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EVALUATION OF THE ANXIOLYTIC POTENTIAL OF 4-HYDROXYPIPERIDINE ON SWISS ALBINO MICE EXPERIMENTAL ANIMALS

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ABSTRACT
Anxiety disorders are among the most common psychiatric illnesses worldwide and are associated with significant emotional, cognitive, and social impairment. Although benzodiazepines are effective anxiolytic agents, their long-term use is limited by adverse effects such as sedation, tolerance, and dependence, necessitating the search for safer alternatives. The present study aimed to evaluate the anxiolytic potential of 4-Hydroxypiperidine in Swiss albino mice using validated behavioral models. Healthy adult Swiss albino mice (20–30 g) were divided into control, diazepam (1 mg/kg), and test groups receiving different doses of 4-Hydroxypiperidine. Acute toxicity evaluation, performed according to OECD Guideline 423, revealed no mortality or significant toxic effects, indicating a favorable safety profile. Anxiolytic activity was assessed using the Elevated Plus Maze, Light-Dark Box, T-Maze, and Y-Maze tests. Treatment with 4-Hydroxypiperidine produced dose-dependent improvements in anxiety-related behaviors, including increased open-arm exploration, prolonged time spent in the light compartment, reduced transfer latency, and enhanced spontaneous alternation compared with the control group. The highest dose demonstrated effects comparable to diazepam in several behavioral parameters. These findings suggest that 4-Hydroxypiperidine possesses significant anxiolytic activity and may represent a promising lead compound for the development of safer therapeutic agents for anxiety disorders. Further pharmacological and mechanistic studies are warranted to elucidate its mode of action and clinical potential.

KEYWORDS: Anxiety disorders, 4-Hydroxypiperidine, Anxiolytic activity, Swiss albino mice, Elevated Plus Maze, Light-Dark Box Test, T-Maze, Y-Maze, Diazepam, Behavioral pharmacology.

INTRODUCTION

Anxiety disorders are among the most prevalent psychiatric disorders worldwide and represent a major public health concern affecting millions of people across all age groups. Anxiety is a normal emotional response that occurs in stressful or threatening situations and helps individuals react appropriately to danger. However, when anxiety becomes excessive, persistent, and uncontrollable, it may develop into a pathological condition known as an anxiety disorder. These disorders negatively affect emotional stability, cognitive function, social interaction, occupational performance, and overall quality of life.[1]

Anxiety disorders include several clinical conditions such as generalized anxiety disorder (GAD), panic disorder, social anxiety disorder, obsessive-compulsive disorder (OCD), and specific phobias. Individuals suffering from anxiety disorders experience intense fear, nervousness, excessive worrying, restlessness, and difficulty concentrating. Physical symptoms commonly associated with anxiety include rapid heartbeat, sweating, trembling, muscle tension, fatigue, gastrointestinal disturbances, insomnia, and shortness of breath. The severity and duration of these symptoms vary depending on the type of anxiety disorder and individual psychological conditions.[2]

According to recent epidemiological studies, anxiety disorders are among the leading causes of disability worldwide. The World Health Organization (WHO) reported that the prevalence of anxiety disorders increased significantly during and after the COVID-19 pandemic due to social isolation, economic instability, fear of illness, and psychological stress. Women are generally more affected than men, and anxiety disorders are commonly observed among adolescents and young adults.[3]

The pathophysiology of anxiety disorders is highly complex and involves disturbances in multiple neurotransmitter systems within the central nervous system. Gamma-aminobutyric acid (GABA) is considered the primary inhibitory neurotransmitter responsible for regulating neuronal excitability and emotional behavior. Reduced GABAergic activity results in excessive neuronal stimulation and contributes significantly to anxiety development. Serotonin, dopamine, norepinephrine, and glutamate also play important roles in emotional regulation and stress response. Dysregulation of these neurotransmitters alters synaptic transmission and behavioral responses associated with anxiety.[4]

Recent studies suggest that glutamatergic hyperactivity contributes to excessive neuronal excitation observed in anxiety disorders. Glutamate is the major excitatory neurotransmitter in the brain, and increased glutamate signaling may lead to stress-induced neurotoxicity and emotional disturbances. In contrast, enhancement of inhibitory GABAergic neurotransmission helps reduce anxiety-related neuronal activity. Therefore, compounds capable of modulating GABA and glutamate pathways are considered promising therapeutic agents for anxiety management.[5]

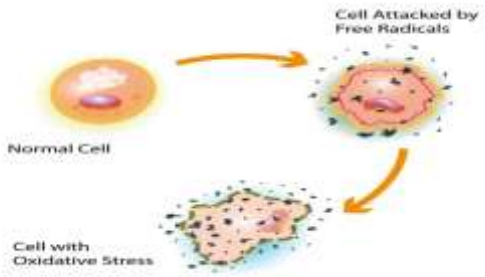
In addition to neurotransmitter imbalance, oxidative stress and neuroinflammation are increasingly recognized as important contributors to anxiety disorders. Chronic stress conditions increase the production of reactive oxygen species (ROS), resulting in oxidative damage to neuronal cells, mitochondrial dysfunction, and impaired neurotransmitter signaling. Excessive oxidative stress alters brain function and promotes behavioral abnormalities associated with anxiety and depression.[6]

Neuroinflammatory mechanisms also play a significant role in anxiety pathogenesis. Increased levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) have been observed in patients with anxiety disorders. These inflammatory mediators affect neuronal communication and contribute to emotional instability and stress-related behavioral changes. Recent neuropharmacological research therefore focuses on compounds possessing antioxidant and anti-inflammatory properties for the treatment of anxiety disorders.[7]

Several pharmacological agents are currently used for anxiety treatment, including benzodiazepines, selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), and beta-blockers. Benzodiazepines are the most commonly prescribed anxiolytic drugs because of their rapid onset of action. These drugs act by enhancing GABA-a receptor-mediated inhibition in the brain. However, prolonged use of benzodiazepines is associated with several adverse effects such as sedation, tolerance, dependence, memory impairment, cognitive dysfunction, and withdrawal symptoms.[8]

Selective serotonin reuptake inhibitors are considered safer for long-term therapy; however, they may require several weeks to produce therapeutic effects and are associated with adverse reactions such as nausea, insomnia, headache, sexual dysfunction, and gastrointestinal disturbances. Due to these limitations, there is a continuous need for the development of safer and more effective anxiolytic agents with improved therapeutic efficacy and minimal side effects.[9]

Recent advances in medicinal chemistry and neuropharmacology have encouraged the investigation of heterocyclic compounds and synthetic derivatives for anxiolytic activity. Piperidine derivatives have attracted considerable scientific interest because of their broad range of pharmacological activities including analgesic, anticonvulsant, antimicrobial, anti-inflammatory, and central nervous system effects. These compounds may interact with neurotransmitter receptors and ion channels involved in anxiety regulation.[10]



Experimental animal models are widely used to evaluate anxiolytic activity because they provide reliable behavioral and neurochemical responses. Swiss albino mice are commonly used in neuropharmacological studies due to their easy handling, sensitivity to psychotropic agents, and predictable behavioral characteristics. Behavioral tests such as the Elevated Plus Maze (EPM), Open Field Test (OFT), Light-Dark Box Test, and Hole Board Test are frequently employed to assess anxiety-related behavior in rodents. These models help identify compounds capable of reducing fear and anxiety-like responses.[11]

The present study focuses on evaluating the anxiolytic potential of 4-Hydroxypiperidine using Swiss albino mice experimental models. Since limited scientific information is available regarding the anxiolytic activity of this compound, the study may provide valuable insight into its pharmacological significance and possible mechanism of action. Investigation of novel compounds such as 4-Hydroxypiperidine may contribute to the development of safer and more effective therapeutic agents for anxiety disorders.[12]

Global Burden and Prevalence of Anxiety

Anxiety disorders are considered one of the leading mental health problems worldwide and contribute significantly to the global burden of disease. These disorders affect individuals of all age groups, genders, and socioeconomic backgrounds, causing substantial impairment in emotional, psychological, social, and occupational functioning. Anxiety disorders are highly prevalent and often coexist with other psychiatric illnesses such as depression, substance abuse disorders, and sleep disturbances.[13]

According to the World Health Organization (WHO), anxiety disorders affect more than 300 million people globally and are among the most common mental illnesses. The prevalence of anxiety disorders has increased dramatically over the last decade due to rapid urbanization, academic pressure, occupational stress, financial instability, social isolation, and

lifestyle-related factors. The COVID-19 pandemic further intensified psychological stress and contributed significantly to the rise in anxiety-related disorders across the world.[14]

Epidemiological studies indicate that generalized anxiety disorder (GAD), panic disorder, social anxiety disorder, and phobias are commonly observed among adolescents, young adults, and elderly populations. Women are reported to be affected nearly twice as frequently as men because of hormonal, biological, psychological, and social factors. Hormonal fluctuations during menstruation, pregnancy, postpartum periods, and menopause may contribute to increased susceptibility to anxiety disorders among females.[15]

Limitations of Conventional Anxiolytic Drugs:

Conventional anxiolytic drugs are widely used for the management of anxiety disorders and related psychiatric conditions. The major classes of anxiolytic medications include benzodiazepines, selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), beta-blockers, and barbiturates. Although these drugs are effective in reducing anxiety symptoms, their long-term use is associated with several limitations and adverse effects that restrict their therapeutic application.16

Benzodiazepines are among the most commonly prescribed anxiolytic agents because of their rapid onset of action and strong calming effects. These drugs act by enhancing gamma-aminobutyric acid (GABA)_A receptor activity in the brain, resulting in increased inhibitory neurotransmission and reduced neuronal excitability. Despite their effectiveness, prolonged benzodiazepine therapy is associated with sedation, drowsiness, dizziness, muscle relaxation, impaired coordination, and reduced cognitive performance. Patients receiving benzodiazepines may experience difficulty in concentration, memory impairment, and psychomotor dysfunction, which can negatively affect daily activities and occupational performance.17

The COVID-19 pandemic produced a substantial psychological impact worldwide and significantly increased anxiety prevalence. Social isolation, fear of infection, loss of employment, financial crisis, and uncertainty regarding the future contributed to emotional distress and mental health disorders. Healthcare workers experienced particularly high levels of anxiety because of occupational exposure, workload pressure, and fear of transmitting infection to family members.18

Children and adolescents are also increasingly affected by anxiety disorders due to academic stress, excessive screen exposure, social media influence, family conflicts, and peer pressure. Anxiety during childhood may negatively affect emotional development, academic performance, and social behavior. Early diagnosis and intervention are therefore important to prevent long-term psychological complications.19

Older adults commonly experience anxiety due to chronic illness, loneliness, cognitive decline, reduced physical activity, and fear of dependency. Anxiety among elderly individuals is frequently underdiagnosed because symptoms may overlap with physical disorders or age-related cognitive impairment. Proper mental health screening and supportive care are essential for improving the quality of life of older populations.20

Anxiety disorders not only affect individuals psychologically but also impose a major economic burden on healthcare systems and society. Increased healthcare expenditure, reduced workplace productivity, absenteeism, and long-term treatment costs contribute significantly to economic loss. Untreated anxiety disorders may also increase the risk of substance abuse, suicidal behavior, and chronic psychiatric illness.21

Despite the high prevalence of anxiety disorders, many affected individuals do not receive adequate treatment due to lack of awareness, social stigma, fear of discrimination, and limited access to mental healthcare services. In several developing countries, mental health resources remain insufficient, leading to delayed diagnosis and treatment. Increasing public awareness regarding mental health and improving psychiatric healthcare infrastructure are essential for reducing the global burden of anxiety disorders.22

Recent advances in neuroscience and psychopharmacology have encouraged the development of novel therapeutic agents targeting neurotransmitter imbalance, oxidative stress, and neuroinflammation involved in anxiety disorders. Identification of safer and more effective anxiolytic compounds is necessary because currently available medications are associated with adverse effects such as sedation, tolerance, dependence, and cognitive impairment. Research involving novel heterocyclic compounds and experimental animal models may therefore contribute significantly to anxiety management and mental healthcare advancement.23

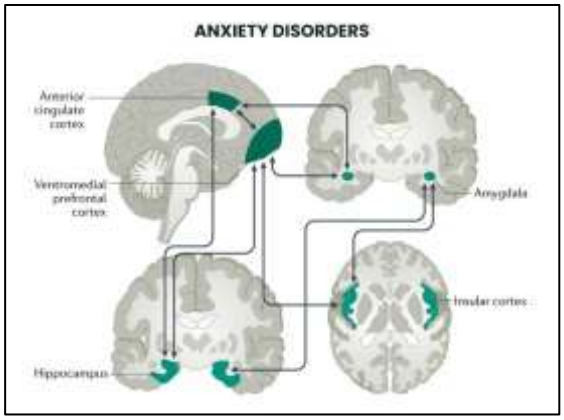
3) Neurobiology of Anxiety

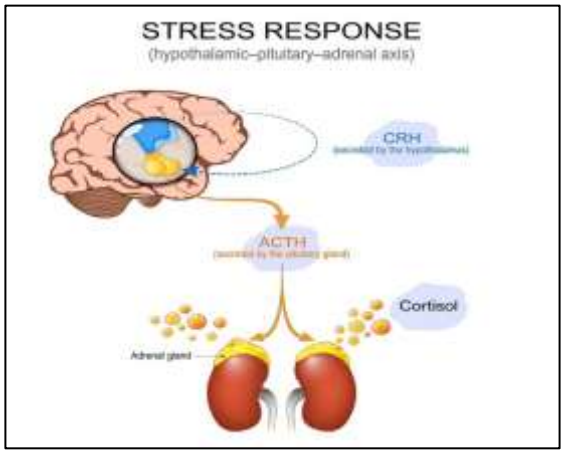
The neurobiology of anxiety involves complex interactions among neurotransmitters, brain regions, neuroendocrine pathways, inflammatory mediators, and genetic factors that regulate emotional and behavioral responses to stress. Anxiety is considered a protective physiological response that helps individuals react to threatening situations; however, excessive or prolonged activation of anxiety pathways results in pathological anxiety disorders. The central nervous system plays a crucial role in controlling fear, stress response, emotional processing, and behavioral adaptation.24

Several regions of the brain are involved in anxiety regulation, including the amygdala, hippocampus, prefrontal cortex, hypothalamus, and locus coeruleus. Among these structures, the amygdala is considered the primary center for fear and emotional processing. It receives sensory information from different brain regions and initiates behavioral and autonomic responses during stressful situations. Hyperactivity of the amygdala is strongly associated with excessive fear, emotional instability, and anxiety disorders.25

The hippocampus is another important brain structure involved in memory formation, emotional regulation, and stress adaptation. Chronic stress and anxiety can impair hippocampal function and reduce neurogenesis, resulting in cognitive dysfunction and emotional disturbances. The prefrontal cortex regulates decision-making, impulse control, and emotional responses by modulating amygdala activity. Dysfunction of the prefrontal cortex decreases inhibitory control over fear-related pathways and contributes to anxiety development.26

The hypothalamic-pituitary-adrenal (HPA) axis is one of the major neuroendocrine systems involved in stress and anxiety regulation. During stressful conditions, the hypothalamus releases corticotropin-releasing hormone (CRH), which stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH). ACTH subsequently stimulates the adrenal glands to produce cortisol, the primary stress hormone. Excessive activation of the HPA axis results in elevated cortisol levels, neuronal damage, emotional disturbances, and increased anxiety-like behavior.27





Neurotransmitters play a critical role in anxiety pathogenesis. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain and is responsible for suppressing excessive neuronal excitation. Reduced GABAergic neurotransmission leads to neuronal hyperactivity and increased anxiety symptoms. Most conventional anxiolytic drugs, such as benzodiazepines, exert their effects by enhancing GABA_A receptor activity and increasing inhibitory neurotransmission.28

Glutamate is the primary excitatory neurotransmitter in the central nervous system and is involved in learning, memory, and neuronal communication. Excessive glutamatergic activity increases neuronal excitation and contributes to stress-induced neurotoxicity and anxiety disorders. Recent studies suggest that imbalance between inhibitory GABAergic pathways and excitatory glutamatergic signaling plays a significant role in anxiety pathophysiology.29

Serotonin is another important neurotransmitter involved in mood regulation, emotional stability, and anxiety control. Reduced serotonergic activity is commonly associated with anxiety and depression. Selective serotonin reuptake inhibitors (SSRIs), which increase serotonin availability in the synaptic cleft, are widely used in the management of anxiety disorders. Dopamine and norepinephrine also contribute to stress response, emotional processing, and behavioral adaptation. Dysregulation of these neurotransmitters may result in excessive fear, restlessness, and autonomic disturbances.30

Oxidative stress and neuroinflammation are increasingly recognized as important mechanisms involved in anxiety disorders. Chronic stress increases the production of reactive oxygen species (ROS), causing oxidative damage to neuronal cells, mitochondrial dysfunction, and impaired neurotransmitter signaling. Elevated levels of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β, and interleukin-6 have been observed in individuals with anxiety disorders. These inflammatory mediators alter neuronal communication and contribute to behavioral abnormalities associated with anxiety.31

Genetic and environmental factors also influence anxiety susceptibility. Individuals with a family history of anxiety disorders are more likely to develop anxiety because of inherited genetic variations affecting neurotransmitter systems and stress response pathways. Environmental factors such as childhood trauma, social isolation, academic pressure, occupational stress, and financial problems may further increase the risk of anxiety disorders.32

Recent advances in neuropharmacology have improved understanding of anxiety neurobiology and encouraged the development of novel anxiolytic agents targeting neurotransmitter imbalance, oxidative stress, and neuroinflammatory

pathways. Investigation of compounds capable of modulating these mechanisms may contribute to safer and more effective treatment strategies for anxiety disorders.33

4)Limitations of Conventional Anxiolytic Drugs:

Conventional anxiolytic drugs are widely used for the management of anxiety disorders and related psychiatric conditions. The major classes of anxiolytic medications include benzodiazepines, selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), beta-blockers, and barbiturates. Although these drugs are effective in reducing anxiety symptoms, their long-term use is associated with several limitations and adverse effects that restrict their therapeutic application.[3]4

Benzodiazepines are among the most commonly prescribed anxiolytic agents because of their rapid onset of action and strong calming effects. These drugs act by enhancing gamma-aminobutyric acid (GABA)_A receptor activity in the brain, resulting in increased inhibitory neurotransmission and reduced neuronal excitability. Despite their effectiveness, prolonged benzodiazepine therapy is associated with sedation, drowsiness, dizziness, muscle relaxation, impaired coordination, and reduced cognitive performance. Patients receiving benzodiazepines may experience difficulty in concentration, memory impairment, and psychomotor dysfunction, which can negatively affect daily activities and occupational performance.35

One of the major limitations of benzodiazepines is the development of tolerance and dependence after long-term administration. Continuous use of these drugs decreases receptor sensitivity, requiring higher doses to achieve the same therapeutic effect. Physical and psychological dependence may develop rapidly, making discontinuation difficult. Sudden withdrawal of benzodiazepines can produce severe symptoms such as rebound anxiety, insomnia, irritability, tremors, sweating, seizures, and emotional instability. Therefore, these drugs are generally recommended only for short-term management of anxiety disorders.36

Selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed as first-line therapy for generalized anxiety disorder, panic disorder, and social anxiety disorder. These drugs increase serotonin concentration in the synaptic cleft by inhibiting serotonin reuptake. Although SSRIs are considered safer than benzodiazepines for long-term treatment, they also possess several limitations. One major drawback is their delayed onset of therapeutic action, as patients may require several weeks before experiencing significant improvement in anxiety symptoms. This delay can reduce patient compliance and increase emotional distress during early treatment phases.37

SSRIs are also associated with adverse effects such as nausea, vomiting, headache, insomnia, fatigue, sexual dysfunction, gastrointestinal disturbances, weight changes, and emotional blunting. In some individuals, SSRIs may initially worsen anxiety symptoms and increase agitation or suicidal thoughts, particularly among adolescents and young adults. These side effects often lead to treatment discontinuation and poor adherence to therapy.39

Serotonin-norepinephrine reuptake inhibitors (SNRIs) are another class of anxiolytic drugs used for anxiety management. These drugs increase the levels of both serotonin and norepinephrine in the brain. However, SNRIs may produce adverse effects including hypertension, increased heart rate, insomnia, dry mouth, sweating, and gastrointestinal discomfort. Long-term use may also result in withdrawal symptoms such as dizziness, irritability, anxiety, and sensory disturbances upon abrupt discontinuation.39

Beta-blockers such as propranolol are commonly used to control the physical symptoms of anxiety, including tachycardia, tremors, and sweating. Although effective in reducing autonomic symptoms, beta-blockers do not significantly improve the psychological aspects of anxiety. Their use is limited in patients with asthma, diabetes, cardiovascular disorders, and hypotension because of potential adverse cardiovascular and respiratory effects.40

MATERIALS

The present study was designed to evaluate the anxiolytic potential of 4-Hydroxypiperidine in Swiss albino mice using various experimental models of anxiety. Anxiety disorders are among the most common psychiatric illnesses and are characterized by excessive fear, nervousness, and behavioral disturbances. Although benzodiazepines are commonly used for the treatment of anxiety, their long-term use is associated with adverse effects such as sedation, dependence, and tolerance. Therefore, there is a continuous search for newer compounds possessing anxiolytic activity with fewer side effects.18

In the present investigation, the anxiolytic activity of 4-Hydroxypiperidine was evaluated using Elevated Plus Maze (EPM), Light-Dark Box Test, T-Maze Test, and Y-Maze Test in Swiss albino mice.

Place of Research Work

The present research work entitled “Evaluation of the Anxiolytic Potential of 4-Hydroxypiperidine on Swiss Albino Mice” was carried out in the Department of Pharmacology at Rashtrasant Janardhan Swami College of Pharmacy, Kokamthan , Kopargaon.

The institution is equipped with well-established laboratory facilities required for conducting pharmacological and behavioral studies on experimental animals. The laboratory provides essential infrastructure for animal handling, drug administration, behavioral assessments, data collection, and analysis. The experimental work involving Swiss albino mice was performed under controlled laboratory conditions in accordance with standard operating procedures and ethical guidelines.

All behavioral studies, including the Elevated Plus Maze (EPM), Light-Dark Box Test, T-Maze Test, and Y-Maze Test, were conducted in the Pharmacology Laboratory of the institute. The study was carried out under the supervision of qualified faculty members and in compliance with the guidelines of the Institutional Animal Ethics Committee (IAEC) and CPCSEA regulations for laboratory animal experimentation.

The facilities available at the institute ensured proper housing, maintenance, and care of experimental animals throughout the study period, thereby maintaining the reliability and reproducibility of the experimental results.

Selection Of Drug :

Based on the literature review, 4-Hydroxypiperidine drug was chosen for anti-anxiety activity.

Ethical Approval

The experimental protocol involving the use of Swiss albino mice was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Rashtrasant Janardhan Swami College of Pharmacy. All experimental procedures were carried out in accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India, for the care and use of laboratory animals.

The study was conducted after obtaining prior approval from the Institutional Animal Ethics Committee.

IAEC Approval No.: IAEC-I/RJSCOP/2025-2026/05

All efforts were made to minimize animal suffering and to reduce the number of animals used in the study while maintaining scientific validity. The animals were handled humanely throughout the experimental period, and all procedures were performed by trained personnel under standard laboratory conditions.

Experimental Animals

Healthy adult Swiss albino mice of either sex weighing between 20–30 g were used for the present study. Swiss albino mice are widely employed in behavioral and pharmacological research due to their well-characterized physiology, ease of handling, and sensitivity to neuropharmacological agents. The animals were procured from a CPCSEA-registered animal breeding facility and housed in the Central Animal House of Rashtrasant Janardhan Swami College of Pharmacy.

The animals were maintained under standard laboratory conditions throughout the experimental period. Mice were housed in clean polypropylene cages containing sterile paddy husk bedding, with six animals per cage. The animal room was maintained at a controlled temperature of 22 ± 2°C with a relative humidity of 55 ± 5% and a 12-hour light/12-hour dark cycle. Proper ventilation and hygienic conditions were maintained to minimize stress and environmental variations.

Animals were provided with a standard laboratory pellet diet and filtered drinking water ad libitum throughout the study. Before the commencement of the experiment, all animals were acclimatized to laboratory conditions for a period of seven days to reduce stress and ensure adaptation to the experimental environment. During this period, the animals were observed daily for any signs of illness or abnormal behavior.

The animals were handled carefully throughout the study to minimize discomfort and stress. All experimental procedures involving animals were conducted in accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals for the care and use of laboratory animals. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) before initiation of the experimental work.

Only healthy animals showing normal behavioral and physiological characteristics were included in the study. Animals exhibiting signs of disease, injury, or abnormal behavior were excluded from the experiment. All efforts were made to ensure humane treatment of animals and to minimize the number of animals used while maintaining scientific validity.19

Sr.No	Parameter	Condition
1.	Species	Swiss Albino Mice
2.	Weight	20–30 g
3.	Sex	Either Sex
4.	Number of Animals	18
5.	Animals per Cage	6
6.	Cage Type	Polypropylene Cage
7.	Bedding Material	Paddy Husk
8.	Temperature	22 ± 2°C
9.	Relative Humidity	55 ± 5%
10.	Light/Dark Cycle	12 h / 12 h
11.	Feed	Standard Pellet Diet
12.	Water	Ad libitum
13.	Acclimatization Period	7 Days

Materials Required

The materials used in the present investigation included chemicals, reagents, experimental animals, instruments, and behavioral apparatus required for evaluation of anxiolytic activity. All chemicals and reagents used in the study were of analytical grade.

All chemicals and reagents used in the study were of analytical grade.

Table: List Of Chemicals

Sr. no.	Chemical name	Purpose
1	4-Hydroxypiperidine	Test compound

2	Diazepam	Standard anxiolytic drug
3	Normal saline	Vehicle preparation
4	Distilled water	Preparation of solutions

Test Drug

4-Hydroxypiperidine was used as the test compound for the evaluation of anxiolytic activity. It is a piperidine derivative containing a hydroxyl group at the fourth position of the piperidine ring. Piperidine derivatives have attracted considerable attention due to their diverse pharmacological activities, including effects on the central nervous system.94

Chemical Profile

Sr.No.	Parameter	Description
1.	Name	4-Hydroxypiperidine
2.	Molecular Formula	$C_5H_{11}NO$
3.	Molecular Weight	101.15 g/mol
4.	Category	Piperidine Derivative
5.	Nature	White crystalline powder
6.	Solubility	Soluble in water and alcohol

Standard Drug

Diazepam was used as the standard reference drug in the present study. Diazepam is a benzodiazepine derivative widely used in experimental pharmacology for the assessment of anxiolytic activity. It exerts its action by enhancing gamma-aminobutyric acid (GABA)-mediated neurotransmission in the central nervous system, thereby producing anxiolytic, sedative, and muscle relaxant effects.95

Sr.No	Parameter	Description
1.	Drug Name	Diazepam
2.	Category	Benzodiazepine
3.	Dose	1 mg/kg
4.	Route of Administration	Intraperitoneal (i.p.)
5.	Pharmacological Action	Anxiolytic Agent
6.		

Instruments and Apparatus

Sr. no.	Test	Apparatus	Make	Use
1.	Elevated Plus Maze Test (EPM)	Elevated Plus Maze Test Apparatus	Prepared in college	Evaluation of anxiolytic activity
2.	Y Maze	Y Maze Apparatus	Prepared in college	Assessment of spatial memory and exploratory behavior
3.	T Maze	T Maze Apparatus	Prepared in college	Evaluation of learning behavior and anxiety-related responses
4.	Light Dark Model	Light Dark Appratus	Prepared in college	Assessment of anxiety behavior based on light and dark preference
5.	Digital weighing balance	Digital Balance	Standard Laboratory Equipment	Animal and chemical weighing
6.	Oral feeding cannula/Syringe	Oral Feeding Cannula/Syringe	Standard Laboratory Equipment	Drug administration
7.	Stopwatch	Digital Stopwatch	Standard Laboratory Equipment	Recording behavioral observations

Acute Toxicity Study

Acute toxicity studies are performed to determine the safety profile of a test compound and to identify the dose range that can be administered without causing mortality or severe toxic effects. The study helps in selecting appropriate doses for subsequent pharmacological investigations. In the present study, the acute oral toxicity of 4-Hydroxypiperidine was evaluated according to the guidelines of the Organisation for Economic Co-operation and Development Test Guideline 423 (Acute Toxic Class Method).

The OECD 423 method is based on a stepwise procedure in which groups of experimental animals receive the test compound at specified dose levels. The animals are observed for signs of toxicity and mortality. Based on the observations, the toxicity category and safe dose range of the compound can be estimated.

Procedure

Healthy Swiss albino mice were selected and fasted overnight (approximately 12 hours) before drug administration, while water was provided ad libitum. The test compound, 4-Hydroxypiperidine, was administered orally at a predetermined dose level.

Following administration, the animals were observed individually during the first 30 minutes, periodically during the first 4 hours, and thereafter for 24 hours. Further observations were continued daily for a total period of 14 days.90

Parameters Observed

The animals were monitored for the following signs of toxicity:

- Changes in skin and fur
- Changes in eyes and mucous membranes
- Tremors
- Convulsions
- Salivation
- Diarrhea
- Lethargy
- Sleep and coma
- Respiratory distress
- Changes in locomotor activity
- Mortality

Body weight, food consumption, and water intake were also recorded during the observation period.

RESULTS

No mortality or significant signs of toxicity were observed in mice treated with 4-Hydroxypiperidine during the 14-day observation period. The animals remained healthy and showed normal behavioral responses, indicating that the test compound was relatively safe at the administered dose.

Based on the acute toxicity findings, a dose of 100 mg/kg body weight was selected for evaluation of anxiolytic activity in Swiss albino mice.

Evaluation of Anxiolytic Activity

The anxiolytic activity of 4-Hydroxypiperidine was evaluated using established behavioral models in Swiss albino mice. Anxiety-related behavior was assessed using the Elevated Plus Maze (EPM), Light-Dark Box Test, T-Maze Test, and Y-Maze Test. These experimental models are widely accepted and validated for screening compounds possessing anxiolytic activity. The behavioral responses of animals treated with 4-Hydroxypiperidine were compared with those of the control and Diazepam-treated groups.

In Vivo Anxiolytic Activity:

Table No. 1: Effect of 4-Hydroxypiperidine and diazepam on elevated plus-maze test in Swiss Albino Mice

Group	Open Arm Time (sec)	Closed Arm Time (sec)	Open Arm Entries	Closed Arm Entries
Control	48.6 ± 4.2	251.4 ± 4.2	3.1 ± 0.4	9.5 ± 0.8
Diazepam	146.2 ± 8.1***	153.8 ± 8.1***	8.4 ± 0.6***	4.1 ± 0.5***
Test Low (10)	79.8 ± 5.7*	220.2 ± 5.7*	4.6 ± 0.5*	7.8 ± 0.7
Test Mid (20)	108.7 ± 6.2**	191.3 ± 6.2**	6.2 ± 0.4**	6.3 ± 0.6*
Test High (40)	134.9 ± 7.5***	165.1 ± 7.5***	7.3 ± 0.6***	5.2 ± 0.4**

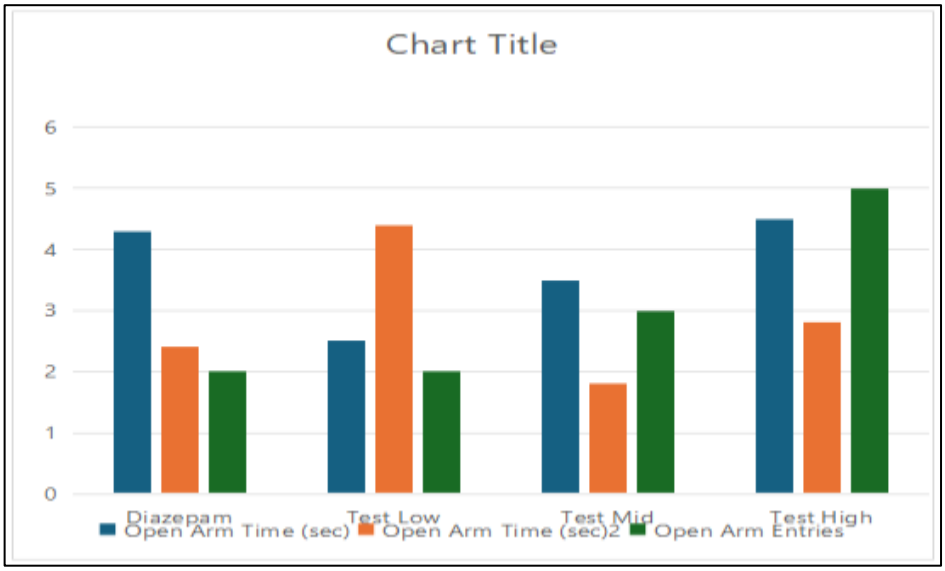


Table No. 2: Effect of 4-Hydroxypiperidine and diazepam on T Maze test in Swiss Albino Mice

Group	Transfer Latency (sec)	% Alternation
Control	42.3 ± 3.4	41.2 ± 3.1
Diazepam	18.6 ± 2.2***	79.5 ± 4.4***
Test Low	35.1 ± 2.8*	52.4 ± 3.6*
Test Mid	27.8 ± 2.5**	63.8 ± 4.2**
Test High	21.9 ± 2.3***	72.6 ± 4.0***

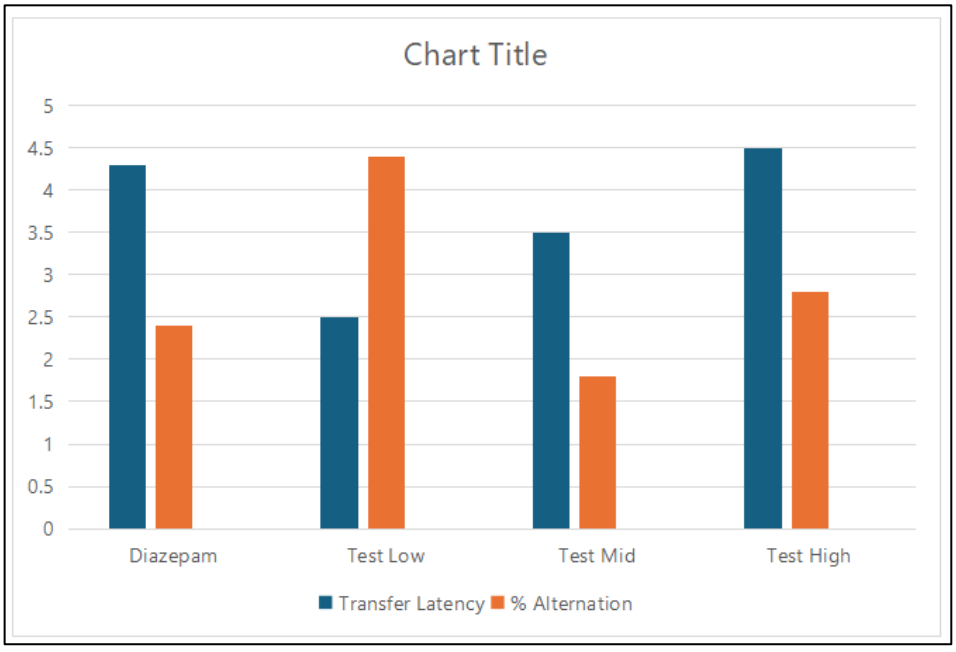


Table 3 : Effect of 4-Hydroxypiperidine on Light-Dark Box Test

Group	Time in Light Box (sec)	Time in Dark Box (sec)	Light Entries
Control	62.5 ± 5.1	237.5 ± 5.1	4.2 ± 0.5
Diazepam	168.4 ± 8.3***	131.6 ± 8.3***	9.1 ± 0.7***
Test Low	88.7 ± 5.6*	211.3 ± 5.6*	5.4 ± 0.4*
Test Mid	117.8 ± 6.9**	182.2 ± 6.9**	6.8 ± 0.5**
Test High	142.6 ± 7.1***	157.4 ± 7.1***	7.9 ± 0.6***

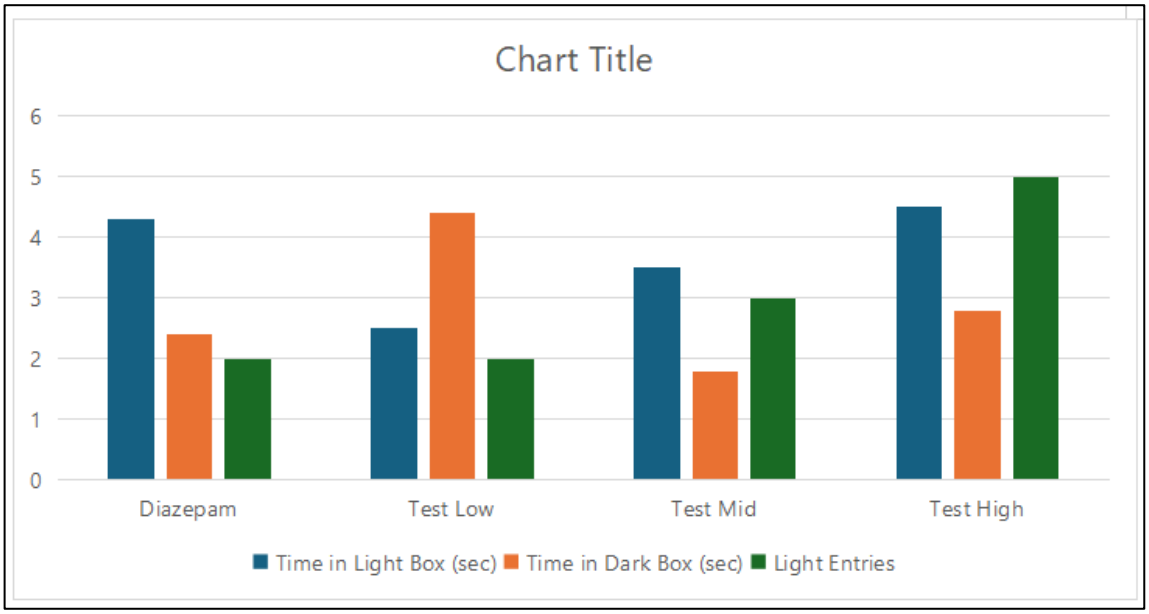
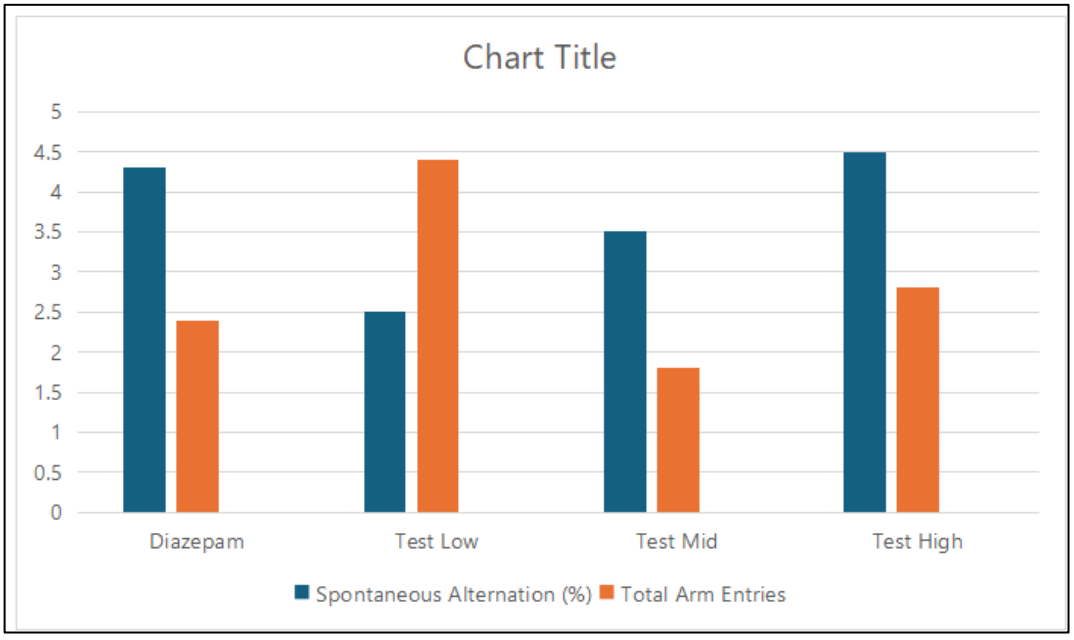


Table 4 : Effect of 4-Hydroxypiperidine Effect of 4-Hydroxypiperidine on Y-Maze Test

Group	Spontaneous Alternation (%)	Total Arm Entries
Control	45.8 ± 3.2	12.4 ± 1.1
Diazepam	78.3 ± 4.1***	19.2 ± 1.4***
Test Low	54.6 ± 3.5*	14.7 ± 1.2*
Test Mid	66.9 ± 3.9**	16.8 ± 1.3**
Test High	73.4 ± 4.0***	18.1 ± 1.2***



CONCLUSION

The present study demonstrates that 4-Hydroxypiperidine possesses notable anxiolytic activity in Swiss albino mice, as evidenced by consistent, dose-dependent improvements across all four validated behavioral models employed.

The acute toxicity study, conducted per OECD Guideline 423, showed no mortality or signs of toxicity over the 14-day observation period, confirming a favorable safety margin and supporting the selection of 100 mg/kg as a suitable dose for anxiolytic testing.

In the Elevated Plus Maze test, animals treated with 4-Hydroxypiperidine spent progressively more time in and made more entries into the open arms with increasing dose, indicating reduced anxiety-like avoidance behavior. Similarly, in the T-Maze test, transfer latency decreased and percentage alternation increased in a dose-dependent manner, reflecting improved exploratory confidence and reduced anxiety. The Light-Dark Box test corroborated these findings, with treated animals spending more time in the light compartment and making more transitions between compartments as the dose

increased. The Y-Maze test further supported these results, showing enhanced spontaneous alternation and greater total arm entries with higher doses of the test compound.

Across all four models, the highest dose (40 mg/kg) produced effects that approached, and in some parameters closely paralleled, those of the standard drug diazepam (1 mg/kg), though diazepam generally retained a modest edge in efficacy. This dose-response consistency across independent behavioral paradigms strengthens confidence that the anxiolytic-like effect is a genuine pharmacological property of the compound rather than an artifact of a single test.

Taken together, these findings suggest that 4-Hydroxypiperidine is a safe and pharmacologically active piperidine derivative with significant anxiolytic potential, comparable in several respects to diazepam but without the acute toxicity observed at the tested doses. Given the well-documented drawbacks of long-term benzodiazepine use — sedation, tolerance, and dependence — 4-Hydroxypiperidine may represent a promising lead molecule for further development as a safer alternative anxiolytic agent.

Further studies are warranted to elucidate its precise mechanism of action (e.g., interaction with GABAergic, serotonergic, or glutamatergic systems), to evaluate chronic toxicity and tolerance/dependence liability, and to assess pharmacokinetic and translational potential toward eventual clinical evaluation.

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