University of Mosul

College of Veterinary Medicine

Assessment of Neuro-Behavioral Effects of Scopolamine in Male Mice

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MSc/Thesis

Veterinary Medicine / Veterinary Pharmacology and Toxicology

Supervised by
Assist. Professor
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1446 A.H **2024 A.D.**

Assessment of Neuro-Behavioral Effects of Scopolamine in Male Mice

A Thesis
Submitted By

Maysam Mohsen Abbas Qanbar Al Mustafa

To
The Council of the College of Veterinary Medicine
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In
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For the Degree of Master of Science
In
Veterinary Medicine / Veterinary Pharmacology and Toxicology

Supervised by
Assist. Professor
Dr. Yamama Zuher Saleh Al-Abdaly

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بسم الله الرحمن الرحيم

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بِحَمْدِهِ ﴾

صدق الله العظيم

سورة الفرقان الآية 58

Supervisor Certification

I certify that this thesis entitled **(Assessment of Neuro-Behavioral Effects of Scopolamine in Male Mice)** was prepared under my supervision at the College of Veterinary Medicine / University of Mosul, as a partial fulfillment of the requirements for the degree of M.Sc. in Veterinary Medicine/ Veterinary Pharmacology and Toxicology.

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Date: / /2024

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We, the members of the Evaluation and Discussion Committee, have reviewed this thesis and examined the student **Maysam Mohsen Abbas** in its contents on 25/9/2024, and we hereby certify that she deserves the degree of M. Sc. in Veterinary Medicine / Veterinary Pharmacology and Toxicology.

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Prof. Dr. Raad Abdulghany Alsanjary
Assistant Dean for Scientific Affairs
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Dean of the College
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Abstract

To study the acute and sub-acute neuro-behavioral toxic effects of scopolamine by evaluating higher brain functions using the open field test and social interaction test in mice, evaluating the autonomic nervous system functions using swimming tests and light-dark tests, besides evaluating the cognitive functions using the negative geotaxis test and hole-board test. Additionally, to investigate some of the underlying mechanisms of the recorded neuro-behavioral effects on mice by measuring certain neurotransmitters which are serotonin (SER), acetylcholine (Ach), and the enzyme of Catechol-O-methyltransferase (COMT).

This study utilized approximately 85-90 male Swiss white mice. The mice weighed between 25-35 grams and were aged around 2 months. The median lethal dose (LD_{50}) of scopolamine was determined intramuscularly.

The effect of time and dose on neuro-behavioral functions were studied through a series of experiments. The experiments included administering low repeated doses daily for two weeks and four weeks in the second and third experiments, respectively, measuring the effect of an acute dose 24 hours post-administration in the fourth experiment, and testing the cumulative effect of a single dose after five days in the fifth experiment. To confirm these results, single doses were administered twice weekly in the sixth experiment. Each experiment used 15 mice, divided into three random groups of five mice. A control group was injected with distilled water and two groups injected intramuscularly with scopolamine. The measured tests included locomotors activity and neuro-behavioral function (open field test, social interaction test, swimming test, negative geotaxis test, hole-board test, and light-dark test). At the end of each experiment, the animals were anesthetized, and blood was collected to

separate plasma for biochemical tests to measure neurotransmitters such as SER, Ach, and COMT enzyme.

The LD_{50} of scopolamine was 148 mg/kg. Neuro-behavioral tests showed increased rearing activity in the 2 mg/kg group and significantly increased in the 4 mg/kg group. Social interaction decreased in both doses. Autonomic nervous system tests showed decreased swimming scores, negative geotaxis time, and head dips. Time spent in the dark decreased in the 4 mg/kg group.

After 4 weeks of daily scopolamine doses, Scopolamine doses significantly increased rearing activity, decreased square crossings, social interaction, swimming scores, negative geotaxis time, head dips, and time spent in the dark after 4 weeks compared to control.

The results of the third experiment showed that administering a single high acute dose of scopolamine (20 mg/kg) The 20 mg/kg group showed increased rearing events, decreased squares crossed, and social interaction, decreased swimming scores, increased negative geotaxis time, and decreased head dips in hole-board tests, while time spent in the dark was significantly reduced.

When single doses of 2 and 4 mg/kg were administered, and the animals were left for 5 days before behavioral measurements, no significant effects of the doses were observed within this period. In the experiment where two low doses of scopolamine were administered weekly, there was no difference in the number of rearing events between the control group and the 2 mg/kg group. The 4 mg/kg group showed a significant increase in rearing activity, decreased square crossings, social interaction scores, swimming scores, and negative geotaxis time, but no significant difference in head dips or time spent in the dark.

Biochemical tests revealed that a single acute high dose of scopolamine led to elevated serotonin levels, reduced acetylcholine levels,

and decreased COMT activity after 24 hours. However, administering a single dose and measuring after 5 days showed no neurotransmitter alterations. Meanwhile, administering two doses per week induced significant changes in serotonin and acetylcholine levels, as well as COMT activity compared to control.

Both sub-acute doses of scopolamine (2 and 4 mg/kg) and the acute 20 mg/kg dose significantly impacted mouse neurobehavior, cognition, and autonomic nervous system responses compared to control. Higher doses generally exhibited more pronounced effects, though differences between doses were often insignificant. The data suggests scopolamine induces neuro-behavioral changes, cognitive impairment, and anxiety-related behaviors in mice. Administering scopolamine every other day for a week notably affected mouse neuro-behavior, with the higher dose (4 mg/kg) showing more pronounced effects on various parameters compared to the lower dose (2 mg/kg) and control. Biochemical tests revealed increased serotonin levels and decreased acetylcholine levels and COMT activity with both daily and acute scopolamine doses.

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List of Abbreviations

Abbreviations	Full name
Ach	Acetylcholine
ADHD	Attention deficit hyperactivity disorder
AUC	Area under the curve
C^{max}	Maximum concentration
COMT	Catechol O methyltransferase
GABA	Gamma aminobutyric acid
LD_{50}	Median lethal dose
SERT	Serotonin transporter
SSRIs	Selective serotonin reuptake inhibitors
T^{max}	Time maximal concentration

Chapter One:

Introduction

Chapter One

Introduction

Bioactive chemical compounds form the cornerstone of modern therapeutic advancements (Dable-Tupas *et al.,* 2023). Among these compounds, scopolamine holds a prominent position due to its diverse effects on the central nervous system, Scopolamine, a tropane alkaloid, has been traditionally utilized as an anesthetic and is currently employed in the treatment of various conditions, including nausea, vomiting, and Parkinson's disease (Kohnen-Johannsen *et al.,* 2019).

Scopolamine, also known as hyoscine, was isolated by Albert Ladenburg in 1880 and is considered one of the most potent hallucinogenic alkaloids found in plants (Oakeley, 2023).

It is a secondary metabolite that is relatively abundant in species like Datura and Hyoscyamus. Historically, scopolamine has been used since ancient times in its purified form for anesthesia and later as an anti-asthmatic and antihistamine. Multiple studies have suggested that scopolamine could be effective, in treating depression and anxiety due to its ability to cause amnesia and hallucinations (Pastore *et al.,* 2015). The primary benefit of using scopolamine in research is its ability to cause memory loss by blocking the system through binding to receptors. Researchers have extensively studied the effects of scopolamine, on memory in animal models (Prabhu *et al.,* 2020).

Scopolamine functions by connecting to brain receptors, which triggers the release of dopamine. Dopamine, a neurotransmitter for memory and learning processes is also affected by scopolamines binding. This interaction boosts dopamine activity potentially leading to

hallucinations and behaviors to those seen in schizophrenia (Kesby *et al.,* 2018).

Moreover, scopolamine is thought to interfere with receptors that normally inhibit activity. This interference could increase activity levels possibly resulting in hallucinations and behaviors resembling schizophrenia. Studies indicate that the relationship between Scopolamine and acetylcholinesterase is complex and can vary depending on factors such as animal species, dosage levels and methods of administration. For instance, research shows that scopolamine appears to influence the behavior of mice and rats (Amann *et al.,* 2010; Xu and Wong, 2018). There's still much to learn about how scopolamine and acetylcholinesterase're connected. However current studies shed light on how this medication can affect the brain and behavior. Scopolamines influence, on memory involves blocking acetylcholine, a neurotransmitter for memory formation (Raja, 2019). When scopolamine gets into the brain it competes with acetylcholine, hinders its receptors from working disrupting the crucial lock and key mechanism for memory formation (Chew, 2007). It is believed that the way scopolamine induces Alzheimer's like symptoms involves its impact on the hippocampus, which serves as the brains memory hub (Kose *et al.,* 2023). The process of memory formation includes stages such as encoding, storage (formation of long term memories) and retrieval. Scopolamine disrupts the encoding phase in the hippocampus preventing information from being stored where it should be (Lana, 2015). Studies suggest that how scopolamine affects behavior and neurology can vary based on factors, like animal species, dosage levels and route of administration. For instance, studies have demonstrated variations, in how scopolamine affects the behavior of mice and rats (Haider *et al.,* 2016).

Understanding the effects of scopolamine can offer insights into the mechanisms that contribute to psychological and behavioral disorders along with the neurological symptoms observed in animals. This understanding may pave the way for developing treatments for these disorders.

1-2: Study objective

1. To examine the prolonged toxic effects of scopolamine by:
    a. Assessing higher brain functions using open field and social interaction tests in mice.
    b. Evaluating autonomic nervous system functions through swimming and light dark tests.
    c. Testing cognitive functions using negative geotaxis and hole board assessments.
2. To explore the underlying mechanisms behind observed effects in mice by analyzing neurotransmitters, like serotonin, acetylcholine and COMT enzyme levels.

Chapter Two:

Review of Literatures

Chapter Two

Review of Literatures

2-1: Overview

Scopolamine, an alkaloid found in plants of Solanaceae family is chemically similar to the atropine and the hyoscyamine. It acts as an analog of acetylcholine, a neurotransmitter. Synthesized for the time in 1959 scopolamine works by blocking acetylcholine receptor in central nervous system and body. This leads to effects on the nervous system and cholinergic signaling both therapeutic and adverse (Decker *et al.,* 2004).

Recognizing its dosage related side effects scopolamine became the drug introduced commercially as the delivery system called Scopoderm TTS® in 1981 (Renner *et al.,* 2005).

Due to its properties research has explored scopolamine for medical uses. It is sanctioned for preventing the nausea and the vomiting linked to the motion sickness and the surgical procedures (Apfel *et al.,* 2010). Initially approved by the U.S. by the Food and the Drug Administration on December 31, 1979. It is currently accessible, in tablets and transdermal delivery formats (Zhong *et al.,* 2018).

2-2: Chemical structure: C17H21NO4

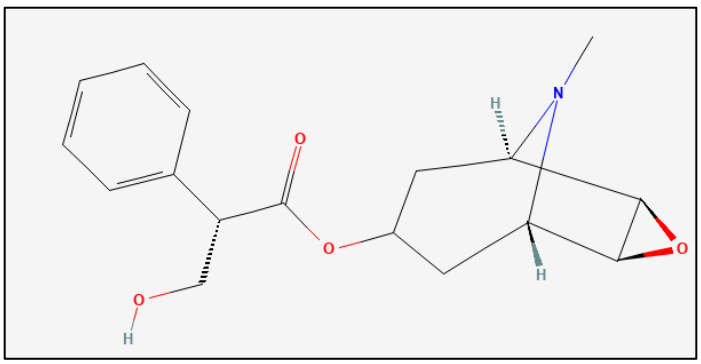

Figure 2-1: Chemical Structure (National Center for Biotechnology Information, 2024).

2-2-1: Synonyms for scopolamine

6,7-Epoxyhyoscyamine, Hyoscine, Scopolamine, Scopolamine Hydrobromide.

Figure 2-2: Plant of Datura species (the source of scopolamine) (Stoyanova, 2013).

2-2-2: Plant source of scopolamine and its toxicity

Intentional or unintentional poisoning caused by smoking Datura species and ingesting 4 to 5 grams of leaves has increased. One gram of scopolamine is enough to kill a child (equal to approximately 125 (in corn) found in (*Datura stramin*), which was discovered by German Albert Ladenburg in 1881 to cause hallucinations or severe poisoning. When the plant is young, the ratio of scopolamine to atropine is about 3:1; after flowering, this ratio reverses, with the amount of scopolamine changing with age, and under traditional, there has been a reasonable degree of knowledge of Datura, controlling the loss of individual seeds by approximately 0.1 million of atropine (Abdulraheem, 2022). Destructive dose (approximate lethal dose): Approximate lethal dose in adults is >10 mg atropine (atropine), or >2-4 mg scopolamine (scopolamine) (Prema, 2018).

2-2-3: Antidote

In the case of Datura or scopolamine poisoning, the hospital is immediately required to carry out treatment that primarily stops cleaning the stomach, anesthesia by benzodiazepine injection, and, as in all cases of anticholinergic poisoning, physostigmine can be given as an antidote if they occur in severe cases (Vigneshwaran, 2022).

2-3: Pharmacodynamics

Scopolamine works by competitively inhibiting muscarinic receptors, altering parasympathetic nervous system function and acting on smooth muscles that respond to acetylcholine but lack cholinergic innervation (Brown and Taylor, 2006). Scopolamine, when administered as a patch, is constantly released over three days and can be detected in urine for up to 108 hours (Gupta and Babu, 2013). Its use is not applicable to patients with closed-angle glaucoma and should be used cautiously to patients with open-angle glaucoma due to its tendency to elevate intraocular pressure (Stein *et al.,* 2024). Neurological and psychiatric effects, including exacerbated psychosis, seizures, seizure-like psychotic reactions, and cognitive impairment (Weiner *et al.,* 2012).

2-4: Mechanism of action

The most widely accepted and significant mechanism of action for selective serotonin reuptake inhibitors (SSRIs) is the inhibition of the serotonin transporter (SERT) located at the presynaptic terminal. SSRIs' drugs primary function is to block SERT, which increases synaptic levels of serotonin by up to sevenfold (Roberts *et al.,* 2020). This increase in 5-HT concentration subsequently activates a number of postsynaptic 5-HT receptors in distinct brain areas, leading to altered serotonin signaling, which is believed to alleviate depressive states. Furthermore, high

extracellular (serotonin) 5-HT concentrations cause a negative response mechanism relating to 5-HT1A autoreceptor, which regularize 5-HT levels in synaptic cleft (Artigas, 2013). SSRIs are widely used to treat major depressive disorder and other neurological and psychiatric conditions, obsessive-compulsive disorders, panic disorders, alcoholism, obesity, migraines, and chronic pain (Dresler *et al.,* 2019).

Sertraline and paroxetine are used to treat panic attacks, obsessive-compulsive disorder, post-traumatic stress disorder, social anxiety disorder, and premenstrual dysphoric disorder, a severe form of premenstrual syndrome (Chandraiah, 2018). Fluoxetine helps eating disorders and slow-channel congenital myasthenic syndromes (Dejthevaporn *et al.,* 2022).

The unifying symptom in these circumstances is a higher amount of Ach than in normal animals (Chandraiah, 2018; Cunnane *et al.,* 2020). This structured overview provides a scientific and comprehensive understanding of scopolamine, including its chemical nature, pharmacodynamics, and therapeutic applications, supporting further exploration of its neuro-behavioral effects (Grassi and Fucile, 2014; Volgin *et al.,* 2018).

2-5: Toxicokinetics

The way scopolamine is processed in the body differs depending on how it's taken. When 0.5 mg of scopolamine is given orally to individuals it leads to a peak concentration (Cmax) of 0.54 ± 0.1 ng/ml after, about 23.5 ± 8.2 minutes with an area under the curve (AUC) of 50.8 ± 1.76 ng*min/mL; however, its absolute bioavailability is around 13 ± 1% due to initial metabolism, in the liver (Chen, 2023). On the hand when the same dose of scopolamine is administered through infusion over a span of 15 minutes it results in a higher Cmax of approximately 5.01 ± 0.42 ng/mL

within just about 5 minutes and an AUC of 369.5 ± 2.3 ng*min/mL. (Renner *et al.,* 2005).

The AUC of 158.2 ng*min/mL was obtained from subcutaneous injection of 0.4 mg scopolamine, which produced a Cmax of 3.28 ng/mL, a Tmax of 14.7 minutes, and an AUC of 158.2 ng*min/mL. Similarly, 0.5 mg of scopolamine administered intramuscularly produced a Cmax of 0.98 ± 0.18 ng/mL, a Tmax of 18.5 ± 4.8 minutes, and an AUC of 81.4 ± 11.3 ng*min/mL (Chen, 2023). Nasal administration of 0.4 mg scopolamine demonstrated rapidity, with a Cmax of 1.69 ± 0.24 ng/mL, a Tmax of 2.2 ± 3 minutes, and an AUC of 166 ± 22 ng*min/mL. Additionally, nasal scopolamine demonstrated superior bioavailability compared to oral scopolamine at 84± 12% (Chen, 2023).

The transdermal patch was created to provide therapeutic plasma concentrations over an extended period of time due to dose-dependent adverse effects. Following application of the patch, scopolanide can be detected after four hours and reaches its maximal concentration (Cmax) after twenty-four hours. Total levels of free and conjugated scopolamine exceed 355 picograms/mL, with an average plasma concentration of 87 picograms/mL (Mahee, 2023).

2-6: Volume of distribution

Scopolamine's distribution volume is not clearly established. Shipley *et al.* (1994) found that a 15-minute intravenous infusion of 0.5 mg scopolamine produced a volume of distribution 141.4 ± 1.7 L\kg.

2-7: Binding to plasma protein

In humans, scopolamine may attach to plasma proteins reversibly. In rats, scopolamine binds to plasma proteins at a comparatively low rate of 10% (Chen, 2023).

2-8: Metabolism

Scopolamine metabolism in humans is poorly understood, despite the discovery of many metabolites in animal studies. The enzyme responsible for scopolamine metabolism is unknown, however oxidative demethylation linked to CYP3A4 activity is proposed (Manna *et al.,* 2020). The contemporaneous intake of grapefruit juice dramatically affected scopolamine pharmacokinetics, showing that CYP3A4 is responsible for at least some of the oxidative demethylation (Trenaman *et al.,* 2021).

2-9: Route of elimination

After oral treatment, roughly 2.6% of the unmodified scopolamine is excreted in urine. In comparison, the transdermal patch approach recovers less than 10% of the whole dose in urine over 108 hours, either as intact scopolamine or metabolites. Less than 5% of the whole dose is retrieved intact (Gupta and Babu, 2013).

2-10: Half-life

Scopolamine plasma concentrations decrease linearly with a half-life of 9.6 hours after removing the transdermal patch system (Wu *et al.,* 2015; Nigg and Beier, 2019). The half-life of scopolamine varies depending on the route of administration, with intravenous, oral, and intramuscular administration having similar half-lives of 68.8 ± 1.0, 63.8 ± 1.4, and 69.3 ± 8.1 minutes, respectively.

2-11: Clearance

An intravenous infusion of 0.50 mg scopolamine resulted in a clearance rate of 81.3 ± 1.54 L/hour, but subcutaneous therapy produced a lower clearance rate of 0.15-0.18 L/hour (Kretzing *et al.,* 2011). After administering 0.50 mg of scopolamine the clearance rate was measured at 81.3 ± 1.54 L/hour whereas the subcutaneous method showed a clearance rate ranging from 0.15 to 0.18 L/hour (Kretzing *et al.,* 2011).

2-12: Use of scopolamine in animals

To prevent animals from feeling unwell and vomiting scopolamine is administered to them during motion sickness. while they are under anesthesia for surgery (Renner *et al.,* 2005). When animals suffer from stomach problems such as nausea, vomiting or diarrhea scopolamine is employed to alleviate their symptoms (Silveira *et al.,* 2019). Additionally, scopolamine is used to animals to manage movement disorders, like Parkinsons disease (Chen *et al.,* 2022). Scopolamine is also applied in animals for pest control purposes targeting rodents and insects (Imam *et al.,* 2018).

Examples of how scopolamine's utilized in animals include:

- Helping cats and dogs avoid feeling sick and throwing up during air travel.
- Treating diarrhea caused by stomach inflammation in cats and dogs.
- Managing movement disorders in horses.
- Controlling rodents on farms.

2-13: Toxicity

A scopolamine overdose can include lethargy, drowsiness, coma, disorientation, agitation, hallucination, seizure, visual difficulty, dry skin, dry mouth, reduced bowel sounds, urine retention, tachycardia,

hypertension, and supraventricular arrhythmias. In certain cases, overdose symptoms may resemble those of withdrawal after cessation. However, withdrawal symptoms such as bradycardia, headache, nausea, stomach cramps, and sweating can help differentiate between these choices (Ebert, 2013).

For the use of high doses of scopolamine, the optimal time to take behavioral measurements post-treatment is after 48 hours. This is because scopolamine is a long-Acting drug and takes longer to reach its peak levels in the brain. Additionally, a high dose of the drug may take more time to exert its full effects (Tavares, 2022).

- It is important to note that scopolamine can be toxic to animals and should be used with caution. Potential side effects in animals include:
  - Vertigo
  - Sleepiness
  - Xerostomia
  - Vision problems
  - Costiveness
  - Urination problems
  - Difficulty breathing
  - Hallucinations
  - Confusion

In some cases, scopolamine can be fatal to animals (Boden and Andrews, 2017).

2-14: Neurotransmitters influencing neural behavior and motor activity

2-14-1: Serotonin

Serotonin (5-HT) is a key neurotransmitter in regulating mood and anxiety. Decreasing in serotonin levels in the brain are linked with increased feelings of anxiety and depression. Medications such as selective serotonin reuptake inhibitors (SSRIs) rise serotonin and are used to treat anxiety disorders (Pourhamzeh *et al.,* 2022).

2-14-2: Norepinephrine

Norepinephrine (or noradrenaline) is involved in the stress response and alertness. High levels of norepinephrine can lead to increased anxiety. Norepinephrine systems play a role in fight-or-flight responses, which are directly associated with feelings of anxiety (Sugama and Kakinuma, 2021).

2-14-3: Dopamine

Dopamine is related to reward and motivation but can also play a role in anxiety. An imbalance in dopamine levels can affect anxiety and social behavior Certain areas of the brain that rely on dopamine, like the tegmental area (VTA) interact with regions linked to anxiety, such as the amygdala (Zarrindast and Khakpai, 2015).

2-14-4: Gamma aminobutyric acid (GABA)

Gamma aminobutyric acid (GABA) serves as the neurotransmitter in the brain playing a vital role in reducing neuronal excitability and easing anxiety. Drugs that boost activity like benzodiazepines are utilized in anxiety treatment for their ability to soothe neural activity (Murala *et al.,* 2022).

2-14-5: Glutamate

Glutamate acts as the excitatory neurotransmitter in the brain. Heightened glutamate levels can be connected with increased anxiety. Maintaining a balance between glutamate and GABA is crucial for regulating mood stability and managing anxiety. Through delving into these neurotransmitters, researchers can gain insights into the mechanisms at play in anxiety and work towards developing new treatments for related disorders (Murala *et al.,* 2022).

2-14-6: Acetylcholine

Acetylcholine plays a role as a neurotransmitter involved in bodily functions such as movement, learning and memory. It is predominantly present in both the central and peripheral systems. Acetylcholine is essential, for functions including; Movement; Acetylcholine triggers muscle contraction that facilitates movement.

Learning and Memory; Acetylcholine is important, for memory and learning. It also helps in controlling hormone secretion, heart rate, blood pressure intestinal movements and the release of fluids according to Klinkenberg *et al.* (2011).

2-15: Acetylcholine related conditions

In the past, scopolamine was utilized as a remedy, for drug addiction like heroin and cocaine. Patients received doses of scopolamine until they reached a state of delirium. This therapy spanned two to three days after which pilocarpine was administered. Following recuperation patients no longer felt the urge for the drug they were dependent on (Brust, 2014).

2-16: COMT enzyme

The COMT, abbreviation of Catechol O methyltransferase is essential, for breaking down Catecholamine like dopamine, norepinephrine and epinephrine. Lower COMT activity can have an effect on the levels of these neurotransmitters, which in turn can influence behavior significantly (Semenova et *al.,* 2017).

2-16-1: Roles of COMT

Methylation of Catecholamines; COMT methylates catecholamines, which lowers their effects and aids in their removal, from the body. Control of Neurotransmitter Levels; Through methylating of catecholamines, COMT assists in managing their levels in the brain. Influence on cognitive and behavioral processes; It is thought that COMT contributes to behavioral functions, like memory, learning and emotions (Kiss and Soares-da-Silva, 2014).

2-16-2: Impact of COMT Val/Met variants on catecholamine breakdown and brain function:

- **COMT Val:** Refers to the allele containing the amino acid Valine at position 158 (or 108 in the membrane-bound form) of the protein. This form of the enzyme is more efficient at breaking down catecholamines (such as dopamine) at a faster rate. Therefore, individuals with this variant tend to break down neurotransmitters more quickly, leading to lower dopamine levels in the brain (Bastos *et al.,* 2017).
- **COMT Met:** Refers to the allele containing the amino acid Methionine at the same position. This form of the enzyme is less efficient at breaking down catecholamines, meaning that neurotransmitters like dopamine persist longer in the brain. This

could lead to psychological and behavioral effects due to the prolonged presence of dopamine (Martens, 2019).

- These differences may affect processes like attention, learning, and memory, as dopamine levels in areas like the prefrontal cortex are important for these functions.

2-16-3: The link, between COMT and psychological conditions

- **Attention deficit hyperactivity disorder (ADHD):** People with the COMT Val variation may have a likelihood of ADHD.
- **Schizophrenia:** those with the COMT Met variation may face an increased risk of developing schizophrenia.
- **Bipolar disorder**: The COMT Val variation could be linked to disorder (Yadav *et al.,* 2021). COMT, an enzyme found in the body, plays a role in breaking down and modifying various chemicals essential for nervous system function like dopamine, epinephrine and norepinephrine. These chemicals play a role in controlling transmission, mood and responses to environmental stressors (Osuch *et al.*, 2004).

The impact of COMT on activity is related to its breakdown of dopamine, which helps regulate dopamine levels in the brain (Witte and Flöel, 2012). This influence can affect mental characteristics such as:

1. **Stress:** The connection between dopamine levels and mood/stress responses can influence how individuals respond to environmental stressors. Reduced COMT activity may lead to increased dopamine accumulation potentially causing anxiety or stress (Syed and Nemeroff, 2017).
2. Dopamine is known to have an impact on learning and memory functions. Fluctuations in dopamine levels can influence a persons

capacity to acquire and retrieve information effectively (Wimber *et al.*, 2011).

Moreover, dopamine is closely linked to motivation and reward mechanisms meaning that changes in its levels can shape how individuals react to incentives and drive (Landau *et al.,* 2009).

In terms of attention and focus, dopamine is thought to play a role in directing focus and priming attention. Therefore, alterations in dopamine levels may affect an individual's ability to concentrate and stay focused (Miendlarzewska *et al.,* 2016).

Overall, the impact of COMT on functions is influenced by factors such as genetics and the surrounding environment. It forms part of a network of elements that collectively shape an individual's behaviors and responses to environmental stimuli and challenges (Rutter *et al.,* 2006).

2-16-4: Low level of COMT

Having levels of COMT signifies that the amount of catechol O methyltransferase in the bloodstream is lower than usual. This change can affect both the system and behavior in ways such as:

1. Increased dopamine levels; when COMT is at a level dopamine might build up more in the brain since it is not being broken down effectively.
2. Affect mood, stress levels, motivation, learning abilities and memory functions differently for each individual and situation (Martens, 2019).
3. Enhanced motivation and focus; Elevated dopamine levels could boost motivation. Enhance the ability to focus and concentrate.
4. Mood effects; Higher dopamine levels may have an influence on mood by promoting feelings of contentment and joy. However, they could also contribute to heightened stress and anxiety in scenarios.

5. Impact on learning and memory; Increased dopamine levels have the potential to influence learning and memory capacities, which might affect attention span and concentration (Volkow *et al.*, 2019).

This comprehensive knowledge about neurotransmitters enzymes like COMT and their functions within the system plays a role in developing therapeutic approaches, for various neurological and psychological conditions.

2-16-5: Effects of low COMT activity on drug therapies

People, with levels of COMT activity might have reactions to drugs that impact dopamine and norepinephrine levels. The effectiveness of antidepressants such, as SSRIs and serotonin norepinephrine reuptake inhibitors may be influenced by reduced COMT activity (Zai, 2021).

2-17: Genetic studies and experiments

Studies in genetics have revealed that changes in the COMT gene affect the activity of enzymes. Individuals carrying mutations that result in low COMT activity exhibit different emotional and cognitive responses. Experiments on genetically modified mice lacking COMT have shown increased dopamine levels and behaviors associated with stress and anxiety. Reduced COMT activity can lead to increased dopamine and norepinephrine levels in specific brain regions, affecting a wide range of neuro-behaviors (Esmaiel *et al.,* 2020).

2-18: Scopolamine and Alzheimer's disease

Despite the cholinergic theory suggesting that Alzheimer's disease is characterized by defects in acetylcholine synthesis enzymes (choline acetyltransferase), many studies have focused on the enzyme acetylcholinesterase, nicotinic acetylcholine receptors, or muscarinic

receptors. Furthermore, choline acetyltransferase has not been successfully regulated through artificial modification (Chen *et al.,* 2022). Due to the difficulty in retrieving enzymatic components from external sources and the highly selective blood-brain barrier, targeting receptor modulation with biologically active plant components is essential (Wu *et al.,* 2023).

This explains the effectiveness of numerous pharmacological investigations on memory impairment that have used scopolamine-induced amnesia as a research model. Scopolamine's impact on the cholinergic pathway closely resembles the cognitive impairment found in alzheimer's disease. Scopolamine-induced amnesia has been extensively employed in scientific studies. Some of these studies explore the effects of the cholinergic system on some studies focus on cognition and alzheimer's disease, while others look on receptor response modulation via nicotinic or muscarinic regulation. Most studies, however, seek to determine the pharmacological potential of specific plant extracts that could be utilized as alternative or novel treatments for Alzheimer's disease (Khesmakhi *et al.,* 2024).

Chapter Three:

Materials and Methods

Chapter Three

Materials and Methods

3-1: Animals

For this study, approximately 85-90 male Swiss albino mice were used, bred in the animal house of the College of Veterinary Medicine, University of Mosul. The weights of the mice ranged from 25-35 grams, and their ages were approximately 2 months. The mice were housed in suitable cages designed specifically for this purpose, with free access to water and feed. Environmental conditions, including ventilation, temperature, lighting, and bedding, were adequately maintained. The feed was sourced from local animal feed production facilities.

3-2: Drugs, materials, and equipment used in the study

Table (3-1): Drugs, materials, and equipment used in the study:

Substance/Equipment	Source
Scopolamine injection (1%)	Pioneer Company, Sulaymaniyah, Iraq
Distilled Water	Iraq
Laboratory tools (various sizes of syringes, anticoagulant tubes, Eppendorf tubes, gloves, medical cotton)	China
Spectrophotometer	Jen-way Company, China
Centrifuge	Chalice Company, England
Sensitive balance	Adam Company, Turkey
Water bath	Electro. Mag Company, Turkey

Open field box	Locally Made
Negative geotaxis	Locally Made
Light-dark box	Locally Made
Swimming pool	Locally Made

3-3: Diagnostic kits used

Table (3-2): Diagnostic kits used and its sources:

Source	Kit Name
American company Elabscience	Serotonin
American company Elabscience	Acetylcholine
American company Elabscience	COMT enzyme

3-4: Preparation of doses

Scopolamine drug ampules containing (1%) was obtained from Pioneer company, Sulaymaniyah, Iraq. Distilled water was used to dilute the drug according to the required dose based on the weight of the animal.

3-5: Blood sample collection

After completing the neuro-behavioral experiments, the mice were anesthetized with ether, and blood was collected from the orbital sinus using fine capillary tubes. The blood was placed in specialized tubes containing anticoagulant to extract plasma. The plasma samples were centrifuged at 3000 rpm for 15 minutes, then stored in special plastic tubes and frozen at -20°C until biochemical laboratory tests were performed.

3-6: Ethical approval

The ethical approval number for this study was UM.VET.2023.035, dated 10/15/2023.

3-7: Experiments

3-7-1: First experiment: Determination of the median lethal dose (LD_{50}) of scopolamine in mice

Preliminary experiments were used to determine the initial doses administered to the animals, using the up-and-down method (Dixon, 1980). The mice were injected intramuscularly (IM), and a fixed incremental or decremental value was applied to the doses. After administering various doses of scopolamine, the (LD_{50}) was calculated. Following the determination of the median lethal dose, several initial doses were selected for application in behavioral experiments. Fixed doses of 2 and 4 mg/kg IM were chosen for daily or intermittent injections, while single acute doses of 10 and 20 mg/kg were used for single-dose injection experiments. These doses were selected based on their lack of direct impact on neuro-behavioral performance (latent effect doses).

3-7-1-1: Neuro-behavioral tests

The experiments included testing the effects of time duration and dose on neuro-behavioral performance. Low repeated doses were administered daily for 2 and 4 weeks in the second and third experiments. The fourth experiment measured the effect of a single acute dose 24 hours post-administration. The fifth experiment tested the cumulative effect of a single scopolamine dose by observing the animal for 5 days post-dose before conducting neuro-behavioral tests. To confirm the results of the previous experiment, the sixth experiment involved administering single doses twice weekly. The neuro-behavioral tests studied across all experiments included:

A. Behavioral tests for higher brain functions

- **Open field test:** Rearing and Square Crossing Number (Figure 3-1).

  This experiment involved 15 mice divided into 3 groups, each containing 5 mice, randomly assigned. The first group served as the control and was injected with distilled water (0 dose). The second and third groups were injected intramuscularly with scopolamine at doses of 2 and 4 mg/kg body weight, respectively. The doses were selected based on preliminary experiments conducted before the study commenced. After two weeks from the start of the experiment, tests for locomotor activity and neuro-behavioral function were conducted on the animals. These tests included the following:

- **Locomotor activity and neuro-behavioral test in the open field**

  A square wooden box (35 x 35 x 25 cm) with a floor divided into 25 equal squares was used. A video camera recorded each animal's activity individually in an isolated room. The test measured the number of squares crossed with all four paws and the number of times the animal reared on its hind legs. Each animal was placed in the center of the box for 3 minutes. The floor was cleaned with cotton and alcohol after each test to avoid influencing the next animal tested. This test assesses the general locomotor activity of the mice inside the box (Figure 3-1) (Calhoun, 1963; Gil-Bea *et al.,* 2007). After this test, the animal was subjected to the next test.

Figure 3-1: Open field box.

- **Social interaction:**

  The extent of interaction of each mouse with a control group mouse inside the open field box was measured. A video camera recorded each animal's interaction with the control animal, including activities such as contact, licking, sniffing, or playing. The highest rank was 5 levels (Crawley, 2007).

B. Evaluation of the involuntary nervous system

- **Swimming score:** Muscular strength and fatigue were measured using the swimming test, which depends on the neural and functional integration of various brain regions. A special plastic swimming pool (60 x 30 x 40 cm) was used, filled with water to a depth of 30 cm at room temperature to avoid the effect of water temperature on performance. Each animal remained in the pool for 60 seconds. The scoring system for this test included the following ranks (Figure 3-2) (Schapiro *et al.*, 1970; Vorhees *et al.*, 1979):

- 0: Nose underwater.
- 1: Nose at or above the water surface.
- 2: Nose and peak of head at or above water line, with ears submerged.
- 3: As in 2, but water level at mid-ear.
- 4: As in 3, but water level at the base of the ear.

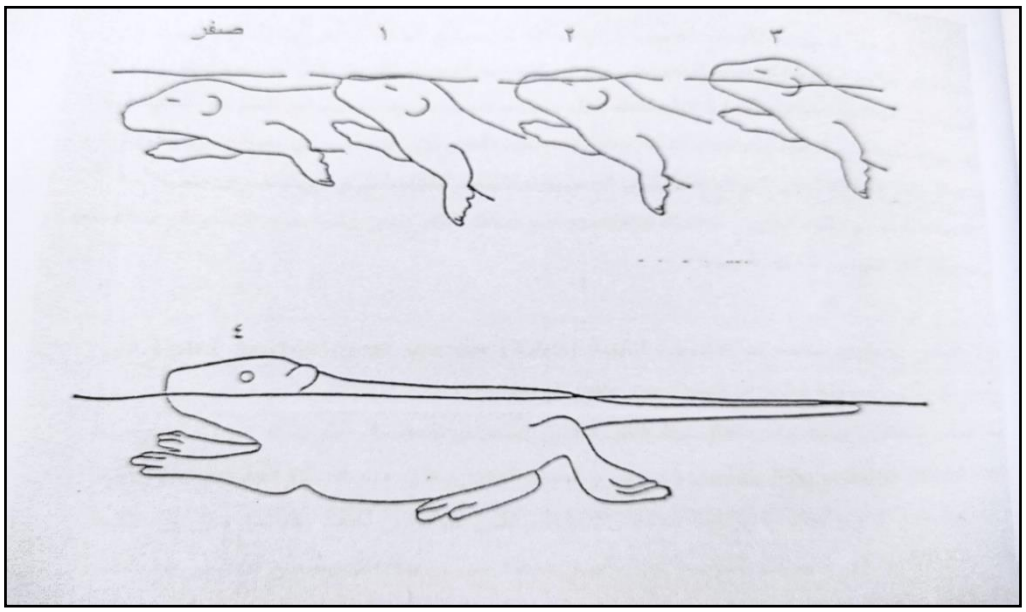

Figure 3-2: Swimming score in mice.

C. Behavioral tests for measuring cognitive impairment

- **Negative geotaxis:** A 45-degree inclined wooden surface was used. The test measured the time taken for the animal to turn 180 degrees from a head-down position to head-up. The maximum allowed time was one minute (60 seconds) for each animal. This test assesses vestibular function and neuromuscular activity (Figure 3-3) (Altman and Sudarshan, 1975; Feather-Schussler and Ferguson, 2016).

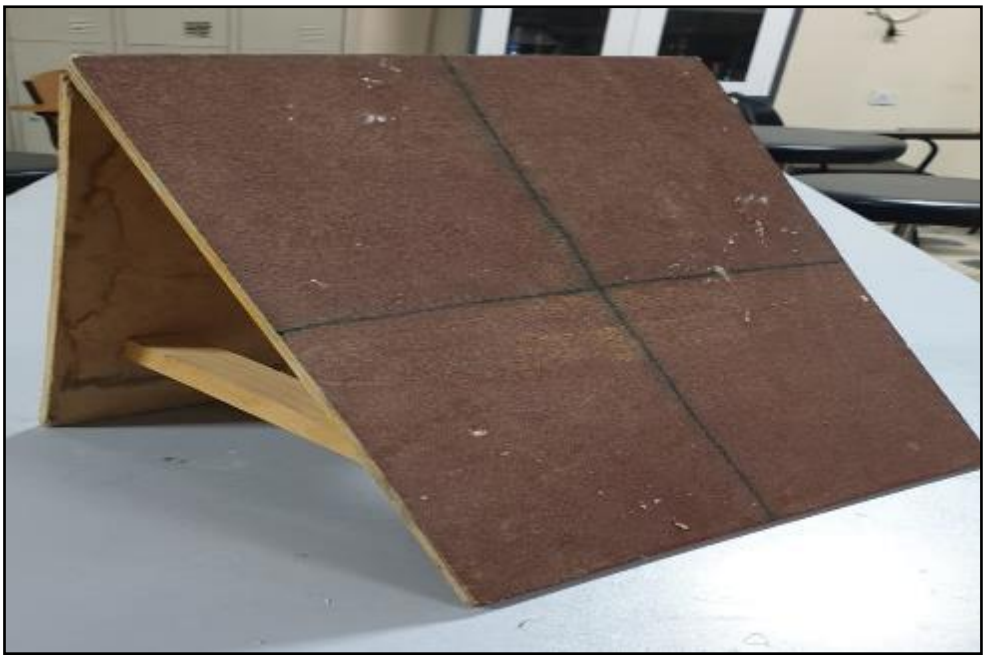

Figure 3-3: Negative geotaxis test.

- **Hole-board test:** A wooden surface with a 30 cm radius and 20 cm height containing 8 circular holes was used. The test involved observing the animal and counting the number of times it inserted its head into the holes. The test duration was 3 minutes per animal, assessing the animal's interest and environmental awareness (Figure 3-4) (File and Wardill, 1975; Rebai and Djebli, 2008).

Figure 3-4: Hole-board test.

- **Time in dark:** The light-dark box test (Crawley and Goodwin, 1980) was used to assess anxiety-like behavior in mice. The box was divided into two compartments: a light compartment (46 x 27 x 30 cm) illuminated with white light and a dark compartment (46 x 27 x 30 cm) with black walls. A small gate allowed passage between compartments. A mobile phone camera placed approximately 50 cm above the box recorded the animal's activity. The mouse was situated in the dark side and permitted to examine for 3 minutes. The duration expended in each section was recorded. The dark time percentage was determined by applying the formula; Dark time = (time, in dark) /180 sec. × 100 (Figures 3-5 and 3-6).

Figure 3-5: Light –dark apparatus.

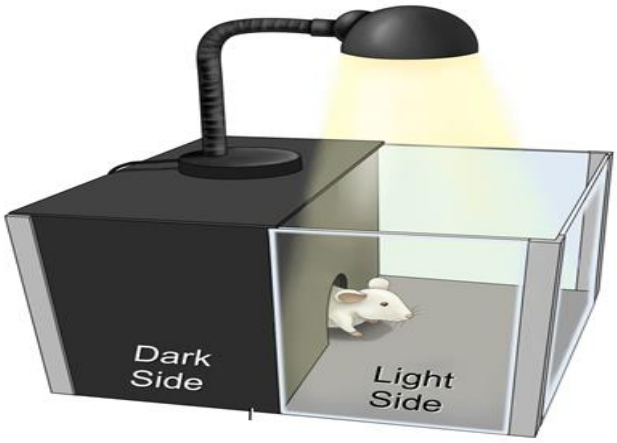

Figure 3-6: Light –dark test.

3-7-2: Second experiment: Effect of daily dose for two weeks of scopolamine in some neuro-behavioral tests

Fifteen male mice were split into three groups, each consisting of five animals. The first group acted as the control. Received an injection of distilled water (0 dose). The second and third groups were given injections of scopolamine at doses of 2 mg/kg and 4 mg/ IM body weight, respectively. After a two week treatment period neuro-behavioral assessments were conducted.

3-7-3: Third experiment: Effect of scopolamine in daily doses for four weeks in some neuro-behavioral tests

Fifteen male mice were split into three groups, each consisting of five animals. The first group acted as the control. Received a dose of water (0 dosage). The second and third groups were given injections of scopolamine intramuscularly at dosages of 2 and 4 mg/kg body weight, respectively. After four weeks of treatment neuro-behavioral assessments were conducted.

3-7-4: Fourth experiment: Impact of acute high single dose treatment with scopolamine on some neuro-behavioral tests

Fifteen male mice were split into three groups, each consisting of five animals. The first group acted as the control. Received a dose of water (0 dosage). The second and third groups were given injections of scopolamine intramuscularly at dosages of 10 and 20 mg/kg body weight, respectively. After 24 hr of treatment neuro-behavioral assessments were conducted.

3-7-5: Fifth experiment: Effect of single dose of scopolamine then neuro-behavioral measurement after 5 days

Fifteen male mice were split into three groups, each consisting of five animals. The initial group acted as the control. Received a dose of water (0 dosage). The second and third groups were given injections of scopolamine intramuscularly at dosages of 2 and 4 mg/kg body weight, respectively. After 5 days of treatment neuro-behavioral assessments were conducted.

3-7-6: Sixth experiment: Effect of administering scopolamine once every two days during the week

The experiment included 15 mice that were split into three groups with each group comprising five mice randomly selected. The first group performed as the control and was injected with distilled water (0 dose). The second and third groups were injected intramuscularly with scopolamine at doses of 2, 4 mg/kg body weight, respectively. The doses were administered twice weekly, and neuro-behavioral and locomotor activity tests were conducted after one week.

3-7-7: Seventh experiment: Biochemical tests

After the completion of each experiment, the animals were anesthetized for blood collection and plasma separation, as previously described, for the following biochemical tests:

3-7-7-1: Measurement test

Principle of the test: The ELISA kit employs the Competitive-ELISA principle, in which ST/5-HT competes with a predetermined amount for specific locations on the Biotinylated Detection Antibody. Excess samples are washed, Avidin coupled with Horseradish Peroxidase is added, followed by a TMB substrate solution and a color change measurement.

The concentration of ST/5-HT is calculated by comparing the optical density of the samples to the standard curve.

3-7-7-1-1: Serotonin levels

1. The serotonin levels after a two-week daily dosing of scopolamine.
2. The serotonin levels after daily doses of scopolamine for 4 weeks.
3. Serotonin levels after 24 hours of administering single doses of scopolamine.
4. Serotonin levels after 5 days of administering one dose per week.
5. Effect of two doses of scopolamine for one week on serotonin levels.

The ELISA kit employs the Competitive-ELISA principle, in which ST/5-HT competes with a predetermined amount on a microplate. Excess is rinsed away, and Avidin conjugated with Horseradish Peroxidase is applied to every well. A TMB substrate solution is added, and the color change is detected spectrophotometrically. The concentration is calculated by comparing the OD to a standard curve (Orzelska-Górka *et al.,* 2023).

3-7-7-1-2: Acetylcholine levels

1. The level of acetylcholine after daily doses of scopolamine for two weeks.
2. The level of acetylcholine after daily doses of scopolamine for four weeks.
3. The level of acetylcholine after 24 hours of treatment with a single dose of scopolamine.
4. The level of acetylcholine after 5 days of stopping treatment with a single dose of scopolamine.
5. The level of acetylcholine after two doses weekly treatment with scopolamine.

The ELISA kit employs the Competitive-ELISA principle, in which acetylcholine (Ach) competes with a predetermined amount on a microplate. Excess conjugate and unbound samples are rinsed, followed by the addition of Avidin conjugated with Horseradish Peroxidase and TMB substrate solution. The change, in color is measured using a spectrophotometer and the concentration of ACH is determined by comparing the samples to a curve (Wang *et al.,* 2020).

3-7-7-1-3: COMT levels

1. The level of COMT after two weeks treatment with scopolamine doses.
2. The level of COMT After four weeks of treatment with scopolamine doses.
3. The level of COMT following acute single dose of treatment.
4. The level of COMT in the body after five days of single dose of scopolamine.
5. The level of COMT after two doses of scopolamine for one week.

This kit uses a microplate pre-coated with COMT antibody, biotin-conjugated antibody, and Avidin conjugated with Horseradish Peroxidase to measure color change and determine COMT concentration (Zhou *et al.*, 1997).

3-8: Statistical analysis

The samples were statistically analyzed using SPSS software at a p-value of $p \leq 0.05$. The one-way ANOVA and LSD were used to compare parametric data between groups. For non-parametric data analysis, the fisher test for percentages and the Kruskal-Wallis test for ranks were used (Pallant, 2020).

Chapter Four:

Results

Chapter Four

Results

4-1: First experiment: Determination of the i.m median lethal dose (LD_{50}) of scopolamine in mice

The LD_{50} of scopolamine, determined through intramuscular injection in mice, is found to be 148 mg/kg. LD_{50} describe the dose at which 50% of the experimental subjects are predicted to die because of the administered substance (Table 4-1).

Table (4-1): Determination of the i.m median lethal dose (LD_{50}) of Scopolamine in mice

Dose (mg/kg)	**Outcome**
172	X
138	0
172	X
138	0
172	X
	Total animals:5
	Increase or decline dose: 34 mg\kg
	LD_{50}: 148.166 mg/kg, IM
Toxic signs: tremors, paralysis, and then death.	
X represent die animal, 0 represent survive animal	

4-2: Second experiment: Effect of daily dose for two weeks of scopolamine in some neuro-behavioral tests

4-2-1: Behavioral tests for higher brain functions

4-2-1-1: Open field test (rearing and squares crossed number) (Table 4-2)

- **Rearing**

  There is a rise in rearing activity in 2 mg/kg scopolamine group compared to the control, but it is not significant. In addition, significant increase in rearing activity in the 4 mg/kg scopolamine group compared to the control ($p \leq 0.05$). There is a significant difference in rearing activity between the 2 and 4 mg/kg scopolamine.

- **Squares crossed number**

  There is reduction in crossing numbers in the 2 mg/kg scopolamine group compared to control, and it is statistically significant ($p \leq 0.05$). There is a significant decline in crossing numbers in the 4 mg/kg scopolamine group compared to the control ($p \leq 0.05$). In addition, results show no significant difference in crossing numbers between the 2 and 4 mg/kg scopolamine groups ($p \leq 0.05$).

4-2-1-2: Social interaction\ score

Results recorded a decline in the median social interaction score in the 2 mg/kg scopolamine group compared to the control, and it is statistically significant ($p \leq 0.05$). There is a decline in the median social interaction score in the 4 mg/kg scopolamine group comparative to the control, and it is statistically significant ($p \leq 0.05$). The result recorded no significant difference in the median social interaction score between the 2 and 4 mg/kg scopolamine groups ($p \leq 0.05$).

Both doses of scopolamine (2 mg/kg and 4 mg/kg) significantly affect the behavior of mice compared to the control. The higher dose (4 mg/kg)

generally has more pronounced effects on behavior, particularly in terms of rearing activity and social interaction.

Table 4-2: Behavioral tests for higher brain functions after daily doses for two weeks of scopolamine in mice.

Groups	Open field		Social interaction \score-3 min
	Rearing\3 min	Squares Crossed number\3min	
Control	19.21±2.2	130±2	5
Scopolamine (2 mg\kg)	21.11±3.2	73±8 *	4*
Scopolamine (4 mg\kg)	45.20±2.5*A	50.14 ±3.2 *A	4*

Data as mean ± SE, data represented as median, p≤0.05, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg group.

4-2-2: Evaluation the involuntary nervous system's

The data in Table 4-3, recorded comparing the mean values for swimming score, between the control group and the scopolamine-treated groups.

Swimming score

There is a significant decline in the swimming score in the 2 mg/kg scopolamine group compared to the control group ($p \leq 0.05$). Similarly, there is a significant decline in the swimming score in the 4 mg/kg scopolamine group comparative to the control group ($p \leq 0.05$). There is no significant difference in the swimming score between the 2 mg/kg and 4 mg/kg scopolamine groups ($p \leq 0.05$).

Table 4-3: Evaluation the involuntary nervous systems after daily doses for two weeks of scopolamine in mice.

Groups	Swimming score
Control	4
Scopolamine (2 mg\kg)	2*
Scopolamine (4 mg\kg)	2*

Data as mean ± SE, data represented as median, p≤0.05, each group of 5 animals, *Represent significant difference from control.

4-2-3: Behavioral tests for measuring cognitive impairment in Table (4-4).

To analyze the data presented in Table (4-4), we will compare the mean values for negative geotaxis time, Hole-Board Test, and time spent in the dark between the control group and the scopolamine-treated groups.

- **Negative geotaxis time**

The results show significant increase in negative geotaxis time in 2 mg/kg scopolamine group comparative to the control ($p \leq 0.05$). Similarly, there is a significant increase in negative geotaxis time in the 4 mg/kg scopolamine group comparative to the control. There is no significant difference in negative geotaxis time between the 2 mg/kg and 4 mg/kg scopolamine groups ($p \leq 0.05$).

- **Hole-board test**

There is a significant decline in poking test duration in the 2 mg/kg scopolamine group comparative to the control. Results recorded a significant decline in poking test duration in the 4 mg/kg scopolamine group comparative to the control group ($p \leq 0.05$). In Hole-Boardtest we

recorded no significant difference between the 2 mg/kg and 4 mg/kg scopolamine groups ($p \leq 0.05$).

- **Time in dark**

There is no significant difference in the time spent in the dark between the 2 mg/kg scopolamine group and the control. In addition, there is a significant decline in the time spent in the dark in the 4 mg/kg scopolamine group comparative to the control. There is no significant difference in the time spent in the dark between the 2 mg/kg and 4 mg/kg scopolamine.

Both doses of scopolamine (2 mg/kg and 4 mg/kg) significantly affect cognitive function in mice comparative to the control group, as indicated by changes in negative geotaxis time and head poking test duration. However, there is no significant difference in the time spent in the dark between the control group and the 2 mg/kg scopolamine group. Additionally, the higher dose (4 mg/kg) significantly declines the time spent in the dark comparative to the control group. There is no significant difference in cognitive impairment between the two doses of scopolamine.

Table (4-4): Behavioral tests for measuring cognitive impairment after daily doses for two weeks of scopolamine in mice.

Groups	Negative geotaxis\sec	Hole-Board test \3 min	Time in Dark\3 min
Control	2.31 ±1.52	16.83 ±3.12	62%
Scopolamine (2 mg\kg)	5.25 ± 1.33*	12.42 ±2.08*	58 %
Scopolamine (4 mg\kg)	5.65 ±1.22*	13.08±2.44*	52%*

Data as mean ± SE, data represented as median, $p \leq 0.05$, each group of 5 animals, *Represent significant difference from control.

4-3: Third experiment: Effect of scopolamine in daily doses for 4 weeks in some neuro-behavioral tests

The data presented in Table (4-5), for comparing the mean values for the Open Field Test (Rearing and Crossing Number) and assess the median values for the Social Interaction Score after daily doses for 4 weeks in mice.

- **Open field test (Rearing)**

There is a significant increase in rearing activity in the 2, 4 mg/kg scopolamine group comparative to the control. There is a significant rise in rearing activity in the 4 mg/kg scopolamine group comparative to the 2 mg/kg scopolamine.

- **Open field test (Squares crossed number)**

There is a significant decline in crossing numbers in the 2 mg/kg scopolamine group comparative to the control group ($p \leq 0.05$). There is a significant decline in crossing numbers in the 4 mg/kg scopolamine group comparative to the control group ($p \leq 0.05$). The result recorded a significant decline in crossing numbers in the 4 mg/kg scopolamine group comparative to the 2 mg/kg scopolamine group ($p \leq 0.05$).

- **Social interaction score**

There is a significant decline in the median social interaction score in the 2 mg/kg scopolamine group comparative to the control group ($p \leq 0.05$). There is a significant decline in the median social interaction score in the 4 mg/kg scopolamine group comparative to the control group ($p \leq 0.05$). There is a significant decline in the median social interaction score in the 4 mg/kg scopolamine group comparative to the 2 mg/kg scopolamine group ($p \leq 0.05$).

Doses of scopolamine (2 mg/kg and 4 mg/kg) significantly affect the behavior of mice comparative to the control group after daily doses for 4 weeks. The higher dose (4 mg/kg) generally has more pronounced effects on behavior, particularly in terms of rearing activity, crossing numbers, and social interaction. The higher dose resulting in more severe impairments.

Table (4-5): Behavioral tests for higher brain functions after daily doses for 4 weeks in mice.

Groups	Open field		Social interaction\ score
	Rearing\3 min	Squares Crossed number\3min	
Control	19.21±2.2	130.5±20.23	+5
Scopolamine (2 mg\kg)	28.21±3.0*	75.13±8.42 *	+3*
Scopolamine (4 mg\kg)	35.19±2.03 *A	35.13±3.12 *A	+1*A

Data as mean ± SE, data represented as median, p≤0.05, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg.

4-3-1: Evaluation the involuntary nervous systems: (Table 4-6).

To compare the mean values for swimming score after daily doses for 4 weeks in mice.

- **Swimming score**

There is a significant decline in swimming score in the 2 mg/kg scopolamine group comparative to the control. The result shows a significant decline in swimming score in the 4 mg/kg scopolamine group comparative to the control. There is no significant difference in swimming score between the 2 mg/kg and 4 mg/kg scopolamine.

Table (4-6): The involuntary nervous system is after daily doses for 4 weeks in mice.

Groups	Swimming score\3 min
Control	4
Scopolamine (2 mg\kg)	3*
Scopolamine (4 mg\kg)	3*

Data as mean ± SE,data represented as median, p≤0.05, each group of 5 animals, *Represent significant difference from control.

4-3-2: Behavioral tests for measuring cognitive impairment: (Table 4-7).

To analyze the data presented in Table (4-7), the mean values for negative geotaxis time, head poking test duration, and time spent in the dark after daily doses for 4 weeks in mice.

- **Negative geotaxis time**

The test showed a significant increase in negative geotaxis time in the 2 mg/kg scopolamine group comparative to the control. There is a significant increase in negative geotaxis time in the 4 mg/kg scopolamine group comparative to the control. There is no significant difference in negative geotaxis time between the 2 mg/kg and 4 mg/kg scopolamine.

- **Hole-board test**

The data recorded a significant decline in head poking test time in the 2 mg/kg scopolamine group comparative to the control. There is a significant decline in head poking test in the 4 mg/kg scopolamine group comparative to the control. There is no significant difference in head poking test between the 2 mg/kg and 4 mg/kg scopolamine.

- **Time in dark**

There is a significant decline in the time spent in the dark in the 2 mg/kg scopolamine group comparative to the control. Results show a significant decline in the time spent in the dark in the 4 mg/kg scopolamine group comparative to the control. a significant decline was recorded in the time spent in the dark in the 4 mg/kg scopolamine group comparative to the 2 mg/kg scopolamine group ($p \leq 0.05$).

Both doses of scopolamine (2 mg/kg and 4 mg/kg) significantly affect cognitive function in mice comparative to the control group after daily doses for 4 weeks. The higher dose (4 mg/kg) generally has more pronounced effects on negative geotaxis time, poking test duration, and time spent in the dark.

Table (4-7): Behavioral tests for measuring cognitive impairment after daily doses for 4 weeks in mice

Groups	Negative geotaxis\sec	Hole-Board test \3 min	Time in Dark\3 min
Control	4.15 ±1.21	16.31 ±3.12	70%
Scopolamine (2 mg\kg)	10.31 ± 2.40*	10.26 ±1.16*	50 %*
Scopolamine (4 mg\kg)	10.11 ±3.41*	11.50±2.19*	36%*A

Data as mean ± SE, data represented as median, $p \leq 0.05$, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg.

4-4: Fourth experiment: Impact of acute high single doses treatment with scopolamine in some neuro-behavioral tests

4-4-1: Behavioral tests for higher brain functions: In table (4-8)

The mean values for the Open Field Test (Rearing and squares Crossing Number) and assess the median values for the Social Interaction Score after 24 hours of acute single doses of scopolamine in mice.

Open field test:

- **Rearing**

There is a significant increase in rearing activity in the 20 mg/kg scopolamine group comparative to the control and 10 mg/kg ($p \leq 0.05$).

- **Crossing number**

There is a significant decline in crossing numbers in the 20 mg/kg scopolamine group comparative to the control and 10 mg\kg ($p \leq 0.05$).

- **Social interaction score**

There is a significant decline in the median social interaction score in the 20 mg/kg scopolamine group comparative to the control.

Table (4-8): Behavioral tests for higher brain functions after 24 hours of acute single doses of scopolamine in mice

Groups Control	Open field		Social interaction\score-3 min
	Rearing\3 min	Squares Crossed number\3min	
Control	20.21±2.11	140.13±22.51	+5
Scopolamine (10 mg\kg)	22.14±2.10	139.42±7.19	+4
Scopolamine (20 mg\kg)	35.40±3.12*A	100.11±12.31*A	+3*

Data as mean ± SE, data represented as median, $p \leq 0.05$, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 10 mg\kg.

4-4-2: Evaluation the involuntary nervous systems: In table (4-9)

To analyze the data presented in Table (4-9), we will compare the mean values for swimming score, and tail suspension duration after 24 hours of acute single doses of scopolamine in mice.

- **Swimming score**

There is no significant difference in swimming score between the control group and the 10 mg/kg scopolamine. In addition to recorded a significant decline in swimming score in the 20 mg/kg scopolamine group comparative to the control group ($p \leq 0.05$). There is no significant difference in swimming score between the 10 mg/kg and 4 mg/kg scopolamine.

Table (4-9): Evaluation the involuntary nervous system's after 24 hour of acute single doses of scopolamine in mice

Groups	Swimming score\3 min
Control	4
Scopolamine (10 mg\kg)	4
Scopolamine (20 mg\kg)	3*

Data as mean ± SE, data represented as median, $p \leq 0.05$, each group of 5 animals, *Represent significant difference from control.

4-4-3: Behavioral tests for measuring cognitive impairment: Table (4-10)

The mean values for negative geotaxis time, head poking test duration, and time spent in the dark after 24 hours of acute single doses of scopolamine in mice.

- **Negative geotaxis time**

The data show a significant increase in negative geotaxis time in the 20 mg/kg scopolamine group comparative to the control group and 10 mg\kg.

- **Hole-board test**

The result recorded a significant decline in head poking in the 20 mg/kg scopolamine group comparative to the control group and 10 mg\kg.

- **Time spent in dark**

There is a significant decline in the time spent in the dark in the 20 mg/kg scopolamine group comparative to the control group and 10 mg\kg.

Table (4-10): Behavioral tests for measuring cognitive impairment and anxiety after 24 hours of acute single doses of scopolamine in mice

Groups	Negative geotaxis	Hole-Board test	Time in Dark
Control	4.20 ±1.28	16.25 ±3.1	75%
Scopolamine (10 mg\kg)	5.00 ± 2.25	16.31 ±4.52	77 %
Scopolamine (20 mg\kg)	15.51± 1.24*A	6.52±12*A	41% *A

Data as mean ± SE, data represented as median, $p \leq 0.05$, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg.

4-5: Fifth experiment: Effect of single dose of scopolamine then neuro-behavioral measurement after 5 days

4-5-1: Behavioral tests for higher brain functions: Table (4-11)

- **Open field test**

The mean values for the Open Field Test (Rearing and Crossing Number) and assess the median values for the Social Interaction Score after weekly single doses of scopolamine, then measured after 5 days in mice.

- **Rearing**

There is no significant difference in rearing activity between the control group and the 2 mg/kg scopolamine. There is no significant difference in rearing activity between the control group and the 4 mg/kg scopolamine. There is no significant difference in rearing activity between the 2 mg/kg and 4 mg/kg scopolamine.

- **Squares crossed number**

There is no significant difference in crossing numbers between the control group and the 2 and 4 mg/kg scopolamine group ($p \leq 0.05$). There is no significant difference in crossing numbers between the 2 mg/kg and 4 mg/kg scopolamine.

- **Social interaction score**

There is no significant difference in the median social interaction score between the control group and the 2 and 4 mg/kg scopolamine. There is no significant difference in the median social interaction score between the 2 mg/kg and 4 mg/kg scopolamine.

After weekly single doses of scopolamine followed by measurement after 5 days, there were no significant differences observed in the behavioral tests for higher brain functions (Open Field Test) and social interaction comparative to the control group. Both doses of scopolamine (2 mg/kg and 4 mg/kg) did not produce significant alterations in the measured parameters in this experimental setup.

Table (4-11): Behavioral tests for higher brain functions after weekly two doses of scopolamine then measure after 5 days in mice

Groups	Open field		Social interaction\score
	Rearing\3 min	Squares Crossed number\3min	
Control	22.16±2.15	146.40±20.21	5
Scopolamine (2 mg\kg)	19.20±3.20	150.23±8.09	5
Scopolamine (4 mg\kg)	20.29±7.29	139.98±10.39	5

Data as mean ± SE, data represented as median, $p \leq 0.05$, each group of 5 animals.

4-5-2: Evaluation the involuntary nervous system's: Table (4-12)

The mean values for swimming score, and tail suspension duration after a single dose of scopolamine, then measured after 5 days in mice.

- **Swimming score**

There is no significant difference in swimming score between the control group and the 2 and 4 mg/kg scopolamine. There is no significant difference in swimming score between the 2 mg/kg and 4 mg/kg scopolamine.

Table (4-12): Evaluation the involuntary nervous systems after single dose of scopolamine then measure after 5 days in mice.

Groups	Swimming score\3 min
Control	4
Scopolamine (2 mg\kg)	4
Scopolamine (4 mg\kg)	4

Data as mean ± SE, data represented as median, $p \leq 0.05$, each group of 5 animals.

4-5-3: Behavioral tests for measuring cognitive impairment: Table (4-13)

The mean values for negative geotaxis time, poking test duration, and time spent in the dark after a single weekly dose of scopolamine, and then measured after 5 days in mice.

- **Negative geotaxis time**

There is no significant difference in negative geotaxis time between the control group and the 2 and 4 mg/kg scopolamine. There is no significant difference in negative geotaxis time between the 2 mg/kg and 4 mg/kg scopolamine.

- **Hole-board test**

There is no significant difference in head poking between the control group and the 2 and 4 mg/kg scopolamine. In addition, there is no significant difference in head poking between the 2 mg/kg and 4 mg/kg scopolamine.

- **Time in dark test**

There is no significant difference in the time spent in the dark between the control group and the 2 and 4 mg/kg scopolamine. In addition, there is no significant difference in the time spent in the dark between the 2 mg/kg and 4 mg/kg scopolamine.

After a single weekly dose of scopolamine followed by measurement after 5 days, there were no significant differences observed in the behavioral tests for cognitive impairment (negative geotaxis and poking test) and time spent in the dark comparative to the control. Both doses of scopolamine (2 mg/kg and 4 mg/kg) did not produce significant alterations in the measured parameters in this experimental setup.

Table (4-13): Behavioral tests for measuring cognitive impairment after two doses weekly of scopolamine then measure after 5 days.

Groups	Negative geotaxis\sec	Hole-Board test \3 min	Time in Dark\3 min
Control	3.99 ±1.83	17.10 ±3.01	62 %
Scopolamine (2 mg\kg)	4.03 ± 2.12	15.98 ±8.02	64 %
Scopolamine (4 mg\kg)	4.21 ±3.43	16.37±12.23	53 %

Data as mean ± SE, data represented as median, $p \leq 0.05$, each group of 5 animals.

4-6: Sixth experiment: Effect of giving scopolamine in a one dose every two days for a one week on some neuro-behavioral tests

4-6-1: Behavioral tests for higher brain functions: Table (4-14)

The mean values for the Open Field Test (Rearing and Squares Crossed Number) and assess the median values for the Social Interaction Score after one dose every two days for one week in mice.

Open field test

- **Rearing**

There is no significant difference in rearing activity between the control group and the 2 mg/kg scopolamine. While found a significant increase in rearing activity in the 4 mg/kg scopolamine group comparative to the control. There is a significant increase in rearing activity in the 4 mg/kg scopolamine comparative to the 2 mg/kg scopolamine.

- **Head crossing number**

There is no significant difference in crossing numbers between the control group and the 2 mg/kg scopolamine. While there is, a significant decline in crossing numbers in the 4 mg/kg scopolamine group

comparative to the control. There is a significant decline in crossing numbers in the 4 mg/kg scopolamine group comparative to the 2 mg/kg scopolamine.

- **Social interaction score**

The results shows no significant difference in the median social interaction score between the control group and the 2 mg/kg scopolamine. There is a significant decline in the median social interaction score in the 4 mg/kg scopolamine group comparative to the control. In addition to no significant difference was found in the median social interaction score between the 2 mg/kg and 4 mg/kg scopolamine. After one dose every two days for one week in mice, both doses of scopolamine (2 mg/kg and 4 mg/kg) significantly affect the behavior of mice comparative to the control group. The higher dose (4 mg/kg) generally has more pronounced effects on rearing activity and crossing numbers.

Table (4-14): Behavioral tests for measuring cognitive impairment after two doses in week of scopolamine then measure after 5 days.

Groups	Open field		Social interaction\score -3 min
	Rearing\3 min	Squares Crossed number\3min	
control	18.21±2.64	138.87±20.73	5
Scopolamine (2 mg\kg)	19.97±88 .7	128.03±8.23	5
Scopolamine (4 mg\kg)	36.23±1.46 *A	86.43±12.20 *A	3*A

Data as mean ± SE,data represented as median, p≤0.05, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg.

4-6-2: Evaluation the involuntary nervous system's: Table (4-15)

The mean values for swimming score and tail suspension duration after one dose every two days for one week in mice.

- **Swimming score**

There is no significant difference in swimming score between the control group and the 2 mg/kg scopolamine. In addition to decline in swimming score in the 4 mg/kg scopolamine, group comparative to the control. There is a significant decline in swimming score in the 4 mg/kg scopolamine group comparative to the 2 mg/kg scopolamine group ($p \leq 0.05$).

Table (4-15): Evaluation the involuntary nervous systems after one dose every two days for a one week in mice.

Groups	Swimming score\3 min
Control	4
Scopolamine (2 mg\kg)	4
Scopolamine (4 mg\kg)	3*

Data as mean ± SE, data represented as median, $p \leq 0.05$, each group of 5 animals, *Represent significant difference from control.

4-6-3: Behavioral tests for measuring cognitive impairment: Table (4-16)

The mean values for negative geotaxis time, poking test duration, and time spent in the dark after one dose every two days for one week in mice.

- **Negative geotaxis time**

There is no significant difference in negative geotaxis time between the control group and the 2 mg/kg scopolamine. While there is a significant increase in negative geotaxis time in the 4 mg/kg scopolamine group comparative to the control. There is a significant increase in negative geotaxis time in the 4 mg/kg scopolamine group comparative to the 2 mg/kg scopolamine.

- **Hole-board test**

There is no significant difference in poking test duration between the control group and the 2,4 mg/kg scopolamine group.

- **Time in dark test**

There is no significant difference in the time spent in the dark between the control group and the 2 mg/kg scopolamine. Also, found a significant decline in the time spent in the dark in the 4 mg/kg scopolamine group comparative to the control.

Table (4-16): Behavioral tests for measuring cognitive impairment after one dose every two days for a one week in mice.

Groups	Negative geotaxis\sec	Hole-Board test \3 min	Time in Dark\3 min
Control	5.00 ±1.12	16.87 ±3.50	80%
Scopolamine (2 mg\kg)	5.12 ± 3.75	17.34 ±4.31	77%
Scopolamine (4 mg\kg)	10.68 ±3.24*	18.12±2.9	60%*

Data as mean ± SE, data represented as median, $p \leq 0.05$, each group of 5 animals, *Represent significant difference from control.

4-7: Seventh experiment: Biochemical tests

4-7-1: Serotonin levels

4-7-1-1: The serotonin levels after a two-week daily dosing of scopolamine: (Table 4-17)

The statistical analysis suggests that after daily doses of scopolamine for 2 weeks 2 and 4 mg/kg Scopolamine group exhibited a significant increase in serotonin levels comparative to the control.

Table (4-17): Serotonin level after a two-week daily dosing of scopolamine.

Groups	Serotonin ng/ml
Control group	213.458± 50.31
Scopolamine (2mg\kg)	488.866 ±65.12*
Scopolamine (4mg\kg)	696.911±67.25*A

Data as mean ± SE, data represented as median, p≤0.05, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg.

4-7-1-2: The serotonin levels after daily doses of scopolamine for 4 weeks: (Table 4-18)

The statistical analysis suggests that after daily doses of scopolamine for 4 weeks 2 and 4 mg/kg Scopolamine group exhibited a significant increase in serotonin levels comparative to the control.

Table (4-18): Serotonin level after daily doses of scopolamine for 4 weeks.

Groups	Serotonin ng/ml
Control group	213.458± 40.22
Scopolamine (2mg\kg)	425.101± 39.34*
Scopolamine (4mg\kg)	446.452±56.29*

Data as mean ± SE, data represented as median, p≤0.05, each group of 5 animals, *Represent significant difference from control.

Based on the table (4-19), the statistical analysis for the serotonin levels after 24 hours of giving single doses of scopolamine can be summarized as follows:

Control group: Mean serotonin level was 213.458 with a standard error (SE) of 20.35.

Scopolamine 10 mg/kg group: Mean serotonin level was 248.581 with SE of 32.11, marked with * indicating statistical significance comparative to control.

Scopolamine 20 mg/kg group: Mean serotonin level was 373.959 with SE of 51.20, marked with *A indicating statistical significance comparative to control and scopolamine. The statistical significance was determined as $p \leq 0.05$, indicating a significant difference in serotonin levels between the control group and both scopolamine-treated groups.

This statistical analysis suggests that after 24 hours of giving single doses of scopolamine, both the 10 mg/kg and 20 mg/kg Scopolamine groups exhibited significant increases in serotonin levels comparative to the control group. Additionally, the 20 mg/kg group showed a significance comparative to the control and 10 mg/kg group.

Table (4-19): Serotonin level after 24 hours of giving single doses of scopolamine.

Groups	Serotonin ng/ml
Control group	213.458±20.35
Scopolamine (10 mg\kg)	248.581± 32.11
Scopolamine (20 mg\kg)	373.959± 21.20*A

Data as mean ± SE, data represented as median, $p \leq 0.05$, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg.

4-7-1-3: Serotonin levels after 5 days of giving one dose per week: (Table 4-20)

This statistical analysis suggests that after 5 days of giving one dose per week, the Scopolamine 2 and 4 mg/kg group exhibited no significant rise in serotonin levels comparative to the control group.

Table (4-20): Serotonin level after 5 days of giving one dose per week.

Groups	Serotonin ng/ml
Control group	213.458± 40.50
Scopolamine (2 mg\kg)	250.911± 29.80
Scopolamine (4 mg\kg)	237.938± 50.4

Data as mean ± SE, data represented as median, p≤0.05, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg.

The statistical analysis for the serotonin levels after two doses of scopolamine for one week can be recorded as follows (table 4-21). Both the 2 mg/kg and 4 mg/kg Scopolamine groups exhibited significant increases in serotonin levels comparative to the control. Additionally, the 4 mg/kg group showed a significance comparative to the 2 mg/kg group.

Table (4-21): Effect of two doses of scopolamine for one week on serotonin level.

Groups	Serotonin ng/ml
Control group	213.458± 20.11
Scopolamine (2 mg\kg)	262.606± 40.21
Scopolamine (4 mg\kg)	348.178± 30.11*A

Data as mean ± SE, data represented as median, p≤0.05, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg.

4-7-2: Acetylcholine levels

4-7-2-1: Acetylcholine level after daily doses of scopolamine for two weeks: (Table 4-22)

The statistical significance was determined as p≤0.05, indicating a significant difference in acetylcholine levels between the control group and both scopolamine-treated groups.

This statistical analysis suggests that after daily doses of scopolamine for two weeks, both the 2 mg/kg and 4 mg/kg Scopolamine groups exhibited significant declines in acetylcholine levels comparative to the control group. Additionally, the 4 mg/kg group showed a higher level of significance comparative to the 2 mg/kg group.

Table (4-22): Acetylcholine level after a daily doses of scopolamine for two weeks

Groups	Acetylcholine ng/ml
Control group	187.5±20.13
Scopolamine (2mg\kg)	150.647±51.35*
Scopolamine (4mg\kg)	123.435±19.6*A

Data as mean ± SE, data represented as median, p≤0.05, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg.

4-7-2-2: Acetylcholine level after daily doses of scopolamine for four weeks: (Table 4-23)

The statistical significance was determined as p≤0.05, indicating a significant difference in acetylcholine levels between the control group and both treated groups the 2 mg/kg and 4 mg/kg Scopolamine groups exhibited significant declines in acetylcholine levels comparative to the control group. Additionally, the 4 mg/kg group showed a higher level of significance comparative to the 2 mg/kg group.

Table (4-23): Acetylcholine level in daily doses of scopolamine for 4 weeks.

Groups	Acetylcholine ng/ml
Control group	187.5±19.50
Scopolamine (2mg\kg)	145.029±40.10*
Scopolamine (4mg\kg)	90.799±20.30*A

Data as mean ± SE, data represented as median, p≤0.05, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg.

4-7-2-3: Acetylcholine level after a 24-hour treatment of a single dose of scopolamine: (Table 4-24)

The statistical significance was determined as p≤0.05, indicating a significant difference in acetylcholine levels between the control group and both scopolamine-treated groups. This statistical analysis suggests that after a 24-hour treatment of a single dose of scopolamine, both the 10 mg/kg and 20 mg/kg Scopolamine groups exhibited significant declines in acetylcholine levels comparative to the control group. Additionally, the 20 mg/kg group showed a higher level of significance comparative to the 10 mg/kg group.

Table (4-24): Acetylcholine level after 24 hours of treatment of a single doses of scopolamine.

Groups	Acetylcholine ng/ml
Control group	187.5±35.20
Scopolamine (10 mg\kg)	157.336±43.11*
Scopolamine (20 mg\kg)	104.703±40.20*A

Data as mean ± SE, data represented as median, p≤0.05, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg.

4-7-2-4: Acetylcholine level after 5 days of stopping treatment with a single dose of scopolamine: (Table 4-25)

The statistical analysis suggests that after 5 days of stopping treatment with a single dose of scopolamine, there were no significant differences in acetylcholine levels between the control and the groups previously treated with scopolamine (both 2 mg/kg and 4 mg/kg).

Table (4-25): Acetylcholine level after 5 days of stopping treatment with single dose of scopolamine.

Groups	Acetylcholine ng/ml
Control group	187.5±30.21
Scopolamine (2 mg\kg)	185.318±29.11
Scopolamine (4 mg\kg)	186.567±40.13

Data as mean ± SE, $p \leq 0.05$, each group of 5 animals.

4-7-2-5: Acetylcholine level after two doses weekly treatment with scopolamine: (Table 4-26)

The statistical significance was determined as $p \leq 0.05$, indicating a significant difference in acetylcholine levels between the control group and the Scopolamine 4 mg/kg group.

This statistical analysis suggests that after two doses weekly treatment with scopolamine, the 4 mg/kg Scopolamine group exhibited a significant decline in acetylcholine levels comparative to the control group. However, no significant difference was observed in the 2 mg/kg Scopolamine group comparative to the control group.

Table (4-26): Acetylcholine level after two doses weekly treatment with scopolamine.

Groups	Acetylcholine ng/mL
Control group	187.5±29.11
Scopolamine (2 mg\kg)	175.12±53.32
Scopolamine (4 mg\kg)	116.796±35.20*

Data as mean ± SE, data represented as median, p≤0.05, each group of 5 animals, *Represent significant difference from control.

4-7-3: The level of COMT enzyme

4-7-3-1: The level of COMT enzyme after daily doses treatment for two weeks: (Table 4-27)

The statistical significance was determined as p≤0.05, indicating a significant difference in COMT levels between the control group and both scopolamine-treated groups.

This statistical analysis suggests that after daily doses treatment for two weeks, both the 2 mg/kg and 4 mg/kg Scopolamine groups exhibited significant declines in COMT levels comparative to the control group. Additionally, the 4 mg/kg group showed a higher level of significance comparative to the 2 mg/kg group.

Table (4-27): The level of COMT enzyme in a daily doses treatment for two weeks.

Groups	COMT nmol/ ml
Control group	0.156± 0.014
Scopolamine (2mg\kg)	0.100±0.020*
Scopolamine (4mg\kg)	0.080±0.021*A

Data as mean ± SE, data represented as median, p≤0.05, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg.

4-7-3-2: The level of COMT enzyme after daily doses treatment for four weeks: (Table 4-28)

The statistical significance was determined as $p \leq 0.05$, indicating a significant difference in COMT levels between the control group and the Scopolamine 4 mg/kg group.

This statistical analysis suggests that after daily doses treatment for four weeks, the 4 mg/kg Scopolamine group exhibited a significant decline in COMT levels comparative to the control group. However, no significant difference was observed in the 2 mg/kg Scopolamine group comparative to the control group.

Table (4-28): The level of COMT enzyme in a daily doses treatment for 4 weeks.

Groups	COMT nmol/ ml
Control group	0.156±0.020
Scopolamine (2 mg\kg)	0.140±0.0308
Scopolamine (4 mg\kg)	0.086±0.021*A

Data as mean ± SE, data represented as median, $p \leq 0.05$, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg.

4-7-3-3: The level of COMT enzyme after 24 hours of a single dose of treatment: (Table 4-29)

This statistical analysis suggests that after 24 hours of a single dose of treatment, the 20 mg/kg Scopolamine group exhibited a significant decline in COMT levels comparative to the control group. However, no significant difference was observed in the 10 mg/kg Scopolamine comparative to the control.

Table (4-29): The level of COMT enzyme after 24 hours of a single dose of treatment.

Groups	COMT nmol/ ml
Control group	0.156±0.031
Scopolamine (10 mg\kg)	0.164±0.041
Scopolamine (20 mg\kg)	0.050±0.033*A

Data as mean ± SE, data represented as median, p≤0.05, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg.

4-7-3-4: The level of COMT enzyme after 5 days of stopping treatment with a single dose of scopolamine: (Table 4-30)

This statistical analysis suggests that after 5 days of stopping treatment with a single dose of scopolamine, no significant difference was observed in the 2,4 mg/kg Scopolamine comparative to the control.

Table (4-30): The level of COMT enzyme after 5 days of stopping treatment with single dose of scopolamine.

Groups	COMT nmol/ ml
Control group	0.156±0.06
Scopolamine (2 mg\kg)	0.157±0.04
Scopolamine (4 mg\kg)	0.150 ±0.03

Data as mean ± SE, data represented as median, p≤0.05, each group of 5 animals.

4-7-3-5: The level of COMT enzyme after two doses of scopolamine for one week: (Table 4-31)

The statistical significance was determined as p≤0.05, indicating a significant difference in COMT levels between the control group and the Scopolamine 4 mg/kg group. This statistical analysis suggests that after two doses of scopolamine for one week, the 4 mg/kg Scopolamine group

exhibited a significant decline in COMT levels comparative to the control group. However, no significant difference was observed in the 2 mg/kg Scopolamine group comparative to the control.

Table (4-31): The level of COMT enzyme after two doses of scopolamine for one week.

Groups	COMT nmol/ ml
Control group	0.156±0.03
Scopolamine (2 mg\kg)	0.155±0.09
Scopolamine (4 mg\kg)	0.066±0.05*A

Data as mean ± SE, data represented as median, $p \leq 0.05$, each group of 5 animals, *Represent significant difference from control, A: Represent significant difference from 2 mg\kg.

Chapter Five: Discussion

Chapter Five

Discussion

The discussion offers insights into the toxicity and behavioral impacts of scopolamine in mice. Determining the LD_{50} value provides information on its lethality with a dose for half of the tested animals set at 148 mg/kg. Studies on animals demonstrated a scopolamine LD_{50} of 1880 mg/kg and 1270 mg/kg well as a subcutaneous LD_{50} of 1650 mg/kg and 296 mg/kg in mice (Shim *et al.,* 2022). It's important to consider that LD_{50} values can vary based on factors such as species, method of administration and individual sensitivity. Applying these findings to toxicity necessitates research and deliberation due to biological variations between species (Lane *et al.,* 2023).

The extended use of scopolamine at doses ranging from 2 to 4 mg/kg, IM over periods of 2 to 4 weeks along with a term regimen of weekly doses at 4 mg/kg exhibited comparable effects on neuro-behavioral functions. These effects included reduced squares crossed increased rearing behavior, decreased interaction, swimming episodes, heightened negative geotaxis response, reduced curiosity levels and increased time spent in darkness. The findings indicated that the impact of a dose of scopolamine (10 mg/kg) was milder in comparison to the impacts of repeated doses (2 and 4 mg/kg) given over a period of 2 to 4 weeks. This difference can be attributed to factors involving adjustments, cognitive effects and alterations in neurotransmitters. When exposed to repeated doses over a timeframe (2-4 weeks) the nervous system gradually adapts to the ongoing interference leading to lasting modifications, in receptor function and neurotransmitter levels (De Bartolomeis *et al.,* 2015).

Repeated use of scopolamine causes a change in the sensitivity of muscarinic receptors and may affect the speed of their response and change their numbers, and thus this is reflected in neurological behavior and motor activity (Navarria *et al.,* 2015). Also, the response of the nervous system to the rest of the neurotransmitters, such as (dopamine, serotonin, and norepinephrine), is more severe and this is a compensatory mechanism for the decrease in acetylcholine (Haider *et al.,* 2016).

If scopolamine is given sub-acutely, a kind of habituation occurs, and obtaining the same effect requires larger doses to achieve that balance in the nerve signal (Kokane *et al.,* 2020). The effect of acute doses of treatment is strong and sometimes temporary depending on the type of treatment, while the effects of repeated administration of treatment are more permanent due to the effect of neuroconditioning in the brain (Desai *et al.,* 2022). Scopolamine works by competing with muscarinic receptors, and the response, as current experiments have shown, depends on the dose and duration of treatment (Chen, 2023). Most of the observed behavioral effects are due to the fact that scopolamine has an effect on neurotransmitters, especially serotonin and acetylcholine in different parts of the brain (Chen and Yeong, 2020). As for the absence of behavioral changes when treatment was stopped for 5 days, this may be because the drug was filtered from the plasma and had no traces left in the tissues.

One of the reasons for decreased motor activity and a decrease in the number of squares crossed is increased Cholinesterase enzyme activity, which breaks down acetylcholine, causing its effectiveness to be removed. this leads to a decrease in motor and muscle activity in animals as proven by previous studies (Johnson *et al.,* 2022). The appearance of signs of anxiety and stress in animals was observed in our study through an increase in the number of standing times and numerous climbing attempts

associated with treatment with scopolamine. This may be due to the fact that scopolamine has affected in a certain way the mechanism of action of serotonin, which has a role in the emergence of these effects (Dempsey and Kavanagh, 2023). The decrease in social interaction activities and swimming pools may be due to the decrease in acetylcholine levels that were measured in this study, and this is reflected in the animal's decreased passion for playing or contact between them, as well as muscle weakness and rapid fatigue in the animals (Hayes, 2022). The significant effect with both doses of scopolamine indicates that the drug reaches a stage of saturation at what is called the threshold level (Tan *et al.,* 2022). To adjust the direction of its body against gravity, the animal needs neuromuscular balance, and vestibular function is involved in part of this effect (Blair, 2021; Birmann *et al.,* 2023).

The animal's nervous activity is usually affected by the function of the frontal cortex and the hippocampus, as well as the amygdala. These parts of the brain are usually active when the animal faces strange environmental conditions, such as exploring a new environment that differs from its original environment. It may also be due to its role in the speed of perception and the desire to explore in the animal. This is why we see that the behavior of inserting the head into holes It has decreased in most behavioral experiments conducted on animals (Chao *et al.,* 2020; Ouyang *et al.,* 2020; Šimić *et al.,* 2021). Among the tests that explore the animal's condition in terms of depression and anxiety is the light and dark test, in which the time spent by the animal on each side is tested. The longer the period of time the animal stays on one side, it indicates the influence of a certain nervous behavior. For example, a decrease in the period of time the animal stays in the dark indicates a state of nervous disorder and tension. In animals with scopolamine, this effect increased with increasing doses

given, and the significant increase in serotonin levels may have a role in the emergence of anxiety and tension that appeared in the animals in this study, and this is consistent with another study that proved this result (Tavares, 2022; Poli *et al.,* 2024).

The significant decrease in squares and increased number of stances observed in both scopolamine-treated groups suggests a decrease in locomotor activity or exploratory behavior, consistent with the results of previous experiments. This persistent decrease in squares over the four-week period may indicate persistent impairment in motor coordination or changes in motor control resulting from chronic scopolamine administration (Abdelghany *et al.,* 2022), Cognitive (Kose *et al.,* 2023). When examining the effects of acute single doses of scopolamine on neuro-behavioral tests, with particular emphasis on standing frequency, decrease in squares in the open field test, and social interaction 24 hours after treatment. Scopolamine (20 mg/kg) significantly affected the behavior of mice. The higher dose (20 mg/kg) had generally more pronounced effects on behavior, confirming the rapid effects of scopolamine on higher brain functions and social behavior (Mansouri *et al.,* 2021). When an acute dose of scopolamine is administered, a rapid and strong effect occurs on acetylcholine receptors, leading to immediate disruption of their functions. However, the nervous system quickly regains its balance after the effects of an acute dose subside (Li *et al.,* 2024). Acute doses of scopolamine often produce rapid but temporary effects, such as hallucinations, confusion, and short-term memory loss. In contrast, chronic doses can lead to sustained effects on memory and behavior due to persistent neuronal changes (Constanzo *et al.,* 2020).

Anxious behavior was also shown by increasing the number of times it stood on its back legs in the open field test, indicating the state of mental disorder that the animal suffers from (Abdelghany *et al.,* 2022).

The dynamic effect of the drug through metabolism in the liver, filtering and excretion through the kidneys plays a role in the length of time the drug remains in the body. Some drugs are also stored in the body and released into the bloodstream in small doses. Therefore, such drugs continue to have effects even after stopping (Bereda, 2022). Giving it while it was found in the treatment experiment with a single dose, then leaving the rats for 5 days without treatment, and then conducting behavioral tests, that scopolamine's effect does not last for long periods at low doses and does not have a permanent behavioral effect. This is consistent with the fact that the effects recorded at relatively low doses were transient and depend on the period for which the administration continues. The drug is in it (Talebi *et al.,* 2021), as the experiment of giving two doses weekly showed a continued effect on neurobehavior, which confirms that the half-life of scopolamine is approximately 28 hours, as proven by another study (Tancheva *et al.,* 2023).

Experiments with biochemical tests showed that the level of serotonin showed a significant increase in the sub acute trials of 2 and 4 mg/kg for 2 and 4 weeks, as well as in the trial treated with the acute single dose of 20 mg/kg and then measuring after 24 hours, as well as the trial of giving two weekly doses of scopolamine. These previous results are consistent with what has been recorded regarding neuro-behavioral differences, as the level of serotonin is at a critical rate without increase or decrease, and the body's various neurochemical mechanics control the adjustment of these levels with incoming nerve signals (Gulecha *et al.,* 2020). A normal level of serotonin has beneficial effects. For the body, it control the feeling of

happiness and the desire for social communication and natural interaction between animals, and this was observed in control group animals (Wo *et al.,* 2021; Naseri *et al.,* 2023). While the treated groups showed an unprecedented increase in the level of serotonin, this confirms the reliability of the results of neuro-behavioral tests and the appearance of anxiety and tension in the animals, and indicates the role of the mechanism of action of scopolamine in preventing the selective capture of serotonin from the synaptic cleft, which leads to the appearance of high concentrations of it in the plasma (Taheri Zadeh *et al.,* 2021).

While the results of the experiment of giving a single dose and then taking the measurement after 5 days was that there was no significant difference in the level of serotonin, and this confirms what was previously explained, that the drug was metabolized and disappeared from the blood 5 days after the first dose (Wo *et al.,* 2021; Naseri *et al.,* 2023). When measuring the level of acetylcholine, it was found that there was a difference in its concentration in the form of a significant decrease compared to the control group in the sub acute trials of 2 and 4 mg/kg for a period of 2 and 4 weeks, as well as in the trial treated with the acute single dose of 20 mg/kg, then the measurement was performed after 24 hours, as well as the trial. given two weekly doses of scopolamine. These effects are a reflection of what was not observed in neuro-behavioral tests, as acetylcholine is a neurotransmitter that has an effective role in the motor activity and nervous behavior of the animal, and each increase or decrease in its level has a different behavioral reflection, as its increase leads to an increase in motor activity and an increase in the number of squares. Its increase often indicates the presence of inhibition of cholinesterase yeast, which is responsible for its breakdown (Gulecha *et al.,* 2020; Gott *et al.,* 2024). While its decrease causes lethargy in the animal, muscle weakness,

and lack of motor activity, and may be suppressed by an increase in the activity of cholinesterase yeast, which acts as a neutralizer. Its effectiveness (Naseri *et al.,* 2023).

In our experiments, the role of scopolamine had the greatest role in influencing this neurotransmitter, as one of the mechanisms of scopolamine is to compete in binding to the muscarinic receptors and prevent them from binding to the acetylcholine transporter, and at the same time it affects the activation of acetylcholine yeast, causing a decrease in its concentration in the body (Chen, 2023). While the effect of scopolamine on the level of acetylcholine was insignificant 5 days after giving a single dose, this indicates that the activity of neurotransmitters returned to its level after leaving treatment with low doses for 5 days as a result of the decrease in the drug in the plasma (Wo *et al.,* 2021).

To search for one of the possible mechanisms for the effect of different neurotransmitters when giving scopolamine, the enzyme COMT, which is responsible for breaking down some neurotransmitters such as epinephrine, norepinephrine, and serotonin, was measured. It was found that the relationship exists, as a decrease in the level of this enzyme was shown in many sub acute experiments at 2 and 4 mg/kg and for periods of 2 and 4. Weeks, as well as in the experiment of treating with a single acute dose of 20 mg/kg, then measuring after 24 hours, as well as the experiment of giving two weekly doses of scopolamine.

The deficiency or inhibition of COMT leads to a significant increase in some neurotransmitters such as serotonin, epinephrine, and norepinephrine, and these changes have neuro-behavioral repercussions, as was observed in our previous neuro-behavioral experiments (Khushboo *et al.,* 2022).

The level of COMT is involved in catecholamine pathways (Guzmán *et al.,* 2022; Tavares, 2022). These previous results indicate that scopolamine, as has been proven in previous studies, has an effect on many neurotransmitters with complex and overlapping mechanisms that require many studies to determine the underlying causes of these effects (Guzmán *et al,* 2022; Khushboo *et al.,* 2022; Tavares*,* 2022).

Chapter Six: Conclusions and Recommendations

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6-1: Conclusions

1. Scopolamine, with an LD_{50} of 148 mg/kg I.M, affects higher brain functions, the autonomic nervous system, and cognitive and memory functions at doses of 2 and 4 mg/kg in sub-acute treatments lasting 2-4 weeks.
2. A single acute dose of scopolamine 10 & 20 mg\kg has a strong but temporary effect, while daily low doses maintain a steady drug level, leading to sustained effects.
3. Acute treatment with a 20 mg/kg dose has a more significant impact than a 10 mg/kg dose after 24 hours, as observed in various behavioral tests.
4. A high acute dose's impact after 24 hours is less than that of daily low doses over 2-4 weeks due to differences in pharmacodynamics and pharmacokinetics.
5. The toxicological effects of 2 and 4 mg/kg doses disappear 5 days after stopping treatment, but persist when administered twice a week, highlighting the importance of dosage and duration.
6. Sub-acute treatment with scopolamine increases serotonin levels and decreases acetylcholine and COMT enzyme levels, suggesting long-term modulation of neurotransmitter systems and lasting effects on neurological functions.

6-2: Recommendations

1. Exploring the biochemical mechanisms affecting neurotransmitter systems, with a particular focus on serotonin levels, acetylcholine, and COMT enzyme.
2. Examining the impact of repeated low-dose scopolamine treatment on neuroplasticity and brain adaptation over time.
3. Study the withdrawal effects and recovery processes after discontinuing scopolamine treatment.
4. Exploring the role of genetic variability in the response to scopolamine treatment. Studies could involve different animal strains or genetically modified models to identify genetic factors that influence drug efficacy and toxicity.
5. Evaluate neuroprotective agents or interventions that can be co-administered with scopolamine to reduce its negative effects on the brain and neurotransmitter systems.
6. Study the effects of different administration routes (such as oral, intraperitoneal, transdermal) on the Toxicokinetics of scopolamine.

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Appendices

Appendices

Table 1. Median lethal dose (Dixon, 1980).

The second part of the series	K: Represent the test series that begins as follows					Standard error (LD-50)
	O	OO	OOO	OOOO		
XOOO	0.157 -	0.154 -	0.154 -	0.154 -	OXXX	σ 0,61
XOOX	0.878 -	0.861 -	0.860-	0.860 -	OXXO	
XOXO	0.701	0.737	0.741	0.741	OXOX	
XOXX	0.084	0.169	0.181	0,182	OXOO	
XXOO	0.305	0.372	0.380	0.381	OOXX	
XXOX	0.305 -	0.169 -	0.144 -	0.142 -	OOXO	
XXXO	1.288	1.500	1.544	1.549	OOOX	
XXXX	0.555	0.897	0.985	1,000	OOOO	
	X	XX	XXX	XXXX	The second part of the series	
	K: Represent the test series that begins as follows					

الخلاصة

دراسة التأثيرات السمية السلوكية العصبية الحادة وتحت الحادة للسكوبلامين من خلال تقييم وظائف الدماغ العليا باستخدام اختباري الميدان المفتوح والتفاعل الاجتماعي للفئران و تقييم وظائف الجهاز العصبي اللاإرادي بإجراء اختبارات السباحة واختبار الضوء والظلام وتقييم الوظائف الادراكية باستخدام اختبارات الانتحاء الأرضي السالب و اختبار ادخال الراس في الثقوب وأخيرا الوقوف على بعض الاليات الكامنة للتأثيرات السلوكية العصبية المسجلة في الفئران من خلال قياس بعض النواقل العصبية مثل السيروتونين والاستيل كولين وانزيم COMT.

استخدم لهذه الدراسة ذكور الفئران السويسرية البيضاء بعدد تقريبي يعادل 85-90 فأرا ربيت في بيت الحيوان التابع لكلية الطب البيطري جامعة الموصل، كانت اوزان الفئران 25-35 غرام وبأعمار تراوحت 2 شهر. اوجدت الجرعة المميتة الوسطية للسكوبولامين عند الحقن العضلي وكانت 148 ملغم\كغم.

تمت دراسة تأثير الزمن والجرعة على الوظائف العصبية السلوكية من خلال سلسلة تجارب. شملت التجارب إعطاء جرع منخفضة بشكل يومي لمدة أسبوعين وأربعة أسابيع في التجربتين الثانية والثالثة على التوالي، وقياس تأثير جرعتين حادتين بعد 24 ساعة في التجربة الرابعة، واختبار التأثير التراكمي لجرعة مفردة بعد 5 أيام في التجربة الخامسة. وللتأكيد، أعطيت جرع مفردة مرتين أسبوعياً في التجربة السادسة. استخدم في كل تجربة 15 فأراً مقسمة إلى 3مجاميع عشوائية (5 فئران لكل مجموعة): مجموعة سيطرة حقنت بالماء المقطر، ومجموعتين حقنتا بالسكوبولامين عضلياً. شملت الاختبارات المقاسة النشاط الحركي والسلوك العصبي (اختبار الميدان المفتوح، اختبار التفاعل الاجتماعي، اختبار السباحة، اختبار الانتحاء الأرضي السالب، اختبار الصفيحة المثقبة، واختبار الضوء والظلام). بعد نهاية كل تجربة، تم تخدير الحيوانات وسحب الدم لفصل البلازما واستخدامها في اختبارات كيموحيوية لقياس النواقل العصبية مثل السيروتونين، الأسيتيل كولين، وإنزيم COMT.

كانت قيمة LD_{50} 148 ملغم /كغم عند الحقن بالعضلة. أظهرت الاختبارات العصبية السلوكية للفئران بعد الجرع اليومية من السكوبولامين بجرعة 2 و4 ملغم/كغم لمدة أسبوعين زيادة في عدد مرات الوقوف في مجموعة 2 ملغم/كغم مقارنة بمجموعة السيطرة، ولكنها لم تكن ذات دلالة إحصائية، وكانت الزيادة في مجموعة 4 ملغم/كغم كبيرة .($p \leq 0.05$) انخفض عدد المربعات المقطوعة بشكل ملحوظ في مجموعتي السكوبولامين مقارنة بمجموعة السيطرة، كما انخفض

التفاعل الاجتماعي بشكل ملحوظ في كلا الجرعتين مقارنة بمجموعة السيطرة. في اختبارات الجهاز العصبي اللاإرادي، انخفضت درجات السباحة بشكل ملحوظ في كلا الجرعتين ($p \leq 0.05$)، وزاد وقت الانتحاء الأرضي السالب بشكل ملحوظ، وانخفضت عدد مرات إدخال الرأس في الثقوب في اختبار اللوحة المثقبة بشكل ملحوظ في كلا الجرعتين مقارنة بمجموعة السيطرة. الوقت الذي قضته الفئران في الظلام انخفض بشكل ملحوظ في مجموعة 4 ملغم/كغم ($p \leq 0.05$) ولم يكن هناك فرق بين جرعة 2 ملغم/كغم ومجموعة السيطرة.

بعد 4 أسابيع من الجرع اليومية للسكوبولامين، زاد نشاط الوقوف بشكل ملحوظ في كلا الجرعتين مقارنة بمجموعة السيطرة، وكان الزيادة أكثر وضوحًا في الجرعة الأعلى. انخفض عدد المربعات المقطوعة بشكل ملحوظ في كلا الجرعتين، وكان الانخفاض أكثر وضوحًا في الجرعة الأعلى. انخفض التفاعل الاجتماعي ودرجات السباحة بشكل ملحوظ، وزاد وقت الانتحاء الأرضي السالب، وانخفضت عدد مرات إدخال الرأس في اللوحة المثقبة بشكل ملحوظ في كلا الجرعتين مقارنة بمجموعة السيطرة. الوقت الذي قضته الفئران في الظلام انخفض بشكل ملحوظ في كلا الجرعتين مقارنة بمجموعة السيطرة.

أظهرت نتائج التجربة الثالثة أن إعطاء جرعة عالية مفردة حادة من السكوبولامين (20 ملغم/كغم) زاد عدد مرات الوقوف بشكل معنوي مقارنة بمجموعة السيطرة، مع انخفاض معنوي في عدد المربعات المقطوعة والتفاعل الاجتماعي. كما سجلت المجموعة انخفاضاً معنوياً في مرتبة السباحة وزمن الانتحاء الأرضي السالب، وانخفاضاً في عدد مرات إدخال الرأس في الثقوب مقارنة بمجموعة السيطرة. لم يكن هناك فرق معنوي بين الجرعتين في هذا الاختبار. كان البقاء في الظلام منخفضاً بشكل معنوي في مجموعة 20 ملغم/كغم مقارنة بمجموعة السيطرة.

اما في تجربة إعطاء الجرع المفردة 2 و4 ملغم/كغم ثم ترك الحيوانات لمدة 5 أيام وبعدها اخذ القياسات السلوكية فلم يظهر أي تأثير معنوي للجرع المستخدمة بهذه المدة.

في تجربة إعطاء جرعتين منخفضتين من السكوبولامين أسبوعياً، لم يكن هناك فرق في عدد مرات الوقوف بين مجموعة السيطرة ومجموعة 2 ملغم/كغم. سجلت مجموعة 4 ملغم/كغم زيادة معنوية في نشاط الوقوف مقارنة بمجموعتي السيطرة و2 ملغم/كغم. كما سجلت مجموعة 4 ملغم/كغم انخفاضاً معنوياً في عدد المربعات المقطوعة ودرجة التفاعل الاجتماعي ودرجة السباحة وزمن الانتحاء الأرضي السالب مقارنة بالمجموعتين الأخرى. لم يكن هناك فرق معنوي في عدد مرات إدخال الرأس بين مجاميع السيطرة و2 ملغم/كغم و4 ملغم/كغم. سجل وقت البقاء في الظلام انخفاضاً معنوياً في مجموعة 4 ملغم/كغم مقارنة بمجموعة السيطرة.

أظهرت الاختبارات الكيميائية أن الجرعة العالية الحادة للسكوبولامين الفردية أدت إلى ارتفاع في مستويات السيروتونين، وانخفاض في مستويات الأسيتيل كولين، وانخفاض في نشاط COMT بعد مرور 24 ساعة. ومع ذلك، لم تظهر أي تغييرات في النواقل العصبية عند إعطاء جرعة واحدة وقياسها بعد 5 أيام. في الوقت نفسه، أدت إعطاء جرعتين في الأسبوع إلى تغييرات كبيرة في مستويات السيروتونين والأسيتيل كولين، وكذلك في نشاط COMT مقارنة بمجموعة السيطرة.

اثرت كل من جرعتي السكوبولامين (2 و4 ملغم/كغم) في التجارب تحت الحادة والحادة بجرعة 20 ملغم/كغم بشكل كبير على سلوك الفئران، ووظائف الإدراك، واستجابات الجهاز العصبي اللاإرادي مقارنة بمجموعة السيطرة. الجرع الأعلى عمومًا أظهرت تأثيرات أكثر وضوحًا، على الرغم من أن الفروق بين الجرع في نفس التجربة كانت في كثير من الأحيان غير معنوية. تشير البيانات إلى أن السكوبولامين يسبب تغييرات في السلوك وتدهورًا في الإدراك وسلوكيات مرتبطة بالقلق لدى الفئران. كما أظهر إعطاء السكوبولامين بجرعة كل يومين لمدة أسبوع تأثيرًا ملحوظًا على سلوك الفئران، حيث أن الجرعة الأعلى (4 ملغم\كغم) أظهرت تأثيرات أكثر وضوحًا على مختلف المعايير مقارنة بالجرعة الأقل (2 ملغم\كغم) ومجموعة السيطرة. أخيرًا، أظهرت الاختبارات الكيميائية زيادة ملحوظة في مستويات السيروتونين وانخفاض ملحوظ في مستويات الأسيتيل كولين ونشاط COMT مع كل من الجرع اليومية للسكوبولامين لمدة أسبوعين وأربعة أسابيع، والجرعة الحادة بمقدار 20 ملغم.

تقييم التأثيرات السلوكية-العصبية للسكوبولامين في ذكور الفئران

رسالة تقدمت بها

ميسم محسن عباس قنبر المصطفى

إلى

مجلس كلية الطب البيطري في جامعة الموصل

وهي جزء من متطلبات نيل شهادة الماجستير

في اختصاص الطب البيطري / الادوية والسموم البيطرية

بإشراف

الأستاذ المساعد الدكتورة

يمامة زهير صالح العبدلي

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جامعة الموصل
كلية الطب البيطري

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