People's Democratic Republic of Algeria

Ministry of Higher Education and Scientific Research

Ministère de l'Enseignement Supérieur et de la Recherche Scientifique

Echahid Cheikh Larbi Tebessi University–Tebessa-

Faculty of Exact Sciences and Natural and Life Sciences

Department of Applied Biology

Field of Natural and Life Sciences

End-of-study dissertation

Presented for the purpose of obtaining the Master's degree

In: Biological Sciences

Option: Applied Biochemistry

Presented by:

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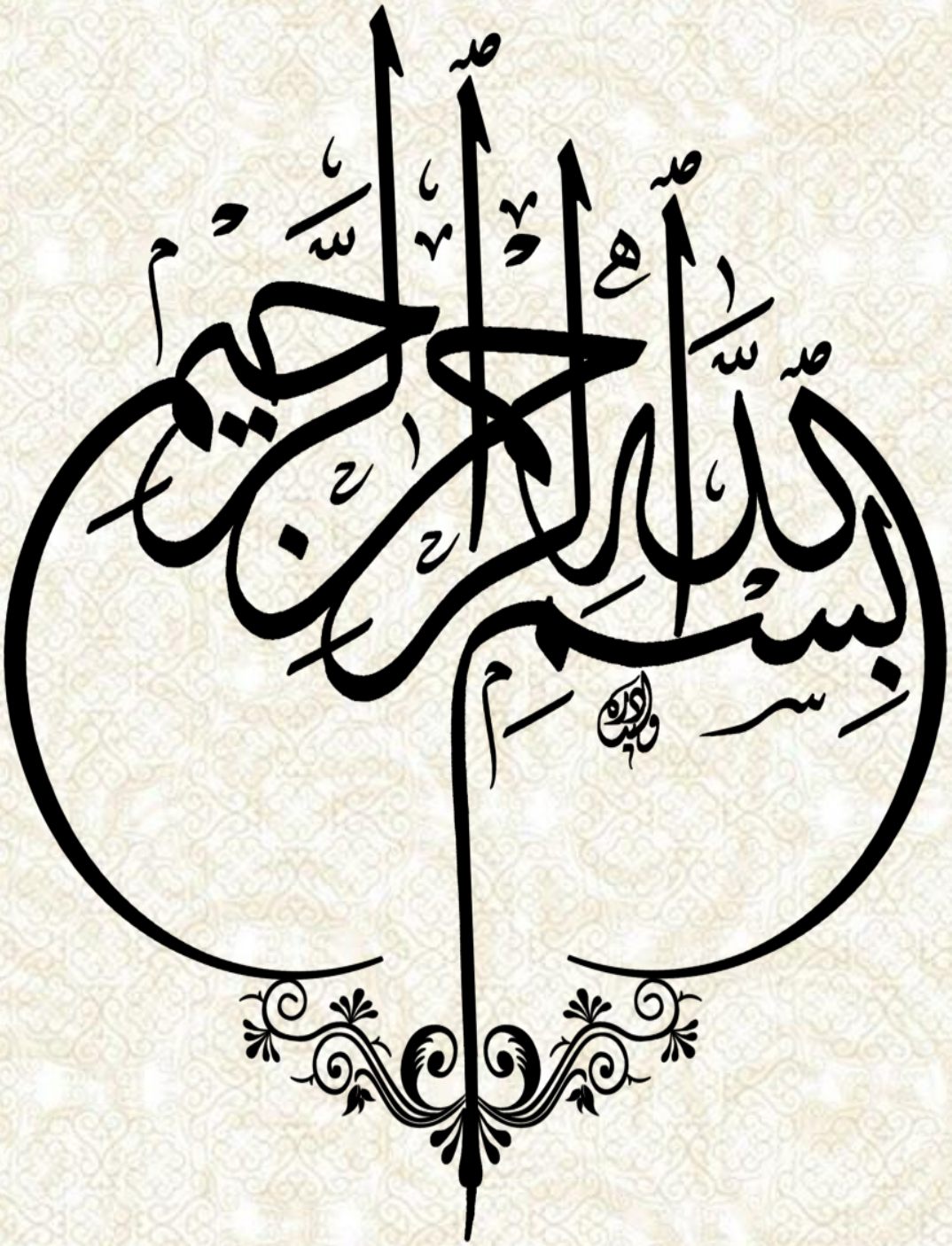
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Possible protective effect of *Urtica dioica* against neurotoxicity of lambda- cyhalothrin in rat

In front of jury:

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Defense date : 04 / 06 / 2025


بسم الله الرحمن الرحيم

Acknowledgements

***Alhamdulillah**. The successful completion of this work reflects the collective dedication and assistance of numerous individuals and organizations, to whom we are deeply grateful.*

***First and foremost,** we extend our heartfelt thanks to the distinguished members of the jury for their invaluable input in this research. Their insightful feedback has been crucial in enhancing the quality and thoroughness of this study. We also sincerely appreciate the jury panel for dedicating their time, knowledge, and expert guidance during the evaluation process.*

***Special thanks** go to Dr. **Gasmi Salim** and Dr. **Messaadia Amira** for their diligent review and assessment of this research. Their critical evaluations and recommendations have greatly improved the study's content and methodology.*

***Furthermore**, we are deeply grateful for the unwavering support and expert guidance of our supervisor, Dr. **Guedri Kamilia**, throughout this project. Her mentorship and encouragement have been instrumental in helping us overcome the challenges and intricacies of academic research.*

***We also extend our sincere thanks** to Echahid Cheikh Larbi Tebessi University for its steadfast support, provision of essential resources, and cultivation of an environment that promotes scholarly research and academic excellence.*

***Dear Limami Maroua and Ben Djazia Rania,** we are incredibly grateful for your profound impact throughout my graduation career. Your invaluable guidance during my classes and continuous advisory were instrumental. Moreover, we truly appreciate your steadfast support during my laboratory work and the meticulous editing of our master's thesis. Thank you for consistently boosting our confidence.*

***Furthermore,** we express our profound gratitude for the invaluable contributions made by Rebai Raounek, , Roched Eddine Manel, Sayeda Nardjes, and Kherchi Donia. Their expertise, assistance, and collaboration have greatly enhanced the scope and depth of our research efforts.*

***We are particularly thankful** for the commitment and cooperative spirit demonstrated by our colleagues, whose relentless endeavors have been crucial in advancing this research.*

***Lastly**, we wish to convey our heartfelt appreciation to our families and friends for their steadfast support and encouragement throughout this journey.*

***We extend our thanks** to all who have played a role in the realization of this research project. Your support and guidance have been essential, and we are sincerely grateful for the opportunity to learn and develop through this experience.*

Dedication

To the cherished memory of my grandfathers, whose presence I deeply miss and whose wisdom continues to guide me. May God grant them eternal mercy and the highest place in heaven; I only wish they were here to share this moment.

To my beloved parents and family, your unwavering love, support, and sacrifices have been my constant inspiration. This achievement is as much yours as it is mine.

And to my incredible friends and colleagues, thank you for the laughter, encouragement, and shared journey that made this path more enjoyable.

TRIKI OKBA

Dedication

I humbly dedicate this work to

My cherished parents, whose love,

meticulous guidance, and lessons in diligence and perfectionism have been foundational.

May God grant them continued health and well-being.

To my dear fiancée, for your unwavering support.

To my entire family and all my cherished friends.

To all those who are dear to me.

Finally, I wish to express my profound gratitude

to every individual who contributed,

directly or indirectly, to the successful

completion of this endeavor.

MERAHBAOUI ALA EDDINE

Abstract

The intensive use of pesticides in recent years has led to the emergence of numerous ecological, environmental, and health problems. Indeed, the presence of these substances poses potential health risks. Herbal medicine plays an important role in treating the components of several diseases. Nettle (*Urtica dioica*) is considered a valuable plant thanks to its bioactive compounds such as formic acid and its richness in flavonoids. In this context, our study was conducted to investigate the neurotoxic effect of a pyrethroid insecticide, Lambda-cyhalothrin (LCT), and the possible protective power of *Urtica dioica* extract (URT) in rats.

An ethnobotanical study was conducted to assess the recognition and use of this plant in our region, and an in vivo study was conducted on twenty female rats of the Wistar strain, which were divided into four groups and treated orally for 30 days: control group; group treated with LCT (6.3 mg/kg); URT-treated group (100 mg/kg) and a co-treatment group. At the end of the experiment, we performed a battery of behavioral tests (FST, EPM, OF) and the rats were decapitated and their blood was collected for hematological and biochemical analysis (blood glucose), and the brain was carefully removed and preserved for the antioxidant markers.

The results show that LCT induced, a loss of body weight, hematological disturbances (Leukocytosis, thrombocytopenia and anemia), hyperglycemia, an increase in brain protein levels, a decrease in brain GSH and GST activities, and behavioral disorders (Reduction in locomotion and exploratory performance, anxiogenic and depression-like behaviors). In contrast, administration of *Urtica dioica* significantly attenuated these alterations: improvement in the activity of brain antioxidant markers, stabilization of hematological parameters, partial normalization of behavior, and reduction of hyperglycemia.

These results suggest an anxiolytic, antidepressant, and antioxidant effect of *Urtica dioica* against LCT-induced damages.

Key words: Lambda-cyhalothrin, *Urtica dioica*, Neurotoxicity, Behavior, Rat**.**

Résumé

L'utilisation intensive des pesticides ces dernières années a entraîné l'émergence de nombreux problèmes écologiques, environnementaux et sanitaires. En effet, la présence de ces substances, présente des risques potentiels pour la santé. La phytothérapie joue un rôle important dans le traitement des composantes du plusieurs maladies. L'ortie (*Urtica dioica)* est considérée comme une plante précieuse grâce à ses composés bioactifs tels que l'acide formique et sa richesse en flavonoïdes. Pour cela, cette étude a été réalisées pour investiguer l'effet neurotoxique d'un insecticide pyréthrinoide Lambda-cyhalothrine (LCT), et le pouvoir protecteur possible de l'extrait d'*Urtica dioica* (URT) chez les rats.

Une étude ethnobotanique a été réalisée afin d'estimer la reconnaissance et l'utilisation de cette plante dans notre région et une étude in vivo effectué sur Vingt rats femelles de la souche Wistar qui ont été répartis en quatre groupes et traités oralement pendant 30 jours : groupe témoin ; groupe traité par LCT (6.3 mg/kg) ; groupe traité par URT (100 mg/kg) et un groupe de Co-traitement. À la fin de l'expérimentation, nous avons réalisé une batterie des tests comportementaux (FST, EPM, OF) et les rats sont décapités et leur sang a été collecté pour l'analyse hématologique et biochimique (Glycémie), et cerveau a été soigneusement prélevé et conservé pour l'étude d'activité des antioxydants.

Les résultats montrent que la LCT a induit d'une part, une perte du poids corporelle, des perturbations hématologiques (Leucocytose, thrombopénie et anémie), une hyperglycémie, une élévation du taux de protéines cérébrales et une diminution d'activité des de GSH et GST cérébrales, et d'autre part des troubles comportementaux (hypo-locomotion et réduction de l'exploration, augmentation du degré d'anxiété et de dépression). .

En revanche, l'administration de *Urtica dioica* a permis d'atténuer de manière significative ces altérations : amélioration de l'activité des marqueurs antioxydants cérébrale, stabilisation des paramètres hématologiques, normalisation partielle du comportement et diminution de l'hyperglycémie.

Ces résultats suggèrent un effet anxiolytique, antidépressive et antioxydant d'*Urtica dioica* contre les dommages induisent par la LCT.

Mots clés : Lambda-cyhalothrin, *Urtica dioica*, Neurotoxicité, Comportement, Rat.

الملخص

أدت كثرة استخدام المبيدات الحشرية في السنوات الأخيرة إلى ظهور العديد من المشاكل البيئية والصحية. في الواقع، فإن وجود هذه المواد يشكل مخاطر صحية محتملة. يلعب طب الأعشاب دورًا مهمًا في علاج العديد من الأمراض. يعتبر نبات القراص نباتًا قيمًا بفضل مركباته النشطة بيولوجيًا مثل حمض الفورميك وثرائه بالفلافونويد. ولتحقيق هذه الغاية، أجريت هذه الدراسة للتحقيق في التأثير السام على الأعصاب لمبيد حشري من مجموعة البيرثرويد، الامداسيهالوثرين، والقدرة الوقائية المحتملة لمستخلص القراص على الفئران.

أجريت دراسة إثنونباتية لتقييم التعرف على هذا النبات واستخدامه في منطقتنا، وأجريت دراسة حية على عشرين فأر أنثى من سلالة ويستار، والتي تم تقسيمها إلى أربع مجموعات عولجت عن طريق الفم لمدة 30 يومًا: المجموعة الشاهدة؛ المجموعة التي عولجت بـ المبيد الحشري (6.3 مجم/ كجم)؛ مجموعة عولجت بـالقراص (100 ملغ/كغ) ومجموعة أخرى عولجت بالعلاج المشترك. في نهاية التجربة، أجرينا مجموعة من الاختبارات السلوكية (FST، EPM، OF)، وقتلت الفئران وجُمعت دماؤها للتحليل الدموي والكيميائي الحيوي (سكر الدم). بعد ذلك، قُطعت رؤوس الفئران، وأُزيلت أدمغتها بعناية وحُفظت لدراسة معايير الإجهاد التأكسدي. أظهرت النتائج أن العلاج بالمبيد الحشري أدى، من جهة، إلى فقدان الوزن، واضطرابات دموية (زيادة عدد الكريات البيضاء، وزيادة عدد الخلايا الليمفاوية، وزيادة عدد الخلايا الوحيدة، وقلة الصفيحات الدموية، وفقر الدم)، وارتفاع سكر الدم، وزيادة مستويات بروتين الدماغ، وانخفاض نشاط مضادات الأكسدة (GSH، GST)، ومن جهة أخرى، اضطرابات سلوكية (ضعف الحركة وقلة الاستكشاف، وزيادة مستويات القلق والاكتئاب). في المقابل، أدى تناول القراص إلى تخفيف هذه التغيرات بشكل ملحوظ: تحسن في نشاط مؤشرات مضادات الأكسدة في الدماغ، واستقرار المؤشرات الدموية، وتطبيع جزئي للسلوك، وانخفاض في ارتفاع سكر الدم.

تشير هذه النتائج إلى تأثير القراص كمضاد للقلق، ومضاد للاكتئاب، ومضاد للأكسدة ضد الضرر الناتج عن العلاج الكيميائي طويل الأمد.

الكلمات المفتاحية : لامدا-سيهالوثرين، القراص، السمية العصبية، السلوك، الجرذان.

List of abbreviations

- **ANOVA** – Analysis of Variance
- **BBC**– Solution Coomassie Brilliant Blue
- **BSA** – Bovine Serum Albumin
- **CDNB**– chloro diitrobenzen
- **DLM**– minimum lethal dose
- **DMT**– dimethyltryptamie
- **DNA**– deoxyribonucleic acid
- **DTNB** – 5,5'-Dithiobis-2-nitrobenzoic acid
- **EDTA** – Ethylenediaminetetraacetic acid
- **EPM** – Elevated Plus Maze
- **FST** – Forced Swimming Test
- **GABA** – Gamma-aminobutyric acid
- **GC-MS** – Gas Chromatography-Mass Spectrometry
- **GPx** – Glutathione peroxidase
- **GSH** – Glutathione
- **GST** – Glutathione S-transferase
- **Hb** – Hemoglobin
- **Hct** – Hematocrit
- **HDL**– High-density lipoprotein
- **HPLC** – High-Performance Liquid Chromatography
- **IGF** – insulin-like growth factor
- **ISO** – international organization standaidization
- **LCT** – Lambda-cyhalothrin
- **LD50** – Lethal Dose for 50% of the population
- **LPO**– lipid peroxidation
- **Mcp1** – Monocyte chemoattractant protein-1
- **NACL** – sodium chloride

- **OF** – Open Field
- **PAL**– phenylalanine ammonia-lyase
- **PLT**– Platelet
- **PS** – Pyrethroid Pesticides
- **RBC** – Red Blood Cells
- **RNA** ribonucleic acid
- **ROS** – Reactive Oxygen Species
- **TNB** – Thionitrobenzoic acid
- **TNF-α** – Tumor Necrosis Factor alpha
- **TR** – Thyroid hormone receptors
- **UDA**– Urtica Dioica Agglutinin
- **URT** – *Urtica dioica*
- **WBC** – White Blood Cells
- **WHO** – World Health Organization

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Introduction

Introduction

Pesticides are lipophilic substances known for their neurotoxic effects and relatively low toxicity in mammals **(Saoudi *et al*., 2021).** Their widespread use in agriculture has led to significant environmental contamination, contributing to various health issues. Exposure to different pesticide classes, including organochlorines (OCs), organophosphorus (OP), carbamates, and pyrethroids, has been associated with a range of adverse effects, including neurological disorders, carcinogenicity, respiratory complications, dermatological conditions, and reproductive abnormalities **(Akoto *et al*., 2015).** Among these, pyrethroids have been reported to cause alterations in hematologic, biochemical, and reproductive parameters **(El-Demerdash *et al*., 2004).**

Lambda-cyhalothrin (LCT), a type II a synthetic pyrethroid, is extensively utilized in both agricultural practices and domestic pest management. Nonetheless, its neurotoxic impact on mammals, particularly in rodent models, is well-established. LCT interferes with neuronal activity by extending the activation of sodium channels, which leads to oxidative stress, lipid peroxidation, and mitochondrial impairment, ultimately resulting in apoptosis and neurobehavioral impairments **(Soderlund, 2012; Nasuti *et al*., 2013).** Given the scarcity of pharmacological options to alleviate its neurotoxic effects, natural antioxidants like Urtica dioica have been identified as potential neuroprotective agents.

For centuries, medicinal plants have played a crucial role in traditional medicine, primarily due to their antioxidant properties, which help mitigate oxidative damage **(Pincemail *et al*., 2002).** *Urtica dioica (U. dioica)* is one such plant, known for its therapeutic applications. Research has demonstrated its efficacy in treating nasal and menstrual hemorrhages, nephritis, anemia, and hematuria, as well as its blood-purifying properties. Additionally, it has been investigated for its potential benefits in managing diabetes, atherosclerosis, cardiovascular diseases, and prostate cancer **(Ghasemi *et al*., 2019).** *U. dioica* is also widely used in traditional medicine for conditions such as allergies, renal stones, burns, internal bleeding, and diabetes **(Dar *et al*., 2013)**. In Algeria, it has been employed for alleviating stomachaches, rheumatic pain, colds, and coughs, while its application extends to hypertension, allergic rhinitis, and cardiovascular diseases. The plant's diuretic properties contribute to blood pressure reduction by lowering vascular tension **(Boyrie, 2016).** Moreover, studies have reported that the aqueous extract of *U. dioica* exhibits hypoglycemic effects, and the hydroalcoholic extract of its leaves has been shown to enhance insulin secretion in rats **(Hailemeskel & Fullas, 2015).** Given these

pharmacological properties, the present study aims to evaluate the protective potential of *U. dioica* extract against LCT-induced neurotoxicity, and rats' oxidative stress.

PART ONE: BIBLIOGRAPHIC SYNTHESIS

Chapter 1: Pesticide (Lambda-cyhalothrin)

I.1. History of pesticides

Pesticides, often referred to as plant protection products, encompass a range of organic or inorganic chemicals that consist of one or more active ingredients along with supplementary compounds such as diluents or adjuvants **(Tayade, 2013).** The term "pesticide" is derived from the English word "pest," which denotes harmful animals, insects, or plants, combined with the suffix “cide,” originating from the Latin “caedo, cadere,” meaning “to kill.” Consequently, the term pesticide encompasses all substances designed to manage organisms considered undesirable **(Lushchak *et al*., 2018).**

Prior to the onset of the Second World War, agricultural pesticides primarily consisted of mineral or plant-based substances, including arsenic, copper, zinc, manganese, lead, pyrethrum, rotenone, and nicotine sulfate **(Štajn *et al*., 1997).** The 19th century marked the beginning of significant advancements in the chemical industry, which facilitated the development of numerous synthetic pesticide compounds, with over a thousand now registered. Between 1945 and 1985, global pesticide consumption experienced a twofold increase approximately every decade. This surge can be attributed to population growth and the corresponding demand for enhanced crop yields to satisfy escalating food requirements, thereby driving the widespread adoption of pesticides across various categories **(Davis *et al*., 2017; Zhang *et al*., 2021).**

I.2.Classification of pesticides

The current market offers a wide array of pesticides, each differing significantly in their chemical composition, functional groups, and biological activity, which complicates their classification. Various methods exist for categorizing pesticides **(Kojima *et al*., 2004),** with the most straightforward and inclusive approach being the division into broad categories based on their target organisms: herbicides, fungicides, and insecticides. Beyond these primary categories, there are additional products such as acaricides (targeting mites), nematicides (targeting nematodes), rodenticides (targeting rodents), taupicides (targeting moles), molluscicides (primarily targeting slugs and snails), corvicides (targeting crop pests, particularly ravens), and repellents. From a chemical perspective, certain pesticides are identified by the presence of halogenated compounds, such as chlorine or bromine, while others contain functional groups of oxygen, nitrogen, and phosphorus, which influence their reactivity, metabolism, and degradation. The modes of action of these substances are equally varied and often multifaceted for individual compounds. Consequently, a universally accepted classification system for pesticides remains elusive **(Lushchak *et al*., 2018).**

Table 1 presents the principal categories of commonly utilized pesticides along with the key chemical families associated with these categories .

Table 1: Some chemical families of pesticides and their main targets (INSERM. 2013)

Chemical families	Examples of active substances	Ranking by target
Organchlorés	DDT, Chlordane, Lindane, Dieldrine, Heptachlore	Insecticides
Organophosphorés	Malathion, Parathion, Chlorpyrifos, Diazinon	Insecticides
Pyréthrinoides	Perméthrine, Deltaméthrine, Lambda-cyhalothrine	Insecticides
Carbamates	Aldicarbe, Carbaryl, Carbofuran, Méthomyl	Insecticides
	Asulame, Diallate., Terbucarbe, Triallate	Herbicides
	Benthiavalicarbe	Fongicides
Dithiocarbamates	Mancozèbe, Manèbe Thirame, Zinèbe	Fongicides
Phtalimides	Folpel, Captane, Captafol	Fongicides
Triazines	Atrazine, Simazine, Terbutylazine	Herbicides
Phénoxyherbicides	MCPA, 2,4-D, 2,4,5-T	Herbicides
Chloroacétamides	Alachlore, Métolachlore	Herbicides
Pyridines-bipyridiliums	Paraquat, Diquat	Herbicides
Aminophosphonates	Glycine Glyphosate	Herbicides

I-3-Intended for use in the environment

Pesticides are introduced into the environment mainly through various agricultural practices. Once they enter the ecosystem, certain categories of pesticides may accumulate or transform into contaminants. The Community of EU Member States has observed that these substances can migrate through soil via runoff or percolation, as well as through atmospheric processes such as precipitation and evaporation. For nearly five decades, pesticides have been detected across all environmental compartments that lack remediation. These chemicals and their metabolites can be found in surface waters, groundwater, the atmosphere, and precipitation, as well as in agricultural products such as fruits, vegetables, grains, and livestock. Annually, over 2.5 million tonnes of pesticides are utilized globally, with a mere 0.3%-0.5% achieving their intended effect on target organisms, while the remaining 99.5% contribute to environmental pollution **(Yekeen *et al*., 2016).** There are several factors contributing to pesticide contamination, including leaching due to rainfall, which transports agrochemicals into surface and groundwater **(Triegel & Guo, 2018)**. Additionally, improper use of substances and techniques, such as incorrect calibration of sprayers and excessive application rates, plays a significant role **(Tobi *et al*., 2011).** Accidental spills, such as the rinsing or disposal of containers that previously held agrochemicals, also contribute to this issue **(A.-T. Mossa & Mohafrash, 2023).**

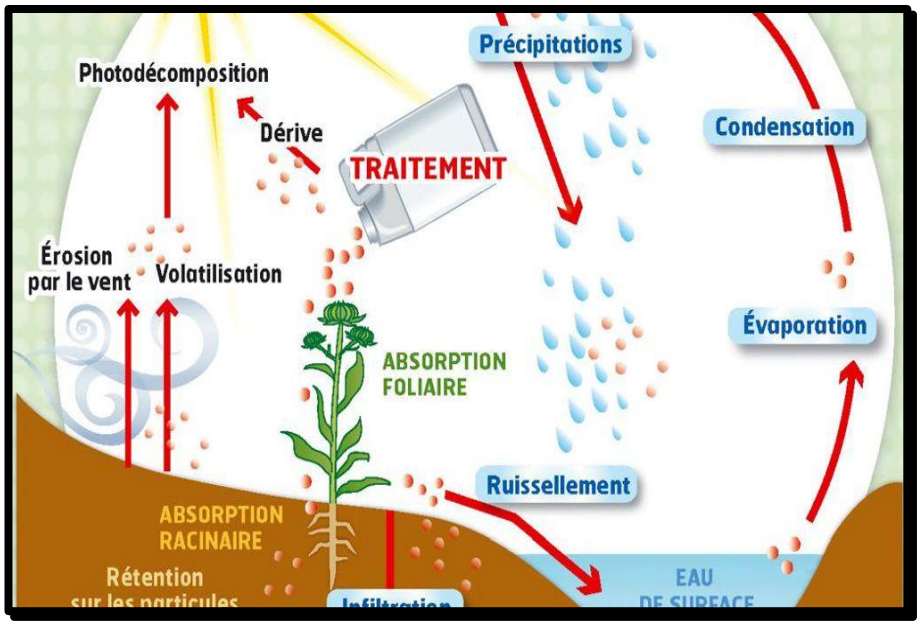


Figure 1 : Different modes of environmental contamination by pesticides **(Web 1)**

I-4-Routes of exposure to pesticides

Pesticides can enter the body through oral, dermal, and respiratory pathways, with the most severe instances of poisoning arising from ingestion. Children are particularly vulnerable

to such poisoning due to their propensity to place objects and their fingers in their mouths. However, adults who smoke or consume food without first washing their hands after handling pesticides are also at risk of exposure **(Tudi *et al*., 2022).**

Among individuals who utilize pesticides, the dermal route typically serves as the primary pathway for the entry of these substances into the body **(Nurulain *et al*., 2017).**

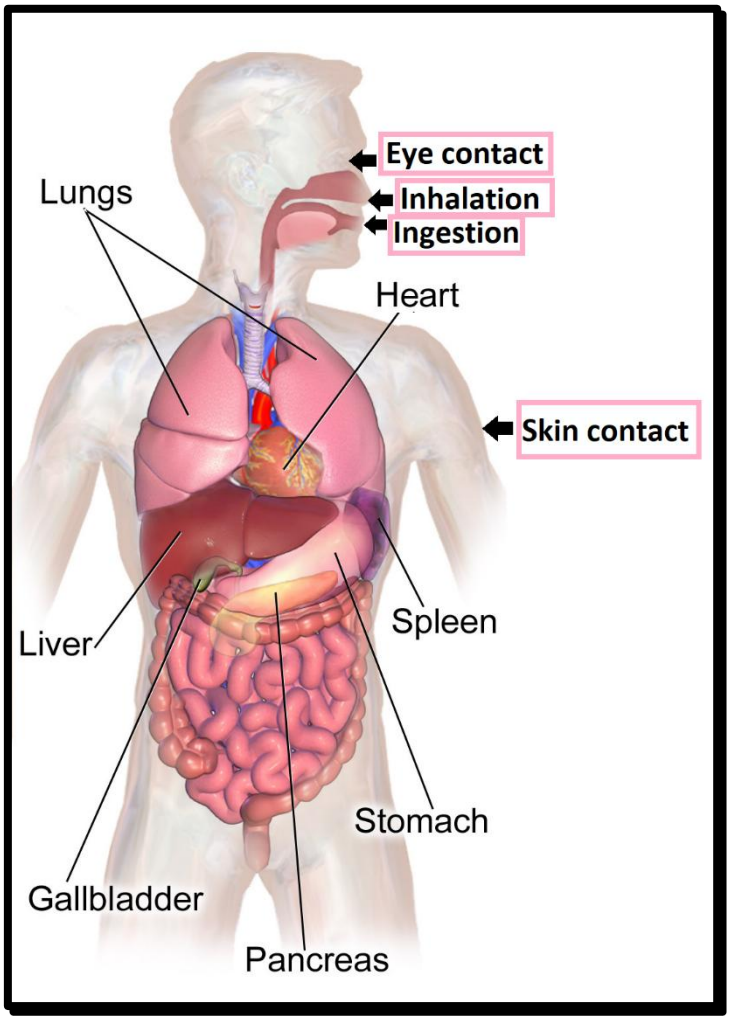


Figure 2 : The main routes of exposure to pesticides **(Web 2)**

II-Synthetic pyrethroids: a new generation of pesticides

II.1.Definition

Pyrethroid pesticides are synthesized from pyrethrins, which are naturally occurring insecticidal compounds found in pyrethrum, a substance extracted from chrysanthemum flowers. These pesticides are chemically classified as esters of chrysanthemic acid (ethyl 2,2-dimethyl-3-(1-isobutenyl) cyclopropane-1-carboxylate). Since their introduction in the 1980s, pyrethroid pesticides have gained global acceptance due to their remarkable efficacy and comparatively low toxicity relative to organophosphate and carbamate pesticides. Functioning as neurotoxins, pyrethroid pesticides specifically interact with the receptor sites of voltage-gated sodium channels. Their mechanism of action involves binding to these sodium channels, leading to excitatory paralysis in insects. Additionally, they induce alterations in membrane potential, resulting in a persistently hyperexcitable state in nerve cells. Consequently, insects subjected to these pesticides experience "knockdown," a condition characterized by temporary incapacitation **(Ahamad & Kumar, 2023).**

II.2.Chemical structure

Pyrethroids possess chiral centers and double bonds, resulting in the existence of multiple conformers. The isomeric specificity of the pyrethroids employed varies from one active substance to another, allowing for different combinations of isomers (formulations) for a single active ingredient. For instance, the market offers various forms such as dcis, trans allethrin, d-trans allethrin, and allethrin, which is a mixture of eight stereoisomers. Permethrin and cypermethrin, among the most significant, typically appear in a cis/trans ratio of approximately 40/60; however, this ratio may vary depending on the formulation. The isomeric form (cis versus trans), along with the presence of a nitrile group at the Cα position, affects the efficacy, the nature of the effects produced, and the biotransformation of these compounds. The presence or absence of a nitrile group at Cα is one of the criteria utilized by **(Dar *et al*., 2021)**to categorize pyrethroids into two distinct types. Type I refers to a pyrethroid lacking a nitrile group at Cα, while Type II is designated for those that contain a nitrile group at this position. Although this classification is conceptually useful, it is not without limitations, particularly for newer, more complex molecules whose in vivo effects do not always correlate with their chemical structure **(Davare *et al*., 2015).**

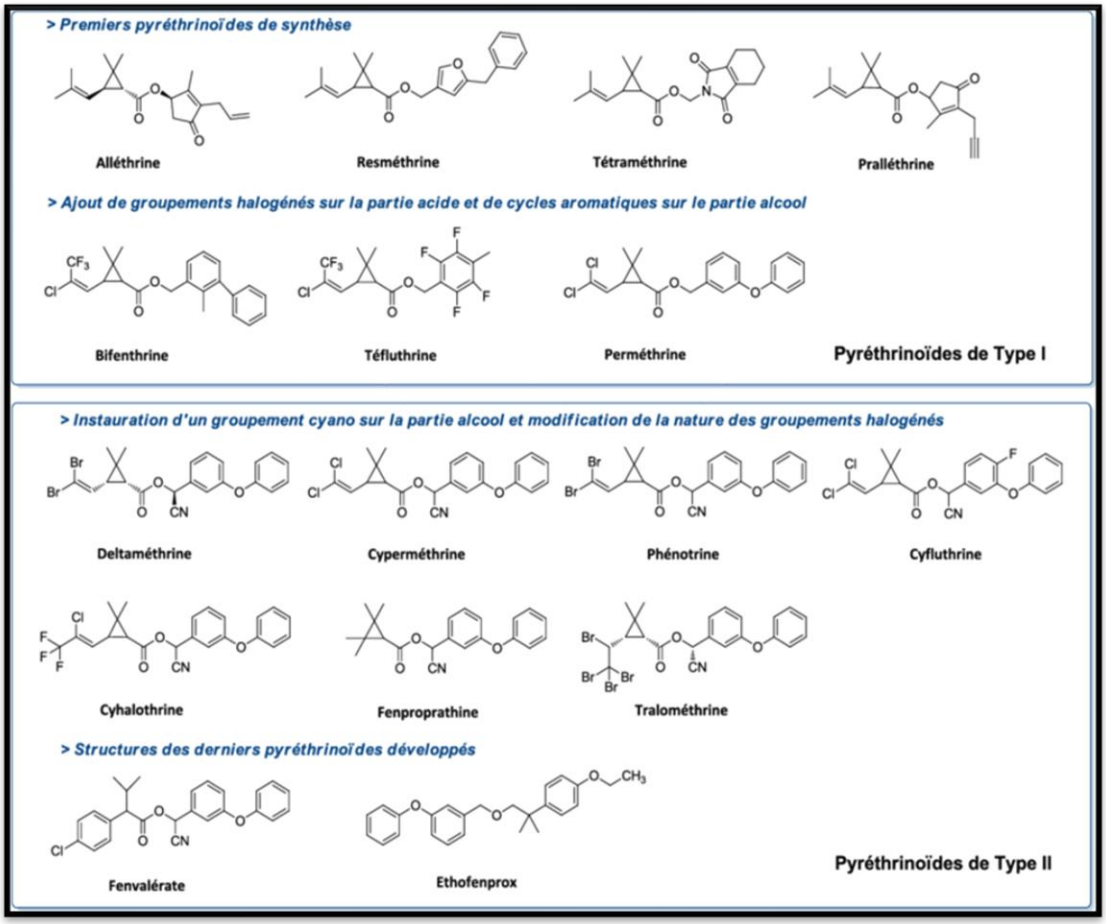


Figure 3: Structural evolution of synthetic pyrethroids **(Web 3)**

III. Lambda-cyhalothrin

III.1. Definition

Lambda-cyhalothrin (LCT) is classified as a second-generation pyrethroid. This chemical is designed for the management of animal parasites and agricultural pests, functioning either to exterminate or to inhibit the growth of these organisms. **(Chau & Smith, 2018).** Consequently, it poses significant toxicity risks to both human health and the environment. It is imperative to adhere to the recommended dosages and application methods for which the product is officially registered. These lipophilic insecticides serve as alternatives to organophosphorus compounds, owing to their reduced volatility and rapid metabolic **(Chau & Smith, 2018).**

III.2. The active substance and the formulated

Lambda-cyhalothrin is the ISO common name for the reaction product comprising equal quantities of (R)-α-cyano-3-phenoxybenzyl (1S,3S)-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-

2,2dimethylcyclopropanecarboxylate and (S)-α-cyano-3-phenoxybenzyl(1R,3R)-3-[(Z)-2-chloro-3,3,3trifluoropropenyl]-2,2 dimethylcyclopropanecarboxylate or of (R)-α-cyano-3-phenoxybenzyl(1S)-cis-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2dimethylcyclopropanecarboxylate and (S)-α-cyano-3-phenoxybenzyl(1R)-cis-3-[(Z)-2-chloro-3,3,3trifluoropropenyl]-2,2 dimethylcyclopropanecarboxylate (IUPAC). The representative formulated products for the evaluation were 'Karate 10CS (A12690B)', a capsule suspension (CS) containing 100 g/L lambda-cyhalothrin (9.43 % w/w) and 'Kaiso sorbie 5% EG (CA2352)', an emulsifiable granule (EG) containing 50 g/kg lambda-cyhalothrin for the Syngenta Task Force (STF), while 'Lambda-Cyhalothrin 100 CS (HAG 400 01 I)', a capsule suspension (CS) containing 100 g/L lambda-cyhalothrin (9.87 % w/w) and 'Lambda 50 EC (LC50-2)', an emulsifiable concentrate (EC) containing 50 g/L lambda-cyhalothrin for the Task Force Lambda (TFL) **(EFSA, 2014)**

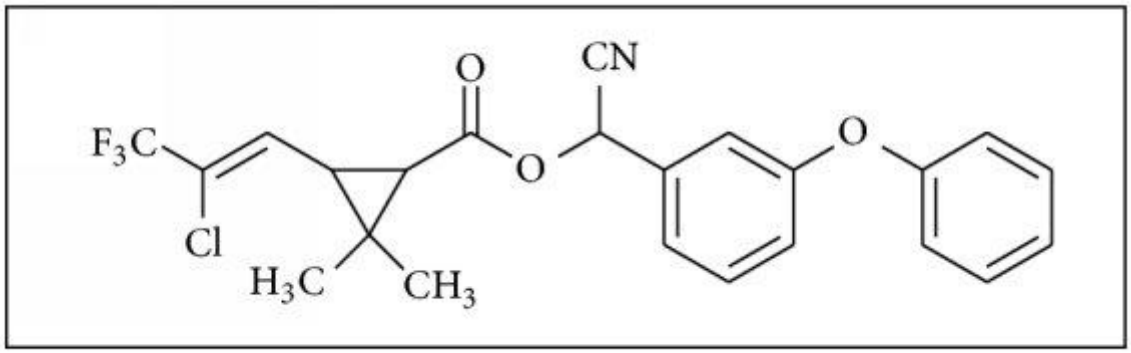


Figure 4: Chemical structure of lambda-cyhalothrin **(Web 4)**

III.3. Physico-chemical property

The primary physical and chemical properties of lambda-cyhalothrin are detailed in Table 2

Table 2: Physical, chemical, and environmental properties of lambda-cyhalothrin **(Ming He *et al.*,2008)**

CAS number	**91465-08-6**
US EPA PC Code	128897
CA DPR Chem Code	2297
Molecular formula	$C_{23}H_{19}ClF_3NO_3$
Molecular weight (g/mol)	449.9
Density (g/mL at 25°C)	1.33
Melting point (°C)	49.2
Boiling point (°C at 0.2 mmHg)	187-190
Vapor pressure (mPa at 20°C)	0.0002
Henry's law constant (Pa-m³/mole)	0.018
Water solubility (mg/L at 20°C)	0.005
Solubility in other solvents (e.g., acetone) (mg/L)	>500,000
Octanol-water partitioning (log K_{OW} at 20°C)	7.00
Hydrolysis half-life (d)	
PH 5	Stable
PH 7	Stable
PH 9	8.66
Photolysis half-life (d)	
Water at pH 5 and 25°C	24.5
Soil	53.7
Bioconcentration factor (BCF) (fish)	2,240
Soil adsorption K_{OC} (cm³/g)	247,000-330,000
Soil degradation half-life (d)	
Aerobic soil	42.6
Aquatic degradation half-life (d)	
aerobic aquatic	21.6

III.4. Toxicokinetics of lambda-cyhalothrin

Toxicokinetics focuses on elucidating and comprehending the journey of a molecule within the biological system. Initially, the molecule undergoes absorption, subsequently entering the bloodstream, where it is distributed throughout various organs. Enzymatic activity then transforms the parent molecule into metabolites. Ultimately, both the original molecule and its metabolites are eliminated from the body through feces or urine. This entire sequence of events aligns with the processes of absorption, distribution, metabolism, and excretion (ADME) of the molecule **(Côté & Bouchard, 2024).**

III.1.4.1. Absorption

Synthetic pyrethroids (PS) are primarily absorbed in occupational settings through inhalation, occurring rapidly in the form of dust or aerosols. Additionally, a minor portion is

absorbed through the dermal route, accounting for less than 1% of the applied dose. In contrast, the gastrointestinal route facilitates the absorption of approximately 40 to 60% of the administered dose **(Côté & Bouchard, 2024).**

Following oral administration of lambda-cyhalothrin, peak plasma concentrations are observed approximately 3 to 6 hours post-ingestion for both metabolites, namely cis-3- (2-chloro-3,3,3-trifluoroprop-1-en-1-yl) -2,2- dimethyl cyclo propane carboxylic acid (CFMP) and 3-PBA. The plasma half-lives for these metabolites are recorded at 6 hours for 3-PBA and 8 hours for CFMP. In cases of dermal exposure, peak plasma levels are reached roughly 4 to 10 hours after the cessation of contact, with plasma half-lives of 7 to 8 hours for 3-PBA and 11 hours for CFMP **(Bossou *et al*., 2020)** The oral bioavailability of lambda-cyhalothrin has also been estimated to be 67 ± 9% in rats. Although it has been less extensively researched, inhalation absorption is believed to be both rapid and significant **(Anadón *et al*., 2006).**

III.1.4.2. Distribution

Once xenobiotics enter the bloodstream, they are disseminated throughout various tissues and organs, where they may produce effects that are either beneficial or detrimental, be stored, or be eliminated. The distribution of xenobiotics within the body is significantly influenced by three primary factors: the blood flow to the specific organ or tissue, the ability of the xenobiotic to exit the bloodstream and enter the cells of the targeted organ, and the affinity of the xenobiotic for particular tissues or organs. Organs with high vascularization, such as the brain and visceral organs, facilitate the swift delivery of xenobiotics via the bloodstream **(Bossou *et al*., 2020; Zoumenou *et al*., 2015).** The affinity of xenobiotics for a given tissue is predominantly determined by the physicochemical properties and composition of that tissue. Additionally, the transfer of xenobiotics from the blood to tissues is also contingent upon the effectiveness of biological membranes in functioning as barriers**(Bossou *et al*., 2020; Zoumenou *et al*., 2015).**Certain barriers, such as the blood-brain barrier and the placental barrier, are highly effective in permitting only the passage of very lipophilic compounds. Once a xenobiotic enters the bloodstream, it can bind to plasma proteins with varying degrees of strength and reversibility. Albumin molecules, which are abundant in plasma, serve as a significant storage site for certain xenobiotics, particularly pharmaceuticals. Although the binding to plasma proteins is reversible, it restricts the distribution of these substances from the vascular compartment to other tissues. Additionally, individual-dependent factors can influence the distribution of xenobiotics, including age, hydration levels, the ratio of lean body mass to

adipose tissue, and specific pathophysiological conditions such as shock, chronic heart failure, severe burns, and infectious states **(Bossou *et al.*, 2020; Zoumenou *et al.*, 2015).**

III.1.4.3.Metabolism

Metabolism plays a crucial role in the management of chemical toxicity. For instance, substances that are initially non-toxic can be converted into highly toxic metabolites, while toxic compounds may be transformed into metabolites that are either less harmful or more detrimental **(Xu *et al.*, 2023).** To elucidate the toxicological mechanisms associated with lambda-cyhalothrin, this review concentrates on its primary metabolites, the metabolic enzymes that facilitate these reactions, and the associated metabolic pathways. Additionally, it highlights the significance of oxidative stress in the context of lambda-cyhalothrin toxicity. The principal metabolites of lambda-cyhalothrin, along with the metabolic enzymes involved in these reactions,. Various metabolic processes occur in lambda-cyhalothrin under the influence of CYP450 enzymes, carboxylesterases (**CarEs**), and Bacillus species, leading to the formation of distinct metabolites **(Xu *et al.*, 2023).**

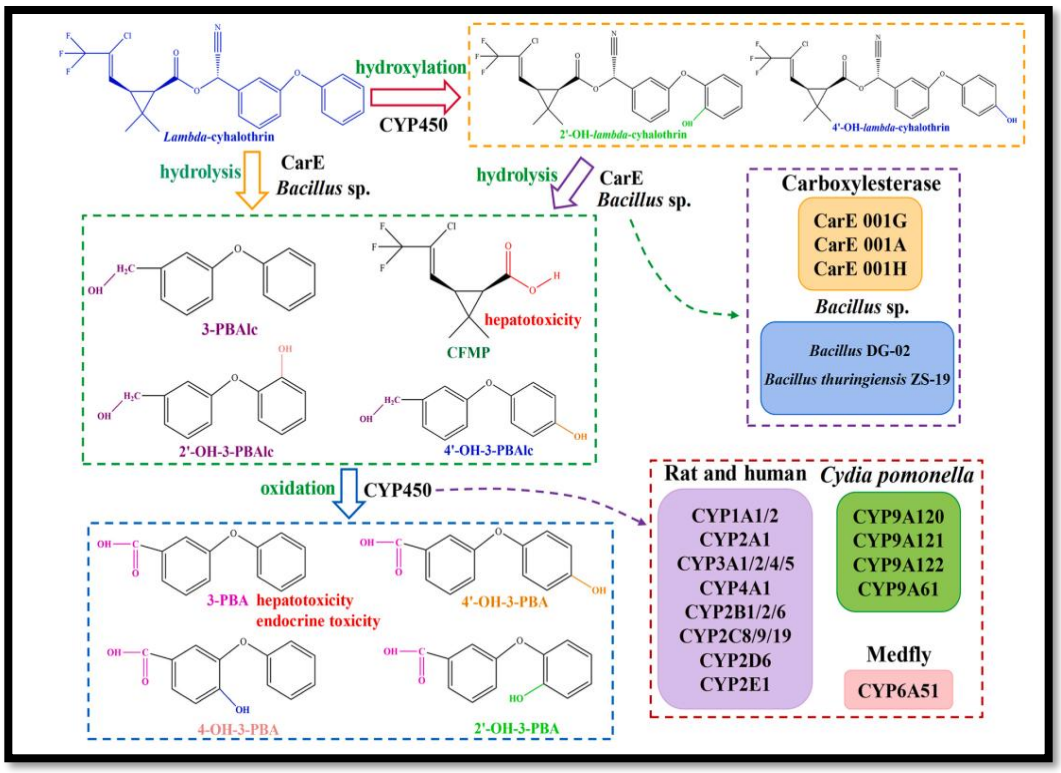


Figure 5*:* The primary metabolites of lambda-cyhalothrin and the key metabolic enzymes that participate in its metabolic processes **(Xu *et al.* 2023)**

III.1.4.4 Elimination

This phenomenon pertains to the removal of the compound, including both the parent molecule and its metabolites, from the organism. The pathways for elimination may include renal, fecal, pulmonary, as well as excretion through sweat or breast milk. The kidneys serve as the primary organ responsible for this elimination process. In terms of metabolism, the renal clearance of the compound is assessed, which facilitates the correlation between the concentrations of the compound in the blood and urine. Approximately 50% of the administered dose is excreted as urinary metabolites, while fecal elimination accounts for between 10% and 26% of the absorbed dose **(Aouey *et al*., 2019).** A study conducted on rats demonstrated that lambda-cyhalothrin exhibited a plasma half-life of 7 hours following intravenous injection and 10 hours after oral administration **(Martínez *et al*., 2018;Khemiri *et al*., 2017).**

IV. Toxicity of lambda-cyhalothrin

IV.1. Neurotoxicity

Lambda-cyhalothrin has the potential to disrupt the nervous system's functions, chemistry, and structure due to its lipophilic nature and ability to cross the blood-brain barrier (BBB). Insecticides like λ-cyhalothrin are known to cause neurotoxicity by interfering with neurotransmitter chemical transmission **(Imam *et al*., 2025).** This study focused on GABAergic interneurons and the inflammatory responses of astrocytes and microglia, which play a crucial role in maintaining the brain's neurochemical and physiological balance. Microglia, the brain's resident macrophages, are essential for preserving normal brain function by continuously monitoring the brain parenchyma to ensure homeostasis **(Imam *et al*., 2025).** When exposed to external threats, injury, or stress, microglia release cytokines and other proteins to facilitate cell repair and regeneration. These responses can be protective, involving either M1 or M2 type microglia. M1 microglia produce pro-inflammatory cytokines and proteins such as TNF-α and IL-1β, while M2 microglia generate neuroprotective substances like insulin-like growth factor 1 (IGF1) and brain-derived neurotrophic factor (BDNF). However, uncontrolled microglia activation often results in the release of free radicals, proinflammatory cytokines, and chemokines **(Imam *et al*., 2025).** The research revealed that lambda-cyhalothrin induces neuroinflammation and microglia activation in both males and females. However, the effects vary between sexes, potentially due to differences in glial cell numbers across brain regions or hormonal factors. Additionally, the impact of lambda-cyhalothrin exposure varies across hippocampal regions, with the CA1 region appearing particularly vulnerable **(Imam *et al*., 2025).** Recent research has suggested that pyrethroids may

have contrasting effects on neurodevelopment. While they exhibit neurotrophic properties, studies have also shown that pyrethroids can increase both the density and length of the spinal column **(Andersen *et al*., 2022).**

IV.2. Oxidative stress

Oxidative stress is recognized as the primary mechanism behind LCT toxicity. Unfortunately, pancreatic β-cells are particularly vulnerable to oxidative stress due to their high intrinsic production of reactive oxygen species (ROS) and inadequate expression of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). At the molecular level, the presence of mitochondria within membranes makes them a primary target for pyrethroid-induced toxicity. Oxidative damage to β-cells disrupts the mitochondria's ability to connect glucose stimulation to insulin secretion during hyperglycemia, leading to impaired insulin production **(Sakr & Rashad, 2023).** Additionally, excessive ROS production worsens pyrethroid-induced mitochondrial dysfunction by increasing endoplasmic reticulum (ER) stress. This ER stress disrupts Ca2+ homeostasis in mitochondria, further exacerbating mitochondrial dysfunction, generating more ROS, and ultimately causing β-cell exhaustion and loss. In two recent studies, oxidative stress has been identified as a key factor in β-cell dysfunction and loss in the pancreas of rats treated with LCT and DMT **(Sakr & Rashad, 2023).**

IV.3. The blood effects

The LCT-treated group showed a significant increase in serum glucose levels and a decrease in insulin levels. These findings align with previous studies where oral exposure to low (1/40 of LD50) and high (1/4 of LD50) doses of LCT over three months in rats caused notable hyperglycemia and hypoinsulinemia, observed after the first, second, and third months of treatment. Additionally, LCT administration in rabbits significantly raised blood glucose levels. Similarly, a 60-day exposure to DLM in rats led to a marked increase in blood glucose and a noticeable decline in insulin levels. Furthermore, hyperglycemia induced by CYP has also been reported in Indian major carp (Labeo rohita) **(Sakr & Rashad, 2023).**

Chapter 2: Medicinal plants for therapeutic use: *Urtica dioica L (Nettle)*

II.1. Phytotherapy

II.1.1. Definition

The term phytotherapy is derived from two Greek roots: "phuton,"meaning "plant," and "therapeia," which translates to "treatment" or "healing with plants" (**Kamou & Benhadj, 2018).** Phytotherapy represents a field of study that bridges ancient practices and contemporary science, and can be characterized as an allopathic approach aimed at preventing and addressing specific functional disorders or pathological conditions through the use of plants or herbal formulations **(Wichtl & Anton, 2003).** The plants utilized in this practice are referred to as "medicinal plants," whose active compounds play a crucial role in a significant portion of medical treatments and healthcare products **(Hans, 2007).** In recent years, this form of treatment has undergone substantial development and is increasingly gaining popularity among the public **(Oueledelhachemi, 2012).**

II.1.2. Types of phethotherapy

Herbal medicine can be categorized into three distinct types. The first type is traditional herbal medicine, which has ancient roots and relies on the empirical virtues of plants **(Clément, 2005).** The second type, pharmacological herbal medicine, is characterized by its focus on active ingredients. Lastly, clinical herbal medicine integrates elements from both traditional and pharmacological approaches **(Carillon, 2009).** The World Health Organization (WHO) notes that traditional herbal medicine is widely utilized in various developing nations **(Bouzouita, 2016).**

II .1.3. Medicinal plants

Medicinal plants are characterized by their therapeutic properties and have been integral to traditional medicine, serving as the foundation for numerous pharmaceuticals **(Biyiti *et al*., 2004).** The efficacy of these plants is attributed to their chemical constituents, which may include primary or secondary metabolites, as well as the synergistic effects of various compounds present within them **(Sanago, 2006).** These plants can be utilized through various methods, including decoction, maceration, and infusion. Furthermore, different parts of the plants, such as roots, leaves, or flowers, may be employed for medicinal purposes **(Dutertre, 2011).**

II.2. *Urtica dioica L (Nettle)*

II.2.1. Description

Nettle is a perennial herbaceous plant distinguished by its unique single-celled hairs. The plant typically reaches heights ranging from 30 to 150 centimeters. Its stem is sturdy, upright, hairy, unbranched, and has a square cross-section. In its juvenile stage, the stem appears green, transitioning to a reddish-purple hue as it matures **(Ghedira *et al*., 2009).**The leaves are arranged oppositely, exhibiting an oval, elongated shape with serrated edges and pointed tips. Both the leaves and stems are covered with stinging hairs. Nettle is dioecious, meaning it has distinct male and female plants **(Ghedira *et al*., 2009).** The flowers, which are unisexual and quite small, bloom from June to September and are found in branched clusters within the leaf axils. The female flower is characterized by a greenish hue and features a unilocular, uniovulate ovary, topped with a style and a brush-like stigma. In contrast, the male flower is yellowish, possessing four stamens with long, flexible filaments that are folded within the flower bud. The resulting fruit is an oval achene containing numerous tiny seeds that are brownish to blackish. The root system consists of a taproot that branches into fine rootlets, facilitating the nettle tuft's spread **(Ghedira *et al*., 2009).**

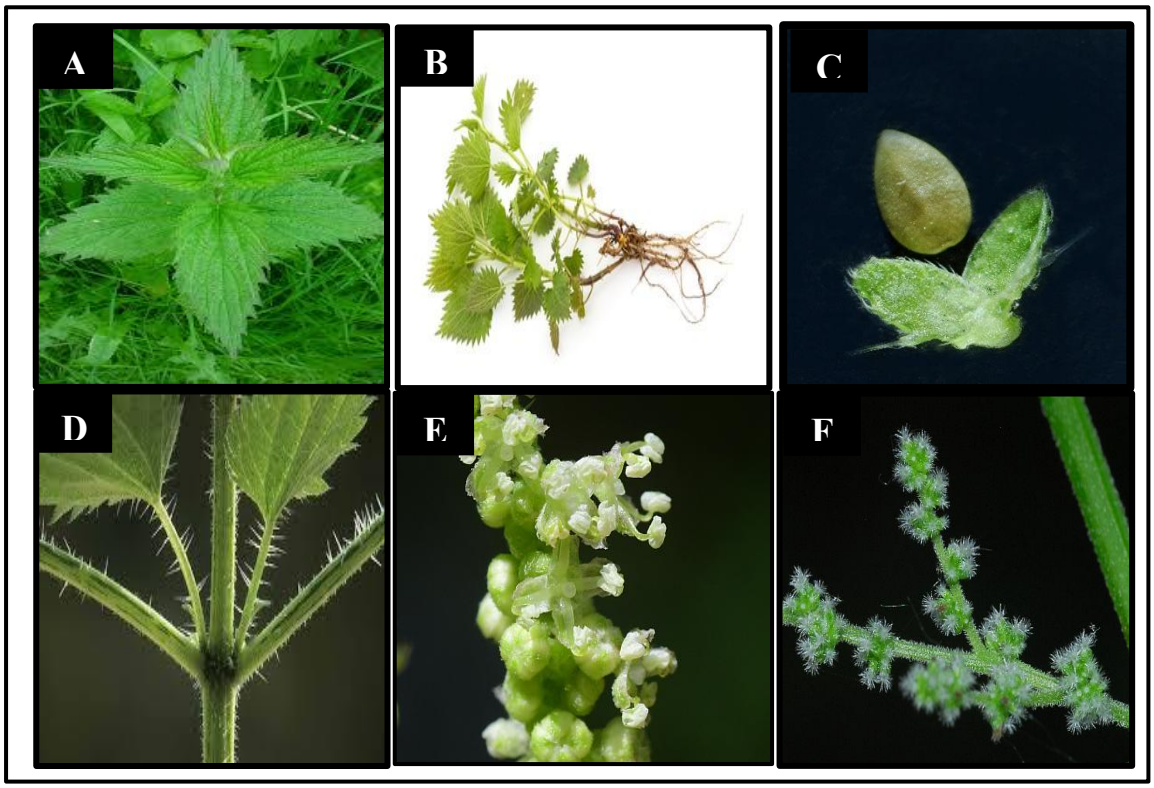


Figure 6 *:* *Urtica dioica*, a: leaves, b: root , c: fruit , d: stem , e: male flowers(Staminate), f: female flowers (Pistillate)

II.2.2. Denomination:

The nomenclature *Urtica* is derived from the Latin term uro or urere, signifying "I burn," which references the plant's stinging hairs that cause significant irritation upon contact. The designation *dioica* originates from the term dioecious, indicating that male and female flowers are found on distinct plants (**Geneviève, 2009)**.

Latin name: *Urtica dioica L*. Syn.

French names: Ortie dioïque, grande ortie, ortie piquante, ortie haute.

English names: Nettle, Common Nettle, Stinging Nettle, Tall Nettle, Slender Nettle, Greater Nettle (**Geneviève, 2009).**

II.2.3 Taxonomy

Kingdom- Plantae: Plants.

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class- Magnoliopsida: Dicotyledons.

Subclass: Hamamelidae.

Order: Urticales.

Family- Urticaceae: Nettle family.

Genus: *Urtica*

Species: *Urtica dioica L.,* - stinging nettle **(Le Moal &Truffa-Bachi, 1988).**

II.2.4. Traditional uses

Urtica dioica has a longstanding tradition of application in home remedies and as a component of a nutritious diet. The powdered leaf extract is recognized for its antihemorrhagic properties, effectively mitigating excessive menstrual bleeding and nasal hemorrhages. This plant has been employed in the treatment of various conditions, including arthritis, anemia, and hay fever, and has been utilized as a diuretic, astringent, and blood tonic in traditional medicine. Historically, a tea brewed from the leaves of *U. dioica* has served as a cleansing tonic and blood purifier. Externally, the plant is applied to address skin issues, gout, sciatica, neuralgia,

hemorrhoids, and hair-related problems **(Bombardelli & Morazzoni, 1997).** Herbalists globally have utilized this plant for centuries. In the first century, Greek physicians such as Pedanius Dioscorides and Galen documented the diuretic and laxative effects of *U. dioica* leaves, noting their efficacy in treating asthma, pleurisy, and spleen disorders. Additionally, *nettle* leaves are recognized as a nutritious supplement and are often employed as an aid for weight loss **(Ji, 2009).**

II.2.5. Phytochemistry

The seeds and leaves of *U. dioica* contain vitamins, minerals and amino acids. Chemical enthusiasm for *U. dioica* was fortified by reports that they cause bothering when interacts with skin. The leaves of *U. dioica* have sharp spines with stinging hairs that contains histamine and formic corrosive, which causes irritation **(Krystofova *et al*., 2010).** Many mixes were beforehand confined by various specialists on *U. dioica,* viz., phytocsterols **(Miraj, 2016)**, lignans **(Miraj, 2016),** carotenoids **(Csir, 1989),** greasy acids **(Grieve, 1931),** phenolics **(Miraj & Kiani, 2016),** and so forth. The leaves of *U. dioica* are being utilized a s home grown tea for the treatment of various afflictions, for example, hypertension, kind prostatic hyperplasia and so forth the leaves were accounted for to contain caffeic corrosive, chlorogenic acid **(Miraj & Kiani, 2016),** high substance of chlorophyll and other pigments **(Almasi *et al*., 2016),** Phytochemical literature on *U. dioica* gave a frail sign for the nearness of alkaloids, there are no reports distributed somewhere else. Rhizomes of *U. dioica* are found to contain agglutin, a progression of long chain amino corrosive linkages **(Miraj, 2016).** The structure of agglutin was additionally affirmed as an individual from proteins family with two hevein like spaces display in each subunit **(Shabani *et al*., 2015).** Studies on phytochemical examinations demonstrated the leaves and rhizomes are rich in phenolic compounds **(Miraj, 2016).** It was accounted for to contain distinctive cinnamic corrosive subsidiaries, coumarins and homovanillyl liquor, a portion of the phenolic mixes were considered in derivatized shape utilizing trimethyl silyl esters in GC-MS analysis **(Khadem *et al*., 2015).** The derivatization of phenolic mixes yielded 34 mixes and structures were interpretated by utilizing mass fragemntation, maintenance time and contrasted and industrially avaliable mixes. A portion of the mixes were recognized as homovanillyl liquor, vanillin, vanillic corrosive, (+) isolaricini ¬ resinol, (-) secolaricinresinol. Methylated subordinates of phenolic compounds and flavonoids from U. dioica and with different plants were prepared by utilizing methyl iodide, and subjected chromatographic examination. In light of derivatization, GC-MS examination of *U. dioica* methanloic leaf separate brought about the nearness of 3.0 ng/mg of homo vanillic corrosive,

2.5 ng/mg of vanillic corrosive, 43.65 ng/mg of 4-hydroxy cinnamic corrosive and 574 ng/mg of ferulic acid **(Yildirim *et al*., 2012).** Aqueous concentrate of U. dioica leaves yielded two noteworthy divisions on preparative HPLC investigation utilizing Sephadex segment. One of the parts was found to contain a blend of sugars and proteins called glycoprotein **(Namazi *et al*., 2012).** Blumenthal *et al*, were made an endeavor to think about the connection amongst protein and starch by hydrolysing with sodium hydroxide and sodium borohydride. Results demonstrated that the protein and sugar are associated with by means of serine and O-galactosidic linkages, promote methylation showed the bother glycoproteins were extended and shown the nearness of 3,5 and 2, 5 di-o-methyl arabinoses is connected with 1-2, 1-3 and 1-4/5 arabinose, while unbranched galactose are associated essentially 1-3 and some 1-4 and 1-6 linkages. However the second fractiopn was unsuccesssful in its methylation process **(Namazi *et al*., 2012).** The underlying foundations of *U. dioica* demonstrated the nearness of trans neo-olivil, a lignans glycoside **(Mahlangeni *et al*., 2016).** The leaves of U. dioica were utilized as a part of creature cultivation generally, various reports demonstrated the carotenoids and chlorophyll are utilized to improve full gainful of animals **(Mahlangeni *et al*., 2016).** The conventional strategies for extraction from plant material incorporate steam refining, soxhlet permeation utilizing natural solvents, maybe these methodology have loads of disadvantages, for example, debasement, loss of organically dynamic mixes, duration and so forth. Sovova *et al* used supercritical liquid extraction utilizing fluid CO2 inorder to disconnect carotenoids and chlorophyll from the leaves of *U. dioica* **(Patel *et al*., 2013).** HPLC investigation of concentrates resluted most extreme yields of chlorophyll a, chlorophyll b, beta carotene, and lutein. This report proves the nearness of plentiful chlorophyll and beta carotene in the leaves of *U. dioica* **(Patel *et al*., 2013).**

II.2.6. Nutritional value

Nettle leaves are abundant in proteins, lipids, carbohydrates, vitamins, minerals, and trace elements. Proteins constitute approximately 30% of the dry weight **(Pradhan *et al*., 2015).** Furthermore, the protein profile of nettle leaves significantly meets the amino acid needs, especially the essential amino acids required by humans **(Rutto *et al*., 2013; Wetherilt, 1992).** Regarding the mineral content, nettle leaves may comprise up to 20% of the dry mass **(Pradhan *et al*., 2015; Chrubasik *et al*., 2007).** These leaves are particularly rich in minerals such as iron, zinc, magnesium, calcium, phosphorus, and potassium. Additionally, the presence of trace elements like cobalt, nickel, molybdenum, and selenium has been identified in the leaves **(Mihaljev *et al*., 2014).**

II.3. Pharmacological properties

Nettle is a nutritionally rich plant with diverse pharmacological benefits. Its abundance of vitamins (B1, C, E) and trace elements (iron, zinc, selenium, manganese) enhances immune function and provides strong antioxidant properties, helping to combat infections and reduce the risk of cardiovascular diseases and immune deficiencies **(Rafajlovska *et al*., 2001).** Nettle also shows anti-tumor effects and supports cardiovascular health due to its high potassium content. Its mineral richness (calcium, potassium, silicon, iron) makes it beneficial for bone health, particularly in cases of osteoarthritis and osteoporosis**(Rafajlovska *et al*., 2001).**The iron and vitamin C content improve iron absorption, aiding in the treatment of anemia, while magnesium may help reduce stress. Zinc contributes to its anti-inflammatory action**(Rafajlovska *et al*., 2001).** Additionally, nettle contains high levels of chlorophyll (4.8 mg/g dry leaves), which supports detoxification, digestion, and healing. Thanks to its high protein, essential amino acids, and micronutrient content, nettle is also a valuable dietary supplement, especially for vulnerable groups like malnourished children, pregnant women, and the elderly **(Rafajlovska *et al*., 2001).**

II.3.1. Anti-inflammatory activity

Scientific investigations have demonstrated that nettle can mitigate inflammatory responses through various mechanisms. These mechanisms lead to a decrease in the production of lipid mediators and pro-inflammatory cytokines. Specifically, leaf extracts have been shown to inhibit the activity of enzymes involved in the arachidonic acid pathway, particularly cyclooxygenases COX-1 and COX-2, thereby obstructing the synthesis of prostaglandins and thromboxane **(Roschek *et al*., 2009).** Furthermore, multiple studies indicate that the leaf extract reduces the secretion of interleukins IL-2 and IL-1β, as well as interferon γ (IFNγ) and tumor necrosis factors TNF-α and TNF-κ (**Konrad *et al*., 2005; Yilmaz *et al*., 2014).** Moreover, the aqueous extract derived from nettle roots exhibits anti-inflammatory properties. Research conducted by Wagner revealed that a polysaccharide fraction from this extract demonstrates an inhibitory effect on induced edema in rat paws, comparable to that of indomethacin **(Wagner *et al*., 1994).**

II .3.2. Antioxidant activity

Numerous investigations have demonstrated that both methanolic and ethanolic extracts derived from leaves exhibit significant antioxidant properties against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical **(Pourmorad *et al*., 2006).** Additionally, research conducted on rats subjected to tetrachloromethane (CCl4) treatment revealed that nettle not only reduced lipid

peroxidation but also enhanced the functionality of the antioxidant defense system, thereby providing a protective effect against hepatotoxicity. This antioxidant capability is primarily associated with the presence of phenolic compounds **(Kataki *et al*., 2012).**

II .3.3. Immunomodulatory activity

Numerous investigations suggest that extracts derived from nettle possess the capability to influence immune system functionality. Research involving the aerial components of nettle was conducted on mice, utilizing an ethanolic extract administered orally at two distinct dosages (50 and 100 mg/kg) over a period of 14 days. The results indicated a notable reduction in the activities of enzymes such as cytochrome P450, lactate dehydrogenase (LDH), and NADPH-cytochrome P450 reductase, while antioxidant enzyme activities exhibited a significant increase **(Ozen & Korkmaz, 2003).** Additionally, the plant demonstrated a modulating effect on enzymatic activities in the kidneys, lungs, and stomach, specifically affecting glutathione-S-transferase, superoxide dismutase, and catalase **(Ozen & Korkmaz, 2003).** The immunomodulatory properties of nettle are further attributed to compounds such as quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside, and isorhamnetin-3-O-glucoside found in its aerial parts **(Bhuwan *et al*., 2014; Akbay *et al*., 2003**). Moreover, the immunomodulatory effects of UDA extracted from the roots, have been corroborated by various studies that detail its influence on T lymphocytes, macrophages, thymocytes, and the secretion of TNFα (**Chrubasik *et al*., 2007).**

II .3.4. Anti-infectious properties:

Numerous studies have underscored the antibacterial properties of various extracts from Urtica dioica in relation to specific bacterial strains. One investigation involving nine bacterial species namely *C. koseri, E. aerogenes, E. coli*, Micrococcus luteus, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, and Streptococcus pneumonia demonstrated that the aqueous extract derived from the aerial parts of the plant effectively inhibited the growth of these bacteria, with the exception of certain strains of Pseudomonas aeruginosa **(Gulcin *et al*., 2004).** Additionally, a separate study examining 38 microorganisms provided compelling evidence of the bactericidal properties of organic extracts from the aerial parts of nettle, attributing this antibacterial activity to the phenolic compounds present in the plant (**Modarresi *et al*., 2012).** Furthermore, the antiviral potential of nettle has been assessed in vitro **(Uncini *et al*., 2005),** revealing a significant and selective inhibitory effect of Urtica dioica extracts on the intracellular replication of human immunodeficiency virus (HIV-1 and HIV-2), respiratory syncytial virus (RSV), and

cytomegalovirus (CMV) **(Balzarini *et al*., 1992).** The antifungal activity against certain pathogenic fungi has also been validated **(Gulcin *et al*., 2004 ; Hadizadeh *et al*., 2009).**

II .3.5. Antidiabetic activity

A research investigation aimed at assessing the in vivo antidiabetic properties has demonstrated the hypoglycemic effects of aqueous extracts derived from nettle leaves in diabetic rat models. This phenomenon can be attributed to the suppression of glucose absorption in the intestines **(Bnouham *et al*., 2003).** Additionally, investigations focused on the islets of Langerhans have established that nettle exerts a stimulating influence on insulin secretion, which is associated with a reduction in blood sugar levels. Experiments conducted on both normal and diabetic rats following the intraperitoneal administration of the aqueous extract have corroborated these findings **(Farzami *et al*., 2003).**

II .4. Toxicity

Toxicological investigations have determined that the median lethal dose (LD_{50}) of the aqueous extract from the leaves, when administered intraperitoneally to mice, is 3.5 g/kg **(Hadizadeh *et al*., 2009).** In contrast, the LD_{50} for the hydro-alcoholic extract of the leaves, when given orally, is 5.77 g/kg **(Farahpour & Khoshgozaran, 2015**). Studies focusing on the roots have indicated that the LD50 values following intravenous administration of the aqueous extract and infusion of the roots in rats are 1.721 g/kg and 1.929 g/kg, respectively **(Baraibar *et al*., 1983**). Additionally, the LD50 for hydro-alcoholic extracts administered via intraperitoneal injection is reported to be 600 mg/kg **(Pourahmadi *et al*., 2014).**

PART TWO: EXPERIMENTAL SECTION

Material and Methods

I.1. Materials

I.1.1 Animal material

The present study's biological material consists of 20 Albino female Wistar-strain *Rattus rattus* obtained from the Pasteur Institute in Algiers, which are weighted between 105-120 g and aged between 4- 6 weeks old. They Characterized with their huge head, short ears, red globular eyes, and long tail.



Figure 7 : Experimentation Rats

I.1.2. Plant material

The medicinal plant utilized in this experiment is *Urtica dioica*, a member of the Urticaceae family collected from Bakkaria (TEBESSA), Algeria during February 2025.



Figure 8: *Urtica dioica plant*

I.1.3. Pesticide

Lambda-cyhalothrin (LCT), a synthetic pyrethroid insecticide with the trade name "KARATE", chemical name CAS (R1S)-a-cyano-3-(phenoxyphenyl)methyl-(1S1R)-cis-3-(2-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane-carboxylate, CAS Registry Number 91465-08-06.



Figure 9: Lambda-cyhalothrin pesticide

I.2. Methods

I.2.1. Ethnobotanical survey

A survey utilizing individual questionnaires was conducted among a sample of residents in the TEBESSA region from February to March 2025. The questionnaire was disseminated to participants through Google Forms and directly question to citizens.

The aim of this survey is to gather extensive information regarding the prevalence of *Urtica dioica* within the indigenous community. The questionnaire comprises two parts:

- Part 1: focused on the personal details of the participants.
- Part 2: addressed inquiries related to the plant, including its methods of consumption, applications, and daily usage.

The information obtained was systematically processed using Google Forms and Microsoft Excel, and subsequently illustrated through various graphical formats.

I.2.2. Animal care

The rats were divided into four groups, with five rats in each group. They underwent a 15-day acclimatization period in the animal facility of the biology department, where the ambient temperature was maintained at 25 °C and a natural light cycle of 12 hours of light and 12 hours of darkness was observed. The rats were housed in plastic cages equipped with stainless steel lids and provided with water bottles of 250 ml capacity, which were lined with a layer of bedding that was replaced daily prior to the commencement of the observation. They were fed a standard diet, with water renewed daily.

I.2.3. Weight measurement

Weight measurement is carried out on the rats every day on a regular basis during the breeding period, either during adaptation (for the evaluation of possible changes by food or place (external factors) or during treatment (to evaluate the effects of pesticides) using electronic scales.

I.2.4. Choice of dose

In this study, we used a pesticide (lambda cyhalothrin) at a dose of 6,3 mg/kg/**d (Ghosh *et al*., 2018),** and the extract of *Urtica dioica* at a dose of 100 mg/kg/d **(Dhouibi *et al*., 2021)**,they administered chronically orally each day for 30 days.

Thus, 04 lots of 05 rats in each were distributed as follows:

- **Control groups (C):** Rats received distilled water by gavage (0.5 ml/day).
- **Lambda-cyhalothrin groups (LCT):** Rats received pesticide by gavage (6.3 mg/kg/d) during 30 days.
- ***Urtica dioica* group (URT):** Rats received plant by gavage (100 mg/kg/d) during 30 days.
- **Pesticide + plant (LCT+URT):** Rats received a combination of pesticide (6.3mg/kg) + plant (1.5 ml/day) by gavage.

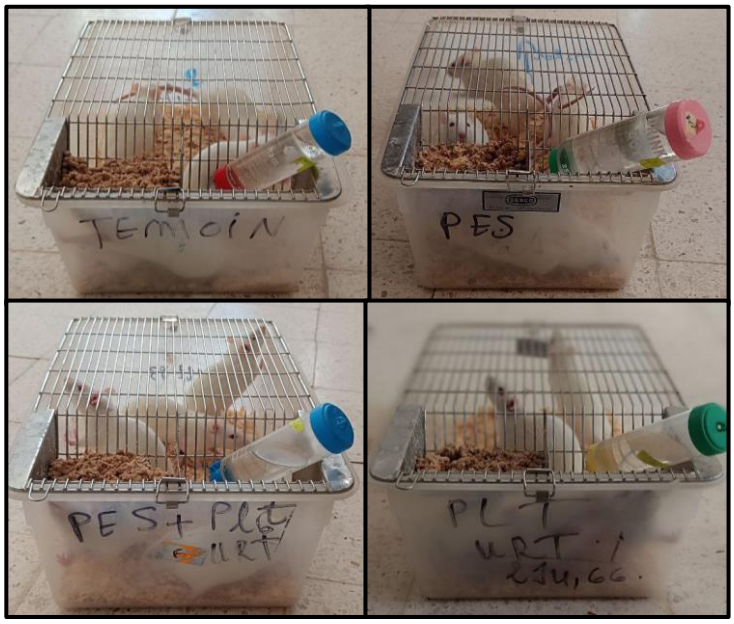


Figure 10: Rats repartition groups



Figure 11: Rats gavage technique

I.2.5. Plant decoction extract

The leaves of the fresh plant *U. dioica* were dried at room temperature for a week. 16g of *U. dioica* leaves powder were boiled with 400 ml of distilled water for 30 min with occasional stirring. Let it

steep for two hours. Bring the mixture to a boil, then simmer for 10 minutes. The decoction preparation was filtered and the extracts were stored at +4°C.

Note: This mixture should only be used within 48 hours



Figure 12: Preparation of plant decoction

I.2.6. Behavioral study

This study is conducted in our laboratory to assess the impact of LCT and *U. dioica* on loco-motor activity, anxiety-like depression behavior. Various tests have been designed, including the forced swimming test (FST), the elevated plus maze (EPM), and the open field (OF). The animals' behaviors were captured and documented using video recording equipment on the 28th day of treatments.

I.2.6.1. Forced swimming test (FST)

FST serves as a behavioral model to assess the efficacy of antidepressant therapies **(Porsolt *et al.*, 1977).** This model is applicable to both rats and mice, although the procedural specifics vary by species. The test involves placing a rat individually in a 40 cm high and 30 cm wide aquarium, designed to prevent escape by clinging to the edges.

The aquarium is filled with water at a temperature of 25°C, reaching a height of 35 cm to ensure that the rat cannot utilize its hind limbs to remain afloat, thereby compelling it to swim (Figure 8). Following an initial vigorous activity phase (adaptation period), the control animal ceases swimming and exhibits a state of immobility, indicative of a despair response. The rat is deemed stationary when it floats horizontally, engaging only in minimal movements necessary to keep its head above water. The FST is conducted in two phases for rats: the pre-test (FST1) and the test (FST2), with a 24-hour interval during which treatment is administered. In the pre-test, the rat is submerged in the aquarium for 15 minutes, during which it cannot escape, resulting in immobility at the session's conclusion. The following day, the rat is reintroduced to the aquarium for a 5-minute duration, during which the duration of immobility is recorded. Effective antidepressant treatment is indicated by a reduction in immobility time specifically on the test day **(Porsolt *et al.*, 1978).** Recently, an enhancement of the test has been validated, which not only assesses immobility but also evaluates two active behaviors—swimming and climbing—that contribute to the reduction of immobility. The variables measured include: immobility time (in seconds), swimming time (in seconds), and climbing time (in seconds).

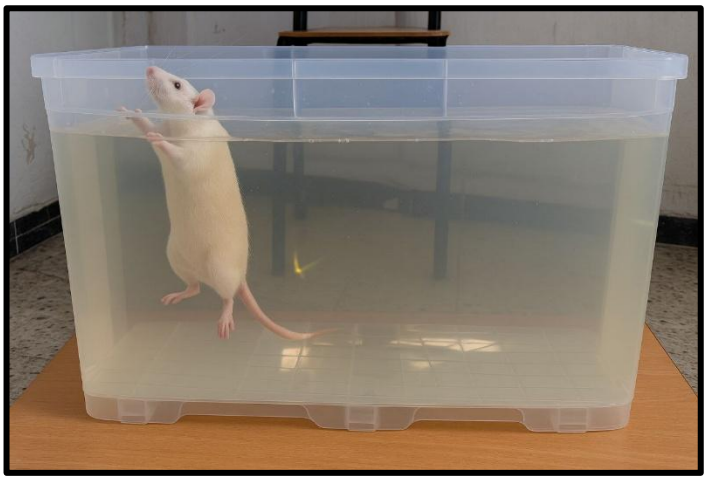


Figure 13 : Forced swimming test (FST)

I.2.6.2. Elevated plus maze test (EPM)

EPM is a widely studied test to highlight the properties anxiolytics or anxiogenic pharmacological compounds. The device consists of a raised maze in the shape of a cross with two open arms (50 × 10cm) and two closed arms (50 × 10 × 45 cm). The device is located at a height of 50 cm above the soil **(Patin *et al.*, 2005).** Each rat is placed individually in the center of the EPM directed towards one of the open arms and its behavior in free exploration is recorded and examined for 5 min. A visit was counted when the rat had all four legs in an arm

The time spent and the number of entries in the open and closed arms are measured. The experiment exploits the conflict, in rodents, between fear of open spaces and the desire to explore a new environment. Closed arms represent security, while open arms offer exploratory value. An anxious animal will have naturally tended to prefer closed and dark spaces over open ones and enlightened. Thus, behavioral anxiety is measured by the degree of avoidance of spaces open to the labyrinth. At the end of each session, the animal is returned to its cage and the device is cleaned with an alcoholic solution.

The variables measured are: time spent in the center (second), time spent in open arms (second), time passed to the distal and proximal part of the open arm (second), number of entries to the open arm, time spent in closed arm (second), time spent at distal and proximal part of closed arm (second), number of entries in closed arm, Redressement number.



Figure 14: Elevated plus maze test (EPM)

I.2.6.3. Open field test (OF)

OF test was conducted to evaluate variations in emotional reactivity among rodents (**Hall., 1934**),. This test facilitates the examination of locomotor activity and exploratory behavior in rats. Specifically, the OF consists of a Plexiglas enclosure measuring (70 cm × 70 cm × 40 cm), with its floor segmented into central and peripheral zones. Each rat is individually placed in the center of the apparatus and allowed a 5min exploration period **(Brenes Sáenz *et al*., 2006).** A rat exhibiting anxiety towards the exploratory environment tends to favor the peripheral zones while avoiding the central area. Each testing session is recorded on video, and the apparatus is

sanitized with an alcoholic solution after each use to eliminate any residual odors from previous subjects that could influence the results.

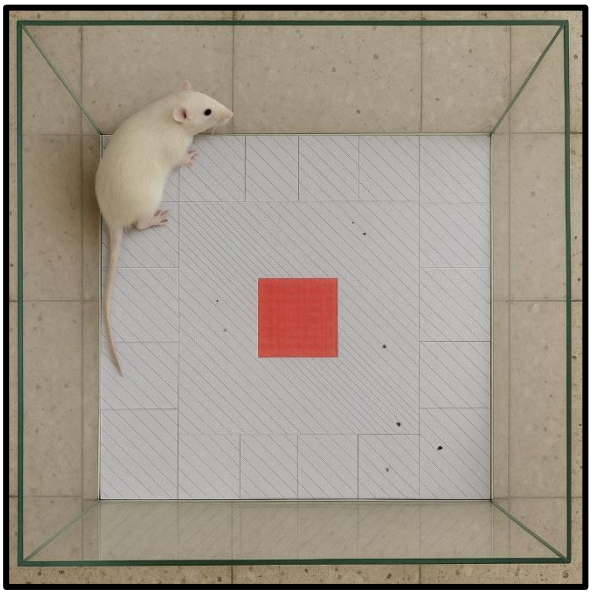


Figure 15: Open field test (OF)

Note: The data was processed using Any-Maze version 7.49

I.2.7. Sampling

I.2.7.1. Blood Sampling

The blood is taken from rats via the ophthalmic route, using a hematocrit capillary tube through the retro-orbital sinus at the level of the orbital vein. We collect blood in an EDTA tube for hematological parameters.

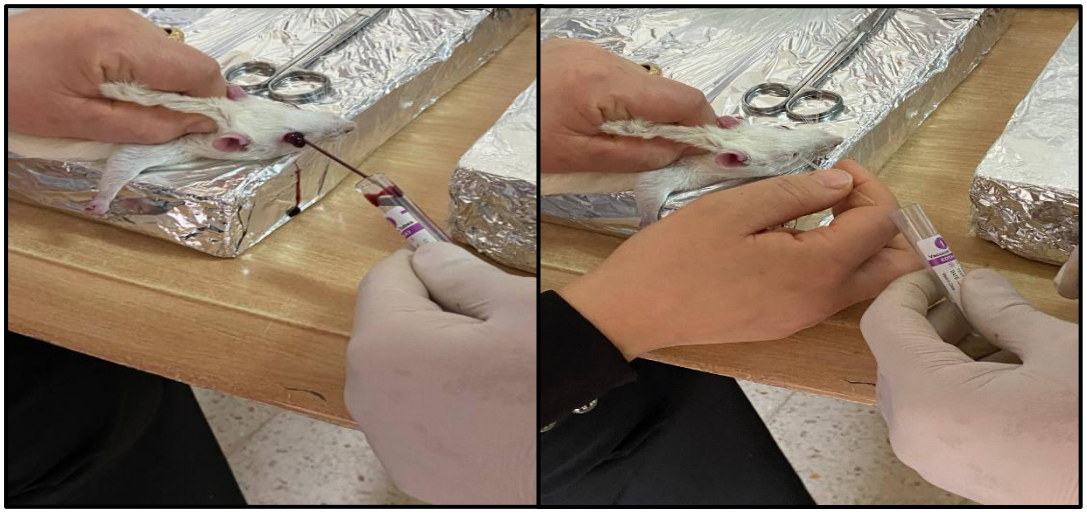


Figure 16: Blood Sampling using a hematocrit capillary tube

I.2.7.2. Sacrifice and brain removing

At the end of the experimental period, the animals are sacrificed by decapitation and the brain were removed quickly and rinsed in cold wash buffer (NaCl 9%), then dried at low temperature (4°C) with semi-absorbent paper and weighed using a precision balance (SCALTEC SBC 51).

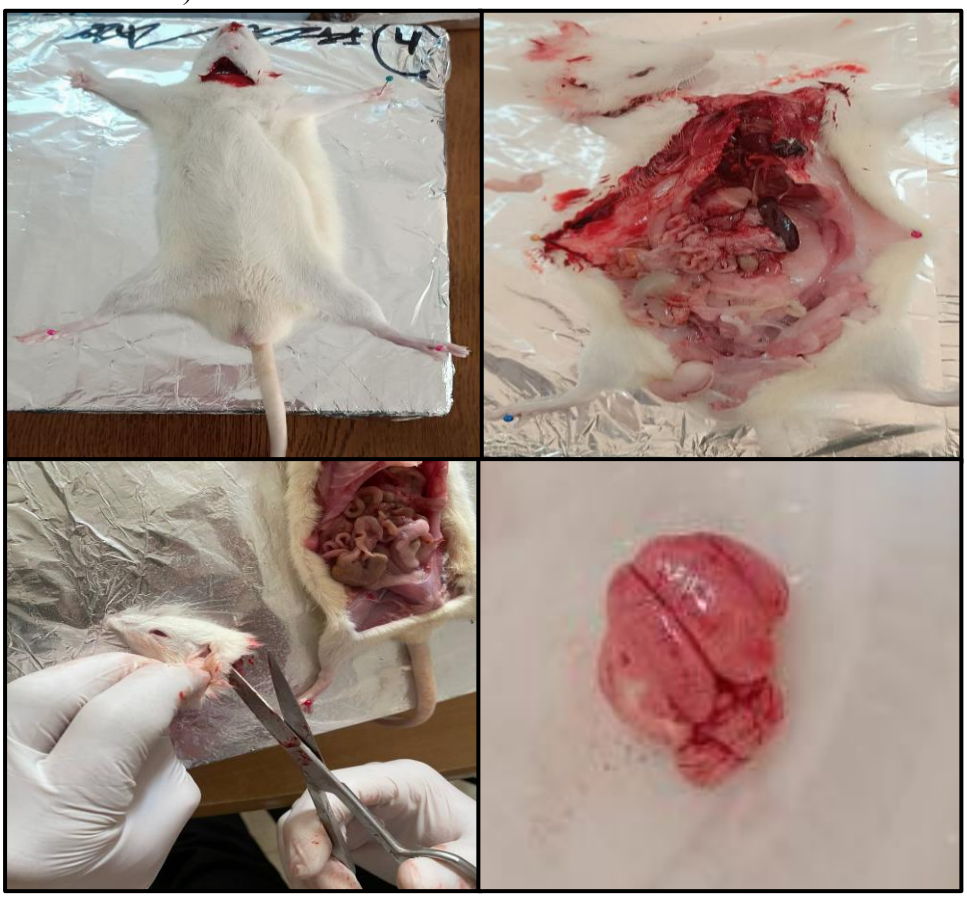


Figure 17: Sacrifice and organs sampling

I.2.8. Hematological parameters

- **Principle**

The blood analysis was conducted using a type counter automaton (Mindray BC-30S) that assessed 19 parameters. This evaluation aimed to quantify the cellular components of the blood, including the counts of red blood cells, white blood cells, and the levels of hemoglobin, lymphocytes, monocytes, and granulocytes. The analysis was performed on blood samples preserved in tubes containing EDTA or heparin, and the counting process took place at the Bouguerra Boulaaras Hospital in Bakkaria, Tebessa, Algeria.



Figure 18: Automaton counter of CBC .

I.2.9. Study of biochemical parameters

Blood glucose measurement

- **Principle**

The measure of blood glucose was carried out by a glucometer which uses test strips. These are intended for in-diagnosis use in vitro for blood glucose testing. They are designed to measure glucose in the capillary whole blood. The test strip contains glucose oxidase, an enzyme that oxidizes glucose in the blood and produces d-gluconic acid and hydrogen peroxide.

- **Mode of operation**

- The reader starts automatically by simply inserting the strip Reactivates Accu-Chek (in the direction of the arrows and up to the stop).
- The symbol of a drop flashes.
- Place the drop of blood on the orange drop zone of the strip. The result appears in 5 seconds. Blood glucose is given in g/dl.



Figure 19 : Blood glucose measurement.

I.2.10. Brain antioxidant activities

a. Glutathione S-transferase (GST) assay

- **Principle**

The assessment of GST activity involves providing the enzyme with a substrate, typically 1-chloro, 2.4-dinitrobenzene (CDNB), which reacts readily with various forms of GST and glutathione. The conjugation of these two components leads to the production of a novel molecule that exhibits light absorption at 340 nm. The methodology employed in this investigation for the determination of GSTs is based on the work of **(Habig *et al*., 1974),** which entails introducing the GSTs present in the homogenate into a mixture of GSH and CDNB at a temperature of 37°C and a pH of 6.5. The change in optical density resulting from the GSH-CDNB complex is recorded over a period of 1 minute, repeated for a total duration of 5 minutes at a wavelength of 340 nm.

- **Preparation of the homogenate**

100 mg of tissue + 1 ml phosphate buffer (0.1 M pH 6.5) homogenized cold grinding.

- **Solutions preparation**

- Phosphate buffered saline preparation (0.1 M pH 6.5)

- Solution (A): Dissolve 35.8 grams of sodium hydrogen phosphate (Na2HPO4) in 1 liter of distilled water.

- Solution (B): Dissolve 13.8 grams of sodium dihydrogen phosphate (NaH2PO4) in 1 liter of distilled water.
- Combine 61.5 ml of solution A with 438.5 ml of solution B, and incorporate 21.39 g of sucrose.

-CDNB solution preparation (0.02 M)

- Dissolve 202.55 mg of CDNB in 50 ml of absolute ethanol.

-GSH solution preparation (0.1 M)

- Dissolve 153.65 mg of GSH in 50 ml of distilled water.

❖ **Operateur mode**

Table 3: GST dosage mode.

Reagents used	Blank (µl)	Sample (µl)
phosphate-buffered saline (0.1 M, pH 6.5)	850	850
CDNB (0.02 M)	50	50
GSH (0.1 M)	100	100
Surnageant	-	20
Distilled water	20	-

The activity of GST expressed in CDNB nanomoles per minute per milligram of protein (nmol CDNB/min/mg prot) as follows:

$$\mathbf{GST\ (nmol\ CDNB/min/mg\ prot)} = \frac{\mathbf{\Delta\ DO\ sample} - \mathbf{\Delta\ DO\ Blanc}}{\boldsymbol{\varepsilon}\ .\ \mathbf{L}\ .\ \mathbf{mg\ prot}}$$

Δ DO sample – Δ DO blanc : mean sample OD per minute – mean Blank OD per minute

ε : CDNB molecular extinction coefficient,

ε CDNB = 9.6 mM-1.cm-1.

L : Optical path of the vessel = 1 cm.



Figure 20: Assay of GST activity preparation

b. Protein assay in a sample according to (Bradford & Ward, 1976)

- **Principle**

This method employs Coomassie blue as a reagent that interacts with arginine in acidic environments, and to a lesser degree with histidine, lysine, and aromatic amino acids. Coomassie blue attaches to proteins through non-covalent bonds, including hydrogen bonds, hydrophobic interactions, and ionic interactions. The peak absorption wavelength shifts from 465 nm (red) to 595 nm (blue) **(Bradford & Ward, 1976).**

- Bradford Reagent, BBC Solution (Coomassie Brilliant Blue)

100 mg of BBC G250 is dissolved in 50 ml of ethanol (95%), cover the beaker with aluminum.

- Agitation for 02 hours.
- Add 100 ml of orthophosphoric acid (85%) + 850 ml of distilled water to obtain a 1L solution.
- Filter and store for up to 1 month at 4°C temperature and away from light..

➢ **Preparation of the calibration range**

- Stock solution of BSA (Bovine serum albumin)

01 mg of BSA + 01 ml of distilled water → Agitation

❖ **Mode of operation**

- Repair 06 tubes of different composition, after stirring, read the optical density at 595 nm:

Table 04. The number of tubes and compositions.

Tubes	Compositions
01	4 ml BBC + 100 µl of distilled water + 0 µl BSA
02	4 ml BBC + 80 µl of distilled water + 20 µl BSA
03	4 ml BBC + 60 µl of distilled water + 40 µl BSA
04	4 ml BBC + 40 ul of distilled water + 60 µl BSA
05	4 ml BBC + 20 ul of distilled water + 80 µl BSA
06	4 ml BBC + 0 ul of distilled water + 100 µl BSA

This range enables us to formulate the equation **y=ax+b** for determining the protein concentration within the sample.

❖ **Mode of operation**

- Collect 0.1 ml (100 µl) of the sample (supernatant),.
- Add 4 ml of Bradford reagent, mix thoroughly.
- Shake and let stand 5 min for color stabilization.
- Measure the optical density at 595 nm relative to the blank, which consists of 4 ml of BBC and 0.1 ml of distilled water. The resulting optical density is then represented on a calibration curve. The concentration of protein is assessed by comparing it to a standard range of bovine serum albumin (BSA) at 1 mg/ml, maintained under identical conditions.



Figure 20: Protein assay preparation

c. Reduced glutathione assay according to (Weckbecker & Cory, 1988)

Principle:

The assessment of GSH activity is conducted using the colorimetric technique established by**(Weckbecker & Cory, 1988)** . This method relies on the oxidation of GSH by 5,5'-Dithiobis-2-nitrobenzoic acid (DTNB), resulting in the formation of thionitrobenzoic acid (TNB), which exhibits an absorbance peak at 412 nm.

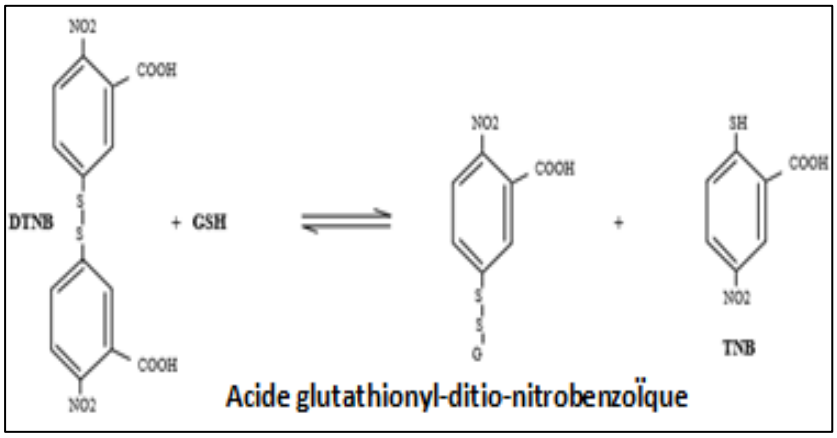


Figure 21: GSH reaction mechanism **(Baker, *et al*., 1990).**

➢ **Protocol**

- Utilizing an IKA VORTEX GENIVS 3 agitator, a combination of 0.8 ml of homogenate and 0.2 ml of 0.25% sulfosalicylic acid is prepared, followed by immersion of the mixture in an ice bath for a duration of 15 minutes.
- Subsequently, it was centrifuged at 1000 revolutions per minute for a duration of 5 minutes.
- A 0.5 ml aliquot of the supernatant is combined with 1 ml of a tris/EDTA buffer (0.02 M, pH 9.6), which is prepared by dissolving 63.04 g of tris and 7.4448 g of EDTA in 1000 ml of distilled water, and supplemented with 0.025 ml of DTNB (0.01 M), derived from dissolving 3.96 g of DTNB in 1000 ml of absolute methanol.
- Following a 5-minute incubation period at ambient temperature, the absorbance is measured at a wavelength of 412 nm using a spectrophotometer, with distilled water serving as the blank reference.

The concentration of GSH is estimated using the following formula:

$$\mathbf{GST} = \left(\frac{\mathbf{DO}}{\mathbf{13.1}} \mathbf{X} \frac{\mathbf{Vd}}{\mathbf{Vh}} \mathbf{X} \frac{\mathbf{Vt}}{\mathbf{Vs}}\right) \mathbf{/mg\ of\ proteines}$$

GSH: Micromole of hydrolysed substrate per mg protein (nM/mg protein).

OD: The absorbance reading by the spectrophotometer.

13.1: Molar extinction coefficient for the thiol group (-SH).

Vd: Total volume of solutions used in deproteinization: 1 ml [0.2 ml ASS + 0.8 ml homogenate].

Vh: Volume of the homogenate used in deproteinization: 0.8 ml.

Vt: Total volume in the vessel: 1.525 ml [0.5 ml supernatant + 1 ml tris/EDTA + 0.025 ml DTNB].

Vs: Volume of the supernatant in the tank: 0.5 ml.



Figure 22: GSH assay preparation

I.2.11. Statistical analysis

The results are presented as means accompanied by their standard deviations (m ± s) and are illustrated through tables and histograms. Statistical analysis of the results was conducted using a one-way analysis of variance (ANOVA), with Tukey's test employed to compare the treated groups against the control group and the LCT group. All statistical analyses were performed using Minitab 17.1 and GraphPad Prism version 10 was used for the graphs. The threshold for statistical significance was set at $p<0.05$.

*: Significant difference (p 0.05).

**: Highly significant difference (p 0.01).

***: Very highly significant difference (p 0.001).

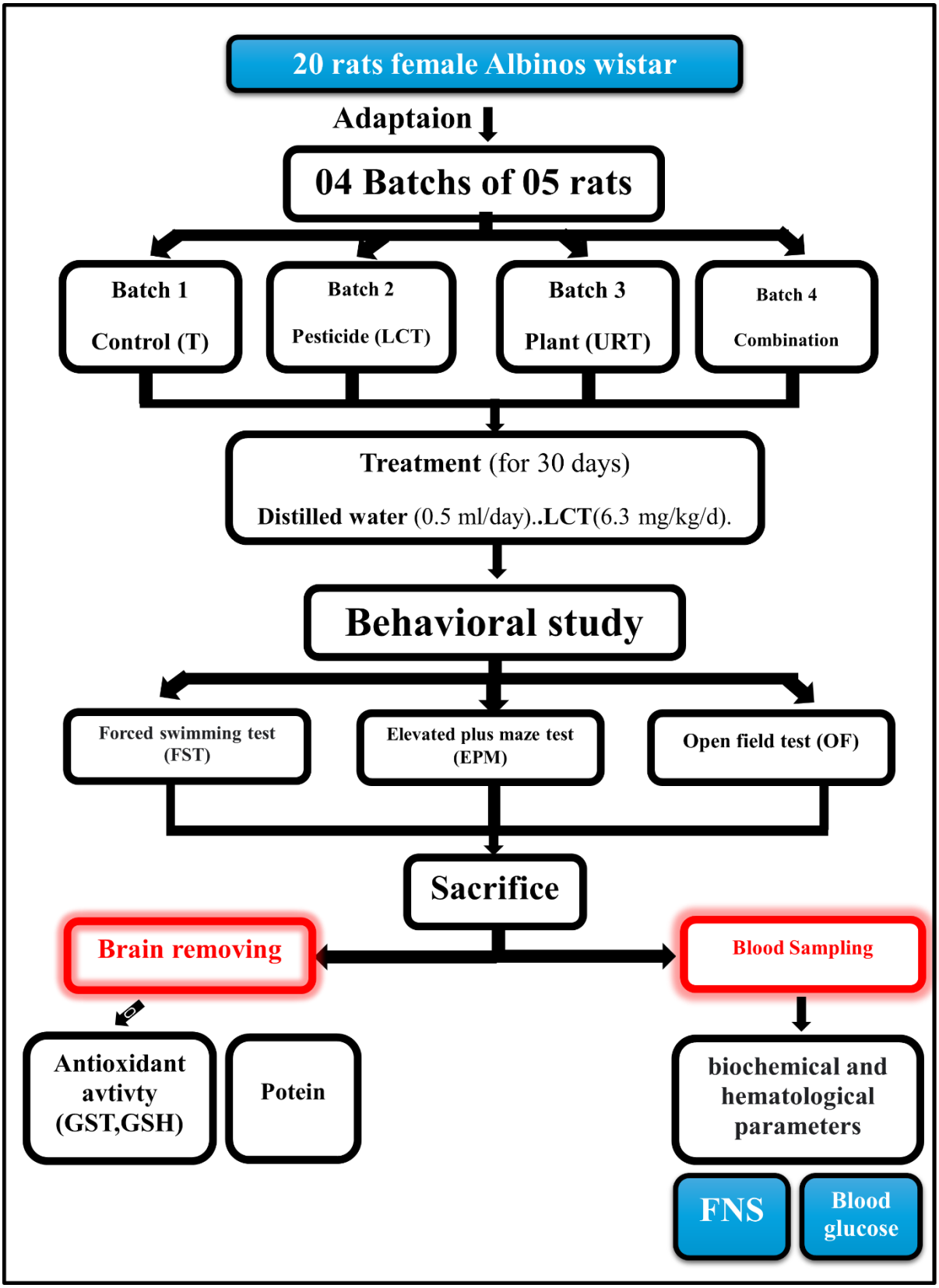


Figure 24: Summary diagram of the experimental protocol

Results and discussion

Results and discussion

II.1.Ethnobotanical study

The research encompassed the gathering of data through an ethnobotanical survey, which was subsequently documented in a Microsoft Excel database and analyzed to assess the proportions of different variables, including gender, age, education level, family status, occupation, consumption patterns, uses, and daily intake of *Urtica dioica*, especially in relation to the treatment of various illnesses. The sample analyzed comprised 207 individuals: 52 men and 154 women, potentially reflecting trends or variations in behavior concerning the utilization of the plant in the region of TEBESSA.

II.1.1. Results of sociodemographic information

Our results indicate that most our population (63%) know about Urica, compared to (37%) who don't and that the predominance is for women with a ratio of (74.8%) against (25.2%) for men. *Urtica dioica* is utilized across all age groups, but is particularly prevalent among individuals aged 18-35 years, accounting for (81.6%). In contrast, its usage is minimal among those aged between (55-75) years at (1.5%), (14.1%) for the 35-55 age group, and non-existent for individuals aged 75 years and older. Furthermore, the consumption of *Urtica dioica* is limited to (2.4%) among individuals younger than 18 years. The herb is predominantly used by single individuals, who make up (80.5%) of the sample, while married individuals constitute (17.1%). Additionally, a significant majority of the users hold a university degree, representing (92.6%). In terms of employment status, the data reveals that 38.6% of respondents are unemployed, (22.8%) are wage earners, (14.1%) engage in private business activities, and (1.1%) are retired.

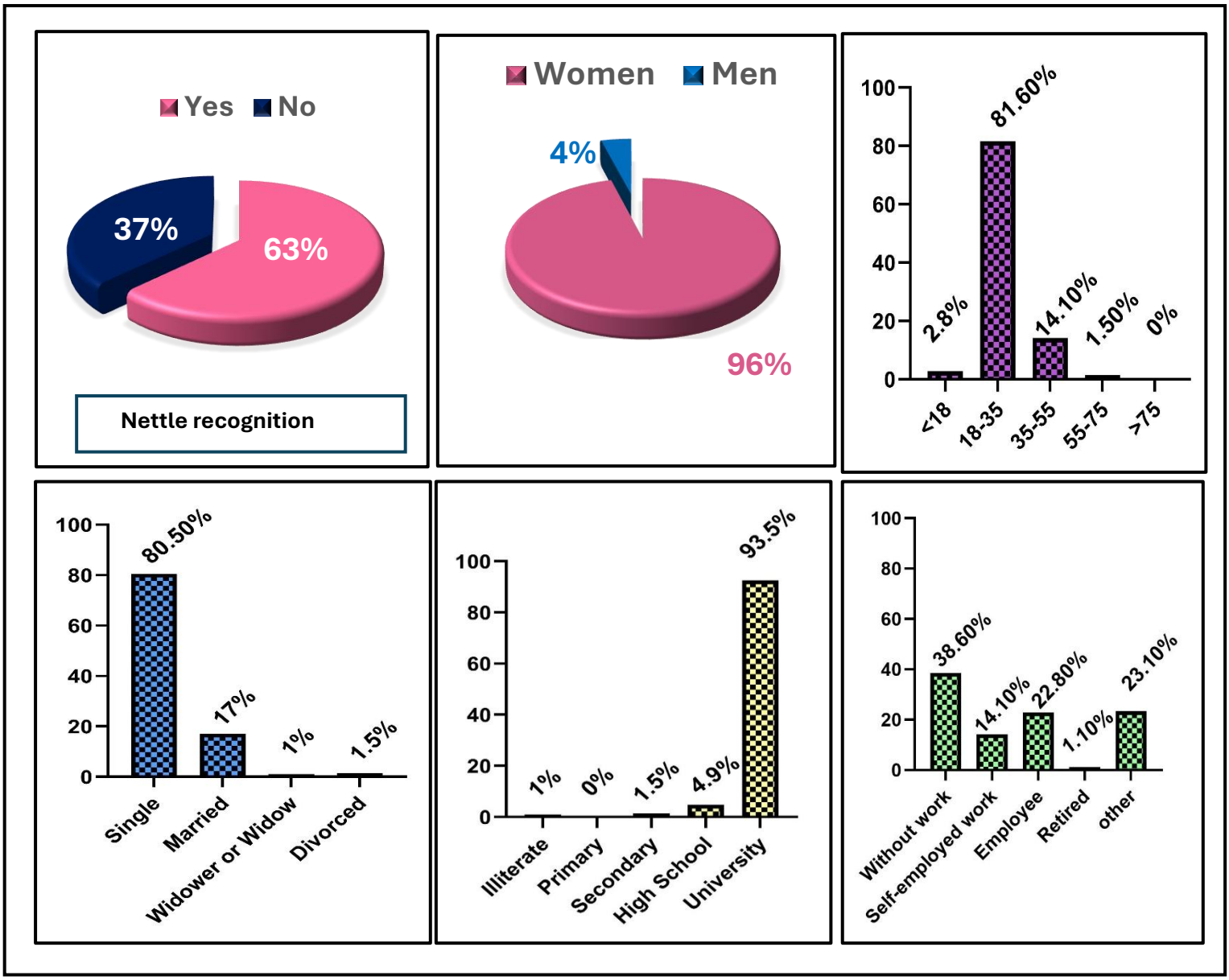


Figure 23: Socio-demographic results

II.1.2. Mode of consumption of *Urtica dioica*

77.4% of people drink Nettle in the form of tea, while (22.6%) choose to consume it in powdered form.

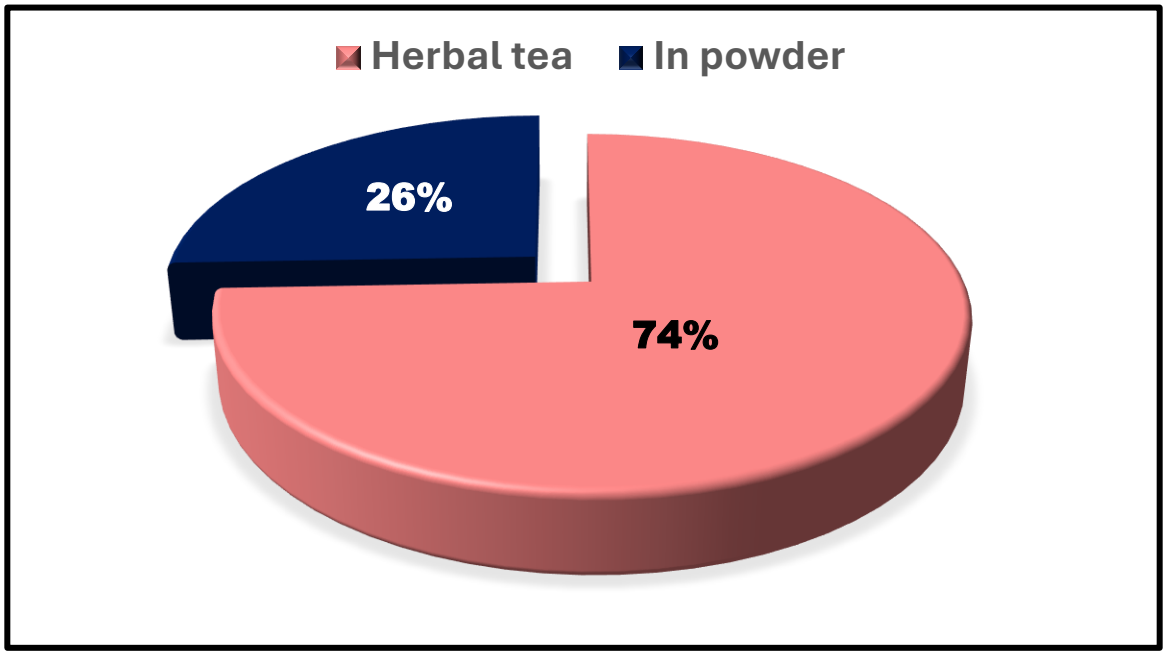


Figure 24: Mode of consumption of *U. dioica*

I.1.3. Uses of *Urtica dioica*

According to the results obtained, (69.1%) of the survey participants consume Urtica for therapeutic purposes, while (30.9%) use it for culinary purposes

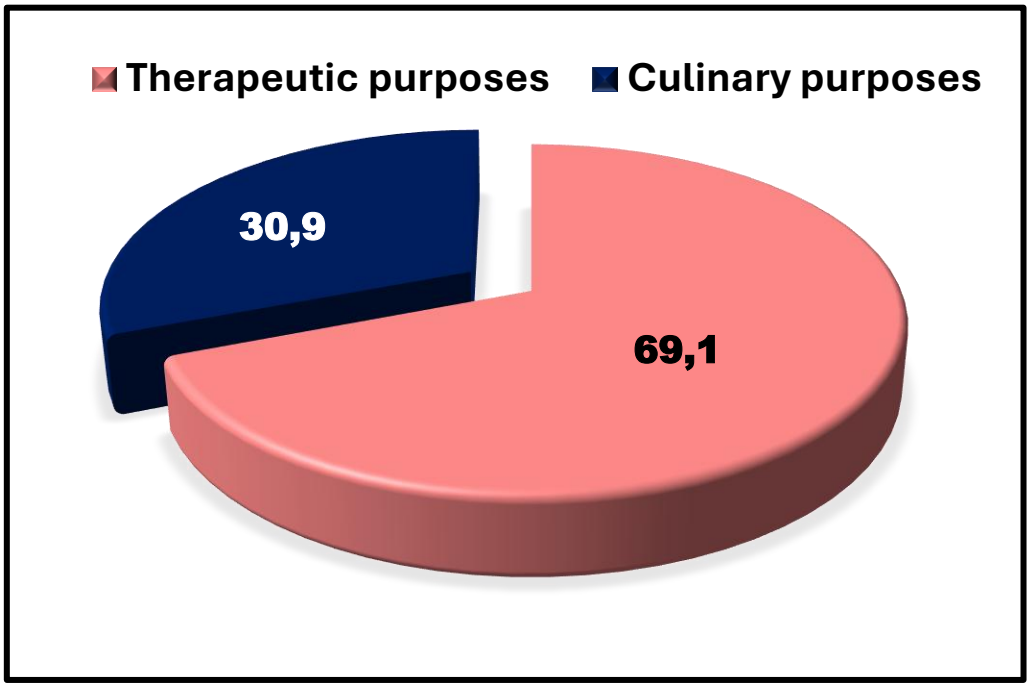


Figure 25: Uses of *U. dioica*

I.1.4. Daily intake of *Urtica dioica*

The survey revealed that the majority of respondents take Urtica once a day, representing (93.1%) of the sample. Only (3.4%) of participants reported taking two doses per day, also (3.4%) for three doses per day.

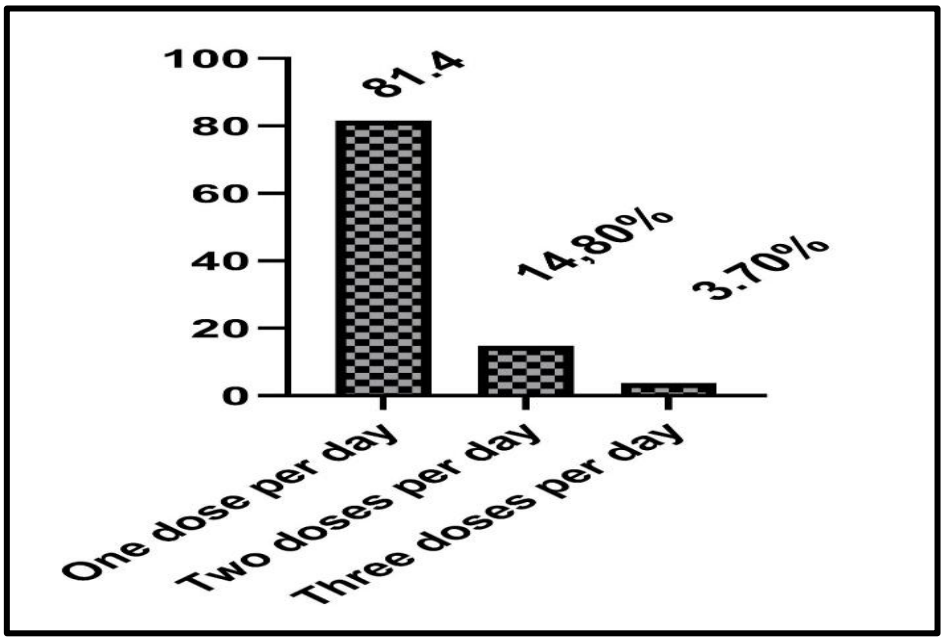


Figure 26: Daily intake of *U. dioica*

Urtica dioica, commonly known as stinging nettle, has been the subject of ethnobotanical studies across various regions, highlighting its diverse uses in traditional medicine, food, and textiles **(Singh & Kali, 2019; Devkota *et al*., 2022).** These studies aim to document traditional knowledge and practices associated with the plant by indigenous people **(Shonte & Woldetsadik, 2022)**. In Algeria, *Urtica dioica* is traditionally used for various medicinal purposes. An ethnobotanical survey focusing on the herbal medicines used by nomadic peoples in the Algerian steppe identified medicinal plants and their uses **(Miara *et al*., 2018).** Additionally, the plant is used in Algerian traditional medicine for treating many diseases **(Taïbi *et al*., 2021) .** In the Aurès Mountains, (72%) of households utilize nettle, predominantly among women (65%) and older adults (58%), highlighting its significance in home healthcare **(Rebbas *et al*., 2012).** In Tlemcen, (68%) of traditional healers recommend nettle, with a greater prevalence in rural communities (76%) compared to urban settings (42%), emphasizing the dependence of rural populations on herbal treatments **(Miara *et al*., 2019).**

U. dioica is utilized in various forms throughout Algeria, showcasing its adaptability in traditional customs. The predominant preparation method is through decoctions or infusions

(55%), which are generally made by boiling 3–5 grams of dried leaves in 200 mL of water, mainly employed to treat rheumatism or urinary tract problems **(Boudjelal *et al*., 2013).** Cooked greens (45%) are the second most favored option, where young leaves are boiled as a substitute for spinach or added to soups, especially in Kabylie, with families consuming them 2–3 times a week **(Boudjelal *et al*., 2013).** Another significant preparation method is poultices (32%), in which fresh leaves are crushed and applied externally to relieve joint pain, as noted in Tlemcen **(Miara *et al*., 2019).**

Quantitative information regarding the daily intake of Nettle in Algeria is still scarce; however, empirical research and traditional knowledge offer valuable insights into its usage patterns. For medicinal applications, a daily consumption of 1–2 cups (150–200 mL) of leaf infusion is frequently advised for chronic ailments such as rheumatism or urinary issues, particularly noted in the Aurès Mountains **(Rebbas *et al*., 2012).** In terms of nutrition, families in areas like Kabylie typically consume 50–100 grams of cooked nettle leaves per serving, usually incorporated into meals 2–3 times a week to benefit from its vitamin and mineral properties **(Boudjelal *et al*., 2013).** Despite the advantages, 78% of consumers refrain from extended use (>3 months) due to traditional warnings regarding possible side effects, including gastrointestinal discomfort or nutrient imbalances, which reflects a culturally rooted focus on moderation **(Meddour *et al*., 2020).**

Nettle has been used to treat conditions such as arthritis, constipation, and diabetes **(Rawat *et al*., 2020)**. Nettle root is employed in treating urological disorders related to benign prostatic hyperplasia **(Subba & Pradhan, 2022),** and the leaves are used for treating arthritis and rheumatism **(Subba & Pradhan, 2022).**

II.2. In vivo study

II.2.1.Biological parameters

II.2.1.1.Effect of Lambda-cyhalothrin and *Urtica dioica* treatment on body weight

This study evaluated the effects of LCT, URT, and their combination on a physiological parameter over four weeks. In our results we show an increase in body weight in Control, LCT and URT-treated groups for 21 days and a decrease above the 3rd week, but we see a severe reduction in LCT+URT-treated group from the first week of treatment.

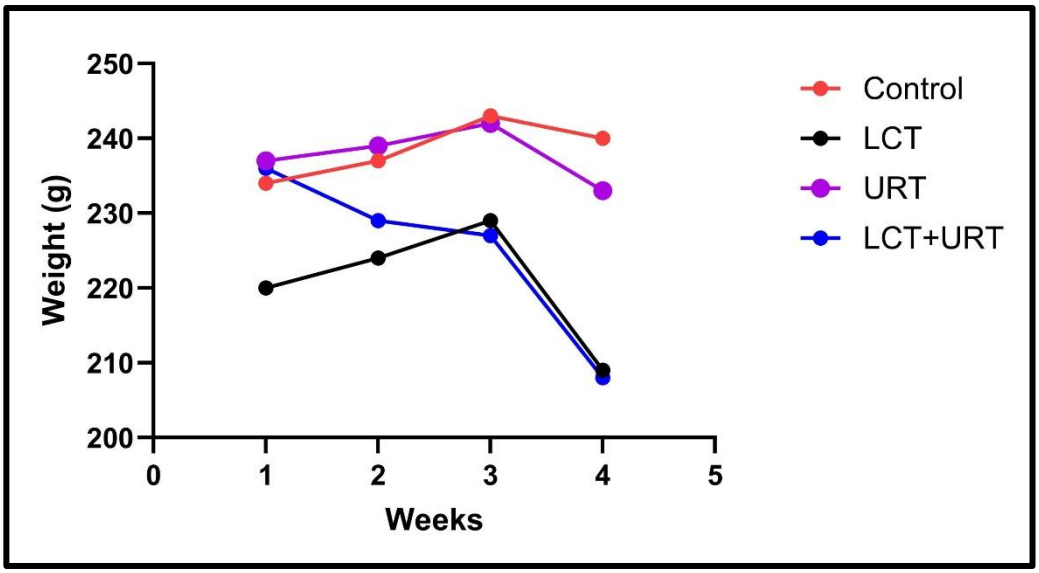


Figure 27: Variation of body weight in control and treated groups

Body weight variation is a key indicator of the animal's overall physiological state. Our results show a progressive decrease in body weight in control, *Urtica* and LCT treated-groups. In LCT treated group, this weight loss may be related to a decrease in appetite or an alteration in metabolism induced by the insecticide, as reported in other studies on the toxic effects of pesticides in mammals **(El-Sheikh & Galal, 2015;Settar *et al*., 2023)**. Indeed, the toxic action of lambda-cyhalothrin can affect the intestinal absorption of nutrients or cause transient anorexia in exposed animals, which would explain the observed weight **loss (Ball & Chhabra, 1981 ; El-Demerdash *et al*., 2004 ; Mongi *et al*., 2011; Mossa *et al*., 2014).** Furthermore, *Uritica dioica* is recognized for its anti-obesity properties and its potential in the regulation of carbohydrate and lipid metabolism. Our results are therefore in agreement with several studies having shown that this medicinal plant induces weight loss in treated subjects **(Kaçak *et al*., 2024).** It has been established that *U. dioica* significantly suppressed the expression of Brca1, Brca2, Fas, Lpl, Dgat1 and Mcp1 genes, resulting in significant changes in lipid metabolism, and inflammation. Molecular docking analyses showed that *U. dioica* components have strong binding affinities with target proteins. In particular, the interactions between Dgat1-Isorhamnetin rutinoside (-10.3 kcal/mol), Fas-Quercetin acetyl rutinoside (-10.3 kcal/mol), Lpl-Apigenin hexoside (-9.2 kcal/mol) and Mcp1-Quercetin acetyl rutinoside (-8.6 kcal/mol) were notable **(Eren *et al*., 2025).**

II.2.1.2. Effect of Lambda-cyhalothrin and *Urtica dioica* on absolute brain weight

The study evaluated the effects of Lambda-cyhalothrin (LCT) and *Urtica dioica* (URT) on absolute brain weight. The control group (T) showed a mean brain weight of (1.703 ± 0.084). While the LCT group exhibited a slight decrease (1.6450 ± 0.051) and the URT group a marginal increase (1.7426±0.062), these changes were not statistically significant (ns). The LCT+URT combination group displayed a brain weight closer to control values (1.682 ± 0.041), but this difference also lacked significance.

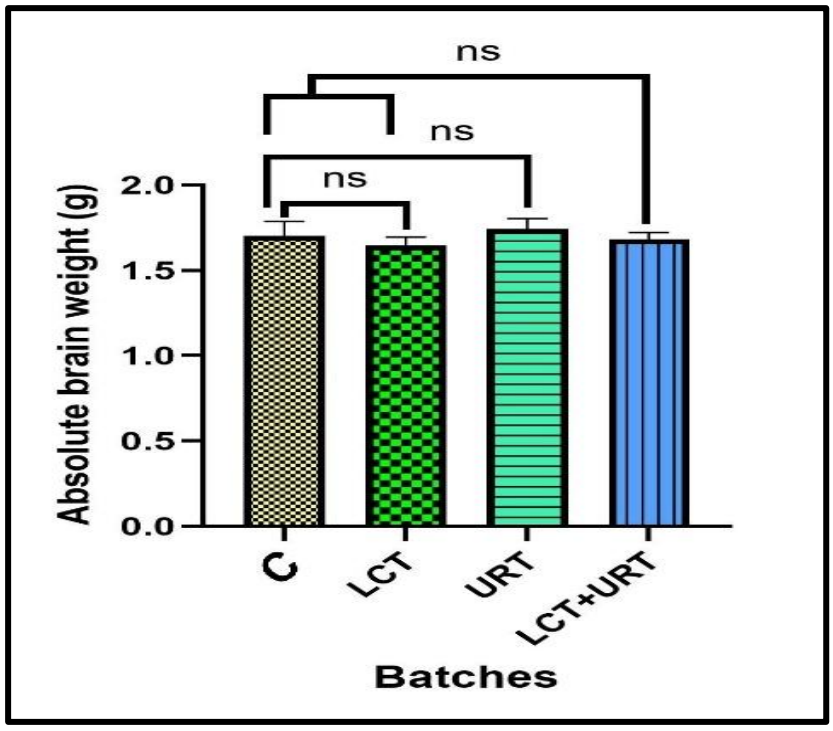


Figure 30: Variation of absolute brain weight in control and treated group

The study by **(Elbetieha *et al.*, 2001)** reported a similar result, indicating that exposure to synthetic pyrethroids such as LCT did not consistently lead to significant changes in brain weight, especially at lower doses. *Urtica dioica* has been acknowledged for its antioxidant and protective attributes. Research by **(Tahri *et al.*, 2000)** revealed that URT extract enhanced various physiological parameters in rodents. Additionally, **(Gülçin *et al.*, 2004)** established that URT contained substantial amounts of phenolic and flavonoid compounds, which may play a role in its protective effects. Consequently, the slight normalization of brain weight observed in the LCT+URT group implies that URT might have alleviated the potential neurotoxic effects associated with LCT.

II.2.2. Hematological parameters

II.2.2.1.Effect of Lambda-cyhalothrin and *Urtica dioica* on count blood cells (CBC)

The various treatments had a notable impact on multiple hematological parameters. The LCT group exhibited a significant rise in white blood cell (WBC) count when compared to the control group (T), whereas the URT group did not demonstrate any significant variation. The combination of LCT and URT resulted in a substantial increase in WBC count relative to both T and LCT. Regarding lymphocytes, LCT significantly raised levels in comparison to T, while URT showed no significant influence. Nevertheless, the LCT+URT combination significantly enhanced lymphocyte levels compared to both T and LCT. Monocyte levels were significantly elevated in the LCT group compared to T, with URT showing no significant effect. The combination of LCT and URT also significantly increased monocyte levels compared to both T and LCT. In terms of red blood cell (RBC) counts, there was a significant decrease in the LCT group compared to T, while URT led to a significant increase in RBC levels. The LCT+URT combination exhibited a significant reduction in RBC counts compared to both T and LCT. Hemoglobin (Hb) concentration significantly decreased in the LCT group, whereas URT significantly increased it. The LCT+URT group also showed a significant increase in Hb compared to LCT. Hematocrit (Hct) levels significantly decreased in the LCT group compared to T, remained stable in URT, and increased significantly in the combination group compared to LCT. Platelet (PLT) counts significantly decreased in the LCT group, remained unchanged in URT, and increased significantly in the LCT+URT group compared to both T and LCT

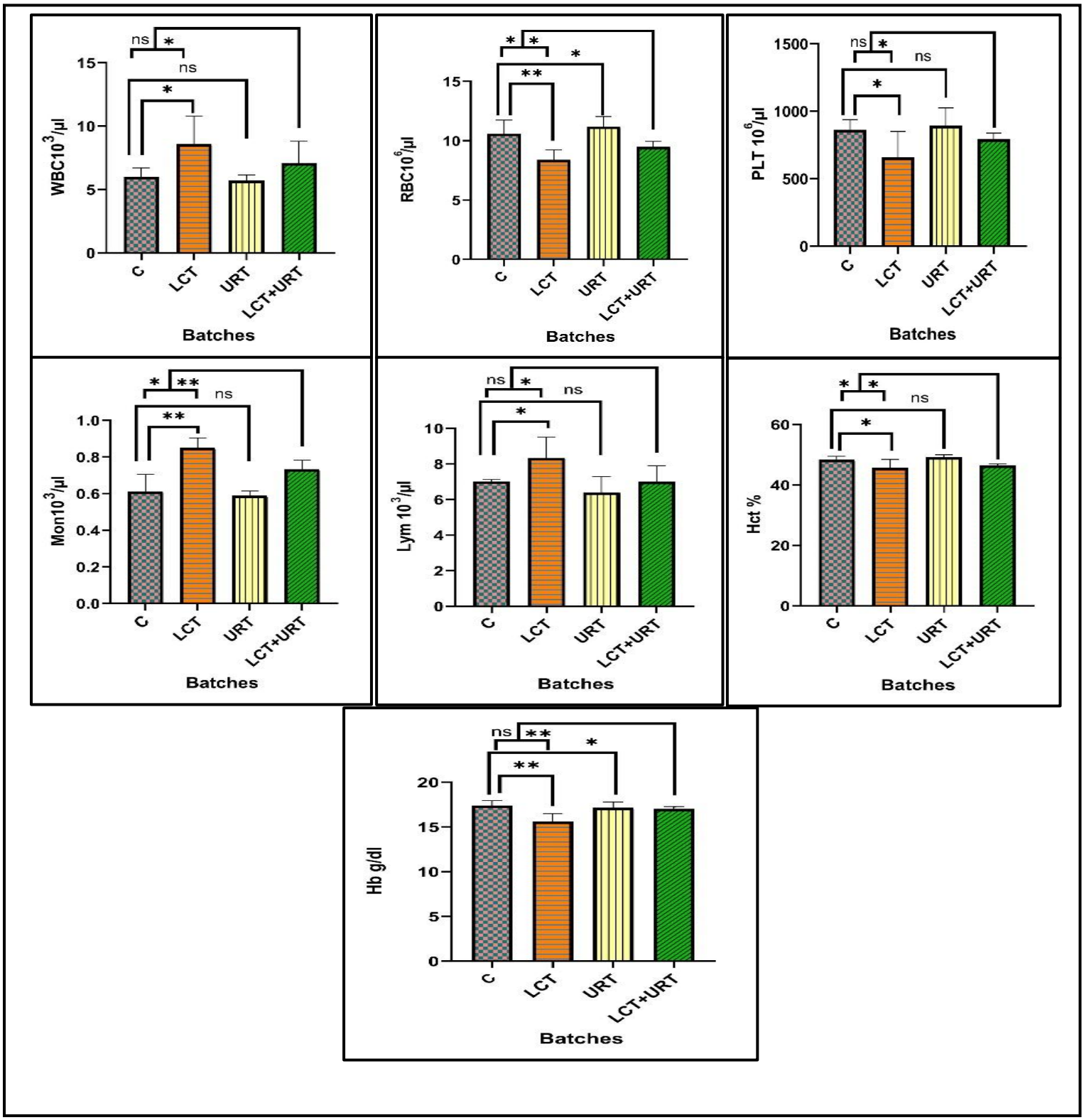


Figure 31: Variation of hematological parameters in control and treated groups

Exposure to LCT led to notable changes in hematological parameters**.** The administration of LCT resulted in a notable elevation of white blood cell, lymphocyte, and monocyte counts, reflecting an immune-enhancing effect, which is consistent with earlier research on pro-inflammatory agents **(Assar *et al*., 2023)**. Conversely, it induced a significant decrease in red

blood cell, hemoglobin, hematocrit, and platelet levels, indicating potential hematotoxicity **(Afolabi *et al*., 2019).** In contrast, URT did not produce significant changes in most hematological parameters but did enhance red blood cell and hemoglobin levels, suggests a potential role in stimulating red blood cell production **(Di zaye *et al*., 2013) .** Urtica dioica, has been investigated for its potential hematological effects, including its influence on blood cell counts and hematopoiesis (Golalipour & Khori, 2007). Several studies have explored Urtica dioica's impact on leukocytes, and platelets, as well as its broader effects on blood health and related conditions **(Zouari Bouassida *et al*., 2017).** The combination of LCT and URT resulted in an increase in immune cell counts and partially counteracted the declines in red blood cells, hemoglobin, and platelets, suggesting a compensatory or protective interaction **(Sorauf *et al*., 2014)**. In summary, URT seemed to alleviate some of the negative hematological impacts of LCT, reinforcing its potential as a protective adjunct treatment.

II.2.3. Biochemical parameters

II.2.3.1.Effect of Lambda-cyhalothrin and *Urtica dioica* on Glucose level

The blood glucose results revealed distinct variations among the groups. The control group maintained a baseline glucose level of (0,980 ±0,268). The LCT group showed a significant increase ($p<0.05$) compared to the control, indicating hyperglycemic effects. In contrast, the URT group displayed no significant difference from the control. Notably, the LCT+URT combination reduced glucose levels compared to LCT alone (b*, $p<0.05$), suggesting URT counteracted LCT hyperglycemic impact.

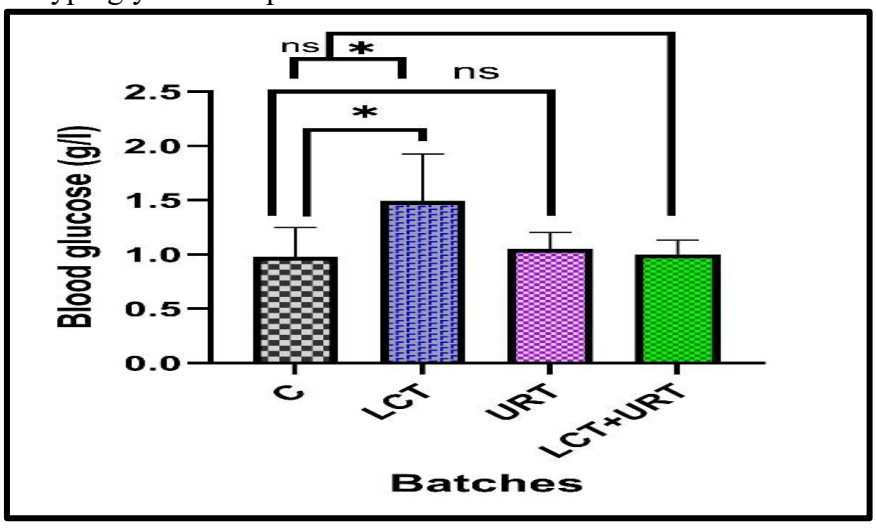


Figure 32: Variation in blood glucose level in the control and treated groups

In our results, LCT induced hyperglycemia, previous research indicates that LCT exposure can disrupt glucose homeostasis and cause related metabolic alterations **(Yang *et al*., 2022;Kalinovich *et al*., 2020)**, LCT exposure in mice leads to lipid accumulation in the liver, which is associated with AMPK inactivation **(Yang *et al*., 2022).** Disruption of hepatic lipid metabolism can indirectly affect glucose regulation.

Conversely, U. dioica, has been widely studied for its potential antihyperglycemic effects **(Omar & Mohammed, 2024**; **Jafar Gola *et al*., 2006)** . Traditional medicine has long utilized U. dioica for managing diabetes, and recent research aims to validate these uses and elucidate the underlying mechanisms **(Haouari *et al*., 2018;Mehri *et al*., 2011)**.

II.2.3.2. Effect of Lymbda-cyhalothrin and *Urtica dioica* on cerebral protein level

Our results shows that LCT significantly increased protein levels compared to control group, URT-treated group alone did not significantly alter protein levels, but its combination with LCT significantly decreased protein concentration compared to LCT-treated group.

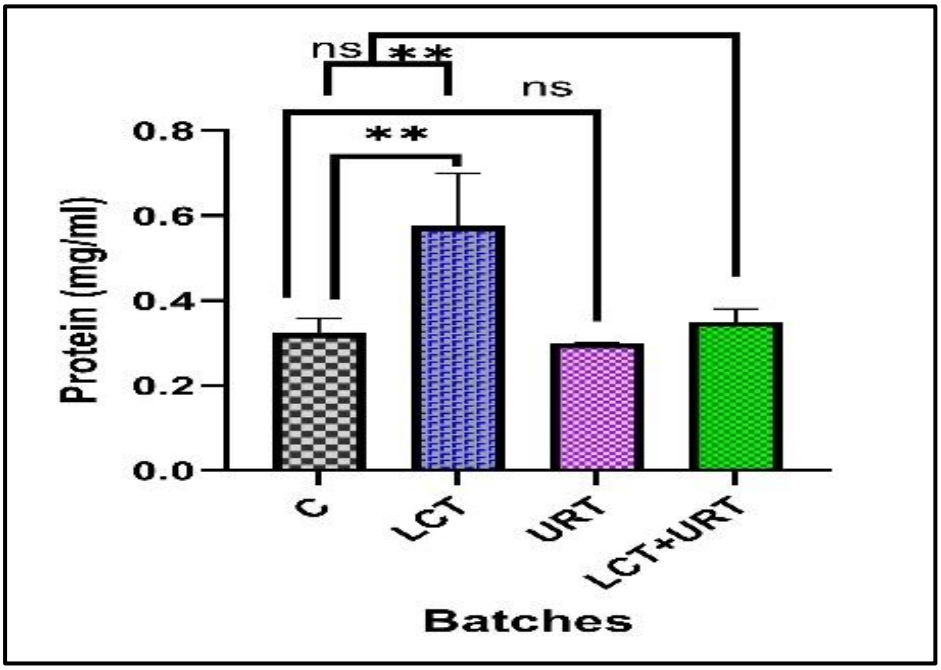


Figure 33: Variation of brain protein concentration in control and treated groups

Results show a significant increase in total protein concentration in the LCT-treated group, who may reflect oxidative damage leading to protein denaturation, aggregation, or upregulation of stress-related proteins. Similar increases in protein content under oxidative stress conditions have been documented and are often linked to cellular responses to toxic insults **(Türk, 2010).** Also, protein levels in the URT-treated group remained statistically comparable to controls,

suggesting an absence of stress-induced protein dysregulation. Most notably, co-treated LCT+URT group resulted from a significant attenuation of LCT-induced protein elevation was normalized relative to the LCT group. Previous Research suggests that *U. dioica* can impact oxidative stress, inflammation, and neuronal function, all of which are linked to protein expression and regulation in the brain **(Namazi *et al*., 2022).**

II.2.3.3. Effect of Lambda-cyhalothrin and *Urtica dioica* on cerebral antioxidant activities

Our results show that LCT significantly reduced both GST and GSH brain activities, indicating oxidative damage. URT alone significantly increased both GST and GSH. Beside, LCT+URT- treated group revealed an enhancement of GSH and GST compared to LCT-treated group.

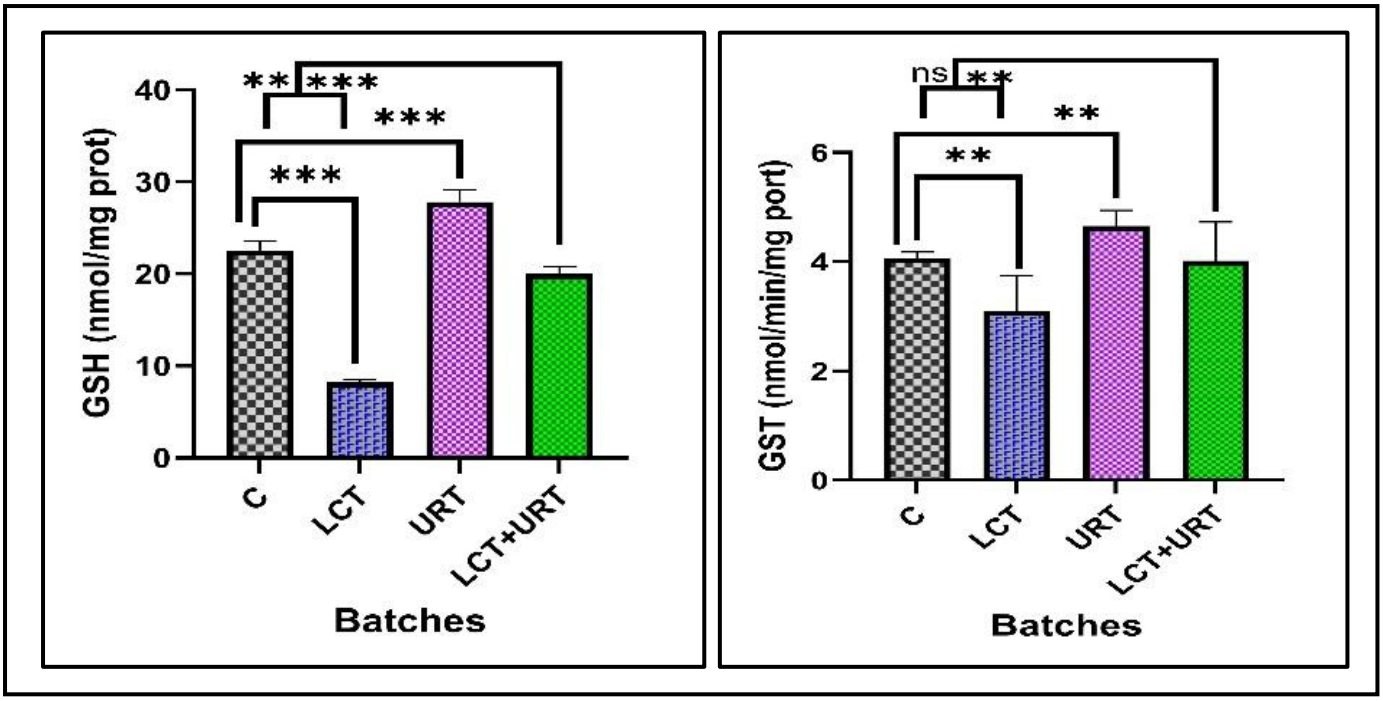


Figure 34: Variation of GST and GSH in the control and treated groups

GSH and GST are often used as biomarkers to evaluate the toxic effects of various environmental pollutants, including heavy metals and pesticides **(Zhang *et al*., 2021**; **Khalil *et al*., 2020)**. GST are a family of enzymes that catalyze the conjugation of GSH to a wide range of electrophilic compounds, including xenobiotics and products of oxidative stress **(Zhang *et al*., 2021**;**Pillai *et al*., 2024)**. This conjugation facilitates the detoxification and excretion of these harmful substances. GSH, a tripeptide, acts as a major intracellular antioxidant and plays a key role in protecting cells from oxidative damage by directly scavenging reactive oxygen species

(ROS) and serving as a substrate for GSTs and glutathione peroxidases (GPx). The current findings demonstrate that exposure to Lambda-cyhalothrin induced significant decrease in the brain glutathione and glutathione S-transferase activities. It has been reported that Lambda-cyhalothrin induces oxidative stress in various organisms, leading to the depletion of GSH and GST activities **(Xu *et al*., 2023**; **Houda *et al*., 2022**; **Fetoui *et al*., 2010)** . This oxidative stress is a primary mechanism of LCT toxicity **(Xu *et al*., 2023)**.Studies on *Helix aspersa* (snail) have shown that exposure to lambda-cyhalothrin leads to oxidative stress, evidenced by decreased levels of GSH and GST **(Houda *et al*., 2022).**

Interestingly, *Urtica dioica* (URT) alone enhanced antioxidant status, as shown in our study by increasing both GST and GSH levels, consistent with its known antioxidant and cytoprotective properties. URT contains a variety of bioactive compounds, such as flavonoids, vitamins C and E, and polyphenols, which have been shown to scavenge free radicals and upregulate endogenous antioxidant enzymes (**Gülçin *et al*., 2004 ; Mahmood *et al*., 2010).**

Most notably, co-treated LCT+URT group resulted a significant attenuation of LCT-induced effects. GST and GSH decrease were partially or fully restored. These findings support the protective role of *Urtica dioica* against pesticide-induced neurotoxicity, likely through its ability to bolster antioxidant defenses and maintain cellular homeostasis **(Ahmad *et al*., 2018).**

II.2.3. Behavioral study

II.2.3.1.Effect of Lambda-cyhalothrin and *Urtica dioica* on Forced Swimming Test parameters

FST revealed clear behavioral differences among the experimental groups. The LCT-treated rats showed a significant increase in immobility time and a decrease in both swimming and climbing time, indicating a strong depression-like behavior effect. In contrast, the URT-treated group displayed behavior comparable to controls, suggesting no adverse impact. Importantly, the URT+LCT-treated group showed a partial reversal of LCT-induced depression, with reduced immobility and improved swimming and climbing times compared to the LCT group. These findings suggest that URT co-administration attenuated the depressive-like behaviors induced by LCT, pointing to a potential protective or therapeutic effect.

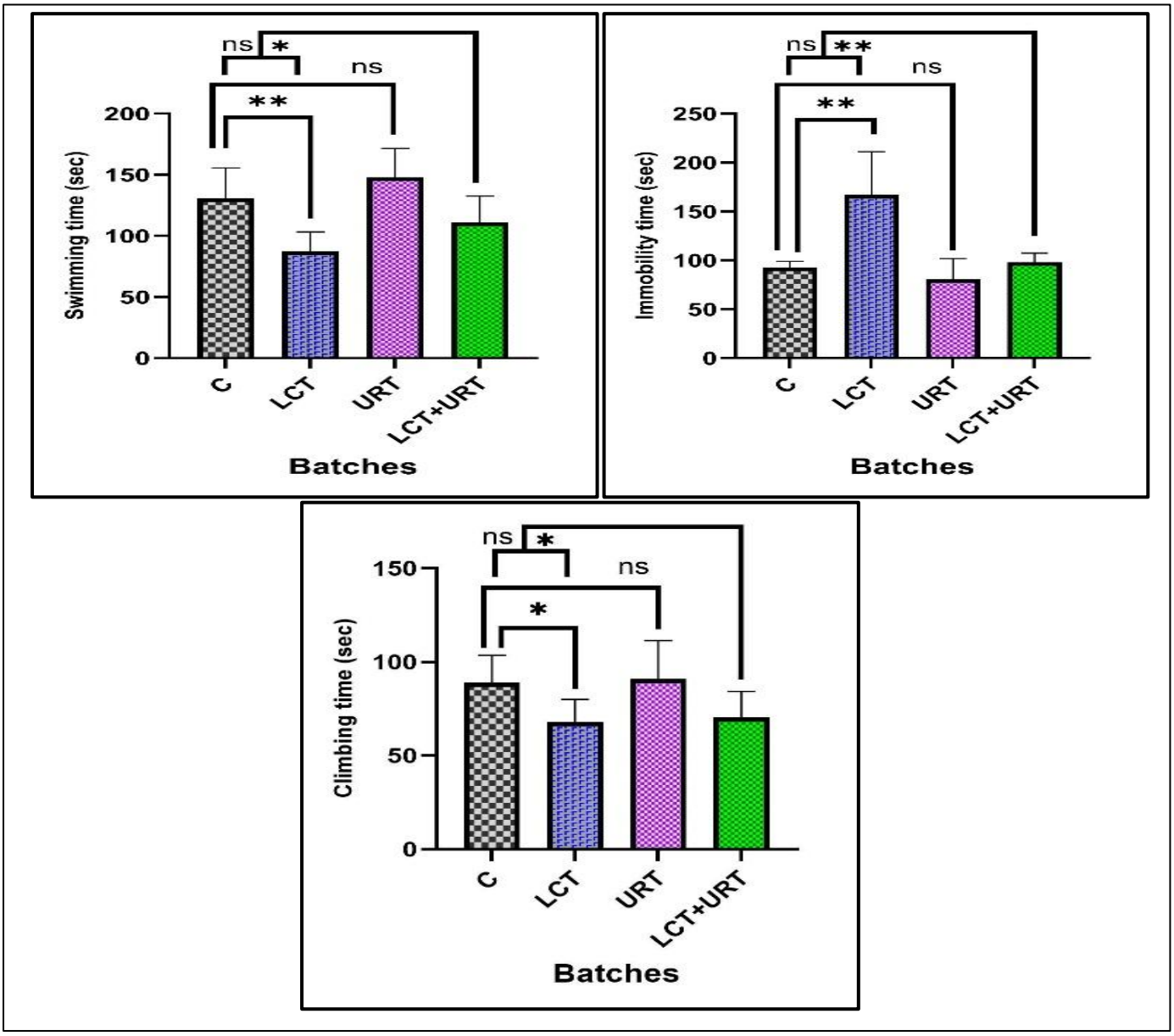


Figure 35: Variation of FST parameters in the control and treated groups

Depression is a state of low mood and avoidance of activity or reduction of biological function. Therefore, the assessment of behavioral activities has become a useful approach for the diagnosis of depression. It is one of the leading causes of morbidity in the growing world in men and women **(Takasaki *et al*., 2013).** Furthermore, the results of the forced swimming test showed that exposure to LCT causes a significant increase in the immobility time of animals. These results prove the depressant effect of this LCT. Previous studies **(Takasaki *et al*., 2013)** conducted on mice exposed to deltamethrin and permethrin showed a decrease in immobility time in rats exposed to deltamethrin (5 and 10 mg/kg) and not those exposed to

permethrin (10 mg/kg). An in vitro study carried out on rat cortical neuronal cells exposed to deltamethrin activates the gene expression of this factor (BDNF) 100 times more than in controls and this through signaling pathways involving calcium as a second messenger **(Meijer *et al*., 2014).**

Urtica dioica, also known as stinging nettle, has demonstrated antidepressant effects in several studies, particularly in the context of diabetes-related depression and general mood regulation **(Patel *et al*., 2018).** Additionally, research has investigated the plant's protective effects against various toxic substances, including those relevant to pesticide exposure **(Dhouibi *et al*., 2021**; **Albogami, 2023**; **Mahmoud *et al*., 2023)**. While direct studies on *U. dioica* counteracting pesticide-induced depression are limited, we can analyze its potential based on related findings. The antidepressive effect of U. dioica can be connected with a plethora of different molecular mechanisms, but the role of its components against inflammation and oxidative stress plays a fundamental role. Neuroprotective efficacy of Netelle can be achieved by exhibiting various mechanisms such as antioxidant activity, inhibition of inflammation and preventing accumulation of polyubiquitinated protein aggregates in brain and enhancing protective signaling. It has been demonstrated that U. dioica has provided neuroprotective activity via modulation of different inflammatory and biochemical markers (TNF-α, IL-1β, NF-kB, GSH, CAT, etc.) and highlights the significant potential in the management of neuroinflammation **(Semwal *et al*., 2023).**

II.2.3.2.Effect of Lambda-cyhalothrin and *Urtica dioica* on the Elevated Plus Maze Test

EPM results revealed distinct anxiety-related behavioral patterns across groups. The LCT group displayed heightened anxiety: it spent less time in open arms (5.26 ± 3.10 sec vs. Control's 52.30 ± 18.79 sec; $p<0.001$), avoided distal open arms (1.20 ± 0.45 sec vs. 16.72 ± 5.21 sec; $p<0.001$), and made fewer open-arm entries (2.00 ± 0.45 vs. 24.40 ± 1.22; $p<0.001$). The URT group showed no significant anxiety-related changes (e.g., open-arm time: 45.64 ± 18.15 sec, ns). However, the LCT+URT combination exacerbated anxiety: open-arm time dropped further (13.98 ± 8.84 sec, $p<0.01$ vs. Control; $p<0.001$ vs. LCT), and closed-arm time increased (215.92 ± 24.84 sec vs. LCT's 279.96 ± 14.81 sec; $p<0.001$*). URT failed to mitigate LCT's effects and worsened avoidance (e.g., distal closed-arm time rose to 152.64 ± 46.96 sec, **$p<0.01$* vs. LCT). Overall, LCT induced anxiety, URT remained neutral, but their combination reduced anxiety unexpectedly, necessitating further research.

Table 4: Variation of EPM parameters in control and treated rats

Parameter/Groups	C	LCT	URT	LCT+URT
Time spent in center (sec)	29,360 ± 9,267	14,900 ± 6,405 a*	27,34 ± 17,25 a^{ns}	24,62 ± 7,66 a^{ns}, b*
Time spent in open arms (sec)	52,30 ± 18,79	5,26 ± 3,10 a***	45,64 ±18,15 a^{ns}	13,98 ± 8,84 a**, b***
Time spent in distal open arms (sec)	16,720 ± 5,213	1,200 ± 0,447 a***	7,600 ± 1,817 a**	5,000 ± 2,345 a**, b***
Time spent in proximal open arms(sec)	36,54 ±16,24	3,66 ± 5,05 a***	30,68 ± 5,38NS a^{ns}	10,16 ± 2,33 a**, b***
Number of entries in open arms	24,400 ± 1,216	2,000 ± 0,449 a***	21,000 ± 1,581 a^{ns}	9,400 ± 1,140 a*, b***
Time spent in closed arms (sec)	199,18 ± 24,12	279,96 ± 14,81 a***	172,38 ± 18,02 ns	215,92 ± 24,84 a^{ns}, b***
Time spent in proximal closed arms(sec)	112,76 ± 29,10	54,22 ± 19,60 a**	98,84 ± 18,37 a^{ns}	65,04 ± 29,93 a*, b^{ns}
Time spent in distal closed arms (sec)	96,38 ± 21,38	225,52 ± 26,05 a***	68,46 ± 15,47 a*	152,64 ± 46,96 a*, b**
Number of entries in closed arms	28,60 ± 12,542	7,800 ± 4,438 a***	30,80 ± 11,12 a_{ns}	17,80 ± 10,21 a^{ns}, b*

(*p < 0.05, ** p< 0.01 , *** p< 0.001, ns: non significante (a vs C; b vs LCT, n=5)

The Elevated Plus Maze test revealed that Lambda-cyhalothrin induced anxiety-like behavior in rats. LCT-treated mice spent significantly less time in open arms, in their distal open arms, and displayed fewer open-arm entries compared to controls, indicating a clear anxiogenic effect. These results aligned with **(Wolansky & Harrill, 2008),** who reported that pyrethroid insecticides like LCT disrupted neurotransmitter balance and increased oxidative stress, exacerbating anxiety in rodents. Conversely, *U. dioica* alone showed no significant differences from the control group in open-arm exploration, suggesting a neutral or mild anxiolytic effect.

This supported earlier studies by **(Farzami *et al*., 2003)** , which proposed that URT's neuroprotective properties might stem from GABAergic modulation or antioxidant activity. Beside, the LCT+URT-treated group reduced anxiety-related behaviors induced by LCT. Previous findings on mice has demonstrated that *U. dioica* extracts can reduce anxiety-like behavior. For example, one study using a mice model found that methanolic extracts of *Urtica urens*, a related species, exhibited anxiolytic activity in hole board and light-dark box tests **(Doukkali *et al*., 2015)**. The efficacy of the plant extract (100–400 mg/kg) was comparable to that of diazepam (1 mg/kg), a standard anxiolytic drug **(Doukkali *et al*., 2015)**.

II.2.3.3. Effect of Lambda-cyhalothrin and *Urtica dioica* on the Parameters of the open field (OF) Test

The open field test revealed significant behavioral changes across the groups. Rats treated with LCT traveled a significantly shorter distance than controls ($p<0.01$), while URT- treatment significantly increased locomotion. However, rats in the URT+LCT group covered less distance than those treated with URT alone, suggesting a possible interaction effect. Immobilization time increased significantly in the LCT group, while URT alone had no effect. The URT+LCT-treated group showed greater immobility than the URT group.

LCT-treated rats spent more time in the periphery and significantly less time in the center compared to controls. URT treatment increased time spent in the center, but this effect was reduced in the URT+LCT group. Similarly, center entries decreased with LCT, increased with URT, and then declined again in the URT+LCT group.

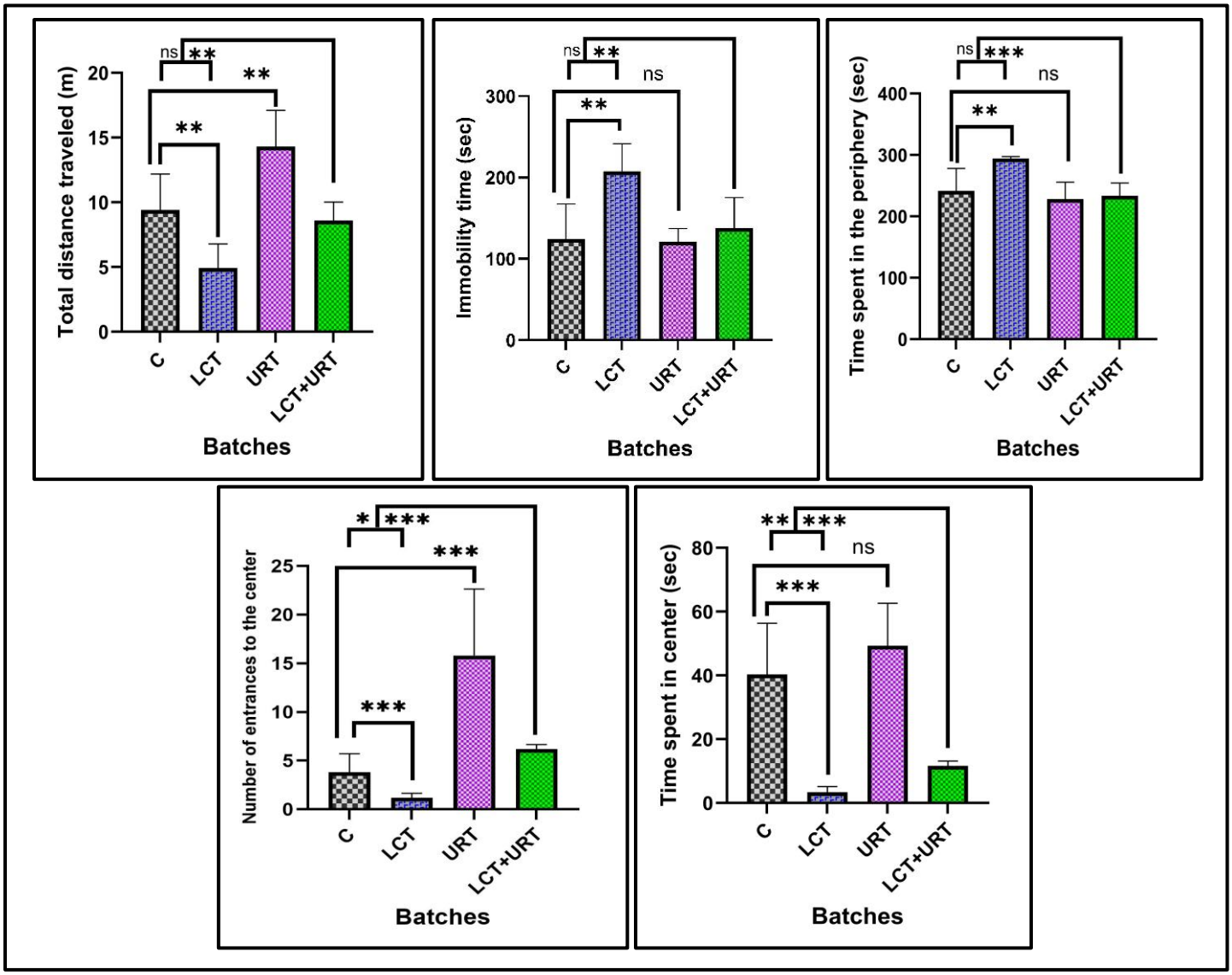


Figure 28: Variation in OF parameters in control and treated rats

The open field test assesses locomotor activity, anxiety-like behavior, and exploratory behavior in a novel environment **(Choleris, 2001)**. locomotors activity such as total travelled distance; immobility time and exploring parameter like number of entries and time spent in center, time spent in periphery were assessed using the Open Field Test (OF). Although, in our study, the administration of LCT showed reduce locomotor and exploratory activity of rats. Our results were agree with funding of **(Rao *et al*., 2024),** who mentioned that exposure to LCT can induce a range of behavioral alterations, reflecting neurotoxic effects and potential impacts on the nervous system.

The administration of *U. dioica* to LCT-treated group significantly modulated locomotor and exploratory disorders induced by LCT, suggesting an excitatory effect. Its due to the presence of neuroactive phytochemicals in nettle, such as acetylcholine and serotonin, could modulate

neurotransmitter systems involved in motor control **(Izunwanne *et al*., 2024)**, Serotonin, for example, plays a crucial role in modulating locomotor activity, and its interaction with specific receptors in the central nervous system can influence movement patterns **(Stewart *et al*., 2019).** Our results agree with the study of **(Babiec *et al*., 2024).**

Conclusion and perspective

Conclusion and perspectives

This research was conducted to investigate the neuroprotective capabilities of *Urtica dioica*, a plant recognized for its medicinal and antioxidant attributes, against the neurotoxic effects caused by lambda-cyhalothrin (LCT), a widely utilized pyrethroid pesticide. The experimental design encompassed behavioral assessments, biochemical and hematological evaluations, along with an ethno-botanical survey, there by offering a comprehensive perspective on both traditional knowledge and scientific substantiation.

The results obtained indicated that exposure to LCT significantly modified various physiological, behavioral, and hemato-biochemical parameters in rats. These changes included weight reduction, depression and anxiety-like behaviors, hyperglycemia and reduced GSH and GST activities.

Conversely, the administration of *Urtica dioica* demonstrated a significant capacity to alleviate damages induced by LCT. The plant extract enhanced levels of antioxidant enzymes, reduce anxiety and depression degree's, rectified glycemic, and promoted hematological equilibrium. The concurrent administration of *U. dioica* with LCT indicated a synergistic or modulatory effect that contributed to mitigating the neurotoxic consequences of the pesticide.

These findings imply that *Nettle* presents potential as a neuroprotective agent, likely attributable to its abundant phytochemical composition, which encompasses flavonoids, phenolic and formic acid knows for their antioxidant and anti-inflammatory properties.

To build on these findings, future studies should:

- ✓ Identify active compounds in *U. dioica* using advanced chromatographic techniques (HPLC, GC-MS).
- ✓ Explore its molecular mechanisms via gene/protein expression related to neuroprotection.
- ✓ Confirm antioxidant potential with DPPH or FRAP assays.
- ✓ Use in silico tools to predict molecular interactions.
- ✓ Assess long-term effects and develop potential therapeutic formulations.

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Webographic

1-https://www.lienhorticole.fr/2012/article/782268/pesticides-horticoles-l-eau-l-air-et-les-sols-impactes (11-02- 2025)

2-https://query.libretexts.org/Francais/Sciences_de_l%27environnement_%28Ha_et_Schleiger%29/04%3A_Les_humains_et_l%27environnement/4.04%3A_Sant%C3%A9_environnementale/4.4.04%3A_Toxicologie_environnementale (11-02- 2025)

3-https://www.researchgate.net/figure/Evolution-structurale-des-pyrethrinoides-de-synthese_fig1_291147739 (12-02- 2025)

4-https://www.researchgate.net/figure/Chemical-structure-of-lambda-cyhalothrin_fig1_282161320 (12-02- 2025)

Annexes

Annexes

Questions of the ethnobotanical survey *Urtica dioica* (القراص)

Q1. Do you know *Urtica dioica*?

Yes ☐ No ☐

Q2. Do you consume *Urtica dioica*?

Yes ☐ No ☐

Q3. If not, does anyone in your surroundings consume it?

Yes ☐ No ☐

Q4. If yes, how do you use it?

Tea ☐ In powder ☐

Personal Information:

Gender: Female ☐ Male ☐

Age: <18 ☐ 18-35 ☐ 35-55 ☐ 55-75 ☐ >75 ☐

Marital Status:

Single ☐ Married (e) ☐ Widowed (e) ☐ Divorced (e) ☐

Education Level:

Illiterate ☐ Primary ☐ Secondary ☐ High School ☐

University ☐

Profession:

Unemployed ☐ Private activity (Merchant, Farmer…) ☐ Employee ☐

Retired Housewife ☐

Other, specify: .....

Chronic Illnesses: Yes ☐ No ☐

If yes: Diabetes ☐ HTA ☐ Cholesterol ☐ Other ☐

Q7. What is it used for?

Therapeutic use. ☐ Culinary use. ☐

Other, specify: .....

Q9. What is the amount you take?

One dose per day ☐ Two doses per day ☐ Three doses per day. ☐

Q10. How do you rate the effectiveness of the plant?

Excellent ☐ Good ☐ Average ☐ None ☐

Q11. Does the plant have any side effects?

Yes. ☐ No. ☐ Don't know. ☐

If yes, please specify ……

Q12. Do you mix the plant with other plants?

Yes ☐ No. ☐

If yes, please specify ……

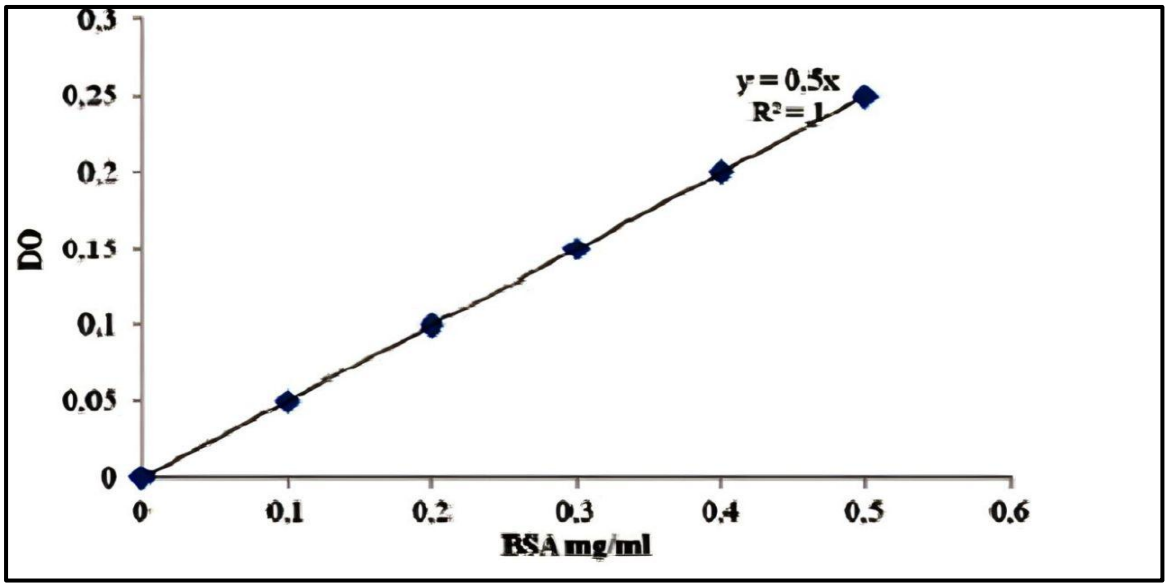


Figure : Calibration curve for bovine serum albumin