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Protective effect of *Gymnema sylvestre* leave against neurotoxicity of lambda-cyhalothrin in experimental rats

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Abdelmoutaleb

Dedication

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EL Mountassar Billah



Abstract

The demographic explosion of the world population and its food needs are the main reasons that have led to a dramatic increase in the production and use of pesticides that can cause the appearance of several diseases. Currently, research is focused on medicinal plants considered as a potential source of multiple phytotherapeutic substances endowed with antioxidant activities. *Gymnema sylvestre (GYM)*, commonly known as Gurmar, is famous for containing bioactive compounds which made it eligible for use as a medicinal plant. Therefore, the purpose of this study was to investigate the potential of *G. sylvestre* leaf extract against the toxic effect of Lambda-cyhalothrin (LCT) a synthetic pyrethroid pesticide in rat.

An ethnobotanical study was conducted to assess the recognition and the use of this plant in our region, and an in vivo study was carried out on twenty female rats Wistar, which were divided into four groups and treated orally for 30 days: control group; LCT treated-group (6.3 mg/kg); GYM-treated group (400 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, brain was carefully removed and preserved for the antioxidant activities.

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

In conclusion, *Gymnema sylvestre* exhibits an anxiolytic, antidepressant, and antioxidant effects against LCT-induced brain damage and hemato-biochemical disturbance in rat.

Keywords: *Gymnema sylvestre*, Lambda-cyhalothrin, Neurotoxicity, Behavior, Antioxidant Ezymes.

Résumé

L'explosion démographique de la population mondiale et ses besoins alimentaires et sanitaires sont les principales raisons ayant conduit à une augmentation spectaculaire de la production et l'utilisation des pesticides susceptibles d'engendrer l'apparition de plusieurs maladies. Actuellement, les recherches s'orientent vers les plantes médicinales considérées comme source potentielle de multiples substances phytothérapiques douées d'activités antioxydantes. Le *Gymnema sylvestre* (GYM), communément appelé Gurmar, est réputé pour contenir des composés bioactifs qui le rendent éligible à une utilisation comme plante médicinale. Par conséquent, l'objectif de cette étude était d'évaluer le potentiel d'un extrait de feuille de *G. sylvestre* contre l'effet toxique de la lambda-cyhalothrin (LCT), un pesticide pyréthroïde synthétique, chez le rat.

Une étude ethnobotanique a été menée pour estimer la reconnaissance et l'utilisation de cette plante dans notre région, et une étude in vivo a été réaliser sur vingt rates femelles de la souche Wistar, divisées en quatre groupes et traitées par voie orale pendant 30 jours : groupe témoin ; groupe traité par LCT (6,3 mg/kg) ; groupe traité par GYM (400 mg/kg) et groupe de co-traitement. À la fin de l'expérience, nous avons réalisé une batterie de tests comportementaux (FST, EPM, OF). Les rats ont été décapités et leur sang a été prélevé pour des analyses hématologiques et biochimiques (glycémie) et leur cerveau a été soigneusement prélevé et conservé pour l'étude des paramètres du stress oxydatif.

Les résultats montrent que la LCT a induit une perte de poids, des troubles hématologiques (leucocytose, lymphocytose, monocytose, thrombocytopénie et anémie), une hyperglycémie, une augmentation des taux de protéines cérébrales et une diminution de l'activité des enzymes antioxydantes (GSH, GST), ainsi que des troubles comportementaux (diminution de la locomotion et l'exploration, augmentation des niveaux d'anxiété et de dépression). En revanche, l'administration de *G. sylvestre* a significativement atténué ces altérations : amélioration de l'activité des marqueurs antioxydants cérébraux, stabilisation des paramètres hématologiques, normalisation partielle du comportement et réduction de l'hyperglycémie.

En conclusion, *Gymnema sylvestre* présente un effet anxiolytique, antidépresseur et antioxydant contre les dommages cérébrales et les troubles hémato-biochimiques induits par le LCT chez le rat.

Mots-clés: *Gymnema sylvestre*, Lambda-cyhalothrine, Neurotoxicité, Comportement, Enzymes Antioxydants.

الملخص

يُعدّ الانفجار السكاني العالمي واحتياجاته الغذائية من الأسباب الرئيسية التي أدت إلى زيادة هائلة في إنتاج واستخدام المبيدات الحشرية التي قد تُسبب ظهور أمراض متعددة. تُجرى حاليًا أبحاث موجهة نحو النباتات الطبية التي تُعتبر مصدرًا محتملًا للعديد من المواد العلاجية النباتية ذات النشاط المضاد للأكسدة. يُعرف نبات الجيمنيما (GYM)، المعروف باسم الجورمار، باحتوائه على مركبات نشطة بيولوجيًا تجعله مؤهلًا للاستخدام كنبات طبي. لذلك، كان الهدف من هذه الدراسة تقييم فعالية مستخلص أوراق الجيمنيما في مواجهة التأثير السام لمبيد لامبدا-سيهالوثرين (LCT)، وهو مبيد بيريثرويدي اصطناعي 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 بـــ المبيد الحشري (6.3 مجم / كجم)؛ مجموعة عولجت بـــالجمنيما (400 ملغ/كغ) ومجموعة أخرى عولجت بالعلاج المشترك. في نهاية التجربة، أجرينا مجموعة من الاختبارات السلوكية ، وقتلت الفئران وجُمعت دماؤها للتحليل الدموي والكيميائي الحيوي (سكر الدم). بعد ذلك، قُطعت رؤوس الفئران، وأُزيلت أدمغتها بعناية وحُفظت لدراسة معايير الإجهاد التأكسدي.

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الكلمات المفتاحية: جيمنيما ، لامدا سيهالوثرين، السمية العصبية، السلوك، انزيمات مضادة للاكسدة.

List of Abbreviations

- **ABTS**: 2,2’-Azinobis(3-ethylbenzthiazoline-6-sulfonic acid)
- **ANOVA**: Analysis of Variance
- **B. pumilis**: Bacillus pumilis
- **B. subtilis**: Bacillus subtilis
- **BBC**: Coomassie Brilliant Blue
- **BMI**: Body Mass Index
- **BSA**: Bovine Serum Albumin
- **CBC**: Complete Blood Count
- **CDNB**: 1-Chloro-2,4-Dinitrobenzene
- **DDT**: Dichlorodiphenyltrichloroethane
- **DNT**: 2,4-dinitrothiazole
- **DPPH**: 2,2-diphenyl-1-picrylhydrazyl
- **DTNB**: 5,5'-Dithiobis-2-nitrobenzoic Acid
- **E. coli**: Escherichia coli
- **EDTA**: Ethylenediaminetetraacetic Acid
- **EPA**: Environmental Protection Agency
- **EPM**: Elevated Plus Maze
- **FST**: Forced Swimming Test
- ***G. sylvestre***: *Gymnema sylvestre*
- **GC-MS**: Gas Chromatography-Mass Spectrometry
- **GSH**: Glutathione
- **GST**: Glutathione-S-Transferase
- **GYM**: *Gymnema sylvestre*

- **Hb**: Hemoglobin
- **HPLC**: High-Performance Liquid Chromatography
- **IL-1b**: Interleukin 1 Beta
- **LC-MS**: Liquid Chromatography-Mass Spectrometry
- **LCT**: Lambda-Cyhalothrin
- **NaCl**: Sodium Chloride
- **NTE**: Neuropathy Target Esterase
- **OF**: Open Field
- **P. aeruginosa**: Pseudomonas aeruginosa
- **P. vulgaris**: Proteus vulgaris
- **PBS**: Phosphate Buffered Saline
- **PLT**: Platelets
- **RBC**: Red Blood Cells
- **ROS**: Reactive Oxygen Species
- **S. aureus**: Staphylococcus aureus
- **S. paratyphi**: Salmonella paratyphi
- **S. typhi**: Salmonella typhi
- **S. typhimurium**: Salmonella typhimurium
- **SD**: Standard Deviation
- **TBA**: Thiobarbituric Acid
- **TCA**: Trichloroacetic Acid
- **Tukey's Test**: Statistical Test for Comparing Means
- **WBC**: White Blood Cells

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Introduction

Pesticides are substances employed to prevent, eliminate, repel, or mitigate pests, targeting insects, rodents, weeds, and a variety of other undesirable organisms. Ideally, the harmful action of pesticides would be highly specific to unwanted species; however, most pesticides lack selectivity and are generally toxic to numerous non-target species, including humans. The adverse effects of pesticides on human health encompass a range of areas; some compounds may only cause mild skin irritations, while others can impact liver or lung functions. Certain pesticides are carcinogenic, while others may induce reproductive toxicity or possess endocrine-disrupting properties. Additionally, many pesticides exhibit neurotoxic effects. Insecticides, particularly those that kill insects by disrupting their nervous systems, also produce neurotoxic effects in humans, such as those seen with carbamate insecticides **(Costa et al, 2008).**

Exposure to pesticides can occur in two primary ways: directly through their production or professional and domestic use, and indirectly via air, contact with contaminated surfaces or through the consumption of water and food products. Depending on the context, either specific populations or the general public may be affected by occupational exposure, with these substances entering the body through three main routes: dermal, digestive (or oral), and respiratory. Typically, the most significant route is the oral pathway, which results from the ingestion of food and non-food items (such as dust) **(Baldi et *al*., 2013)**.

Pyrethroids are insecticides that are synthetic analogs of pyrethrins, which are natural compounds derived from chrysanthemum flowers. They exhibit high solubility in fats and are chemically unstable, categorized into two classes: type I and type II pyrethroids. Lambda cyhalothrin, a type II pyrethroid insecticide, is the most widely used globally and is known to induce the formation of free radicals and disrupt antioxidant defense systems **(Anadon et al., 2006).**

On the other hand, use of natural phyto-compounds is beneficial for protection against the radiation damage, as they are less toxic compared to the synthetic compounds at their optimum protective dose levels **(Bhatia et al., 2008).** *Gymnema sylvestre* is a conventionally used medicinal plant described as a medication for diabetes mellitus, diuretic problems, stomachic, used in ayurvedic, and homeopathic systems of medicine **(Bera et al., 2015).** Gymnemagenin is the simplest form of Gymnemic acid, which is the major active compounds of *G. sylvestre*, which helps in detoxification of drugs and toxic-compounds.

In light of these findings, we have chosen to adopt an experimental approach focused on investigating the effects of *Gymnema sylvestre* on the brain toxicity and neurobehavioral

disorders induced by Lambda-cyhalothrin in Wistar rats. To achieve this objective, this thesis is structured into two main sections :

The first section provides essential background information necessary for understanding the experimental work, while the second section details the materials utilized, the methodology employed, and presents the results along with their discussion.

PART ONE:

BIBLIOGRAPHIC SYNTHESIS

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 **(Zhang et al., 2011 ; Davis et al., 2017).**

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 01: Some chemical families of pesticides and their main targets **(INSERM. 2013)**

Chemical Families	Examples of Active Substances	Classification by Target
Organochlorines	DDT, Chlordane, Lindane, Dieldrin, Heptachlor	Insecticides
Organophosphates	Malathion, Parathion, Chlorpyrifos, Diazinon	Insecticides
Pyrethroids	Permethrin, Deltamethrin, Lambda-cyhalothrin	Insecticides
Carbamates	Aldicarb, Carbaryl, Carbofuran, Methomyl	Insecticides
	Asulam, Diallate, Terbucarb, Triallate	Herbicides
Dithiocarbamates	Benthiavalicarb	Fungicides
	Mancozeb, Maneb, Thiram, Zineb	Fungicides
Phthalimides	Folpet, Captan, Captafol	Fungicides
Triazines	Atrazine, Simazine, Terbutylazine	Herbicides
Phenoxy herbicides	MCPA, 2,4-D, 2,4,5-T	Herbicides
Chloroacetamides	Alachlor, Metolachlor	Herbicides
Pyridines–bipyridyliums	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 **(Mossa & Mohafrash, 2023).**

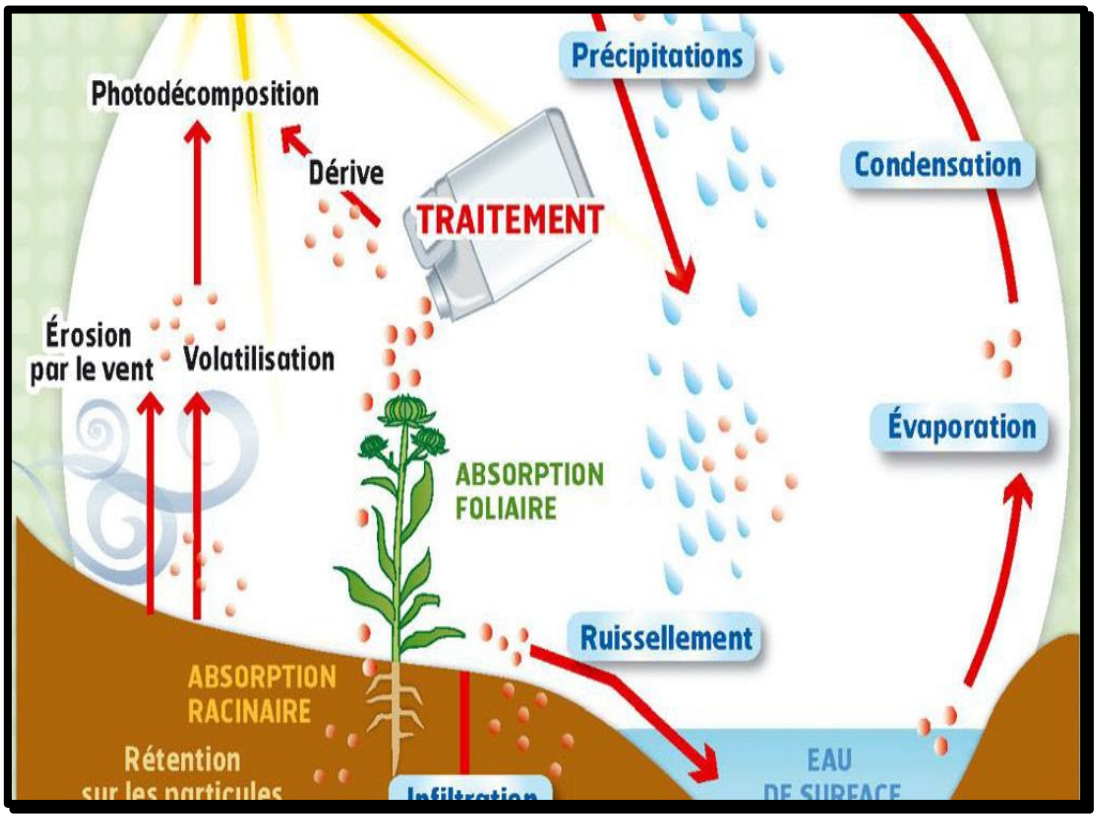


Figure 1: Different modes of environmental contamination by pesticides **(web 1)**

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).**

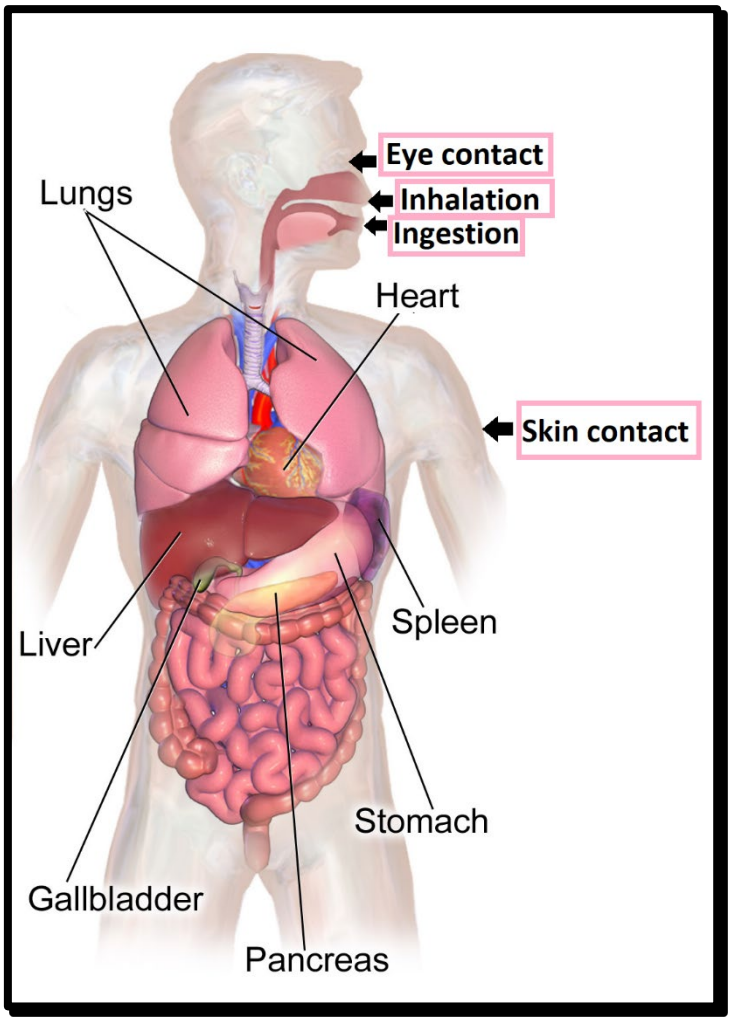


Figure 2: The main routes of exposure to pesticides **(web 2)**

I.5 Synthetic pyrethroids

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).**

I.6 Lambda-cyhalothrin: new generation of synthetic pyrthroids

I.6.1 Definition

Lambda-cyhalothrin (LCT) is a second-generation pyrethroid**,** his chemical is designed to combat harmful animal and plant pests that threaten crops, functioning either by killing or repelling these pests. Consequently, it is considered toxic and poses risks to both human health and the environment. It is essential to adhere to the recommended dosages and the specific applications for which the product is approved **(Hamadi et *al* ., 2008)**

I.6.2 Chemical Classification and Structure

Lambda-cyhalothrin is composed of a 1:1 blend of two stereoisomers: (S)-α-cyano-3-phenoxybenzyl-(Z)-(1R,3R)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl) 2,2dimethylcyclopropanecarboxylate and (R)-α-cyano-3-phenoxybenzyl-(Z)-(1S,3S)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate. The compound was initially identified by **(Robson and Crosby in 1984)** and subsequently introduced to Central America and the Far East in 1985 by ICI Agrochemicals, which is now known as Syngenta.

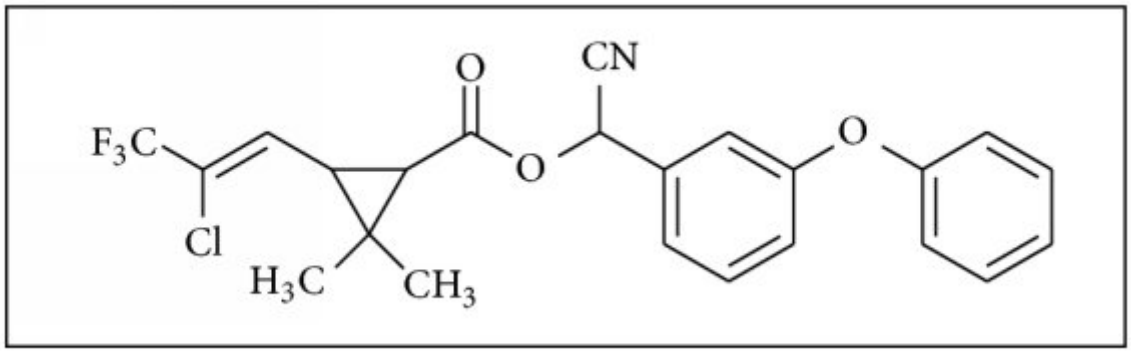


Figure 3: Chemical structure of lambda-cyhalothrin **(web 3)**

I.5.3 Chemical Classification

Table 02: Physico-Chemical Properties of Lambda-Cyhalothrin **(INERIS, 2011)**

Parameters	**Values**
Molecular formula	$C_{23}H_{19}ClF_3NO_3$
Chemical family	Type II synthetic pyrethroids
Molecular weight [g/mol]	449.85 ± 0.023
Water solubility [mg/L]	4.10^{-3} at 20°C and pH 5 5.10^{-3} at 20°C and pH 6.5 4.10^{-3} at 20°C and pH 9.2
Vapor pressure [Pa]	2.10^{-7} at 20°C
Henry's law constant [Pa·m³/mol]	0.02 at 20°C
Log Octanol-Water Partition Coefficient (log Kow)	7
Organic carbon adsorption coefficient (Koc) [L/kg]	157,000 (average value) Range: 38,000–345,000

I.5.4 Commercial and Agricultural Uses

Lambda-cyhalothrin (LCT) is a synthetic pyrethroid that is employed in various applications such as pest control in residential settings, agriculture, food protection, disease management, and the control of pathogenic vectors. It has been extensively utilized to combat a range of insects, including aphids, Colorado potato beetles, and larval stages of lepidopterans **(Fetoui et al., 2010; Al-Amoudi, 2018).**

In the context of animals, pests pose significant threats to agriculture, public health, residential areas, and gardens. Within agricultural settings, target crops include cotton, cereals, hops, ornamental plants, potatoes, and various vegetables, with pest control efforts aimed at managing aphids, beetles, and lepidopteran pests. Pyrethroids serve as crucial tools in public health management, where they are employed to control cockroaches, mosquitoes, ticks, and flies, all of which can act as disease vectors. The residential use of pyrethrin products has seen an increase, particularly following the ban on organophosphate products containing chlorpyrifos or diazinon, which are used against flies, lice, mosquitoes, and ticks **(He et al., 2008).**

I.7 Toxicokinetic and Toxicodynamic of lambda-cyhalothrin

I.7.1 Toxicokinetic

A. **Absorption**

The primary routes of absorption for pyrethroid pesticides include the digestive, pulmonary, and dermal pathways. Evidence suggests that pyrethroids are rapidly absorbed through inhalation, as indicated by the detection of urinary metabolites within 30 minutes post-exposure **(Leng et al., 1997).** Literature regarding agricultural workers' exposure to pyrethroids indicates that absorption may occur during handling or while performing treatment operations in the fields **(Leng et al., 1996).** An assessment of absorption among these agricultural workers handling lambda-cyhalothrin has been conducted **(Naravaneni & Jamil, 2005).**

B. Distribution

Lambda-cyhalothrin is distributed throughout various tissues. Average elimination half-lives of approximately 35 and 18 hours have been observed in the hypothalamus, in contrast to 13 and 12 hours in the liver, following oral and intravenous exposure, respectively. Given this distribution pattern, it has been established that the highest concentration of lambda-cyhalothrin is found in the hypothalamus **(Anadon, 2006).**

C. Metabolism

Biotransformation, also known as metabolism, pertains to the chemical and biochemical alterations of xenobiotics. This process serves as a defense mechanism that enables the organism to prevent the accumulation of foreign substances, particularly lipophilic compounds, which could disrupt natural biological functions. The metabolic reactions associated with pyrethroid insecticides, as outlined in various studies, primarily involve oxidation of alcohol and acid fractions, hydrolysis of ester bonds, and conjugation reactions. Pyrethroids are rapidly degraded into carboxylic acids and hydrolysable metabolites, which are subsequently excreted in urine **(Khemiri, 2017).**

In the case of lambda-cyhalothrin, this compound is cleaved to yield three main metabolites: 3-phenoxybenzoic acid (3-PBA), 3-(4-hydroxy)-phenoxybenzoic acid (4OH-3PBA), and 3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropane carboxylic acid (CFMP) **(Kaneko et Miyamoto, 2001).**

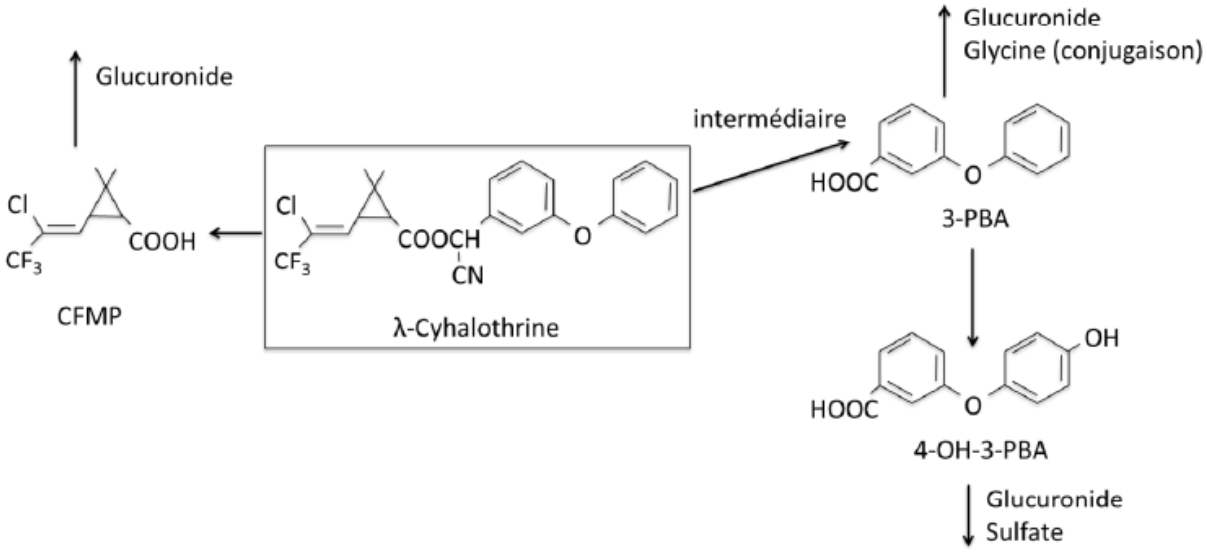


Figure 4: Metabolism of Lambda-cyhalothrin in mammals (**Khemiri, 2019)**

D. **Elimination**

This phenomenon pertains to the removal of compounds (parent molecules and/or metabolites) from the body. Elimination pathways may include renal, fecal, pulmonary routes, or occur through sweat or breast milk. Nevertheless, the kidneys serve as the primary organ for elimination. Similar to metabolism, the renal clearance of the compound is assessed, which facilitates the correlation between its concentrations in 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., 2017).** A study conducted on rats indicated 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).**
The urinary metabolites of lambda-cyhalothrin that have already been identified and can be utilized for biological monitoring of exposure include 3-phenoxybenzoic acid (3-PBA) and cis-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropane carboxylic acid (CFMP).

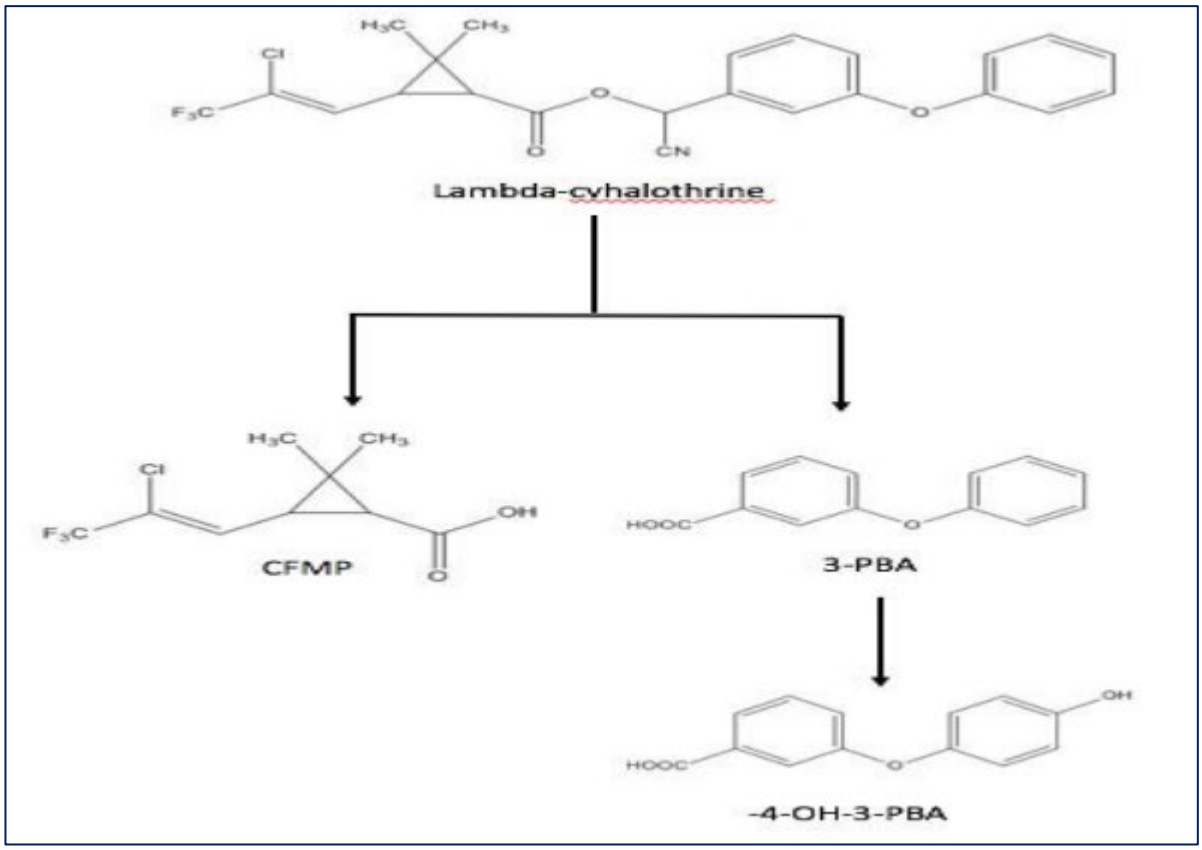


Figure 5: Structure of lambda-cyhalothrin and its metabolites **(Khemiri, 2017)**

I.7.2 Toxicodynamic

Lambda-cyhalothrin acts as an axonic poison, targeting both the peripheral and central nervous systems of insects **(Li-Ming He et al., 2008)**. Its primary site of action is the voltage-gated sodium channels located in the membranes of nerve cells, which are a key target of pyrethroids **(Li-Ming He et al., 2008)**. Specifically, lambda-cyhalothrin inhibits the closing of these sodium channels, thereby disrupting the normal function of the nervous system. It penetrates the insect's cuticle and interferes with nerve signal transmission within minutes, leading to feeding cessation, loss of muscle control, paralysis, and eventually death **(Zoumenou et al., 2015).**

I.8 Toxicity

I.8.1 Neurotoxicity

Lambda Cyhalothrin exhibits neurotoxic properties due to its ability to disrupt the transmission of neuronal signals. Its mechanism of action involves the inhibition of voltage-gated sodium channels in the membranes of nerve cells, as illustrated in Figure 04. By keeping these channels open, it initiates a cascade of electrical impulses that lead to depolarization, ultimately disturbing the normal functioning of the nervous system. This disruption can result

in various symptoms, including involuntary movements and, in severe cases, death **(Li-Ming et al., 2008).**

These compounds are also recognized for their effects on various channels and receptors, including calcium channels and chloride channels. Furthermore, some research indicates that pyrethroids or their metabolites may function as genotoxic agents or endocrine disruptors **(Lawrence & Casida, 1983; Davies et al., 2007).**

I.7.2 Immunotoxicity

The impact of lambda-cyhalothrin on immune response has been investigated in rabbits exposed to this insecticide through their diet. The immune response was assessed by measuring delayed-type hypersensitivity reactions to tuberculin. Significant reductions were observed in total serum proteins, albumin, globulin (particularly gamma globulin), the albumin/globulin ratio, and the body weight/organ ratios for the spleen and thymus as the dosage of the insecticide increased. Additionally, various pathological changes in the liver were noted. Lambda-cyhalothrin was found to suppress both humoral and cell-mediated immune responses in animals exposed to the tested concentrations, depending on the dosage **(Morgan & Osman, 2007).**

I.8.3 Effects on food and water consumption

It has been noted that oral exposure to lambda-cyhalothrin in rats resulted in a significant reduction in food intake, water consumption, and body weight. Comparable findings were observed in animals exposed to various pyrethroid compounds, such as deltamethrin and fenvalerate **(Kilian et al., 2007).**

I.8.4 Oxidative stress

- Lambda-cyhalothrin, like most pyrethroids, is a hydrophobic compound, indicating that its interaction with biological membranes may be associated with alterations in proteins and phospholipids **(Michelangeli et al., 1990).**
- Recent research highlights the role of oxidative stress as a mechanism of toxicity for type II synthetic pyrethroids in various rat tissues, with lambda-cyhalothrin appearing to accumulate in biological membranes and induce oxidative damage **(Michelangeli et al., 1990).**
- Specific indicators of such damage, including elevated levels of reactive oxygen species in the liver and lipid peroxidation, were observed 60 days following repeated daily exposure in rats **(Aouey et al., 2017),** suggesting that lambda-cyhalothrin or its metabolites cause cytotoxic damage to hepatocytes, with a significant correlation found between the levels of lambda-cyhalothrin metabolites, reactive oxygen species, and lipid peroxidation in exposed rats.

I.8.6. Hematotoxicity

Furthermore, the research conducted by **(Fetoui et al., 2008)** revealed alterations in hematological parameters in rats exposed to lambda-cyhalothrin, specifically a reduction in red blood cells leading to the development of anemia. These findings corroborate earlier studies involving rats exposed to other pyrethroids, such as alpha-cypermethrin **(Manna et al., 2005).** Additionally, a study on rabbits indicated that lambda-cyhalothrin may cause a decrease in both red and white blood cell counts, as well as reductions in hemoglobin and lymphocyte concentrations in the blood **(Basir et al., 2011).**

I.8.7 Nephrotoxicity

Oxidative damage to the kidneys is characterized by the generation of reactive nitrogen species, including peroxynitrite, nitric oxide, and hydroxyl radicals (OH), which arise from the degradation products of lambda-cyhalothrin **(Kale et al., 1999).** These radicals compromise the integrity of the cell membrane, leading to lipid peroxidation and subsequent destabilization **(Štajn et al., 1997).** Research conducted by **(Fetoui et al. 2010)** revealed a significant reduction in enzymatic activity (including catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, and glutathione S-transferase) within renal tissues, indicating renal impairment. Furthermore, histopathological examinations have demonstrated that lambda-cyhalothrin causes multiple hemorrhagic foci, tubular dilation in the proximal tubule, desquamation of tubular cells, infiltration of inflammatory cells, and swelling of the tubules in the kidney **(Fortin et al., 2009).**

I.8.8 Liver toxicity

Numerous studies have demonstrated the connection between exposure to pyrethroid insecticides and liver damage, as the liver serves as the primary site for the metabolism of all pesticides. The accumulation of lambda-cyhalothrin metabolites (CFMP and 3-PBA) in hepatic tissues is significantly associated with damage to hepatocytes **(Khemiri, 2017).**

II.1 Introduction

Plants play a significant role in the exploration of drug discovery and serve as a primary source for contemporary medicine. Approximately 25% of current pharmaceuticals are derived from plant origins, yet only 5-15% of plant species have been thoroughly examined for their medicinal properties. In recent times, researchers globally have focused on natural plants, herbal remedies, phytomedicines, and functional foods, leading to the identification of valuable therapeutic potentials, including antidiabetic, anticancer, immunomodulatory, antiobesity, lipid-lowering, anti-inflammatory, and antibacterial activities. Among the noteworthy medicinal plants is *Gymnema sylvestre*, which belongs to the Apocynaceae family and has been traditionally utilized for treating various ailments. This wild herb is native to regions such as India, Africa, Australia, and China **(Khan et al., 2019).**

II.2 Definition

Le Gymnéma ou *Gymnema Sylvestris* est une plante grimpante qui appartient à la famille des apocynacées et qui comprend de nombreuses plantes dont le laurier-rose par exemple. Cette plante se présente sous forme d'un buisson rampant avec des fleurs en ombelles de couleur jaune ou blanche et peut atteindre jusqu'à 3 mètres de haut. Il existe diverses appellations pour désigner le gymnéma : Gurmar ; Periploca de bois ; Shardunika. Le gymnéma est originaire des forêts tropicales indiennes et est actuellement cultivé un peu partout dans le monde : les pays de l'ouest de l'Afrique, la Chine, l'Australie, le Japon et le Vietnam. Son utilisation en médecine ayurvédique remonte à des milliers d'années. Les Indiens l'utilisent pour ses vertus contre le diabète sucré, contre les infections microbiennes et pour son efficacité dans la protection de la santé cardiovasculaire **(Le moal., 2023).**

II.3 Taxonomy & Vernacular Names

II.3.1 Taxonomic Classification: (Gupta et al.,2023)

-Kingdom: Plantae
-Subkingdom: Tracheobionta
-Division: Magnoliophyta
-Class: Magnoliopsida
-Subclass: Asteridae
-Order: Gentianales
-Family: Asclepiadaceae
-Genus: *Gymnema*
- Species: *sylvestre*

II.3.2 Vernacular Names

Table 3: Different names of *G. sylvestre* in different languages **(Di Fabio et al., 2013).**

Languages	Names
English	Gymnema, Cowplant, Australian cowplant, Gurmari, Gurmarbooti, Gurmar, Periploca of the woods, Meshasringa, Gemnema Melicida, Gimnema, Gur-Mar, Gymnema montanum, Gymnéma, Gymnéma Sylvestre, Miracle plant, Periploca sylvestris, Shardunika, Vishani, Ram's horn, Miracle fruit, Merasingi, Small Indian ipecac, Sugar destroyer
Bengali	Mera-Singi
Sanskrit	Meshashringi, Madhunashini, Ajaballi, Ajagandini, Bahalchakshu, Karnika, Chakshurabahala, Kshinavartta
Marathi	Kavali, Kalikardori, Vakundi
Hindi	Gurmar, Merasingi
Gujrathi	Dhuleti, Mardashingi
Telugu	Podapatri
Tamil	Adigam, Cherukurinja, Sarkarikolli

II.4 Botanical Description & Traditional Uses

II.4.1 Geographical distribution

G. sylvestre is a climbing woody plant (see figure 1) indigenous to regions such as India, Malaysia, Sri Lanka, Indonesia, Japan, Vietnam, and the southwestern part of the People's Republic of China **(Sharma, 2010).** This species exhibits slow growth and is widely distributed across Asia, Australia, and tropical Africa **(Kanetkar et al., 2007).** It can also be found in habitats such as Banda, Konkan, the Western Ghats, and the Deccan region **(Sharma, 2010).** The plant thrives at elevations ranging from 300 to 700 meters and is capable of colonizing coastal plains, scrub jungles, and thickets **(Vijayakumar & Prabhu, 2014).** High humidity levels are particularly beneficial for its growth, making *G. sylvestre* predominantly found in tropical or subtropical climates and forested hills. As a climbing species, it relies on external structures for support during its growth **(Tiwari et al., 2014).**



Figure 6: Geographical representation of *G. sylvestre* around the world (Parveen et al., 2015)

II.4.2 Morphology and anatomy

a) Leaves of *G. sylvestre*

The leaves are simple in structure, featuring a short petiole and a green hue **(Gurav et al., 2007; Sharma, 2010).** They are arranged oppositely and exhibit either an elliptic or ovate shape **(Sharma, 2010).** In terms of dimensions, the leaves typically measure between 3.2 to 6.5

cm in length and 3 to 16 mm in width **(Patel, 2017).** Additionally, these leaves are noted for their distinctive aroma, accompanied by a mild bitter and astringent flavor **(Sharma, 2010).**



Figure 7: Leaves of *G. sylvestre* **(Mey, 2019)**

b) Flowers of *G. sylvestre*

The flowers are diminutive and exhibit a yellow hue. Additionally, the follicles possess a cylindrical form with a lanceolate apex, reaching lengths of up to 3 inches **(Gurav et al., 2007).**



Figure 8: Flower of *G. sylvestre* **(Ken Fern, 2014)**

c) Roots of *G. sylvestre*

This plant features roots that are covered in hair **(Rajashekar et al., 2016).**



Figure 9: Roots of *G. sylvestre* **(Devi & Srinivasan, 2008)**

d) Stem of *G. sylvestre*

The transverse section of the stem exhibits a rounded marginal line, similar to that of the roots. Microscopic analysis reveals a robust epidermis accompanied by multicellular trichomes. The cork is composed of cortical cells, while the phloem resembles a perforated wall that contains phloem parenchyma **(Sharma, 2010).**



Figure 10: Stem of *G. sylvestre* **(Ali, 2020)**

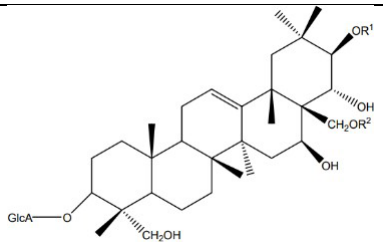
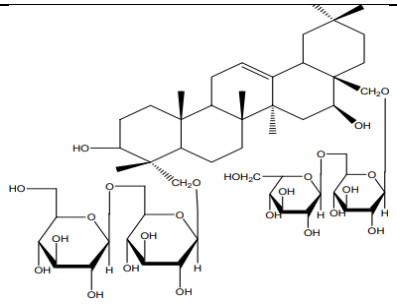
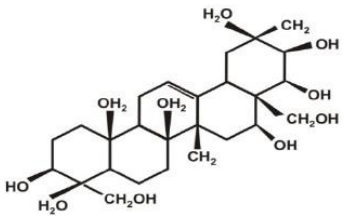
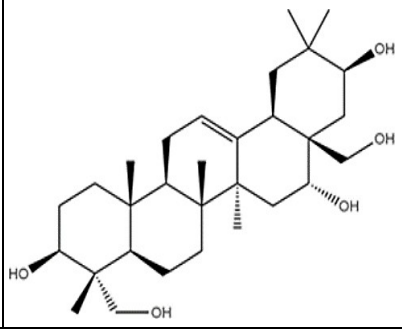
II.4.3 Traditional Uses

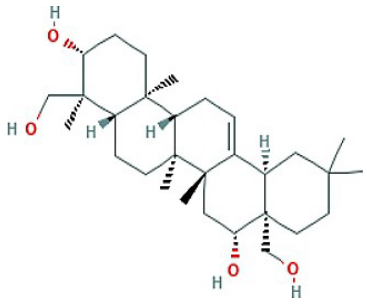
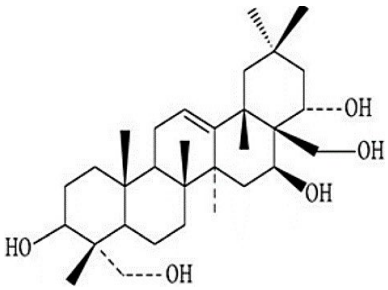
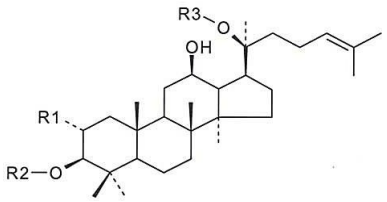
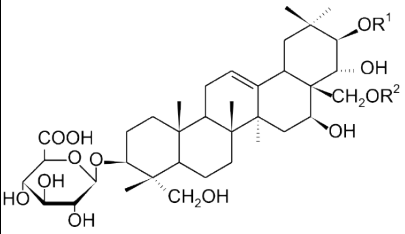
Gymnema sylvestre has been utilized as a sugar inhibitor, primarily due to its leaves, which impair the perception of sweetness. This herb has been employed in the treatment of glycosuria and various urinary disorders. Numerous studies have highlighted its diverse properties, including bitterness, astringency, thermogenic effects, anti-inflammatory capabilities, digestive support, liver tonification, diuretic action, stomachic benefits, stimulation, anthelmintic properties, laxative effects, cardiotonic benefits, antipyretic effects, and uterine tonification. It has shown efficacy in managing conditions such as dyspepsia, constipation, hepatitis, hemorrhoids, kidney and bladder stones, heart disease, asthma, bronchitis, amenorrhea, conjunctivitis, and leukoderma **(Saneja et al., 2010).**

II.5 Phytochemical Constituents

Several studies have identified and documented various phytochemical compounds within the plant. It has been noted that the plant encompasses a diverse array of secondary metabolite classes, which include flavonoids, phytosterols, anthraquinones, and a predominantly intricate blend of saponins referred to as triterpene saponins **(Karale & Karale, 2017; Mey, 2019).**

Table 4: Classification of triterpenoid saponins of *Gymnema*

Class	Classiffication	Basic structure	References
Oleanane Saponins	Gymnemic acids I-XVIII, Gymnemic acids A,B,C,D and F		**(Tiwari et al., 2014)**
	Gymnema saponins I-V		**(Mey, 2019)**
	Gymnemagenin I-V		**(Vaidya, 2011)**
	Gymnestrogenin		**(Dash et al., 2018)**

	23-hydroxylongispinogenin		("PubChem Compound Summary for CID 13322806, 23-Hydroxylongispinogenin.," 2021)
	Gymnemanol		**(Dash et al., 2018)**
Damm arene saponi ns	gypenoside XXVIII, XXX, VII, LV, LXII, LXIII		**(Laha & Paul, 2019)**
	Gymnemosides A, B, C, D, and F		**(Tiwari et al., 2014)**

II.6 Phytoconstituents

Table 5: Important phytoconstituents of ***G. sylvestre***

Phytoconstituents	Percentage
Organic acid	5.5% (Gurav et al., 2007; Sharma, 2010)
Calcium oxalate	7.3% (Gurav et al., 2007; Sharma, 2010)
Lignin	4.8% (Gurav et al., 2007; Sharma, 2010)
Cellulose	22% (Gurav et al., 2007; Sharma, 2010)
Crude fat	5.80% (Sharma et al., 2017)
Crude protein	10.94% (Sharma et al., 2017)
Crude fiber	11.50% (Sharma et al., 2017)
Total carbohydrates	54.89% (Sharma et al., 2017)

II.7 Minerals of *G. sylvestre*

Table 6: Minerals of *G. sylvestre* **(Sharma et al., 2017)**

Minerals	Mg per 100g
Calcium	1542.63
Magnesium	592.40
Chromium	2.70
Zinc	21.80
Copper	12.71
Iron	36.91

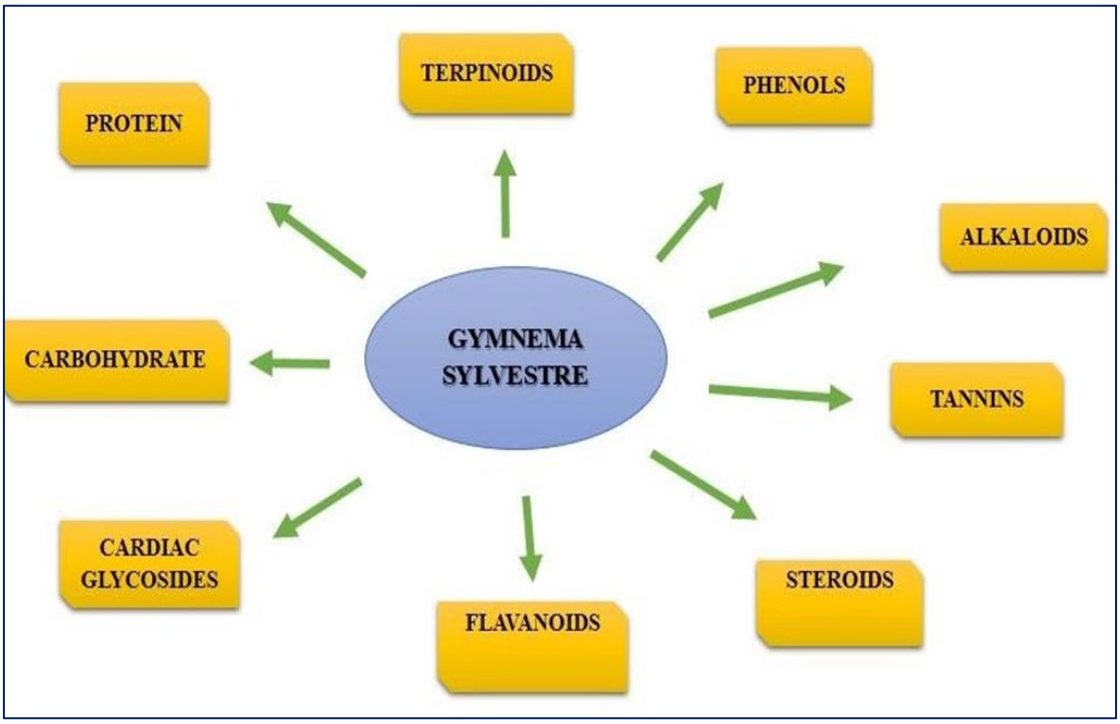


Figure 11: Classes of Phytochemical constituents present in *G. sylvestre* (Khan et al., 2019).

II.8 Pharmacological Properties

II.8.1 Anti-obesity

G. Sylvestre is believed to aid in weight loss, potentially by diminishing cravings for sugary foods and regulating blood sugar levels. Research indicates that the gurmarin peptide inhibits the perception of sweet and bitter tastes, thereby decreasing the desire for sweets. A standardized extract of *G. sylvestre*, when combined with niacin-bound chromium and hydroxycitric acid, has been assessed for its anti-obesity effects by tracking variations in body weight, body mass index (BMI), appetite, lipid profiles, serum leptin levels, and the excretion of urinary fat metabolites. The findings suggest that this combination of *G. Sylvestre* extract, hydroxycitric acid, and niacin-bound chromium may function as a safe and effective weight loss solution, contributing to reductions in excess body weight and BMI while also supporting healthy blood lipid levels (**Saneja et al., 2010).**

II.8.2 Anti-oxidant activity

Preliminary phytochemical analysis and in vitro antioxidant evaluation of *Gymnema sylvestre*. leaf extract were conducted. The extract, prepared with 55% alcohol, was tested using various in vitro antioxidant assays, including DPPH radical scavenging activity, superoxide radical scavenging activity, ferric reducing power, and hydrogen peroxide scavenging activity. The total antioxidant capacity was measured at 17.54 mg/g, expressed in terms of ascorbic acid.

The significant antioxidant activity observed in the plant may be attributed to the presence of acidic compounds, flavonoids, phenols, saponins, tannins, and triterpenoids identified during the preliminary phytochemical screening **(Rachh et al., 2009)**

II.8.3 Immuno modulatory activity

The immunomodulatory properties of the plant were evaluated through various assays, including neutrophil motility, chemotaxis, phagocytosis of heat-killed Candida albicans, and nitroblue tetrazolium reduction tests. The aqueous leaf extract notably enhanced phagocytic activity and chemotactic movement at a concentration of 25 µg/ml. Additionally, the intracellular reduction of nitroblue tetrazolium to formazan was significantly elevated at a concentration of 50 µg/ml, indicating the neutrophils' capacity to kill. Collectively, these findings demonstrate the immunomodulatory effects of *G. sylvestre* **(Jitender et al., 2009)**.

II.8.4 Antidiabetic Activity

The initial scientific validation of the use of *G. sylvestre* in managing diabetes in humans dates back nearly a century, when it was shown that the leaves of *G. sylvestre* can decrease glucose levels in urine among diabetic patients. In a study conducted on animals, Paliwal et al. found that gurmar leaf powder had beneficial and promising effects on blood glucose levels. No negative health impacts were reported among the subjects, leading to the conclusion that gurmar powder is effective in reducing both fasting and postprandial blood glucose levels. Additionally, Sugihar et al. explored the antihyperglycemic properties of a crude saponin fraction and five triterpene glycosides extracted from the methanol extracts of *G. sylvestre* **(Saneja et al., 2010).**

II.8.5 Antimicrobial Activity

The ethanolic extract derived from the leaves of *G. sylvestre* demonstrated significant antimicrobial properties against Bacillus pumilis, B. subtilis, Pseudomonas aeruginosa, and Staphylococcus aureus, while exhibiting no activity against Proteus vulgaris and Escherichia coli. Additionally, both the aqueous and methanolic extracts of *G. sylvestre* leaves displayed moderate efficacy against the three pathogenic species of Salmonella: Salmonella typhi, S. typhimurium, and S. paratyphi. Among these extracts, the aqueous variant exhibited superior activity against the Salmonella species. Furthermore, ethanolic, chloroform, and ethyl acetate extracts from the aerial parts of *G. sylvestre* have also been reported to possess antibacterial effects against *P. vulgaris, E. coli, P. aeruginosa, Klebsiella pneumoniae, and S. aureus* **(Saneja et al., 2010).**

II.8.6 Radio protective activity

The radioprotective properties of Gymnemic acid were assessed in Swiss albino mice subjected to radiation exposure. Biochemical changes in the liver were observed. Gymnemic acid was found to reduce lipid peroxidation, safeguard against the depletion of endogenous glutathione (GSH), enhance protein levels, and demonstrate a notable hepatoprotective effect in comparison to both normal and control groups **(Bhatia et al., 2010).**

II.8.7 Anti-Inflammatory Activity

Activity The aqueous extract of *G. sylvestre* leaves was investigated for evaluation of anti-inflammatory activity in rats at a dose 200, 300 and 500 mg/kg in carrageenin-induced paw oedema and cotton pellet method. The aqueous extract at 300 mg/kg decreased the paw oedema volume by 48.5% with in 4 h after administration, while the standard drug phenylbutazone decreased the paw oedema volume by 57.6% when compared with the paw oedema volume of control. The aqueous extract at the dose of 200 mg/kg and 300 mg/kg produced significant reduction in granuloma weight, when compared to control group **(Saneja et al., 2010).**

II.8.8 Hypolipidaemic Activity

The administration of leaf extracts to hyperlipidaemic rats over a two-week period resulted in a dose-dependent decrease in elevated levels of serum triglycerides (TG), total cholesterol (TC), very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) cholesterol. The effectiveness of this treatment was found to be comparable to that of the established lipid-lowering medication clofibrate **(Saneja et al., 2010).**

PART TOW:

EXPERIMENTAL SECTION

I.1. Materials

I.1.1 Animal material

The primary biological subjects utilized in this research are twenty female Wistar strain rats, sourced from the Pasteur Institute in Algiers. These specimens weigh between 105 and 120 grams and are aged between 4 to 6 weeks. Characterized by their large heads, short ears, red, round eyes, and elongated tails, these nocturnal rodents are extensively used across various fields of experimental research.

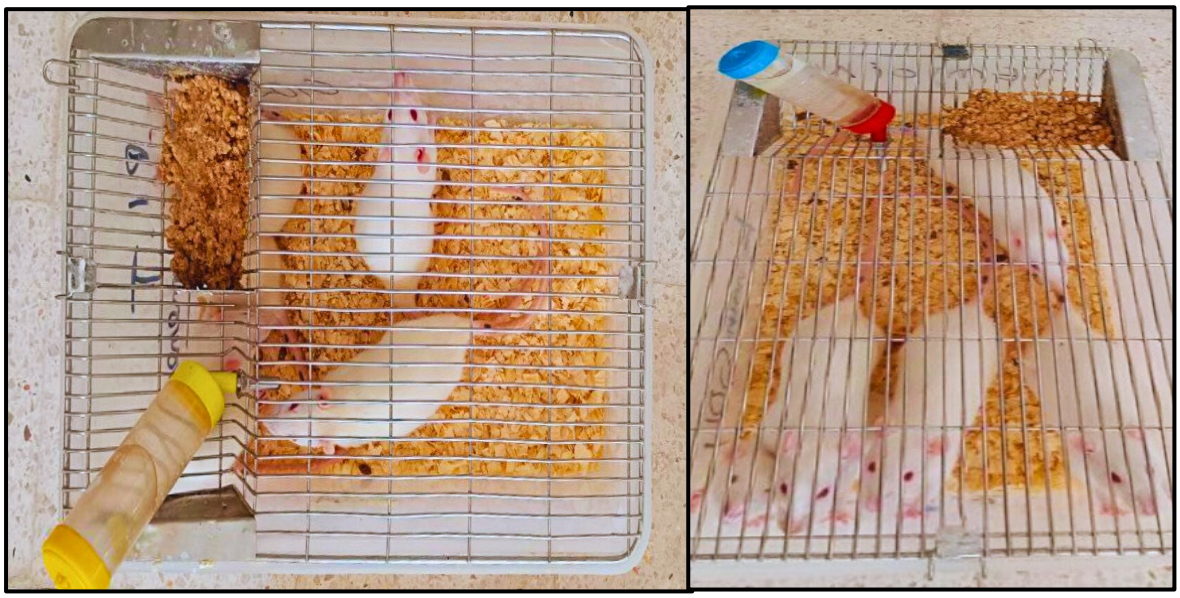


Figure 12: Experimentation Rats**.**

I.1.2. Vegetal material

The medicinal plant utilized in this experiment is *Gymnema sylvestre*, a member of the Asclepiadaceae, it used in this experimental work is a medicinal species belonging to the Gymnacea family, and as its leaves are the most used, it was chosen for this experimental study. The plant studied was purchased from an herbalist and identified by a botanist from the University of Bechar.



Figure 13: *Gymnema sylvestre* 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 14. Lambda-cyhalothrin pesticid

I.2. Methods

I.2.1. Ethnobotanical survey

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

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

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

I.2.2. Animal care

The rats were categorized into four distinct groups, each consisting of five rats. They experienced a 21-day acclimatization phase within the animal facility of the biology department, where the temperature was consistently maintained at 25 °C, and a natural light cycle of 12 hours of illumination followed by 12 hours of darkness was adhered to. The rats were accommodated in plastic cages that featured stainless steel lids and were supplied with 250 ml capacity water bottles, which were lined with bedding that was replaced on a daily basis before the observation period began. They were provided with a standard diet, and their water was replenished each day.

I.2.3 Weight measurement

Weight measurements are conducted daily on the rats throughout the breeding period, either during the adaptation phase (to assess potential changes due to food or environment (external factors)) or during the treatment phase (to evaluate the impact of pesticides), utilizing electronic scales.

I.2.4 Dose choice

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 *Gymnema sylvestre* at a dose of 400 mg/kg/d **(Pachiappan et al., 2023)** ,they administered orally each day for 30 consecutive days.

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

- **Control groups (C):** Rats received water by gavage (0.5 ml/day).
- **Lymbda-cyhalothrin groups (LCT):** Rats received pesticide by gavage (6.3 mg/kg) during 30 days.
- ***Gymnema sylvestre* group (GYM):** Rats received plant by gavage (400 mg/kg) during 30 days.
- **Pesticide + plant (LCT+GYM):** Rats received a combination of pesticide (6.3mg/kg) + plant (400 mg/kg) by gavage during 30 days.



Figure 15: Rats groups distribution .



Figure 16: Rat gavage technique

I.2.5. Plant decoction extract

10g of *Gymnema sylvestre* leaves powder were boiled with 200 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 was stored at +4°C.

Note: This mixture should only be used within 48 hours



Figure 17: 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 locomotor 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).

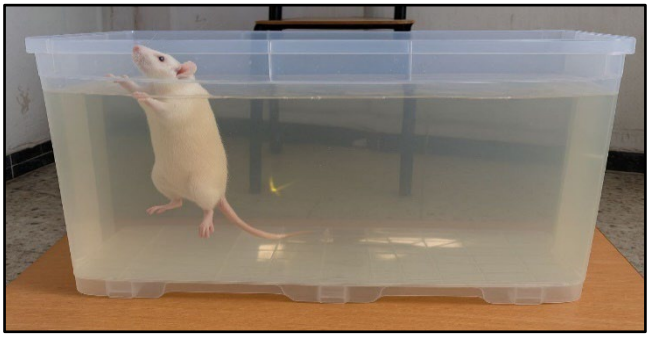


Figure 18: Forced swimming test (FST).

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

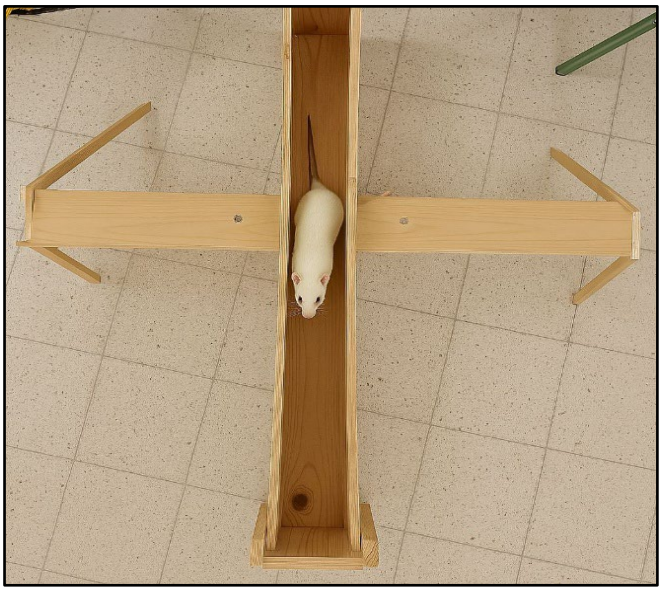


Figure 19. Elevated plus maze test (EPM).

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.

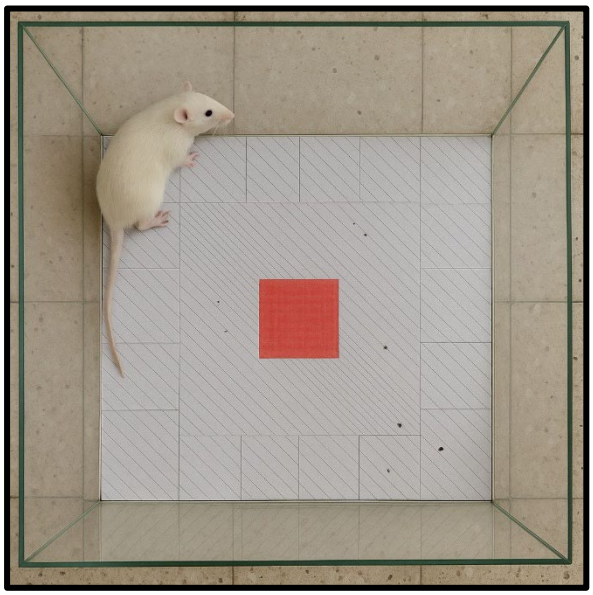


Figure 20. Open field test (OF).

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

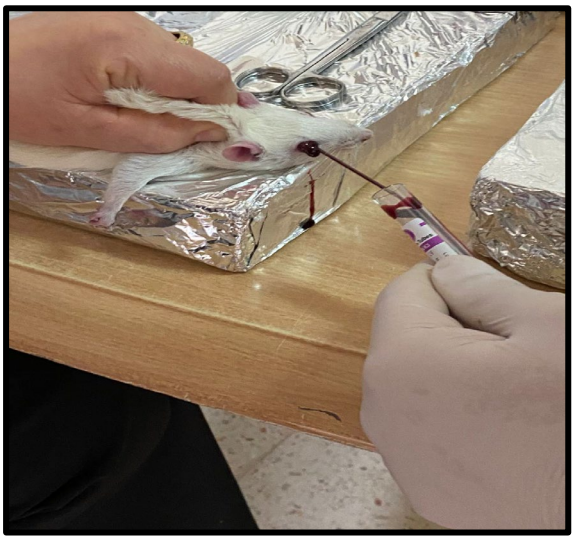


Figure 21: Blood Sampling using a hematocrit capillary tube.

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).

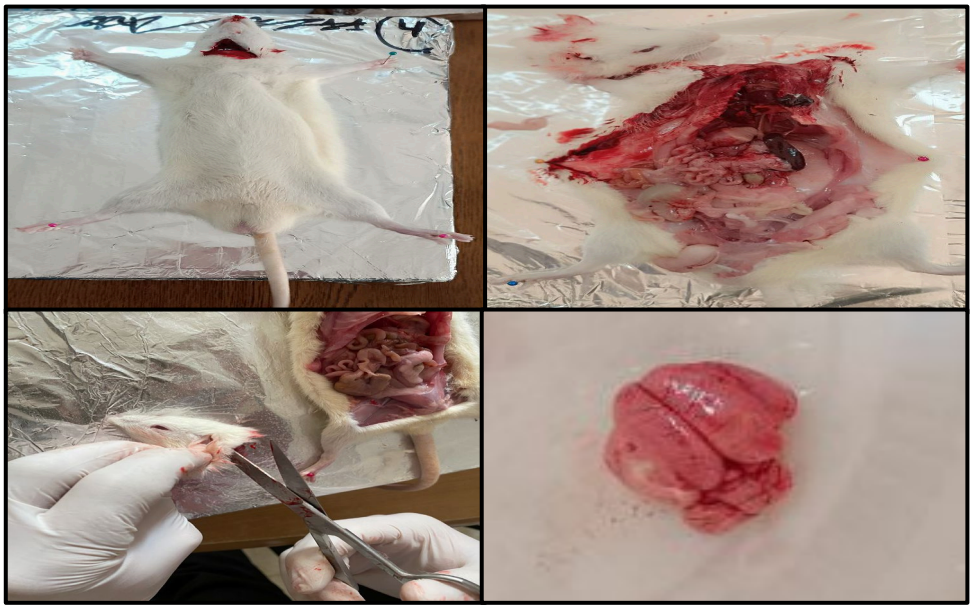


Figure 22: Sacrifice and organs sampling.

I.2.8. Hematological parameters

- **Principe**

The blood analysis was carried out utilizing a type counter automaton (Mindray BC-30S) that evaluated 19 parameters. This assessment was intended to measure the cellular constituents of the blood (CBC), which included the counts of red blood cells, white blood cells, as well as the levels of hemoglobin, lymphocytes, monocytes, and granulocytes. The analysis was conducted on blood samples stored in tubes containing EDTA, and the counting procedure occurred at the Bouguerra Boulaaras Hospital in Bakkaria, Tebessa, Algeria.



Figure 23: Automaton counter of CBC

I.2.9. Biochemical parameters

1.2.9.1. Blood glucose measurement

- **Principe**

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, a enzyme that oxidizes glucose in the blood and produces D-gluconic acid and hydrogen peroxide.

- **Operating mode**

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 24: Blood glucose measurement.

1.2.9.2. 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

- **Operating mode**

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

Table 07: 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.

❖ **Operating mode**

- 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.

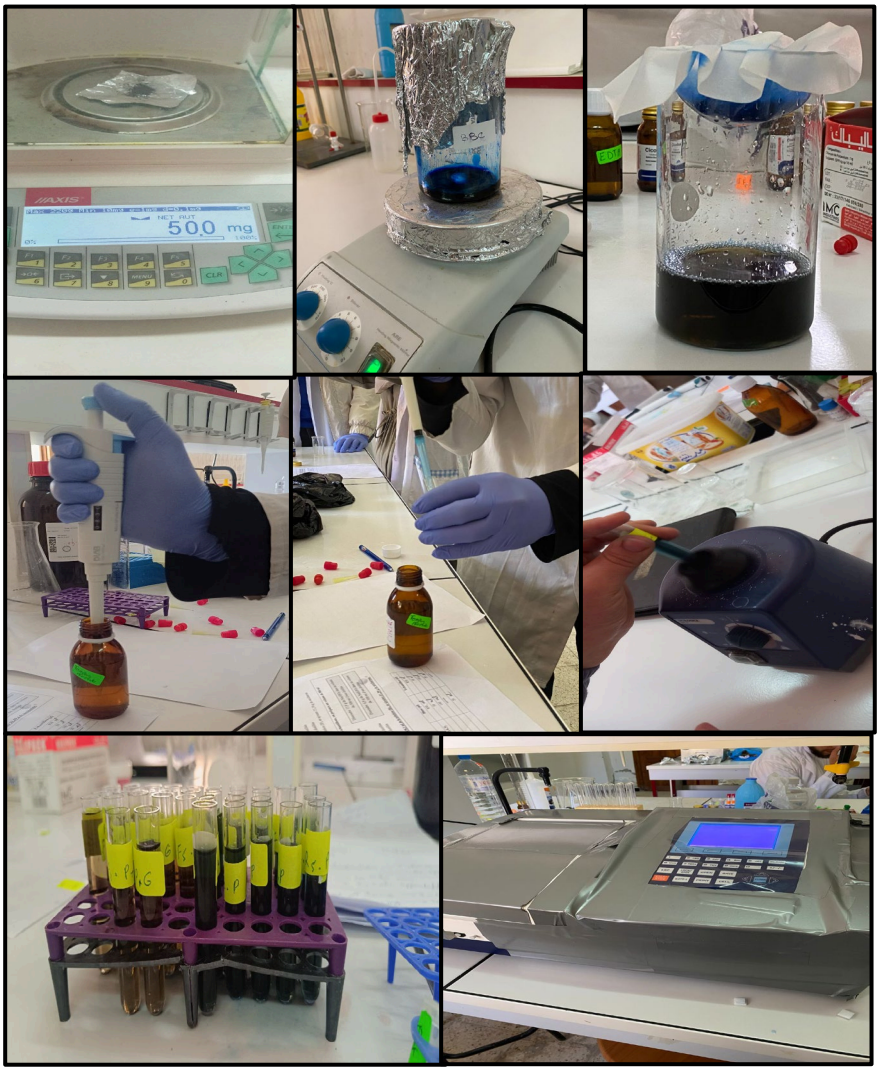


Figure 25: Protein assay preparation

I.2.10 Brain antioxidants enzymes activity

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.

- **Homogénat preparation**

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.

Table 08: GST dosage mode.

Les réactifs utilizes	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:

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

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.

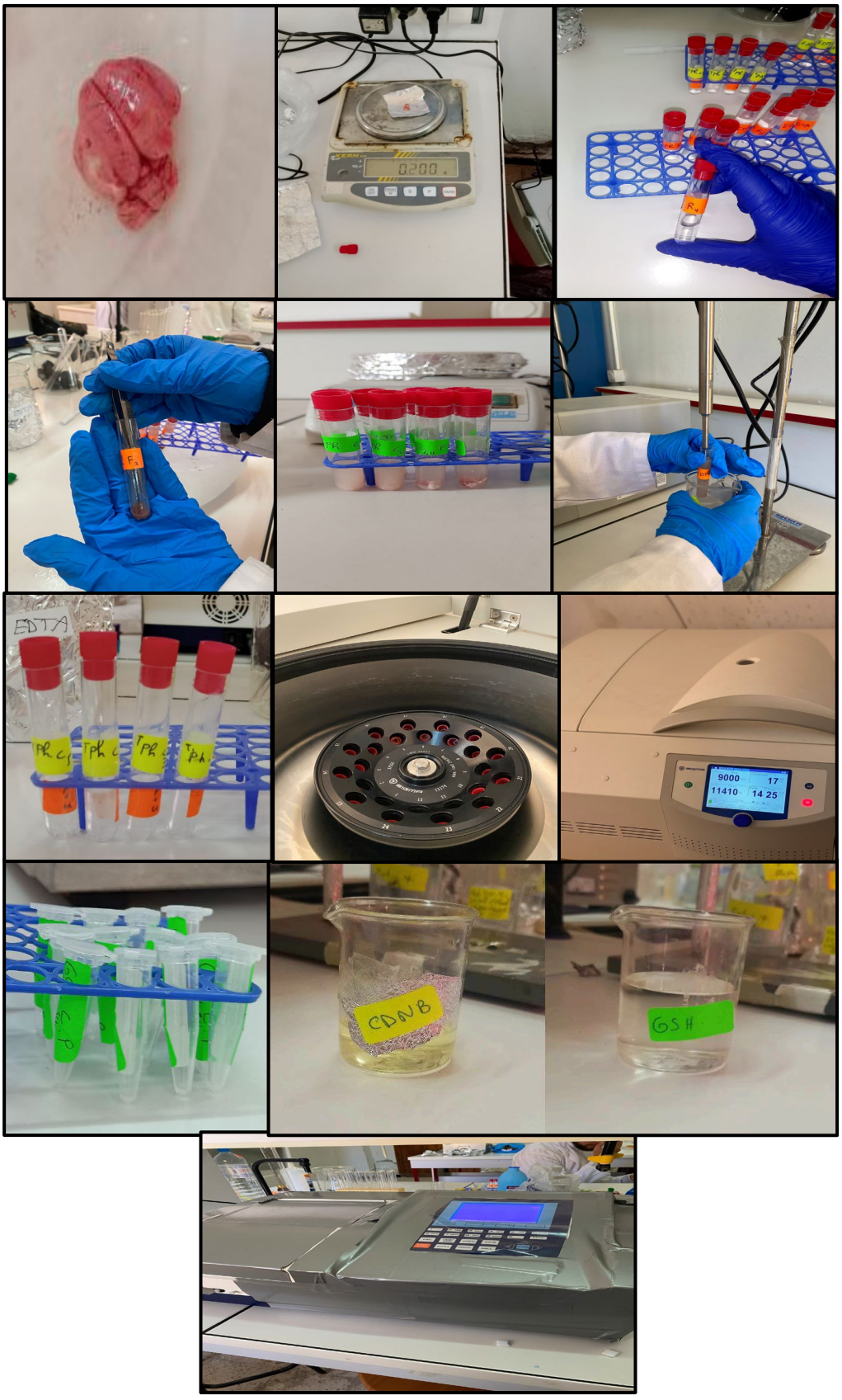


Figure 26: Assay of Glutathione S-Transferase activity preparation

b. Reduced glutathione assay according to Wechberker and Cory, (1988)

- **Principle**

The assessment of GSH activity is conducted using the colorimetric technique established by (**Wechberker & 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.

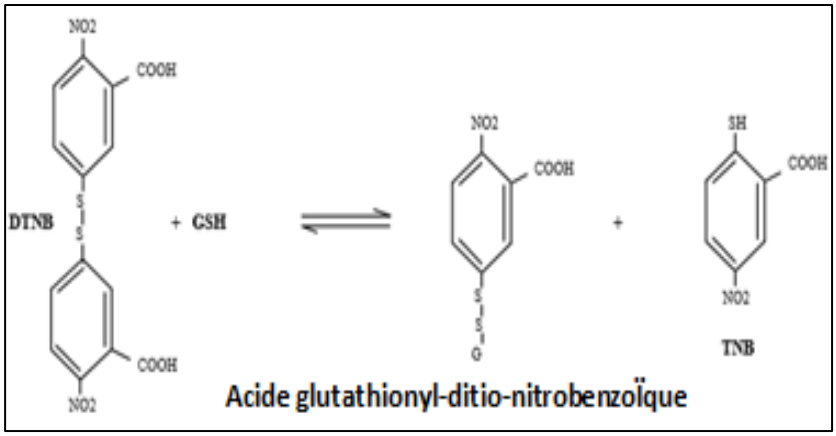


Figure 27 . GSH reaction mechanism **(Baker, et al., 1990).**

- **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).



Figure 28: GSH reduced glutathione 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 LCT group. All statistical analyses were performed using Minitab 17.1 and using 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 ($p< 0.001$).

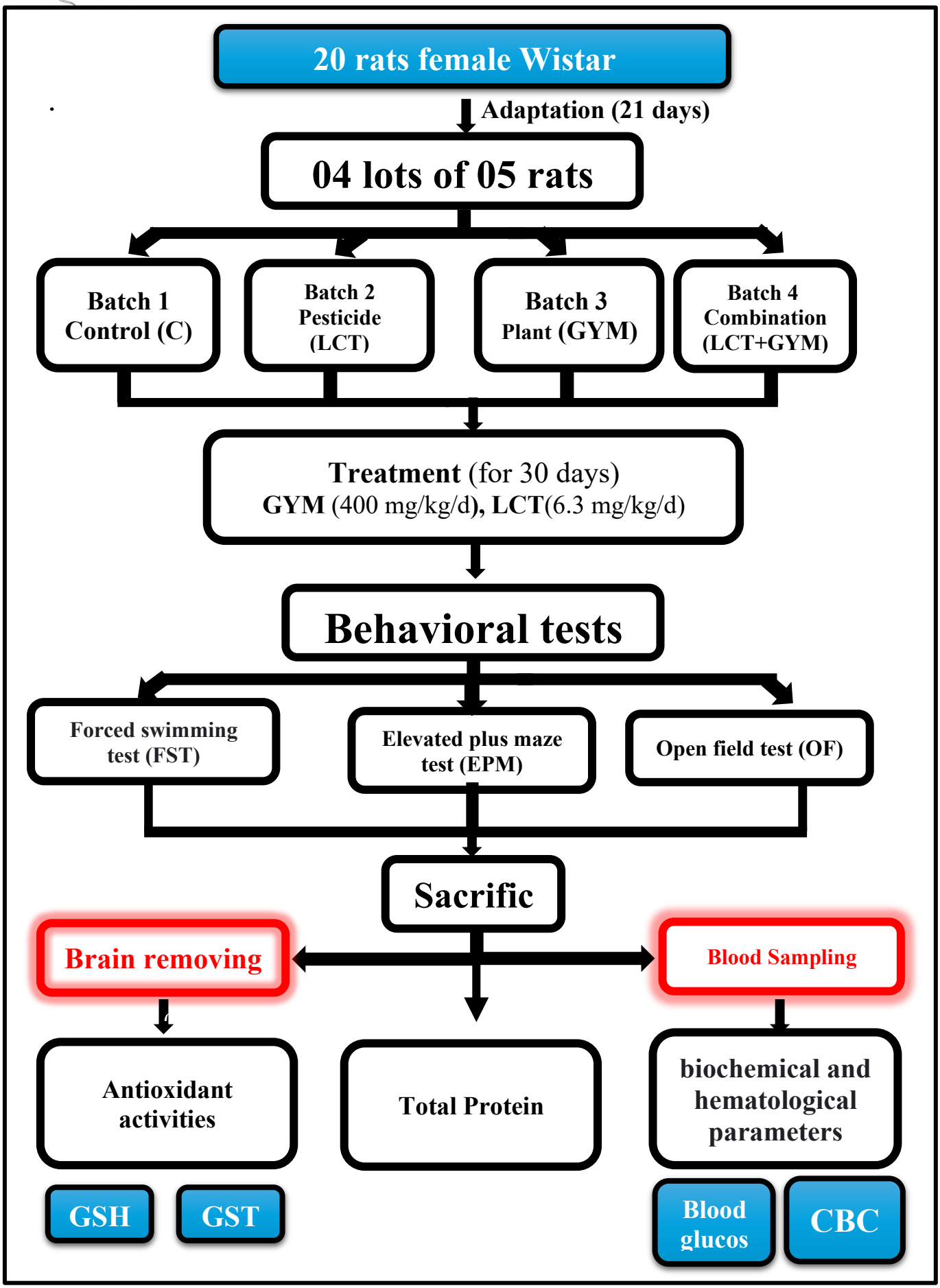


Figure 29: Summary diagram of the experimental protocol

II.1 Ethnobotanical Study

The study involved collecting data from an ethnobotanical survey, which was recorded in a Microsoft Excel database and then analyzed to measure the proportions of various variables such as gender, age, education level, family status, occupation, consumption pattern, uses, and daily intake of *Gymnema sylvestre*, particularly in the treatment of illnesses. The sample studied consisted of 79 individuals: 13 men and 64 women, which may indicate trends or differences in behavior regarding the use of the plant.

II.1.1. Results of sociodemographic information

The results obtained show that 77.2% of participants do not know *Gymnema sylvestre*, while only 22.8% are familiar with the plant. Women are predominant compared to men, with a percentage of 83.1% versus 16.9%. *G. sylvestre* is also used by all ages, with a strong predominance for 18–35 years (87%), 55–75 years (1.3%), 35–55 years (3.9%), and 0% for 75 years and older. Plant use is only 7.8% for those under 18 years old. *G. sylvestre* is widely used by single people, representing 90.8% of the sample, compared to brides, who represent only 9.2%. Also, the majority have a university degree (93.5%). Regarding the professional activity, there are people without work (50.8%), wage earners (10.80%), those who have a private activity (4.6%), and housewives (1.5%).

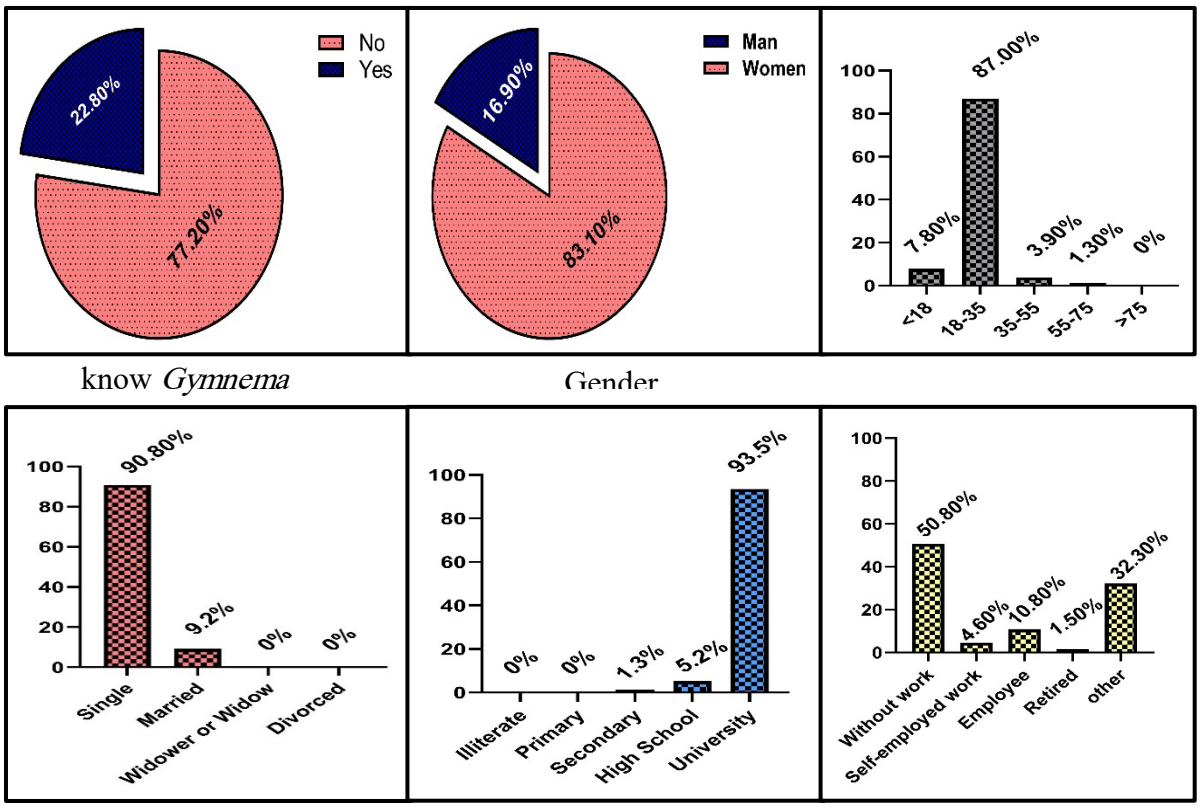


Figure 30: Sociodemographic results

II.1.2. Mode of consumption of Gymnenma

76.2% of individuals who consume *Gymnema* herbal tea prepare it in the form of herbal tea, whereas 23.8% prefer to take it in powdered form.

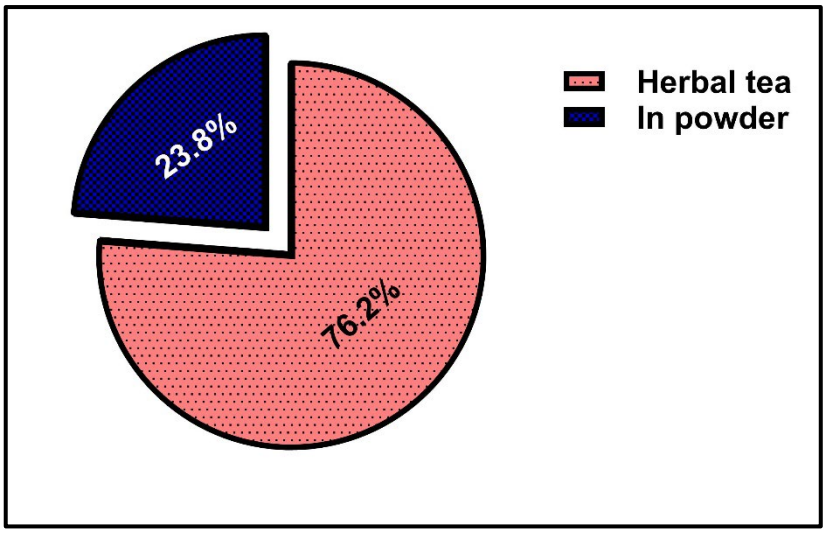


Figure 31: Mode of consumption of *Gymnema sylvestre*

II.1.3. Uses of *Gymnema*

According to the results obtained, 84.2% of the survey participants consume *Gymnema* for therapeutic purposes, while 15.80% use it for culinary purposes.

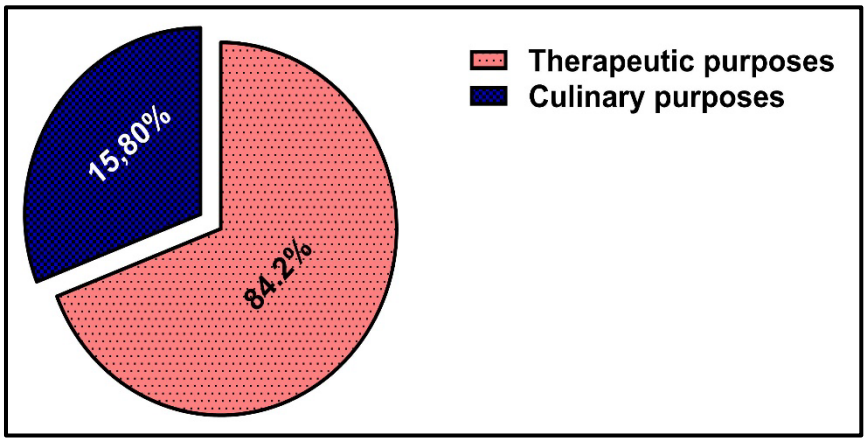


Figure 32: Uses of *Gymnema sylvestre*

II.1.4. Daily intake of *Gymnema*

The survey revealed that the majority of respondents take *Gymnema* once a day, representing 81.5% of the sample. Only 14.8% of participants reported taking two doses per day, and 3.70% for three doses per day.

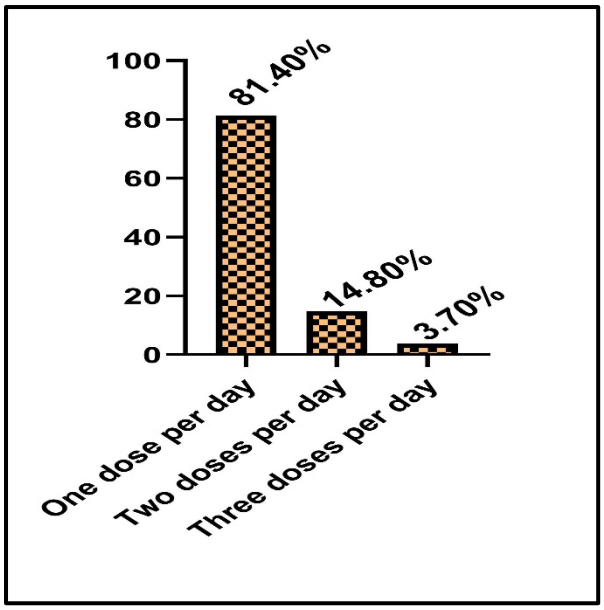


Figure 33: Daily intake of *Gymnema sylvestre*

The results of the ethnobotanical survey on *Gymnema sylvestre* show that most people in the study (77.2%) did not know the plant, while only a small number (22.8%) recognized it, suggesting that knowledge about this medicinal herb is not common among the surveyed population . Women were much more likely to be in the study than men (83.1% versus 16.9%), which matches findings in other ethnobotanical studies that show women often play a bigger role in using and sharing plant knowledge **(Sõukand et al., 2020)**. Most users were young adults aged 18–35 (87%), and hardly any were over 75 (0%), which might mean that older people are not using or sharing this knowledge as much as younger people **(Reyes-García et al., 2013)**. A large number of users were single (90.8%) and had a university degree (93.5%), but many were also unemployed (50.8%), which could mean that people look for natural remedies when they have less money or more free time **(Ceuterick et al., 2008)**. Most people prepared *Gymnema* as herbal tea (76.2%) and used it for medicine (84.2%) rather than for cooking (15.8%), and the most common way to take it was once a day (81.5%), showing that people use it regularly for health. These results suggest that *Gymnema sylvestre* is mainly used by young, educated women who are single and may not have steady jobs, but more research is needed to understand why these groups use it the most.

II.2 Biological parameter

II.2.1 Effect of Lambda-cyhalothrin and *Gymnema sylvestre* on body weight

The results show that the control and *Gymnema*-treated groups maintained relatively stable body weight over time, indicating normal physiological growth. In contrast, the groups treated with Lambda-cyhalothrin, either alone or in combination with *Gymnema*, exhibited a noticeable decline in body weight by the end of the study period, suggesting treatment-related effects on metabolic balance.

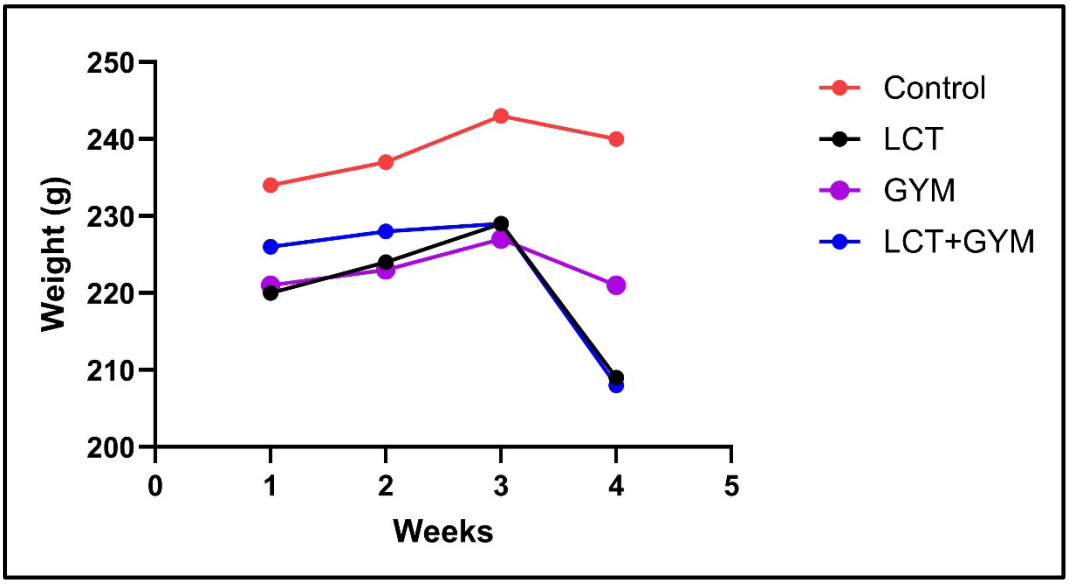


Figure 34: Variation of body weight in control and treated groups

Body weight variation is a key indicator of the animal's overall physiological state. Our results show a progressive increase in body weight during 21 days and an decrease above 2rd week in LCT, GYM and combined 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 and 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 and Chhabra, 1981; El-Demerdash et al., 2004; Saoudi et al., 2011; Mossa et al., 2014).**

In contrast, *G. sylvestre* supplementation was associated with weight reduction. Several studies have reported that *Gymnema* extracts reduce body weight by 4-6% in moderately obese subjects and animal models, likely through mechanisms involving suppression of sweet taste receptors, reduced sugar cravings, and decreased caloric intake **(Healthline, 2018).**

II.2.2Effect of Lambda-cyhalothrin and *Gymnema sylvestre* on body weight gain

The results presented in the table reveal notable differences in body weight changes across treatment groups. The control group (C) showed a modest weight gain (+19.33%), indicating normal physiological development in the absence of treatment. Conversely, the LCT treated-group experienced significant weight loss (–35.33%), highlighting the negative impact of Lambda-cyhalothrin on body weight. The GYM group exhibited nearly stable weight (–0.67%), suggesting minimal effect from *Gymnema* alone. The most pronounced weight loss was observed in the LCT + GYM group (–59.33%), indicating a potential compounded or synergistic effect when both substances were administered.

Table 09: Variation of weight gain in control and treated groups

Groups	C	LCT	GYM	LCT+GYM
Initial weight (g)	234.6 ± 18,23	220 ±8.09	221.8 ± 17.58	226.4 ± 25.51
Final weight (g)	240.4 ± 22.72	209.4 ± 8.79	221.6 ± 20.40	208.6 ± 19.57
Gain (%)	19.33	-35.33	-0.67	-59.33

The marked reduction in weight gain in rats treated with Lambda-cyhalothrin aligns with prior studies demonstrating the compound's metabolic toxicity. LCT, a synthetic pyrethroid, has been associated with oxidative stress and disruptions in lipid and carbohydrate metabolism, which may explain the significant body weight reduction observed **(Saad et al., 2019)**. *Gymnema sylvestre,* known for its hypoglycemic and anti-obesity properties, produced a negligible effect on body weight in isolation, which corresponds to findings from clinical and experimental studies showing its weight-stabilizing capacity **(Shanmugasundaram et al., 1990; Baskaran et al., 1990)**. However, when combined with LCT, *Gymnema* did not counteract the weight loss and may have exacerbated the metabolic disturbances, possibly due to additive or overlapping mechanisms affecting energy balance.

II.2.3 Effect of Lymbda-cyhalothrin and *Gymnema sylvestre* on absolute brain weight.

The study evaluated the effects of Lambda-cyhalothrin, *Gymnema*, and their combination on absolute brain weight. The control group showed a mean brain weight of 1.703 ± 0.084 g.The LCT group exhibited a slight decrease (1.645 ± 0.051 g), while the *Gymnema* group showed a minor increase (1.720 ± 0.070 g); however, both changes were not statistically significant (ns). Similarly, the combination group (LCT+*Gymnema*) had a mean brain weight of 1.668 ± 0.058 g, showing no significant difference compared to either the control or LCT group.

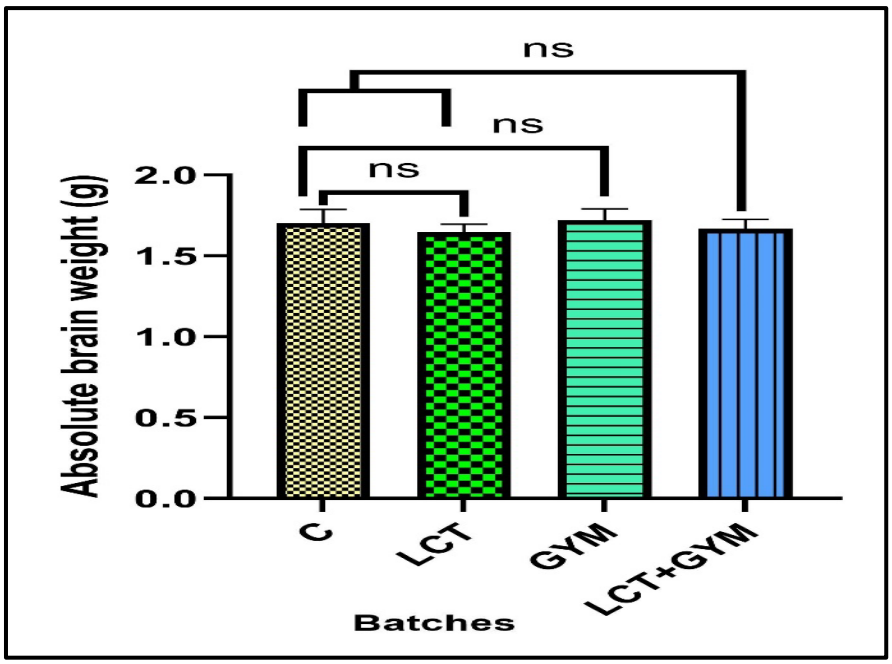


Figure 35: Variation of brain Absolut weight in control and treated groups

The current study observed no statistically significant changes in absolute brain weight among rats treated with Lambda-cyhalothrin, *G. sylvestre*, and their combination compared to the control group. These findings align with previous research indicating that LCT exposure does not significantly affect brain weight but may influence other neurological parameters **(Dhuriya et al., 2017)**

Similarly, while *G. sylvestre* has been recognized for its neuroprotective properties, particularly in diabetic models, its impact on brain weight appears minimal. Research has demonstrated that gymnemic acid, a compound derived from *Gymnema*, can restore and improve brain vascular characteristics in diabetic rats, suggesting potential neuroprotective effects without significant alterations in brain weight **(Sandech et al., 2021)**.

II.3 Hematological study

II.3.1 The effect of Lymbda-cyhalothrin and *Gymnema sylvestre* on blood count cells

The study assessed the impact of Lambda-cyhalothrin (LCT), *Gymnema sylvestre* (GYM), and their combination on hematological parameters in rats. LCT significantly increased white blood cells (WBCs), lymphocytes, and monocytes ($p < 0.05$ or $p < 0.01$) while decreasing red blood cells (RBCs), hemoglobin (Hb), hematocrit (Hct), and platelets (PLT) compared to control group. Beside, *G. sylvestre* alone showed no significant changes in studied parameters. However, in the combination group (LCT+GYM), several values (WBCs, lymphocytes, monocytes, RBCs, Hb, Hct, and PLT) showed intermediate values and significant improvements compared to LCT treated-group, indicating a partial protective effect of plant.

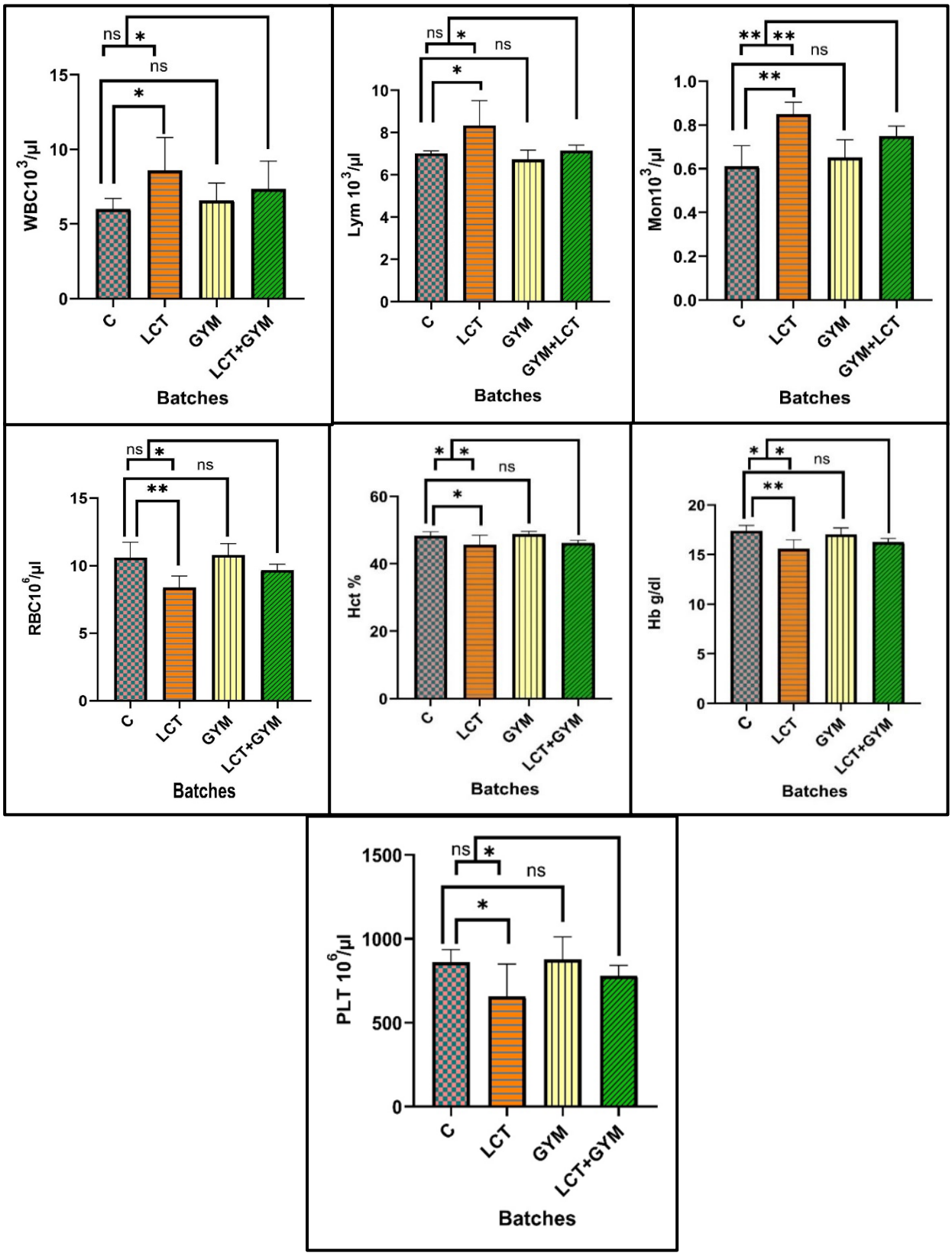


Figure 36: Variations in hematological parameters in control, stressed and treated rats

Exposure to lambda-cyhalothrin resulted in a significant decrease in red blood cell (RBC) count, hemoglobin concentration, and hematocrit compared to the control group. These findings are consistent with previous studies indicating that pyrethroid insecticides can induce anemia and suppress erythropoiesis, likely through oxidative stress and bone marrow toxicity **(El-Sayed et al., 2023; Ali et al., 2017).**

In contrast, treatment of LCT group by *G. sylvestre* led to a notable improvement in these hematological indices. Specifically, there was a partial or complete restoration of RBC count, hemoglobin, and hematocrit values toward normal levels. This effect is supported by literature demonstrating the hematoprotective and antioxidant properties of *Gymnema*, which can mitigate toxin-induced oxidative damage and support hematological recovery **(Kumar et al., 2014; Shanmugasundaram et al., 2011).** Also, this improvement could be related to the immunomodulatory properties of *G. sylvester,* as it is recognized as an immune stimulant with antioxidant and anti-inflammatory properties **(Vasudeva et al. 2024*).*** *G. sylvestre* extract showed a regulatory effect on lymphocytes in diabetic rats. Diabetes led to an increase in lymphocyte count, due to the hyperglycemic state that promotes susceptibility to infections. However, after treatment with the extract, this count decreased to approach normal values. This reduction reflects the hypoglycemic effect of G. sylvestre, which, by restoring glycemic balance, limits the excessive immune response and thus contributes to the stabilization of lymphocyte counts **(Thakur &Padmadeo, 2019).**

II.4 Biochemical study

II.4.1 Effect of Lymbda-cyhalothrin and *Gymnema sylvestre* on Blood sugar

The study assessed the effects of LCT, GYM, and their combination on blood glucose levels. The control group had a glucose level of (0.980 ± 0.268). LCT significantly increased blood sugar (1.492 ± 0.435, $p < 0.05$ vs. control), while GYM showed a non-significant decrease (0.750 ± 0.468). The combination group (LCT+GYM) showed values close to the control (0.986 ± 0.238 g/L), with no significant difference compared to the control (ns), but a significant decrease compared to LCT alone ($p < 0.01$).

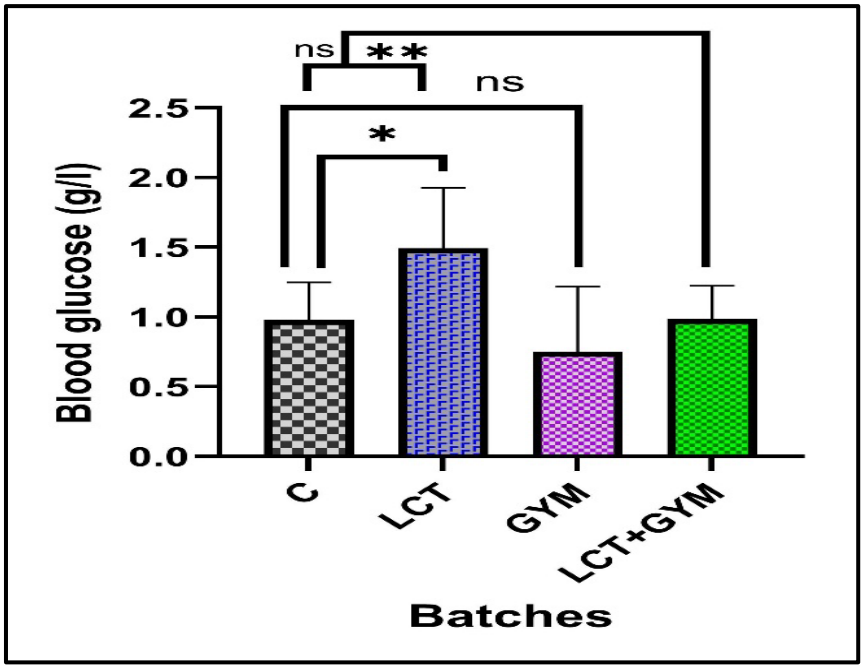


Figure 37: Variation in blood glucose in the control group and the treated groups

The present findings demonstrate that exposure to Lambda-cyhalothrin (LCT) results in a significant increase in blood glucose levels compared to control animals, suggesting a potential hyperglycemic effect. This observation is supported by previous studies indicating that LCT and other pyrethroids can disrupt pancreatic function and glucose metabolism, potentially via oxidative stress and inflammatory mechanisms **(Ahmed et al., 2019).**

Conversely, administration of *Gymnema sylvestre* resulted in a slight, non-significant reduction in blood glucose, corroborating earlier research highlighting the antidiabetic and hypoglycemic properties of *Gymnema* through insulin stimulation and glucose uptake enhancement **(Leach, 2007).**

Interestingly, the combined treatment group (LCT+GYM) showed glycemia values comparable to control, with a statistically significant reduction compared to LCT-treated group

($p < 0.01$). This suggests that *Gymnema* may exert a protective or antagonistic effect against LCT-induced hyperglycemia. Similar interactions have been reported where Gymnema mitigated chemically-induced glucose disturbances in animal models **(Bhat et al., 2018)**. Furthermore, *Gymnema* also exerts an effect by delaying the absorption of glucose into the blood. Indeed, the atomic structure of gymnemic acids in the taste buds is similar to that of sugar molecules, which allows them to bind to the receptors of the taste buds and prevent their activation by sugars present in food. Similarly, in the intestine, these acids bind to the receptors located on the surface, thus blocking the absorption of glucose and leading to a reduction in blood sugar levels **(Sahu et al., 1996).**

II.4.2 Effect of Lymbda-cyhalothrin and *Gymnema Sylvester* on cerebral protein concentration

The study evaluated total brain protein level in rats treated with LCT, *Gymnema*, GYM, and their combination. The control group recorded a mean protein level of 0.324 ± 0.034. A significant increase was noted in the LCT group (0.576 ± 0.123, $p < 0.01$), while GYM treated group reveled a non-significant decrease (0.302 ± 0.027). The LCT+GYM-treated group showed a moderate significant increase (0.392 ± 0.042) versus control ($p < 0.05$) and LCT groups ($p < 0.01$).

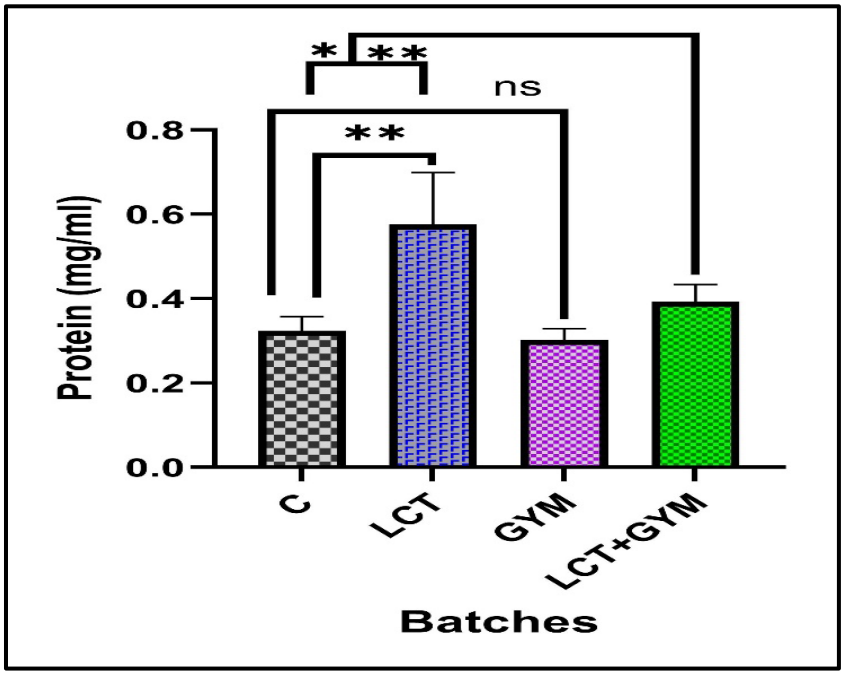


Figure38: Variation of protein concentration in control and treated groups

Exposure to Lambda-cyhalothrin resulted in a significant increase in total brain protein content, suggesting either protein accumulation due to oxidative stress or possible

compensatory biosynthesis. Previous studies have reported that pesticide exposure can lead to altered protein metabolism and enzyme induction in neural tissues **(Abdel-Daim et al., 2014).** Conversely, *G. sylvestre* did not significantly alter protein levels, indicating minimal impact on protein turnover or synthesis under the tested conditions. Interestingly, co-treatment with *Gymnema* moderated the protein increase seen with LCT alone, suggesting a potential modulatory effect. This may reflect the antioxidant and anti-inflammatory properties of *Gymnema*, which could reduce cellular stress and restore normal protein homeostasis **(Rachh et al., 2010).** This result is consistent with the observations of **Pawar et al. (2011),** who reported that *G. sylvestre* stimulates protein synthesis and improves metabolism in diabetic animals.

II.4.3 Effect of Lymbda-cyhalothrin and *Gymnema sylvestre* on cerebral antioxidant activities

a. Glutathione S transferase (GST) activity

GST activity significantly decreased in the LCT-treated group (3.104 ± 0.636, $p < 0.01$), whereas GYM induced a non-significant increase (4.452 ± 0.420). The LCT+GYM combination produced a partial restoration (3.844 ± 0.140), showing significant differences compared to both the control ($p < 0.05$) and LCT groups ($p < 0.05$).

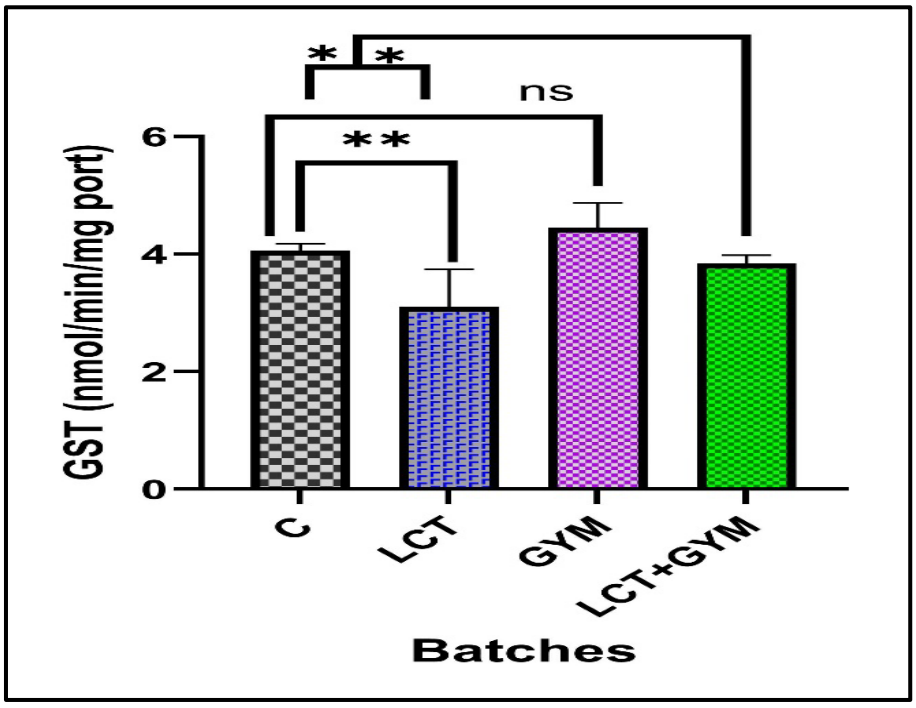


Figure39: Variation of GST activity in control and treated groups

GST activity, a key marker of detoxification and antioxidant defense, was significantly reduced following LCT exposure. This reduction may reflect LCT-induced oxidative stress that impairs enzymatic systems responsible for neutralizing electrophilic compounds **(Kumar et al., 2018).** Our results are in agreement with **Alsadee et al., (2024),** who found a significant decrease in GSH when rats were treated with a low dose of LCT.

In contrast, the *Gymnema*-treated group exhibited normal GST levels, which may indicate the herb's capacity to support antioxidant enzyme function.

The LCT+GYM-treated group demonstrated improving GST activity relative to LCT-treated group, this beneficial effect can be explained by the stimulation of detoxification enzymatic pathways thanks to its richness in phenolic compounds, flavonoids, quercetin and kaempferol, have shown a significant association with anti-oxidation and protection against endothelial cell death inhibited by reactive nitrogen species **(Onanong et al., 2024).** This result is in agreement with the work of **Karthic et al. (2012),** who reported that *G. sylvestre* potentiates the activity of antioxidant , including GST, thus participating in the protection of tissues against oxidative stress. This finding aligns with other studies showing that plant-based antioxidants help stabilize GST activity under toxic stress **(Lounis et al., 2021).**

b. Reduced glutathione (GSH) activity

The control group showed a mean brain GSH level of 22.544 ± 1.032. LCT caused a drastic and highly significant reduction (8.250 ± 0.270, $p < 0.001$) compared to control, while GYM increased GSH levels above control (25.152 ± 0.757, $p < 0.01$). The combination group showed intermediate values (17.598 ± 2.630), significantly lower than control ($p < 0.01$) and significantly higher than LCT ($p < 0.001$).

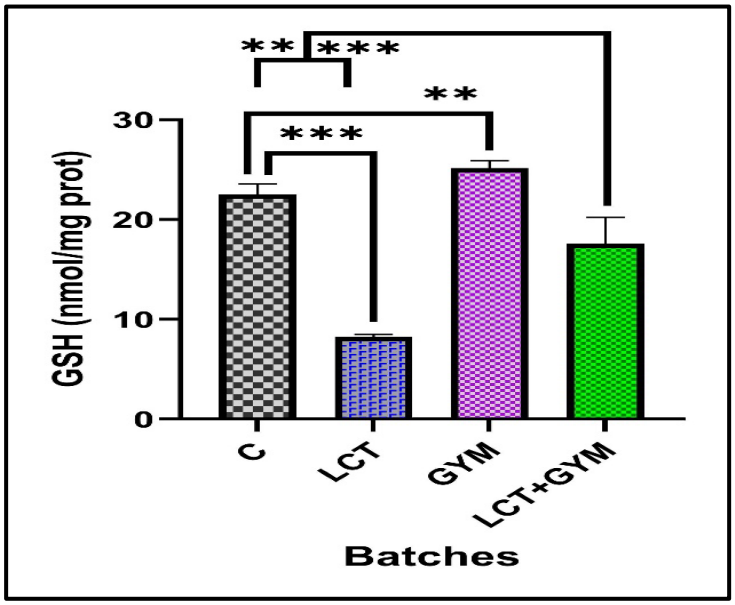


Figure 40: Variation of GSH activity in control and treated groups

Glutathione (GSH) is a critical antioxidant in the brain, safeguarding neural tissues from oxidative stress. Exposure to neurotoxic agents like lambda-cyhalothrin (LCT), a type II pyrethroid pesticide, has been shown to disrupt this balance. In a study **by Ansari et al. (2012),** developing rats exposed to LCT exhibited significant reductions in GSH levels across various brain regions, including the hippocampus and cortex. This depletion was accompanied by increased lipid peroxidation and protein carbonylation, indicating heightened oxidative stress and potential neuronal damage.

Gymnema sylvestre is renowned for its antioxidant properties, attributed to its rich phytochemical composition, including gymnemic acids, flavonoids, and saponins. In a study **by Ibrahim (2022),** rats treated with *G. sylvestre* leaf extract demonstrated enhanced antioxidant enzyme activities, including catalase and superoxide dismutase, in the brain. While the study focused on cisplatin-induced neurotoxicity, the findings suggest that *G. sylvestre* may bolster the brain's antioxidant defenses, potentially mitigating oxidative damage. Phytochemical studies on *Gymnema sylvestre* have shown that its extract contains flavonoids, which are known for their antioxidant properties **(Khan et al., 2019).** According to **Kumar et al. (2014),** flavonoids are capable of activating intracellular antioxidant signaling pathways, thereby stimulating the production of endogenous antioxidants such as glutathione (GSH). Additionally, these compounds have been identified as activators of Nrf2, a key transcription factor involved in regulating the antioxidant response **(Hamid et al., 2020; Wang et al., 2019).** Under conditions of oxidative stress, Nrf2 activation promotes its translocation into the nucleus, where it binds to the antioxidant response element (ARE), leading to the expression of cytoprotective genes such as GSTA **(Lin et al., 2023).**

II.5 Behavioral study

II.5.1 Effect of Lymbda-cyhalothrin and *Gymnema Selvester* on the Elevated Plus Maze Test

The study evaluated anxiety-related behaviors across various groups utilizing the elevated EPM methodology. The GYM group demonstrated a significantly greater duration in the center area (78.92 sec) compared to the LCT group (14.90 sec), suggesting a decrease in anxiety levels. Additionally, the GYM group spent more time in the open arms (26.74 sec) relative to the LCT group (5.26 sec), while the GYM+LCT group exhibited a slightly reduced duration (12.20 sec), indicating a partial alleviation of anxiety. In the distal open arms, the GYM group also displayed a greater presence (7.20 sec) compared to the LCT group (1.20 sec). The frequency of entries into the open arms was notably higher in the GYM group (18.60) than in the LCT group (2.00), reflecting enhanced exploratory behavior. In contrast, the LCT group allocated more time to the closed arms (279.96 sec), which is typically linked to heightened anxiety, whereas the GYM group showed a decrease in closed-arm duration (187.44 sec). Collectively, the GYM intervention seemed to reduce anxiety-related behaviors, while the LCT alone appeared to an anxiogenic effect.

Parameter/Groups	T	LCT	GYM	GYM+LCT
Time spent in center (sec)	29,360 ± 9,267	14,900 ± 6,405 a*	78,92 ± 46,21 a*	27,94 ± 6,68 a^{ns}, b*
Time spent in open arms (sec)	52,30 ± 18,79	5,26 ± 3,10 a***	26,74 ± 7,12 A*,	12,20 ± 2,94 a**, b***
Time spent in distal open arms (sec)	16,720 ± 5,213	1,200 ± 0,447 a***	7,200 ± 1,924 a**	4,800 ± 0,837 a**,b***
Time spent in proximal open arms(sec)	36,54 ± 16,24	3,66 ±5,05 a***	20,04 ± 3,16 a*	8,10 ± 6,57 a**,b*
Number of entries in open arms	24,400 ± 1,21	2,000 ± 0,449 ***a	18,600 ± 1,140 a^{ns}	7,800 ± 6,301 a*,b**
Time spent in closed arms (sec)	199,18 ± 24,12	279,96 ± 14,81 a***	187,44 ± 42,06 a^{ns}	226,18 ± 42,17 ans,b**
Time spent in proximal closed arms(sec)	112,76 ± 29,10	54,22 ± 19,60 a**	89,54 ± 24,03 a^{ns}	67,30 ± 17,89 a**, b^{ns}
Time spent in distal closed arms (sec)	96,38 ± 21,38	225,52 ±26,05 a***	88,88 ± 18,63 a^{ns}	172,08 ± 62,08 a*, b*
Number of entries in closed arms	28,60 ± 12,542	7,800 ± 4,438 a***	25,20 ± 23,68 ns	11,000 ± 2,550 a**, b^{ns}

(*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 and in its 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, GYM-treated group demonstrated importante time spent in the open arms and in the center of the elevated plus maze (EPM), along

with a higher frequency of entries into the open arms, which implies enhanced exploratory behavior and diminished anxiety levels. Notably, the combination of GYM and LCT (GYM+LCT) seemed to partially mitigate anxiety like behavior observed in the LCT group alone, as this cohort displayed intermediate results in both open and closed arm measures. This finding suggests a potential protective effect of Gymnema against the anxiogenic impact of LCT, *Gymnema sylvestre* extract has shown anti-inflammatory and antioxidant activities **(Jangam et al., 2023).** Anxiety and stress are often linked to increased inflammation and oxidative stress in the brain. By mitigating these factors, Gymnema may exert anxiolytic effects. Unfortunately, no studies have been conducted on the effect of *Gymnema sylvestre* on anxiety-like behavior.

II.5.2 Effect of Lymbda-cyhalothrin and *Gymnema Selvestre* on Open Field Test parameters

The study evaluated locomotor and anxiety-related behaviors in rats using the open field (OF) test following treatment with Lambda-cyhalothrin (LCT), Gymnema sylvestre (GYM), or their combination (GYM+LCT). The control group exhibited a total distance traveled of 9,409 m. Exposure to LCT led to a marked reduction in locomotor activity (4,932 m), whereas the GYM group showed a pronounced increase (13,255 m), suggesting a stimulatory effect. The GYM+LCT group demonstrated partial recovery (7,118 m), with values closer to the control than to LCT alone. In terms of immobility time, LCT exposure significantly increased the duration (207.50 sec) compared to the control (123.98 sec), indicating depressive-like behavior. Both GYM (123.68 seconds) and GYM+LCT (160.02 sec) groups exhibited reduced immobility, with GYM restoring it to control levels and GYM+LCT indicating partial improvement. Time spent in the periphery, a measure of anxiety, was highest in the LCT group (294.04 sec), whereas both GYM (230.02 sec) and GYM+LCT (238.98 sec) groups showed lower durations, more comparable to the control (241.58 sec), suggesting reduced anxiety. Regarding time spent in the center area, LCT-treated rats displayed severely reduced engagement (3.36 sec), whereas GYM-treated rats spent more time (47.56 sec), indicating reduced anxiety and increase of exploration. The GYM+LCT group (10.20 sec) again reflected partial recovery. The number of center entries followed a similar pattern: the LCT group had the fewest (1.20), while the GYM group had the most (14.40), and the GYM+LCT group and showed moderate activity (5.40). Overall, LCT administration induced anxiety-like and depressive-like behaviors, characterized by reduced movement, increased peripheral activity,

avoidance of the center area. In contrast, *Gymnema* treatment reversed many of these effects, and when combined with LCT, partially alleviated the behavioral impairments.

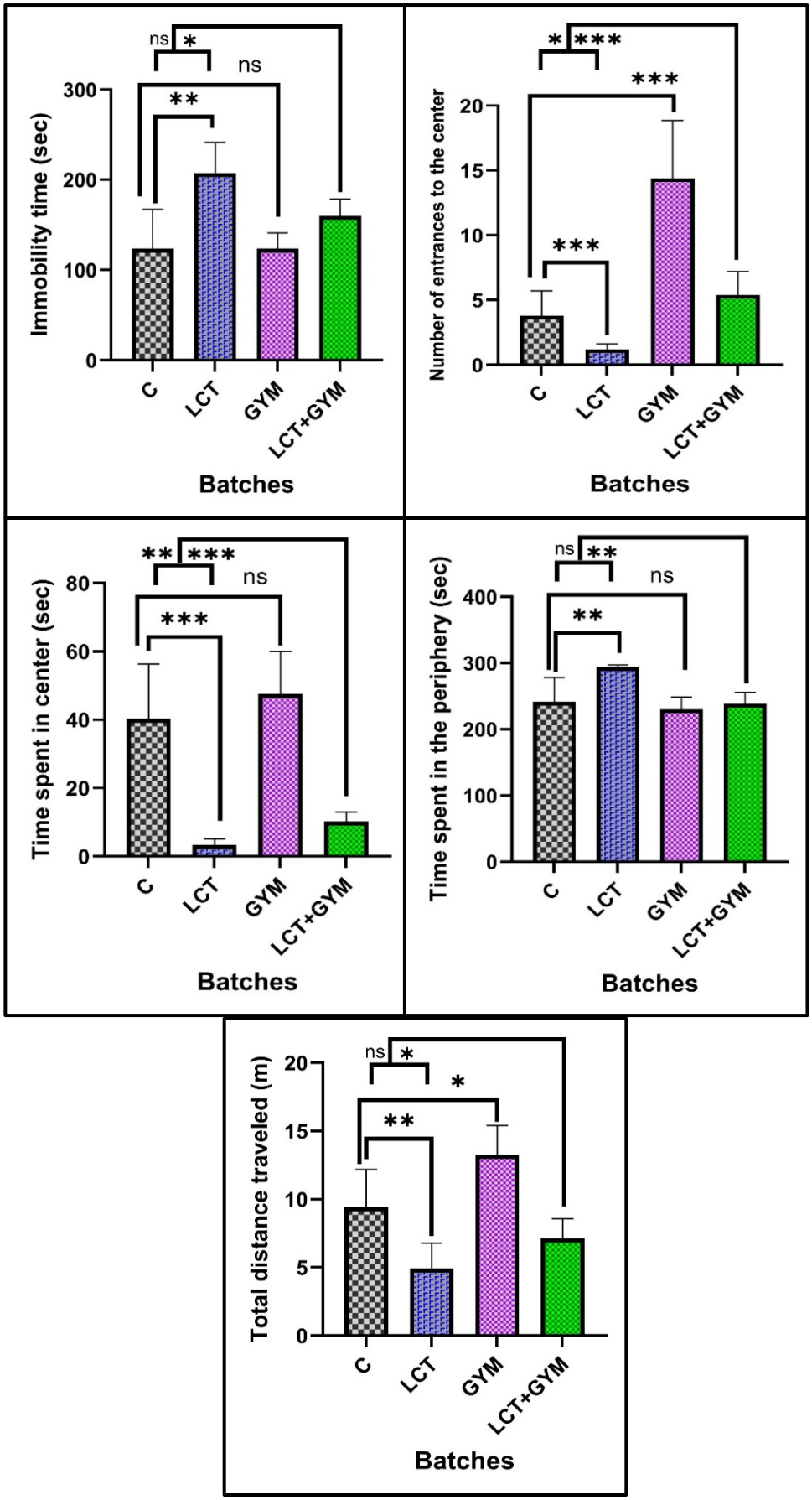


Figure 41: Variation of OF test parameters in control and treated groups

Open Field Test used to measure locomotor activity, exploratory behavior, and anxiety-like behavior (**Reis et al., 2020; Park et al., 2023).** In our study, the administration of LCT showed reduce locomotor and exploratory activity of rats. These findings are consistent with previous literature identifying pyrethroids like LCT as disruptors of neuronal function, leading to anxiety and reduced exploratory behavior **(Shafer et al., 2005; Soderlund et al., 2002).** Conversely, GYM-treated rats demonstrated enhanced locomotor activity and exploratory behavior, with outcomes comparable to or even surpassing control levels. This aligns with reports of *Gymnema* neuroactive properties, including adaptogenic, and antioxidant effects **(Rachh et al., 2010; Panda & Kar, 2012).** Notably, the GYM+LCT group showed partial behavioral restoration compared to LCT alone, particularly in locomotion and center exploration, suggesting that GYM may mitigate some of the neurobehavioral toxicity induced by LCT. However, not all parameters fully returned to control levels, indicating that while *Gymnema* exerts a protective influence, it may not completely reverse pesticide-induced impairments. Studies on diabetic rats suggest that *Gymnema sylvestre* can influence general well-being and activity levels, which indirectly affect locomotion **(Muzaffar et al., 2023; Tchouanka et al., 2022)**. While specific locomotion parameters are not always the primary focus, improvements in metabolic health can lead to increased physical activity.

II.5.3 Effect of Lymbda-cyhalothrin and *Gymnema Sylvestre* on the Forced Swimming test parameters

The study examined depressive-like behaviors using the FST in rats treated with Lambda-cyhalothrin (LCT), *Gymnema sylvestre* (GYM), or their combination (GYM+LCT). The control group exhibited an immobility time of 92.80 ± 6.22 sec. LCT treatment significantly increased immobility time (167.00 ± 44.10 sec, **$p < 0.01$ vs. control**), suggesting enhanced behavioral despair. GYM treatment alone slightly reduced immobility (83.40 ± 21.29 sec), with no significant difference from the control, while the GYM+LCT group showed a partial reduction (116.80 ± 22.44 seconds), which was significantly different from both the control ($p < 0.05$) and LCT groups ($p < 0.05$). Regarding swimming time, the control group spent 130.60 ± 24.94 seconds swimming. This parameter decreased significantly in the LCT group (87.20 ± 16.02 seconds, $p < 0.01$ vs. control), while the GYM group exhibited a slight, non-significant increase (133.40 ± 22.63 sec). The GYM+LCT group showed intermediate swimming activity (109.80 ± 20.13 sec), significantly improved compared to LCT ($p < 0.05$), but not significantly different from the control. In climbing behavior, the control group recorded

89.00 ± 14.51 seconds. LCT significantly reduced climbing time (68.00 ± 12.06 sec, $p < 0.05$ vs. control), while the GYM group increased it to 95.80 ± 13.26 seconds (ns). The GYM+LCT group (75.60 ± 15.47 sec) showed a partial restoration, with significant improvement compared to LCT ($p < 0.05$). Collectively, these results suggest that LCT induces depressive-like behavior, while GYM exerts antidepressant-like effect, and its co-administration with LCT alleviates some of the LCT-induced deficits.

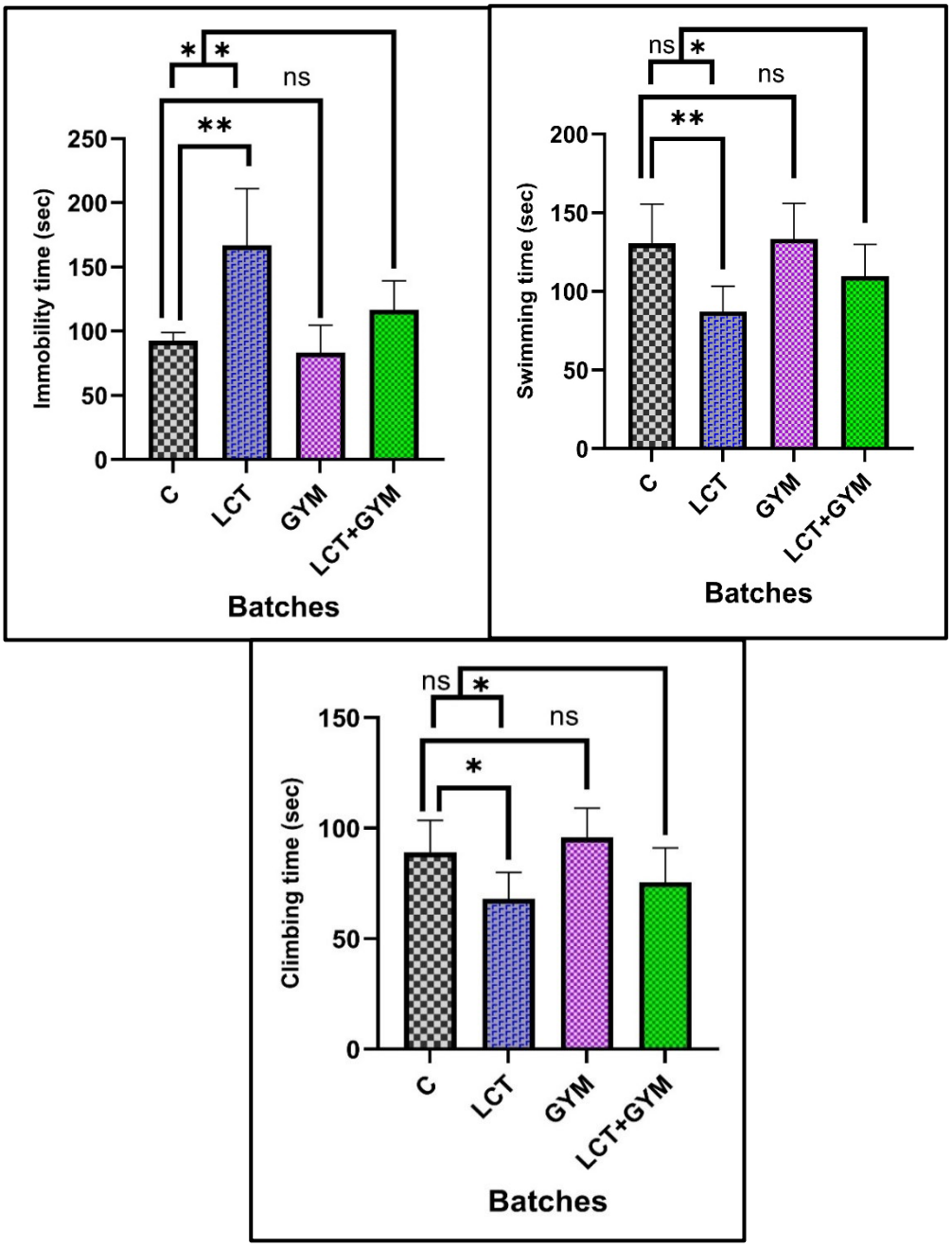


Figure 42: Variation of FST parameters in control and treated groups

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 FST results reinforce the behavioral toxicity of Lambda-cyhalothrin, particularly its capacity to induce depressive-like symptoms, as evidenced by increased immobility and reduced active behaviors such as swimming and climbing. These findings align with earlier research indicating that pyrethroid insecticides can disrupt neurotransmitter systems involved in mood regulation **(Shafer et al., 2005; Soderlund et al., 2002).**

In contrast, *G. sylvestre* treatment led to a reduction in immobility and enhancement of swimming and climbing time, resembling antidepressant-like effects. These outcomes are consistent with the known adaptogenic and neuroprotective properties of GYM **(Rachh et al., 2010; Panda & Kar, 2012).** Depression is often associated with imbalances in neurotransmitter levels and impaired brain function. Research explores the potential of various compounds and herbal medicines to modulate these factors **(Cao et al., 2024).** Some research suggests that compounds in *Gymnema sylvestre* may interact with neurotransmitter pathways. For example, saponins, which are found in GYM **(Xinyong et al., 2013),** have been shown to interact with neurotransmitter-receptor systems in the brain **(Chen et al., 2024).** Though not directly serotonin or GABA, these interactions indicate a potential for broader neurological effects. The GYM+LCT group exhibited behavioral performance that was significantly better than LCT alone in all measured parameters, indicating a partial reversal of LCT-induced behavioral despair. While the combined treatment did not fully restore control-level behavior, the improvements suggest a therapeutic role for GYM in mitigating pesticide-induced depression-like behavior.

CONCLUSION AND PERSPECTIVES

Conclusion and Perspectives

The widespread use of pesticides, such as lambda-cyhalothrin, to control harmful organisms in agriculture represents a serious threat to both human and animal health due to their neurotoxic effects. In this study, we aimed to better understand the harmful effects of this pesticide on the nervous system and explore a natural protective alternative through the use of *Gymnema sylvestre*, a medicinal plant known for its antioxidant and anti-inflammatory properties.

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

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

To complete this study and deepen the understanding of the mechanisms involved, it would be relevant to:

- Identify the bioactive compounds responsible for the neuroprotective effect using chromatographic methods such as HPLC, GC-MS, or LC-MS.
- Evaluate the antioxidant activity of *Gymnema sylvestre* extract using additional assays such as DPPH, ABTS, or FRAP.
- Assess the expression of molecular markers associated with neurotoxicity such as IL1b, caspase, and neurotransmitter
- Conduct an **in silico** study to model the interaction of active compounds with brain molecular targets.

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Webographic

1-https://www.lienhorticole.fr/2012/article/782268/pesticides-horticoles-l-eau-l-air-et-les-sols-impactes (15-02- 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 (21-02- 2025)

3-https://www.researchgate.net/figure/Chemical-structure-of-lambda cyhalothrin_fig1_282161320 (22-02- 2025)

Annexes

Questions of the ethnobotanical survey *Gymnema sylvestre* (الجورمار)

Q1. Do you know *Gymnema*?
Yes ☐ No ☐

Q2. Do you consume *Gymnema*?
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 form. ☐

Personal Information:
Gender : Fem ☐ 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?
Excellente ☐ 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 ……

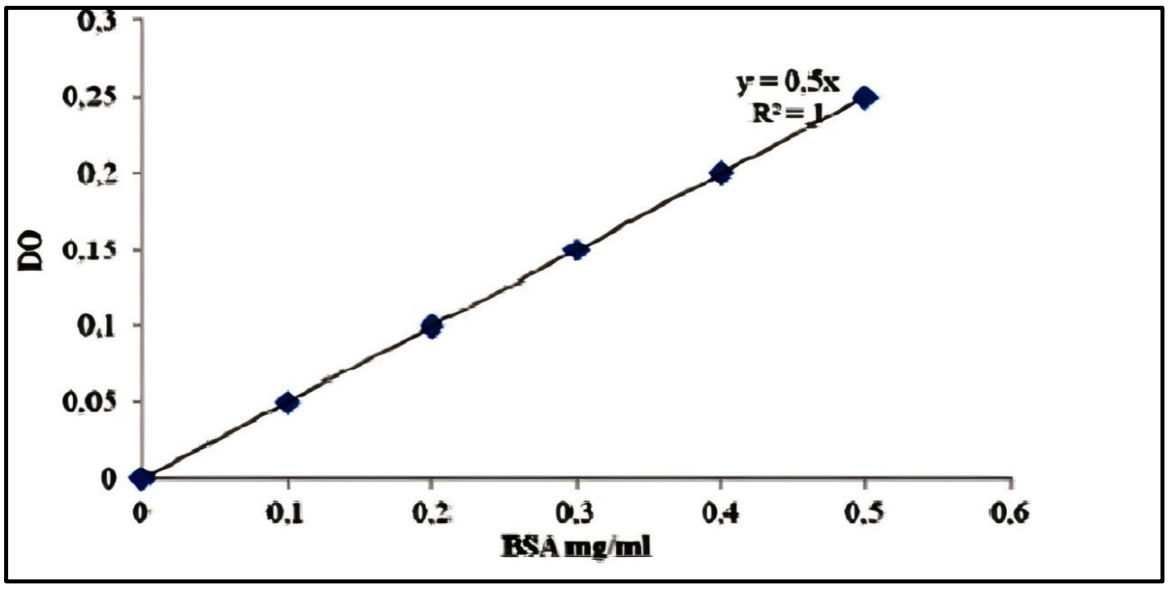


Figure : Calibration curve for bovine serum albumin