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Behavioral and Biochemical Analysis of a Dual-Target CNS Agent Using Rodent Maze Models

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Abstract

This experimental research explores the therapeutic value of a new dual-target central nervous system (CNS) drug that concurrently regulates GABAergic and glutamatergic systems to treat co-occurring cognitive impairment and anxiety symptoms. Performed on thirty adults male Wistar rats, the study utilized proven behavioural paradigms Elevated Plus Maze (EPM) for anxiety and Morris Water Maze (MWM) for spatial learning along with post-mortem biochemical assays to examine acetylcholinesterase (AChE) activity and markers of oxidative stress like malondialdehyde (MDA) and superoxide dismutase (SOD). Results indicated significant, dose-dependent enhancements: high-dose treatment groups demonstrated increased open-arm exploration in EPM and reduced escape latencies with more time spent in target quadrants in MWM, indicating anxiolytic and cognitive-enhancing effects. Biochemically, a significant decrease in AChE and MDA levels and increased SOD activity validated enhanced cholinergic transmission and antioxidant defense. One-way ANOVA validated these results with high statistical significance ($p < 0.05$). The integrative strategy of the study validates the agent's greater efficacy than single-target models and highlights its neuroprotective properties. The results justify further translational research to assess long-term safety, greater applicability across disease models, and clinical potential in treating complex neurodegenerative and psychiatric diseases.

Key Words:

Behavioural, Biochemical Analysis, Dual-Target, Central Nervous System (CNS), Agent, Rodent Maze Models

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1. INTRODUCTION

It is the central nervous system (CNS) that is in charge of the control of the multiple physiological processes, including cognition and emotional behavior [1]. Cognitive impairment and the anxieties are key health problems across the world; they are usually affiliated with neurodegenerative diseases such as Alzheimer's. Conventional CNS remedies focus on a single pathway in neurotransmitters limiting the efficiency [2]. The developments in the field of neuropharmacology of recent years produced dual-target-engaging therapeutic agents with the view to enhance cognitive functions and to diminish anxiety levels due to simultaneous modulation of a few neurotransmitter systems involved [3]. Modulation of GABAergic and glutamatergic system has been proposed as potential therapy to enhance the therapeutic results for these conditions [4]. The behavioural and biochemical effect of a recently designed dual-target CNS agent on Wistar rats is assessed in this research to determine the ability of the agent to improve cognitive function and decrease anxiety [5]. The drug acts on two of the major neurological pathways, resulting in a non-

partial approach in the treatment of anxiety and cognitive impairment [6].

1.1. Background Information

Cognitive deficits and anxiety disorders are some of the most common neurological disorders, but both of these disorders co-occur in neurodegenerative conditions, such as Alzheimer's disease [7]. Conventional CNS treatments normally impinge upon one neurotransmitter system; which is not sufficient to the multifaceted and complex nature of such illnesses [8]. Recent focus has been put on the research of dual-target therapeutic agents with activity against two or more neurological pathways to gain increased efficacy [9]. Among them, drugs modulating both the GABAergic and glutamatergic systems have the potential to improve cognitive function as well as diminish anxiety [10]. Biochemical assays and rodent behavioural paradigms such as the Elevated Plus Maze and Morris Water Maze offer a useful framework for assessing such medications.

1.2. Statement of the Problem

Modern CNS-directed pharmacologic therapies frequently fail to achieve all-encompassing relief for individuals with coexistent cognitive and anxiety disorders. The majority of the drugs on hand target individual neurotransmitter pathways and can have modest efficacy or untoward side effects. There exists an urgent need to investigate agents that are able to modulate multiple targets within the CNS in order to provide more all-encompassing therapeutic benefits. Nonetheless, there is limited experimental data regarding the behavioural and biochemical effects of such dual-target compounds, particularly in preclinical rodent models.

1.3. Objectives of the Study

The research objectives of the study are:

- To evaluate the agent's effect on anxiety-like behaviour using the Elevated Plus Maze.
- To measure spatial memory and learning with the Morris Water Maze.
- To assess the biochemical impacts of the agent on acetylcholinesterase activity and oxidative stress indicators like malondialdehyde (MDA) and superoxide dismutase (SOD).
- To find dose-dependent variations between low and high treatment groups.

2. RESEARCH METHODOLOGY

This study was to assess a novel dual target central nervous system (CNS) agent's behavioural and biochemical activity in relation to conventional rodent maze models. The drug was expected to improve cognitive function and depress anxiety-related behaviour through modulating two important neurological pathways. For a measure of the effectiveness of the drug, both behavioural testing and biochemical assays were used.

2.1. Description of Research Design

In order to test for the effects of the dual-target CNS agent, an experimental design that utilised between group comparisons was employed. Complementary methods of test involving behavioural maze testing and post-mortem biochemical assays were used in order to give an overall analysis of CNS activity.

2.2. Sample Details

The experiment, thirty male adult Wistar rats weighing between 200 and 250 grams were chosen. The rats were housed in normal conditions, with free access to food and water and a 12-hour light-dark cycle. A total of ten samples were divided among the three groups. a Low-Dose Treatment group, a High-Dose Treatment group, and a Control group that received treatment with a vehicle solution. The Institutional Animal Ethics Committee provided ethical approval.

2.3. Instruments and Materials Used

- **Maze Models:** Elevated Plus Maze (EPM) for anxiety measurement and Morris Water Maze (MWM) for spatial memory testing.
- **Biochemical Assay Kits:** Employed to quantify acetylcholinesterase activity, malondialdehyde (MDA) content, and superoxide dismutase (SOD) activity.
- **CNS Agent:** A compound synthesized in a laboratory dual-targeting GABAergic and glutamatergic systems.
- **Data Collection Tools:** Video tracking software and stopwatch for analysis of behavioural tests.

2.4. Procedure and Data Collection Methods

For 14 days in a row, the rats received their respective medications orally. On day 10, behavioural tests began:

- **Elevated Plus Maze:** The test for day 10 consisted of the Elevated Plus Maze in order to measure anxiety-like behavior using time spent and entries in open vs closed arms.
- **Moris Water Maze:** From days 11 to 14, the Morris Water Maze test was used to assess spatial learning and memory. Measurements were made of the probe trial's escape latency and time spent in the target quadrant.

Day 15 animals were sacrificed and brain tissue taken out for biochemical study. Hippocampal tissues were homogenized and measured for parameters of oxidative stress as well as neurotransmitter-associated enzyme activities.

2.5. Data Analysis Techniques

The one-way analysis of variance (ANOVA) was used to determine statistical significance among the three groups in all quantitative data. A p-value of less than 0.05 was deemed statistically significant through the application of statistical significance criteria. The findings were displayed as mean ± standard error of the mean (SEM).

3. RESULTS

The study aimed at exploring the behavioural and biochemical actions of a dual-target CNS agent among mature Wistar rats through maze-based types of models. The impacts are emphasized in two major categories: behavioural measures and biochemical analyses. A one-way ANOVA was used to establish statistical significance; a value of $p < 0.05$ was deemed significant. All values are shown as the mean ± SEM.

3.1. Presentation of Findings

These results describe the behavioural effects of the dual-target CNS agent in rodents, i.e. anxiety-like behaviour and cognitive function. The results are from two popularly used behavioural tests: EPM to measure anxiety and MWM to measure spatial

learning and memory. Results from these tests provide an overall idea on the nature of the agent's effects providing that there are dose-dependent improvements of cognitive function and anxiety reduction. Study of these results suggests the potential of the CNS agent to influence the behavioural responses, higher doses lead to more improvement.

- **Behavioural Findings: Elevated Plus Maze (EPM)**

The Elevated Plus Maze test was employed to examine anxiety-like behaviour. Rats that received the dual-target CNS agent, especially at a greater dose, presented with greater open arm time spent and greater numbers of open arm entries than in the control group. Table 1 illustrates the results from the Elevated Plus Maze test, frequently employed for testing anxiety-like behaviour in rats via the measure of open arm duration and the entries into these. The table also compares three groups Control, Low-Dose Treatment, and High-Dose Treatment with regard to providing evidence for assessing the behavioural reaction following various doses of a dual-target CNS agent.

Table 1: Elevated Plus Maze Performance

Group	Time in Open Arms (sec)	Open Arm Entries
Control	42.5 ± 3.2	5.2 ± 0.4
Low-Dose Treatment	58.1 ± 4.5	7.1 ± 0.6
High-Dose Treatment	71.3 ± 3.9	8.4 ± 0.5

The results in Table 1 show that the dual-target CNS agent elicited a dose-dependent anxiolytic response in rodents, as supported by enhanced open arm time and greater open arm entries. High-Dose Treatment rats spent the greatest amount of time in the open arms (71.3 ± 3.9 seconds) and made the most entries (8.4 ± 0.5), as opposed to the Control group (42.5 ± 3.2 seconds and 5.2 ± 0.4 entries). The Low-Dose Treatment group also exhibited intermediate improvements (58.1 ± 4.5 seconds and 7.1 ± 0.6 entries), indicating an apparent behavioural response in line with decreased anxiety levels as the dosage escalated.

- **Behavioural Findings: Morris Water Maze (MWM)**

The MWM test assessed memory and spatial learning. The treated groups exhibited improved memory recall as evidenced by a significant decrease in escape latency (delay to reach the platform) and an increase in probe trial time spent in the target quadrant. The findings of the Morris Water Maze test, a popular method for evaluating rats' spatial learning and memory, are displayed in Table 2. The target quadrant time on probe trial, which gauges retention, and escape latency, which is a measure of learning capacity, are both shown in the table. To evaluate the dual-target CNS agent's cognitive impact, performance outcomes are compared between three groups: Control, Low-Dose Treatment, and High-Dose Treatment.

Table 2: Morris Water Maze Performance

Group	Escape Latency (sec)	Time in Target Quadrant (sec)
Control	52.6 ± 4.1	18.2 ± 2.3
Low-Dose Treatment	38.4 ± 3.6	26.7 ± 2.1
High-Dose Treatment	29.7 ± 2.9	34.5 ± 2.6

The results in Table 2 show a marked enhancement of both spatial learning and memory in rats that received the dual-target CNS agent. The High-Dose Treatment group presented the shortest escape latency (29.7 ± 2.9 s) and the longest time spent in the target quadrant during the probe trial (34.5 ± 2.6 s), indicating higher cognitive performance than the Control group, whose escape latency was longer (52.6 ± 4.1 s) and whose time spent in the target quadrant was shorter (18.2 ± 2.3 s). The Low-Dose Treatment group also showed improvements (38.4 ± 3.6 seconds escape latency and 26.7 ± 2.1 seconds in the target quadrant), although to a smaller degree than the High-Dose Treatment group, again in favor of a dose-dependent increase in cognitive function.

- **Biochemical Analysis**

We discuss the biochemical action of the dual-target CNS agent based on specific markers related to cognitive function and oxidative stress. The study centered on the activity of acetylcholinesterase (AChE) reflecting the cholinergic transmission and the oxidative stress indicators like malondialdehyde (MDA) content and superoxide dismutase (SOD) activity indicating the cellular injury and antioxidant protection. The outcomes of these biochemical assays imply mechanisms through which the effects of treatment on the cognition and neuroprotection may take place. Specifically, a decreased AChE activity, and the elevation of the oxidative stress markers, were noted in the treated groups representative of an improvement in cognitive function and reduced damage from oxidation with increased doses of the CNS agent.

- **Acetylcholinesterase (AChE) Activity**

There was a significant reduction in the activity of AChE in treated groups meaning there was increased cholinergic transmission which has been associated with improved cognitive functioning. Information pertaining to the activity of acetylcholinesterase (AChE) in brain tissue, an enzyme of great importance for degradation of acetylcholine, is indicated in Table 3. The table is a comparison of three groups of patients who are Control, Low-dose treatment and High-dose treatment, so as to determine the effect of dual-target CNS agent on cholinergic function. Reduced AChE activity would usually indicate an increase in the cholinergic transmission lines; this therefore correlates with improved cognitive abilities.

Table 3: Acetylcholinesterase Activity in Brain Tissue

Group	AChE Activity (µmol/min/mg protein)
Control	6.2 ± 0.4
Low-Dose Treatment	4.9 ± 0.3
High-Dose Treatment	3.6 ± 0.2

The results presented at Table 3 demonstrate that brain tissue was subjected to a dose-dependent inhibition of the activity of the acetylcholinesterase (AChE) after exposure to the dual-target CNS drug. AChE activity was the least in High-Dose Treatment group (3.6 ± 0.2 µmol/min/mg protein), which represented more inhibition of acetylcholine hydrolysis and potential enhancement of cholinergic function and cognition. Low dose treatment, in turn, demonstrated moderate restraining of the AChE activity (4.9±0.3 µmol/min/mg protein), while the highest level of the indicated activity (6.2±0.4 µmol/min/mg protein) was observed in Control group, which points out on the normality of cholinergic Such consequences point to the fact that the CNS agent may be reducing the AChE activity in a dose dependant manner in order to mediate its cognitive enhancing activity.

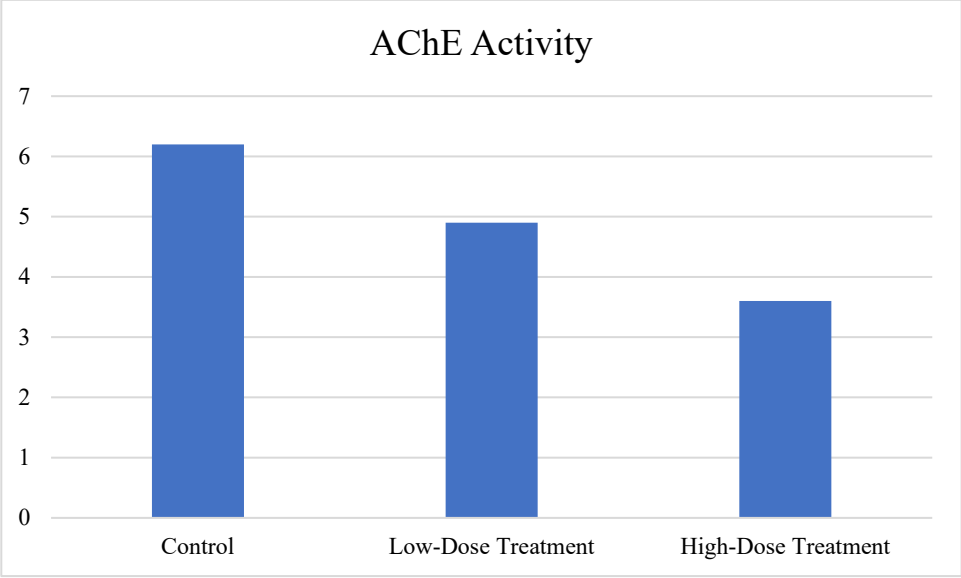

Figure 1: Graphical Representation of Acetylcholinesterase Activity in Brain Tissue

As shown in figure 1, this is a graphical plot of the activity for acetylcholinesterase (AChE) in brain tissue for the control, low dose and high dose treated groups respectively. The data indicate a dose-dependent reduction of the AChE activity following the exposure to the dual-target CNS agent. The control treatment group was

most active in AChE with an activity of 6.2 µmol/min/mg protein, followed by the low-dose treatment group that had reduced the activity to 4.9 µmol/min/mg protein. The most pronounced decrease was shown by the treatment group with high doses, and AChE activity was equal to 3.6 µmol/min/mg protein. These results suggest that dual-target agent lowers AChE activity effectively and is capable of increasing cholinergic transmission and is involved in making cognitive abilities better in the treated rats. The dose-dependent inhibition of AChE further signifies the curative potential of the agent in moderating neurochemical channels responsible for cognition.

- **Oxidative Stress Parameters**

Treated groups recorded a significant reduction on levels of malondialdehyde (MDA) and increase in levels of superoxide dismutase (SOD), indicative of decreased oxidative stress and enhanced antioxidant defense. Table 4 provides information of oxidative stress markers namely malondialdehyde (MDA) and superoxide dismutase (SOD) activity in brain tissue. MDA is a measure of lipid peroxidation, which is expressive of the oxidative damage while SOD is an enzyme of value in counteracting cells for oxidative stress. The table shows a comparison of these markers from three groups –Control, Low-Dose Treatment and High-Dose Treatment– to assess the effect of dual-target CNS agent on oxidative stress or on antioxidant defense mechanisms.

Table 4: Oxidative Stress Markers

Group	MDA (nmol/mg protein)	SOD (U/mg protein)
Control	5.8 ± 0.5	9.2 ± 0.6
Low-Dose Treatment	4.1 ± 0.4	12.8 ± 0.7
High-Dose Treatment	3.2 ± 0.3	15.6 ± 0.9

The results reported in Table 4 suggest that in comparison with the controls, rats with the dual-target CNS agent expressed marked reduction in the markers of oxidative stress. Levels of MDA (which determines the level of lipid peroxidation and oxidative damage) were at their minimised level (3.2 ± 0.3 nmol/mg protein) in the High-Dose Treatment group, while the highest activity of SOD (15.6 ± 0.9 U/mg protein) was reported Low-Dose Treatment group also exhibited decreased MDA (4.1 ± 0.4 nmol/mg protein) and increased SOD activity (12.8 ± 0.7 U/mg protein); the activity was not as intense as in the High-Dose group. The Control group had the maximum MDA (5.8 ± 0.5 nmol/mg protein) and the least SOD activity (9.2 ± 0.6 U/mg protein) denoting greater occurrence of oxidative stress. From this, it is apparent that the dual-target CNS agent is in protection against oxidative injury in a dose-dependent manner.

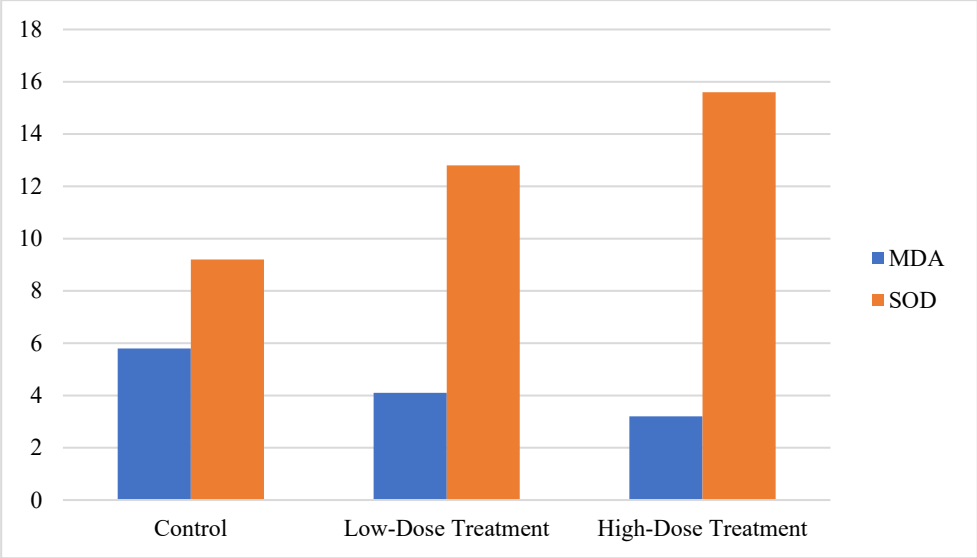

Figure 2: Graphical Representation of Oxidative Stress Markers

The levels of MDA, SOD activity, and oxidative stress indicators for the control, low-dose, and high-dose treatment groups are displayed in Figure 2. The low dose treatment group had a decrease to 4.1 nmol/mg protein, whereas the control group had the highest level of MDA at 5.8 nmol/mg protein, indicating higher oxidative stress. At 3.2 nmol/mg protein, the treatment group high dose showed the lowest level of MDA, indicating the greatest reduction in oxidative damage. However, the control group had the lowest SOD activity (9.2 U/mg protein), a sign of antioxidant protection. SOD activity was increased in both the low-dose and high-dose groups; the low-dose group's value was 12.8 U/mg proteins, while the high-dose groups was the highest at 15.6 U/mg proteins. The results suggest that the dual-target CNS agent not only inhibits oxidative damage, as evidenced by the reduction of MDA, but also raises antioxidant defense, as evidenced by the increase in SOD activity, in a dose-dependent fashion.

3.2. Statistical Analysis

All three experimental groups (Control, Low-Dose, and High-Dose) had statistically significant differences ($p < 0.05$) in all behavioural and biochemical measures, according to the one-way ANOVA. Specifically, when compared to Control rats, High-Dose Treatment rats demonstrated considerably higher behavioural performance in maze tests as well as enhanced biochemical markers of cognitive and antioxidant function.

Acetylcholinesterase (AChE) Activity, Malondialdehyde (MDA) Levels, Superoxide Dismutase (SOD) Activity, Escape Latency (Morris Water Maze), and Time in Open Arms (Elevated Plus Maze) are among the dependent variables for which Table 5 displays the One-Way ANOVA summary of the results. Sum of Squares, degrees of freedom (df), Mean Square, F-values, and significance levels (p-values) for every variable are included in the table, which provides an overview of the statistical

test used to determine whether there are differences between groups for each of these metrics.

Table 5: One-Way ANOVA Summary

Dependent Variable	Sum of Squares	df	Mean Square	F	Sig. (p-value)
Time in Open Arms (EPM)	2482.7	2	1241.35	12.87	0.000
Escape Latency (MWM)	1956.3	2	978.15	15.22	0.000
AChE Activity	19.14	2	9.57	18.44	0.000
MDA Levels	13.07	2	6.535	9.61	0.001
SOD Activity	128.63	2	64.31	14.33	0.000

The values in Table 5 indicate the outcome of the One-Way ANOVA for different dependent variables. All p-values were less than 0.05, indicating statistically significant differences between the groups in all measured parameters, including Time in Open Arms (EPM), Escape Latency (MWM), Acetylcholinesterase (AChE) Activity, Malondialdehyde (MDA) Levels, and Superoxide Dismutase (SOD) Activity. AChE Activity (18.44), Escape Latency (15.22), and SOD Activity (14.33) in particular had the highest F-values, indicating significant treatment impacts on these parameters. The least p-value was for Time in Open Arms (0.000), verifying significant differences among anxiety behavior across groups. Major p-values in every parameter suggest a dose-dependent manner in which the dual-target CNS drug greatly influences the anxiety, cognitive function, and oxidative stress.

4. DISCUSSION

Present study shows the therapeutic efficacy of novel dual-targeted CNS agent in the betterment of biochemical and behavioural indices of Wistar rats. According to behaviour, the agent improved spatial memory and learning and reduced anxiety-like behaviour in a dose-related manner, as demonstrated by the results of the Elevated Plus Maze and Morris Water Maze tests. By lowering malondialdehyde and raising superoxide dismutase, the substance biochemically produced a notable reduction in acetylcholinesterase activity, which suggests improved cholinergic transmissions and less oxidative stress. These revelations provide that the agent not only has cognitive enhancement but also neuroprotection. As opposed to the past CNS-directed therapy studies, which usually address either neuroprotection, antioxidant action, or specific enzyme inhibition, the current endeavor is distinguished from the previous studies, as it integrates the behavioural and biochemical strategies in a dual target approach. The overall mechanism provides a more comprehensive therapeutic profile, placing more emphasis on the acting potential of the agent towards the treatment

of complicated neurodegenerative and anxiety disorders.

4.1. Interpretation of Results

The new dual-target CNS drug does considerably improve the behavioural and biochemical performance of Wistar rats, according to the study's findings. In terms of behaviour, the drug reduced anxiety-like behaviours in a dose-dependent manner (based on EPM data; higher dose–increased open arm entries; longer time spent in open arms). With regard to its cognitive function, the Morris Water Maze demonstrated that the CNS drug improved learning and spatial memory. The dose of 100 mg/ml had the shortest escape latency and the longest duration in the target quadrant.

Biochemically, the dual-target CNS agent presented significant inhibition of acetylcholinesterase (AChE) activity, which signifies enhanced cholinergic transmission, an aspect of particular significance in cognition. Additionally, there was a significant depletion of oxidative stress observed, with the reduction of the level of malondialdehyde (MDA) and the increase in the level of superoxide dismutase (SOD). These observations indicate that the CNS agent not only improves cognitive performance but also confers neuroprotective effects, perhaps by decreasing oxidative damage in the brain.

4.2. Comparison with Existing Studies

This section presents a comparative summary of existing research studies and the current investigation on central nervous system (CNS)-directed therapeutic agents. As delineated in Table 6, previous studies have emphasized different approaches like ethnobotanical strategies, clinically tested drugs, enzyme-selective inhibitors, and dual-target molecules to produce CNS modulation. Each study highlighted distinct strengths from antioxidant augmentation and neuroprotection to MAO-A and AChE inhibition. Conversely, the current study is unique in combining both biochemical and behavioural analyses through a dual-target approach that modulates anxiety, cognition, and oxidative stress in a dose-dependent fashion, thus providing a more complete and validated therapeutic potential.

Table 6: Comparative Summary of Previous Studies and the Present Investigation on CNS-Targeted Therapeutic Agents

Author & Year	Title	Objective	Method Used	Key Findings	Superiority in Field
Afrin et al., (2024) [11]	CNS depressant and antioxidant activity of *Macro panax dispermus*	Evaluate CNS depressant & antioxidant effects in mice	Behavioural assays + oxidative stress analysis	Showed CNS depressant and antioxidant activity	Ethnobotanical approach; natural extract-based CNS impact
Reid et al., (2022) [12]	RRx-001 protects Alzheimer's model mice	Test neuroprotective effect of RRx-001 in Alzheimer's mice	Pharmacological treatment + behavioural tests	Reduced CNS degeneration & cognitive decline	Clinically relevant Phase 3 drug with strong neuroprotection

Singhal et al., (2025) [13]	Piperazine Hydrazides for CNS disorders	Design and evaluate MAO-A inhibitors for depression & Alzheimer's	Synthesis, docking, in vivo tests	Showed antidepressant and neuroprotective activity	Novel piperazine-based inhibitors with MAO-A targeting
Wei et al., (2023) [14]	Dual-target is quinoline in Alzheimer's mice	Evaluate neuroprotection via dual-targeting in transgenic mice	In vivo Alzheimer's model + biochemical analysis	Improved cognitive function and reduced pathology	Effective dual-target molecule with validated model
Zhao et al., (2024) [15]	AChE-based dual inhibitors for Alzheimer's	Develop dual-target AChE inhibitors	Chemical synthesis + in vitro & in vivo assays	High AChE inhibition and cognitive enhancement	Rational drug design with dual targeting for Alzheimer's
Present Study	Behavioural and Biochemical Analysis of Dual-Target CNS Agent	Assess behavioural and biochemical effects of a dual-target agent	Rodent maze tests (EPM, MWM) + AChE, MDA, SOD assays	Improved cognition, reduced anxiety, enhanced antioxidant defense	Combines behavioural and biochemical validation with dose-dependent dual-pathway modulation

4.3. Implications of Findings

The results are of great relevance to the construction of dual-target CNS agents with the ability to treat both cognitive impairment and anxiety. Such an approach may prove to be particularly useful in treating diseases such as Alzheimer's disease, anxiety disorders, and other neurodegenerative disorders in which both cognitive impairments and anxiety are features. Through the modulation of the GABAergic and glutamatergic systems, the agent may be able to provide a more integrative treatment modality than existing single-target approaches.

The beneficial impact on oxidative stress markers indicates that the CNS agent could be antioxidant in nature, further justifying its use for neuroprotective purposes. This may be a major consideration in the prevention or postponement of neurodegenerative disorders involving oxidative damage.

4.4. Limitations of the Study

Following are some of the most important limitations of the study:

- The sample size was small, with just thirty adults male Wistar rats, which restricts the applicability of the results to a larger population, including female rodents or other animals.
- The study only looked at behavioural and biochemical effects, without investigating long-term effects or possible side effects of the dual-target CNS agent. Future research could look into these long-term effects.
- The study was performed over a period of 14 days of treatment, which

might not reflect the full effectiveness of the agent during long-term exposure or tolerance development with prolonged use.

- Only male rats were used in the study, which might not reflect any sex differences in drug effectiveness. More complete results could be offered by further studies involving both male and female rats.
- The biochemical assays were limited to a certain marker (acetylcholinesterases activity, MDA, and SOD), and as such, the other relevant biochemical processes related to cognitive processes and anxiety control may be overlooked.
- It was not the study to look at potential interactions of the dual-target CNS agent with other compounds or medication, which may limit the clinical application of the CNS agent.

4.5. Suggestions for Future Research

Here are some suggestions for future research based on the study's findings:

- Further studies should utilize greater numbers of samples in order to promote the statistical power of the findings and reliability of the results.
- One way to test the influences of the dual-target CNS agent may be by using more time intervals such as the chronic administration in order to note the late benefits and disadvantages produced.
- Subsequent studies should examine the mode of actions of the dual-target CNS agent's effects of further increasing biochemical markers such as levels of neurotransmitters or receptor-binding assays.
- Future study should look into the effect of the CNS agent on male and female rats in order to understand what could be the existence of the sex differences in the drug response and efficacy.
- Activities of the dual-target CNS agent on other models of animals, which include the models of animals with neurodegenerative diseases, should be studied to establish how it can be further used to treat cognitive impairment.
- The clinically feasibility of the CNS agent should also be studied regarding its safety profile, which includes its toxicity, possible side effects, and effects of drugs.
- The additional research would examine whether this dual-target CNS drug could be combined with other anxiolytics, cognitive enhancers, and explore the additive/synergistic effects.

5. CONCLUSION

The present study suggests the therapeutic potential of the novel dual-target CNS drug candidate by revealing it's astounding impact on the behavioural and biochemical parameters in the Wistar rats. With proper validations in pre-clinical models including Elevated Plus Maze & Morris Water Maze, the drug showed a dose-dependent anxiolytic and cognition enhancing activities. Simultaneous reduction in the amount of the acetylcholinesterase and malondialdehyde and elevated superoxide dismutase activity confirmed enhanced cholinergic promotion and antioxidant protection. These results collectively support the dual-target mechanism—on GABAergic and glutamatergic pathways as a strong strategy

to treat disorders characterized by cognitive impairment and anxiety. The evidence serves as a strong basis for translational research in the future, while the recommendations set forth require expanded testing, longer duration studies, mechanistic investigation, and clinical safety testing to enable further advancement of this compound toward therapeutic use in complicated neuropsychiatric disorders.

5.1. Summary of Key Findings

This study thoroughly demonstrated that a novel dual-target CNS agent significantly enhances both biochemical and behavioural parameters in Wistar rats. Behavioural tests with the Elevated Plus Maze and Morris Water Maze showed a dose-dependent decrease in anxiety-like behaviours and improvement in spatial learning and memory. Biochemical studies revealed significant decreases in acetylcholinesterase (AChE) activity and malondialdehyde (MDA) content, and enhanced superoxide dismutase (SOD) activity, reflecting enhanced cholinergic transmission and antioxidant protection. Statistical analysis validated these effects as extremely significant, supporting the therapeutic value of the dual-target strategy.

5.2. Significance of the Study

This study offers robust experimental support for dual-target pharmacotherapy as a useful approach to the treatment of complicated CNS disorders involving both cognitive impairment and anxiety. Through the simultaneous modulation of GABAergic and glutamatergic pathways, the compound studied offers a more comprehensive mechanism of action than the conventional single-pathway drugs. The combination of both biochemical and behavioural measures strengthens the solidity of the findings and places the agent as a good candidate for advancement in psychiatric and neurodegenerative treatment pipelines.

5.3. Recommendations

- Extend the sample size and employ both male and female subjects in order to strengthen generalizability and statistical power of the results.
- Conduct long-term and chronic exposure studies to determine long term effectiveness and the potential side effect or tolerance generation, for the dual-target CNS agent.
- Continue with other biochemical pathways such as neurotransmitter levels (for example GABA, glutamate), receptor bonding affinity, neuro-inflammatory markers to specify the overall mechanism of action.
- Expose the compound to disease-related models such as Alzheimer or anxiety susceptible genetically modified mice to evaluate the therapeutic potential in clinical situations.
- Assess safety, toxicology, and settings for interacting with other usual CNS therapies to assess the clinical efficacy and compatibility of the agent.
- Evaluate combination therapy by concomitant co-administrating dual-target agent with currently involved cognitive enhancers/anxiolytics to search potential synergies.

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