Democratic and popular Algerian republic
Ministry of Higher Education and Scientific Research

Larbi Tebessi University – Tebessa –

Faculty of Exact Sciences and sciences of Natural and Life

Department of Applied Biology

Field : Natural and life sciences

MEMORY

Presented for obtaining the Academic Master's degree

In: Biological sciences

Option: pharmaco-toxicology

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Entitled :

Assessment of melissa *officinalis* L in oxidative stress and biochemical parameters after a neonicotinoid toxicity in rats

Jury :

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الملخص

الهدف من هذه الدراسة هو تقييم السمية العصبية لإيميداكلوبريد نيونيكوتينويد بجرعتين مختلفتين من إيميداكلوبريد (5 و 50 مجم / كجم / يوم) والتأثير الوقائي والعلاجي لمستخلص ميليسا (10 مغم / كغم / يوم) ضد هذه السمية لمدة 40 يومًا على المستوى السيتوبلازمي بالدماغ في فئران ويستار.

استهدفت تجاربنا عدة مجالات أساسية للتجربة، بدءًا من عمل الإنزيمات الخلوية وموت الخلايا. تنقسم دراستنا إلى العديد من المجالات المهمة للدراسة ، ودراسات معاملات الإجهاد التأكسدي (MDA، GST) ، ومعايير فيزيولوجية (نسبة الوزن النسبي للعضو). تقييم معاملات الناقل العصبي (AchE) وتأثيرات الإيميداكلوبزيد على المؤشرات الأيضية (بروتينات. سكريات. دهون) في الدماغ كله.

تظهر نتائجنا أن إيميداكلوبريد يسبب آثارًا ضارة على الكائن الحي ، مما يؤدي إلى حدوث تغييرات في توازن الإجهاد التأكسدي الأنزيمي وغير الأنزيمي ، مما يترجم إلى اضطراب في مستوى MDA ، ونشاط GST ، وزيادة نشاط المؤشرات البيولوجية للأكسدة في الدماغ ككل. وأن شدة السمية تزداد مع زيادة الجرعة ، وهذا ما بينته النتائج المتباينة بين الجرعتين 5 و 50 مجم / كجم / يوم.

.يعمل مستخلص المليسا على تحسين توازن إزالة السموم وتقليل الآثار الضارة لإيميداكلوبريد. يبدو أن المليسا من مضادات الأكسدة الفعالة في تقليل الاختلال بين تكوين الجذور الحرة وأنظمة مضادات الأكسدة في الجسم.

الكلمات المفتاحية: الإجهاد التأكسدي ، نيونيكوتينويد ، إيميداكلوبريد. ،ميليسا أوفيسينال .

Résumé

L’objectif de la présente étude était d’évaluer la neurotoxicité d’un néonicotinoïdes imidaclopride à deux doses d’Imidaclopride (5 & 50 mg/kg/jour) Pendant 40 jours au niveau cytoplasmique et l'effet préventif et curatif de l'extrait de Mélissa (10 mg / kg / jour) contre cette toxicité chez les rats *Wistar* .

Nos expériences ont ciblé plusieurs domaines d'expérimentation de base, à commencer par le travail des enzymes cellulaires et la mort cellulaire. Notre étude est divisée en plusieurs importants axes d’études, études des paramètres de stress oxydatif (MDA, GST), paramètres morphologies (Poids relative d’organe %). L’évaluation des paramètres de neurotransmetteurs (AchE) et L’effets de l’Imidaclopride (IMI) sur les paramètres métaboliques (lipides, protéines, glucides) dans le cerveau total.

Nos résultats montrent que L'imidaclopride provoque des effets nocifs sur l'organisme se traduit par des altérations dans le bilan de stress oxydatif enzymatique et non enzymatique qui traduit par une perturbation de taux de MDA, l’activité de GST , et par une augmentation de l'activité des indicateurs biologiques d'oxydation dans le cerveau en globale. Et que l’intensité de toxicité augmente avec l’augmentation de la dose, et c’est ce que les résultats ont montré entre les deux doses 5 et 50 mg/kg/jour.

L'extrait de mélisse a amélioré l'équilibre de détoxication et atténué les effets nocifs de l'imidaclopride. La mélisse semble être un antioxydant efficace pour réduire le déséquilibre entre la formation de radicaux libres et les systèmes antioxydants dans le corps.

Mots clés : Stress Oxydant, Néonicotinoïdes, Imidaclopride. Mélissa *officinal*.

Abstract

The objective of the present study was to evaluate the neurotoxicity of an Imidacloprid neonicotinoid at two doses of Imidacloprid (5 & 50 mg/kg/day) for 40 days at the cytoplasmic level and the preventive and curative effect of Melissa extract (10 mg/kg/day) against this toxicity in *Wistar* rats.

Our experiments targeted several basic areas of experimentation, starting with the work of cellular enzymes and cell death. Our study is divided into several important areas of study, studies of oxidative stress parameters (MDA, GST), morphology parameters (relative organ weight %). Evaluation of neurotransmitter parameters (AchE) and the effects of Imidacloprid (IMI) on metabolic parameters (lipids, proteins, carbohydrates) in the whole brain.

Our results show that Imidacloprid causes harmful effects on the organism, resulting in alterations in the balance of enzymatic and non-enzymatic oxidative stress, which translates into a disturbance in the level of MDA, the activity of GST, and an increase of the activity of biological indicators of oxidation in the brain as a whole. And that the intensity of toxicity increases with increasing dose, and this is what the results showed between the two doses 5 and 50 mg/kg/day.

Lemon balm extract improved detoxification balance and lessened the harmful effects of Imidacloprid. Lemon balm appears to be an effective antioxidant in reducing the imbalance between the formation of free radicals and the antioxidant systems in the body.

Keywords: Oxidative Stress, Neonicotinoids, Imidacloprid. Melissa *officinalis*.

Acknowledgements

We extend our heartfelt thanks to everyone who contributed to the production of this work, starting with ***Pr. Rashid,*** *who helped us a lot in the memorandum and correcting mistakes*

I express my thanks to all the members of the jury:

*- Mme.****Djermene*** *the Examiner*

-Mr. ***Goudjil*** *the prisident*

. We also thank Dr. ***Sarra Zouaoui****, who worked a lot and stayed up more than us in this modest work. We also thank- I would also like to address all my gratitude to Elham who also gave us a helping hand*

We would like to thank the honorable attendees for coming with us on this special day for us, the day of producing the fruit of our labors all these years, the fruit of reaping and reaping the fruits of hard work.

Our joy is beyond description here, it is a very special feeling for us

Dedication

Praise be to God until the praise reach its end. Praise be to God, who enabled us to complete our memoran and accomplish it in this form.

thank every one who helped us from near or far.

eginning with my great and generous parents, my mother who gave birth to and worked hard in my upbringing and stayed up my sick nights, my mother my sweet smile to my mother who provided me with everything I needed without asking, and who swore to start in her name all my works and my father who knocked the stone and did all the hard work for me, my studies, who suffered more than me To the man who raised me and gave me life, my father with you I live and with you I die To my brothers who helped me with everything I needed, Bilal Yassin Munther and my dear sisters, my dear ones, Salwa Yamina Maryam Khadija and Faryal. Thank you for all the help and happiness you have given me to everyone near and far.

To all the friends you brought together by chance or not, Faith Hana, Iman Rayan. Everyone whom I mentioned in my diary and did not mention them in my tongue, you are in the heart.

My friends,zohour and Hassna, shared the suffering of writing and every small and big thing in accomplishing this scientific research

And lastly, a heartfelt thank you to everyone I knew, and I mentioned them,

hassna.m + hassna.s+ zouhor

Abreviations list

BSA : Bovine Albumin Serum

° C : Celsius

Ca $^{++}$: Calcium ++

Ca2 + : Calcium ion

CDNB : 1-chloro, 2.4-dinitrobenzene

Cu : Copper

$C_9H_{10}CIN_5O_2$: Imidaclopride

Cl : Chlor

DDT : Dichloro-Diphenyl-Trichloroethane

DTNB : 5, 5'-dithiobis (2-nitrobenzoic acid or Ellman's reagent

DO : Optical density

EFSA : European Food Safty Agency

ECHA : European Chemical Agency

EDTA : Ethylene-Diamine-Tetraacetic Acid

Fd : Dilution factor

Fe : Iron

GSSG : Oxidized Glutathione

H_2 : Dihydrogen

HO : hydroxyl.

HO_2 • : Radicalhydroperoxyle

Hcl : hydrochloric acid

HOCl : Hypochloric acid

H_2O_2 : Hydrogen peroxide

k : kelvin unit international thermodynamic temperature system

LC_{50} : Lethal concentration 50

LD_{50} : Lethal dose 50

mAChRs : muscarinic-type acetylcholine receptors

M : Mole

M-1cm- : Mole-1 centimeter-1

Mn : Manganese

nm : Nanometer

NO : Nitric oxide

NOAEL : No observed adverse effect level

NaoH : Sodium hydroxide

NO_3 : Peroxynitrite

nAChR : nicotinic acetylcholine receptors

(**nAChRs)** : post-synaptic nicotinic acetylcholine receptors

OH. : Hydroxyl radical

O_2- : The superoxide anion

PAMP : Molecular Pattern Associated with a Pathogen

PPSE : Excitatory Post-synaptic Potential

ROO • : Pyroxyl radicals

ROOH : Hydroperoxydelipidique

SNC : Central Nervous System

SNP : Peripheral Nervous System

TCA : Trichloroacetic acid

TBA : Thiobarbituric acid

List of figuers

List of tables

Tables of Contents

Partical side

Results

Discussion


Introduction

Introduction

Introduction

Before evolution, life was simple dependent on agriculture, as it was a difficult process that used on lab or and animals but, that was not enough for the quality of production, it was mostly crops spoil due the harmful in sects, it causes pathology for living begins the protection of agriculture one of the most important operations it often requires a chemical treatment such as pesticides.

Pesticides are used widely in agriculture to control insects all over the world with mor than 10,000 commercial formulations of approximately 450 pesticidal compounds currently in use **(Yousef et *al*., 2003)**. Neonicotinoid insecticides, as a replace ment of organophosphates, have been used globally for the treat ment of agricultural pests The most common neoincotinoid insecticides include imidacloprid, acetamiprid, nitenpyram, and clothianidin **(Tian, 2016).**

In particular .Imidacloprid is the most used active ingredient of the neo nicotinoid insecticides, and its activity is focused on whole plant protection. It acts against Homopteran insects, such as rice hoppers or aphids, as well as against some other insects such as thrips, whiteflies, termites, turf insects, and some beetles. This compound is most commonly used on rice , maize , sunflowers , rape , potatoes , sugar beets , vegetables , and fruits crops **(Biever et *al*., 2002).**

Imidacloprid interferes with the transmission of stimuli in the insect's nervous system by causing a blockage in the nicotinergic neuronal pathway. This pathway is more common in insects than in warm blooded animals, making the chemical more toxic to insects than to warm blooded animals **(Okazawa et *al*., 2002).** However, a similar a284 subunit of the nicotinic acetylcholine receptor (nAchR), The brain is particularly vulnerable to the oxidative damage because of its richness of peroxidable fatty acid , its high - energy requests and its relatively weak antioxidant power **(Carole, 2011).** This irreversible molecular damage is the first cause of the neurodegenerative diseases **(Adams et *al*., 1990).**

The harmful effects and pathologies, social reactions behavior can be corrected by medicinal plants; Many medicinal plants have several therapeutic activities because most often they contain several active principles whose effects are additive and / or complementary. This phenomenon also explains why in phytotherapy, several plants or parts of plants are frequently used in combination in order to obtain an optimal effect.

Introduction

Among the main plants of our study, the Mélissa officialisse known for its richness in secondary metabolism products and particularly in essential oils and tenin alkaloids **(Baba, 1999).** The active ingredients in lemon balm are contained in essential oil. Due to its relaxing, sedative and antispasmodic properties, lemon balm is particularly effective in combating stress and anxiety, as well as in treating digestive disorders of nervous origin (**Newman et *al*., 2000)** We find an extract of lemon balm in the Carmine lemon balm water, which is a reputed alcohol ate indicated as antispasmodic, stimulating, for nervous affections, palpitations or even the lack of appetite **(Debuigne, 2013)**. Herbal teas because of its very pleasant taste, or in hydro-alcoholic extract to benefit from the benefits of its essential oil **(Morel, 2017).** It is found in several drugs **(Vidal, 2018)**

The objective of our experimental work is centered on the evaluation of the sub-acute toxicity of the new neonicotinoid insecticide "Imidacloprid" and the opposite effect of lemon balm in the oral *wistar* rat. To this end, we studied ourselves studying its impact on body weight on certain biochemical parameters as well as on the essential organ the brain. Confirmation of the optimal correction of Melissa is done by analyzing biochemical, oxidative stress and their implication on the structural integrity of brain cells and we evaluated the preventive effects of Melissa from the results obtained, we divided the work into 2 main parts, one theoretical and the other practical we will look at them in the content.

Bibliographic part

Chapter 01:

Generality

Chapter 01: Generality

I. Generality

1. Definition of Oxidative Stress

A popular definition of oxidative stress can be found an imbalance between the production and manifestation of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. In a textbook on oxidative stress published in 1985 by Sies, a very similar definition can be found: "the imbalance between oxidants and antioxidants potentially leading to damage." In any case, distinct issues are suggested in these definitions: a production of reactive or activated forms of oxygen (ROS) with potential toxicity and the presence of detoxifying systems **(Tissier, 2011).**

2. Physiological Roles of Oxidative Stress

The production and release of ROS are involved in several physiological pathways, and the intracellular concentrations of further enhance the production of ROS by the xanthine oxidase and NOX enzymes, respectively. Intense exercise or exercise in untrained individuals is more likely to be associated with increased oxidative stress, as compared with regular and mod-erately intense aerobic physical activity. Not surprisingly, a beneficial role of antioxidant supplementation before exercise has not been confirmed, even though antioxidant supplementa-tion decreases the measurable levels of biomarkers of oxida-tive stress. **(Tissier, 2011).**

3. The consequences of oxidative stress

The damage induced by ROS are: lipid peroxidation, protein oxidation, DNA mutations. These alterations can lead to a loss of function and integrity, or even cell death, in particular by apoptosis (programmed cell death). ROS initiate apoptosis by activating the opening of the permeability transition pore. **(Tissier, 2011).**

3.1.Lipid peroxidation

The first targets of ROS are lipids, especially those present in cellular and subcellular membranes. Membranes rich in polyunsaturated fatty acids (PUFAs) are very sensitive to oxidation due to their high degree of unsaturation **(Hulbertl, 2005; Pamplona et *al.*, 2000).** The oxidation of lipids generates lipid peroxides, which are very reactive. Lipid peroxidation induces a decrease in membrane fluidity, permeability and excitability **(Hong et *al.*, 2006).** All of this can lead to apoptosis if the damage is severe **(McMichael, 2007).** Lipid peroxidation of erythrocyte membrane constituents has serious consequences and can lead to severe oxidative hemolysis **(Gallo et *al*., 2013).**

3.2. Oxidation of proteins

Similar to the oxidation of lipids, proteins are also susceptible to being oxidized by ROS. This oxidation causes the introduction of a carbonyl group into the protein **(Levine, 2002).**

3.3. Lésions sur l'ADN

Les lésions des ERO sur l'ADN des cellules sont principalement provoquées par le radical hydroxyle. Le monoxyde d'azote peut également participer à une fragmentation de l'ADN. Les dommages oxydatifs de l'ADN peuvent aboutir à une mutagénèse, une perturbation des processus de réplication, de transcription et de traduction provoquant un arrêt des synthèses et pouvant mener à la mort cellulaire **(Lenzi, 2011).** Le stress oxydant accélère et amplifie toutes les lésions spontanées produites sur l'ADN **(Tissier, 2011).**

3.4 Biochemical consequences of oxidative stress

Excessive production of free radicals causes damage to biological molecules such as DNA, proteins and lipids **(Loft and all., 2008).**

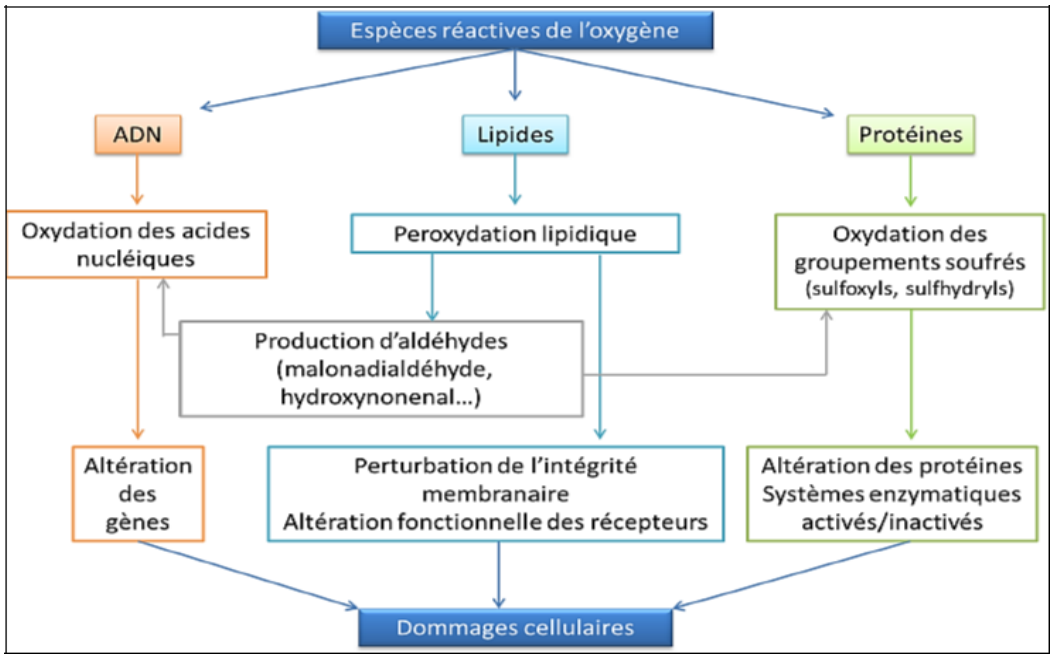


Figure 01: The different target of Reactive Species Oxygen **(Lof t et *al.*,2008).**

4. Antioxidant system

Antioxidants was defined as substances that are able, at relatively low concentrations, to compete with other oxidizable substrates and, thus, to significantly delay or inhibit the oxidation of these substrates **(Dröge, 2002).** According to Shi and their collaborators **(2001),** there are several lines of defense as follows:

(i) inhibiting the formation of active oxygen species and free radicals,

(ii) (ii) preventing chain initiation by scavenging initiating radicals,

(iii) (iii) repairing, de novo and clearance of oxidatively damaged lipids, proteins and DNA.

4.1. Enzymatic antioxidant systems

4.1.1. Catalase

Catalase is also responsible for removing H_2O_2 by transformation into H_2O and O_2. Unlike GPx, the affinity of catalase for H_2O_2 is high only when hydrogen peroxide levels are increased **(Mates et *al*, 1999; Powers et Lennon, 1999).** This enzyme is abundant in the liver and red blood cells. It is found preferentially in the peroxisomes and in smaller quantities in the cytosol

4.1.2. Glutathione peroxidase and glutathione reductase

These two enzymes are located in the cytosol and in the mitochondria. Glutathione peroxidase is a selenoenzyme (Se-GPx) that plays a very important role in the detoxification of hydrogen peroxide. Glutathione reductase (GR), on the other hand, has the role of regenerating GSH from GSSG while using NADPH as a cofactor **(Martínez, 1995; Sorg, 2004).**

4.2. Non-enzymatic antioxidant systems

4.2.1. Glutathione

Reduced glutathione (GSH), reduces hydrogen peroxide and/or organic peroxides through the reaction catalyzed by glutathione peroxidase (GPx). It can also reduce radicals formed by the oxidation of vitamins E and C, thereby lowering levels of lipid peroxidation **(Power et Lennon, 1999; Packer et *al.*, 1997).** The reduced glutathione/oxidized glutathione (GSH/GSSG) ratio is often used as a marker of oxidative stress because the greater the flow of H2O2, the more reduced glutathione is consumed and oxidized glutathione increased **(Ji et *al.*, 1992).**

4.2.2. Vitamin E and vitamin C

Vitamins E (α-tocopherol) and C (ascorbic acid) seem to be most important in the fight against oxidative stress. Since vitamin E is fat-soluble, it binds to membranes and can thus sequester free radicals preventing the propagation of lipid peroxidation reactions **(Evans, 2000; Packer et *al*, 1997**).

The water-soluble vitamin C is found in the cytosol and in the extracellular fluid; it can directly capture O2•- and OH•. It can also reduce the α-tocopherol radical and thus allow better efficacy of vitamin E **(Evans, 2000; Packer et *al*., 1997).**

II. Organ (brain)

1. General information on the nervous system

The nervous system is a complex network of nerves and cells that carry messages to and from the brain and spinal cord to various parts of the body. The nervous system includes both the Central nervous system and Peripheral nervous system. The central nervous system is made up of the brain and spinal cord, and the peripheral nervous system is made up of the Somatic and the Autonomic nervous systems **(Georges et *al*., 2008)**.

2. Definition

The brain is an amazing three-pound organ that controls all functions of the body, interprets information from the outside world, and embodies the essence of the mind and soul. Intelligence, creativity, emotion, and memory are a few of the many things governed by the brain. Protected within the skull, the brain is composed of the cerebrum, cerebellum, and brainstem. The brain receives information through our five senses: sight, smell, touch, taste, and hearing - often many at one time. It assembles the messages in a way that has meaning for us, and can store that information in our memory. The brain controls our thoughts, memory and speech, movement of the arms and legs, and the function of many organs within our body. **(Imbert, 2006; Verkhratsky et Butt, 2007; Bear et *al*., 2016).**

3. Anatomy and physiology of the brain

The brain is composed of the cerebrum, cerebellum, and brainstem:

Cerebrum: is the largest part of the brain and is composed of right and left hemispheres. It performs higher functions like interpreting touch, vision and hearing, as well as speech, reasoning, emotions, learning, and fine control of movement.

Cerebellum: is located under the cerebrum. Its function is to coordinate muscle movements, maintain posture, and balance.

Brainstem: acts as a relay center connecting the cerebrum and cerebellum to the spinal cord. It performs many automatic functions such as breathing, heart rate, body temperature, wake and sleep cycles, digestion, sneezing, coughing, vomiting, and swallowing. **(Marieb, 2005).**

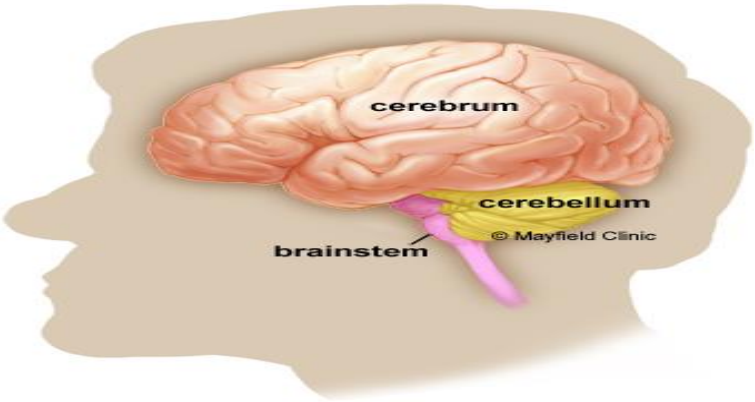


Figure 02: The brain has three main parts: the cerebrum, cerebellum and brainstem. **(William, 1981; Pascal, 2010).**

4. Right brain – left brain

The cerebrum is divided into two halves: the right and left hemispheres They are joined by a bundle of fibers called the corpus callosum that transmits messages from one side to the other. Each hemisphere controls the opposite side of the body. If a stroke occurs on the right side of the brain, your left arm or leg may be weak or paralyzed. **(Christensen et *al*., 2013)**

Chapter 02:

Pesticides

Chapter 02: Pesticides

Generality

A pesticide is any substance used to kill, repel, or control certain forms of plant or animal life that are considered to be pests. Pesticides include herbicides for destroying weeds and other unwanted vegetation, insecticides for controlling a wide variety of insects, fungicides used to prevent the growth of molds and mildew, disinfectants for preventing the spread of bacteria, and compounds used to control mice and rats. Because of the widespread use of agricultural chemicals in food production, people are exposed to low levels of pesticide residues through their diets. Scientists do not yet have a clear understanding of the health effects of these pesticide residues. **(National institute of environmental Health).**

I. Pesticides

1. Definition of Pesticides

Any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances that may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies.**(Bygu's, 2022).**

2. Classification

Classification of pesticides is according to:

- The types of pests they kill :

- Insecticides – Insects
- Herbicide – Plants
- Rodenticides – Rodents (rats & mice)
- Bactericides – Bacteria
- Fungicides – Fungicide
- Larvicides – Larvae. **(Tomizawa et Casida, 2011)**

- Depending on how biodegradable they are:

- **Biodegradable Pesticides**

Biodegradable pesticides are those that can be broken down into harmless compounds by microbes and other living organisms within less period of time.

- **Non-Biodegradable Pesticides**

Few pesticides are known as non-biodegradable, also called persistent pesticides. The most long-lived pesticide materials include aldrin, parathion, DDT, chlordane, and endrin, they take a long period of time to break down. These pesticides can survive in the soil for over 15 years or more**. (Tomizawa et Casida, 2011)**

- Another way of thinking about pesticides is considering the chemical pesticides extracted from a common source or some production method (Chemical pesticides) :

- **Organophosphates**

Many organophosphates are insecticides that impact on the nervous system by compromising the enzyme that regulates the neurotransmitter. **(Tomizawa et Casida, 2011)**

- **Carbamate**

Carbamate pesticides affect the nervous system by compromising the enzyme that regulates the neurotransmitter similar to the organophosphates, but carbamate enzyme effects are usually reversible. **(Tomizawa et Casida, 2011)**

- **Organochlorine Insecticides**

This type was common in the early years when pesticides came into the market. Many countries have banned organochlorine insecticides from their markets because of their impacts and persistence on health and the environmental factors (e.g., DDT, chlordane and toxaphene). **(Tomizawa et Casida, 2011)**

- **Pyrethroid**

There are synthetic variants of pyrethrin, a naturally occurring pesticide present in chrysanthemums (Flower). Their development is such a way they can maximize their environmental resilience. **(Tomizawa et Casida, 2011).**

- **Herbicides**

The commercial production of sulfonylureas herbicides was for weed control like flupyrsulfuron-methyl-sodium, ethoxysulfuron, chlorimuron-ethyl, bensulfuron-methyl, azimsulfuron, and amidosulfuron, rimsulfuron, pyrazosulfuron-ethyl. **(Tomizawa et Casida, 2011).**

3. Use of Pesticide and their harmful

- Pesticides are used to control organisms that are considered to be harmful, or pernicious to their surroundings.
- Insecticides can protect animals from illnesses that can be caused by parasites such as fleas.
- Pesticides can prevent sickness in humans that could be caused by mouldy food or diseased produce. Herbicides can be used to clear roadside weeds, trees, and brush.
- They can also kill invasive weeds that may cause environmental damage. Herbicides are commonly applied in ponds and lakes to control algae and plants such as water grasses that can interfere with activities like swimming and fishing and cause the water to look or smell unpleasant.
- Uncontrolled pests such as termites and mould can damage structures such as houses. Pesticides are used in grocery stores and food storage facilities to manage rodents and insects that infest food such as grain**. (Jeschke et *al*. 2011).**

4. Mechanism of action of Pesticide

The chemical structure of an insecticide generally defines its target site and its mode of action at that target site Mode of action, alternatively, is defined as the action of an insecticide at its target site. In other words, the mode of action of an insecticide is the way in which it causes physiological disruption at its Most of the insecticides commonly used by PMPs can be technically classified as neurotoxins — i.e., their target site within the target organism is some aspect of the nervous system. **(Slide, 2021).**

To understand the mode of action of insecticides that target the insect nervous system, it is important to have a basic understanding of how the nervous system operates. In insects, the nervous system is composed of a series of highly specialized, interconnected cells, along which travel electrical charges called impulses (see Figure 1). Impulses are driven by the movement of electrically charged sodium, potassium and chloride ions into and out of nerve cells. The uninterrupted transmission of impulses along this series of cells is required for a

nervous system to function properly. In insects, prolonged or irreversible disruption of a normal-functioning nervous system will result in death. **(Slide, 2021).**

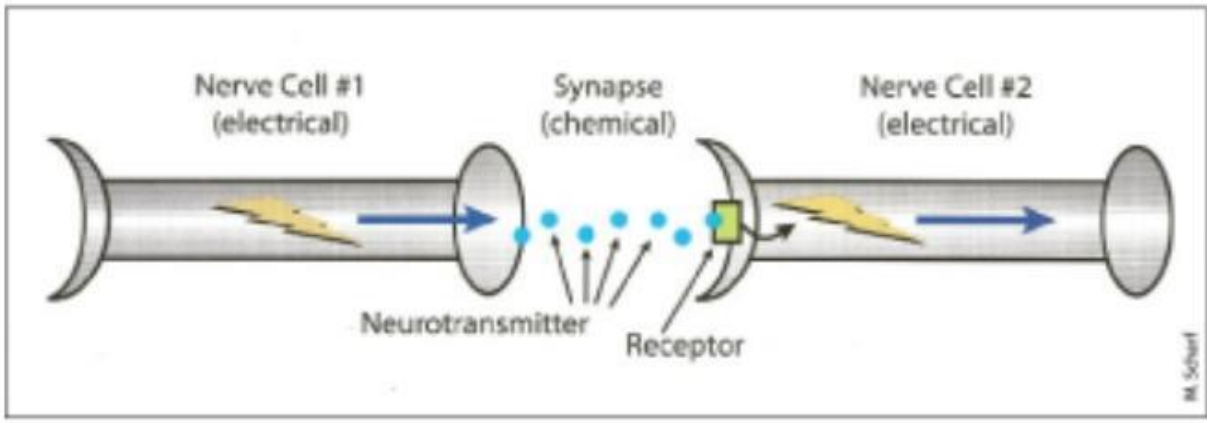


Figure 03: The nervous system in insects is composed of a series of interconnected cells, **(Slide, 2021).**

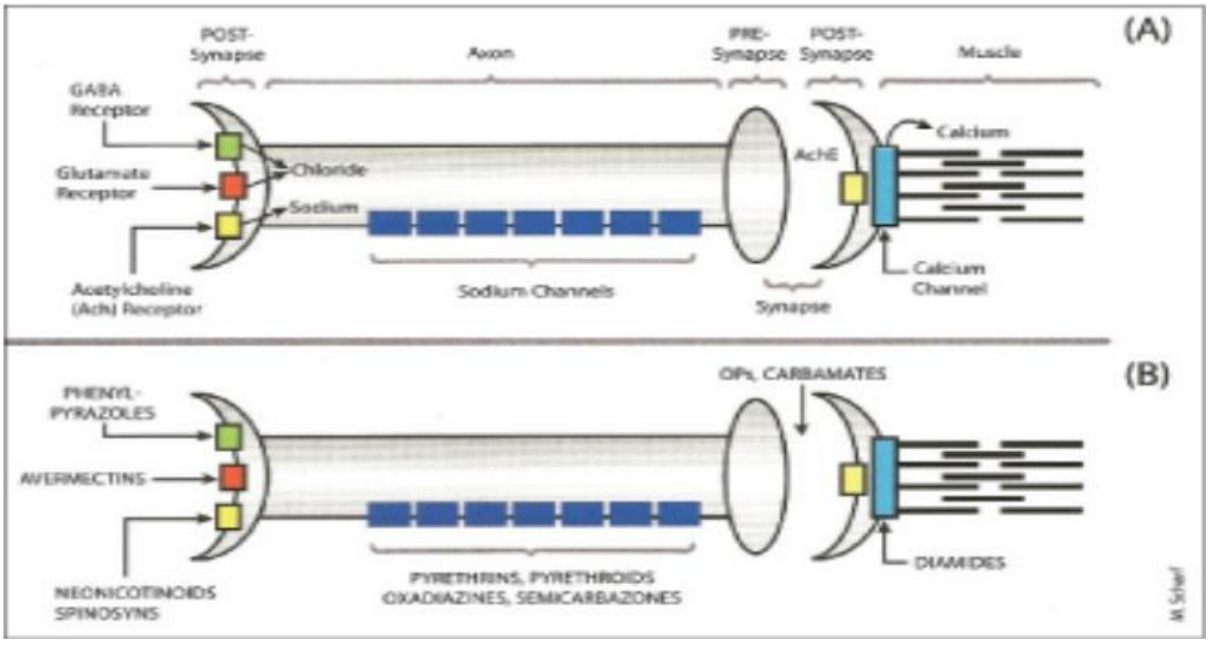


Figure 04: Neurological target site of various insecticide classes used by the urban and structural pest management industry **(Slide, 2021).**

5. Impact of Pesticide on Environmental and human health

5.1. Health effects

The health effects of pesticides depend on the type of pesticide. Some, such as the organophosphates and carbamates, affect the nervous system. Others may irritate the skin or eyes. Some pesticides may be carcinogens. Others may affect the hormone or endocrine system in the body. **(Upa, 2021; Vnite D States, Envirenement Production A geng).**

Pesticides are toxic chemicals designed to be deliberately released into the environment. Although each pesticide is meant to kill a certain pest, a very large percentage of pesticides reach a destination other than their target. Pesticides easily contaminate the air, ground and water when they run off from fields, escape storage tanks, are not discarded properly, and especially when they are sprayed aerially.**(Pesticides Action New work Uk, 2017).**

a) Water

Pesticides can be found in rain, ground water, streams, rivers, lakes and oceans.

There are four major ways that pesticides can reach the water:

• It can drift outside of the area of where it was sprayed.

• It can leach through the soil.

• It can be carried

• Orit may be spilled accidentally.**(Pesticides, Action, New work Uk, 2017).**

b) Soil

The use of pesticides decreases the general biodiversity in the soil. Soil quality is higher without chemicals and this allows for higher water retention, necessary for plants to grow Pla Soi **(Pesticides Action New Work Uk,2017).**

Nitrogen fixation, which is necessary for the growth of many large plants, is hindered by pesticides that can be found in soil. This can lead to a large decline in crop yields. Application of pesticides to crops that are in bloom can kill honeybees, which act as pollinators. This also decreases crop pollination and reproduction.

c) Animals

Animals may be poisoned by pesticide residues that remain on food after spraying. An application of pesticides in an area can eliminate food sources that certain types of animals need, causing the animals to relocate, change their diet, or starve. Poisoning from pesticides can even make its way up the food chain ; for example, birds can be harmed when they eat insects and worms that have consumed pesticides **(Pesticides Action New Work Uk, 2017).**

d) Aquatic Life

Fish and other aquatic biota may be harmed by pesticide-contaminated water. Application of herbicides to bodies of water can cause plants to die, diminishing the water's oxygen and suffocating the fish. Repeated exposure of some pesticides can cause physiological and behavioural changes in fish that reduce populations, such as abandonment of nests, decreased immunity to disease, and increased failure to avoid predators.

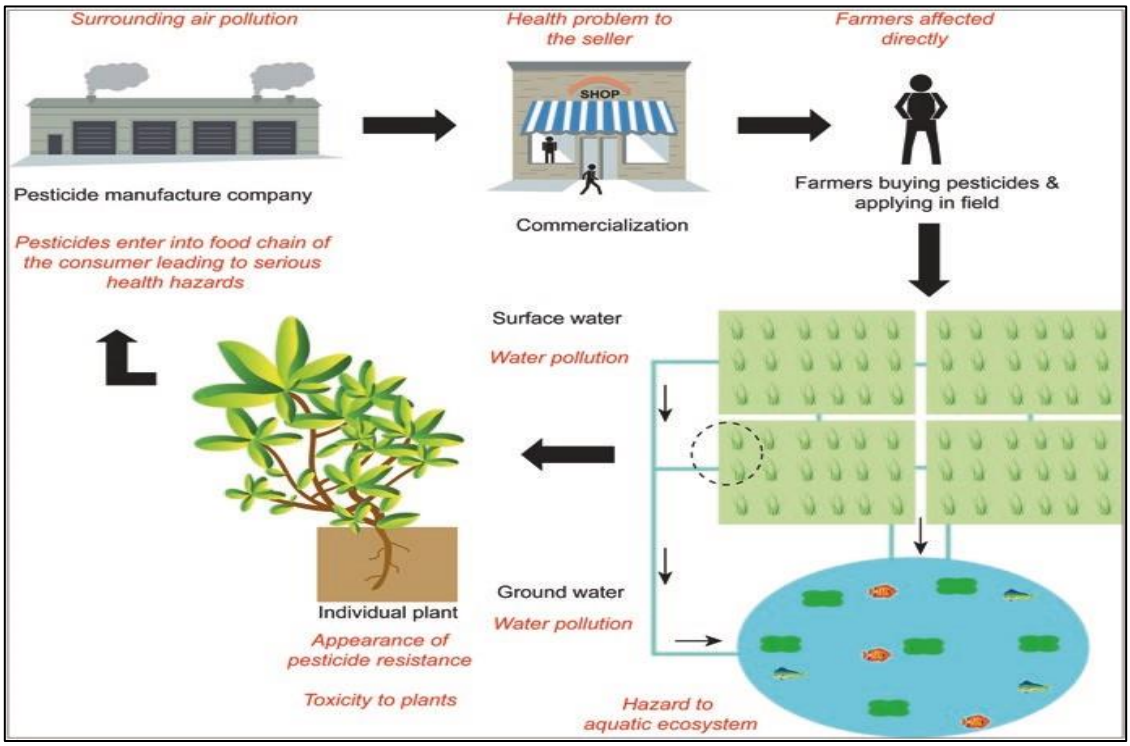


Figure 05: Effect of pesticides in the environment. **(Kagaba, 1997).**

II. Neonicotinoids

1. Definition

Neonicotinoids are a group of insecticides derived from nicotine isolated from the tobacco plant (Nicotiana tabaccum) which presents insecticidal activity and has been used extensively as natural insecticide. The developing road from nicotine to neonicotinoids was long and complicated. Neonicotinoids effective control of insect pests and helminthic parasites has been achieved by targeting invertebrate (insect) nAChRs.**(Matsuda et *al*, 2005; Tomizawa et Casida, 2003-2005).**Introduction of the 6-chloro-3pyridylmethyl and nitroimine moieties led to the development of the first type of nicotinic insecticide called imidacloprid.**(Kagaba, 1997).**

2. The different types of Neonicotinoids

Neonicotinoids are used in over 120 countries and have 140 different crop uses.**(Lundin, et *al*, 2015).** There are 8 neonicotinoids that are commercially available : imidacloprid, thiacloprid, clothianidin, thiamethoxam, acetamiprid, nitenpyram, dinotefuran, and sulfoxaflor.**(Enveronmental protection 2015).**

3. Toxicity of Neonicotinoids

3.1. Poisoning Humans

Published studies regarding human exposure to neonicotinoids and toxicity is quite rare in spite of its widespread use. One case reports of attempted suicide (22-year-old male with clinical toxicity due to intentional ingestion of 30mL of imidacloprid at a concentration of 17.8%, described signs of toxicity including fever, drowsiness, dizziness, vomiting, disorientation, an palpitation. Although the initial tachycardia could be attributed to nicotinic effects of the compound, it is unclear if the bradycardia was due to toxic effects or metabolic disturbances. The vomiting again could have been secondary to gastric irritation ; however, this was not evaluated with an upper gastrointestinal scopy.**(Wismer, 2004).**

Lastly a case report, includes a previously healthy 35-year-old male farmer (body weight, 85kg) was found by his family to be drowsy with severe nausea and copious vomiting. The patient reported that approximately 350mL of imidacloprid was ingested in a suicide attempt about 30min previously. Disorientation, drowsiness, dizziness, and palpitations had developed by the time of arrival at a local hospital (1h after ingestion) and copious vomiting, which progress to coma, tachycardia, hypertension, apnostic respiration,

mydriasis with sluggish reaction to light, fever, leukocytosis, hypokalemia, hypernatremia, and finally bradicardia and cardiopulmonary arrest **(They, 1999).**

3.2.Poisoning Animals

There is limited published information detailing adverse effects of imidacloprid in dogs and cats. Because the drug is bitter tasting, oral contact may cause excessive salivation. Signs of poisoning from imidacloprid are similar to nicotinic signs, including lethargy, vomiting, diarrhea, hypersalivation, initial tremors, muscle weakness, and ataxia. Acute oral ingestions can cause salivation and vomiting. At very high oral imidacroprid exposures may lead to lethargy, vomiting, diarrhea, salivation, muscle weakness, and ataxia, which are all indicative of nicotinic signs. **(Frontiers et Bioscience, 2008).** Other signs of exposure at high doses are tremors, uncoordinated gait, and reduced motility/activity, apathy onset of signs in minutes, fast reversible.**(Lonare et *al*, 2014).**

III. Imidaclorprid

1. Definition

Imidacloprid (IMI) is one of the major representatives of the new generation of neonicotinoid insecticides. It was patented for the first time in 1985 by Bayer and was placed on the market in 1991.It is a nicotine derived compound (neonicotinoid) with a large potential distribution due to its agonistic action on insect nAChRs and its selective toxicity to insects over vertebrates.**(Matsuda et *al.*, 2001; De Mrc et *al*, 2007).** IMI [1-[(6-chloro-3 pyridynil) methyl]-N-nitro-2- imidazolidinimine] has the molecular formula(C9H10ClN5O) , with a molecular weight of 255.7 g mol-1. In appearance, it consists of colourless crystals .

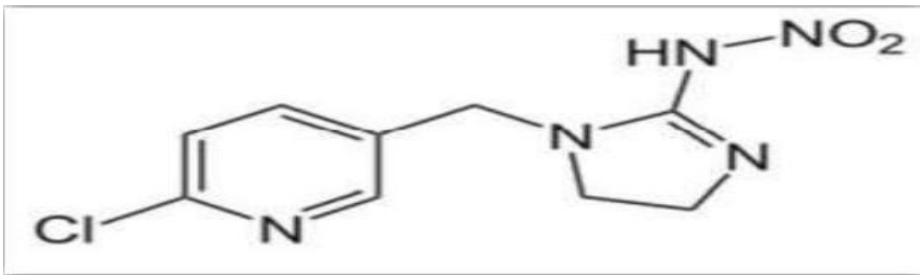


Figure 06: Chemical Structure of Imidacloprid (https://www.chemsrc.com)

Chapter 02: Pesticides

This component is a relatively new pest control substance, which is having the fastest growing sales worldwide.**(Millar et Denholm, 2007).** and is generating increasing concern on its possible impacts on natural ecosystems.**(Tomizawa, Tomlin, 1994; et Casida, 2005).** IMI‟s physicochemical properties render it useful for a wide range of application techniques, including foliar, seed treatment, soil drench, and stem application. **(Tomlin).** It is a versatile, broad-spectrum, systemic insecticide with activity against sucking insects (eg aphids, leafhoppers, whiteflies, and termites), several species of Coleoptera, Diptera, Lepidoptera and parasites on a different types of crops.**(Hazardous, 2006).**In addition, it is also applied as veterinary medicine against parasites and fleas in dogs and cats.

2. Physico chemical Property of Imidacloprid

- Imidacloprid is made up of colorless crystals with a slight but characteristic odor.**(Hazardous, 2006).**
- Vapor pressure.**(Olive et *al.*, 2005).** 3 x 10-12 mmHg at 20 °C
- Octanol-Water Partition Coefficient (Kow)…[24]: 0.57 at 21 °C
- Henry's constant**(Hazardous, 2006).**: 1.7 x 10-10 Pa·m3/mol
- Molecular weight**(Hazardous, 2006).**: 255.7 g/mol
- Solubility (water) **(Hazardous, 2006).**: 0.61 g/L (610 mg/L) at 20 °C
- Soil Sorption Coefficient (Koc).**(G Ag, 1999).**

3. Metabolism

- Mammals metabolize imidacloprid in two major pathways discussed below. Metabolism occurs primarily in the liver **(Klein et *al.*, 1999).**
- In the first pathway, imidacloprid may be broken by oxidative cleavage to 6-chloronicotinic acid and imidazolidine. Imidazolidine is excreted in the urine, and 6-chloronicotinic acid undergoes further metabolism via glutathione conjugation to form mercaptonicotinic acid and a hippuric acid. **(Klein et *al.*, 1999); (Klein, 1987).**
- Imidacloprid may also be metabolized by hydroxylation of the imidazolidine ring in the second major pathway **(Klein et *al,* 1999); *(*Klein, 1987).**
- Metabolic products from the second pathway include 5-hydroxy and olefin derivatives **(Fossen, M, 2006).**

4. Mode of action

4.1. target Organisms

Imidacloprid is designed to be effective by contact or ingestion.**(Hazardous, 2006).** It is a systemic insecticide that translocates rapidly through plant tissues following application Imidaclopride. **(Hazardous, 2006)**.**(Fossen, 2006).** acts on several types of post-synaptic nicotinic acetylcholine receptors in the nervous system. **(Buckingham et *al.*, 1997; Matsuda et *al.*, 2005).**In insects, these receptors are located only within the central nervous system. Following binding to the nicotinic receptor, nerve impulses are spontaneously discharged at first, followed by failure of the neuron to propagate any signal.**(Flattum et *al.*, 2001).**Sustained activation of the receptor results from the inability of acetylcholinesterases to break down the pesticide**(Matsuda et *al.*, 2005).**This binding process is irreversible.**(Sheets *al*., 2001).**

4.2.Non-target Organisms

Imidacloprid's mode of action is similar on target and non-target beneficial insects including honeybees, predatory ground beetles and parasitoid wasps. **(Fossen, 2006).**However, imidacloprid is ineffective against spider mites and nematodes..**(Hazardous, 2006)**. Mammalian nicotinic receptors are made up of a number of subtypes**(Sheets, 2001).**In contrast to insects, these receptors are present at neuromuscular junctions as well as in the central nervous system…However, the binding affinity of imidacloprid at the nicotinic receptors in mammals is much less than that of insect nicotinic receptors **(Matsuda et *al.*, 2005).**

- This appears to be true of other vertebrate groups including birds. .**(Tomizawa and Tomlin, 1994; Casida, 2005).(Labelle Review Manual, U.S Envrennemental).**
- The blood-brain barrier in vertebrates blocks access of imidacloprid to the central nervous system, reducing its toxicity. **(Sheets, 2001).**

5.Imidaclorpride toxicity

Imidacloprid is very low in toxicity via dermal exposure.**(Gerais et *al).***and moderately toxic if ingested.**(Thjyssen et *al*).**but upon inhalation, its toxicity is variable. Its dust is considered slightly toxic but the aerosol form is highly toxic. The LC50 for inhalation is 0.05 mg/L of aerosol form & acute oral LD50 for moderate toxicity is 50–500 mg/kg.**(Wu, 2001).**

5.1 Animal toxicity

A very high oral dose may lead to lethargy, vomiting, diarrhea, salivation, muscle weakness and ataxia .Other features at high doses are uncoordinated gait, tremors, and reduced

activity**.**On acute exposure the signs of toxicity appear and disappear rapidly, with most resolving within 24 h of the exposure. If a lethal dose has been administered, death occurs within 24 h **(Huang et *al.*, 2006).**

5.2Human toxicity

There is a dearth of literature about human toxicity from imidacloprid. Few case reports of attempted suicides described signs of toxicity such as drowsiness, dizziness, vomiting, disorientation and, fever. **(Wu, 2001; Shsadnia, 2008)**. A 69-year-old woman who ingested a formulated product containing 9.6% imidacloprid suffered severe cardiac toxicity with complaints of disorientation, sweating, vomiting, increased heart and respiratory rates and died 12 h after the exposure**. (Agarwal et Srinvas,2007).** A 24-year-old man who accidentally inhaled a pesticide containing 17.8% imidacloprid while working on his farm was disoriented, agitated, incoherent, sweating and breathless following the exposure.

6. Relationship between the Pesticids Neonicotinoids (imidacloprid) and neurotoxicity

Insecticides play a relevant role in the control of insect pests. All of the chemical insecticides in use today are neurotoxicants, and act by poisoning the nervous systems of the target organisms. The taget sites for insecticides in insects. **(Frontiersbioscience,2008).**

1. Cholinesterase inhibitors (organophosphates and carbamates), followed by the pyrethroids, and by other more recently develope compounds, such the neonicotinoids. The organochlorine compounds were widely used until most of them were.**(Frontiersbioscience,2008).**
2. Banned in the mid-1970s ; however, some are still used in certain countries, and exposure of the general population still occurs .In an**(Frontiers bioscience ,2008)**
3. oral subacute neurotoxicity study rats were exposed to imidacloprid (45 and 90mg/kgbw) for the period of 28 days, produced significant decreased in spontaneous locomotor activity (SLA) and stimulates pain threshold along with pathological changes in the brain. The following neurotoxicity indicators (salivation, lachrymation, piloerection diarrhea, dyspnea, tremor, convulsion, paralysis) were taken as clinical signs of toxicity. AChE, ATPase and serum biochemical parameters such as creatine kinase, lactate dehydrogenase, sorbitol dehydrogenase and alkaline phosphatase levels were significantly decreased as result of imidacloprid exposure. Imidacloprid caused a significant decrease in antioxidant enzymes activity and non-enzymes level with increase in lipid peroxidation. The imidacloprid induced brain damage of biochemical markers and neurotoxicity in exposed rats **(Tomizawa and casida ,2003).**

Chapter 03:

Medicinal Plants

Chapter 03: Medicinal Plants

I. Medicinal plants

1. Definition

A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. This description makes it possible to distinguish between medicinal plants whose therapeutic properties and constituents have been established scientifically, and plants that are regarded as medicinal but which have not yet been subjected to a thorough scientific study.

A number of plants have been used in traditional medicine for many years. Some do seem to work although there may not be sufficient scientific data (double-blind trials, for example) to confirm their efficacy. Such plants should qualify as medicinal plants. The term 'crude drugs of natural or biological origin' is used by pharmacists and pharmacologists to describe whole plants or parts of plants which have medicinal properties **(Sofowora, 2008; Evans, 2008).**

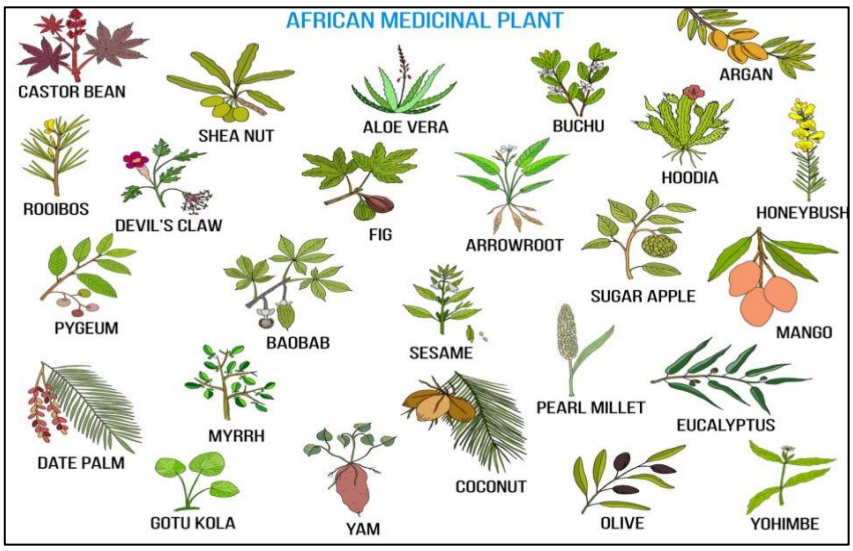


Figure 07: Medicinal Plants **(vectorstick. Com**)

2. Uses and Pharmacological Activity of Medicinal Plant

- Medicinal plants have been known as immune stimulants for thousands of years.
- The application of medicinal plants as natural and innocuous compounds has potential in aquacultures an alternative to antibiotics and immune prophylactics.
- The growing interest in these plants has increased world-wide because they are easy to prepare, cheap, and have few side effects on animals and the environment.
- A wide range of medicinal plants such as herbs, spices, seaweeds, herbal medicines, herbal extracted compounds, traditional Chinese medicines, and commercial plant-derived

products has been studied in various aquatic animals. The whole plant or its parts viz. roots, leaves, seeds, flowers or extract compounds can be used**.**

- The extraction process is simple, with ethanol and methanol being commonly used. Various chemicals used to extract compounds may lead to different degrees of effects on aquatic animals**.**
- The dosages and duration of time varies and the optimal levels have not been considered. Medicinal plants show their main properties as growth promoters, immune enhancers, where they act as antibacterial and antiviral agents to the host immune system **(modifiedfrom Velderrain-Rodríguez et *al*., 2014).**

3. Secondery metabolites of medicinal plants

Secondary metabolites (SMs) of medicinal plants are the material basis of their clinically curative effects. They are also important indicators for evaluating the quality of medicinal materials. However, the synthesis and accumulation of SMs are very complex, which are affected by many factors including internal developmental genetic circuits (regulated gene, enzyme) and by external environment factors (light, temperature, water, salinity, etc.). Currently, lots of literatures focused on the effect of environmental factors on the synthesis and accumulation of SMs of medicinal plants, the effect of the developmental growth and genetic factors on the synthesis and accumulation of SMs still lack systematic classification and summary. Here, we have given the review base on our previous works on the morphological development of medicinal plants and their secondary metabolites, and systematically outlined the literature reports how different environmental factors affected the synthesis and accumulation of SMs **(Yanqun, 2020).**

II. Phenolic compound's

1. Definition

They are compounds that have one or more hydroxyl groups attached directly to an aromatic ring. Phenol is the structure upon which the entire group is based. The aromatic ring in this case is, of course, benzene **(modifiedfrom Velderrain-Rodríguez et *al.*, 2014).**

The phenols are in many ways similar to alcohols of aliphatic structures where the hydroxyl group is attached to a chain of carbons. The phenolic hydroxyl group, however, is influenced by the presence of the aromatic ring. Because of the aromatic ring, the hydrogen of the phenolic hydroxyl is labile, which makes phenols weak acids **(Laura, 2019).**

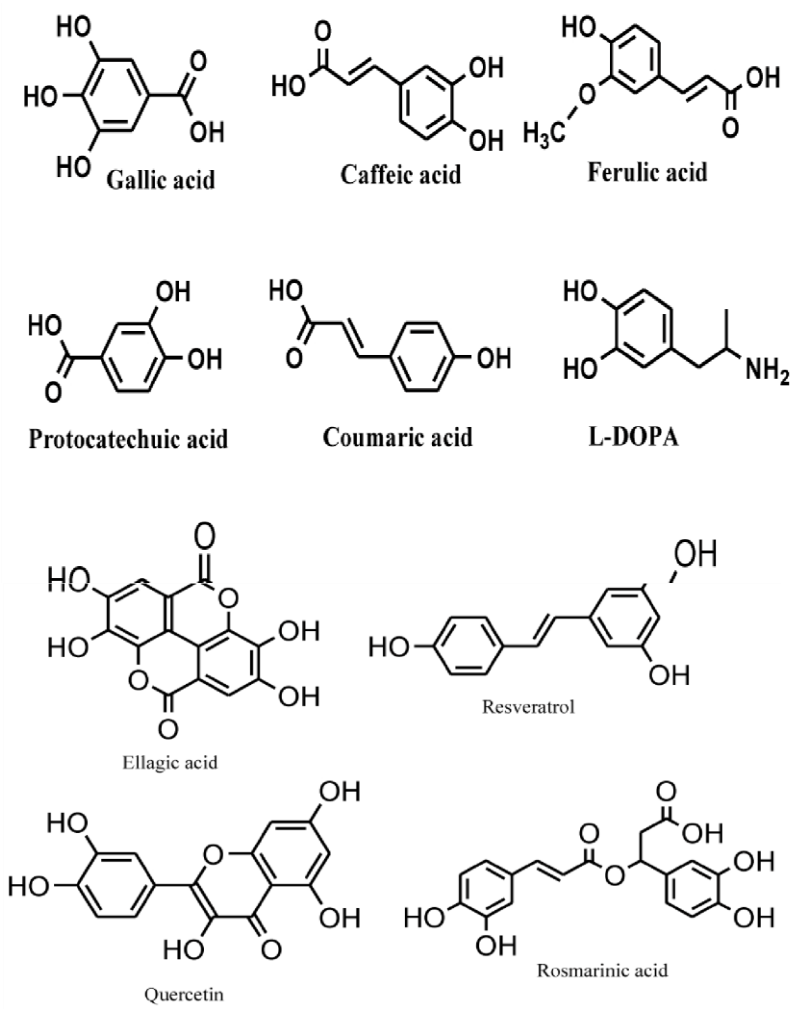


Figure 08: Common phenolic compounds in plants comprise an aromatic ring, bear one or more **(Velderrain-Rodríguez et *al*., 2014).**

hydroxyl Common phenolic compounds in plants comprise an aromatic ring, bear one or more hydroxyl substituents and range from simple phenolic molecules to highly polymerized compounds (modified substituents and range from simple phenolic molecules to highly polymerized compounds **(Velderrain-Rodríguez et *al*., 2014).**

2 . Health Benefits of Phenolic Compounds

-Many studies have reported the advantages of phenolic compounds, such as anti-aging, anti-studies have reported the advantages of phenolic compounds, such as anti-aging, anti-inflammatory, antioxidant and anti-proliferative agents. In addition to the adjustment of the above, there antioxidant and ant proliferative agents. In addition to the adjustment of the above,are relevant antioxidant enzymes to counter oxidants**(shukitt hale et *al*., 2008).**

-Phenolic compounds, including stress-linked phytochemicals, have been related to favorable compounds, including stress-linked phytochemicals, have been related to favorable mpacts,

which are caused by the consumption of fruits and vegetables, particularly due to their which are caused by the consumption of fruits and vegetables, particularly due to their antioxidantantioxidant activity **(Heima et *al*.,2002).**

3 . Classification and Properties of Phenolic Compounds

- Polyphenols have been a feature of plants since their early appearance. These compounds, also called secondary metabolites, are indeed crucial for many important functional aspects of plant life, including structural roles in different supportive or protective tissues, involvement in defence strategies, and signalling properties, particularly in the interactions between plants and their environment. Collectively, higher plants synthesise several thousand different known phenolic compounds, and the number of these which have been fully characterized is continually increasing **(Magalhaes et *al*., 2009).**
- The term "polyphenol" includes more than 8,000 compounds with great structural diversity (although each has at least one aromatic ring with one or more hydroxyl groups). They can be divided into 10 different classes depending on their basic chemical structure.

Table 01: shows the main families of phenolic compounds, most of which are found in nature associated with mono- or polysaccharides (glycosides) or functional derivatives such as esters or methyl esters. Moreover, the main sources where phenolic compounds are found have been classified **(Boudet, 2007).**

Carbon Numbers	Class	Sources
1. C6 2. C6-C1 3. C6-C2 4. C6-C3 5. C6-C4 6. C6-C1-C6 7. C6-C2-C6 8. C6-C3-C6 9. (C6-C3)2 10. (C6-C1)11	11. Simple phenols 12. OH 13. Benzoquinones 14. Benzoic acid 15. Acetophenones 16. Phenylacetic acid 17. Cinnamic acid 18. Phenylpropene 19. Coumarins 20. Chromones 21. Naphthoquinones 22. Xanthones 23. Stilbenes 24. Anthraquinones 25. Flavonoids 26. Lignans,neolignans 27. Hydrolysable tannins 28. Lignins	29. Cranberry, cereals 30. Apple, abricot, banana, 31. Cauliflower 32. Apple, apricot, banana, 33. Cauliflower 34. Carrot, celery, citrus, parsley 35. Nuts 36. Mango, Mangosteen 37. Grapes 38. Widely distributed 39. Sesame, rye, wheat, flax

Table 1: Classification of families of phenolic compounds **(Boudet, 2007).**

- The most abundant polyphenols in the diet are phenolic acids (benzoic and cinnamic acids), and flavonoids (30 and 60% of the total, respectively) **(Escarpa et *al*., 2006).**
- On the one hand, phenolic acids occur in different forms in plants, including aglycones (free phenolic acids), esters, glycosides, and/or bound complexes. These different forms of phenolic acids show variable suitability for different extraction conditions and vary in their susceptibility to degradation **(Naczk et *al*., 2006).**
- On the other hand, the common structure of flavonoids consists of two aromatic rings linked by three carbons that usually form an oxygenated heterocycle. In plants, flavonoids can be found as aglycones, although they are usually found as glycosides

contributing to the colour (blue, scarlet, orange) of leaves, flowers, and fruits. Phenolic compounds are found not only in fruits and vegetables but also can be found in legumes, cereals, nuts, medicinal plants, spices, and beverages (e.g. tea, wine, and beer). Furthermore, flavonoids can be subdivided in 13 classes: chalcones, dihydrochalcone, auron, flavones, flavonols, dihydroflavonol, flavanones, flavanols (catechins), flavandioles or leucoanthocyanidins, anthocyanidins (its glycoside is called anthocyanin), isoflavononas, flavonoids, and condensed tannins or proanthocyanidins **(Escarpa et *al*., 2006).**

- According to the epidemiological studies, the intake of phenolic compounds is inversely correlated with the risk of coronary heart disease **(Ross et al., 2009).** In the human body, these phytochemicals are thought to provide health benefits by several mechanisms, including: (1) free-radical scavenging; (2) protection and regeneration of other dietary antioxidants (i.e. vitamin E); and (3) chelating of pro-oxidant metal ions. The species and levels of phenolic compounds vary dramatically among plants, and their different structures or levels are likely to have different functional properties – Besi **(Magalhaes et *al*., 2009) , (Fang et *al*., 2007).**
- the general properties of the compounds, a number of polyphenolic compounds, especially catechins, have been found to be potent antioxidants and to be effective in preventing cancer **(Fang et *al*., 2005).**while tannins have been reported to exert other physiological effects; e.g. they can reduce blood pressure, accelerate blood clotting, lower serum-lipid levels, modulate immune responses and cause liver necrosis **(Fang et *al*., 2007)**.As mentioned above, it is impossible to separate the close relationship between the structure and properties of polyphenolics. The structure of phenolic compounds is a key determinant of their radical scavenging and metal-chelating activity. For example, in the case of phenolic acids, the antioxidant activity depends on the numbers and positions of the hydroxyl groups in relation to the carboxyl functional group. Thus, the antioxidant activity of phenolic acids increases the higher the degree of hydroxylation **(Escarpa et *al*., 2006).**

4. The strategy of using antioxidant activity of medicinal plants in prevention of diseases

Oxidative stress, caused by reactive oxygen species, plays an important role in many chronic and degenerative diseases, such as atherosclerosis, ischemic heart disease, cancer, diabetes mellitus, neurodegenerative diseases and ageing **(Azizova, 2002).** The body's non-enzymatic antioxidant defence system includes some antioxidants, such as vitamin C,

vitamin E, vitamin K and glutathione. Some synthetic antioxidants, widely used in food industry to protect food from oxidation and spoiling, are harmful because of their potential toxicity and carcinogenicity **(Botterweck *et al.*, 2000).** However, natural antioxidants in fruits and vegetables are inversely related with the risk of the chronic diseases mentioned above **(Leifertand, 2008).** Natural antioxidants, therefore, provide alternative strategy to prevention as well as treatment of these diseases. Phenolic compounds because of their oxidative activity are potential agents for preventing and treating many oxidative stress-related diseases. The antioxidant activity of polyphenols is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators and reductants of ferryl hemoglobin **(Kratchanova *et al.*, 2010).** Some medicinal plants possess more potent antioxidant activity than common dietary plants **(Cai *et al.*, 2004).** Therefore, their extract, if not toxic, can serve as food additive and can be used for disease prevention **(Liu, 2003; Liu *et al.*, 2008).**

III. Millissa *officionalis* L

1. Definition

Melissa officinalis L. Is a perennial plant in the family Lamiacae. It occurs naturally in the Mediterranean and West Asia. Moreover, it is cultivated widely in Europe and North America. Melissais known for its characteristic lemon taste and aroma **(Moradkhani et *al.*,2010).** This herb is also known under other names as lemon balm, balm, common balm or balm mint. Notes about lemon balm come from the antiquity when it was described by among others -Hippocrates and Dioskurides. In the middle Ages, Avicenna (980-1037) recommended it to strengthen the heart, and Paracelsus (1493-1541) prepared “the elixirs of life” containing the lemon balm. Melissa officinalis has been traditionally known for being able to restore youth, support weakened people and prevent baldness. In herbal medicine, leaf, herb and essential oil of lemon balm are used **(Senderski, 2009).** Melissa officinalis due to its numerous health promoting properties, apart from medical use, is also increasingly used as an ingredient in supplements and functional foods. A compilation of the latest data on the therapeutic properties of lemon balm will facilitate its application also in food products.

Figure 09 : Melissa ofitionalise citronnelles (**Jaime-jardiner. Ouest France. Fr)**

2. Taxonomy

Melissa officinalis L. belongs to:

Kingdom: Plantae,

Subkingdom: Tracheobionta,

Superdivision: Spermatophyta,

Division: Magnoliophyta,

Class: Magnoliopsida,

Subclass: Asteridae,

Order: Lamiales,

Family: Lamiaceae,

Genus: Melissa,

Species: officinalis L.,

Subspecies: officinalis **(Balasundran et al., 2006), (Wu et al., 2008).**

3.Botany

- is a perennial lemon-scented herb belonging to the Lamiaceae **(Awad et *al*., 2009).**
- According to "The Plant List" Melissa officinalis L. is the only accepted name for the plant, with nine synonyms including "Melissa officinalis subsp. altissima (Sm.) Arcang., Melissa officinalis var. altissima (Sm.) K.Koch, Melissa M. officinalis, also known as lemon balm, common balm or sweet balm,
- officinalis var. cordifolia (Pers.) K.Koch, Melissa officinalis var. Foliosa Briq., Melissa officinalis var. graveolens (Host) Nyman, Melissa offi- cinalis var. hirsuta K. Koch, Melissa officinalis subsp. officinalis, Melissa officinalis var. romana (Mill.)

Woodv. and Melissa officinalis var. villosa Benth.". There is also an infraspecific taxon of the species Melissa officinalis L.: Melissa officinalis subsp. inodora Bornm. **(The Plant List, 2013).**

- Melissa officinalis grows to the height of 30–125 cm, with soft short hairs surrounding all parts. The stem is erect, branched, usually glabrous and quadrangular. Leaves are petiolate, ovate, to 6 cm long, 3 cm broad, the upper cuneate, the lower cordate at base, crenate-toothed, subglabrous, sometimes with glandular hairs or punctuate glands beneath **(Komarov, 1977).** Flowers are white or pale pink consisting of small clusters of 4–12 blossoms in the summer. It has two stamens and four lobed ovaries forming 1–4 nutlets. The seeds are very small about 1–1.5 mm long, with ovate dark brown or black color. Lemon balm can rapidly grow at a temperature range of 15–35 °C, and requires 500–600 mm precipitation well distributed throughout the growing season, otherwise it should be irrigated **(Saeb et Gholamrezaee, 2012).**
- M. officinalis has a hairy root system with many lateral roots, which makes the plant more adaptable to different environmental conditions. The upper parts of the plant die off at the start of winter but new shoots re-emerge from roots at the beginning of spring **(Turhan, 2006).**
- M. officinalis grows worldwide but its origin has not been well defined. However, the Eastern Mediterranean region, Western Asia and Southern Europe, Caucasus and Northern Iran are considered as areas of origin **(Sousa et *al*., 2004; Fernandes, 1973).**

4. The chemical composition

Fresh herbs contain phenolic compounds, l-ascorbic acid, carote- noids, flavonoids and terpenoids. Lemon balm leaves are rich in flavonoids (0.5 % dry weight) consist of quercitrin (a derivative of quercetin), ramnocitrin, luteolin and its derivatives (luteolin 7-*o*-*β*- d-glucuronopyranoside, luteolin 3'-*o*-*β*-d-glucuronopyranoside, apigenin 7-*o*-*β*-d-glucopyranoside, and luteolin 7-*o*-*β*-d-gluco- pyranoside-3'-*o*-*β*-d-glucuronopyranoside). The major components among terpenoids are neral. geranyl acetate, ursolic acid and tan- nins **(Moradkhani et *al*., 2010; Miraj et *al*., 2017).** 0.087 g/100 g of caffeic acid and 21.15 g/100 g of rosmarinic acid were detected in the hydroethanolic extract of lemon balm leaves and phenolic com- pounds constituted 33.97% **(OżarOwski et *al*., 2016).**

Components	Retention time	Mass percentage
Beta-ocimene Z	1020	0.2
Beta-ocimene E	1032	0.1
Citronellal	1086	0.01
Neral	1145	43.8
Geraniol	1121	5.3
Geranial	1246	52
Thymol	1258	7.9
Carvacrol	1274	0.8
Citronellyl formate	1276	0.2
Geranyl acetate	1362	2.3
Germacrene D	1375	0.3
Beta- caryophyllene	1424	13.5
Alpha-humulene	1575	0.7
Caryophyllene oxide	1575	0.3
Globulol	1581	6.8
Humulene epoxide	1617	0.3
5-cedranone	1629	0.2
Total		89,01

5. Therapeutic properties of Lemon balm (Melissa *officinalis* L.)

On the basis of the literature review, the results indicating the health promoting properties of lemon balm were collected Antioxidant properties − mostly the therapeutic properties of lemon balm based on antioxidant activities

Due to the high content of flavonoids, Melissa *officinalis* has anti- oxidant properties. *In vitro* studies confirmed that 100-500 μg/ml of hydro ethanolic extract from the herb had a cyto protective activity against the toxic effects of hydrogen peroxide on human umbilical vein endothelial cells. The extract also reduced amount of hydrogen peroxide in intra- and extracellular fluids. This may be important in the prevention of cardiovascular diseases **(Safaeian et *al*., 2016).**

6 . Antimicrobial properties

In vitro tests, the essential oil of Melissa officinalis leaves had an inhibitory effect on the growth of Gram-positive (Staphylococcus aureus, Bacillus subtilis, Listeria monocytogenes) and Gram-nega- tive (Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneu- moniae, Salmonella enterica) bacteria, yeasts (Candida albicans, Saccharomyces cerevisiae) and fungi (Fusarium oxysporum spp., Mucor ramannianus) **(abdellatif et *al*., 2014).**

7. Effect on memory and neurodegenerative diseases

Numerous studies are directed to the action of Melissa *officinalis* in the prevention of memory disorders and neurodegenerative diseases. Melissa *officinalis* and rosmarinic acid can help patients with Alz- heimer's disease via inflammatory and neuroprotective effects as well as inhibition of the acetylcholine esterase activity and β-amyloid plaque formation in brain **(Mahboubi, 2019).**

8.Application in sleep disorders

Melissa officinalis is a frequently used sleeping remedy for the el- derly (lutoMSki, 2000). Melissa also acts on the quality of sleep in people who do not present this problem **(Cerny and Schmid, 1999).**

- **Anxiolytic and anti-depressant properties**

Lemon balm leaf is a frequently used herbal drug with anxiolytic and anti-depressant effect. It lowers the level of anxiety, facilitates falling asleep, prevents gastrointestinal disorders on the mental background and has sedative properties. It is usually recommended to consume infusions from 2-3 g of leaves 3 times a day **(NOwak, 2009).**

- **Application in gynecology**

Lemon balm is used in menstrual disorders, such as lack of or irregular menstruation, in menstrual pain and during excessive bleeding **(kaPczyński, 2000).**

- **Application in gastroenterology**

Lemon balm can be used in the irritable bowel syndrome. In an in vitro study, it was demonstrated that essential oil and citral (one of the main components of the oil) have the effect of relieving intestinal spasms caused by potassium chloride, serotonin and acetylcholine **(Sadraei et *al*., 2003).**

9.Theprotective effects of Melissa officinalis

- -Total extract and acidic fraction were not attributed to their anticholinesterase activity. Acidic fraction showed more potent protective effect compared to the total extract.
- -Leading to the fact that polyphenolic compounds and terpenoic acids were the most effective components in the total extract concentrated in this fraction E **(Lopez, 2009).**
- **Effect** of Melissa officinalis on hypoxia induced neuronal death in a cortical neuronal culture system as in vitro model and transient hippocampal ischemia as in vivo model was investigated. Results showed that Melissa officinalis could be considered as a protective agent in various.
- Neurological diseases associated with ischemic brain injury The **(Bayat et *al*., 2012).** neuroprotective effects of Melissa officinalis was investigated against neuron toxicity in hippocampal primary culture induced by 3,4-methylenedioxymethamphetamine (MDMA) or ecstasy.

Pratical side

Chapter 01:

matrial and method

Chapter 01: matrials and methods

I. Materials and methods

1. Animal treatments

For the realization of our experimentation, we used an experimental model animal Adult male "*Wistar* albino rats", were obtained from API (Algeria Pasteur institute). Weighing around 240-300 g. Animals are divided into a control and treatment group.

Upon arrival, the rats were housed, 10 per cage. Animals were maintained under a daily 12-h light/dark cycle at a constant temperature (24 ± 4 °C), relative high humidity of 60 ± 10%, with free access to food and water. Under standard hygienic conditions.

Figure 10: Adult male "*Wistar* albino rats".

Figure 11: housing rats in cages during the experimental study.

In this study, Rats were adapted for **04** weeks before the indicated treatments. Animals have been divided into **05** groups of **10** rats each.

Housed in cages of large dimensions (50 × 35 × 20 cm) with double bottles excluding any crowding effect. Treatment was carried out by the administration of the prepared solutions by gastric gavages using a probe attached to a syringe during 40 days:

- **Group I: Control group (CT):** receiving only distilled water (1ml) by gavage for 40 days.
- **Group II (IMI D1 group):** Daily receiving aqueous solution of Imidacloprid. (Dose1= 5 mg/kg/day).
- **Group III (IMI D2 group):** Received Dose 2= 50 mg/kg/day of Imidacloprid.
- **Group V:** Rats treated with pesticide (imidacloprid) 5 mg / kg / day + lemon balm extract 10 mg/kg/day.
- **Group IV:** Rats treated with pesticide (imidacloprid) 5 mg / kg /day + lemon balm

extract 10 mg/ kg/day.

All experiments were carried out in conformity according to International Guidebook on Treatment, and Use of Laboratory Animals. This study was conducted toxicology Laboratory Faculty of applied biology of Tébessa University, Algeria.

2. Chemical product

In this work, we used Imidacloprid which is part of the family of neonicotinoids which is systemic insecticide her chemical formula $C_9H_{10}ClN_5O_2$ for the treatment of rats in solution form with two (02) doses (5 and 50 mg) , we brought it powder than we mixed it with distilled water to get the Imidacloprid solution.

3. Plant materiel

During treatment, we used the aqueous extract of Melissa Officinalis dilute in distilled water to facilitate oral gavage it is available in the laboratories of our university the dose eligible is 10mg /kg.

Figure 12: Melissa *officinalis* extract.

4. Sacrifice and brain extraction

At the end of the xenobiotic administration period (Imidacloprid) of 40 days, the animals were sacrificed by decapitation; the brains were carefully excised and immediately rinsed in physiological solution, then dried at low temperature (4 ° C) with a semi-absorbent paper. The brains are weighed and then stored at -80 ° C until analysis for the determination of following parameters: **(Carbohydrate, Proteins, Lipids, MDA, GST and AChE).**

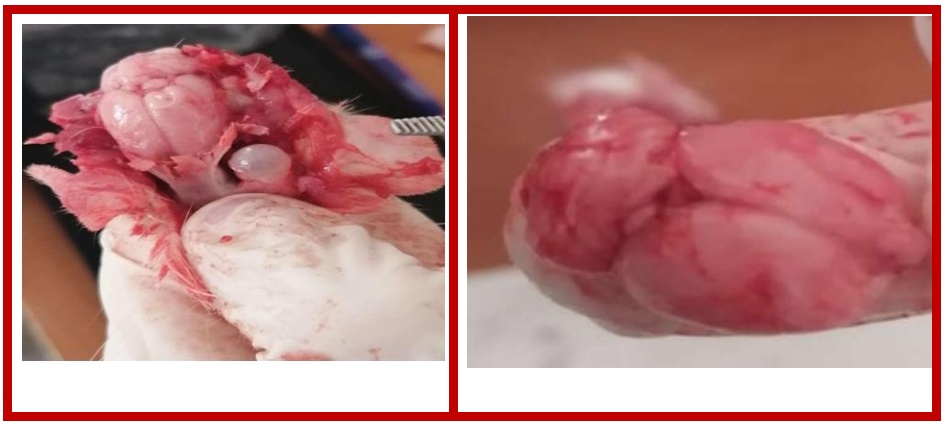


Figure 13: Removal of brain.

5. Estimation of the relative weight of the brain

The relative weight of the brains extracted from the rats (PRC [g / 100 g of body weight]) is calculated relative to the total weight of the rat according to the following formula:

$$\text{RWB (g/100gofTW)} = \text{WB/TWx100}$$

RWB: Relative weighs of Brains (g).

TW: Total weight of rat (g).

WB: Weigh to f Brain.

6. Preparation of cytosolic samples

One gram of nervous tissue was homogenized in 2 ml of phosphate buffered saline solution (PBS; pH 7.4). Then the homogenates were centrifuged at 3000 rpm for 15 min at 4°C and the resulting supernatant was used for the determination of the level of MDA and the enzymatic activity of GST.

30 days of adaptation

Group n° 1 :

10 control rats

Force-Feeding by distillate water.

Group n° 2: 10 rats treated with 5mg/kg/day

Of IMI

Group n° 3: 10 rats treated with 50mg/kg/day

Of IMI

Group n° 4:

10 rats

Treated with 5mg/kg/j

of IMI+10mg/kg/j of Melissa

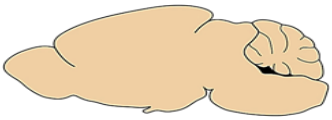
Group n° 5: 10 rats

Treated with 5mg/kg/j

Of IMI+10mg/kg/j of Melissa

Brain Sacrifices And recovery

After 40 days of treatment



Estimation of the relative weight of the brain

Biochemical parameters

- **Lipids**
- **Proteins**
- **Carbohydrate**

Oxidative stress parameters

- **MDA**
- **GST**

Neurotransmitter estimation

Acetyl cholinesterase(Ache)

Figure 14: Explanatory diagram of experimental Protocol

7 . Evaluation of biochemical parameters

- **Carbohydrate dosage**

The determination of the total soluble carbohydrates was carried out according to the method of (**Dubois, 1956**). The extraction of soluble sugars consists in putting 100µl of homogenate in test tubes then adding 2 ml of 80% ethanol, the whole is left 48h. The assay is done by total evaporation of the alcohol by placing the test tubes in a water bath at 70 ° C. After cooling, the volume of each test tube is made up to 20ml with distilled water, then 1ml of the solution is taken and 1ml of 5% phenol is added, taking care to shake well, then 2ml of acid is added. Concentrated sulfuric acid in tubes previously placed in an ice bath. Finally everything is left to rest for 25min, and then we proceed to the read at 490nm wavelength. The calculation of the actual concentrations is done by the equation deduced from the calibration range prepared from a stock glucose solution.

- **Lipids dosage**

Tissue lipids are evaluated according to the method (**Goldsworthy et *al., 1972***), using 200µl of homogenate in 5ml of 20% trichloroacetic acid (TCA), and ground and filtered this mixture; and directly applied centrifugation at 5000 rpm for 10min. The pellet is kept in a tube containing 1ml of the Ether / Chlorophorme mixture, and after centrifuging this mixture at 5000t/min for 10min, 100µl of the supernatant is taken, to which 1ml of sulfuric acid is added and after stirring the tubes in a water bath at 100 ° C for 10min. After cooling, the sample is taken again using a 200 µl micropipetteof the extract to which is added 2.5ml of the 85% sulfophosphovanillin mixture (0.38g vanillin + 195ml orthphosphoric acid + 55ml H2O) and left this mixture 30min "in the dark", the reading at a wavelength of 530nm. The calculation of the actual concentrations is made from the equation deduced from the calibration range carried out from a stock solution prepared using oil of sunflower (Appendices).

- **Protein dosage**

The method used for the determination of proteins is that of **(Bradford, 1976**) which uses the BSA as standard, on the same sample used to measure the lipids, the pellet from the second centrifugation to which 1 ml of NaOH (0.1N) was added and stirred energetically for protein dissolution. Then, we take, by means of a micropipette, a volume of 100µl to which is added 4ml of the BBC reagent (Brilliant Blue of Coumassie) (50mg BBC + 50ml of 85% or

thophosphoric acid and make up to 500ml with distilled water). Thus a blue color develops and the samples are passed directly for reading at a wavelength of 595nm. The concentration is calculated by the equation deduced from the calibration range produced from a solution of bovine serum albumin (Appendices).

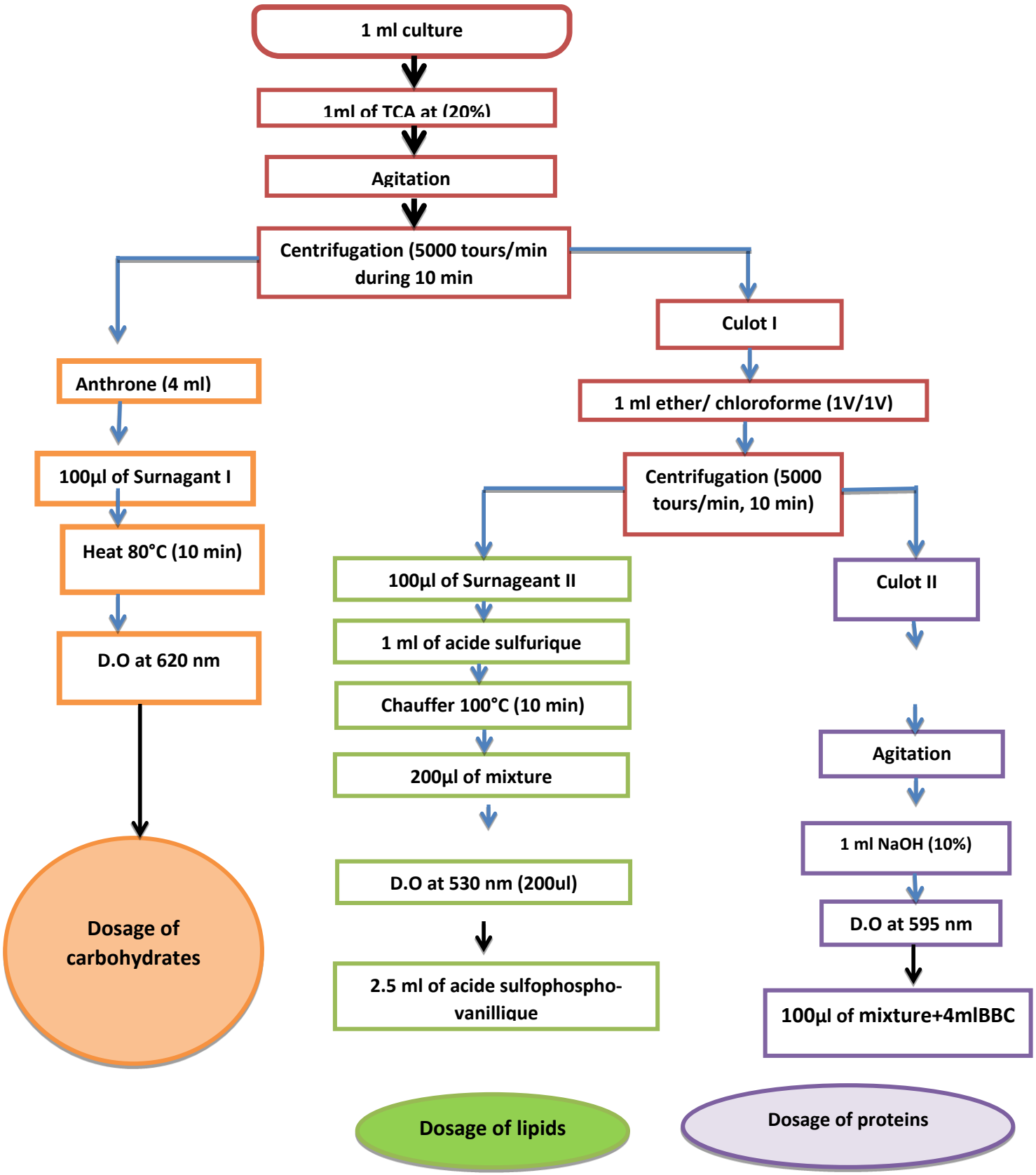


Figure 15: Method of extraction and determination of the main biochemical constituents (carbohydrate lipid and protein).

8. Evaluation of oxidative stress parameters

- **Dosage of Malondialdehyde (MDA)**

MDA is one of the products terminated during the breakdown of polyunsaturated fatty acids (PUFA) mediated by free radicals.

- Malondialdehydes (MDA) are assayed according to the method of (**Eserbauer et *al*.,1992)** This method is based on the colorimetric measure of the reaction from the acid thiobarbituric (TBA) and the malondialdehyde (MDA) in an acidic and hot medium (100 ° C) giving a red brown product whose color is obtained at a wavelength of 530 nm.

II. Experimental Protocol

- Prepare the homogenates from 200 mg of organ with TP homogenization buffer (pH 7.4);
- Centrifuge at 3000 rpm for 10 min;
- Take 375 µl of supernatant;
- Add 150 µl of TBS solution buffer (50 mM Tris, 150 mM NaCl pH 7.4);
- Add 375 µl of TCA-BHT solution (TCA 20%, BHT 1%);
- Agitator and centrifuge at 1000 rpm for 10 min;
- Take 400 µl of supernatant;
- Add 80 µl of 0.6 M HCl;
- Add 320 µl of Tris-TBA solution (Tris 26 mM, TBA 120 mM);
- Mix and incubate in a water bath at a temperature of 80 ° C for 10 min;
- Read the optical density at λ = 530 nm.
- The absorbance is directly proportional to the amount of MDA formed, thus giving an accurate assessment of the peroxidized lipids.

1. Assaying of the activity of glutathione S-transferase (GST)

The measurement of glutathione S-transferase (GST) activity is determined according tothe method of **(Habig et *al*., 1974),** It is based on the conjugation reaction between GST and asubstrate,CDNB(1-Chloro2,4-dinitrobenzene)inacofactorglutathione(GST),theconjugationleadstotheformationofamoleculenovelle;1-S-Glutathionyl2-4Dinitro benzene to measure GST activity.

The value of the optical density measured is directly proportional to the amount of conjugate formed ,it self-related to the intensity of the GST activity. The samples are homogenized in 1ml of phosphate

buffer (0.1M, pH6). The homogenate is centrifuged at14,000 rpmfor30min and the recovered supernatan will serve as a source of enzymes.

The assay consists in reacting 200 µl of the supernatant with 1.2 ml of the CDNB (1mM), GSH (5 mM) mixture [20.26 mg CDNB, 153.65 mg GSH, 1 ml of ethanol, 100 ml of phosphate buffer (0.1M, PH 6)]. The absorbance reading is taken for one minute and every 15sec at a wavelength of 340 nm against a blank containing 200 µl of distilled water replacing the amount of supernatant.

2. Experimental protocol

Homogenization with 1 ml of phosphate buffer (0.1 M, pH 06).

- The homogenate is centrifuged at 14000 rpm for 30 min and the recovered supernatant will serve as the source of enzymes.
- The assay consists in reacting 200 µl of the supernatant with 1.2 ml of the CDNB (1 mM), GSH (5 mM) mixture [20.26 mg CDNB, 153.65 mg GSH, 1 ml ethanol, 100 ml phosphate buffer (0.1 M, pH 06)].
- The absorbance reading is carried out for one minute and every 15 seconds at a wavelength of 340 nm against a blank containing 200 µl of distilled water replacing the amount of supernatant.
- The absorbance is read at 340 nm after 30 s in 3 min intervals.

3. Neurotransmitter estimation

Determination of acetylcholinesterase (AChE)

The most common AChE acetylcholinesterase assay (**Ellman et *al*.,1961**) is to provide the enzyme with a substrate, lectylecholine, the hydrolysis of which releases thiocholine and acetic acid. the sample is homogenized in 1 ml of detergent solution (38.03 mgethylethylene glycol tris- β-aminoethyl ether NN N'N, 1 ml triton X100% 5.845g NaCl , 80 ml tris buffer 10 mm) using an ultrasonic homogenizer and then centrifuges at 5000 rpm for 5 min, the supernatant is used immediately for the measurement of AChE activity. the AChE assay steps are as follows:

- 100 µl of supernatant + 100 µl of DTNB (0.1 M, ph = 8) (39.6 mg of DTNB, 15 mg CO_3 Na, in 1 ml tris (0.1 M, ph7) and 1 ml of tris buffer (0.1 M , ph 7)
- After 5 minutes of rest necessary to exhaust the spontaneous reaction, 100 µl of acetylthiocholine substrate (118mg Ach without 5ml distilled water)
- The densities are read at 412nm every 4min for 20min.

4. Statistic study

The results obtained were expressed by the average of six repetitions (mean ± deviation type), and to better visualize using *the Excel 2018* office to represent these results in the form of graphs and histograms. Statistical analysis was performed using *the Minitab® software18.1*. The significance of the difference between the control batch and the treated batches is verified using *the Du* and the comparison result as following **:** *nette test*

- P> 0.05 = the difference is not significant,

- (*) 0.05> P> 0.01 = the difference is significant,

- (**) 0.01> P> 0.001 = the difference is highly significant,

- (***) P <0.001 = the difference is very highly signifi

Chapter 02

Results

I. Effect of Imidacloprid (IMI) and Melissa *Officinalis* Extract *(M)* on Growth parameters

1. Relative weight of the brain (RBW)

For monitoring changes in relative brain weights period we observed a non-significant decrease (p> 0.05) in the batch treated with Imidacloprid at a dose (5 mg / kg / day), and a very highly significant decrease (P <0.001) in the batch treated with the same pesticide in deferent dose (50mg / kg / day), Also, a highly significant increase (0.01> P> 0.001) was observed in the batch treated by the 5mg / kg / day dose combination of Imidacloprid and Melissa *Officinalis* extract, and a significant decrease (0.05> P > 0.01) in the batch treated with the 50 mg / kg dose combination of Imidacloprid and Melissa *Officinalis* extract .

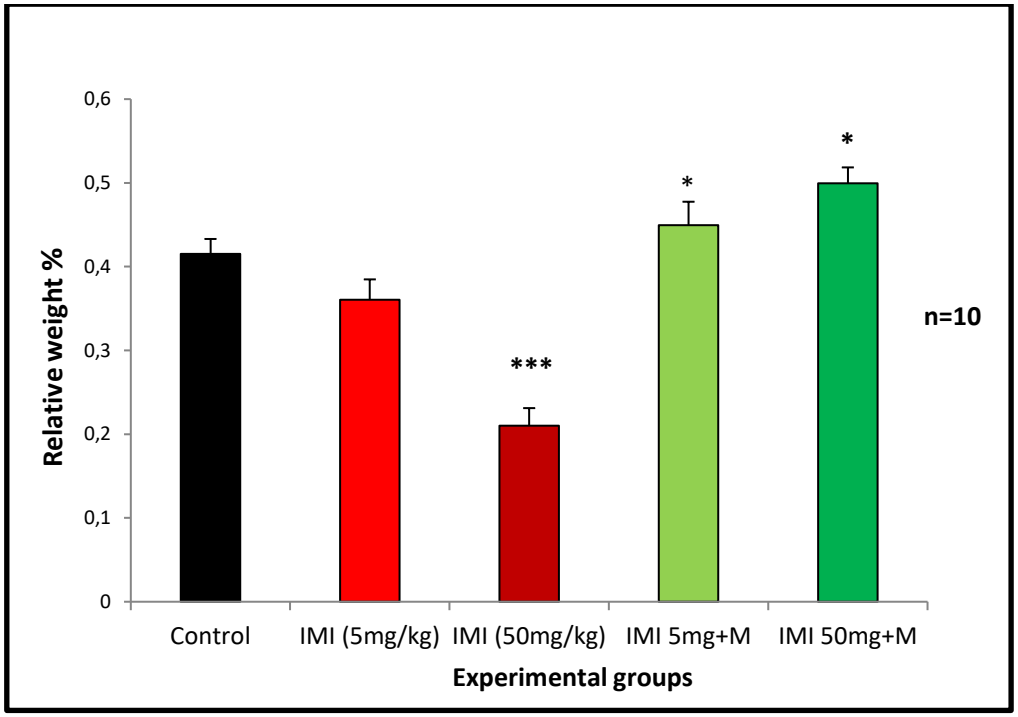


Figure 16: Evaluation of relative brain weights in rats treated with Imidacloprid and Melissa *Officinalis* extract compared to controls rats

(p≤0.01): highly significant (**), (p≤0.001): very highly significant (***),(0.05> P > 0.01)significant ,P> 0.05: not significant (ns).

2. Effects of Effect of Imidacloprid (IMI) and Melissa *officinalis* Extract (M) on biochemical parameters in the total brain

1. Carbohydrate

The results of the evaluation of the variation in the level of carbohydrates in the brain are shown in figure15.Statistical analysis of these results shows that the administration of IMI causes a very highly significant increase ($p \leq 0.001$) in both groups which are treated by 5and50mg/kg/day of IMI compared to the control group.

Also, a highly significant increase decrease ($0.01 > P > 0.001$) was observed in the batch treated by the 50mg / kg / day dose combination of Imidacloprid and Melissa *Officinalis* extract, and a significant decrease ($0.05 > P > 0.01$) in the batch treated with the 5 mg / kg dose combination of Imidacloprid and Melissa *Officinalis* extract .

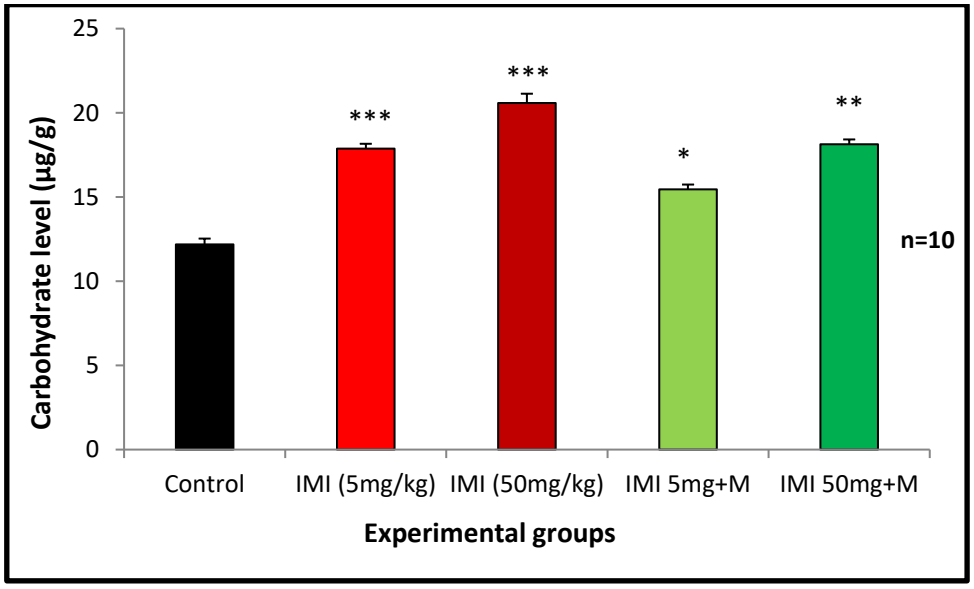


Figure 17: Variation in carbohydrate levels in control and treated rats after 40 days of treatment..

($p \leq 0.01$): highly significant (**), ($p \leq 0.001$): very highly significant (***), ($0.05 > P > 0.01$) significant, $P > 0.05$: not significant (ns).

3.3.Lipids

The results of the evaluation of the variation in the level of lipid in the brain are shown infigure 18. Statistical analysis of these results shows that the administration of IMIcauses a highly significant decrease (0.01> P> 0.001) in the grouplk treated by 5 mg/kg/day of IMI and also a very highly signify ant decrease (p≤0.001)in the group treated by 50mg/kg/day of IMI compared to the control group.

Also, a significant decrease (0.05> P> 0.01) in the batch treated with the 50/5 mg / kg / day dose combination of Imidacloprid and extract of Melissa *Officinales*

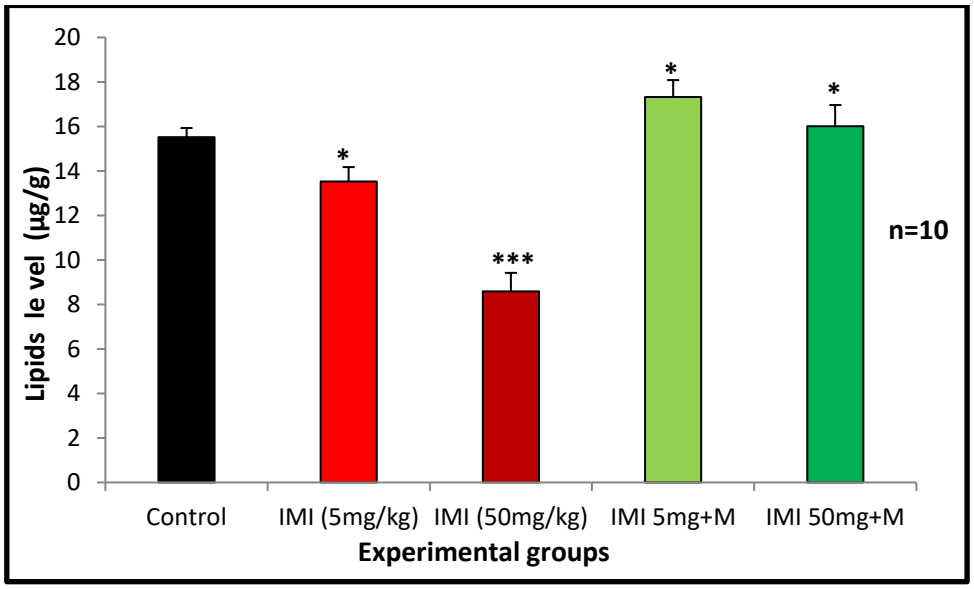


Figure 18: Variation in lipids levels in control and treated rats after 40 days of treatment.

. (p≤0.01): highly significant (**), (p≤0.001): very highly significant (***), (0.05> P > 0.01) significant, P> 0.05: not significant (ns).

3.4.Protein

The Results of the evaluation of the variation in the level of protein in the brain are shown in figure17. Statistical analysis of these results shows that the administration of IMIcauses a hightly significant increase (0.01> P> 0.001) in the group treated by 5 mg/kg/day ofIMI and a very hightly significan increase (p≥0.001)in the group treated by 50mg/kg/day of IMI compared to the control group.

Also, there was a significant decrease (0.05> P> 0.01) in the batch treated with the 5mg / kg dose combination of Imidacloprid and Melissa *Officinalis* extract, and a non-significant increase. P> 0.01 in the batch treated with the 50 mg / kg / day dose combination of Imidacloprid and extract of Melissa *Officinales.*

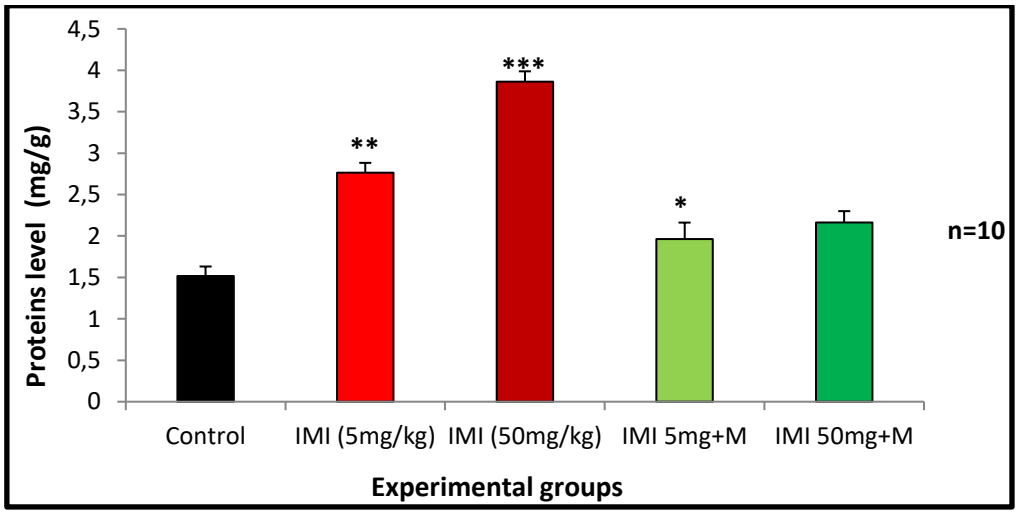


Figure 19: Variationin protein levels in control and treated rats after 40 days of treatment.

(p≤0.01): highly significant (**), (p≤0.001): very highly significant (***),
(0.05> P > 0.01) significant, P> 0.05: not significant (ns).

3. Effect of Imidacloprid (IMI) and Melissa Officinalis Extract (M) on cytosolic redox status in total brain

a. Glutathione-s-transferase (GST)

The results of the evaluation of the variation in the level of GST in the brain are shown infigure20. Statistical analysis of these results shows that the administration of IMI causes a very hightly significant increase ($p \leq 0.001$) in the group treated by 50 mg/kg/day of IMI and hightly significant increase ($p \leq 0.001$) in the group treated by 5 mg/kg/day of IMI compared to the control group.

And we observe a significant increase in the batch treated by the dose combination 50 mg / kg of Imidacloprid and extract of Melissa *Officinalis*, and highly significant increase in the batch treated by the dose combination 5mg / kg of Imidacloprid and extract of Melissa *Officinalis*.

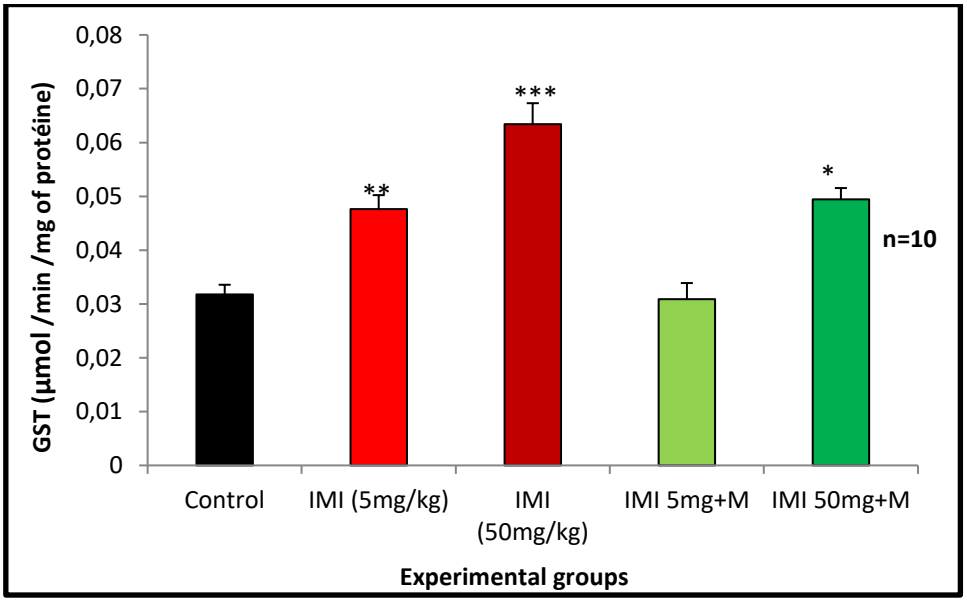


Figure 20: Variation in GST levels in control and treated rats after 40 days of treatment.

($p \leq 0.01$): highly significant (**), ($p \leq 0.001$): very highly significant (***), ($0.05 > P > 0.01$) significant, $P > 0.05$: not significant (ns).

b. Malondialdehyde (MDA)

The results of the evaluation of the variation in the level of MDA in the brain are shown infigure19. Statistical analysis of these results shows that the administration of IMIcauses a significant increase (0.05>P>0, 01) of the MDA level in the total brain respectively inthe treated with 5 mg/kg/day of IMI when compared to the control group and a very highly significant increase (P>0.001)in brain Malondialdehyde levels in rat streated with 50mg/kg/day compared to the control.

Also, a non-significant decrease (p> 0.05) is observed in the batch treated by the combination of dose 5 mg / kg of Imidacloprid and the extract of Melissa *Officinalis*, and significant decrease (0.05>P> 0 , 01) in the batch treated by the 50 mg / kg dose combination of Imidacloprid and extract of Melissa *Officinalis*

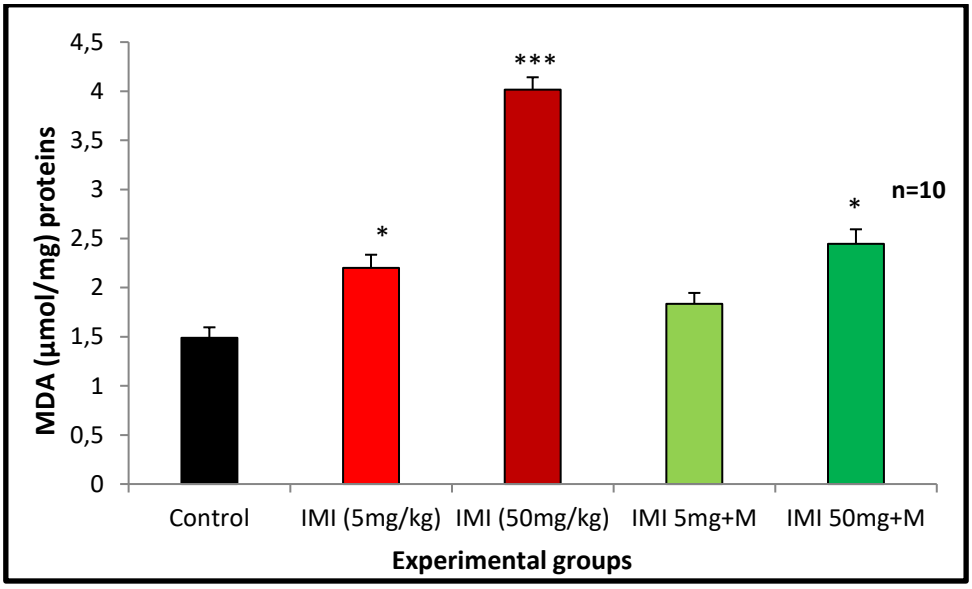


Figure 21: Variation in MDA levels in the brains of control and treated rats after 40 days of treatment.

(p≤0.01): highly significant (**), (p≤0.001): very highly significant (***), (0.05> P > 0.01) significant, P> 0.05: not significant (ns).

4. Effects of Imidacloprid (IMI) and Melissa Officinalis Extract (M) on Neurotransmitters in the total brain

The results obtained during the evaluation of the activity of acetylcholine esterase in the total brain in rats treated with Imidacloprid (IMI) in (figure 22).Statistical analysis of these results shows that the administration of IMI causes no significant decrease (P>0.05) in the group treated groups by 5mg/kg/day compared to the control, and a very highly significant (P> 0.001) decrease in the second group treated by 50 mg/kg/day compared with control group.

Also, a non-significant decrease (p> 0.05) is observed in the batch treated by the combination of dose 5 mg / kg of Imidacloprid and the extract of Melissa *Officinalis*, and significant decrease (0.05>P> 0 , 01) in the batch treated by the 50 mg / kg dose combination of Imidacloprid and extract of Melissa *Officinales* compared with control group.

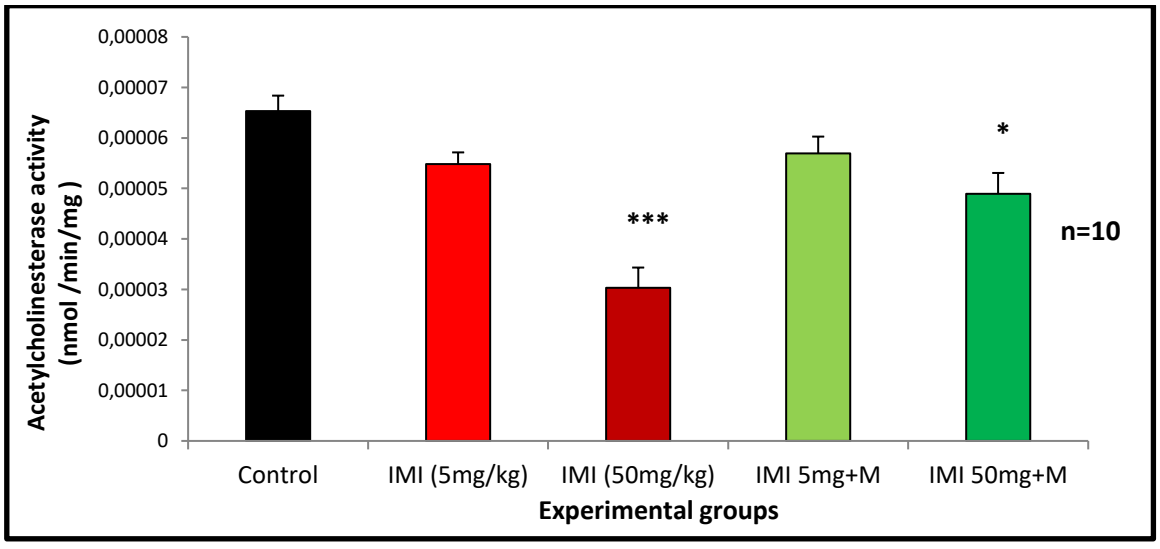


Figure 22: Variation in Acetylcholin esterase levels in control and treated rats after 40 days of treatment..

(p≤0.01): highly significant (**), (p≤0.001): very highly significant (***), (0.05> P > 0.01) significant ,P> 0.05: not significant (ns).

Chapter 03 :

discussion

Discussion

Several studies show the impact of neonicotoid insecticides on health, one of which conducted on rats suggests that neonicotinoids could adversely affect human health,(**Derriche, 2012)**

Oxidative stress is one of the main mechanisms of toxicity associated with a variety of Xenobiotic in the environment, among which are pesticides and phytosanitary products (**Lauvverys et *al*., 2007; Lukaszewicz, 2008; Michael et *al*., 2016).**

The present study was carried out to evaluatethe protective role of the extract of Melissa *Officinalis*10 mg/kg/day against the neurotoxic effect and biochemical alteration as a result of imidacloprid (IMI) exposure (5 and 50mg/kg body weight; orally) as stressor disturbance of metabolism, which corresponds to the stress response.

1. Effect of Imidacloprid and lemon balm extract on relative weight

The results of the evaluation of the weight parameters suggest that the administration of Imidacloprid causes a significant decrease in the body growth of the different groups of rats. This reduction can be translated by the disturbance of cellular metabolism under the effect of the oxidative stress generated by the ROS observed in this study, as well as by other chemical mediators such as certain pro-inflammatory cytokines that the organism can release after the toxic effects. Pesticides**(Carole et Harve, 2011; Viviana, 2015),** who reported a reduction in food consumption in male rats that occurred with sub-chronic toxicity. Furthermore, the use of Melissa has shown an improvement in these animal weight parameters. This could be the consequence of its antioxidant power by normalizing intracellular redox homeostasis and restoring the psychic state of animals **(Cliona et *al*., 2011; Toumi et *al*., 2016).**

Furthermore, the use of lemon balm has shown an improvement in the relative weight of the brain. This could probably be explained by the reduction in the accumulation of free radicals induced by the antioxidants present in lemon balm (**Ronat, 2001).**Decrease is improved by the administration of lemon balm thanks to its digestive, antioxidant and anti-inflammatory property**. (Iserin, 2001).**

2. Effect of neonicotinoids on antioxidant activity and the protective effect of Melissa *Officinalis* extract

2.1. Effect of imidacloprid and lemon balm extracton the level of Malondialdehyde (MDA)

MDA is the main compound resulting from the formation of various aldehydes toxic to the organism following the oxidative degradation of polyunsaturated fatty acids in cell membranes **(Maiza et *al*., 2011).**

In our work, an increase in MDA has been observed in the brain, which suggests that an increase in lipid peroxidation may be a consequence of the exhaustion of GSH **(Birsen, 2011).** Our results are consistent with the work carried out on mice treated with a lethal dose (14.976 mg / kg) of Imidacloprid **(Kawther et *al*., 2010),** and in *Wistar* rats after sub-acute exposure to acetamiprid **(Chakroun et *all*., 2016; Devan et *all*., 2015)** have shown that acetamiprid increases lipid peroxidation inthe liver of rats. **Kapoor et *al*., (2010)** reported a significant increase in the level of MDA in the liver and kidneys after administration of 20 mg / kg / day of imidacloprid for 90 days in female rats; similarly **(Kapoor et *al*., 2010)** studied the effects of imidacloprid on lipid peroxidation in female rats, and found identical results. In addition,**(Ince et *al*., 2013)** reported that administration of 15 mg / kg / day of imidacloprid for 28 days caused a significant decrease in the level of MDA in mice. And oral administration at sub-lethal doses (0, 5, 2 and 8 mg / kg) of imidacloprid for 90 days leads to an increase in MDA in the reproductive appearance of male rats **(Ramazan Bal et *al*., 2012)**. In addition, **(BirsenAydin 2011)** has shown that exposing rats to 112.5 and 22.5 mg / kg / day for 30 days of thiacloprid (a neonicotinoid insecticide) causes an increase in the level of MDA in all lymphoid organs.

A study carried out by **Ikizler M et *al,* in 2007** proved that the perfusion of the heart with a solution containing Quercetin (composed of lemon balm) for 30 minutes and more strongly by an oral treatment with Quercetin for 1 week reduced the levels of Malondialdehyde in brain tissue after reperfusion by inhibiting the production of EROs.

2.2.Effect on GST activity

Glutathione transferase (GSTs), also known as glutathione S-transferases) are major phase II detoxification enzymes found primarily in the cytosol. In addition to their role in catalysis, the conjugation of electrophilic substrates to glutathione (GSH), these enzymes also perform a range of other functions. They have peroxidase and isomerase activities **(Sheehan et *al.*, 2001).**

The response of GST activity depends on several factors such as the type of xenobiotic, the concentration, the exposure time and the species **(Oruç et Üner, 2000).**

The biochemical results of the group treated with Imidacloprid revealed a very highly significant increase ($P \leq 0.01$) in the enzymatic activity of glutathione S-transferase (GST). Generally the increase in the expression of GST is observed when the cell is stressed (**Di-Monte, 1992).**

In addition, GST is also involved in reducing the damage of ROS in different cells. It represents a family of multifunctional enzymes essentially cytosolic, involved in various operations of transport and intracellular biosynthesis **(Bidlack, 1996; Sauer, 2014; Di-Monte Lavasani, 2002).**

The work of **(Loaner et *al.*, 2014)** showed the establishment of a detoxification system in rats after the administration of a neonicotinoid Imidacloprid orally at two doses 5 and 50 mg / kg / day. In fact, a decrease in the enzymatic activity of glutathione S-transferase compared to the control group. Thus our result is similar to the results in male mice treated with doses of 14.976 mg / kg) of Imidacloprid **(Kawther et *al.*, 2010).**

The results for glutathione S-transferase (GST) activity indicate an increase in the specific activity of this enzyme in rats treated with imidacloprid at a dose of 50mg/kg this result is supported by the study of **(Mikoic et Brcic, 2018)** increase is considered to be one of the fundamental indicators informing about cellular damage caused by SAR **(Gasmi, 2018)** While mice treated with formalized lemon balm dose 10mg/kg shows a decrease.

On the other hand, the administration of the extract Melissa has greatly improved on oxidative stress parameter.

Recently, the role of the Melissa extract as a neuroprotective has been documented in several studies **(Gindin et *al.*, 1995; Benhammou, 2011; Lahouel et *al.*, 2016; Begoule et *al.*, 2017).**

3. Effects of Imidacloprid and Melissa on Metabolites

Membrane lipids, which are particularly rich in polyunsaturated fatty acids (PUFA), represent a privileged target for radical aggression. The more the fatty acid is rich in double bonds, the more it is peroxidable, that is to say degradable by an oxidative process harmful to the cell. Lipids also are the seat of lipid peroxidation. Their oxidation leads to membrane disorganization with changes in fluidity and permeability **(Hiltenbrand, 1999; Durand et *al*.,2013).**

Treatment with Imidacloprid at a different dose (5 and 50 mg / kg / D) for 40 days in rats is induced significant metabolic alterations in the brain by very highly significant reduction in total lipid content in comparison with the control, this decrease can be stimulated by free radicals in case of oxidative stress generated by Imidacloprid.

The decrease in lipid levels in treated snails is the consequence of oxygenated free radicals which are the cause of lipid degradation through β oxidation. Our results are consistent with the results obtained in *Wistar* rats at sub-lethal doses of imidacloprid after 1, 3 and 7 days of treatment **(Radwan et Mohamed, 2013).**

On the other hand, according to the results obtained when using Melissa as a cytoprotective molecule, it turns out to have improved the homeostasis of the biochemical parameters studied in this present work. The treatment of rats with 10 mg / kg / day of the extract of Melissa *officinalis* improve the lipid decrease. For the antioxidant role, vitamin C from Melissa ensures the protection and maintenance of proteins, lipids, enzymes and other antioxidants in their normal form by reducing metal ions and trapping free radicals **(Adimi. 2018).**

Also increasing protein levels after sub-acute exposure of rats to Imidacloprid translates the synthesis of enzymes and defense peptides against the homeostatic imbalance of oxidative stress **(Anadn et *al*., 1991; Benbouzib, 2012; Rouabhi et *al*., 2015).**

- Oxygenated free radicals induce modifications in the primary, secondary and tertiary structures of proteins by the formation of carbonylated protein derivatives via several mechanisms including the fragmentation and oxidation of amino acids **(Gasmi, S. 2018).**
- The significant increase in the level of proteins in a dose-dependent manner to acetamiprid can be explained by the synthesis of enzymes and peptides defending against the homeostatic imbalance of oxidative stress; by inducing the synthesis of

stress proteins in relation to the phenomenon of bio activation / biotransformation on the one hand and on the other hand by lipid peroxidation generated by free radicals **(Anadn et *al.*, 1991; Benbouzib, 2012; Rouabhi et *al.*, 2015) .**In addition, during stress, animals synthesize stress proteins. Thus, the observed increase in protein level could be explained by an increase in the protein synthesis of snails in response to such a stimulus **(Radwan et Mohamed, 2013).** Our results are in good agreement with those reported by **(Radwan et Mohamed, 2013)** who found a significant increase in total protein in *Wistar* rats after exposure to 0.2 LD 50 of imidacloprid for 1, 3 and 7 days. In addition, our results are consistent with work carried out on male rats of the *Wistar* treated with a neonicotinoid insecticide Acetamiprid **(Chakroun et *al.*, 2016).** Other studies have also shown a significant increase in total protein after exposure to imidacloprid-polluted soil in earthworms for one week. An increase in total protein content was also detected in juveniles of P. scaber, after two weeks of feeding on 5 mg imidacloprid / g dry food **(Drobne et *al.*, 2008).** The same effect has been shown by the work of **(Bourbia, 2013)** after the application of commercial insecticides based on thiamethoxam with doses of 0, 25, 50, 100 and 200 mg / L on *Wistar* rats after six weeks of treatment.

4. Effect of imidacloprid and Melissa *officinalis* extract on the Neurotransmitter Acetyl cholinesterase (Ache)

The impact of nicotinic has been reported by **(Bhardwaj et *al.*, 2010)** in the expensive *Wistar* rat after the oral administration of 5 and 50 mg / kg / day of Imidacloprid. Their results showed the inhibition of the specific activity of acetylcholinesterase (AChe). Similar results have been reported by **(Rodrigues et *al.*, 2010)**. **(Banerjee et *al.*, 2014)** have demonstrated the alteration of this key nervous system enzyme after administration of the pendimethalin compound to rats. However, the intra peritoneal injection of Imidacloprid at a dose of 50 mg / kg / day for 40 days causes the rat to increase in the specific activity of this enzyme, thereby inducing alteration of the nervous system and muscular tetany **(Abou-Donia et *al.*, 2008).** Recent work by **(Lonare et *al.*, 2014)** reported a significant decrease in acetyl-cholinesterase in the plasma and brain of rats treated orally with Imidacloprid.

Melissa extract increases acetylcholine production, supports mitochondria in energy production, prevents oxidative damage; increases brain oxygen consumption, and promote

dopamine synthesis. In general, the virtues of this extract are magnificent, going as far as repairing damaged neurons **(Mona et *al.*, 2014; Gao *et al*., 2014).**


Conclusion

Conclusion

Conclusion and perspective

Pesticides, toxic chemicals, pose a real public health problem.

Neonicotinoids are pesticides for agricultural use widely used by farmers. This present work presents a bibliographic study, Through this study, based on the evaluation of the oral toxicity of Imidacloprid at two doses (5 and 50 mg / kg / day) on body weight and nervous system function in *Wistar* rats for 40 days at the cytoplasmic level and the preventive and curative effect of Melissa extract (10 mg/kg/day) against this toxicity, we have shown that the doses tested, especially the high and medium doses, have several harmful effects.

Imidacloprid tested and result in a significant reduction in physiological parameters such as body weight, weight gain and an increase in relative brain weights.

- Imidacloprid tested and lead to a significant reduction in physiological parameters such as relative brain weights.
- On the other hand, the biochemical parameter is also affected by the different treatments with a strong disturbance of the different cellular metabolites including proteins and lipids.
- The pesticide Imidacloprid have also caused alterations in the balance of oxidative stress which results in a disturbance in levels of MDA, the activity of GST.
- Inhibition of neurotransmitter activity (AchE)
- The force-feeding of Melissa extract at a dose of 10 mg / kg / day for 40 days to rats treated with Imidacloprid restored all values to normal, which reflects the protective effect of the extract on the function of nervous systems.
- **In perspective**, it would be interesting to develop this research from an operational point of view by deepening know ledge on:

➢ Investigation of the effect of this dose on the other vital organs

➢ Extend the period of exposure of animals to force-feeding to this insecticide, in order to know whether the disturbances in the oxidant / antioxidant status observed could lead to the appearance of pathologies.

➢ Deepen the study with a histological study aimed at locating the tissue damage caused by pesticides.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