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College of Veterinary Medicine

# Evaluation of the pharmacological effects of Serratiopeptidase on the central nervous system in mice

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**Supervised by**

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**2025 A.D.** **1447 A.H.**

# Evaluation of the pharmacological effects of Serratiopeptidase on the central nervous system in mice

A thesis
Submitted by

**Younes Masoud Abdul Hameed Farhan**

To
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Supervised by
Assistant Professor

**Dr. Ahmed Salah Naser**

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بسم الله الرحمن الرحيم

# وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ
# عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ

(صدق الله العظيم)

سورة هود/الآية ٨٨

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YOUNES M. ALHAMEED

# Abstract

The study aimed to evaluate the neurobehavioral, anxiolytic, antidepressant, anticonvulsant, physiological effects and its effect on general anesthesia of Serratiopeptidase, a proteolytic enzyme, then utilizes a veterinary patient monitor accompanied by continuous monitoring and tracking of vital signs and physiological parameters in mice following single and repeated oral administrations. The research has employed a series of short- and long-term behavioral and physiological tests to evaluate dose-dependent responses at 5 mg/kg, 10 mg/kg, and 20 mg/kg doses, with comparisons to negative (distilled water) and positive controls (sertraline or diazepam). Key findings have demonstrated Serratiopeptidase's multifaceted pharmacological potential, particularly at higher doses, notable impacts on locomotion, anxiety-like behaviors, depression-related immobility, convulsion modulation, and vital organs function under anesthesia.

In the open field test (OFT), a single-dose Serratiopeptidase significantly increases horizontal locomotion (e.g., 93.6 ± 3.52 squares crossed at 20 mg/kg vs. 13.2 ± 1.71 in control, $P \leq 0.05$) and vertical activity (rearing), suggesting enhanced exploratory behavior. Repeated administration over 15 days further has increase horizontal locomotion (96.13 ± 6.65 squares crossed at 20 mg/kg) and vertical exploration (24.25± 1.68 rearing), indicating sustained motor stimulation. The negative geotaxis test (NG) revealed delayed 180° rotation times after a single 5 mg/kg dose (12.6 ± 5.31 seconds vs. 9.8 ± 1.11 in control), while repeated dosing at 20 mg/kg reduced this time (4.80 ± 1.20 seconds), reflecting improved vestibular function. Head pocking test, a measure of curiosity, increased in both single and repeated administration (the repeated doses, 12.20 ± 1.71 counts at 20 mg/kg vs. 10.40 ± 2.83 in control).

In the elevated plus maze test (EPM), a single-dose of Serratiopeptidase at (20 mg/kg) has shown a significant increase in amount of time mice spent in the open arms (187.40 ± 23.92 seconds vs. 111.40 ± 5.97 in negative control, $p \leq 0.05$) and a reduced close-arms entries, indicative of reduced anxiety. Repeated administration reinforced this trend with the 20 mg/kg group spending (120.60 ± 29.79 seconds) in the open arms, surpassing even Sertraline (99.00 ± 20.72 seconds). The light/dark box test has corroborated these findings; the 20 mg/kg dose increases light-side exploration (198.40 ± 26.23 seconds vs. 157.00 ± 19.69 in negative control) superiority over Sertraline, and reduces dark-side preference, highlighting robust anxiolytic effects. Even after 15 days of administration, a significant reduction in anxiety was detected with a preference for periods of staying on the light side, and the opposite on the dark side.

In acute administration, a single dose of SRP at 10 mg/kg has decreased the immobility time of mice in the tail suspension test (TST) by 7% (25.50 ± 9.90 seconds vs. 62.33 ± 21.94 in negative control), outperforming sertraline. However, a biphasic response has emerged at 20 mg/kg (43.50 ± 17.92 seconds). In the repeated doses, 10 and 20 mg/kg by (11%) vs (21% and 22% in controls, respectively). Similarly, the forced swimming test (FST) reveals immobility reduction at 5 mg/kg (15%) and 20mg/kg (14%), though the 10mg/kg group has paradoxically increased immobility by 30%. Administration over 15 days, both 10 and 20 mg/kg doses has demonstrated sustained antidepressant efficacy, reducing TST immobility to (11%) vs. (21% and 22% in controls, respectively) and FST immobility to (6-7%) vs. (29% in negative control), compatible to sertraline.

Repeated administration of Serratiopeptidase has delayed the onset of pilocarpine-induced convulsion in a dose-dependent manner (24.25 ± 0.5 minutes at 20 mg/kg vs. 8.00 ± 2.0 in control, $p \leq 0.05$) and has reduced

convulsion frequency (3.5 ± 0.3 vs. 10.3 ± 2.1 in control). Survival rates have improved to (100%) at 10-20 mg/kg, matching diazepam's efficacy.

Serratiopeptidase has prolonged anesthesia onset (147.40 ± 8.13 seconds at 5mg/kg vs. 72.88±13.50 in control) and duration (192.25 ± 32.81 minutes at 5 mg/kg vs. 119.50 ± 7.22 in controls). Similarly, at (10 and 20mg/kg). Recovery time has increased, peaking at 77.9 ± 14.90 minutes (20 mg/kg). Vital organ monitoring has revealed enhanced oxygen saturation, peaking (97.50 ± 0.42 at 20 mg/kg vs. 86.50 ± 4.21 in control), elevated respiratory rates (19.8 ±2.9 breaths/min at 20 mg/kg vs. 11.0 ± 0.4 in control), and reduced heart rates (83.3 ± 3.2 bpm at 20 mg/kg vs. 101.5 ± 15.2 in control), suggesting an improved oxygen saturation and a mild cardiorespiratory improvement.

In conclusion, Serratiopeptidase exhibits broad-spectrum neuroactive properties, demonstrating anxiolytic, antidepressant, and anticonvulsant efficacy comparable to or superior to standard therapies. Its ability to enhance exploratory behavior, delay convulsions, modulate anesthesia, and improve physiological parameters underscores therapeutic potential for neurological and psychiatric disorder. However, biphasic responses in acute antidepressant tests and variability in higher-dose effects warrant further mechanistic and clinical exploration. These findings position Serratiopeptidase as a promising candidate for repurposing in neuropharmacological, meriting advanced study to optimize dosing and elucidate the molecular pathway.

# Table of Contents

# Lists of Figures

# List of Tables

# List of Abbreviations

| Abbreviation | Full Expression |
|---|---|
| SP/SRP | Serratiopeptidase |
| OFT | Open field test |
| NG | Negative geotaxis test |
| HP | Head pocking test |
| ST | Swimming rank test |
| EPM | Elevated plus maze test |
| LADB | Light and dark box test |
| TST | Tail suspension test |
| FST | Forced swimming test |
| M.W | Molecular weight |
| 5-HT1AR | 5-Hydroxytryptamine 1A receptor |
| CNS | Central nervous system |
| $SpO_2$ | Peripheral capillary oxygen saturation |
| E-15 | Strain bacteria that produce the enzyme |
| EC | Enzyme commission number |
| Asn | Asparagine |
| Gln | Glutamine |
| CysSO3H | Cysteine sulfate |
| Gly | Glycine |
| Arg | Arginine |
| Tyr | Tyrosine |
| His | Histidine |
| Leu | Leucine |
| Ala | Alanine |
| Phe | Phenylalanine |
| Thr | Threonine |
| IL | Interleukin (Cytokine) |
| SSRIs | Selective serotonin reuptake inhibitors |
| CVD | Cardiovascular Disease |
| AchE | Acetylcholinesterase |
| BDNF | Brain-Derived neurotrophic factor |
| AD | Alzheimer's disease |
| GABA | Gamma-Aminobutyric acid |
| COX | Cyclooxygenase |
| TNF-α | Tumor necrosis factor alpha |
| NMDA | N-Methyl-D-Aspartate (Receptor) |
| 5-HT | 5-Hydroxytryptamine (Serotonin) |
| GluN2B | Glutamate (NMDA) receptor subunit |

# CHAPTER ONE

## INTRODUCTION

# Chapter One
# Introduction

## 1-1: Introduction

Serratiopeptidase (SP/SRP) is a potent proteolytic enzyme widely recognized for its ability to reduce inflammation. Also known as Serrapeptase, which is generated by the silkworm associated with the bacterium genus *Serratia marcescens* (Luthra *et al.*, 2022). Since the 1960s, when the Japanese first discovered the silk worm-derived Serratiopeptidase EC 3.4.24.40, it has been employed in modern medicine for potent anti-inflammatory properties with a molecular weight (M.W.) of approximately 52 kilodaltons. (Gupte and Luthra, 2017).

The existence of a zinc atom within its form improves Serratiopeptidase's proteolytic action. SRP optimum activity at pH 9.0 and 40°C, and is completely inactivated at 55°C, with an M.W. ranging from 45-60 kilodaltons (Ethiraj and Gopinath, 2017). Serratiopeptidase has been observed to be susceptible and sensitive to acid hydrolysis at gastric pH, reducing its stability. Therefore, when administered orally in its enteric-coated form, it is absorbed and dissolved in the alkaline pH of the intestine and then distributed through the lymph and plasma. The drug's peak plasma concentrations are within 15 to 30 minutes, with a duration of action (6 hours) in animal. However, the effects of Serratiopeptidase are dose-dependent. The brand name of Serratiopeptidase is Amidase® (Jothieswari *et al*., 2023).

Serratiopeptidase has therapeutic effects including anti-inflammatory, as well as mucolytic, analgesic, thrombolytic and antibiofilm(Anil and Kashinath, 2013; Nair and Devi., 2022).

Serratiopeptidase (SP), has been used in veterinary medicine as a therapeutic agent because it acts as an anti-edematous, fibrinolytic, and anti-inflammatory molecule (Mutzberg, 2021; Anwar *et al*., 2024). On the other hand, there are proven benefits of this enzyme through oral administration in dogs and also, has been reported to be applied in cats due to its anti-inflammatory and analgesic properties; Serratiopeptidase being an interesting alternative for the treatment of these animals without exposing them to additional adverse effects (Salvo *et al*., 2021; Haidary *et al*., 2022). It's worth mentioning that widespread use in avian acts as anti-inflammatory by decreasing the fluids produced by an injury and facilitating their outflow, it may lessen inflammation, effective for respiratory function in treating sinusitis by decreasing mucus secretion and clearing damaged scar tissues, as analgesic by stopping the release of "amines" responsible for pain, this could potentially diminish pain. Thrombolytic and fibrinolytic effects in animal, help improve the cardiovascular system without damaging the inside of the arteries due to dissolving clots forming in the blood, arterial plaque and fibrosis that is a byproduct of the healing process without damaging healthy tissue (Ahmed *et al.*, 2014; Gupte and Luthra, 2017).

Sertraline (SSRIs), is an FDA-approved treatment for both anxiety and depression (Cipriani *et al.*, 2010). It inhibits serotonin reuptake and increases serotonin stimulation of the brain's 1A receptor (5-HT1AR) (Istifli *et al.*, 2018) and has been used in clinical animals' trail (Kurt *et al*., 2000; Sitges *et al*., 2012).

Pilocarpine is a well-known pharmaceutical agent used in the treatment of xerostomia and glaucoma in human (Kinney *et al.*, 2020). It is a muscarinic receptor agonist in animals (Grasing *et al*., 2019)and is

employed in clinical trials to induced seizures in animals used in laboratories (Vezzani, 2009).

The study aims to evaluate the pharmacological effects of Serratiopeptidase on the CNS in mice through the following:

1-Assessing short and long-term neurobehavioral effects involving exploratory behavior, locomotor activity, motor coordination, curiosity, and neuromuscular performance (motor endurance and muscle strength).

2-Assessing short and long-term antianxiety effect.

3-Assessing short and long-term antidepressant effect.

4-Assessing long-term anticonvulsant effect.

5-Assessing its effects on general anesthesia, accompanied by continuously observing and tracking mice's vital signs and physiological parameters such as oxygen saturation ($SpO_2$), respiratory rate, and heart rate monitoring via the multiparameter monitor (veterinary patient monitor). Although widespread use of Serratiopeptidase as an anti-inflammatory and proteolytic enzyme, its neurobehavioral and physiological effects in animal models remain underexplored, warranting further investigation.

# CHAPTER TWO

## LITERATURE REVIEW

# Chapter Two

# Literature Review

## 2-1: Background and History of Serratiopeptidase

Proteolytic enzymes are commonly referred to as proteases. Branching from these enzymes is a specialized group through their expression and classification as a metalloprotease.(Landi *et al.*, 2015; Dhillon *et al.*, 2016). Serratiopeptidase is a proteolytic enzyme produced by the gram-negative *Serratia marcescens* E-15 (*bombyx mori*),(Nageswara *et al*., 2019) .The enzyme is also known as Serratia Peptidase, and Serrapeptase. Its name is systematically named "Peptidase" and the enzyme commission number (EC number) is 3.4.24.40(Mei *et al.*, 2022). However, commercially available products are usually called "Serrapeptase". Serrapeptidase was discovered in the intestinal microflora of silkworms and was isolated and identified in the 1960s(Al-Khateeb and Nusair, 2008; Gupte and Luthra, 2017). Serratiopeptidase was introduced in Japan shortly after its discovery due to its significant anti-inflammatory properties. By the 1960s, injectable enzyme formulations had given way to enteric-coated ones intended for oral use. In the 1980s-1990s, groundbreaking studies from Europe and other countries like Japan revealed that Serratiopeptidase has been identified as the most effective enzyme in decreasing and attenuating inflammation, outperforming all other options. As a result, Serratiopeptidase has become fundamental in treating pain and inflammation for nearly four decades(Gupte and Luthra, 2017).

Although oral administration of Serratiopeptidase has very low toxicity, the medical dosage and pharmacokinetics of Serratiopeptidase in animals have not been conclusively studied(Jadhav *et al*., 2020; Khanwelkar *et al*., 2021)

## 2-2: Chemical and Physical Properties of Serratiopeptidase (SRP)

Serratiopeptidase is a proteolytic enzyme that is secreted during the growth of the enterobacteria, *Serratia* E15, localized in the silkworm's intestine, specifically, in the *Bombyx mori*. Upon entering the intestine of the silkworm, the enzyme is absorbed by the intestine, and biological studies have indicated that this enzyme participates in the prevention of bacterial degradation(Jadhav *et al*., 2020; Koul *et al*., 2021). Serratiopeptidase structure was predicted and validated through being compared to the neutral protease (thermolysin and *Bacillus subtilis* structures). The enzyme has an isoelectric point of 5.3 and it is bind to alpha-2 macroglobulin in bodily fluids. Serratiopeptidase in the blood has a binding ratio about 1:1, indicating that each enzyme molecule binds to a single plasma protein molecule. These are helping it to conceal its antigenicity while maintaining its activity.(Ethiraj and Gopinath, 2017). Serratiopeptidase is one of metalloprotease that containing amino acids about (470). All are important for their proteolytic activity. Notably, it lacks cysteine and methionine (sulfur group). In-vitro, SRP activity is optimally at pH 9.0 and 40°C, but is totally inactivated at 55°C after fifteen minutes (Kumar, 2018).

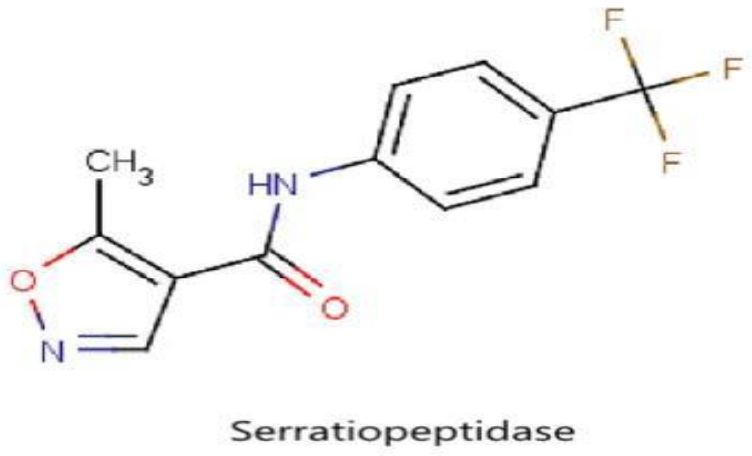


Figure 1: Chemical Structure of Serratiopeptidase (Kumar *et al*., 2023)

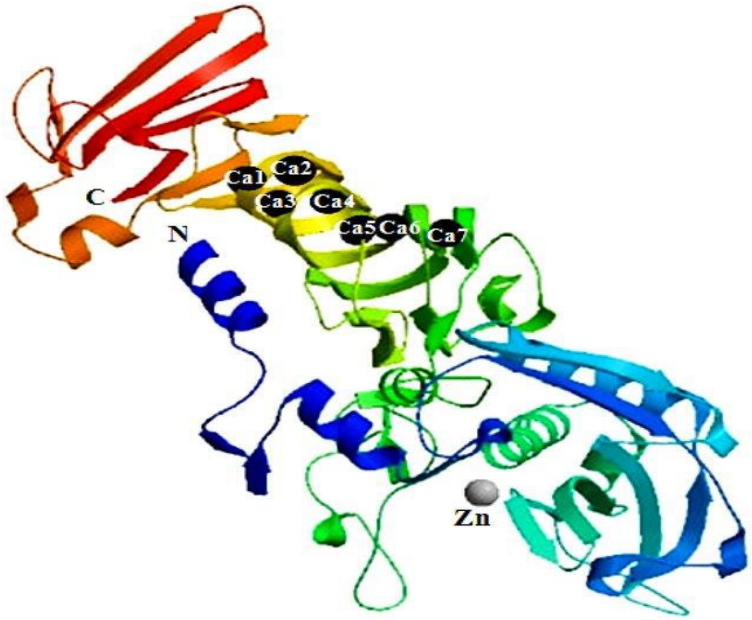


Figure 2: A 3D dimension of Serratiopeptidase(Chander *et al.*, 2021)

## 2-2-1: Enzymatic Activity and Substrate Specificity

Serratiopeptidase, better known as "serralysin" and "serratiapeptase," is a protease produced by *Serratia* spp., a pathogenic group of bacteria that infects various organisms. Although these bacteria produce a large number of complex proteases, none are as potent as *Serratia marcescens*-derived serralysin(Sharma *et al*., 2021; Melchor *et al*., 2024). Serratiopeptidase is synthesized by the fibrinolytic enzymes of the enterobacterium *Serratia marcescens* and is considered to be one of the most traditionally used immunomodulatory drugs in human medicine(Sharma *et al*., 2021). At the industrial level, serrapeptase is obtained from bacteria of the genus *Serratia*, and after being produced, it is extracted from the bacterial culture and purified. From a chemical point of view, it belongs to the superfamily of metalloproteases, which contains zinc in its active sites, allowing the breaking of peptide bonds, specifically the “CysSO3H-Gly, Asn-Gln, Arg-Gly, and Tyr-Tyr as along with the bond between His-Leu, Gly-Ala, Ala-Leu, Tyr-Leu, Gly-Gly, Phen-Tyr, and Tyr-Thr” that determine the primary specificity(Verma, 2020). Serratiopeptidase has a widespread

specificity, and (Gly, Arg, His, Leu, and Phe) are the favored amino acids linked to the mentioned sub-sites. It was discovered that the favored split position (Gly-Phe) and (Serine-His196) are associated with bradykinin (Kaviyarasi *et al*., 2016). Serratiopeptidase is a potent proteolytic enzyme that exhibits very specific substrate abilities. However, the rate of hydrolysis of peptide bonds might vary within the different amino acid compositions(Srivastava *et al*., 2019; Kumar *et al*., 2023). SRP is soluble in water, but insoluble in alcohol(Kulkarni *et al.*, 2022).

## 2-3: Pharmacokinetics

The limited information available about the pharmacokinetic profile of SRP has been obtained from human and laboratory animal studies, often performed using non-validated analytical methods. Nevertheless, SRP, either in pure or pharmaceutical formulations, has been administered to dogs, rabbits, and rats in compliance with ethical and scientific rigour, demonstrating its safety and pharmacological effects(Misraulia *et al*., 2013; Al-Sailawi *et al*., 2024; Mushtaq *et al*., 2024).

Serratiopeptidase has several challenges, such as its limited solubility, susceptibility to pH and temperature fluctuations, and propensity for self-cleavage, all of which could impair its catalytic effectiveness and therapeutic potential(Melchor *et al*., 2024). In general, there have been fears regarding these enzymes being digested or bio-transformed in the stomach, and the quantity of active enzyme accessing the circulatory system and the location of inflammation in the body, a capable of crossing through the GI tract's mucosal barrier and accessing the blood and lymph with unaltered, very large molecules and physiologically functional states (Shete *et al*., 2022).

Serratiopeptidase is an acid-labile drug, and therefore has low stability in the GI tract, although claimed to be effective when taken orally, its

relatively large molecular size leads to low bioavailability(Narayan Hire *et al.*, 2014). The enzyme is sensitive to acidity and it is reasonable that it can be destroyed by acid in the stomach if not shielded. In contrast, enteric-coated pills allow the enzyme to pass preceding the stomach unharmed, and enabling it to be absorbed in the intestine. The presence of Serratiopeptidase in small quantities in urine indicates that the enzyme gets transferred directly from the colon to the circulatory system(Rakshe *et al*., 2022).

**2-3-1: Absorption**

In animals, the absorption of orally administered Serratiopeptidase depends upon the preparation, formulation, the gastrointestinal conditions, and the animal species. Immediately following oral administration of Serratiopeptidase, it is absorbed into the bloodstream, crossing the intestine without undergoing any significant change. Through this process, the enzyme accumulates at elevated levels within inflamed tissues, reaching its maximum concentration approximately one hour after administration. However, due to the fact that the enzyme is a peptide, it faces enzymatic degradation in the GI tract, reducing its ability to penetrate cellular membranes, particularly considering that peptides and proteins are in general hydrophilic. As a result, these factors cause the bioavailability of the enzyme to be reduced when it is in a therapeutic used (Bhagat *et al*., 2013; Tiwari, 2017). Following oral administration, differently formulated Serratiopeptidase products have retained their proteolytic activity in rat serum, indicating early onset of absorption (Yang *et al.*, 2018). The pH- and bile-dependent absorption following oral administration leads to variable serum protease activity time curves and, hence, the effect to retard local and systemic absorption by formulating enteric-coated Serratiopeptidase was tested. (Panthi *et al.*, 2021). However, when administered orally, Serratiopeptidase has different challenges due to the

high likelihood of enzymatic degradation within the GI tract (Kulkarni *et al.*, 2022).

### 2-3-2: Distribution

Post-absorption, the enzyme distributes throughout the tissues. Where it has been found in plasma at concentrations greater than 30 mg/kg and in lymph at concentrations greater than 1 mg/kg after oral administration according to the study conducted on mice(Bhagat *et al*., 2013). Enzyme levels in plasma and lymph are varied according to the amount provided. The highest concentration in the plasma has been attained (15 to 30 minutes) after the treatment, and the duration has been six hours. In the research investigation referred a dosage at (100 mg/kg) produced maximum plasma and lymph concentrations (Bhagat *et al*., 2013). Serratiopeptidase is distributed efficiently to numerous tissues, becoming bioavailable in both lymph and plasma. Then, the enzyme binds in the bloodstream to (alpha-2-macroglobulin). As a result, it is completely free of allergenic effects while preserving its activity as enzyme at both of the systemic and cellular levels (Sharma *et al*., 2021).

Serratiopeptidase is generally accumulated at higher levels in the liver and kidney because of its therapeutic action related to inflammation. Besides the liver and kidney, the enzyme accumulates in various animal organs, including inflamed connective tissues, at different levels of serum or plasma concentration in avian(Abd El-Hamid *et al*., 2014). Furthermore, Serratiopeptidase enzyme has limited membrane permeability in the gut due to the nature of this enzyme as hydrophilic, which requires the oral intake of a very high dosage to produce considerable anti-inflammatory effects (Panthi *et al.*, 2021)

### 2-3-3: Metabolism

In animals, the active form of Serratiopeptidase remains unchanged, and the levels of this enzyme in inflammatory tissues are much higher than in plasma, this process follows its entry into the circulation. In rat blood, the enzyme with “plasma protease inhibitor alpha-1 macroglobulin” produces a complex, and binding proportion of the molarity approximately 1:1. However, in humans both of the particular doses are needed to provide treatment efficacy and the pharmacokinetic details such as the drug's oral bioavailability are published anywhere else(Bhagat *et al*., 2013).

Serratiopeptidase is degraded and eliminated via metabolic pathway in animals, including humans. It is evident that tryptophan and phenylalanine serve as substrates for numerous enzymatic reactions, which result in the structural modification of Serratiopeptidase. The metabolic routes may bring about the formation of either active or inactive intermediates, which can influence the drug concentration at the site of action, thereby contributing to its overall activity. Endogenous metabolizing enzymes that can bioactivate and/or inactivate Serratiopeptidase are present in various animal tissues. Species differences in the relative rates of metabolism through bioactivation and/or bioinactivation of the enzyme could be responsible for interspecies differences in rates of its degradation as well as differences in its therapeutic efficacy. Thus, the knowledge of the enzymes responsible and metabolic pathways may explain target organs for adverse reactions, and the suitability and selectivity of animal species used in safety evaluation studies of Serratiopeptidase(Kumar *et al*., 2023).

### 2-3-4: Excretion

Literature surveys indicate that no considerable work has been done in relation to the excretory aspect of Serratiopeptidase in experimental

animals over the past several years. None of the studies revealed the active transport mechanism involved in the excretion of Serratiopeptidase. In light of the above information, the renal and biliary excretion of Serratiopeptidase in animals is presented(Calogero *et al.*, 2017).

## 2-4: Pharmacodynamics

Serratiopeptidase shows different pharmacological activities that mainly derive from its anti-inflammatory activity. In fact, Serratiopeptidase is the enzyme that comes from the gram-negative bacteria and hydrolyzes proteins of fibrin and others, which have accelerated tissue repair activities and show anti-inflammatory effects resulting from its protease activity(Garg *et al*., 2012; Fadl *et al*., 2013).These properties form the basis for its clinical use to treat inflammations linked to bleeding after surgery, tooth removal, injuries, or fractures and works not only on the site close to its delivery space but is also able to act even intravenously if needed. This is important for post-surgical hemorrhage at a distance from the surgical site. SRP impacts not only through lowering inflammatory molecules, thereby aiding in controlling the influx of inflammatory cells to its affected site, but its most important property is the ability to break down fibrin, which accumulates during the inflammatory processes(Sharma *et al*., 2021) .

Medically, it is produced by nonpathogenic bacteria, and after its production, recycling, and purification, it is capable of performing multiple beneficial pharmacological activities such as decreasing edema, treating infections of the regenerating tissue, and reducing the fibrous compactness in chronic lesions or in the form of scars that result after a wound has healed (Kamenova *et al.*, 2024). By the enzymatic degradation of fibrin covering bacteria, SRP works to successfully combats infections triggered by bacteria that produce biofilms. Its activity enhances antibiotics

accumulation in the infected area as well as boosts the effectiveness of antibiotics significantly. Apart from having a synergistic effect on antibiotics against causative pathogens, SRP can be also administered as an alternative to painkiller and inflammation suppressor medication (NSAIDs), therefore, it would be advisable to use Serrapeptase instead of long-term use of antibiotics in order to reduce the dose and shorten the duration of the same antibiotic and to allow the regrowth of beneficial bacteria(Mecikoglu *et al.*, 2006). This proteolytic enzyme exhibits a one-to-one binding with plasma protein, indicating that each molecule of the enzyme binds to approximately one molecule of plasma protein, primarily alpha-2 macroglobulin found in blood and other body fluids. Its binding conceals its antigenicity(Patel *et al*., 2024).

### 2-4-1: Mechanism of Action

Serratiopeptidase exhibits several mechanisms of action, it is an exceptionally potent inhibitor of bradykinin-induced inflammation that is initially detected in the intestine (Patil and Wagdarikar, 2024). Serratiopeptidase hydrolyses bradykinin, histamine, and serotonin assist in reducing pain and swelling and enhancing microcirculation, thus aiding in the wound healing process, also it allows drainage in inflamed areas by thinning the existing fluid, promoting minimized pain, swelling and tissue repair, SRP speeds up the healing process by exhibiting its special ability to dissolving dead tissue bordering injured area without impacting alive tissue. SRP also organizes inflammatory cytokines and significantly changes cell adhesion molecules, which control the navigation of inflammatory cells to their sites. SRP blood clots dissolving alongside with atherosclerotic plaques is one of the most important mechanisms, this enzyme aids in breaking down fibrin and also targets proinflammatory. It helps prevent the formation of microthrombi in blood vessels, thereby

lowering the risk of thromboembolic events. SRP facilitates mucociliary clearance by reducing neutrophils and changing sputum viscosity. Finally, SRP inhibits forming a biofilm and reduces bacterial adhesion, especially in (*S. aureus, S. epidermidis, and Listeria monocytogenes*), its serves to boost the penetration of antibiotics into the resistant biofilm, raising the response of biofilms to antibiotics(Jadhav *et al*., 2020; Nair and Devi, 2022; Patel *et al*., 2024).

Potential mechanism of action clarifies that Serratiopeptidase shows great affinity for the arachidonic pathway (cyclo-oxygenase I and II), and these are essentially associated with the production of (interleukin, prostaglandin, and thromboxane)(Mauzy, 2021; Nair and Devi, 2022). It is opposite to standard anti-inflammatory medicines since this enzyme cannot bind to lipo-oxygenase. This special mechanism and special affinity refer that it plays a key role in returning tissue to its natural condition, which means keeping the homeostasis (Tiwari, 2017; Nair and Devi, 2022).

**2-4-1-1: Anti-inflammatory Effects**

Inflammation is an important defense mechanism in animal cells against certain damages or infections. An acute inflammatory response is the initial response to various harmful stimuli, production of pro-inflammatory mediators and infiltration and accumulation of neutrophils and macrophages at the inflammatory site which are the main characteristics(Abdulkhaleq *et al.*, 2018). However, chronic inflammation is considered to participate in the development and persistence of various acute diseases and also chronic ones, for example, autoimmune diseases, CVD, some types of tumors, and some degenerative brain diseases(Nigam *et al.*, 2023). In both acute and chronic conditions Serratiopeptidase seems to be useful in treating these conditions, particularly those occurring in the respiratory tract(Nakamura *et al.*, 2003). Serratiopeptidase is used to treat

inflammation instead of standard NSAIDs, and it has been given to minimize swelling and oedema and to promote the reduction of scar tissue, and actively contribute to the healing process of wounds. SRP acts as an anti-inflammatory by modulating cytokines associated with inflammation. It also speeds up the healing because of its ability to breaking-down fibrous tissues surrounding the affected area, all while preserving the integrity of healthy ones(Kamenova *et al.*, 2024). Serratiopeptidase may potentially work by altering cell surface molecule adhesion, thereby directing inflammatory cells towards the area of inflammation (Bhagat *et al*., 2013; Jamal *et al*., 2024) Altering this adhesin serves as an essential part in the progression of arthritis along with various auto-immune illnesses. SRP has no interference with prostaglandin in any of its physiological or functional states; therefore, it is favored in the inflammatory management for example, osteo-arthritis and rheumatoid(Chanalia *et al.*, 2011).

Serratiopeptidase exhibits anti-inflammatory effects in veterinary animals, it effectively treats teat fibrosis in dairy cows by lowering the size of fibrous masses in affected teats, with 80% of the 60 treated animals showing a good clinical response after six days of treatment. SRP can be an effective component of non-antibiotic therapy in cases of teat fibrosis, serving to minimize the size of fibrous masses and enhancing the production of milk from the affected quadrant (Yadav *et al.*, 2018). this indicates that Serratiopeptidase has an anti-inflammatory effect in dairy cows(Nahak *et al*., 2014; Yadav *et al*., 2018; Das *et al*., 2020). According to a rat model, the Serratiopeptidase's anti-inflammatory effect results from a neutrophilic vector. This drug's actions include apoptosis, in addition to the reduction of neutrophils flow to the location of inflammation and also it concurrently reduced of vascular permeability, and clearing of inflammation-related debris(Mammdoh *et al.*, 2022). Another study shows

that SRP somehow promotes resolution of inflammation because it has no impact on the lipo-oxygenase which is in turn catalyzed the production of specialized pro-resolvin mediators (SPMs), crucial participants in resolution (Tiwari, 2017).

**2-4-1-2: Fibrinolytic and Thrombolytic Effects**

Thrombosis of the intravascular and formation of blood clots are the most prevalent reasons of death universally linked with CVD diseases and even strokes. However, fibrinolytic enzymes, commonly referred to as (proteases), are extremely effective in dissolving these clots along with blood circulation restoring without causing any adverse effects. The aforementioned enzymes can additionally be mass-produced at a very low cost(Vachher *et al.*, 2021). As a potent proteolytic enzyme, Serratiopeptidase exhibits remarkable fibrinolytic properties, aiding in the dissolution of the blood clots and plaques associated with atherosclerotic due to its ability breaking down fibrin along with dead and damaged tissue without causing any damaging to healthy and living tissue. It helps prevent the microthrombus fashioning in blood vessels, thereby lowering the risk of thromboembolic events, thrombophlebitis, and other related complications(Jadhav *et al.*, 2020). Serratiopeptidase is capable to degraded the protein that is insoluble referred to as fibrin, which consists of a long fibrous chain-like protein the fact that considering inactive, is referred to as fibrinogen which is a protein with a soluble form that results from the liver and exists in blood plasma. Usually, tissue damage leads to bleeding. Thrombin activity converts fibrinogen to an active form referred to as fibrin at the region of injury. The tissue that is injured or dead is also broken down by SRP, other tissues such as non-living including plaques, mucous, and blood clots. SRP has potability to dissolve and diminish “fatty cholesterol, arterial plaques, among various foreign protein substances”

that remain attached to artery walls. It could potentially be effective in atherosclerosis(Jothieswari *et al*., 2023).

## 2-5: Serratiopeptidase in Clinical Therapy: A Review of Applications, Dosages, and Potential Therapeutics

Serratiopeptidase represents an exciting new category of drugs, while it is a well-known target for inhibition and have already been utilized in clinical practice for numerous decades, it is not commonly regarded as a standalone class of medications(Kadar and PA., 2024). Have potential to address a range of conditions; Cystic and submucosal fibrosis(Artini *et al.*, 2022) post-operative swelling(Krishna *et al.*, 2020; Tamimi *et al.*, 2021; Bapat *et al.*, 2024) sinusitis and bronchitis(Nakamura *et al.*, 2003) carpal tunnel syndrome(Fitzmaurice, 2022), and arthritis(Ateia *et al*., 2018).

### 2-5-1: Clinical Applications in Veterinary Medicine

Serrapeptase is applied in the treatment of inflammatory diseases in diverse animal' species (dog, cat, rabbit, avian and other species) (Misraulia *et al*., 2013; Al-Sailawi *et al*., 2024; Naser and Albadrany, 2024). Serratiopeptidase is given orally septically at a dose of (2 mg/kg) for use in the treatment of oedema and has been shown to greatly decrease the size of oedema in the dog's paw(Misraulia *et al*., 2013). It can be used for the treatment of teat fibrosis in both cattle and Buffalo, helping to enhance milk production and improving overall udder health(Yadav *et al*., 2018). Serratiopeptidase is utilized in avian species, particularly broilers, to enhance growth performance, is used to enhance feed conversion efficiency in infected birds while reducing the inflammation (especially at a dose of two gram/liter). SRP Improve the immune response, thus supporting the effectiveness of newcastle disease vaccination(Ahmed *et al.*, 2014).

Besides, this enzyme is also used for infections, swelling of nasal airways, SRP's ability to modulate sputum viscoelasticity and improve mucociliary transport is advantageous in managing respiratory conditions. In many countries, its clinical evaluation considers inflammatory diseases as a substitution therapy to avoid the risk of using nonsteroidal anti-inflammatory drugs due to side effects(Sharma *et al*., 2021). Unlike the traditional therapy cost, the use of Serrapeptase is relatively cheaper, more efficient and safer. Therefore, Serrapeptase also receives attention in the veterinary animal husbandry field to aim at treating animal inflammatory diseases and infections(Nair and Devi, 2024).

**2-5-1-1: Pain Management**

Pain management is an essential part of animal welfare(Schröter and Mergenthaler, 2021). Many different conditions can cause pain in animals, including infections, trauma, and surgery(Weary *et al.*, 2006). In order to manage pain effectively, we must address its underlying cause. Selective cyclooxygenase-2 inhibitors have robust anti-inflammatory activity and a low risk of gastrointestinal side effects(Ferrer *et al*., 2019). Although NSAIDs control the symptoms of inflammation and pain, while avoiding the use of corticosteroids, although they have fewer side effects, but they have problems of high liver toxicity and low efficacy in many cases(Bindu *et al*., 2020). In many cases, it is important to search for alternative agents; Serratiopeptidase represents a potential alternative for reducing inflammation and increasing antibiotic penetration. Differences between Serratiopeptidase and corticosteroid anti-inflammatory treatment include their therapeutic actions(Tillaeva *et al.*, 2020). SRP diminish pain due to inhibit the release of pain-causing substances known as amines, which play a crucial role in the sensation of discomfort and pain within the animal body(Ahmed *et al.*, 2014). Serratiopeptidase as ointment form applied

topically proved to be beneficial with minimum side effects, it possesses mild analgesic effects in animals (Mammdoh *et al.*, 2022).

### 2-5-1-2: Wound Healing

The process of wound healing is intricate which includes diverse phases, such as hemostasis, inflammation, proliferation, and tissue remodeling(Landén *et al*., 2016). Serratiopeptidase has the potential to promote wound healing in all the four phases, through reforming the skin and reclaiming its temperature in the location of inflammation, it organizes immune cells to flow from the lymph node towards the location of the inflammation and the affected tissue, enhance the microcirculation, suppressing of inflammation and keeping hemostasis(Mutzberg, 2021; Mushtaq *et al*., 2024). The application of SRP enhances and accelerates the healing process in rabbits by optimizing the thickness of the wounds(Rath *et al*., 2011). Serratiopeptidase can be used topically to enhance the healing process, and can be used on rabbits suffering from facial wounds. Topically applied Serratiopeptidase offer the benefit of straightforward access to the site of the affected lesion(Mammdoh *et al*., 2020).

Other possible uses for the Serratiopeptidase that have been referred in the study conducted in rabbits are for skin grafting which is a widely recognized dermatological procedure commonly employed for the heal of wounds resulting from eliminating the defective skin. Serratiopeptidase can be utilized to improve and help the skin grafts, contributing to a rise in the haematological, histological, and aspartate aminotransferase levels in rabbits. (Al-Sailawi *et al.*, 2024).

### 2-5-1-3: Respiratory Conditions

Serratiopeptidase features potent anti-inflammatory effects in airway diseases, including sinusitis, bronchitis(Ammar *et al.*, 2019; Singh *et al.*,

2023). Serratiopeptidase is an effective mucolytic drug that has been used to decrease viscosity related to sputum and facilitate the cleaning of the mucociliary while also decreasing infection and inflammation. Hence relieving signs like cough and congestion are typically associated with respiratory disorders. All of these makes Serratiopeptidase aid in ameliorating hygiene in the airway and help preventing complications(Sharma *et al*., 2021). Serratiopeptidase tends to be active in case of infections that occur in the lung by removing mucus, which causes to spontaneous repair of the body tissue in a way of remodeling dead tissues with those that are healthier, allowing lung functions to return to normal(Uzair *et al.*, 2022). The inclusion of Serratiopeptidase in the therapeutic regimen for the treatment of bronchopneumonia has shown promising results in reducing clinical signs and increasing weight gain(Amin *et al*., 2019). Therefore, Serratiopeptidase can be a good therapeutic in avian medicine, aiding in respiratory distress; in this sense, the utilization of Serratiopeptidase has been advised to alleviate risks associated with chronic respiratory disease as (*Escherichia coli* and *Mycoplasma gallisepticum*)(Ahmed *et al.*, 2014).

### 2-5-2: Potential Therapeutic Applications

Serratiopeptidase can be potentially utilized to treat disorders like diabetes, carotid artery blockage, traumatic swelling, bromyalgia, Crohn's disease, and brocystic breast disease(Venkataprasad *et al*., 2021). However, Serratiopeptidase is an exciting treatment candidate for Alzheimer's disease, which is defined as a gradual and irreversible brain condition characterized by the precipitation of β-amyloid peptides which is in its insoluble form and found in the neuropil, resulting to dementia. A recent study found that the Serratiopeptidase enzyme may effectively disintegrate and minimize amyloid fibrils such as β-amyloid, making it a

promising therapy targeting protein misfolding-related illnesses(Metkar *et al.*, 2024). The histological examination of treated rats' brain tissue verified these findings, Serratiopeptidase might be beneficial in modifying certain parameters associated with Alzheimer's disease. Thus, these enzymes might offer a therapeutic utility for the therapeutic management of Alzheimer's disease (AD). Mentioned in one study through oral administration of SRP to a rat model once daily for forty-five days for AD is proposed, resulting in the greatest drop-in activity of brain acetylcholinesterase, transforming growth factor-beta, and interleukin-6 levels. Furthermore, therapy with SRP caused a considerable increase in the levels of insulin-like growth factor-1 and brain-derived neurotrophic factor(Fadl *et al.*, 2013). A patent was requested regarding a topical formulation comprising Lysozyme and Serratiopeptidase for use in the treatment of udder and uterine inflammation in mammals(Rathi *et al*., 2009).

Serratiopeptidase with its extraordinary catalytic potential, provides valuable benefits for modern medical treatments, The Synergistic action of Serratiopeptidase with Metformin proved safe as well as effective for treating of osteo-arthritis in the knee. This treatment protocol successfully decreases pain together with inflammatory signs(Ateia *et al*., 2018).

### 2-5-3: Insights into the Effective Dosing in Pharmacological Researches

For inflammation and pain relief, Serratiopeptidase, a proteolytic enzyme with anti-inflammatory and mucolytic properties, is usually prescribed at a dose of (10 mg three times a day) on an empty stomach. The dosage range is varied from (15 to 60 mg/day), it depends on the severity, and the treatment duration from two to four weeks in human (Rakshe *et al*., 2022; Jothieswari D *et al*., 2023). For arterial blockage, the

initial dose is three tablets (10 mg each twice a day) for the first month, after which it is lowered to (three tablets once per day)(Rakshe *et al*., 2022). For animals, the typical daily dosage is 1 to 3 tablets (10 mg), and for mucus-related issues, one or two tablets can relieve a sore throat in thirty minutes(Rakshe *et al*., 2022). The maximum recommended dose should not exceed (60 mg/day), Serratiopeptidase is normally used for one week for anti-inflammatory effects and two weeks for mucolytic effects, always preferably under the guidance of a health care professional(Sharma *et al*., 2021; Tamimi *et al*., 2021).

## 2-6: Safety

Serratiopeptidase is regarded as a molecule derived from natural sources that has been utilized for four decades. Therefore, it is normally regarded as safe, also the safety profile of the SRP in various therapeutic applications is backed by various studies that have recorded no side effects and adverse reactions, however, adverse effects caused by this molecule have been indicated in several studies(Al-Khateeb and Nusair, 2008; Ateia *et al*., 2018), though these are uncommon, Buccal space abscess has been reported as one of this molecule's side effects, such adverse effects might be driven by the dosage or could result from interaction with other drugs, comprehensive, scientifically structured controlled clinical trials are desirable to more investigate the safety, it is paramount that clinical adverse event reports do not depend on field data systems alone, but also define safety-related problems in well-defined model species, such safety reports can confirm the selection of the starting animal species and provide proper connection for the species extrapolation applied at the time of veterinary approval(Jadhav *et al*., 2020). Serratiopeptidase is safer than other options for example the corticosteroids and the NSAIDs. Therefore, it can be used as a substitute therapy in those who acquire the intolerance

or contraindications for these other drugs(Sivaramakrishnan and Sridharan, 2018). Due to the great therapeutic potential of Serratiopeptidase in veterinary medicine, the assessment of its safety profile is an essential concern when using the enzyme in companion animals(Jamal *et al.*, 2024). The Serratiopeptidase enzyme has been used worldwide for decades, its safety record is impressive, and no significant adverse effects have been documented during screening due to its high affinity and specificity for its intended targets, enzymes can transform a variety of target molecules into their intended products, so enzyme-based specific medications were created to manage and treat a broad range of diseases(Bapat *et al.*, 2024).

## 2-7: Side Effects

Adverse reactions of Serratiopeptidase are hypersensitivity reactions such as rash and erythema, gastrointestinal symptoms such as diarrhea, anorexia, gastric pain, nausea, and vomiting, and, in extremely rare cases, haemolysis with bleeding tendencies such as epistaxis and sputum with blood; also, pneumonitis, subepidermal bullous dermatosis, and acute eosinophilic pneumonia have been reported with its administration(Rakshe *et al*., 2022). Therefore, for the safe and proper use of Serrapeptase in veterinary medicine, it is important to clarify these effects. However, oral administration of Serratiopeptidase is accompanied by a variety of side effects like anorexia, nausea, and gastrointestinal disturbance. As a result, the topical delivery of SRP represents the advantage of being able to target the enzyme to the site of action, hence minimizing systemic side effects(Nirale and Menon, 2010). Other adverse effects have been reported to be mild to moderate dyspepsia was the most often reported(Garg *et al.*, 2012). Higher risk of bleeding if the medication is used with fish oil, curcumin, garlic, and other herbal supplements(Venkata *et al*., 2017).

## 2-8: Complications and Contraindications

Complications of Serratiopeptidase are usually extremely safe, with severe complications occurring extremely rarely. Nevertheless, taken in high doses, SRP may give rise to serious and potentially issues, including eosinophilic pneumonia in the acute state, paralysis, and oesophagal ulcers, which highlight the importance of careful dosage and monitoring with therapy (Kumar, 2018).

Contraindications for this treatment include patients with blood coagulation disorders and patients with severe liver or kidney dysfunction because these can significantly raise the risk of serious complication(Rakshe *et al*., 2022).

## 2-9: Research Gap on Serratiopeptidase

Although Serratiopeptidase has been extensively studied for its anti-inflammatory, fibrinolytic, and mucolytic properties, there remains a lack of understanding regarding its broader pharmacological potential. Specifically, its neurobehavioral effects—such as those related to anxiety, depression, and seizures—have not been sufficiently investigated, particularly in animal models. In veterinary medicine, its impact on the nervous system, including behavioral and physiological functions, has not been adequately addressed. Additionally, limited information is available on its pharmacokinetics, especially its excretion pathways, and its physiological effects under general anesthesia remain underexplored. This highlights the need for comprehensive studies to clarify the mechanisms of action and therapeutic potential of Serratiopeptidase in neurophysiology and behavior.

# CHAPTER THREE

## MATERIALS AND METHODS

# Chapter Three

# Materials and Methods

## 3-1: Laboratory animals

449 *Swiss albino* male mice aged (2 months), weighing (25 – 30g) were purchased from the laboratory animals house of the College of Veterinary Medicine, University of Mosul, they were accommodated and kept in organized circumstances of (21± 2°C), (12h-12h light/dark) cycles. Water in plastic bottles was provided as drinking water throughout the study; all mice received commercially available diet. Animals were housed in groups of five per cage, with wood shavings used bedding materials, and most of the experiments were conducted during the daytime.

## 3-2: Ethical approval

The study was standardized (the animal use and experimental design) through the care committee which affiliated to the Veterinary Medicine College / Mosul University (approval code no. UM.VET.2024.006).

## 3-3: Instruments

Table (1): List of instruments used in the experiments

| Apparatus | Company |
|---|---|
| Open Field Apparatus | Local made- Mosul / Iraq |
| Head Pocking Apparatus | Local made- Mosul / Iraq |
| Negative Geotaxis Apparatus | Local made- Mosul / Iraq |
| Swimming Apparatus | Local made- Mosul / Iraq |
| Elevated Plus Maze Apparatus | Local made- Mosul / Iraq |
| Light And Dark Apparatus | Local made- Mosul / Iraq |

| Apparatus | Company |
|---|---|
| Tail Suspension Apparatus | Local made- Mosul / Iraq |
| Forced Swimming Apparatus | Local made- Mosul / Iraq |
| Multiparameter Monitor Device (veterinary patient monitor) | YONKER/China |
| Electronic Scale | Great river company/China |
| Stop Watch | iPhone company / United states |
| Video Camera | iPhone company / United states |
| Aluminum Phone Stand | Tripod 3110 company / China |
| Digital Thermometer | ThermoWorks company/United states |

## 3-4: Chemicals

Table (2): List of chemicals used in the experiments

| Item | Conc. | Manufactured by |
|---|---|---|
| Semazin ® (Serratiopeptidase) Cap | 40,000 IU | Bioactive T Company/Bulgaria |
| Ethanol Solution | 70% | Al-Joud Company/Iraq |
| Sertraline Accord® Tab | 50 mg | Accord Company /United Kingdom |
| Apicarpine ® (Pilocarpine HCL) solution | 2% | API Company/Jordan |
| Diazem ® (Diazepam ampoule) | 10 mg | Deva Holding A.Ş/Turkey |
| xyla® (xylazine injection) | 20 mg | Intercheme/Holland |
| Ketamine fresenius® (ketamine HCL) | 50 mg | Fresenius kabi/Germany |
| Atrovap® (Atropine sulphate) solution | 1mg/1 ml | VAPCO \| Product manufacturing co.ltd / Jordan |

## 3-5: Dose preparation

To get the needed concentration, the capsule (containing 40,000 IU of Serratiopeptidase) dissolved in 5 ml of distilled water. Each 1 IU equivalent to 0.67 mg. The concentration of the drug is 40,000 IU, meaning that one capsule contains 20 mg. Since three doses were used (5, 10 and 20 mg/kg). The following dilutions were prepared: To obtain a concentration of 10 mg/kg. we take 2 ml from the tube that we prepared previously (20 mg/kg) and add 2 ml of distilled water to it. To obtain a concentration of 5 mg/kg. we take 1 ml from the tube that we prepared previously (20 mg/kg) and add 3 ml of distilled water to it. In all experiments, Serratiopeptidase was administered orally, and a waiting period was one hour before initiating any test.

## 3-6: Experiments

### 3-6-1: Assessment neurobehavioral effects of a single oral dose after one hour in mice treated with Serratiopeptidase

Twenty mice were divided into four groups and treated as follows:

The first group was treated orally with distilled water (control).

The second group was orally administered Serratiopeptidase 5 mg/kg.

The third group was orally administered Serratiopeptidase 10 mg/kg.

The fourth group was orally administered Serratiopeptidase 20 mg/kg.

#### 3-6-1-1: Open Field Test (OFT)

It is used to exploring anxiety-like behavior in rodents, defined as a square box that is measured (50x50x35cm, Length × width × height) layout the floor of the box with lines dividing it into twenty-five squares of

the same size (10x10 cm, Length × width). Transferred animals to the laboratory for an hour before treatment for adapting, the open field apparatus cleaned with 70% ethanol and dried with cotton and the duration was 5 minutes, the test conducted after one hour of administration (Stojanović *et al*., 2017). A video camera was utilized to record the following criteria

1-Number of squares or lines that the animal crosses with all its limbs (Horizontal activity)

2-Number of times rearing on the back legs (Vertical activity)

3-Number of fecal balls

4-Number of times urinate

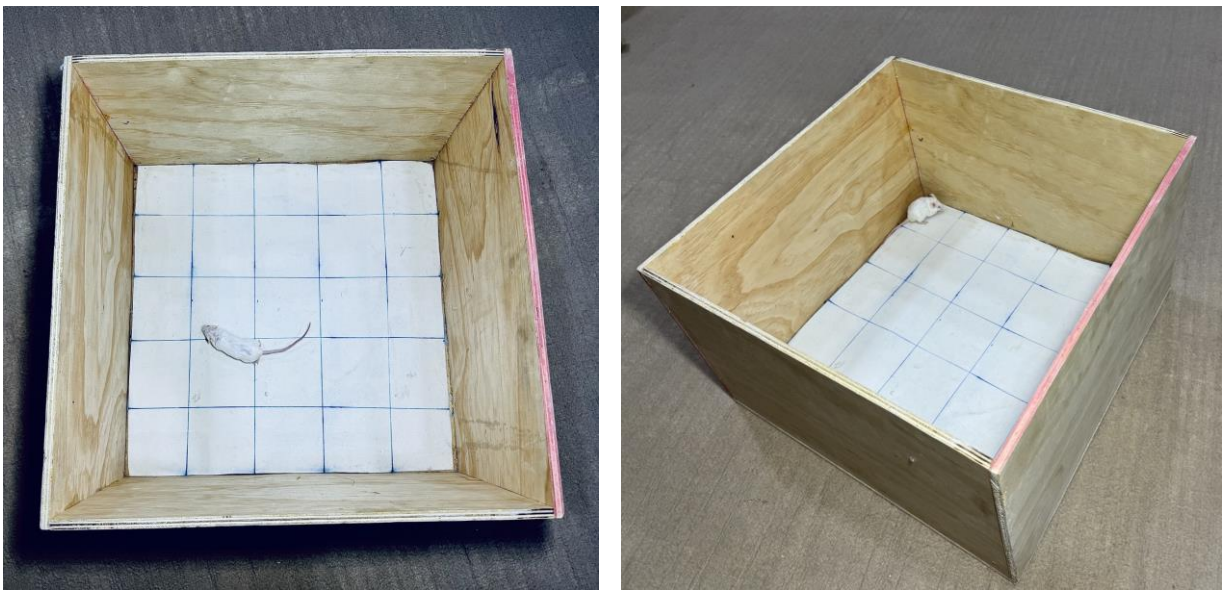

Figure (3): Open field test

### 3-6-1-2: Negative Geotaxis Test (NG)

Negative geotaxis test is composed of a wooden surface inclined covered with rough cloth sloping at a 45-degree angle. The test depends on

time taken by animal to turn 180 degrees with its entire body to the top of the slope which involves putting animal with its head upside down, the duration and cut-off time of test is (60 seconds), the test conducted after one hour of administration. The NG test was carried out by timing how long it took the animal to spin around. The NG evaluates the sensory motor response and determines geogravitational stimuli by measuring the vestibular function(Kreider and Blumberg, 1999; Ruhela *et al.*, 2019; Hassan and Al-Baggou, 2023).

Figure (4): Negative geotaxis test

### 3-6-1-3: Head Pocking Test (HP)

The test is conducted on a plastic surface that is 20 cm high and 30 cm in diameter. It has ten round holes. To conduct the test the animal was observed, and the number of efforts to enter the head into the holes was calculated. Three minutes are allotted for the animal test.

The animal's level of curiosity and familiarity with its surroundings were assessed by this test following one hour of administration(Takeda *et al*., 1998; Mustafa and Al-Baggou, 2020; Al-Abdaly *et al*., 2021).

Figure (5): Head pocking test

**3-6-1-4: Swimming Rank Test (ST)**

The amount of muscle strength and feeling of fatigue was measured using a swimming test, as the test depends on the extent of neural and functional integration in the different parts of the brain. A special plastic swimming pool with dimensions of (40×25×30 cm, Length × width × height) was used in the test, and water was placed in it at a height of 30 cm and a temperature 30 ºC, the room was intended to exclude the effect of water temperature on the animal's performance (the duration of each animal's stay in the tank was 3 minutes and initiation period after one hour of administration). (Mohammad, 1986; Khudhair, 2024). As follows

Grade Zero: If the nose is under water.

Grade 1: The nose is at or above the water level.

Grade 2: The nose and top of the head are at or above the water level, with the ears remaining in the water.

Grade 3: As in 2, except that the water reaches the middle of the ear.

Grade 4: As in 3 except that the water reaches the base of the ear.

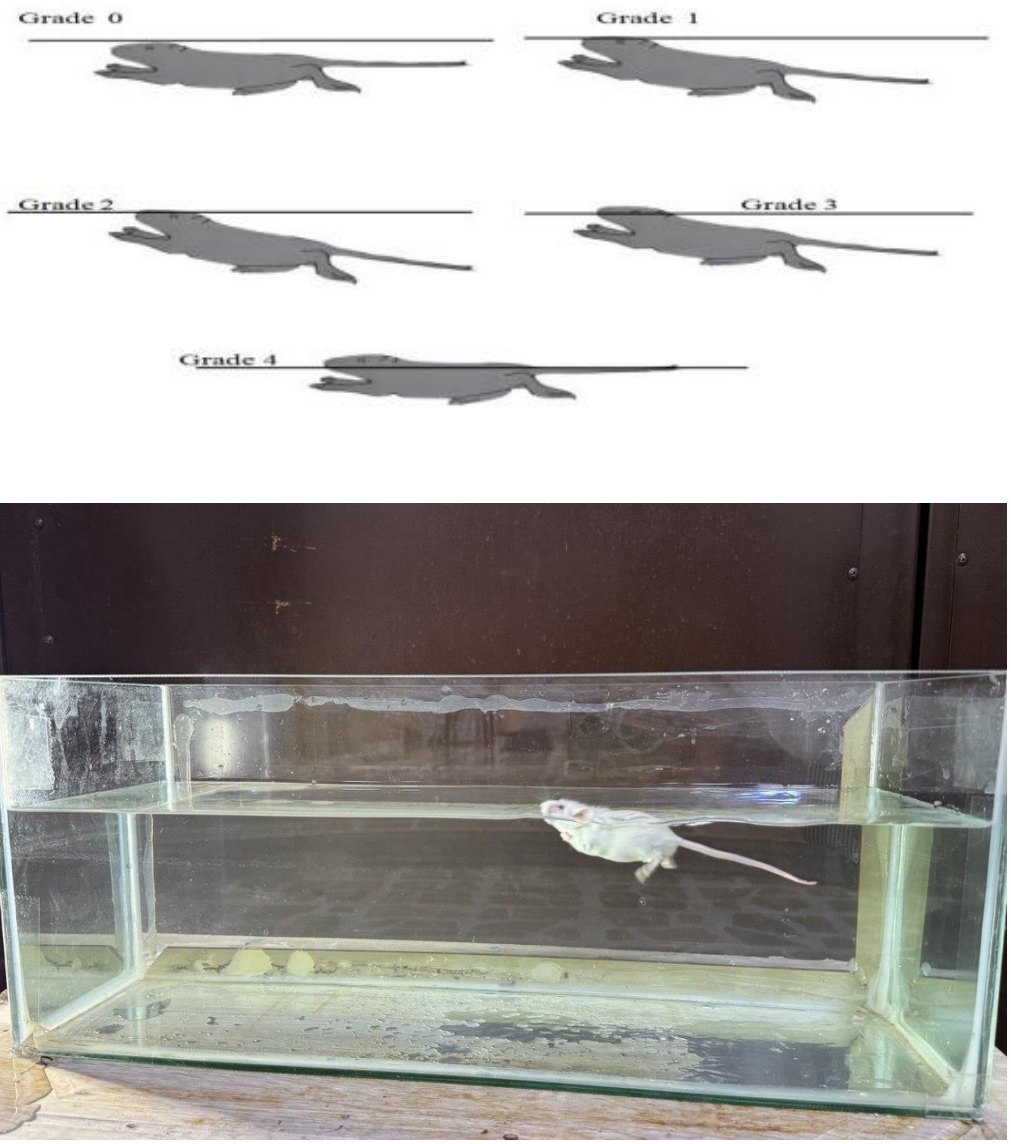


Figure (6): Swimming rank test

**3-6-2: Assessment of neurobehavioral effects of repeated oral doses of Serratiopeptidase (after 15 days of administration) in mice using open field test, negative geotaxis test and head pocking test**

Twenty mice were divided into four groups and treated as follows:

The first group was treated orally with distilled water (control).

The second group was orally administered Serratiopeptidase 5 mg/kg.

The third group was orally administered Serratiopeptidase 10 mg/kg.

The fourth group was orally administered Serratiopeptidase 20 mg/kg.

### 3-6-3: Assessment of anti-anxiety effects of a single oral dose after one hour in mice treated with Serratiopeptidase

Twenty-five mice were divided into five groups and treated as follows:

The first group was treated orally with distilled water (the negative control).

The second group was administered a single oral dose of Sertraline 10mg/kg, assessment was conducted after four-hours interval (the positive control). (Sitges *et al.*, 2012)

The third group was orally administered Serratiopeptidase 5 mg/kg.

The fourth group was orally administered Serratiopeptidase 10 mg/kg.

The fifth group was orally administered Serratiopeptidase 20 mg/kg.

#### 3-6-3-1: Elevated Plus Maze Test (EPM)

In this experiment, mice's levels of fear and anxiety are assessed. It is composed of two opposite open arms (30 × 5 cm, Length × width) with two opposite closed arms (30 × 5 cm, Length × width) and a small central square (5 × 5 $cm^2$, Length × width) between all arms. The maze was elevated (50 cm) above the ground. The mouse was placed in the central small square and its head was toward the open arms. The duration of the test is 5 minutes and initiation period after one hour of administration, by using a video camera to record. The maze was cleaned with 70% ethanol and cotton(Rodgers and Dalvi, 1997). The following parameters are obtained from anxiety analysis and are performed during each session:

1- Time spent in the open arms.

2- Time spent in the closed arms.

3- Number of times entering the open arms.

4. Number of times entering the closed arms.

Figure (7): Elevated plus maze test

### 3-6-3-2: Light and Dark Box Test (LADB)

This test is used to measure anxiety behavior in mice. Two connected boxes with equal size were employed in the experiment with spacer in between contain an opening and highly lit with 100 w in the light side (figure8), the dimensions of box were (42× 30 × 20 $cm^3$, Length × width × height), While dimensions of an opening (6 × 6 $cm^2$, Length × width). The duration of the experiment was 5 minutes and the test conducted after one hour of administration, the mouse was placed in the center of the boxes inside opening with its head towards the dark side by using a video camera to record the following criteria(Narayanan and Kumar, 2018; Matsuo *et al.*, 2019)

1. Number of times the dark side was entered

2. Number of times the light side was entered

3. Period of staying on the dark side.

4-Periods of staying on the bright side.

The devices were rinsed with ethanol solution and dried with cotton before each test and after each mouse to eliminate previous mouse odor.

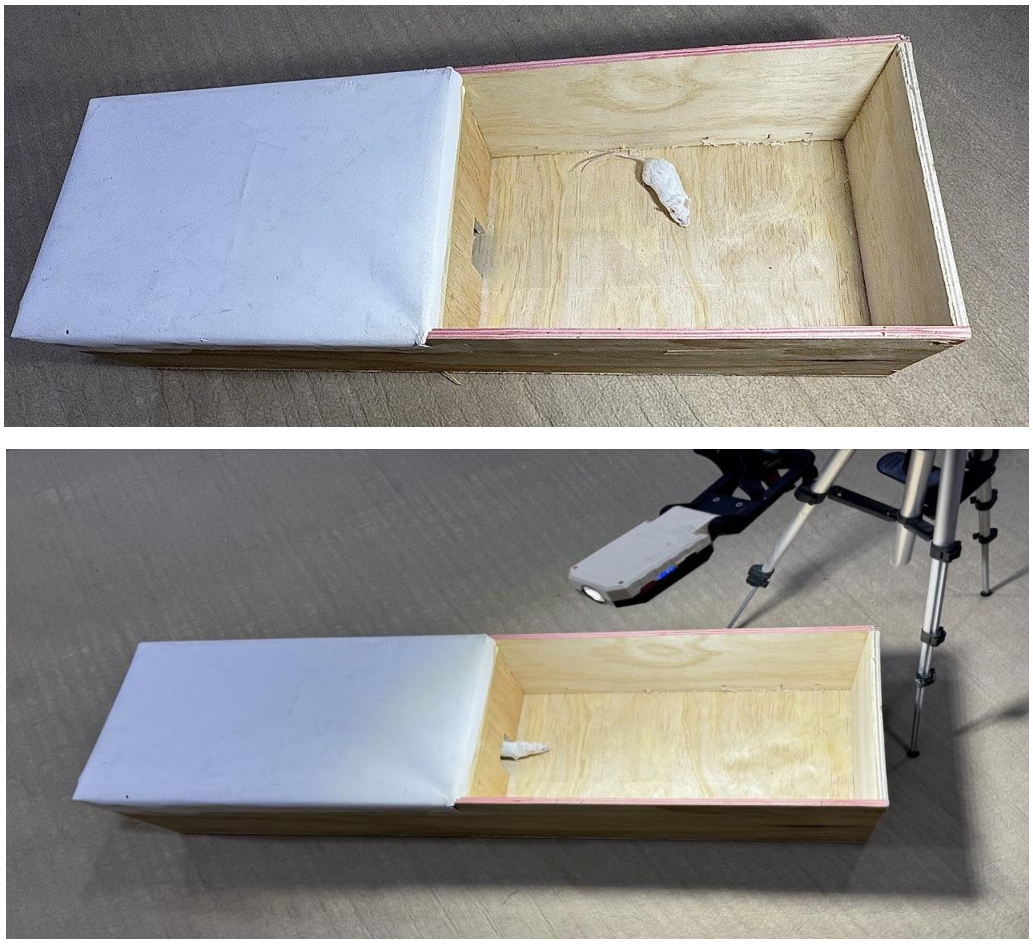

Figure (8): Light and dark box test

**3-6-4: Assessment anti-anxiety effects of repeated oral doses of Serratiopeptidase (after 15 days of continuous daily administration) in mice using elevated plus maze test and light/dark box test**

Twenty-five mice were divided into five groups and treated as follows:

The first group was treated orally with distilled water (the negative control).

The second group was administered a single oral dose of Sertraline 10mg/kg, assessment was conducted after four-hours interval (the positive control). (Sitges *et al.*, 2012)

The third group was orally administered Serratiopeptidase 5 mg/kg.

The fourth group was orally administered Serratiopeptidase 10 mg/kg.

The fifth group was orally administered Serratiopeptidase 20 mg/kg.

### 3-6-5: Assessment anti-depressant effects of a single oral dose after one hour in mice treated with Serratiopeptidase

Twenty-five mice were divided into five groups and treated as follows:

The first group was treated orally with distilled water (the negative control).

The second group was administered a single oral dose of Sertraline 10mg/kg, assessment was conducted after four-hours interval (the positive control). (Sitges *et al.*, 2012)

The third group was orally administered Serratiopeptidase 5 mg/kg.

The fourth group was orally administered Serratiopeptidase 10 mg/kg.

The fifth group was orally administered Serratiopeptidase 20 mg/kg.

#### 3-6-5-1: Tail Suspension Test (TST)

The tail suspension test, which has been described by (Steru *et al.*, 1985; Can *et al.*, 2012) was used to examine depression-like behavior. A closed box with one side left open (20 × 40 × 60 $cm^3$, Length × width ×

height) with one light side that allowed for video recording of the animals made up in test apparatus. Using adhesive tape placed approximately 1 cm from the tip of the mouse's tail, each mouse was hanged by the tail 60 cm above the chamber floor. A video camera records the subsequent behavior for 6 minutes, with the first minute excluded from analysis and the test conducted after one hour of administered.

The following parameters were later determined by analyzing the behavior: total time spent immobile. The total time that each mouse was immobile was measured in seconds, and the percentage of total time per minute was calculated. The term "immobile time" was used in this test to describe the time when the animals were motionless for less than one second.

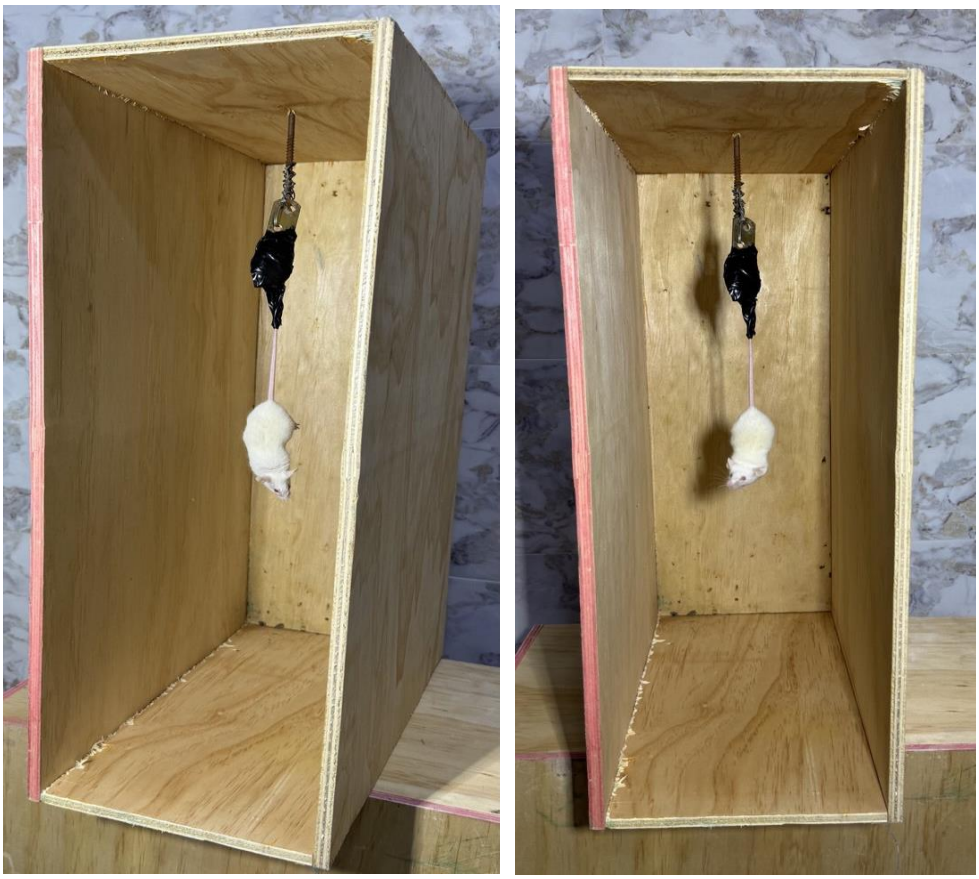

Figure (9): Tail suspension test

### 3-6-5-2: Forced Swimming Test (FST)

Also known as behavioural despair(Browne, 1979), this test was conducted using the following device was made up of 10 x 20 cm clear plastic cylinders that held 7 cm of water at 23°C, every animal spent 6 minutes inside the plastic cylinder, with the first minute excluded from analysis and the test conducted after one hour of administration. Each treatment group's immobility time is represented as a percentage of the average time recorded for the comparable treatments. The forced swimming test is used to measure antidepressant effect in mice

The following parameters were recorded using a video camera

1-Immobility time (seconds)

2-Percentage immobility time

Figure (10): Forced swimming test

### 3-6-6: Assessment anti-depressant effects of repeated oral doses of Serratiopeptidase (after 15 days of continuous daily administration) in mice using tail suspension test and forced swimming test

Twenty-five mice were divided into five groups and treated as follows:

The first group was treated orally with distilled water (control).

The second group was administered a single oral dose of Sertraline 10mg/kg, assessment was conducted after four-hours interval (the positive control). (Sitges *et al.*, 2012)

The third group was orally administered Serratiopeptidase 5 mg/kg.

The fourth group was orally administered Serratiopeptidase 10 mg/kg.

The fifth group was orally administered Serratiopeptidase 20 mg/kg.

### 3-6-7: Assessment of the anticonvulsant effect of repeated oral doses of Serratiopeptidase (after 15 days of continuous daily administration) using pilocarpine-induced seizures in mice

Forty mice were distributed into five groups, as follows:

The first group was administered distilled water orally (negative control).

The second group was administered diazepam at 1 mg/kg intraperitoneal, initiating immediately after injected (The positive control) (Nishimura *et al.*, 1989)

The third group was administered Serratiopeptidase at 5 mg/kg orally.

The fourth group was administered Serratiopeptidase at 10 mg/kg orally.

The fifth group was administered Serratiopeptidase at 20 mg/kg

orally.

### 3-6-7-1: Pilocarpine-induced seizure

To stop peripheral cholinergic activation an hour after dosing in all groups, mice were subcutaneously injected with atropine sulphate, 1mg/kg(Vezzani, 2009), after ten minutes, 200 mg/kg i.p. of pilocarpine was injected to create seizures(Hernandez *et al*., 2002). This test is used to evaluate the effect of Serratiopeptidase after 15 days of oral administration on convulsion induced by pilocarpine (the procedure was initiated immediately after the injection on the day 15) animals were monitored for an hour following the injection of pilocarpine.

We record the followings:

1-Onset of convulsion (seizure)

2-Numbers of convulsions

3-Survival percentage

### 3-6-8: Assessment the effect of repeated oral doses of Serratiopeptidase (after 15 days of continuous daily administration) on general anesthesia by using xylazine and ketamine (respectively) in mice

This test is used to evaluate the effect of Serratiopeptidase after 15 days of oral administration on general anesthesia by administered xylazine and ketamine 10,150mg/kg intraperitoneal on the day 15 (Levin-Arama *et al.*, 2016) which conducted immediately after administered.

Thirty-two mice were distributed into four groups. As follows:

The first group was administered distilled water orally (control).

The second group was administered Serratiopeptidase at 5 mg/kg orally.

The third group was administered Serratiopeptidase at 10 mg/kg orally.

The fourth group was administered Serratiopeptidase at 20 mg/kg orally.

Anesthetic protocol: Xylazine, an α2 receptor agonist provides sedation, muscle relaxation and analgesia. Ketamine, an NMDA receptor antagonist, offers potent analgesia and anesthesia but poor muscle relaxation. when combined they produce a balanced anesthetic state (David *et al*., 2022).

### 3-6-9: Determination the effect of repeated oral doses of Serratiopeptidase (after 15 days of continuous daily administration) under general anesthesia on mice's vital signs and physiological parameters through monitoring using the veterinary patient monitor

Thirty-two mice were distributed into four groups and administered xylazine and ketamine 10,150mg/kg intraperitoneal(Levin-Arama *et al.*, 2016). As follows:

The first group was administered distilled water orally (control).

The second group was administered Serratiopeptidase at 5 mg/kg orally.

The third group was administered Serratiopeptidase at 10 mg/kg orally.

The fourth group was administered Serratiopeptidase at 20 mg/kg orally. (Figure 11).

#### 3-6-9-1: Procedure for using veterinary patient monitor device to continuously observation vital signs in anesthetized male mice after repeated oral administration of Serratiopeptidase

A multiparameter monitor device or patient monitor in veterinary medicine is used to measure and display vital signs and physiological parameters of an animal's health during anesthesia, surgery, or intensive care, through prepared anesthetized mice**.** Ensure the animal is in a stable,

appropriate position for monitoring. Place Sensors like (ECG and RR) attach the electrodes to the right and left arms and one attached to the left leg for monitoring (heart rate and rhythm with respiratory rate**),** while the $SpO_2$ probe is placed the sensor on a non-invasive area, like the right leg(Hiyam *et al*., 2025).

Monitoring anesthetized male mice**:** Observe readings continuously for vital signs and physiological parameters such as:

1. Heart rate (HR)
2. Respiratory rate (RR)
3. Oxygen saturation ($SpO_2$)

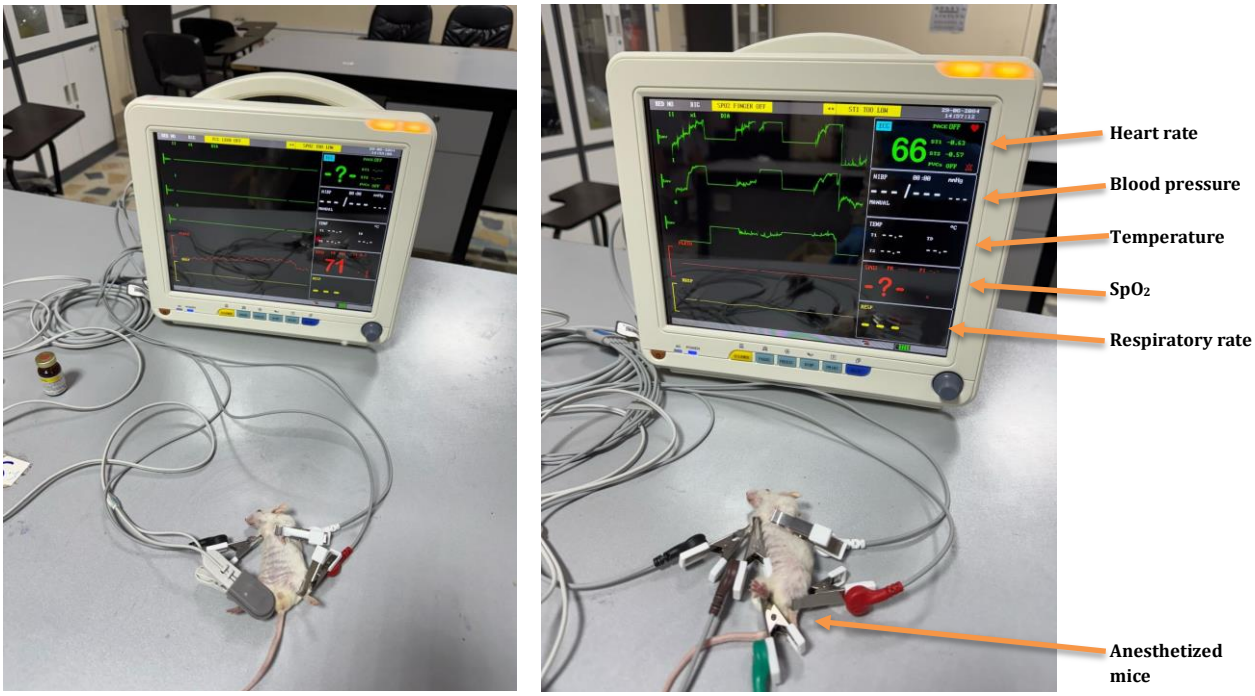


Figure (11): Multiparameter monitor device (veterinary patient monitor)

## Statistical analysis

IBM SPSS Statistic version 27 was employed to statistically analyze the results, and mean ± standard error (mean ± SE) was utilized to expression the results. To determine the likelihood existed any significant differences between the groups, The Mann-Whitney U test was used to

analyze the data obtained from the swimming rank test, while one-way analysis of variance (ANOVA) was employed for all other tests followed by Duncan's multiple comparisons were then performed within probability at ($P \leq 0.05$)(Johnkutty *et al*., 2025).

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# CHAPTER FOUR

## RESULTS

# Chapter Four

# Results

## 4-1: Assessment neurobehavioral effects of a single oral dose of Serratiopeptidase in mice using open field test, negative geotaxis test, head pocking test and swimming rank test (one hour after administration)

In open field test, the number of squares or lines that the animal crosses with all its limbs was significantly increase in (5, 10 and 20 mg/kg groups) compared with the control group. The number of times rearing on the back legs was significantly increase in (5, 10 and 20 mg/kg groups) than in the control group, while the number of fecal balls was increase in all groups, and no difference in the number of times urinate occurred among all groups than control group (Table 3).

**Table 3: Neuro-behavioral effect of a single oral dose after one hour in mice treated with Serratiopeptidase in OFT**

| Parameters / Groups (orally) | Number of squares or lines that the animal crosses with all its limbs (sec) | Number of times rearing on the back legs (sec) | Number of fecal balls | Number of times urinate |
|---|---|---|---|---|
| **Control (Distilled water)** | 13.2 ± 1.71 | 3.6 ± 2.61 | 0 ± 0 | 0 ± 0 |
| **Serratiopeptidase (5mg/kg)** | 90.4 ±12.32* | 20.40 ± 2.06* | 0.4 ± 0.24 | 0.2 ± 0.2 |
| **Serratiopeptidase (10mg/kg)** | 89.8 ± 14.74* | 27.0 ± 3.11* | 0.2 ± 0.20 | 0 ± 0 |
| **Serratiopeptidase (20mg/kg)** | 93.6 ± 3.52* | 22.0 ±2.48* | 0.2 ± 0.20 | 0 ± 0 |

The data are mean ± SE of 5 mice/group/for five minutes

* Significantly different from the data of control group, at ($p \leq 0.05$).

In the negative geotaxis test, recorded an increase in the time required for the animal to turn around and modify its body position by 180º degrees with its entire body to the top in mice treated with 5 mg/kg Serratiopeptidase group compared to control group after one hour of treatment, while 10 mg/kg Serratiopeptidase groups did not record a significant difference after one hour of treatment, with exception that 20 mg/kg decreased at the rotation time. (Table 4)

While in the head pocking test, no noticeable change was recorded in the frequency of times the head was inserted into the holes at 5 mg/kg Serratiopeptidase group compared to the control group. In contrast, 10 and 20 mg/kg Serratiopeptidase groups recorded an increase compared to the control group. (Table 4)

**Table 4: Neuro-behavioral effects of a single oral dose after one hour in mice treated with Serratiopeptidase in the NG and HP test**

| Parameter / Groups (orally) | Negative geotaxis\60 sec | Head pocking \3 min |
|---|---|---|
| **Control (Distilled water)** | 9.8 ± 1.11 | 12.6 ± 1.6 |
| **Serratiopeptidase(5mg/kg)** | 12.6 ± 5.31 | 11.6 ± 2.35 |
| **Serratiopeptidase(10mg/kg)** | 8.6 ±1.91 | 15.8 ± 5.13 |
| **Serratiopeptidase(20mg/kg)** | 6.8 ± 2.13 | 16.8 ± 3.78 |

The data are mean ± SE of 5 mice/group.

In the swimming test, after an hour of treatment, mice treated with 5, 10, and 20 mg/kg Serratiopeptidase groups did not significantly vary from the control group in terms of swimming ability. (Table 5).

**Table 5: Neuro-behavioral effects of a single oral dose after one hour in mice treated with Serratiopeptidase in the swimming rank test**

| Parameter / Groups (orally) | Swimming scores |
|---|---|
| **Control (Distilled water)** | 3.6 ± 0.24 |
| **Serratiopeptidase(5mg/kg)** | 3.2 ± 0.37 |
| **Serratiopeptidase(10mg/kg)** | 3.8 ± 0.20 |
| **Serratiopeptidase(20mg/kg)** | 3.4 ± 0.24 |

The data are mean ± SE of 5 mice/group/ for three minutes

## 4-2: Assessment neurobehavioral effects of repeated oral doses of Serratiopeptidase in mice using open field test, negative geotaxis test and head pocking test (15 days)

In the Open field test, horizontal activity (locomotion): The control group exhibited a baseline horizontal activity of 76.75±7.74 squares crossed. The 5mg/kg group showed increase in horizontal activity (91.25±8.22 squares crossed) compared to the control group. The 10mg/kg group displayed a decrease in horizontal activity (83.25±8.52 squares crossed) compared to the 5mg/kg group, but still higher than the control group. The 20 mg/kg group demonstrated the highest horizontal activity (96.13±6.65 squares crossed).

Vertical activity (Exploration/ Rearing): The 5mg/kg group exhibited an increase in vertical activity (20.75±2.50 rearings) compared to the control. Both the 10 mg/kg (22.38±4.56 rearings) and 20 mg/kg (24.25 ±1.68) groups displayed further increases in vertical activity (Table 6).

**Table 6: The influence of oral Serratiopeptidase (15 days of administration) on motor activity and exploratory behavior in mice**

| Parameters / Groups (orally) | Horizontal activity (Squares crossed/sec) | Vertical activity (Rearings/sec) |
|---|---|---|
| **Control (Distilled water)** | 76.75 ±7.74 | 16.00 ±2.67 |
| **Serratiopeptidase(5mg/kg)** | 91.25 ±8.22 | 20.75 ± 2.50 |
| **Serratiopeptidase(10mg/kg)** | 83.25 ±8.52 | 22.38 ± 4.56 |
| **Serratiopeptidase(20mg/kg)** | 96.13 ±6.65 | 24.25 ±1.68 |

The data are mean ± SE of 5 mice/group/ for five minutes

In the negative geotaxis test, after taking Serratiopeptidase for a long period (15 days) shows neurobehavioral effects. These effects revolved around motor (coordination and response) and this effect appeared clearly at the 20 mg/kg group, as the time required for the animal to rotate 180 degrees around itself was estimated to decrease by half when compared with the control group, decrease at 10 mg/kg, While the 5 mg/kg group showed an increase compared to control group (Table 7).

**Table 7: The influence of oral Serratiopeptidase (15 days of administration) on vestibular function in mice using negative geotaxis test**

| Parameter / Groups (orally) | Negative geotaxis\60 sec |
|---|---|
| **control (Distilled water)** | 8.00 ± 4.27 |
| **Serratiopeptidase (5mg/kg)** | 10.40 ± 2.61 |
| **Serratiopeptidase (10mg/kg)** | 5.20 ± 0.37 |
| **Serratiopeptidase (20mg/kg)** | 4.80 ± 1.20 |

The data are mean ± SE of 5 mice/group.

In the head pocking test, repeated oral administration of Serratiopeptidase for periods of about 15 days was associated with an increase in exploratory behavior (head pocking counts over 3 minutes, in terms of a number of times inserted head into holes) in mice compared to the control group. Higher dose correlating to increased head pocking activity. However, statistical significance was not observed in the data provided (Table 8).

**Table 8: The influence of oral Serratiopeptidase (15 days of administration) on the animal's curiosity with cognitive to surrounding in mice using head pocking test**

| Parameter / Groups (orally) | Head pocking \3 min |
|---|---|
| **Control (Distilled water)** | 10.40 ± 2.83 |
| **Serratiopeptidase (5mg/kg)** | 11.40 ± 2.63 |
| **Serratiopeptidase (10mg/kg)** | 12.00 ± 2.21 |
| **Serratiopeptidase (20mg/kg)** | 12.20 ± 1.71 |

The data are mean ± SE of 5 mice/group.

## 4-3: Assessment anti-anxiety effects of a single oral dose of Serratiopeptidase in mice using elevated plus maze and light/dark box test (one hour after administration)

In EPM test, regarding the time spent, the group that given 20 mg/kg of Serratiopeptidase spent more time with a significant increase in the open arms as opposed to less time with a significant decrease in the closed arms after one hour of treatment compared to the negative control, with no significant change compared to the positive group but still outperforms

even sertraline, demonstrating an anxiety reduction. A similar trend was observed in (5 and 10 mg/kg Serratiopeptidase groups) where a significant increase was seen compared to the negative group, with no significant change compared to the positive group.

Nonetheless, the frequency of times entering the open arms was decreased in both of the 5 and 10 mg/kg Serratiopeptidase groups relative to the negative and positive controls; similar patterns were observed in closed arms aligned with those results from open arms, with a reduction noted in the group administered 5 mg/kg Serratiopeptidase. (Table 9)

**Table 9: Effects of oral Serratiopeptidase on anxiety-related behaviors in mice: Elevated plus maze test after one hour of administration**

| Parameters / Groups (orally) | Time spent in open arms (sec) | Time spent in closed arms (sec) | Number of times entering the open arms | Number of times entering the closed arms |
|---|---|---|---|---|
| **Negative control (Distilled water)** | 111.40 ± 5.97 | 188.60 ± 5.97 | 10.80 ± 1.52 | 10.60 ± 1.16 |
| **Positive control (Sertraline)** | 146.40 ± 17.13 | 153.60 ± 17.13 | 8.80 ± 1.46 | 7.20 ± 0.80* |
| **Serratiopeptidase (5mg/kg)** | 134.60 ± 16.69* | 165.40 ± 16.69* | 6.80 ± 0.73 | 7.80 ± 1.06 |
| **Serratiopeptidase (10mg/kg)** | 137.00 ± 15.10* | 163.00 ± 15.10* | 9.60 ± 1.02 | 9.00 ± 0.89 |
| **Serratiopeptidase (20mg/kg)** | 187.40 ± 23.92* | 112.60 ± 23.92* | 7.60 ± 1.93 | 7.40 ± 1.56 |

The data are mean ± SE of 5 mice/group/ for five minutes

* Significantly different from the data of control group, at ($p \leq 0.05$).

In the light/dark box test, in terms of entry frequency, the results demonstrated at (5 and 20 mg/kg Serratiopeptidase groups) decrease in the

number of times entering to the light side than in both controls, and this reduction was mirrored in the dark side. Significantly was observed at (10 and 20mg/kg) compared to the negative group; however, in the 10 mg/kg Serratiopeptidase group, the results showed an increase in both sides. Periods of staying in dark/light side, the results demonstrated a significant increase in the duration of the animal's stay on the bright side, with a significant decrease on the dark side, the 20 mg/kg dose increases light-side exploration than in the negative control, outperforming over Sertraline, and reduces dark-side preference, highlighting potent anxiolytic effects. Lower doses demonstrated no significant (Table 10).

**Table 10: Effects of oral Serratiopeptidase on anxiety-related behaviors in mice: Light/dark box test after one hour of administration**

| Parameters / Groups (orally) | Number of times the dark side was entered | Number of times the light side was entered | Period of staying on the dark side (sec) | Periods of staying on the bright side (sec) |
|---|---|---|---|---|
| **Negative control (Distilled water)** | 7.40 ± 1.20 | 7.00 ± 1.26 | 143.00 ± 19.69 | 157.00 ± 19.69 |
| **Positive control (Sertraline)** | 8.40 ± 1.24 | 8.40 ± .92 | 180.60 ± 16.93* | 119.40 ± 16.93* |
| **Serratiopeptidase (5mg/kg)** | 6.40 ± 1.86 | 6.80 ± 1.74 | 141.60 ± 29.59 | 158.40 ± 29.59 |
| **Serratiopeptidase (10mg/kg)** | 10.40 ± 1.80* | 10.20 ± 1.77* | 147.80 ± 29.50 | 152.20 ± 29.50 |
| **Serratiopeptidase (20mg/kg)** | 5.80 ± 1.35* | 6.00 ± 1.18* | 101.60 ± 26.23* | 198.40 ± 26.23* |

The data are mean ± SE of 5 mice/group/ for five minutes

* Significantly different from the data of control group, at ($p \leq 0.05$).

## 4-4: Assessment anti-anxiety effects of repeated oral doses of Serratiopeptidase in mice using elevated plus maze and light/dark box test (15 days)

Serratiopeptidase after 15 days of administration, the highest dose of Serratiopeptidase 20mg/kg increased time spent in open arms, surpassing even the positive control. Lower doses (5 and 10 mg) showed intermediate effects, and the opposite effect in the aforementioned doses in the closed arms. A similar trend was observed in open arms entries, the 20 mg/kg group approached baseline levels, while lower doses exhibited reduced entries. This supports its anxiolytic potential (Table 11).

**Table 11: Effects of oral serratiopeptidase on anxiety-related behaviors in mice: Elevated plus maze test after 15 days of administration**

| Parameters / Groups (orally) | Time spent in open arms (sec) | Time spent in closed arms (sec) | Number of times entering the open arms | Number of times entering the closed arms |
|---|---|---|---|---|
| **Negative control (Distilled water)** | 72.00 ± 27.69 | 228.00 ± 27.69 | 6.00 ± 1.78 | 6.80 ± 2.17 |
| **Positive control (Sertraline)** | 99.00 ± 20.72 | 201.00 ± 20.72 | 7.20 ± 2.03 | 7.20 ± 2.03 |
| **Serratiopeptidase (5mg/kg)** | 84.20 ± 28.48 | 215.80 ± 28.48 | 2.60 ± 0.50 | 3.80 ± 0.96 |
| **Serratiopeptidase (10mg/kg)** | 81.80 ± 22.85 | 218.20 ± 22.85 | 4.80 ± 2.10 | 5.80 ± 2.39 |
| **Serratiopeptidase (20mg/kg)** | 120.60 ± 29.79 | 179.40 ± 29.79 | 5.60 ± 1.16 | 5.80 ± 1.11 |

The data are mean ± SE of 5 mice/group/for five minutes.

In the light/dark box test, a commonly used behavioral assay designed to assess anxiety-like behavior in rodents, mice are evaluated based on their preference for the dark, enclosed compartment versus the brightly lit, open area, along with the time spent in either area, the oral administration of Serratiopeptidase after 15 days at dose (20mg/kg) provides the most pronounced and anxiolytic effects, as evidenced by significant increased entries into the light side (6.40 ± 1.69) and the extended time spent there (149.20 ± 32.11 seconds), concurrently with a substantial decrease in the duration of time staying on the dark side (150.80 ± 32.11 seconds).

These parameters collectively suggest that the 20mg/kg dose significantly reduces anxiety, facilitating greater exploratory behavior typically associated with reduced anxiety levels. In comparison to sertraline (the positive control), which also showed efficacy in reducing anxiety, as indicated by increased light side entries and decreased dark side time, its effects were less robust than Serratiopeptidase 20mg/kg.

Lower doses of Serratiopeptidase (5mg/kg and 10mg/kg), demonstrated more limited and anxiolytic effects, with less time spent in the dark side and longer in the bright side, with significantly reduced frequency of entry to both sides, further reinforcing a dose-dependent response.

These findings align with the hypothesis that higher doses of Serratiopeptidase elicit stronger anxiolytic action, thereby offering enhanced behavioral outcomes. (Table 12).

**Table 12: Effects of oral Serratiopeptidase on anxiety-related behaviors in mice: Light/dark box test after 15 days of administration**

| Parameters / Groups (orally) | Number of times the dark side was entered | Number of times the light side was entered | Period of staying on the dark side (sec) | Periods of staying on the bright side (sec) |
|---|---|---|---|---|
| **Negative control (Distilled water)** | 6.40 ± 1.43 | 5.40 ± 1.43 | 214.00 ± 25.80 | 85.60 ± 26.11 |
| **Positive control (Sertraline)** | 9.40 ± 1.07* | 8.60 ± 1.02* | 188.00 ± 15.54 | 112.00 ± 15.54 |
| **Serratiopeptidase (5mg/kg)** | 4.00 ± 0.70* | 3.40 ± 0.92* | 214.20 ± 23.73 | 85.80 ± 23.73 |
| **Serratiopeptidase (10mg/kg)** | 5.20 ± 0.58* | 4.60 ± 0.67* | 179.00 ± 15.37 | 118.60 ± 14.92 |
| **Serratiopeptidase (20mg/kg)** | 7.00 ± 1.61* | 6.40 ± 1.69* | 150.80 ± 32.11 | 149.20 ± 32.11 |

The data are mean ± SE of 5 mice/group/ for five minutes

* Significantly different from the data of control group, at ($p \leq 0.05$).

## 4-5: Assessment anti-depressant effects of single oral dose of Serratiopeptidase in mice using tail suspension test and forced swimming test (one hour after administration)

In the tail suspension test, the negative control group exhibited the longest immobility time, while the positive control (sertraline) showed reduced immobility (16%). Notably, mice treated with 10 mg/kg Serratiopeptidase displayed a marked decrease in immobility time (7%), outperforming both controls and indicating a pronounced antidepressant like effect. However, the 20mg/kg dose group exhibited an unexpected

increase in immobility (12%) compared to the 10mg/kg group, suggesting a biphasic response where higher doses may attenuate efficacy. Standard error values highlighted variability across groups, with the 10mg/kg dose showing the lowest variability. These findings imply that Serratiopeptidase, particularly at 10mg/kg, may possess acute antidepressant activity (Table 13).

**Table (13): Acute antidepressant-like effects of a single oral dose of Serratiopeptidase in mice: Tail suspension test outcomes after one hour**

| Parameters / Groups (orally) | Immobility time (sec) in tail suspension test | Percentage of immobility time |
|---|---|---|
| **Negative control (Distilled water)** | 62.33 ± 21.94 | 17% |
| **Positive control (Sertraline)** | 56.83 ± 16.66 | 16% |
| **Serratiopeptidase (5mg/kg)** | 58.33 ± 16.05 | 16% |
| **Serratiopeptidase (10mg/kg)** | 25.50 ± 9.90 | 7% |
| **Serratiopeptidase (20mg/kg)** | 43.50 ± 17.92 | 12% |

The data are mean ± SE of 6 mice/group/for five minutes

In the forced swimming test, the negative control group (distilled water) exhibited the highest immobility time (48%), while the positive control showed a (22%) reduction. Mice treated with 5mg/kg and 20 mg/kg Serratiopeptidase displayed a decrease in immobility time (15% and 14%, respectively), with the 20mg/kg dose achieving statistical significance

compared to the negative control. However, the 10 mg/kg Serratiopeptidase group demonstrated an unexpected increase in immobility time of approximately 30% in the measured parameter, suggesting a potential biphasic or non-monotonic dose-response relationship (Table 14).

**Table (14): Acute antidepressant-like effects of a single oral dose of Serratiopeptidase in mice: Forced swimming test outcomes after one hour**

| Parameters / Groups (orally) | Immobility time (sec) in Forced swimming test | Percentage of immobility time |
|---|---|---|
| **Negative control (Distilled water)** | 144.20 ± 39.42 | 48% |
| **Positive control (Sertraline)** | 67.20 ± 30.83 | 22% |
| **Serratiopeptidase (5mg/kg)** | 45.60 ± 17.90 | 15% |
| **Serratiopeptidase (10mg/kg)** | 89.40 ± 19.64 | 30% |
| **Serratiopeptidase (20mg/kg)** | 40.80 ± 16.39* | 14% |

The data are mean ± SE of 5 mice/group/for five minutes

* Significantly different from the data of control group, at ($p \leq 0.05$).

## 4-6: Assessment anti-depressant effects of repeated oral doses of Serratiopeptidase in mice using tail suspension test and forced swimming test (15 days)

In TST, the doses of 10mg/kg and 20mg/kg show the most substantial reduction in immobility time, suggesting a strong potential for the antidepressant-like effects. Both Serratiopeptidase doses exhibit the same decrease (11%) in immobility compared to either control (21%) the

negative control and (22%) the positive control, even exceeding sertraline, a known antidepressant. In contrast, Serratiopeptidase 5mg/kg (19%) demonstrates a less pronounced effect, though still lower than the negative control. Higher doses (10mg/kg and 20mg/kg) showing similar, if not superior, effects to the positive control, positioning Serratiopeptidase as a promising candidate in the context of antidepressant therapies (Table 15).

**Table 15: Antidepressant-like effects of oral dose of Serratiopeptidase in mice: Tail suspension test outcomes after 15 days of administration**

| Parameters / Groups (orally) | Immobility time (sec) in tail suspension test | Percentage of immobility time |
|---|---|---|
| **Negative control (Distilled water)** | 64.40 ± 24.14 | 21% |
| **Positive control (Sertraline)** | 66.40 ± 18.52 | 22% |
| **Serratiopeptidase (5mg/kg)** | 56.20 ± 13.40 | 19% |
| **Serratiopeptidase (10mg/kg)** | 33.60 ± 20.91 | 11% |
| **Serratiopeptidase (20mg/kg)** | 33.00 ± 11.67 | 11% |

The data are mean ± SE of 5 mice/group/for five minutes

The forced swimming test demonstrates an antidepressant effect over 15 days of administration. The antidepressant effect appeared in the form of a significant decrease in immobility time at groups (10 mg/kg and 20mg/kg), as percentage (6% and 7%, respectively) compared to negative control (29%), with no significant effect compared to positive control (8%). Reflecting the outcomes in TST, higher doses elicit a stronger antidepressant effect, thus supporting its therapeutic importance in the management of depressive disorders (Table 16).

**Table 16: Antidepressant-like effects of oral dose of Serratiopeptidase in mice: Forced swimming test outcomes (15 days)**

| Parameters / Groups (orally) | Immobility time (sec) in Forced swimming test | Percentage of immobility time |
|---|---|---|
| **Negative control (Distilled water)** | 85.80 ± 23.11 | 29% |
| **Positive control (Sertraline)** | 24.80 ± 10.69* | 8% |
| **Serratiopeptidase (5mg/kg)** | 62.20 ± 17.17 | 21% |
| **Serratiopeptidase (10mg/kg)** | 18.80 ± 8.26* | 6% |
| **Serratiopeptidase (20mg/kg)** | 22.40 ± 11.26* | 7% |

The data are mean ± SE of 5 mice/group/for five minutes

* Significantly different from the data of control group, at ($p \leq 0.05$).

## 4-7: Assessment of the anticonvulsant effect of repeated oral doses of Serratiopeptidase in mice (15 days)

Administration of Serratiopeptidase for 15 days demonstrated significant effects on pilocarpine-induced convulsions in mice. Compared to the negative control, all Serratiopeptidase doses delayed the onset of convulsions with higher doses (10 and 20 mg/kg) showing greater delay than the 5 mg/kg dose. However, the positive control (diazepam) exhibited the longest delay, and all Serratiopeptidase groups remained significantly shorter than those observed in the diazepam group. The number of convulsions decreased significantly in the entire treatment groups ($p \leq 0.05$ vs. negative control), with (10 and 20 mg/kg) showing stronger suppression than diazepam though statistical significance relative to diazepam was not

explicitly marked. Survival rates varied: the 5mg dose group had the lowest survival (50%), while the 10 and 20mg/kg doses matched diazepam (100%) survival. The negative control showed (75%) survival (Table 17).

**Table 17: Dose-dependent effects of 15-day Serratiopeptidase administration on pilocarpine-induced convulsions in mice**

| Parameters / Groups | Onset of convulsion (minute) | Number of convulsions (minute) | Survival percentage |
|---|---|---|---|
| **Negative control (Distilled water)** | 8.00±2.0 | 10.3±2.1 | 75% |
| **Positive control (diazepam 1mg/kg IP)** | 32.75±3.2* | 5.8±1.2* | 100% |
| **Serratiopeptidase (5mg/kg)** | 14.50±2.7*$^{A}$ | 4.5±0.6* | 50% |
| **Serratiopeptidase (10mg/kg)** | 24.50±2.4 *$^{AB}$ | 3.5±0.4* | 100% |
| **Serratiopeptidase (20mg/kg)** | 24.25±0.5*$^{A\,B}$ | 3.5±0.3* | 100% |

The data are mean ± SE of 8 mice/group/for one hour.

* Significantly different from the data of control group, at ($p \leq 0.05$).

A Significantly different from the data of positive control, at ($p \leq 0.05$).

B Significantly different from the data of group of 5mg/kg, at ($p \leq 0.05$).

## 4-8: Determination the effect of repeated oral doses of Serratiopeptidase on general anesthesia and its effect on vital organs as monitored using a veterinary patient monitor

### 4-8-1: Anesthesia test: Interpretation the effect of Serratiopeptidase on general anesthesia after 15 days of administration

Administration of Serratiopeptidase for 15 days significantly altered xylazine-ketamine – induced anesthesia parameters in mice. Compared to

the control group, the 5 mg/kg and 10 mg/kg doses delayed the onset of anesthesia (147.40 ± 8.13 and 137.40 ± 17.72 seconds, respectively vs. control: 72.88 ± 13.50 seconds; $p \leq 0.05$) and prolonged the duration of anesthesia (192.25 ±32.81 and 195.75 ± 26.66 minutes, respectively vs. control: 119.50 ± 7.22 minutes; $p \leq 0.05$). In contrast, the 20 mg/kg dose partially reversed these effects, showing a shorter onset (107.38 ± 8.05 seconds, $p \leq 0.05$ vs. 5 mg/kg group) and duration (120.50 ± 14.74 minutes, comparable to control but $p \leq 0.05$ vs. 5 mg/kg group). Recovery time remained unaffected across all doses (from 55.3 ± 9.38 to 77.9 ± 14.90 minutes vs. control: 59.0 ± 5.82 minutes; $p > 0.05$). In summary, Serratiopeptidase produced modulation: lower doses(5-10mg) were associated with significant prolongation of both anesthesia induction and maintenance, while the highest dose (20mg) attenuated these effects, but still statistically difference compared to the control group (Table 18).

**Table 18: The effect of Serratiopeptidase (15 days of administration) on the anesthesia by xylazine and ketamine (10, 150 mg, I.P)**

| Parameters / Groups (orally) | Onset of anesthesia (second) | Duration of anesthesia (minute) | Recovery of anesthesia (minute) |
|---|---|---|---|
| **Control (Distilled water)** | 72.88±13.50 | 119.50±7.22 | 59.0±5.82 |
| **Serratiopeptidase (5mg/kg)** | 147.40±8.13* | 192.25±32.81* | 55.3±9.38 |
| **Serratiopeptidase (10mg/kg)** | 137.40±17.72* | 195.75±26.66* | 64.5±9.69 |
| **Serratiopeptidase (20mg/kg)** | 107.38±8.05$^{A}$ | 120.50±14.74$^{AB}$ | 77.9±14.90 |

The data are mean ± SE of 8 mice/group.

* Significantly different from the data of control group, at ($p \leq 0.05$).

A Significantly different from the data of group 5mg/kg, at ($p \leq 0.05$).

B Significantly different from the data of group of 10mg/kg, at ($p \leq 0.05$).

### 4-8-2: Effects of Serratiopeptidase on vital organ (vital signs and physiological parameters) by using a veterinary patient monitor device

oxygen saturation ($SpO_2$): As the Serratiopeptidase dosage increases, oxygen saturation improves significantly, with the 20mg/kg Serratiopeptidase group showing the highest average oxygen saturation. The differences are statistically significant when compared to the control group. Respiratory rate (Breaths per minute): The respiratory rate tends to rise with the increasing doses of Serratiopeptidase, with the 20mg/kg group showing the highest average respiratory rate. However, a significant increase was observed at the 5mg/kg and 20mg/kg groups. Heart rate (Beats per minute): The heart rate decreases significantly at all doses, suggesting a potential calming or slowing effect on the heart rate. From this standpoint, oxygen saturation increases significantly with higher doses, with the 20mg/kg group showing the most improvement. (Table 19)

**Table 19: The effect of Serratiopeptidase (15 days of administration) on the vital organ (physiological parameters) indices by xylazine and ketamine (10, 150 mg, I.P) as monitored by veterinary patient monitor**

| Parameters / Groups (orally) | Oxygen saturation ($SpO_2$, %) | Respiratory rate (Breaths per minute-bpm) | Heart rate (Beats per minute-bpm) |
|---|---|---|---|
| **Control (Distilled water)** | 86.50 ±4.21 | 11.0±0.4 | 101.5±15.2 |
| **Serratiopeptidase (5mg/kg)** | 92.00 ±1.83 | 17.5±2.9* | 84.0±1.8 |
| **Serratiopeptidase (10mg/kg)** | 95.25±0.81* | 15.8±1.1 | 82.3±5.6 |
| **Serratiopeptidase (20mg/kg)** | 97.50±0.42* | 19.8±2.9* | 83.3±3.2 |

The data are mean ± SE of 8 mice/group.

* Significantly different from the data of control group, at ($p \leq 0.05$).

# CHAPTER FIVE

## DISCUSSION

# Chapter Five

# Discussion

The comprehensive investigation into the neurobehavioral, anxiolytic, antidepressant, anticonvulsant, and physiological effects of Serratiopeptidase in murine models has yielded a wealth of data. These findings not only underscore the multifaceted pharmacological potential of this proteolytic enzyme but also raise intriguing questions about its mechanisms of action, dose-response relationships, and therapeutic applicability. Below, we synthesize these results, contextualize them within existing literature, and explore their implications for future research and clinical translation.

## I- Neurobehavioral Effects of a Single SP Dose

### 1. Open Field Test (OFT): Enhanced Locomotor and Exploratory Activity

The OFT results demonstrated a significant increase in horizontal locomotion (crossed squares) and vertical exploration (rearing) across all SP-treated groups compared to controls. This suggests a stimulatory effect of SP on exploratory behavior. Elevated locomotion and rearing are classically associated with reduced anxiety or increased curiosity in rodents, though hyperactivity can also reflect stress or CNS excitation(Yen, 2012).

The lack of a linear dose-response relationship in rearing (peak at 10 mg/kg vs. 20 mg/kg) implies that higher doses may saturate receptor systems involved in vertical exploration or activate competing pathways. For instance, SP's anti-inflammatory properties—mediated through proteolytic activity—could indirectly modulate neurotransmitter systems

such as dopamine or serotonin, which regulate motor activity and reward-seeking behavior(Darbandi *et al.*, 2024). Alternatively, SP might influence neurotrophic factors like Brain-derived neurotrophic factor (BDNF), which enhance synaptic plasticity and exploratory drive(Wu *et al.*, 2023).

The slight increase in fecal pellets (a marker of stress) in treated groups, albeit statistically insignificant, raises questions about SP's dual role. While increased locomotion suggests reduced anxiety, fecal pellets may indicate mild gastrointestinal or emotional stress(Accarie and Vanuytsel, 2020). However, the absence of significant changes in urination (except a minor increase at 5 mg/kg) argues against pronounced stress responses. Further studies measuring plasma corticosterone levels could clarify this dichotomy.

**2. Negative Geotaxis (NG) Test: Motor Coordination Effects**

In the NG test, the 5 mg/kg SP group showed prolonged latency to reorient, suggesting transient motor impairment or anxiety-like behavior at lower doses. In contrast, higher doses (10 and 20 mg/kg) did not differ from controls, indicating potential adaptation or biphasic effects. Negative geotaxis relies on vestibular function, proprioception, and motor coordination, and delays often reflect cerebellar dysfunction or neuromuscular fatigue(Graham *et al*., 2016).

The normalization of latency at higher doses might involve compensatory mechanisms. For example, SP's anti-inflammatory action could mitigate neuroinflammation-induced motor deficits at higher concentrations, whereas lower doses are insufficient to engage these pathways. Alternatively, SP may interact with ion channels or neurotransmitter receptors (e.g., GABA or glutamate). Further electrophysiological studies are needed to explore these hypotheses.

### 3. Head Pocking (HP) Test: Biphasic Effects on Exploratory Behavior

The HP test showed no change on head pocking at 5 mg/kg SP but increased activity at 10 and 20 mg/kg. Head pocking reflects exploratory behavior and curiosity, and its suppression at low doses may indicate mild sedation or anxiety(File and Wardill, 1975). Conversely, higher doses enhanced this behavior, aligning with the OFT findings of increased exploration. The term biphasic response describes a two-phase reaction to a stimulus in which the effect varies with dose or over time, for instance, a medication could suppress a response at high doses while stimulating it at low ones (or vice versa) (Beckon *et al*., 2008), and this pattern parallels studies on substances like caffeine, where low doses induce anxiety and high doses promote arousal(Smith, 2020).

SP's proteolytic activity may degrade proteins that inhibit neurotransmitter release (e.g., synaptotagmin), thereby enhancing synaptic transmission at higher doses. Additionally, SP's ability to reduce cytokines (e.g., TNF-α, IL-6) could alleviate inflammation-induced lethargy, promoting exploration(Session, 2021). The contrasting effects at 5 mg/kg versus higher doses underscore the need for studies to identify thresholds for behavioral modulation.

### 4. Swimming Test: No Impact on Motor Endurance

The swimming test demonstrated no significant differences between SP-treated and control group, indicating preserved motor coordination and endurance. This contrasts with the NG test's transient deficit at 5 mg/kg, suggesting that SP's effects are task-specific rather than globally impairing. Swimming relies on integrated motor and respiratory systems(Lane, 2011), and the absence of deficits implies that SP does not disrupt these pathways acutely.

There has been increasing interest in Serratiopeptidase's central nervous effects over the last few years. Recent research has unveiled a putative role for Serratiopeptidase in ameliorating neuroinflammation-mediated transient or chronic pain and associated neurological disorders(Tiwari, 2017; Jadhav *et al*., 2020).

To circumvent the limitations of in-vitro studies, various animal models are often employed to assess the efficacy of Serratiopeptidase to improve motor function in response to insult in a clinically relevant paradigm(Al-awadhi *et al.*, 2024; Foda *et al.*, 2024). Attenuating motor deficits in animals represents a direct behavior of the enzyme, thereby indicating a lesser degree of subjectivity than the pain/discomfort threshold(Jadhav *et al*., 2020). Several animal behavioral paradigms have been employed to evaluate motor enhancements, including motor coordination-improving tests such as rotarod, staircase, and horizontal/vertical grip strength, as well as tests to indicate improvements in fine and gross motor function(Crawley, 1999). Therefore, the use of various animal models in this section might not only underscore the translation potential of Serratiopeptidase in clinical applications but also its beneficial effects on diverse motor activities. Furthermore, the discussions in this section also provide insight into the biological relevance of employing these in vivo models that imitate human pathologies.

Findings in these in-vivo animal behavioral studies corroborate the beneficial effects of Serratiopeptidase administration by improving motor function. For instance, oral administration of Serratiopeptidase to arsenic-exposed rats improved locomotor activity, increased the number of rearings, and decreased immobility time in open-field test measurements. In sciatic nerve crush-induced neuropathic pain in rats, oral administration of Serratiopeptidase increased coordination and muscle power (Firdaus *et*

*al.*, 2022; Saxena *et al.*, 2022; Naik *et al.*, 2023). When compared to the oxaliplatin-treated group, administration of Serratiopeptidase to the 'reversed' group of rats resulted in the recovery of sciatic function index, foot print study, and decreased expression of motor protein in the sciatic nerve(Jagannathan *et al.*, 2020; Mutzberg, 2021). The intracerebroventricular injection of Serratiopeptidase restored grip strength and prolonged the retention time and latency to lift in comparison with the group in rats, thereby enhancing the fine motor activities in these rats in the training and on the first day of the memory assessment(Naik *et al.*, 2023).

The increase in motor activity and neurobehavioral of mice treated with Serratiopeptidase in our study may be due to its mechanisms in the brain through inhibition of the brain cholinesterase and thus an increase in the concentration of acetylcholine, which has stimulating effects for the central nervous system (CNS) and memory. In the CNS, acetylcholine acts as a fast synaptic neurotransmitter to create direct and indirect excitatory effects on postsynaptic neurons as well as on presynaptic neurons(Ohkuma and Katsura, 2001; Teleanu *et al.*, 2022).

SP degrades pro-inflammatory cytokines and bradykinin, reducing neuroinflammation. This could enhance neuronal excitability and exploratory behavior. By direct neurotransmitter modulation: Proteases can cleave peptide neurotransmitters (e.g., substance P) or modulate receptor activity, altering dopamine or serotonin signaling(Ossovskaya and Bunnett, 2004). And gut-brain axis; Oral SP may influence gut microbiota, producing metabolites (e.g., short-chain fatty acids) that affect CNS function(Ni *et al.*, 2025).

## II- Neurobehavioral Effects of Repeated SP Doses

### 1. Open Field Test (OFT): Sustained Locomotor and Exploratory Enhancement

Repeated SP administration over 15 days reinforced the acute findings of increased horizontal locomotion (squares crossed) and vertical exploration (rearing). The 20 mg/kg group exhibited the highest horizontal activity (96.13 ±6.65), surpassing even the acute 20 mg/kg group (93.6 ±3.52), suggesting cumulative stimulation of locomotor pathways. Vertical activity (rearing) showed (16.00 ±2.67 in controls vs. 24.25 ±1.68 at 20 mg/kg), contrasting with the acute study's peak at 10 mg/kg. This divergence implies that chronic SP exposure may enhance adaptive neuroplasticity or upregulate receptors involved in exploratory behavior, such as dopamine D1 or glutamate NMDA receptors(Session, 2021).

The sustained increase in rearing—a marker of curiosity and reduced anxiety—aligns with SP's proposed anti-inflammatory action. Chronic neuroinflammation is associated with lethargy and reduced exploration, and SP's proteolytic degradation of pro-inflammatory cytokines (for example, IL-6, TNF-α) could alleviate these effects(Louati and Berenbaum, 2015). Furthermore, SP may modulate the gut-brain axis by altering microbiota composition, resulting with increased short-chain fatty acids (SCFAs) being produced, like butyrate, which enhances BDNF expression and synaptic plasticity(Silva *et al*., 2020).

### 2. Negative Geotaxis (NG) Test: Improved Motor Coordination with Chronic Use

In the NG test, repeated SP administration improved motor performance at higher doses. The 20 mg/kg group demonstrated a 40% reduction in latency to reorient (4.80 ±1.20 vs. 8.00 ±4.27 in control), indicating

enhanced vestibular function or motor learning. This contrasts with acute SP exposure, where the 5 mg/kg group showed transient deficits. The normalization and improvement at higher doses suggest that chronic SP treatment may mitigate neuroinflammation-induced cerebellar dysfunction or enhance GABAergic inhibition, which regulates motor coordination(Abd Wahab *et al*., 2019).

The biphasic response (worse performance at 5 mg/kg vs. improvement at 20 mg/kg) mirrors findings with anti-inflammatory agents like minocycline, where low doses insufficiently engage therapeutic pathways. SP's dose-dependent protease activity could degrade inflammatory mediators more effectively at higher concentrations, restoring neuronal homeostasis.

**3. Head Pocking (HP) Test: Exploratory Drive**

Chronic SP administration induced a progressive increase in head pocking (10.40 ±2.83 in control vs. 12.20 ±1.71 at 20 mg/kg), reflecting heightened curiosity. This aligns with OFT results and supports SP's role in reducing neophobia. The lack of statistical significance, however, underscores the need for larger sample sizes. Head pocking is modulated by serotonin (5-HT) and dopamine systems, and SP's potential to cleave peptide neurotransmitters (e.g., substance P) or reduce oxidative stress may indirectly enhance monoaminergic signaling.

Effect on motor activity: The results revealed a significant increase in both horizontal and vertical motor activity in mice treated with Serratiopeptidase compared to the control group. Several potential mechanisms could underlie these observed effects. Serratiopeptidase is known for its anti-inflammatory properties(Nair, 2022). It is possible that subtle, subclinical inflammation could be affecting motor function and

exploratory drive(Jia, 2022; Cordone, 2024), and the anti-inflammatory effect of Serratiopeptidase could be mitigating this. Alternatively, Serratiopeptidase may influence neurotransmitter system involving in motor control and behavior such as dopaminergic or serotonergic systems(Ahmed *et al*., 2013; Naser and Albadrany, 2024). Many studies demonstrated that Serratiopeptidase has an inhibitory effect on brain cholinesterase which is responsible for the degradation of excitatory neurotransmitter acetylcholine(Jadhav *et al*., 2020; Naser and Albadrany, 2024). Future studies exploring these mechanisms are necessary to fully elucidate the underlying processes.

The results of negative geotaxis and head pocking test present a complex picture of Serratiopeptidase's effects on motor coordination and exploratory behavior. The negative geotaxis effect test results suggest a biphasic dose response. While the lower doses (5mg/kg and 10mg/kg) seemed to impair motor coordination (though not statistically significant), the highest dose(20mg/kg) appeared to improve it. This non-linear dose response warrants further investigation to understand the underlying mechanism. At the lower doses 5 and 10mg/kg, the slight increase in time suggests a possible negative effect on motor coordination or muscle strength at these doses. This could be to various reasons such as; mild muscle relaxation, Serratiopeptidase has some anti-inflammatory effects, which might lead to mild muscle relaxation at doses, slightly hindering the mice’s ability to quickly turn over(Malanga and Wolff, 2008; Malm and Borisch, 2015). Another reason, interference with nerve signaling, it’s possible that doses subtly interfere with nerve impulses involved in motor control. High dose 20mg, the significant decrease in time is intriguing. It suggests a potential improvement in motor function. This could be due to;

enhancement of muscle strength or coordination or stimulatory effect on the nervous system(Sheffler and Chae, 2007).

The head pocking reveals a different pattern. The highest dose of Serratiopeptidase lead to increase in head pokes, suggesting an increase exploratory and/or potentially anxiety-related behavior. This finding raises concerns about potential behavioral side effects of higher doses of Serratiopeptidase. High dose 20mg, the substantial increase to 17.4 pocks is particularly noteworthy, this may be due to exacerbates underlying neurological processes, if these mice have an underlying predisposition to repetitive movements (which is common in some mouse strains)(Barr and Barbe, 2002; Moy *et al.*, 2008), the Serratiopeptidase might be exacerbating those tendencies. Other interpretation, induces or unmasks repetitive behavior, it's possible that the high dose is directly inducing these repetitive movements, or unmasking them by affecting certain brain circuits(Rossini and Pauri, 2000; Shadmehr, 2017).

Several factors could contribute to those observed effects. The anti-inflammatory effect of Serratiopeptidase is one of them, the varying effects at different doses could be related to the modulation of inflammatory pathways at different concentrations. Furthermore, Serratiopeptidase may interact with the neurotransmitter system, like dopamine, serotonin, or acetylcholine. Different doses could differentially affect these systems. At higher concentrations Serratiopeptidase can often have (off-target) effects meaning they interact with other systems or processes in the body beyond their primary target, leading to unintended behavioral consequences(Rudmann, 2013).

## III- Anxiolytic Effects of SP

### 1. Acute Anxiolysis: Elevated Plus Maze (EPM) and Light/Dark box Tests

In the acute EPM test, the 20 mg/kg SP group spent significantly more time in open arms (187.40 ±23.92 sec vs. 111.40 ±5.97 in controls) and reduced entries into closed arms. This suggests robust anxiolytic effects at high doses.

The light/dark box test corroborated these findings: the 20 mg/kg group spent 198.40 ±26.23 sec in the light side (vs. 157.00 ±19.69 in controls), indicating reduced aversion to bright environments. Intriguingly, the 10 mg/kg group showed conflicting trends—increased light-side entries but no change in time spent, highlighting task-specific effects. Such discrepancies are common in anxiety models due to differing motivational drives (e.g., exploration vs. risk assessment).

Our study's main strength is that the dose of Serratiopeptidase that is therapeutically effective against experimentally induced anxiety has been demonstrated. The antianxiety effects of the protease enzyme have been investigated using various experimental models. In this regard, several researchers have reported the antianxiety effects exhibited by it. The antianxiety potential of Serratiopeptidase has been demonstrated in an acute restraint stress-induced anxiety test in rat(Bakare and Owoyele, 2021). The anti-inflammatory aspect appears to be the most likely candidate. Serratiopeptidase minimizes discomfort and probable anxiousness by interacting with neurons and perhaps also some neuroprotective chemicals in the brain. It features extremely strong inhibitory potential, is biologically available in the peripheral framework, and quickly crosses the blood-brain barrier(Dhiman and Purohit, 2023).

However, the increase of serotonin and dopamine in the brain brought on during a low dose of Serratiopeptidase suggests modulation in the levels of these key neurotransmitters, which might be the reason for the anti-anxiety action. In the present study, the concentration of 5-HT and the metabolite of dopamine, 5-HIAA, significantly increased in the cortex and hypothalamus of mice(Yardimci *et al.*, 2023).

**2. Sustained Anxiolysis: Enhanced Efficacy Over Time**

Repeated SP administration amplified anxiolytic effects. In the 15-day EPM test, the 20 mg/kg group spent 120.60 ±29.79 sec in open arms (vs. 72.00 ±27.69 in controls), surpassing sertraline (99.00 ±20.72). This suggests SP's anti-inflammatory and neuroprotective effects accumulate over time, potentially stabilizing limbic circuitry (e.g., amygdala-prefrontal connectivity). The light/dark box test further validated this; the 20 mg/kg group spent 149.20 ±32.11 sec in the light side (vs. 85.60 ±26.11 in controls), with efficacy exceeding sertraline (112.00 ±15.54).

The reduction in dark-side engagement aligns with SP's proposed modulation of the hypothalamic-pituitary-adrenal (HPA) axis(Kapoustina and Rouchotas, 2014). Chronic inflammation dysregulates glucocorticoid release, exacerbating anxiety(Hassamal, 2023). By degrading inflammatory mediators, SP may normalize HPA activity, reducing corticosterone levels and anxiety-like behavior.

The anxiolytic effects of Serratiopeptidase were evident in the elevated plus maze and light/dark box tests. These tests assess anxiety-like behavior by measuring exploratory behavior in aversive environments. The increased time spent in the open arms of the elevated plus maze and observed in Serratiopeptidase-treated mice suggest an anxiety reduction.

In a study similar to ours conducted on rats, this study found that bromelain, a proteolytic enzyme, has analgesic and anti-anxiety effects(Bakare and Owoyele, 2021). Aceclofenac alone or in conjunction with Serratiopeptidase was demonstrated in a study to exhibit antidepressant mechanisms through anti-inflammatory activities that lower cytokines that promote inflammation, including IL-6 (Dodiya and Goswami 2022).

Even though the study's findings are encouraging more investigation is required to pinpoint Serratiopeptidase's exact mechanism of action. Additionally, future studies should investigate the long-term effects of Serratiopeptidase and its potential therapeutic applications in the treatment of anxiety and depression.

## IV- Antidepressant-Like Effects of SP

### 1. Tail Suspension Test (TST): Biphasic Dose Response

A single 10 mg/kg SP dose reduced immobility time by (7% immobility vs. 16% in sertraline), indicating acute antidepressant effects. However, the 20 mg/kg group showed reduced efficacy (12% immobility), suggesting receptor saturation or activation of counter-regulatory pathways (e.g., 5-HT2C receptors, which inhibit monoamine release at high concentrations). This biphasic response is observed with SSRIs like fluoxetine, where excessive serotonin release triggers autoreceptor desensitization over time(Poul *et al*., 1995).

### 2. Mechanistic Considerations for Antidepressant Activity

SP's antidepressant effects may stem from:

Since depression is caused by chronic inflammation, SP's ability to lower pro-inflammatory cytokines like IL-1β and TNF-α may aid in restoring

hippocampal (responsible for memory and mood) neurogenesis. This is one of the mechanisms by which SP has antidepressant effects. Furthermore, SP enhances synaptic plasticity (it is the brain's ability to adapt and learn) by promoting the upregulation of brain-derived neurotrophic factor (BDNF) by utilizing proteolytic activity to cleave pro-BDNF into its mature form. Additionally, SP may alter the gut microbiota by boosting the number of Lactobacillus species, which are known to generate precursors of serotonin and gamma-aminobutyric acid (GABA), two chemicals linked to mood regulation.(Dicks, 2022).

**3. Antidepressant Effects: Biphasic Responses and Dose Optimization**

**3.1. Acute Administration: Tail Suspension Test (TST) and Forced Swimming Test (FST)**

A single oral dose of SP demonstrated a biphasic dose-response relationship in both the TST and FST. In the TST, the 10 mg/kg dose reduced immobility time to 7% (vs. 16% in sertraline), indicating acute antidepressant activity. However, the 20 mg/kg group showed reduced efficacy (12% immobility), suggesting receptor saturation or activation of counter-regulatory pathways. Similarly, in the FST, the 5 mg/kg and 20 mg/kg doses reduced immobility to 15% and 14%, respectively, outperforming sertraline (22%), while the 10 mg/kg dose paradoxically increased immobility (30%).

This non-monotonic response aligns with studies on selective serotonin reuptake inhibitors (SSRIs), where excessive serotonin release at higher doses triggers 5-HT2C autoreceptor activation, dampening net serotonin signaling. SP’s proteolytic activity may degrade inflammatory cytokines (e.g., IL-6, TNF-α) that inhibit hippocampal neurogenesis, thereby alleviating depressive behavior. However, at higher doses, SP might cleave

neurotrophic factors like BDNF or overactivated kynurenine pathways, exacerbating depressive phenotypes.

### 3.2. Chronic Administration: Sustained Efficacy

Repeated SP administration over 15 days revealed antidepressant effects. In the TST, both 10 mg/kg and 20 mg/kg doses reduced immobility to 11%, surpassing sertraline (22%). The FST showed even greater efficacy: 10 mg/kg and 20 mg/kg groups exhibited 6% and 7% immobility, respectively, compared to sertraline's 8%. This suggests chronic SP treatment enhances adaptive neuroplasticity, possibly through cumulative anti-inflammatory action or upregulation of monoamine receptors.

The divergence between acute and chronic responses underscores the importance of treatment duration. Chronic inflammation is a hallmark of depression, and SP's sustained protease activity may progressively normalize neuroimmune imbalances, restoring synaptic plasticity(Almond, 2013). Additionally, SP's modulation of the gut-brain axis, via increased short-chain fatty acid (SCFA) production, could enhance serotonin synthesis in the gut, indirectly influencing central serotonin levels.

The antidepressant-like effects of Serratiopeptidase were observed in the forced swimming test. This test measures despair-like behavior by assessing immobility time in a stressful situation. The decreased immobility time in Serratiopeptidase-treated mice suggests an antidepressant-like effect. The mechanisms underlying the anxiolytic and antidepressant-like effects of Serratiopeptidase are not fully understood. However, potential mechanisms have been proposed, including neuroinflammation theory, and Serratiopeptidase may reduce neuroinflammation by inhibiting the release of inflammatory cytokines(Tiwari, 2017). The other suspected mechanism related to

oxidative stress, Serratiopeptidase has a pronounced reducing oxidative stress by scavenging free radicals(Vo *et al*., 2012). The mechanism may be through neurotransmitter modulation, Serratiopeptidase may modulate the levels of neurotransmitters like serotonin, dopamine and GABA, which are involved in mood regulation.

## V- Anticonvulsant Effects: Dose-Dependent Protection

### 1. Pilocarpine-Induced Convulsions

Repeated SP administration (15 days) significantly delayed seizure onset and reduced convulsion frequency. The 10 mg/kg and 20 mg/kg groups delayed onset to ~24.5 minutes (vs. 8 minutes in controls) and reduced convulsions to 3.5 episodes (vs. 10.3 in controls). Survival rates mirrored this trend: 10 mg/kg and 20 mg/kg groups achieved 100% survival, matching diazepam, while the 5 mg/kg group showed 50% survival.

Pilocarpine induces epilepsy by activating M1 muscarinic receptors, elevating intracellular calcium and excitability, which triggers excessive glutamate release, and overstimulating NMDA receptors. This results in excitotoxicity, status epilepticus, neuronal death, oxidative stress, and neuroinflammation involving cytokines, prostaglandins, and bradykinin. Additionally, it alters seizure susceptibility and advancement toward chronic temporal lobe epilepsy (Scorza *et al*., 2009).

SP's anticonvulsant effects likely stem from multiple mechanisms: GABAergic Modulation; SP may enhance GABA synthesis or inhibit GABA transaminase, increasing inhibitory tone(Saurav *et al.*, 2022).

The poor survival in the 5 mg/kg group highlights a critical therapeutic window. Suboptimal dosing may insufficiently engage protective pathways, leaving mice vulnerable to seizure-related mortality.

The study also revealed that Serratiopeptidase possesses significant anticonvulsant activity in the pilocarpine-induced seizure model. Pretreatment with Serratiopeptidase resulted in a significant delay in the onset of convulsions and a reduction in the number of seizures compared with the negative control group. Notably, the 10 and 20 mg/kg doses of Serratiopeptidase exhibited comparable efficacy to diazepam, a commonly used benzodiazepine anticonvulsant, in reducing seizure severity and improving survival rates. These findings suggest that Serratiopeptidase may offer a therapeutic benefit in the management of seizures. The potential mechanism underlying these anticonvulsant impacts may include modulation of neurotransmitter systems(Sills and Rogawski, 2020). Pilocarpine induces seizure by activating cholinergic muscarinic receptors, Serratiopeptidase may interfere with this process by modulation cholinergic neurotransmission or by affecting other neurotransmitter system involved in seizure generation and propagation, such as GABAergic or glutamatergic systems(Akyuz *et al.*, 2021). Neuroinflammation in addition to oxidative stress (by increasing neuronal hyperexcitability, which elevates reactive oxygen species, ultimately causing neuronal damage) has a crucial role in the pathophysiology of seizures(Parsons *et al.*, 2022). Serratiopeptidase has known anti-inflammatory and antioxidant properties that could contribute to its anticonvulsant effects by mitigating these pathological processes. Blood-brain barrier modulation, some studies suggest that Serratiopeptidase may enhance drug delivery across the blood-brain barrier(Tiwari, 2017). This leads potentially facilitate the access of endogenous anticonvulsant substances like GABA or neuropeptide Y, both of which suppress neuronal hyperexcitability and seizure generation, thereby restoring excitatory-inhibitory balance within the brain or improves the clearance of pro-convulsant substances from the brain.

## VI- Anesthesia Modulation: Biphasic Interaction with Xylazine-Ketamine

### 1. Onset and Duration of Anesthesia

Chronic SP administration altered xylazine-ketamine anesthesia parameters. Lower doses (5–10 mg/kg) delayed anesthesia onset (147.4–137.4 seconds vs. 72.9 seconds in controls) and prolonged duration (192–195 minutes vs. 119.5 minutes). In contrast, the 20 mg/kg group showed near-normal onset (107.4 seconds) and duration (120.5 minutes).

This biphasic interaction suggests SP may: Inhibit hepatic enzymes at low doses, slowing ketamine metabolism via cytochrome P450 inhibition. Induce enzyme activity at high doses, accelerating drug clearance (Chen *et al*. 2018)

Modulate NMDA Receptors—key glutamate receptors involved in synaptic transmission: Ketamine acts via NMDA receptor antagonism. SP's may exert proteolytic cleavage on NMDA subunits (e.g., GluN2B) at higher doses might restore receptor function, shortening anesthesia.

### 2. Vital Signs: Oxygen Saturation, Respiratory Rate, and Heart Rate

SP improved oxygen saturation ($SpO_2$) (97.5 at 20 mg/kg vs. 86.5 in controls), likely due to reduced pulmonary inflammation and enhanced alveolar perfusion. Respiratory rate increased at 5 mg/kg (17.5 breaths/min) and 20 mg/kg (19.8 breaths/min), suggesting SP stimulates respiratory centers or reduces airway resistance. Heart rate decreased at 5–10 mg/kg (84–82 bpm vs. 101.5 bpm in controls), possibly via vagal activation or reduced sympathetic outflow, while the 20 mg/kg group showed a slight rebound (83.3 bpm). These systemic effects highlight SP's broad physiological impact, extending beyond the CNS to

cardiopulmonary function (Sharma *et al*., 2021; El-Abd and Ibrahim, 2020; Abd El-Hamid *et al*., 2014)

The study was designed to examine the properties of Serratiopeptidase on anesthesia made by a mixture of xylazine and ketamine in murine. Anesthesia is a critical aspect of numerous medical procedures, and understanding how drug and other substances influence its onset, duration, and recovery is important for optimizing patient care(Feldheiser *et al.*, 2016).

Serratiopeptidase at 5mg/kg and 10mg/kg orally significantly delayed the onset of anesthesia compared to the control group. This suggests that lower doses of Serratiopeptidase might be cooperating with the anesthetic agents to slow the induction of anesthesia. Serratiopeptidase may compete with the xylazine and ketamine receptors, and this can inhibit the binding and delay anesthesia(Goldman *et al.*, 2014). Serratiopeptidase can influence the concentrations of neurotransmitters participating in the process of anesthesia, like GABA and glutamate(Azzam *et al.*, 2023). and thus influence the duration of the anesthetic action. Serratiopeptidase 10mg/kg and 5mg/kg significantly increased anesthesia duration than control. This may indicate that lower doses of Serratiopeptidase are potentiating the anesthetic effects of xylazine and ketamine. Serratiopeptidase has anti-inflammatory activity. Anesthetic sensitivity can be modulated by inflammation. Serratiopeptidase may act because it potentially reduces inflammation, therefore enhances the anesthetic effects and prolonged their duration(Wala, 2017; Mutzberg, 2021). Serratiopeptidase may affect the concentrations of some of the neurotransmitters relevant to general anesthesia which includes GABA and glutamate leading to extended duration of action of the general anesthetics. The 20mg/kg dose showed a trend towards a longer recovery time, but it

was not statistically significant. This suggests that higher doses of Serratiopeptidase might have a greater impact on recovery time, potentially due to a stronger interaction with anesthetic agents or physiological systems. Here, it should be noted that high doses of Serratiopeptidase may have a stimulating effect on the nervous system through its mechanism of inhibiting the Brain cholinesterase, and thus a relative increase in the concentration of acetylcholine, which is a stimulating neurotransmitter(Ahmed *et al*., 2013; Fadl *et al*., 2013). Therefore, high doses led to a reduction in the period of anesthesia. All doses of Serratiopeptidase led to a significant increase in $SpO_2$ level compared to the control group. This suggests that Serratiopeptidase might be improving oxygenation during anesthesia. An increase in respiratory rate could be a compensatory mechanism to maintain adequate oxygen delivery to the tissues(Shah, 2021). A deeper level of anesthesia can depress respiratory drive, and the increased respiratory rate might be an attempt to counteract this effect. Serratiopeptidase might directly influence the respiratory centers in the brainstem, leading to increased respiratory drive(Hosseini *et al.*, 2024). Xylazine and ketamine themselves can cause a decrease in heart rate(Afshar *et al*., 2005; Ullah *et al*., 2017). Serratiopeptidase might be potentiating this effect. Serratiopeptidase could potentially stimulate the parasympathetic nervous system, leading to a decrease in heart rate.

## VII- Mechanistic Synthesis: Bridging Behavior and Physiology

SP's multimodal effects arise from its proteolytic, anti-inflammatory, and microbiota-modulating properties: Proteolytic Activity; Degrades fibrin, cytokines (e.g., TNF-α), and bradykinin, reducing neuroinflammation and edema. Neurotransmitter modulation; Enhances serotonin and GABA signaling via cytokine degradation and gut

microbiota interactions. Gut-brain axis; Increases SCFA production (e.g., butyrate), which upregulates BDNF and reduces intestinal permeability, attenuating systemic inflammation. Enzyme induction/inhibition; Alters drug metabolism (e.g., ketamine) and receptor function (e.g., NMDA, GABA-A). These mechanisms synergize to enhance neuronal excitability (OFT), reduce anxiety (EPM), suppress seizures, and modulate systemic physiology.

# CHAPTER SIX

## CONCLUSIONS

## AND RECOMMENDATIONS

# Chapter Six

# Conclusions and Recommendations

## 6-1: Conclusions

1-Acute and chronic treatment with Serratiopeptidase acts as a strong stimulant on the central nervous system, evidenced by its capacity to enhance motor activity and exploratory behavior.

2-Serratiopeptidase augments both physical and mental activity by stimulating the central nervous system.

3-Serratiopeptidase acts as a neurological agent, akin to movement stimulants, due to its capacity to promote motor activity.

4-Serratiopeptidase may exhibits anti-vertigo properties by improving vestibular function and mitigation symptoms of dizziness and related disorders.

5-Acute and chronic treatment with Serratiopeptidase serves as neuroprotective, neuro-enhancing, or neurodevelopmental effects, as evidenced by enhanced vestibular and motor coordination.

6-Acute and chronic treatment with Serratiopeptidase could be considered a nootropic or cognitive enhancer, as it improves cognitive function such as memory by increasing curiosity and enhancing familiarity with the surroundings.

7-Acute treatment with Serratiopeptidase does not have therapeutic effects in neuromuscular disorders.

8-Acute and Chronic treatment with Serratiopeptidase has anxiolytic-like effects in a mouse model, with the best outcome observed at a dose of 20 mg/kg.

9-Acute and chronic treatment with Serratiopeptidase has an antidepressant effect, evidenced by a considerable reduction in immobility time.

10-Chronic treatment with Serratiopeptidase acts as an anticonvulsant by a considerable reduction in the number of convulsions, increasing its onset and stronger suppression than diazepam.

11-Chronic treatment with Serratiopeptidase acting as a general anesthesia adjuvant has potential to prolong anesthesia, and higher dosages influence both the onset and recovery; hence, cautious dose control is required in clinical applications.

12-Chronic treatment with Serratiopeptidase acts as an optimizer for physiological parameters and vital signs, indicating potential benefits in enhancing respiratory function and managing cardiovascular parameters, increase in respiratory activity and potential calming or slowing effect on the heart rate and modulate systemic physiology, but more study is required to determine the therapeutic relevance and underlying mechanisms of these effects.

13-The findings of this study suggest that the Serratiopeptidase exhibits broad-spectrum neuroactive properties, demonstrating anxiolytic, antidepressant, and anticonvulsant efficacy. Its ability to enhance exploratory behavior and improve physiological parameters underscores therapeutic potential for neurological and psychiatric disorder, which supports its potential applicability in veterinary medicine.

## 6-2: Recommendations

1-Estimation of the pharmacokinetics of Serratiopeptidase by HPLC

2-Measuring hormonal changes following Serratiopeptidase administration orally, such as adrenaline, serotonin and glutamate

3-A Histological study of the effect of Serratiopeptidase in several doses on the stomach, brain, kidneys and liver can be conducted.

4-Evaluate adverse effects and toxic effects of Serratiopeptidase, especially in more higher doses.

5-Genetic analysis of serotonin or dopamine receptors in the brain can be conducted after behavioral tests to link behavioral changes to drug effects on genes.

6-Futher research on different animals' species is recommended to assess its efficacy within clinical veterinary practices.

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# الخلاصة

هدفت الدراسة الى تقييم التأثيرات السلوكية العصبية ومزيلة القلق ومضاد للاكتئاب والاختلاج والفسيولوجية وتأثيرها على التخدير العام، لإنزيم سيراتيوببتيداز، وهو إنزيم محلل للبروتين، ثم استخدمه جهاز مراقبة المريض البيطري مصحوبا بالمراقبة وتتبع المستمر للعلامات الحيوية والمعلمات الفسيولوجية لدى الفئران بعد تجريعه عن طريق الفم بشكل فردي ومتكرر. حيث استخدم البحث سلسلة من الاختبارات السلوكية والفسيولوجية القصيرة وطويلة الامد لتقييم الاستجابات المعتمدة على الجرعة عند جرعات ٥ ملغم/كغم و١٠ ملغم/كغم و٢٠ ملغم/كغم مع مقارنات بالسيطرة السلبية (الماء المقطر) والإيجابية (سيرترالين أو الديازيبام). أظهرت النتائج الرئيسية الإمكانات الدوائية المتعددة الأوجه للسيراتيوببتيداز، خاصة عند الجرعات الأعلى، مع تأثيرات ملحوظة على الحركة، والسلوكيات المشابهة للقلق، وعدم الحركة المرتبطة بالاكتئاب، وتعديل التشنج، ووظيفة الأعضاء الحيوية تحت التخدير.

في اختبار الميدان المفتوح (OFT)، زادت الجرعة الفردية من سيراتيوببتيداز بشكل كبير من الحركة الأفقية (على سبيل المثال، ٩٣.٦ ± ٣.٥٢ مربع تم عبورها عند ٢٠ ملغم/كغم مقابل ١٣.٢ ± ١.٧١ في السيطرة، $P \leq 0.05$) والنشاط العمودية (الوقوف على القوائم الخلفية)، مما يشير إلى تعزيز السلوك الاستكشافي. وقد أدى تكرار الإعطاء على مدى ١٥ يومًا إلى زيادة الحركة الأفقية (٩٦.١٣ ± ٦.٦٥ مربعًا عند ٢٠ ملغم/كغم) والاستكشاف العمودي(٢٤.٢٥ ± ١.٦٨ في التسلق)، مما يشير إلى التحفيز الحركي المستمر. وكشف اختبار الانتحاء الارضي السالب (NG) عن تأخر زمن الدوران ١٨٠ درجة بعد جرعة واحدة ٥ ملغم/كغم (١٢.٦ ± ٥.٣١ ثانية مقابل ٩.٨ ± ١.١١ في السيطرة)، بينما قلل تكرار الجرعات عند ٢٠ ملغم/كغم من هذا الزمن (٤.٨٠ ± ١.٢٠ ثانية)، مما يعكس تحسن الوظيفة الدهليزية، كما زاد اختبار عدد مرات إدخال الرأس في الثقوب، وهو مقياس للفضول، كل من الإعطاء الفردي والمتكرر (الجرعات المتكررة ١٢.٢٠ ± ١.٧١ عدد عند ٢٠ ملغم/كغم مقابل ١٠.٤٠ ± ٢.٨٣ في السيطرة).

في اختبار المتاهة المرتفعة (EPM)، أظهرت الجرعة الفردية من السيراتيوببتيداز عند (٢٠ ملغم/كغم) زيادة كبيرة في مقدار الوقت الذي قضته الفئران في الأذرع المفتوحة(١٨٧.٤٠ ± ٢٣.٩٢ ثانية مقابل ١١١.٤٠ ± ٥.٩٧ في السيطرة السلبية، $p \leq 0.05$) وتقليل الدخول

في الأذرع المغلقة، مما يدل على انخفاض القلق. وقد عزز تكرار الإعطاء هذا الاتجاه مع قضاء مجموعة ٢٠ ملغم/كغم ( ١٢٠.٦٠ ± ٢٩.٧٩ ثانية) في الأذرع المفتوحة، متجاوزة حتى سيرترالين (٩٩.٠٠ ± ٢٠.٧٢ ثانية). وأيد اختبار صندوق الضوء/الظلام هذه النتائج; زادت جرعة ٢٠ ملغم/كغم من استكشاف الجانب المضيء (١٩٨.٤٠ ± ٢٦.٢٣ ثانية مقابل ١٥٧.٠٠ ± ١٩.٦٩ في السيطرة السلبية) متفوقة على سيرترالين، وقللت من تفضيل الجانب المظلم، مما يسلط الضوء على التأثيرات القوية المزيلة للقلق. حتى بعد ١٥ يومًا من الاعطاء، تم الكشف عن انخفاض كبير في القلق مع تفضيل فترات البقاء على الجانب المضيء، والعكس على الجانب المظلم.

في الاعطاء الحاد، قللت الجرعة الفردية من سيراتيوببتيداز عند ١٠ ملغم/كغم من زمن الجمود لدى الفئران في اختبار تعليق الذيل (TST) بنسبة ٧٪ (٢٥.٥٠ ± ٩.٩٠ ثانية مقابل ٦٢.٣٣ ± ٢١.٩٤ في السيطرة السلبية)، متفوقة على سيرترالين. ومع ذلك، ظهرت استجابة ثنائية الطور عند ٢٠ مجم/كجم (٤٣.٥٠ ± ١٧.٩٢ ثانية) في الجرعات المتكررة، ١٠ و٢٠ ملغم/كغم بنسبة (١١٪) مقابل (٢١٪ و٢٢٪ في مجموعتي السيطرة على التوالي). وبالمثل، كشف اختبار السباحة القسرية (FST) عن انخفاض في عدم الحركة عند ٥ ملغم/كغم (١٥٪) و٢٠ ملغم/كغم (١٤٪)، على الرغم من أن مجموعة ١٠ ملغم/كغم زادت بشكل متناقض من عدم الحركة بنسبة ٣٠٪. بعد ١٥ يوماً من الإعطاء، أظهرت جرعات ١٠ ملغم/كغم و٢٠ ملغم/كغم فعالية مستدامة لمضادات الاكتئاب، مما قلل من عدم الحركة في اختبار TST إلى ( ١١٪) مقابل ( ٢١٪ و٢٢٪ في مجموعتي السيطرة على التوالي) وعدم الحركة في اختبار FST إلى( ٦-٧٪) مقابل ( ٢٩٪ في السيطرة السلبية)، وهو ما يتوافق مع السيرترالين.

أدى تكرار إعطاء سيراتيوببتيداز إلى تأخير بداية التشنج الناجم عن البيلوكاربين بطريقة تعتمد على الجرعة (٢٤.٢٥ ± ٠.٥ دقيقة عند ٢٠ ملغم/كغم مقابل ٨.٠٠ ± ٢.٠ في السيطرة، $p \leq 0.05$) وخفض تواتر التشنج (٣.٥ ± ٠.٣ مقابل ١٠.٣ ± ٢.١ في السيطرة). وتحسنت معدلات البقاء على قيد الحياة إلى (١٠٠٪) عند ١٠-٢٠ ملغم/كغم، وهو ما يضاهي فعالية الديازيبام.

وأدى السيراتيوببتيداز إلى إطالة بداية التخدير (١٤٧.٤٠ ± ٨.١٣ ثانية عند ٥ ملغم/كغم مقابل ٧.٢٢ في السيطرة) ومدته (١٩٢.٢٥ ± ٣٢.٨١ دقيقة عند ٥ ملغم/كغم مقابل ١١٩.٥٠ ± ٧.٢٢ في السيطرة). بالمثل، عند (١٠ و٢٠ ملغم/كغم). زاد وقت التعافي ، حيث

بلغ ذروته عند (٢٠ مجم/كجم) ٧٧.٩ ± ١٤.٩٠ دقيقة. وكشفت مراقبة الأعضاء الحيوية عن تحسن في تشبع الأكسجين ويبلغ ذروته (٩٧.٥٠ ± ٠.٤٢ عند ٢٠ ملغم/كغم مقابل ٨٦.٥٠ ± ٤.٢١ في السيطرة)، وارتفاع معدلات التنفس (١٩.٨ ± ٢.٩ نفس/الدقيقة عند ٢٠ ملغم/كغم مقابل ١١.٠ ± ٠.٤ في السيطرة)، وانخفاض معدلات ضربات القلب (٨٣.٣ ± ٣.٢ نبضة في الدقيقة عند ٢٠ ملغم/كغم مقابل ١٠١.٥ ± ١٥.٢ في السيطرة)، مما يشير إلى تحسن تشبع الأكسجين وتحسن خفيف في الجهاز التنفسي القلبي.

في الاستنتاج، يُظهر السيراتيوببتيداز خصائص عصبية واسعة النطاق، مما يدل على فعالية مزيلة للقلق ومضاد للاكتئاب وفعالية مضادة للاختلاج مماثلة أو متفوقة على العلاجات القياسية. تؤكد قدرته على تعزيز السلوك الاستكشافي وتأخير التشنجات وتعديل التخدير، وتحسين المعايير الفسيولوجية على الإمكانات العلاجية للاضطرابات العصبية والنفسية. ومع ذلك، فإن الاستجابات في اختبارات مضادات الاكتئاب الحادة والتباين في تأثيرات الجرعات العالية تستدعي المزيد من الاستكشاف الآلي والسريري. هذه النتائج تضع سيراتيوببتيداز كمرشح واعد لإعادة توجيهه في مجال الأدوية العصبية، مما يستحق دراسة متقدمة لتحسين الجرعات وتوضيح المسار الجزيئي.

# تقييم التأثيرات الدوائية للسيراتيوبيبتايداز على الجهاز العصبي المركزي في الفئران

رسالة تقدم بها

**يونس مسعود عبد الحميد فرحان**

**الى**

**مجلس كلية الطب البيطري في جامعة الموصل**

**في اختصاص الطب البيطري/الأدوية والسموم البيطرية**

**وهي جزء من متطلبات نيل شهادة الماجستير**

بإشراف

**الأستاذ المساعد**

**الدكتور احمد صلاح ناصر**

١٤٤٧هـ ٢٠٢٥م

جامعة الموصل

كلية الطب البيطري

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يونس مسعود عبد الحميد فرحان

رسالة الماجستير

الطب البيطري/ الأدوية والسموم البيطرية

بإشراف

الأستاذ المساعد

الدكتور احمد صلاح ناصر

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