENG, R., GUO, Z., MENG, Y., ZOU, Y., LIAO, W., PENG, Q., ZHONG, H. & ZHAO, W. 2024a. Direct investigations of the effects of nicardipine on calcium channels of astrocytes by Atomic Force Microscopy. *Talanta,* 274**,** 125947.
28. LI, X., ZENG, L., QU, Z. & ZHANG, F. 2024b. Huoxin pill protects verapamil-induced zebrafish heart failure through inhibition of oxidative stress-triggered inflammation and apoptosis. *Heliyon,* 10**,** e23402.
29. LIAO, K. F., HWANG, B. F., LIU, C. S. & LAI, S. W. 2024. Comment on Rotshild et al's "The Risk for Prostate Cancer With Calcium Channel Blockers". *Ann Pharmacother,* 58**,** 441-442.
30. LIMA, A. L. D., SILVA, E. G., CARDOZO, P. L., DA SILVA, M. C. M., KOERICH, S., RIBEIRO, F. M., MOREIRA, F. A. & VIEIRA, L. B. 2024. Isradipine, an L-type calcium channel blocker, attenuates cocaine effects in mice by reducing central glutamate release. *Eur J Pharmacol,* 971**,** 176489.
31. LIU, S., ZHOU, H., KONG, J., CHEN, H., CHEN, L., TANG, M., ZHOU, B., XU, M., CHEN, L. & ZHOU, T. 2024. Verapamil increases susceptibility of colistin-resistant Acinetobacter baumannii to colistin. *Int J Antimicrob Agents***,** 107233.
32. LONGHENA, F., FAUSTINI, G., BREMBATI, V., PIZZI, M., BENFENATI, F. & BELLUCCI, A. 2021. An updated reappraisal of synapsins: structure, function and role in neurological and psychiatric disorders. *Neuroscience & Biobehavioral Reviews,* 130**,** 33-60.
33. MCKEEVER, R. G. & HAMILTON, R. J. 2018. Calcium channel blockers.
34. PADMAPRIYADARSINI, C., SZUMOWSKI, J. D., AKBAR, N., SHANMUGASUNDARAM, P., JAIN, A., BATHRAGIRI, M., PATTNAIK, M., TURUK, J., KARUNAIANANTHAM, R., BALAKRISHNAN, S., PATI, S., KUMAR, A. K. H., RATHORE, M. K., RAJA, J., NAIDU, K. R., HORN, J., WHITWORTH, L., SEWELL, R., RAMAKRISHNAN, L., SWAMINATHAN, S. & EDELSTEIN, P. H. 2024. A Dose-Finding Study to Guide Use of Verapamil as an Adjunctive Therapy in Tuberculosis. *Clin Pharmacol Ther,* 115**,** 324-332.
35. PEREIRA, S., VEERARAGHAVAN, P., GHOSH, S. & GANDHI, M. 2004. Animal experimentation and ethics in India: the CPCSEA makes a difference. *Alternatives to laboratory animals,* 32**,** 411-415.
36. RUSSELL, R. P. 1988. Side effects of calcium channel blockers. *Hypertension,* 11**,** II42.
37. SIEMENS, A. J., KALANT, H., KHANNA, J. M., MARSHMAN, J. & HO, G. 1974. Effect of cannabis on pentobarbital-induced sleeping time and pentobarbital metabolism in the rat. *Biochemical Pharmacology,* 23**,** 477-488.
38. TARAZI, F. I. & SCHETZ, J. A. 2005. *Neurological and psychiatric disorders*, Springer Science & Business Media.
39. TSUJI, R., ISOBE, N. & KAWASAKI, H. 1996. Mechanism of prolongation of pentobarbital-induced sleeping time by empenthrin in mice. *Toxicology,* 108**,** 185-190.
40. TYLER, W. J. 2012. The mechanobiology of brain function. *Nature Reviews Neuroscience,* 13**,** 867-878.
41. WANG, Z., DONG, Z., LI, Y., JIAO, X., LIU, Y., CHANG, H. & GAN, Y. 2024. Verapamil Attenuates the Severity of Tendinopathy by Mitigating Mitochondrial Dysfunction through the Activation of the Nrf2/HO-1 Pathway. *Biomedicines,* 12.

42. WARDAS, B., SCHNEIDER, J. G., KLUGBAUER, N., FLOCKERZI, V. & BECK, A. 2023. Englerin A Inhibits T-Type Voltage-Gated Calcium Channels at Low Micromolar Concentrations. *Mol Pharmacol,* 104**,** 144-153.
43. WEI, S., SU, L. & GAO, Q. 2023. Initiation of diuretics or calcium channel blockers on the top of renin-angiotensin system inhibitors on kidney outcome: which is better? *Kidney Int,* 104**,** 1036-1037.
44. WELSH, L. H., BOSE, J. T. & SAHHAR, H. S. 2023. Calcium Channel Blocker Overdose Causes Acute Respiratory Distress Syndrome and Acute Kidney Injury in a 15-Year-Old Female. *Cureus,* 15**,** e43806.
45. WICKLINE, J. L., SMITH, S., SHIN, R., ODFALK, K., SANCHEZ, J., JAVORS, M., GINSBURG, B. & HOPP, S. C. 2023. L-type calcium channel antagonist isradipine age-dependently decreases plaque associated dystrophic neurites in 5XFAD mouse model. *Neuropharmacology,* 227**,** 109454.
46. WOŁEK, M., MATUSIAK, K., MACHOCZEK, P., PARTYKA, Ł. & ZASADA, W. 2023. Corrected QT interval in electrocardiogram recordings in patients treated with calcium channel blockers. *Postepy Kardiol Interwencyjnej,* 19**,** 171-177.
47. WU, X., GONG, H. & HU, X. 2024. Fluid-solid coupling numerical simulation of the effects of different doses of verapamil on cancellous bone in type 2 diabetic rats. *BMC Musculoskelet Disord,* 25**,** 123.
48. WU, X., HUSSAIN, M., SYED, S. K., SAADULLAH, M., ALQAHTANI, A. M., ALQAHTANI, T., ALDAHISH, A., FATIMA, M., SHAUKAT, S., HUSSAIN, L., JAMIL, Q., MUKHTAR, I., KHAN, K. U. & ZENG, L. H. 2022. Verapamil attenuates oxidative stress and inflammatory responses in cigarette smoke (CS)-induced murine models of acute lung injury and CSE-stimulated RAW 264.7 macrophages via inhibiting the NF-κB pathway. *Biomed Pharmacother,* 149**,** 112783.
49. YANKELEVITCH-YAHAV, R., FRANKO, M., HULY, A. & DORON, R. 2015. The forced swim test as a model of depressive-like behavior. *JoVE (Journal of Visualized Experiments)***,** e52587.
50. ZHAO, M., ZHANG, Z., PAN, Z., MA, S., CHANG, M., FAN, J., XUE, S., WANG, Y., QU, H. & ZHANG, Y. 2023. N-/T-Type vs. L-Type Calcium Channel Blocker in Treating Chronic Kidney Disease: A Systematic Review and Meta-Analysis. *Pharmaceuticals (Basel),* 16.