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Theme :

Hepatic response to imidaloprid and opposite effect of Melissa in vivo of Wistar rats

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Abreviations list

µmol: Micromoles

½ O2: singlet oxygen

ACh :Acetylcholine

AChE: Acetylcholinesterase

ACtch : acetylcholinesterase

ADI: Acceptable Daily Intake

ADP: Adenosine diphosphate

AF : Fatty acid

AMM: A Marketing authorization

ATP: Adenosine triphosphate

bcl-2: Anti-apoptotic proteins

Ca2 +: calcium ion

Cap :Caspase

CAT: Catalase

CO2 :carbon dioxide

CPF : Chlorpyrifos

Cu: Copper

Cyt: Cytochrome

DLM: Deltamethrine

DNAm: Deoxy ribonucleic acid mitochondrial

DNA: Deoxyribonucleic acid

EDTA: Ethylene-Diamine-Tetraacetic Aci

EFSA: European Authority of safety of food

EOA : Activated Oxygenated Species

EPA: Environmental Protection Agency

ERN: Reactive Nitrogen Species

ERO: Reactive Oxygen Species

ESM: Standard error on the mean

fig: figure

GABA: Gamma-aminobutyric acid

GC: gas chromatography

GPx: Glutathione peroxidase

Grx: Glutaredoxin

GSH: Glutathione reduced

GSSG: Oxidized Glutathione

GST: Glutathione – S-transferase

H: hydrogen

H2O2: Hydrogen peroxide

HO: Heme Oxygenase

L °: Free lipid radical

LB: B lymphocyte

LC50: Lethal concentration 50

LD50 :Lethal dose 50

LI: learning index

LOO •: Radical fatty acid peroxide

LOOH :Fatty acid lipoperoxide

LPO : Peroxidized Lipids

MAC: mitochondrial apoptosis-induced channel

MAO: monoamines oxidases

MDA :Malon-dialdehyde acid

mg :Milligrams

mmol: Milimole

mPT: transient mitochondrial permeability

NADPH: Nicotinamide-adeninedinucleotide-phosphate reduced

NK :natural killer cells

NO: Nitric oxide

NOAEL: No observed adverse effect level

O2 •: Radical superoxide (superoxide anion)

P53: Tumor suppressor protein

RE: endoplasmic reticulum

RH: Oxygenated free radical

RNA: Ribonucleic Acid

SN :Nervous system

SNC: Central Nervous System

SOD : Superoxide dismutase

TBA: Thiobarbituric acid

TCA: Trichloroacetic

TGO:aspartate amino transferase

TGP:alanine amino transferase

US EPA :United State Environment Protection Agency

ملخص

الهدف من هذا العمل هو القاء الضوء على الاثار السامة لجرعتين من الايميداكلوبريد و التاثير الوقائي و العلاجي ل مستخلص ميليسا (الحبق) ضد هذه السمية و قد استهدفت تجاربنا عدة محاور اساسية للتجريب ابتداءآ من عمل الانزيمات الخلوية والموت الخلوي.

شملت دراستنا 20 جرذ موزعة على خمسة مجموعات في كل منها (4 جرذان) :المجموعة 1 شاهد المجموعة 02 معالجة الايميداكلوبريد (5 مغ/ كغ /يوم) ,والمجموعة 03 معالجة بجرعة (50 مغ/ كغ /يوم), والمجموعتين الاخيرتان عولجت بالمزيج ايميداكلوبريد و مستخلص ميليسا (الحبق) (ترنجان) (10 مغ/ كغ /يوم) لمدة 20 يوم.

أظهرت نتائجنا أن التعرض للاميداكلوبريد و مزيجها بسبب تأثيرات ضارة على العضوية يترجم بنقص فالوزن مع زيادة وزن الكبد مقارنة مع الشاهد بالاضافة الى ذلك أظهر تحليل معاملات الكبد مع إيميداكلوبريد زيادة نشاط المؤشرات البيولوجية للأكسدة وانخفاض إنزيمات مضادات الأكسدة في العصارة الخلوية للكبد فيما يخص التأثير الميتوكوندري يتجلى من خلال زيادة في حجم و نفاذية الميتوكوندري.

مستخلص الحبق قد حسن توازن إزالة السموم وخفف من التأثيرات الضارة للإيميداكلوبريد. يبدو أن ميليسا (الحبق)مضاد فعال للأكسدة لتقليل عدم التوازن بين تكوين الجذور الحرة والأنظمة المضادة للأكسدة في الجسم.

الكلمات المفتاحية الإيميداكلوبريد و مستخلص ميليسا (الحبق)والإجهاد التأكسدي ، و ومضادات الأكسدة ، ... إلخ

ABSTRACT

The objective of this work is to highlight the toxic effects of two doses of imidacloprid and the preventive and curative effect of Melissa extract against this toxicity. Our experiments have targeted several basic areas of experimentation, starting with the work of cellular enzymes and cell death.

Our study included 20=5*4 rats divided into five groups in each of them (4 Rats): group 1 control group 02 treated with *imidacloprid* (5 mg /kg /days), group 03 was treated with a dose (50 mg /Kg /days), and the last two groups were treated with mixture of *imidacloprid* and extract of *Melissa extract* (10 mg /kg / day) for 21 days.

Our results have shown that exposure to *imidacloprid* and its mixture due to harmful effects on the body results in a decrease in weight with an increase in the relative weight of the liver compared to the control. In addition, an analysis of liver treatments with imidacloprid shows an increase in the activity of biological indicators of oxidation and a decrease in the antioxidant enzymes in the liver cell juices compared to the witnesses . mitochondrial size and permeability.

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

Keywords: Imidacloprid, Melissa extract, oxidative stress, antioxidants.… .etc.

Résumé

L'objectif de ce travail est de mettre en lumière les effets toxiques de deux doses d'imidaclopride et l'effet préventif et curatif de l'extrait de mélisse contre cette toxicité. Nos expériences ont ciblé plusieurs domaines d'expérimentation de base, à commencer par le travail des enzymes cellulaires et la mort cellulaire.

Notre étude a inclus 20=5*4Rats réparties en cinq groupes dans chacun d'eux (4 souris) : le groupe 1 a observé, le groupe 02 traité avec l'imidaclopride (5 mg /kg /jour), le groupe 03 a été traité avec une dose (50 mg /kg /jour), et les deux derniers groupes ont été traités avec le mélange d'imidaclopride Et extrait de mélisse (10 mg / kg / jour) pendant 21 jours.

Nos résultats ont montré que l'exposition à l'imidaclopride et à son mélange due à des effets nocifs sur l'organisme se traduit par une diminution du poids avec une augmentation du poids relative du foie par rapport aux témoins. De plus, une analyse des traitements hépatiques à l'imidaclopride montre une augmentation de l'activité des indicateurs biologiques d'oxydation et une diminution des enzymes antioxydants dans les sucs cellulaires du foie par rapport aux témoins Lors d'une augmentation de la taille et de la perméabilité mitochondriales.

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 : imidaclopride, extrait de mélisse officinal, stress oxydant, antioxydants….etc.

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First of all, we thank our God, the almighty one for having given us the courage, the will and the health in order to accomplish this work.

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Our deep thanks go to all the teachers in the biology department as well as all the members of the administration and the laboratory. Without forgetting all the people who contributed directly or indirectly to the realization of our work.

.

Dedication

I am very happy to dedicate this humble work as a sign of my gratitude and my deep thanks for the following :

To my hero, The captain of the family ship my dad may God keep him.

To the good lady that all families wish to have a Mama, the pillar of our house. .

To my dear sisters Aida and my twin Douaa, Fadia ... may God protect you.

To my brothers ... Abda'Ellah (and his wife Asma) , Mossadak.

To all my friends…

Lamia Gherbi

Dedication

I dedicate this humble work :

to the most precious person in my heart ... a person who has the destiny not to be present in the sweetest moments of my life ... my dear father

May God have mercy on you and make your dwelling a paradise ....

To my beloved mother .. my brothers and sisters .. may God protect you always.

to my friends ...

And to everyone who loves me ...

Loubna

dication

All words cannot express gratitude, love, respect, recognition, it is all simply that: I dedicate this modest work to:

To My tender Mother Elhadba: You represent for me the source of tenderness and the example of devotion which has not ceased to encourage me. You've done more than a mother can do to keep her children on the right path in their lives and studies.

To My very dear Father Elwardi: No dedication can express the love, esteem, devotion and respect that I always have for you. Nothing in the world is worth the efforts made day and night for my education and my well being .This work and the fruit of your sacrifices that you have made for my education and training over these years.

To my very dear husband Tag Chérif: Your sacrifices, your moral and material support allowed me to succeed in my studies. This work is testimony to my gratitude and my sincere and faithful love.

To my dear and adorable brothers and sisters:

Hichem, Aissa, Ramzi

Salwa, Nawel, Sara, Rabab

As a testimony of my fraternal affection, my deep tenderness and gratitude, I wish you a life full of happiness and success and May God, the almighty, protect and guard you.

To all those who feel dear to me and whom I have omitted to

Samiha

Introduction

Neonicotinoids are the most widely used family of insecticides in the world today. Use as an agricultural insecticide is largest share of sales. In early 1990s, *neonicotinoids* acquired a large share of insecticide market. With a turnover exceeding one billion dollars in 2005, they have become the 1st best-selling class of insecticides in the world, with a market share of more than 23% **(Elbert and *al*., 2008 ; Jeschke and *al*., 2011).**

Neonicotinoids have distinction of being among the least persistent insecticides in the soil. **(Gupta and *al*., 2007 ; Hatcher and *al*., 2008).**

Neonicotinoids can also have harmful effects on the natural enemies of insect pests. They can induce an increase in the mortality of predators following the consumption of treated prey and not by contact with the insecticide. **(Vincent and *al*., 2000; Papachristos and Milonas, 2008 ; Moser and Obrycki, 2009).**

Neonicotinoids are agonists of acetylcholine on postsynaptic nicotinic cholinergic receptors. Their irreversible binding to *nAChRs* causes continuous excitation of the membranes of neurons, leading to cell depletion and then death of the animal **(Belzunces and *al*., 2012).** The nature of the bonds involved and the chemical structure of the sites involved are known with precision. The different substituents of molecules of this family are at the origin of a more or less strong affinity for *nAChRs*. At the intracellular level, phosphorylation mechanisms can also modulate this affinity. This is closely linked to insecticide activity. **(Matsuda and *al*., 2001).**

Among *neonicotinoids, imidacloprid* is the major representative, the best-selling insecticide in the world and representing 42% of *neonicotinoid* sales. **(Jeschke and *al*., 2011).**

*Imidacloprid i*s a neurotoxic insecticide that is part of *Neonicotinoid* family. It was the first *Neonicotinoid* marketed in the world in 1991. The success of this class of insecticides is due to the low persistence of these insecticides in the environment, which is accompanied by good efficacy at low dosages, in particular in the field of seed coatings. In addition, these insecticides have a high selectivity towards insects compared to mammals. **(Tomizawa and Casida, 2003)**.

Imidacloprid is the most widely sold *neonicotinoid* (41.5% of *neonicotinoid* market), making it the most widely used insecticide in the world. **(Jeschke and *al*., 2011).**
It is a neurotoxicant which acts specifically on the nicotinic acetylcholine receptors (*nAChRs*) located in the nervous system of insects. **(Thany and *al*., 2007).**

Medicinal plants have always had an important place in the therapeutic arsenal of humanity. According to the World Health Organization (WHO), about *65-80%* of the world's population in developing countries, due to poverty and lack of access to modern medicine, depend mainly on traditional herbal medicines for their primary health care. And despite the remarkable progress in synthetic organic chemistry of the twentieth century, more than 25% of the drugs prescribed in industrialized countries derive directly or indirectly from plants.**(Newman and *al.*, 2000; Calixto, 2005).**

Our *Melissa Officinal* plant is known for its richness in secondary metabolism products and particularly in essential oils and tannin alkaloids… **(Baba Aissa, 1999).**

Active ingredients of Melissa 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 and *al.*, 2000)** We find an extract of *Melissa* in the water of *Melissa* of the Carmine, which is an alcohol reputed indicated as antispasmodic, stimulating, for nervous affections, palpitations or even the lack of appetite. **(Debuigne, 2013).** Melissa will preferably be used in herbal teas because of its very pleasant taste or in hydro-alcoholic extract in order 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 subacute toxicity of the new neonicotinoid insecticide "*Imidacloprid*" and the opposite effect of Melissa in the oral wistar rat. To this end, we are interested in studying its impact on body weight on certain biochemical parameters as well as on the liver.

Our end-of-study work is divided into two parts:

1) The first is a bibliographic study is divided into three chapters:

- Chapter 01: *Neonicotinoid.*

Quite extended, to studies carried out on the toxicity of *Neonicotinoids* in wistar rat by renowned researchers and whose results are benchmarks in the field.

- Chapter 02: *Imidacloprid.*
- Chapter 03: *Melissa officinalis*

2) 2) The second part devoted to the experimental study divided in two

chapiter :

I. A description of the equipment used and the experimental protocol.

II. A presentation of the results obtained followed by their discussion.

First part

Bibliographic

Chapter 01:

Neonicotinoids

1. Characteristics and use of *neonicotinoids*

Neonicotinoids are insecticides very largely used and whose sales do not cease increasing since their appearance on the market in the Nineties. They are agonists of the receivers to the acetylcholine (*nAChR*), which have a selective toxicity of the invertebrates. Their physicochemical characteristics make them very persistent in the environment.

1.1.Framework of use

1.1.1. History

The first molecules belonging to the family of the néonicotinoïdes were synthesized in the Seventies. The term of "*Neonicotinoid*" was proposed by the Japanese researcher Izuru Yamamoto to differentiate this family from old " *nicotinoids"* the plants containing of nicotine, used like insecticides since the XVIIIe century **(Jeschke and Nauen 2008; Matsuda and *al*., 2001)**. Insecticidal capacity of the first molecules tested was however very weak, research was this continued to identify the active chemical groups and to synthesize molecules with a better activity. *Nithiazin* was one of the first molecules of interest having a satisfactory insecticidal activity, a systemic distribution in the plants, and a low toxicity at the vertebrate ones.But it was quickly degraded by hydrolysis or photolysis, making impossible its agricultural use. Approximately *2 000* molecules were tested before the discovery of the imidaclopride by Shinzo Kagabu and its marketing in *1991 by Bayer CropScience*. The family increased then and counts today seven made up marketed: *imidacloprid* and *thiacloprid*(developed by Bayer CropScience), *clothianidine* (*Bayer CropScience and Sumitomo*), *thiamethoxam* (Syngenta), *acetamiprid* (Nipponese Soda), *nitenpyram* (*Sumitomo),* and *dinotefurane* (*Mitsui Chemicals*). Eighth composed, *sulfoxaflor*, recently was put on the market in China and at United States and was examined by European Authority of safety of food (*EFSA*) for approval in the European Union. In China, new compounds *neonicotinoids* are developed and tested (for example, the guadipyr and the huanyanglin) and are close to their marketing. **(Jeschke and *al*., 2011; Simon-Delso and *al*., 2015).**

The arrival of *neonicotinoid*s on the market of the insecticidal pesticides was an immediate success for several reasons **(Jeschke and Nauen, 2008; Simon-Delso and *al., 2015).***

- there were no resistances known to these pesticides at *ravageurs*, contrary to *organophosphores, carbamates and pyrethrinoid.*

- Their physicochemical properties made them more interesting compared to the preceding generations of insecticides: they are active in quantities much smaller than the other families, their use in pelleting of the seeds rather than in pulverization the wasting limits, and they have a systemic distribution.

- They have a low toxicity for the nontarget user and species (others that arthropods) thanks to their very selective mode of action of the insects.

They this became the insecticides most largely used among the 5 great chemical classes, in front of *organophosphores, carbamats, phenylpyrazols and pyrethrinoid* ones. *neonicotinoids* found applications in agricultural medium, garden of amateur and of domestic use to fight against certain vermin, like in the veterinary field with the control of the external parasites of the domestic animals **(Jeschke and *al*., 2011).**

1.1.2. Use

1.1.2.1. Obtaining the AMM

For any marketing of a new product containing *neonicotinoid*, the manufacturer must obtain **a Marketing authorization** (AMM). He must for that submit a showing dossier:

- Farming effectiveness.

- Harmlessness for the Man.

- Harmlessness for the animals and the environment.

- Conformity with the legislations main road and European.

With regard to the bees, the tests of ecotoxicity carried out include/understand: studies in laboratory, leading to the determination of a lethal amount 50 (DL50), as well as studies under tunnel and on the ground using the product with the amount asserted on the cultures concerned or gravitational cultures for the bee **(Clément and *al*., 2002).**

1.1.2.2. Agricultural use

Neonicotinoids represent the family of insecticides today the most used in the world. The use as an insecticide of agricultural use is the most significant share of the sales. They are used in pulverization and coating of seeds for their insecticidal effects against many vermin, even resistant to other families of insecticides, like the plant louses, the thrips, the termites, the aleurodes, the taupins and certain beetles. Their use was very quickly spread in the world on the cultures of rice, cereals, corn, sunflower, potatoes, cotton, beet sugar and the fruit trees and the market gardenings. The modes of use are varied: pelleted seeds, soil stabilization and pulverization mainly, but also application to the base of tree trunks, injection of the tree trunks and the buds, or steeping and chimio-irrigation for the cultures under greenhouse. The use is not only professional, *neonicotinoids* also find in products of domestic use for the fight against certain vermin as the termites, the cockroaches or the ants **(Casida and Quistad, 2004; Jeschke and *al*., 2011; Bonmatin and *al*., 2015; Goulson, 2013).**

1.1.2.3. Veterinary use

Neonicotinoids are fast insecticides and with very selective effects: their use in the domestic animals is interesting. AMMs relate to the infestations with the chips in the cat, the dog and rabbit. They concern *Advantage*®, *Clearspot*®, *Capstar*® and *Midaspot*®. spectrum of action can be supplemented by pyrethrinoid to also act against the ticks, the mosquitos, the phlebotomi and the piqueuses flies in the dog and the cat: one finds in this group *Advantix*®, *Ataxxa*®, *Seresto*®, *Vectra 3D*® *and Tickgard*®. Lastly, an association with the moxidectine widens the spectrum with the internal parasites of the cat, the dog and the pipe cleaner: one finds in this category Advocate® **(ANSES, 2016b; Hovda and Hooser, 2002; Jeschke and *al*., 2011).**

1.2. Physicochemical characteristic

1.2.1. Chemical structure

The structure of *neonicotinoids* is divided into *3* segments (fig. 1) **(Iwasa and *al*., 2004; Jeschke and Nauen, 2008; Jeschke and *al*., 2011*).***

- (i) a group "R1" and "R2", cyclic (*imidacloprid, thiamethoxam, thiacloprid) or acyclic (acetamiprid, nitempyram, clothianidin, dinotefuran*).

- (ii) a cycle "A" of 5 or 6 atoms, with a chlorine atom.

- (iii) a functional group cyano or nitro "[X there]", essential to the insecticidal activity.

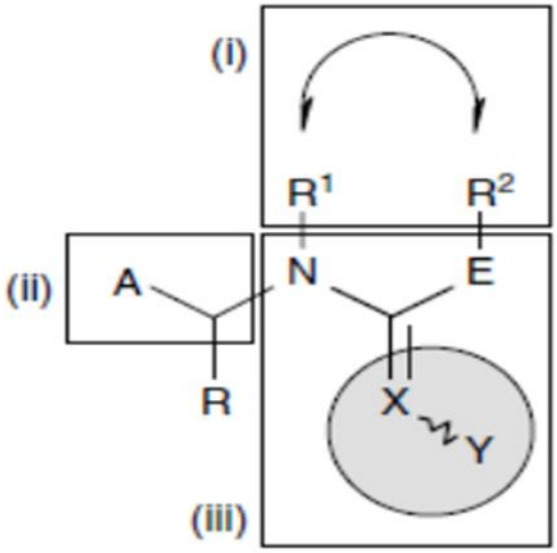


Figure 1: Structure of *Neonicotinoids* **(Jeschke and Nauen, 2008).**

According to this functional group, one can classify the molecules according to property S common physics (fig.1) **(Jeschke and Nauen, 2008)** :

- *nitroimins: thiamethoxam, dinotefuran nithiazin, imidacloprid, clothianidin and nitempyram.*

- *cyanoimins: thiacloprid and acetamiprid.*

The insecticidal capacity is also correlated with the chemical structure **(Iwasa and *al*., 2004; ecourtye and Devillers, 2010; Matsuda and *al*., 2001) :**

- The molecules with a cycle "A" with *5* carbon atoms are less powerful than those with a cycle with *6* atoms.

- The presence of the substituents oxygenates, sulphide or nitrogen increases the insecticidal action.

- Nitrosubstitus molecules present a better insecticidal action than cyanosubstitus.

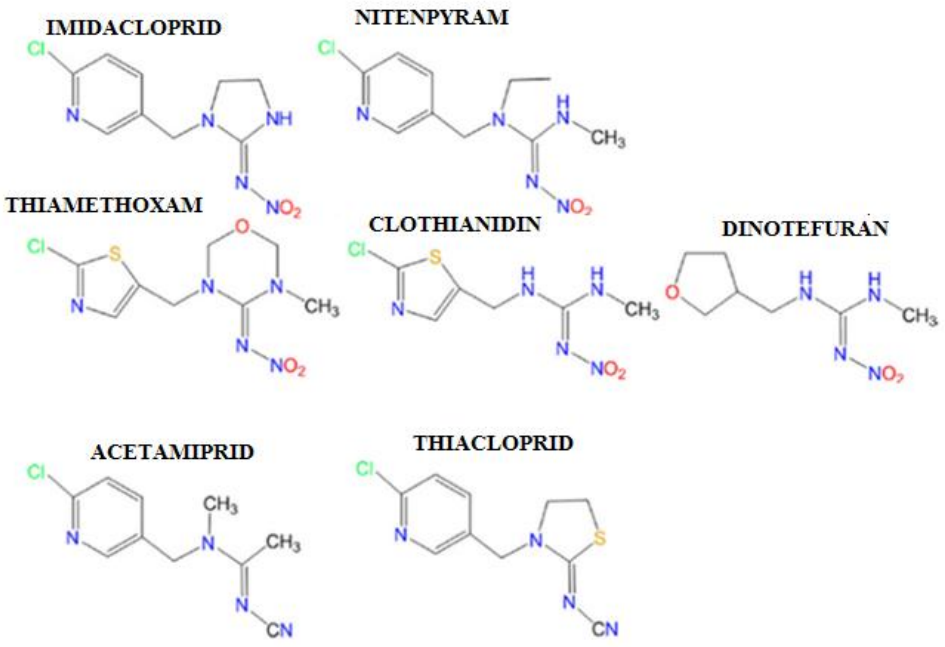


Figure 2: Plane formulas of principal *Neonicotinoids*. According to

(**Simon-Delso and *al*., 2015).**

1.2.2. Physical proprietes

1.2.2.1. Molecular weight

The molecular weight of *neonicotinoids* lies between *250* and *300g/mol*, which in fact of insecticides of low weight: by way of comparison, the weight of the fipronil, another systemic insecticide, is of *440g/mol* **(Bonmatin and *al*., 2015).**

1.2.2.2. Volatility

Neonicotinoids have saturating a vapor pressure ranging between *2,8.10-8* and *0,002* mPa with *25°C*. This in fact of the volatile molecules not very, they contaminates the air during treatments little, and the air contamination lasts little of time after pulverization. **(Bonmatin and *al*., 2015; Fossen, 2006).**

1.2.2.3. Sorption

Sorption characterizes the capacity of connection to particles. *Neonicotinoids* can bind to the particles of the ground, this limiting the phenomenon of scrubbing at the origin of the contamination of water. Several factors come into play: the content of organic matter as well as the content of clay are positively correlated with the sorption of *imidacloprid*, while a low temperature or a weak insecticide concentration decreases sorption. *Neonicotinoids* are also fixed at the particles of the basic marine water or fresh water sediments **(Bonmatin and *al*., 2015; Fossen, 2006; Gervais and *al*., 2010).** However, in the commercial preparations, the addition of many substances modifies the behavior of the active substance: thus scrubbing is higher with all the formulations of the trade compared to *imidacloprid* only **(Bonmatin and *al*., 2015).**

1.2.2.4. Solubility

Solubility in water varies according to molecules, of *184mg/L* for *Thiacloprid* (moderate solubility) with *590g/L* for nitenpyram (high solubility). These values are valid with *20°C* for a *pH* of *7*. Compared to the other families of insecticide, *Neonicotinoids* have a good solubility: as an example, under the same conditions, the solubility of the fipronil is between *1.90* and *3.78mg/L*. **(Jeschke and Nauen, 2008; Bonmatin and *al*., 2015).**

The variations of solubility are correlated with their structure:

- The open compounds are more absorbent than the cyclic compounds

- Concerning the group [X-Y], solubility in water increases with increasing manner [=N-NO2] < [=N-CN] < [=CH-NO2].

Solubility is significant since it conditions their absorption then their diffusion, stages essential to their effectiveness. For example the least water-soluble *neonicotinoids* like *Thiacloprid* or the clothianidine will be adapted for treatment of seeds, the passage membranaine in the roots being better for the lipophilic compounds **(Jeschke and Nauen ,2008; Jeschke and *al*., 2011).**

1.2.3. Persistence in the environment

1.2.3.1. Contamination by aerosols

Neonicotinoids are not very volatile; the contamination of air can occur over a short period at the time of the application of the treatments. The very broad use of pelleted seeds seems to be a method of use surer than pulverization. However, several cases of acute mortality of bees were reported at the time of sowing coated corn seeds. The led studies showed that considerable quantities of *neonicotinoids* are present at the level of the exhaust of the pneumatic seeders (of the order of the µg/m3 with 10m of the seeder), forming a "cloud of toxic dust" **(Fossen, 2006; Tapparo and *al*., 2012; Bonmatin and *al*., 2015)**. These pesticides contaminate in the second time the surrounding vegetation. Thus, of the clothianidine was detected at a rate of 9ng/g on dandelions close to coated corn sowing **(Krupke and *al*., 2012).**

This contamination presents a risk for the bees, on several levels: the toxic cloud of dust directly exposes the butineuses ones to high quantities of *neonicotinoids*, and the contamination of the close vegetation can expose the bees to a nectar or a contaminated pollen **(Bonmatin and *al*., 2015; Sgolastra and *al*., 2012).**

Measures were taken to improve the systems of seeders, by using "deflectors". The objective is to decrease the size of the cloud around the seeder. Certain modifications were also made to the formulation of the seeds treated to limit the erosion and the formation of dust. These points of improvement present limits: the addition of the deflectors can harm the precision in the use of the seeds and increases the working time, and these modifications seem neither significantly to decrease the particulate emission of *neonicotinoids* nor to influence the mortality of the bees near the treated seeds **(Tapparo and *al*., 2012; Bonmatin and *al*., 2015).**

1.2.3.2. Contamination of the ground

1.2.3.2.1. Modes of contamination

The contamination of the grounds can be due to a direct application of *neonicotinoids*, or follow upon the release of the pelleting of the treated seeds **(Bonmatin and *al*., 2015).**

1.2.3.3. Mechanisms and speed of degradation

The contamination decreases then by vegetable absorption, scrubbing and natural degradation of the active molecule. Degradation is influenced by the type of ground, the ultraviolet rays surfaces some, moisture, the temperature and *pH* it is this variable from one

place to another and one finds the half-life shortest in the tropical areas, with a high temperature and a moisture **(Bonmatin and *al*., 2015).**

In spite of these mechanisms of decontamination, *neonicotinoids* can remain present very a long time in the ground: about several me S at several years. According to studies' considered, it arises a great variability in the collected data, which is coherent when one takes into account the factors of variation inherent in the nature of the ground and the weather conditions. Thus, for Imidaclopride, studied molecule, one is located at orders of magnitude going from *100* to *1000* days. The greatest variations are found for Clothianidine, and it is also for this molecule that one has the most raised half-lives: from *148* days with nearly *7000*.

Table 1: Time of half-life in the ground of principal the néonictoinoïdes. Sources : a = (Hladik, Kolpin, Kuivila 2014) ; b = (van der Sluijs and al. 2013) ; c = (Bonmatin and al., 2015) ; d = (Bonmatin and al. 2005) ; e = (Fossen, 2006) ; f = (Goulson, 2013)

Molecule	½ life in the grounds (D)
Acetamiprid	3a ; 31-450f
Clothianidin	545 a ; 148-6931b, f
*Dinotefura*n	82 a ; 75-82f
I*midacloprid*	107c ; 191 a ; 40-997 b ; 188-249c ; 270d ; 26-229e ; 28-1250f
Thiacloprid	15.5 a ; 3.4->1000f
Thiamethoxam	50 a ; 7-353f
Nitempyram	8f

1.2.3.3.1. Levels of contamination

Many studies were interested in the contamination of ground, throughout the world. We can quote the study of **(Bonmatin and al., 2005)**, during which the concentration of *imidacloprid* was analyzed in 74 grounds covering a broad range of climates, types of grounds and husbandries in France. *imidacloprid* was detected in *91%* of the samples *(> 0,1 µg/kg)* whereas only *15%* of the sites had been sown seeds coated during the same year. *imidacloprid* was detected in *97%* of the grounds sown with coated seeds 1 or 2 years before the study. Interesting fact, the concentrations were higher in the grounds which had been treated consecutively during 2 years before the analysis than in those which had received seeds only treated 1 year before the analysis, which indicates that *imidacloprid* can

accumulate in the grounds with the wire of time. This persistence was confirmed by other researchers. This in zones where no culture has been treated for 2 years, *clothianidine* is always.

Detected in the ground Many studies were interested in the contamination of ground, throughout the world. We can quote the study of **(Bonmatin and *al.*, 2005)**, during which the concentration of *imidacloprid* was analyzed in *74* grounds covering a broad range of climates, types of grounds and husbandries in France. *imidacloprid* was detected in *91%* of the samples *(> 0,1 µg/kg*) whereas only *15%* of the sites had been sown seeds coated during the same year. Imidaclopride was detected in *97%* of the grounds sown with coated seeds 1 or 2 years before the study. Interesting fact, the concentrations were higher in the grounds which had been treated consecutively during 2 years before the analysis than in those which had received seeds only treated 1 year before the analysis, which indicates that the imidaclopride can accumulate in the grounds with the wire of time. This persistence was confirmed by other researchers. This in zones where no culture has been treated for 2 years, the clothianidine is always detected in the ground **(Krupke and *al.*, 2012)**. And even six years after the culture of rhododendrons treated with *imidacloprid,* residues were detected with a maximum concentration of *19µg/kg* **(van der Sluijs and *al.*, 2013)**. The analysis of a ground where pelleted seeds had been used 4 to 6 years before showed the presence of *imidacloprid* at a rate of *18µg/kg* **(Schmuck and *al.*, 2001)**. In Ghana, taking away of grounds carried out 4 months at 2 years after the last use *of neonicotinoids* on cocoa cultures show the presence of Imidaclopride in *50%* of the samples, with concentrations of *4.3µg/kg* with *251.4µg/kg*, and clothianidine in *10%* of the samples. The strong contamination noted compared to the other geographical areas is explained partly by a free use of *neonicotinoids* in this country **(Dankyi and *al.*, 2014).**

Persistent contamination of the grounds raises the question of absorption by the following cultures. Several studies measured the contamination of untreated plants cultivated on grounds contaminated by the preceding cultures. **(Schmuck and *al.*, 2001)** did not detect of trace of *nonicotinoids i*n the seedlings of sunflower resulting from seeds untreated cultivated on a ground contaminated with *18µg/kg* (with a limit of detection of *1µg/kg*). Other studies contradict these results: for example of the untreated sunflowers cultivated on a contaminated ground (at a rate of *6µg/kg* of Imidaclopride) contain this pesticide with a concentration of 1 with *2µg/kg* on the level of capitulum **(Bonmatin and *al.*, 2005).**

1.2.3.4. Contamination of water

1.2.3.4.1. Modes of contamination

The water surface and underground can be contaminated by foliar streaming, scrubbing of the grounds, flow, used water, foliar drift of pulverization or dust. Significant contaminations can also be found locally at the time of the treatments, for surface water, during flood of greenhouses **(Bonmatin and *al*., 2015).**

The contamination of water can make following the agricultural use of *neonicotinoids* but also with the urban use, during the treatment of the plants of ornament: the surface water can involve the suspended particles and the particles adsorbed with sediments of small size**.**

1.2.3.4.2. Mechanisms and speed of degradation

Degradation in the water of *neonicotinoids* depends on several factors. In conditions of laboratory, it was shown that the photolysis decreases the half-life of *imidacloprid* to less 3h **(Fossen, 2006).** In conditions of ground, this factor is to be modulated: the light which passes in water varies much according to the depth and the turbidity of water. Degradation is done mainly by hydrolysis, phenomenon much slower. It varies with the temperature: from 547 days with *5°C* at *89* days with *25°C* for a study carried out with the imidaclopride. PH of water between also in account: *Neonicotinoids* are stable for a *pH* ranging between *5* and *7*, and are degraded more quickly with pH basic **(Bonmatin and *al*., 2015; Gervais and *al*., 2010).**

1.2.3.4.3. Levels of contamination

Contamination of water was reported in many countries.In Sweden, a study analyzed the rivers of the zones drained close to market gardenings under greenhouse:

Imidacloprid was present in 36% of the samples, with a maximum content of *9.6µg/L* **(Bonmatin and *al*., 2015)**. In Spain, *imidacloprid* was detected in *58% (2010)* and *17%* of the samples (*2011*), with concentrations at the time of these two years ranging between *2,34* and *19,20 ng/L* **(Masiá and *al.*, 2013)**. In spite of raised frequencies of detection, the measured rates are in conformity with the European standards whose limit is of *0.1µg/L* with regard to drinking water. In the United States, in Iowa, the analysis of the water of brooks of a producing area of corn and soya showed the presence of residues of *neonicotinoids*. In this area of intensive production, it is the clothianidine which was found most frequently (in 75%

of the samples), in front of *Thiamethoxam* (*47%*) then *Imidacloprid* (*23%*). Maximum and median concentrations (maximum concentration; median) of these three molecules followed the same order as their frequency of detection with the c*lothianidine* (*257ng/L; 8.2ng/L*), *Thiamethoxam* (*185ng/L; < 2ng/L*) then *Imidacloprid* (*42.7ng/L; < 2ng/L*) **(Hladik, Kolpin, Kuivila, 2014)**. Another study undertaken in California showed a contamination by *Imidacloprid* in *89%* of the samples **(Starner and Goh, 2012)**. In *2008*, United State Environment Protection Agency (US EPA) detected rates of *0.2* with *0.7µg/L* in water in the State of New York **(Bonmatin and *al.*, 2015)**. A study of the basins of the area of Sydney showed a contamination by *Neonicotinoids* of all the analyzed samples, with *Imidacloprid* in *93%* of the samples (average concentration *0.2µg/L*) and of thiacloprid in *80%* of the samples (average concentration *0.15µg/L*) **(Sánchez-Bayo and Hyne, 2014).**

It is necessary well on taking into account the environment in which these samples are taken: behavior of the farmers, weather of the previous days, products authorized in the countries considered… This, the concentration in *neonicotinoids* is related to precipitations, with an increase after each episode of rain. These values show also a saisonnality, with a peak of contamination of the brooks in May and June, which corresponds to the period of plantation and thus of treatment of the cultures. However, *neonicotinoids* are also detected before this period: they are thus the residues of the treatments of the previous year which are found in the rivers at the time of the cast iron of snows and the rains. After June, the frequency of detection and the concentrations decrease gradually on the remainder of the year **(Hladik, Kolpin and Kuivila, 2014).**

For subsoil waters, the studies are more difficult: no presence of Imidaclopride was still highlighted **(Fossen, 2006)**, however one estimates that time required to have a contamination starting from the contamination of surface water *20* years east **(Bonmatin and *al.*, 2015)**. This, even if *Neonicotinoids* are now used since a little more than 20 years, there is not yet the retreat necessary to evaluate all the consequences in terms of contamination of subsoil waters.

1.3. Mode of action

1.3.1. Cholinergic activity

Cholinergic system represents in the insects the principal exiting transmission channel of the central nervous system **(Palmer and *al.*, 2013).** Acetylcholine is *neurotransmettor* released on the level of the presynaptic membran. The receiver nicotinic with *acetylcholin* (*nAChR*) is

a combination of 5 similar sub-units (homomeric) or different (heteromeric) (fig. 3). These sub-units form a channel with controlled opening, specific of the ions Na+, K+ and Ca2+. receiver is N-final end of a sub-unit, extracellular field: fixing of acetylcholine involves opening of cation channel then a depolarization of nervous cell, at the origin of transmission of nervous signal. **(Casida and Quistad, 2004).**

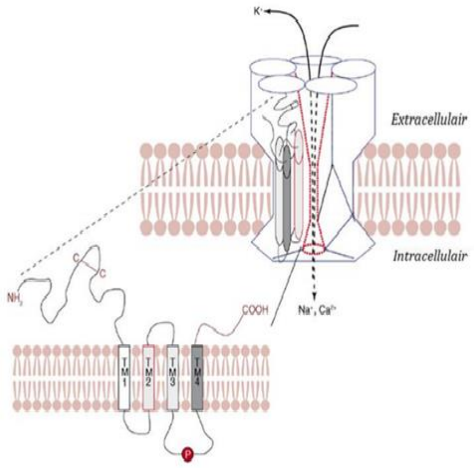


Figure 3 : Nicotinic structure of the receiver to acetylcholine (in top) and of one of its sub-units (in bottom). TM1, 2, 3 and 4 represent the four membran fields of a sub-unit, prolonged by the extracellular ends N-final and COOH. In intracellular position, the site P corresponds to the phosphorylation site implied in the modulation of the nervous messages. According to **(Thany and *al*., 2007)**

Neonicotinoids are agonists of *acetylcholin* on cholinergic receivers nicotinic postsynaptic. Their irreversible connection with *nAChRs* entraine an excitation continues membranes of the neurons, leading to exhaustion of cells then to died of the animal **(Belzunces, Tchamitchian, Brunet, 2012).** Nature of the connections concerned and the chemical structure of implied sites are known with precision.Various substituents of molecules of this family are at the origin of a more or less strong affinity for *nAChRs*. At the intracellular level, phosphorylation mechanisms can also modulate this affinity. This one is closely related to the insecticidal activity **(Matsuda and *al*., 2001)**. Other sites of connection would enter also concerned the activity of neonicotinoids, with for example a role of calcic channels voltage-dependent **(Liu and Casida, 1993; Thany and *al*., 2007; Matsuda and *al*., 2001; Palmer and *al*., 2013; Simon-Delso and *al*., 2015)**. *Neonicotinoids*, like many other insecticides, involve cellular and tissue lesions in the remainder of the organization. Analyses

by immunofluorescence showed that the cells in apoptose were significantly more numerous in larvae of bees nourished during 4 days with 400ppm of *imidacloprid* than in pilot larvae **(Gregorc and Ellis, 2011).**

The degradation of these molecules can be done via *acetylcholin-esterase*, active neither on nicotine nor on *neonicotinoids*. The mechanisms of detoxification on the level of neurons are very limited **(Simon-Delso and *al*., 2015; Casida and Quistad, 2004)**. This involves a prolonged action of insecticide. Moreover, among many the metabolites formed, some are very toxic and can be at the origin of letaux and subletaux effects delayed **(Suchail, Debrauwer, Belzunces, 2004; Simon-Delso and *al*., 2015)**. The metabolisation rests, as at plants, on a phase I dependent on the P450 cytochrom with reactions of oxidation, reduction, or hydrolysis, and phase II of conjugation before excretion **(Casida, Quistad, 2004; Suchail, Debrauwer, Belzunces, 2004; Honda, Tomizawa, Casida, 2006; Casida, 2011).**

1.3.2. Selective action

Vertebrate and invertebrate present differences in structures in the sousunities of the *nAChRs.* This, *neonicotinoids* have a greater affinity for the receivers of insects than for those of the vertebrate ones: at the latter, they act like weak antagonists of *nAChRs* and affinity for these receivers is weak with null. Moreover, the insects present the nervous fabric richest in *nAChRs*. This explains low toxicity at the Vertebrate ones, with however a very variable sensitivity and some significant species like partridge **(Matsuda and *al*., 2001).**

With in the invertebrates, and even within the same species, there are various types of *nAChRs*, having a variable sensitivity to insecticides **(Guez and *al*., 2001; Thany and *al*., 2007)**. For example, one finds at Periplaneta americana, the American cockroach, 2 types of receivers: *nAChR1*, sensitive to *Imidaclopride*, and *nAChR2*, insensitive with *imidacloprid* and sensitive to nicotine, *acetamiprid* and with *thiacloprid*. The variations in the structure of *nAChRs* are related to variations of affinity of the connections which are created between insecticide and its sites of connection. In the bee, the existence of at least two types of *nAChRs* is strongly suspect and could explain differential toxicity between weak amounts and very weak amounts **(Simon-Delso and *al*., 2015)**. Various types of receivers are expressed during the development of the insect: in the bee, it was shown a difference in sensitivity to *neonicotinoids* according to the age, suggesting a maturation of the expression of *nAChRs* **(Guez, Belzunces, Maleszka 2003; Guez and *al*., 2001)**. The studies showed that for

equivalent introduced amounts, the Apis bees will mellifera had not shown any sign of intoxication, whereas the bumblebees Bombus terrestris had modified their food behavior **(Cresswell and *al*., 2012)**. This characteristic is to be taken into account at the time of the study of toxicity at the species nontarget: Apis will mellifera is by far the species most studied, and used like model, but the conclusions at this species always accurately do not reflect the effects at the remainder of pollinating fauna**.** The sensitivity of the invertebrates to *Neonicotinoids* is consequently variable, between the species and within the same species according to its stage of maturation.

1.3.3. Mechanisms of resistance

Resistances to *neonicotinoids* were observed at certain target species like the aleurodes or the Colorado beetles. **(Casida and Quistad, 2004; Goulson, 2013)**.

Several mechanisms at the origin of resistances of the insects to, *neonicotinoids* were highlighted.Genetic changes in the sequences coding for sousunities of *nAChRs* were identified as being at the origin of resistances to *imidacloprid*; *phenomena* of phosphorylation of intracellular fields of *nAChRs* can also desensitize insects **(Thany and *al*., 2007)** ; finally, it was shown that an increase in the synthesis of the enzymes intervening in the metabolisation is responsible for resistances in drosophila **(Casida, 2011).**

In spite of their common target, to date, no cross resistance was shown between *sulfoxaflor* and the others *Neonicotinoids* **(Simon-Delso and *al*., 2015)**. However, it is necessary to note the very recent arrival of this molecule on market, the retreat is not the same one as for the other components of the family.

1.4. Effets on health

1.4.1. Carcinogenic effects

The acute intoxication appears in general immediately or little time after an exposure to a pesticide. The symptomatic signs most often met at the time of an acute intoxication are the cephalgias, nauseas, the vomiting, the dizzy spells, tiredness, the loss of appetite and irritations cutaneous or ocular, difficulties respiratory, convulsions and even coma **(Samuel, 2012)**. These harmful effects can be reversible or irreversible **(Engel and *al*., 2005)**. However the chronic intoxication generally occurs following the absorption repeated of low dose of pesticides for a long length of time. The principal effects of a chronic exposure to the

pesticides are effects on the reproduction, the development, the immune systems and endocriniens as well as carcinogenic effects **(Samuel, 2012).**

1.4.1.1. Carcinogenic effects of neonicotinoids on thyroid gland

Thyroid one is an endocrine gland located at the former face of the neck and controlling, at the vertebrate ones, of many hormonal systems by the secretion of triodothyronine (T3), thyroxine (T4) and calcitonine. The T3 and T4 fix iodine on a protein, under the influence of the TSH, hormone controlling the operation of this gland **(Reynolds and *al.*, 2002)**. In the thyroid one, one finds many small structures round resembling bags which one calls follicules. The follicules produce, store and release the thyroid hormones. The thyroid one is made up various types of cells. The follicules are papered follicular cells, the cells C (also called cells parafolliculairs) which are strewn in all the thyroid one, including between folliculs and in their coating. Among the other cells of thyroid, one counts lymphocytes (a type of white globule) and lubricating cells (called *adipocyts)* **(Reynolds and *al.*, 2002).** (Figure 4).

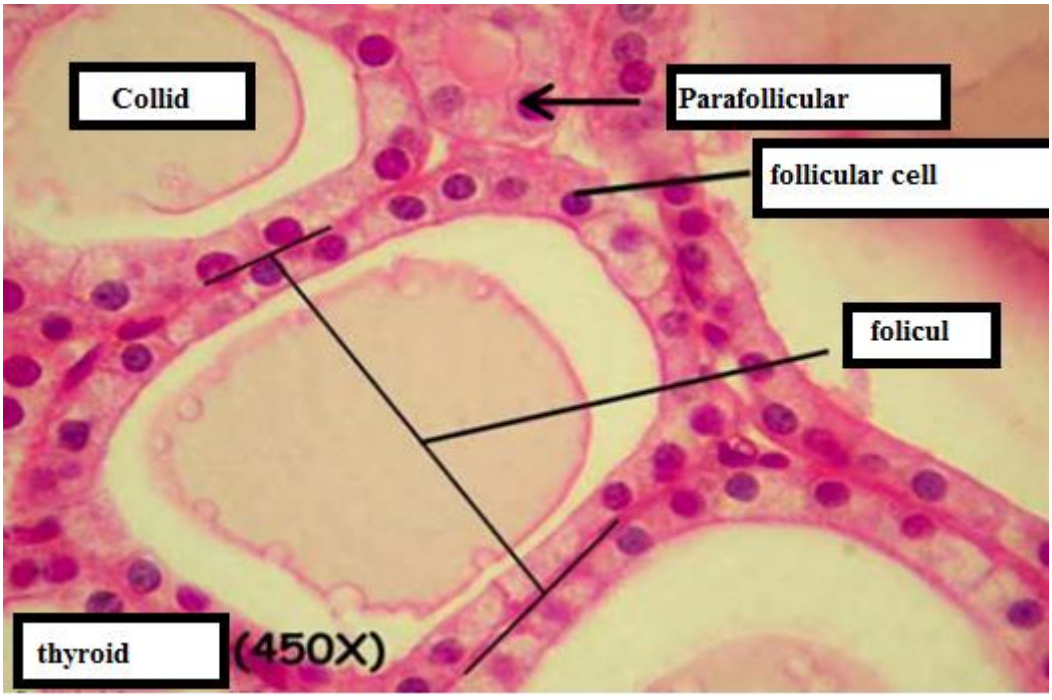


Figure 4 : histology of thyroid the 450x http://cancerthyroide.blogspot.com

Impact of *Neonicotinoids* on this gland with fact the object of several work. The administration of *thiamethoxam* per way oral examination caused a hypertrophy of thyroid gland in rabbits **(Shalaby and *al*., 2010)**. **(Nicolle-Mir, 2011)** showed that the exposure of mal rats to *imidacloprid* entraine a significant increase in the absolute mass of the thyroid one **(Tebourbi and *al*., 2010)** indicated that the injection of another compound *acetamipride* with the rats caused a significant increase in the relative weight of the thyroid one. However an atrophy of thyroid gland translated by the reduction in its absolute mass was to bring back after the treatment of the rats by other *neonicotinoids fipronil* and *Acetamiprid* **(Surks and *al.*, 2004 ; Johnson and *al.*, 2009).**

1.4.2. Non carcinogenic effect of *neonicotinoids*

1.4.2.1. Effect on the reproduction

The reproduction includes/understands the whole of the stages which go from the production of the gamètes conditioning the fertility, until the sexual maturity of the individuals while passing by the fecundation and the nidation of egg, then the embryonic and foetal development. All these stages are characterized by many cellular divisions extremely sensitive to the environmental agents **(Mutigner, 2005)**.

The effects of *neonicotinoids* on the process of reproduction was to prove rabbits expensive, indeed (**Robitaille, 2014)** showed the testosterone increase at subjects exposed to *neonicotinoids* in an agricultural medium. Work of (**Aïna and *al*., 2015)** showed the deterioration of the hormones intervening in the process of reproduction maiîs also the follicular development in rabbits exposed to *Acetamiprid*.

1.4.2.2. Effect hematotoxic of *neonicotinoids*

The immune system is a biological system made up of a coordinated whole of element of recognition and defense which discriminates it to oneself not-oneself. It is made of a system of complex interaction implementing of many bodies, cells and substances different. Osseous marrow and the thymus produce the immunizing cells the lymphocytesr. many pesticides are known to have effects *immunotoxic* **(Riedel and *al*., 1997)**. This of new pesticides are each year developped at the point in order to better correspond to the requirements medical and

from other by each year of new pesticides are withdrawn from the market or put on list of day before from their strong toxicity on the nontarget organizations **(Yahia, 2016)**.

Work of **(Aina and *al*., 2015)** showed the effect of a *neonicotinoids acetamiprid* on the number of the lymphocytes. **(Moullan and *al*., 2016)** showed the increase in the lymphocytes in rabbits exposed to another compound belongs to the family of *Neonicotinoids* the *thiamethoxam*. The effects hematologic of *Neonicotinoids* remains badly known.

1.4.2.3. Effect of *neonicotinids* on the hepatic function

The liver is a body of the digestive system which provides three principal functions storage, redistribution, synthesis and purification at the end of the process of digestion/adsorption **(Desvergne and *al*., 2006)**. The liver is also a body of detoxification which ensures the biotransformation many xenobiotic sight of their elimination **(Desvergne and *al*., 2006)**.

Research on toxicity of a *Neonicotinoid Imidcloprid* brought back deteriorations of the activity of hepatic enzymes and an increase in enzymatic activity of alkaline phosphatase in rats. The results revealed levels significantly raised in transaminases, (TGO) and (TGP) and the rate of bilirubin compared to the witnesses **(Bhardwaj and *al*., 2010)**. (**Mondal and *al*., 2009)** recorded an increase in the rate of bilirubine in the rats treated by a *Neonicotinoids Acetamiprid* by oral way with three concentrations *25, 100* and *200 mg/kg/jour* during *28* days compared to the witnesses (Table 2).

Table 2 : Effect of a *Neonicotinoid, Acetamiprid* on the rate of bilirubine in *rat wistar* **(Mondal and *al*., 2009).**

Parameter	Witnesses	*25mg/kg/ day*	*100mg/kg/ day*	*90mg/kg/ day*
Bilirubine	*1,01±0,10*	*1,17±0,12*	*1,28± 0,08*	*1,75± 0,19*

1.4.2.4. Effect of n*eonicotinoids* on antioxydant activity

Work of (**Lonare and *al*., 2014)** showed the installation of a system of detoxification in the rats after the administration of a *Neonicotinoid*, *Imidacliprid* by way oral examination with two amounts *45* and *90 mg/kg*. Indeed an increase in the activity of two enzymes of

detoxification the GSH and the GST is recorded compar ativement with the witnesses (Table 3).

Table 3: Effect of *Imidacloprid* on the activity of glutathionne (GSH) and the glutathionne S-transférase (GST), managed by oral way with *45 mg/kg* and *90 mg/kg* **(Lonare and *al.*, 2014)**.

Parameters (*µmol/h/mg/ proteins*)	GST	GSH
Control	*0.45± 0.02*	*47.89± 1.24*
Imidacloprid 45mg/kg	*0.41 ±0.02*	*42.31± 1.39*
Imidacloprid 90mg/kg	*0.37± 0.03*	*38.72± 1.76*

1.4.2.5. Neurotoxic effect of *Neonicotinoids*

The impact of *Nenicotinoids* was reported by **(Bhardwaj and *al.*, 2010)** expensive the rat wistar after the administration of 20mg/kg/jour of *Imidacloprid* by way oral examination. Their shown results one the inhibition of the specific activity the acétylcholinestérase (*ACtch*). Similar results were reported by **(Rodrigues and *al.*, 2010)**. **(Banerjee, and *al.*, 2014)** with good shown the deterioration of this key enzyme of nervous system after the administration of a compound pendimethalin with the rats. However the injection intraperitonal of *Imidacloprid* to an amount of *337mg/kg/jour* during *30* days causes in the rat an increase in the specific activity of this enzyme by inducing of this fact deterioration D nervous system and a muscular tetany **(Abou-Donia and *al.*, 2008)**.

Chapter 02 :
Imidacloprid

2. Example on *Neonicotinoids*: *Imidacloprid*

Imidacloprid, 1-*(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-ylideneamin)*, are a *Neonicotinoids* insecticide which belongs to the chemical family of *chloronicotinylnitroguanidin* **(Suchail and *al*., 2009).**

Various commercial nominations are associated for him of which *Admire®, Acidor®* (Agro Chemical Industries), *Gaucho®, Genesis®, Prescribe®* (Gustafson LLC), Marathon®, *Confidate®, Imadate®* **(ChemHandbook, 2002).**

In Algeria, *Imidacloprid* is marketed under the name of *Confidor* ® on the market gardenings, fruit trees and even plants of ornamentation **(Djouber, 2011).**

2.1. Applicability of *Imidacloprid*

In Algeria, this insecticide is largely used on the cultures and fruit trees and for the protection of agricultural harvests targeting the devastating insects including the grasshoppers, the flies, the termites, the ladybirds and the insects of grass of the ground, the plants and the trees **(Djouber, 2011).**

Applicability of IMI is multiple **(Jeschke and *al*., 2010)**, it is applied to the foliage, into the pelleting of the seeds or is injected into the ground or the stems to protect the fruit trees and plants **(Damjana and *al*., 2008)**. Harvests to which IMI are applied include/understand various cereals: rice, corn, fruits, vegetables, sunflower, tobacco, cotton etc **(Kagabu, 1997).**

Imidacloprid are also employed in the treatment of seeds, for lutes against the parasites of grass, of the lawns of household, the parks and the fields sporting **(Struger and *al.*, 2002)**.Its use in veterinary medicine is known, It is introduced into drugs intended for the cats and the dogs **(Struger and *al*., 2002)**.

2.2. Mode of action

Imidacloprid (IMI) was conceived to be effective as well by contacts dermic as by ingestion **(Tomlin, 2006).** It acts on several types of post-synaptic receivers *nicotinic* of acetylcholine in the nervous system **(Buckingham and *al*., 1997; Matsuda and Sattelle, 2005)**. Irreversible connection with the receivers leads to the discharge of the impulses

nervous and the absence of propagation of signal by the neurons **(Schroeder and Flattum, 1984; Larry, 2001).**The constant activation of the receivers results from the incapacity of *Acetylcholinesterase* to degrade pesticide **(Matsuda and Sattelle, 2005).**

In mammals, the receivers nicotinic are divided into several sub-types and are localised as well on the level of the junction *neuromusculair* as in the central nervous system (**Larry, 2001).** However, the affinity of *Imidacloprid* is much higher in the insects than in the mammals **(Tomizawa and Casida, 1999; Alok and *al*., 2013).**

2.3. To become *Imidacloprid* in the environment

Principal ways of dissipation of *Imidacloprid* in the environment are photolysis in aqueous medium, microbial degradation **(Fritz and Hellpointer, 1991)** and absorption by plants **(Scholz, 1992).** *Imidacloprid* are quickly photodegrad in water (half-life of 4 hours**) (Anderson, 1991)** par report/ratio on the ground (171 days half-life).It is stable with hydrolysis with normal *pH*, while it is slowly hydrolized in sterile alkaline solutions **(Yoshida, 1990).** The presence of vegetation increases considerably its rate of degradation in ground **(Scholz, 1992)**. IMI is moderately mobile in ground and its strong persistence generats a contamination of surface water by streaming **(MAPAQ and *al.*, 2012).**

2.4. Pharmacokinetic

Imidacloprid quickly is absorbed by oral way and is distributed in almost all bodies and fabrics **(Larry, 2005).**Son absorption by oral way was estimated at 92-99% in rat **(Brunet and *al*., 2004).**

There are two principal metabolic ways of *imidicloprid* in mammals (**Klein and Brauner, 1990)**.First consists of an oxydative cleavage of *Imidazolidin* and *acid 6-chloronicotinic*, with urinary excretion of fragment of *Imidazolidin*.The fragment nicotinic is degraded by conjugation of glutathion to a derivative of acid mercapturic, then with acid methylmercaptonicotinic, which is combined with glycine to form hippuric acid before its excretion in urine.The second stage of this way of degradation consists of a hydroxylation of ring of *Imidazolidin*, followed drainage of water and the formation of a metabolite unsaturate **(Klein and Brauner, 1991a, B; Karl and *al*., 1991; Klein, 1992).**

Capacity of *imidacloprid* to penetrate fabrics was highlighted after the oral administration of *20 mg/kg* of IMI marked at *C14*.The radioactivity was detected in *13* fabrics and bodies tested

of which gastro-intestinal tract, liver, kidneys, lungs and the heart **(Klein, 1987; Klein;Brauner, 1991a).**

More than *90%* of the amount is eliminated in *24h*, with total excretion in 48h; 80% of amount are excreted in urine in the form of glutathion and of glycine combined with *acidmercaptonicotinic* and *hippuric* acid. The *20%* remainders are eliminated in deposit **(Larry, 2005).**

2.5. Toxicokinetics

2.5.1. Absorbtion

After single administration by cramming of an amount of 1 or *20 mg/kg* or after administration repeated by cramming of an amount of *1 mg/kg* during 14 days in the rat, the *Imidacloprid* is quickly and largely absorbed. The maximum concentration is reached into 1 to *2 hours*. Biodisponibility, calculated on basis of urinary and biliary excretion, is higher than *92 %* in 48 hours.

2.5.2. Distribution

Imidacloprid is quickly and widely distributed in the organization, mainly in liver, the kidneys, the lungs and the skin. It should be noted that the penetration of *Imidacloprid* through hemato-encephalic barrier is relatively limited in rat: among the studied bodies, the brain shows rates of radioactivity weakest 48 hours after an oral or intravenous administration.

2.5.3. Metabolism

Metabolism of *Imidacloprid* is very significant: up to *90 %* of the administered dose are metabolized on level of liver, at least *16* metabolites were identified in the rat. Two principal ways of biotransformation were highlighted in the rat: either oxydative cleavage between the grouping methylene and the cycle *Imidazolidin* followed conjugations, or hydroxylation of the cycle *Imidazolidin*.

2.5.4. Elimination

The excretion is fast and almost complete: *48 hours* after administration of low dose per oral or intravenous way (*1 mg/kg*) or of a strong amount per oral way (*20 mg/kg*), more than *95 %* of the administered dose are excreted: *73-75 %* in the urines and *20-25* % in deposit.

At the time of an administration intra-duodenale of an amount of *1 mg/kg* PC in rat after cannulation of bile duct, biliary excretion represents up to *36 %* of administered dose, testifying to existence of a hepatic cycle entero-.No accumulation is observed.

2.6. General properties of imidacloprid

Imidacloprid has three principal toxicological characteristics is persistence, the systemic action and the neurotoxicity.

2.6.1. Persistence

In the ground, *IMI* are water-soluble, *nonionizabl*, incompetent to adhere to partical and it is not-bird on the ground.Half-life is between one year and two years, it tends to increase with the pH of the ground and the absence of light **(Laramee, 2007).** This insecticide is more persistent in naked ground than in the covered ground of plants.What it round the plant health product more used for control of *Ladybird* Colorado beetle of potato **(Kemp and Rogers, 2002)** and more appropriate to the treatment of seeds (**Fossen, 2006).**

2.6.2. Systemic

Imidacloprid is a systemic product **(Tomlin, 2000)** while crossing the roots towards all the vascular system of the plant through the sap **(Tomizawa, 2000; Fossen, 2006; Mohany and *al.*, 2011).** This property ensures the plant a protection against the ravageurs racinaires and air since it is effective lifting until austade of flowering of the plant **(Al-sayda, 2008).** *Imidacloprid* can be found easily in nectar and pollen **(Suchail and *al.*, 2003)**. ntamination of these last and thus contamination of the cultures untreated **(Laramee, 2007).**

2.6.3. Neurotoxicity

Because of the structural similarity, *Imidacloprid* become neurotoxic by imitation with nicotin on the nicotinic sites of fixing to the receivers of the *acetylcholin* (*nAChR*) **(Benhamou, 2000; Flora and *al.*, 2006; Azevedo and *al.*, 2011).**However, the neurotoxic activity of insecticide is much more specific than that of nicotine **(Tomizawa and Casida, 2003, 2005).** *Imidacloprid* disturb the synaptic transmission of the nervous stimuli, by causing an irreversible blocking of *nAChR*, which leads to accumulation of *acetylcholine* in the synaptic slits **(Yamamoto, 1995 ; Borough, 2006; Herron, 2011).** Like *acetylcholine-esterase* no effect on *Imidaclopride* has, the nerve is continuously surstimule and quickly depolarizes **(Galvis, 2006; Thany, 2010)**.What leads to the rupture of the nervous system, with the

paralysis then with died of insect **(Buckingham and *al.*, 1997; Anatra-Cordone ; Durkin, 2005).**

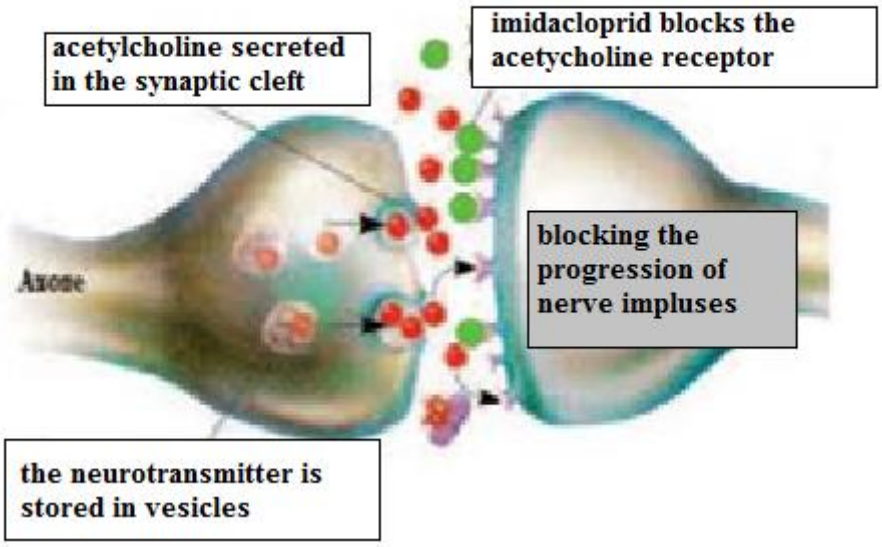


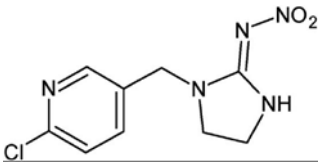
Figure 5: Mode of action of *Imidacloprid* on level of the nervous synapses of insectes **(Asam Riaz, 2011).**

2.7. Principal characteristics of *Imidacloprid*

Imidacloprid is presented in the form of crystalline powder of color blanchatre. As an active substance of phytopharmacological products. *Imidacloprid* must have apurity equal or higher than *970 g/kg* **(appendix of payment EC 1107/2009).**

Imidacloprid is nonvolatile, far from water soluble (*610 mg/L with 20 C*), soluble in the majority of solvents (*dimethylsulfoxyd*, dichloromethan, *2-propanol*, *acetone*…) and not very soluble in aliphatic solvents.

Table 04. physicochemical Properties of *Imidacloprid* **(LARAMEE, 2007 ; AL-SAYEDA, 2008)**

Parameter	**Property**
Chemical formula	C9H10ClN5O2
Structure	
Chemical name	*1-(6-chloro-3-pyridylmethyl)-Nnitroimidazolidin-2-Ylideneamin*
Number CASE	138261-41-3
Propertis	crystals without color with a low characteristic odor
Molecular weight	255,7
Solubility in water	0,61 g L-1 (20 °C)
point melting	Non applicabl
Density	1,54 to 23 °C
Duration of half-life	180 days. This parameter, noted DT50, represents the potential of degradation This active substance, and its speed of degradatin in the ground.
Coefficient de partage octanl/eau	0,57 to 20 °C - pH
DJA	0,06 mg/kg/j
DL50	450 mg/kg

2.8. Effects toxicological

2.8.1. Environmental Toxicity

Imidacloprid is classified by agency of environmental protection in class of toxicity III **(Benbrook, 1991)** seen its persistence in environment, its biological accumulation in food chains **(Sanborn and *al*., 2002)**, in fabrics and the bodies vital **(Fossen, 2006)** and in biological products of the animals such as milk, meat and egg **(Kutches and *al*., 1970; Krohn, 2002; Bonde and *al*., 2008).**

2.8.2. Acute toxicity

Imidacloprid is moderately toxix. *DL50* by oral way is of *450 mg/kg* of body weight in the rat **(Meister, 1995)** and of 131 mg/kg in the mouse **(Kidd and James, 1991).** It is considered nonirritating for eyes and skin (rabbits), and without effect for the guinea-pigs.Concerning toxicity by inhalation in the rat, the airborne CL50 of *Imidacloprid* is > *69 mg/m*^{3} of air in the form of aerosol, and > *5323 mg/m*^{3} of air in the form of dust.These values represent the concentrations to which possible airborne symptoms of poisoning are maximum **(Kidd and James, 1991).**

An acute exposure by oral way of rats and mouse to Imidacloprid caused clinical signs characteristic of the intoxication by nicotine, such as a colouring of the urine, disorders of coordination, tremors, spasms and difficulties .Other symptoms include/understand a reduction in the activity and a lethargy **(Bomann, 1989a)**.The same clinical signs were observed in rats after a *4 hours* exposure to the *imidacloprid* in aerosol **(Pauluhn, 1988a).**

It was deferred, following an accidental inhalation of the IMI, that a farmer expressed severe respiratory disorders as well as gastrointestinaux and neuropsychiatric symptoms **(Alok and *al.*, 2013).**

2.8.3. Chronic toxicity

According to **(Eiben, 1991)**, intoxication with low dose of IMI during *24* months breastfed modifications of serum proportionings and a reduction in the body weight in rat.Various types of tumours were announced in the mouse with a frequent incidence of the liver and thyroid gland **(Bomhard and Rinke, 1994)**.

In the dog, food consumption decreased by *9-14%* in the treated females accompanied by a rise in the plasmatic cholesterol level and an increase in the relative weight in the liver and

brain.Tremors violent one and morphological changes of the liver and the thyroid one were also reported **(Allen and *al*., 1989)**.

2.8.4. Effects mutagenic

Imidacloprid is slightly mutagenic. On *23* analyses of tests of mutagenicity in laboratory, only two showed positive effects: changes on the chromosomes of human lymphocyts and a genotoxicity on the Chinese cells of ovary of hamster.

2.8.5. Neurotoxicity

The effects acute and subchronic of *Imidacloprid* on the nervous system were highlighted starting from the behavioral changes of the rat of which a reduction in the motor activity, tremors, a reduction in the response to the stimuli, a reduction in the reflex of rectification and a hypothermia **(Tomizawa and *al.*, 2001; Shimomura and *al*., 2002)**.

2.8.6. On the hepatic function

Liver is the first site of detoxification of xenobiotic of which pesticides, which it round a body very sensitive to the toxic products **(Guyton, 1995).**

Liver is the site principal of the oral toxicity by *IMI*, which appears by necroses hepatic or hypertrophies; increase in serum rates of transaminase, alkaline phosphatase and/or glutamate deshydrogenase; and the deterioration of the other biochemical parameters, like the uric acid, glucose, cholesterol, total protein and albumin **(Kammon and *al.*, 2010)**.

Work of **(Bhardwadj and *al.*, 2010)** showed that in the rats females of stock wistar, the oral toxicity of the IMI (*20 mg/kg/j*) entraine a significant increase in the serum rate of hepatic enzymes: transaminase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and glutamate deshydrogenas.

Similar results were noted by **(Mohany and *al*., 2011)**, These researchers worked on the rats wistar treated by *1/100* of *DL 50* of *IMI* and they observed deteriorations on level of hepatic parenchyma such as the infiltration of immunizing cells and presence of hepatocytes necrosed, marked by strongly encumbered cores pyknotic, and central sinusoïdes blood.

Work of **(Boukenoui and Mahboubi, 2011)** showed that amounts *1/15* and *1/25* of IMI cause a hepatotoxicity which appears by one necroses or hypertrophies cellular and other signs of ignition (cellular congestion and infiltration).

2.8.7. On reproduction

In the adult male rat, disturbances of index of coupling and a reduction of the viability of spermatozoides as well as the fertility were observed **(Najafi and *al.*, 2010; Ramazan and *al.*, 2012).**

Chapter 03 :

Melissa officinalis

3. *Melissa Officinalis*

3.1. *Lamiaceae family*

Family of *Lamiacees* is a great family including/understanding *3200* to *4000* species divided into *200* to *220* kinds. Among the most known, one can quote mint, thyme, rosemary, the origan, the lavender, the nettle and the hysope. This family of *Lamiacees* is divided into 8 subfamilies: Ajugoideae, Prostantheroideae, Prasioideae, Scutellarioideae, Lavanduloideae, Stachyoideae, Ocimoideae and Catopherioideae. In the subfamily of Stachyoideae, one counts 12 tribes. Among them, the tribe of Saturejeae, it self divided into *5* subgroups: Melissinea, Hyssopinae, Thyminae, Menthinae and Perillinae. That which interests us is the subgroup of Melissinae. She includes/understands the kinds Satureja (Calamintha), Hedeoma, Amadeus and especially the Melissa kind. **(Nathalie, 2001)**.

3.2. Characteristics of *Melissa officinalis*

3.2.1. Definition

Very required by the bees, the name of *Melissa Officinalis* comes from Melissophulon Greek who means "sheet with bees". It is more commonly called citronnelle or melissa-citronnelle, although the true citronnelle one (Cymbopogon nardus) is graminaceous Asian. English names it lemon-balm and German Zitronenmelisse or Melissenkraut. It is a medicinal and aromatic plant, with sheets with citronnees odor and savour **(Kothe, 2007)**.

Figure(6): *Officinalis Melissa .*

(https://jardinage.lemonde.fr/images/dossiers/historique/melisse-184324.jpg)

3.2.2. Botanical description

Very required by the bees, the name of *officinal Melissa* (*Melissa officinalis* comes from the Greek melissophullon who means "sheet with bees" is a medicinal and aromatic plant **(kothe, 2007).**

Melissa has a stem grief generally measuring between *30* and *80* centimetres in height, ramified, drawn up and pushing in tufts (Figure 07) **(Thoby, 2009).**

It is a long-lived also herbaceous plant of the family of *Lamiacees* with sheets with the citronnees odor and savour **(Kothe, 2007).**

The fruit is a tetrakene containing of small brown, sunk seeds and luisantes.

Officinal Melissa can sometimes, in particular if it is gathered in a wild state, being confused with other plants **(Wichtl and Anton, 2003 ; Babulka, 2005).**

Figure 07: Sheets and flowers of *Melissa* https://www.the-passion.fr/blog/wp-content/uploads/2015/08/fleurs-de-m%C3%A9lisse.jpg

3.2.3. Chemical composition of *Melissa*

European Pharmacopeia requires that sheet of dried Melissa have to at least contain *4 %* of total derivatives hydroxycinnamic expressed in the acid rosmarinic. This phenolic compound is indicated for the treatment of the skin troubles like the labial herpes (Herpes simplex) thanks to its anti properties oxidizing and antiviral. Officinal Melissa constitutes a major natural source of AR by its high content (*4 –7 %* of the sheets dry) by comparison with the others *lamiacees* **(Wichtl and Anton, 2003).**

3.2.4. Systematic position

The botanical situation of the species *Officinalis Melissa* is summarized in the following table **(Quezel & Santa, 1962).**

Table 5: Botanical classification of *Melissa*

Reign	Plantae
Division	Magnoliophyta
Classify	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Kind	Melissa
Species	Melisse officinalis.L
Vernacular name	Bleaches on grass with lemon, Citronnelle, Piment of the honey hives, migune

3.2.5. Geographical distribution and habitat

Melissa is present at the wild state in the south of Europe and North America, and in Asia Mineure **(Sari and Ceylan, 2002)** in slightly shaded places such as the edge of a hedge, a wood or a place not cultivated and fresh **(Perrot and Paris, 1971; Wichtl and Anton, 2003)**. It is cultivated in Central and Western Europe, like in the United States **(OMS, 1999a).**

3.2.6. Culture and harvest

After the second year of culture, one can obtain the first normal harvest **(Wichtl and Anton, 2003)**. During the first year, the producer can have only *25 %* of the normal output. One collects the sheets and the stems before flowering it is mean at the end of June at at the beginning of July. The second harvest can be made at the end of August at at the beginning of September. Formerly, the collecting was done with the sickle but today recolteuses mechanics is used, especially when the surface of harvest is large. The drying of the plant is done directly after the end of the gathering.

3.2.6. Use of *Melissa Officinalis*

•**Traditional uses**: This plant is used to cure the wounds, to calm the teeth and to alleviate palpitations. It has a beneficial effect on the moral one. Its use increases longevity.

•**Plant releasing**: *Melissa* is a plant releasing in the event of nervousness of anxiety, irritability and light depression. It alleviates cardiac palpitations of nervous origin and it decreases On emotivity recommends *Melissa* when the anxiety causes digestive disorders such as the distensions, the indigestions, the colics, nauseas and acidity.

• **Herpes**: *Melissa* decreases the frequency of the virus and eliminates its eruptions.

•**A hormonal plant**: *Melissa* calms the hyperexcitability due to the problems of the thyroid one.

•**Other uses**: One employment *Melissa* to look after the insect bites, the cuts and in the fever. **(Adimi leila zade, 2018)**

3.2.7.Effects of *Melissa Officinalis*

3.2.7.1.Effect antioxydant

Certain extracts have a marked antioxydant activity, which is explained rosmarinic presence of acid in the aqueous extracts. **(Teuscher and al., 2005).**

The oxydative stress plays a significant role in certain pathologies like cardiovascular diseases, cancers, the disease of Alzheimer… A study was undertaken on extracts of medicinal plants which have antioxydant properties. Plant studied, Melissa officinalis, Matricaria recutita and Cymbopogon citratus are used with brazil in some neurological pathologies. Antioxydant effects of the compounds phenolic were examined and compared. The results show that Melissa present best antioxydant effects. This study made it possible to show that the extracts of plants could limit the induced reactions of oxidation by the agents pro-oxidants and this to prevent the reactions of peroxidation of lipids. Melissa could this be regarded as an antioxydant agent in the prevention of many neurological diseases related to an oxydative stress **(Peireira and al., 2008).**

3.2.7.2. Anti-inflammatory drug effect

Another study made it possible to determine that the oral supplementation in acid rosmarinic could reduce the symptoms among suffering patients of rhinitis allergic seasonal. The clinical trial consisted in treating during *21 days* each patient maybe with an oral daily catch of 200 Mg or 50 Mg of acid rosmarinic maybe with one placebo. Each day, the intensity of the symptoms was evaluated and the rate of some. Proteins of the ignition was measured thanks to a nasal taking away. Results show that contrary to the placebo, the rosmarinic supplementation in acid A involved a significant reduction in the traditional symptoms of the allergic rhinitis. Moreover, the concentration into polynuclear, neutrophils and eosinophilic also have many decreased in the nasal taking away **(Osakebe and al., 2004)**

3.2.7.3. Effect on proteinic biosynthesis and the cellular division

A study showed that a glycosidic fraction isolated from the aqueous extract from melissa inhibits the proteinic synthesis. Indeed it is of the caffeic acid and a glycoside not identified. The study showed that the coldly prepared caffeic acid solutions had an action less than the solutions preserved several days at 20° C. They have some deduced that derivatives of caffeic acid were formed and taken part in the inhibition of proteinic synthesis. This glycosidic fraction would act by direct interaction on the factor of Ef-2 elongation who causes to prevent his connection with ribosomes and thus to stop peptide elongation **(Chlabicz and Galasinski, 1986).**

The effect anticarcinogenic of the essential oil of melissa is studied on the line of cells Hep-2 (cells derived from pharyngeal human cancer). Molecules of reference in this study were the methotrexate and the vépeside. Essential oil stops division cellular in G1 phase and S, the methotrexate in S and G2 and the vépéside in G1. These results show well a anticarcinogenic effect of the essential oil of Melissa. **(Allahverdiyev and al., 2001)**

3.2.8. Toxicity

No acute or chronic toxicity is announced when Melissa is used like condiment or out of infusion with the usual amounts. No case of allergic reaction is announced **(Teuscher and al., 2005).**

3.3. Properties of the essential oil of Melissa

Melissa has a antibacterienne activity (Shigella sp) and antifongic (Trichophyton sp). It is antioxydant, sensor of free radicals **(Mimica-Dukic and al., 2004)** and antitumor **(Allyne and al., 2004).**

Melissa is also antiviral with respect to Herpes virus I and II It reduces agitation in the insanity **(Ballard and al., 2002).** It has a spasmolytic effect identical to Papaverin **(Sadraei and al., 2003).**

One used herb tea Melissa with other antispasmodic plants (Vervain, Reglisse, Fenouil and Camomile) to treat the colics of the children **(Weizman and Alkrinawi, 1993).**

For the treatment of the sleep disorders of the formidable results were obtained with an extract containing of Melissa, of the Camomile, and Fennel **(Savino and Cresi, 2005).**

An associated extract containing Melissa and of Valerian is used against insomnia is effective more than Triazolam **(Dressing and Riemann, 1992).** Melissa also acts on the quality of the sleep at people not presenting this problem **(Cerny and Schmid, 1999).**

This same combination of plants fights against the sleep disorders of nervous origin in the children of less than 12 years **(Muller and Klement, 2006).**

Second part Practical side

Chapter 01: Materials and Method

1. Material

1.1. Biological material

For our experimentation, we chose to work on the rats,We used 21 male white rats *Rattus* of the Wistar stock, coming from the Pasteur institute of Algiers (Center of ElKouba breedings, Algiers). It is of the mammals of the order of the rodents, largely used in various fields of research.

This works is carried out on the level of the animalery and laboratory of the university Arbi Tebessi de Tebessa.

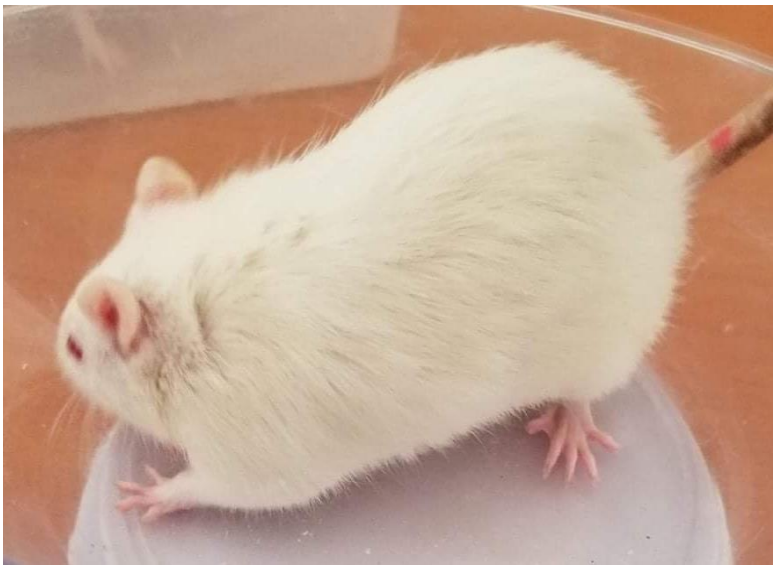


Figure (08): Rat male wistar rats

1.2. Chemical materials

Imidacloprid, 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-ylideneamin, are a *Neonicotinoids* insecticide which belongs to the chemical family of the *chloronicotinylnitroguanidin* **(Suchail and *al*., 2009).**

2. Methods of breeding and treatment

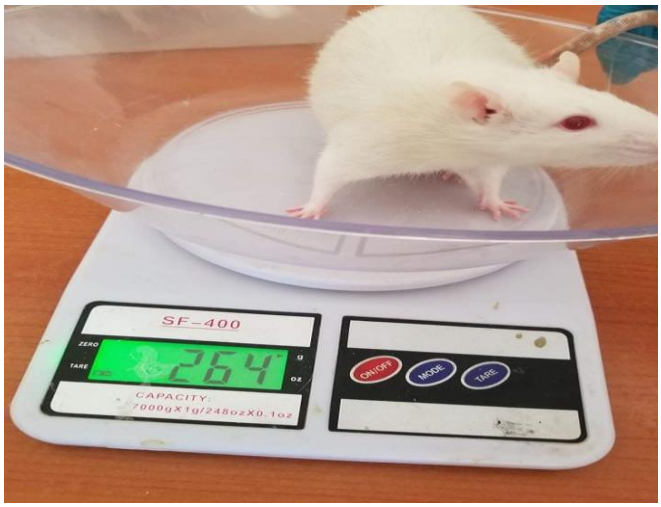
2.1. Description and breeding

For realization of our experimentation, we took as a biological model 20 male white rats *Rattus*. All the rats are weighed and their weight even between (2,08kg and 2,9kg). They placed individually in polyethylene batches, they are devises in 05 batches of *04* individuals for one period of 2 months adaptation in the conditions of the animalery; at a temperature of 25° C± 2° C and a natural photoperiod. The cages were cleaned and the daily changed litter.

Figure (09): Conditions of breeding of *the Rattusrattus* rats

2.2. Measure weight

The measurement of weight is taken on the rats any day throughout breeding, that is to say during the adaptation or the treatment using precision balance.



Figure(10): The measurement of weight of the rats using an precision balance.

2.3. Treatment

After the period of adaptation we began the treatment. The period of treatment is during *21* days, it is by oral way according to their body weights. This the allotment and treatment in our experimentation realized like following (**fig 13**):

N°1 batches: contains 04 rats as pilot no treatment undergoes.

N°2 batches: 04 rats for treatment by *Imidacloprid* has amount of 5 mg/kg/days contains.

N°3 batches: 04 rats for treatment by *Imidacloprid* has amount of 50 mg/kg/days contains.

N°4 batches: 04 rats for treatment by *Imidacloprid* has amount of 5 Mg contains + Melissa has amount of 10 mg/kg/days.

N°5 batches: 04 rats for the treatment by *Imidacloprid* has amount of 50 Mg contains + the extract of Melissa has amount of 10 mg/kg/days.

Figure (11): treatment of rat per oral way.

2.4. Sacrifice and taking away of the livers

After *21* days of the treatment, one sacrificed the rats. The taking away of the bodies was made on the level animalery of the university of Tebessa, the livers are récupéréset rinsed in a solutiond' water physiology then weighed and preserves in the refregidator of laboratory.

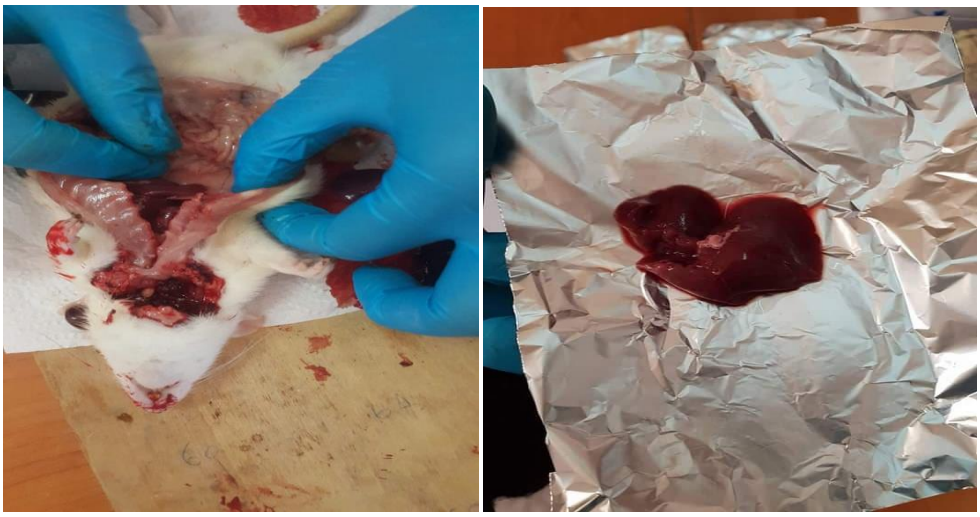


Figure (12): Isolation of liver.

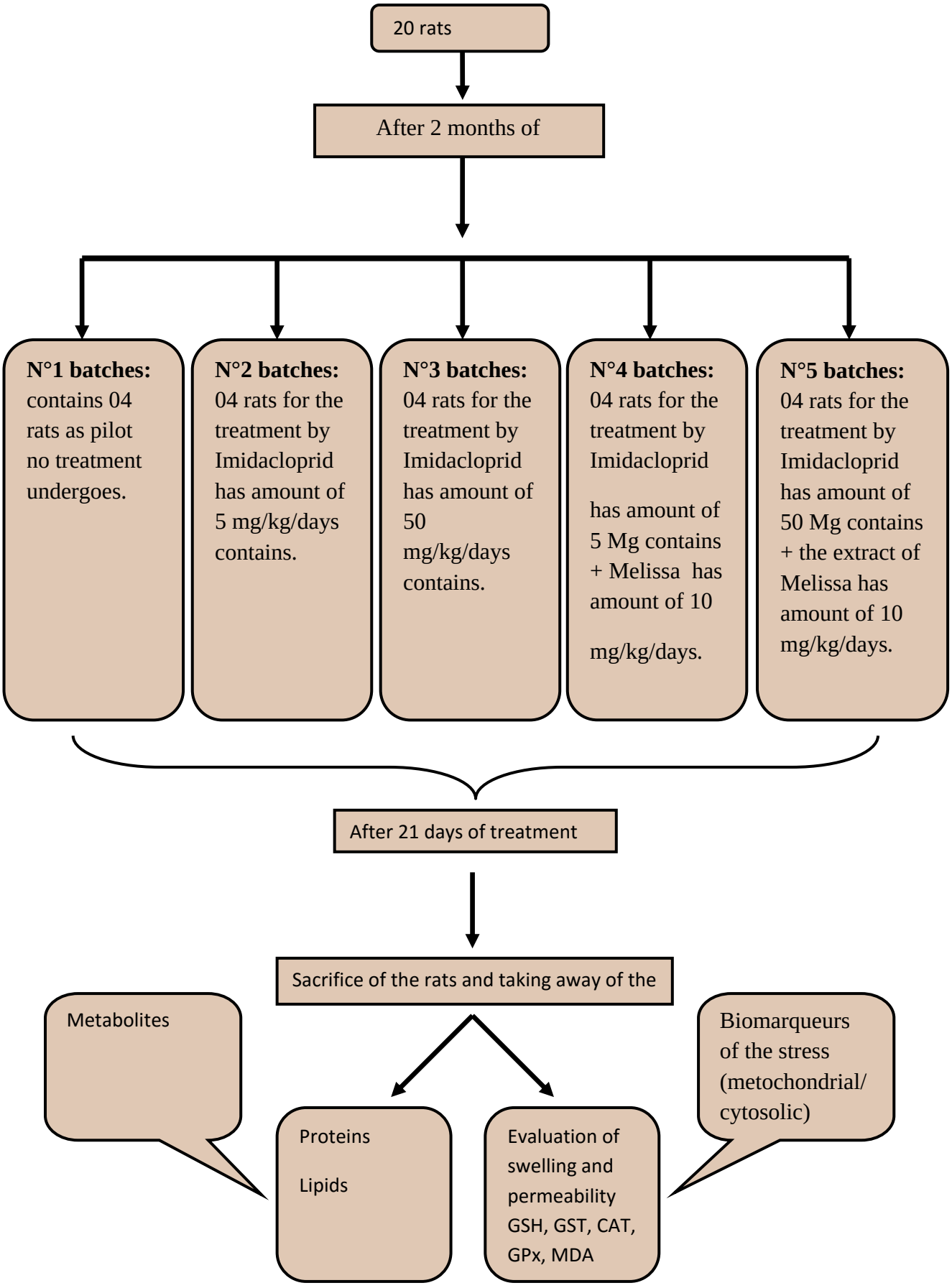


Figure (13): Summary diagram of the experimental protocol.

2.5. Estimation of relative weight of liver

The relative weight of liver extracted rats (PRF *[* g/100g of body weight]) is calculated compared to the total weight of the rat according to the formula according to:

WL: Weight of the liver (g).

TW: Total weight of rat (g).

RWL: Relative weight of liver (%).

2.6.Preparation of samples cytosolic

fabric 1g hepatic was homogenized in 2ml of solution of plug phosphates saline (PBS; pH 7.4). Then the homogenates were centrifuged with 3000t/min during 15min with 4°C and the resulting supernatant was used for the determination of the paramettres of the oxydative stress.

2.7. Extraction and proportioning of metabolites

The extraction of different metabolites was carried out according to the process of **(Shibko and *al*., 1966)**. The control samples and treaties are crushed using a magnetic crusher in the trichloracetic acid (TCA) with 20% (200 Mg of body 1ml of TCA). After the first centrifugation (5000 tours/min, during 10min). To base I, one adds 1 ml of mixture éther/chloroforme (1V/1V) and after the one second centrifugation (5000 tours/mn, during 10mn), one obtains supernatant II and base II, survives it II will be used for the proportioning of the lipids according to method **(Goldsworthy and *al*., 1972)** and base II, dissolves in NaOH (0,1.mM), will be used for proportioning of proteins, according to **(Bradford, 1976**).

2.8. Preparation of suspensions mitochondrials

The extraction of Mitochondries is done according to the method described by **(Rustin and *al*., 1994)**, it is about a purification by differential centrifugation. Briefly, after decapitation of the rats, the livers are quickly taken and to immerse in plug TSE (10m M sorting, 250 mm sucrose, 0,1m Mr. EDTA, pH 7,2 with 4C°). The hepatic fabrics are cut finely and postérisés in 3,5ml of TSE, which allows the destruction of the cells and the release of Mitochondries. The recovered homogenate is centrifuged to 10000 rpm during 10 min thus allowing the elimination of the large cellular remains. The recovered base is centrifuged second once at 10000 rpm during 10min. The supernatant resulting from two centrifugations are recovered and centrifuged to 14000 rpm with 4 C° during 10min. The base obtained is resuspendu in 1ml of the TSE and centrifuged with 14000t/mn during 10min. The base resulting from this last centrifugation is resuspendu in 1ml plug TS (250mM sucrose, 50 mm sorting, pH 7,2 to

20 C°) and centrifuged during 10min with 14000 t/mn. The final base made up of Mitochondries, and is recovered in 500 µ L of plug TS to obtain the suspension Mitochondrial fraiche whose fraction will be useful directly in the evaluation of the structural and functional integrity of Mitochondris, the remainder is preserved at -80° C for the continuation of proportionings.

2.9. Preparation of matrix mitochondrial

Suspension mitochondrial freeze-is defrosted from *6* to *8* times with increased and repeated poterisation, in order to burst the mitochondries. After centrifugation of *10min* with *9600t/min*, the supernatant is used like source of the parameters of stress **(Lahouel and *al.*, 2015).**

2.10.Evaluation of swelling and permeability mitochondriales

According to the method of **(Crystal and *al.*, 1996)**, we carried out the estimate of the permeability mitochondriale based on the rate of the ions of Ca++ which crossed their membranes, this permeability followed by an increase of the size of mitochondrion detected to a wavelength of 540 Nm during 3 minutes and each 30sec.

3. Methodes of proportioning

3.1. Metabolic parameters

3.1.1. Proportioning of lipids

The tissue lipids are evaluated according to method **(Goldsworthy and *al.*, 1972)**, one uses 200 µl homog énat in 5 ml of acid trichloroacétiqu E 20% (TCA), one crushed and filtered this mixture;and directly applied a centrifugation to 5000t/min during 10min. The base is kept in tube contains 1ml Ether/Chlorophorme mixture, and after centrifuged this mixture 5000t/min has during 10min, one takes 100 µl supernatant, to which one adds 1ml sulphuric acid and puts of them after agitation the tubes in a Marie bath at 100° C during 10min.

After cooling, one preleve once again by means of a micropipette 200µlde the extract to which one addition E 2.5ml of the mixture sulfophosphovanillinic with 85% (0.38g vanilline+195ml acid orthphosphorique+55ml H_2O) and left this 30min mixture with the darkness,la reading with a wavelength 530nm.

The calculation of the real concentrations is done starting from the equation deduced from the range of calibration realized starting from a solution prepared mother enutilisant of the sunflower oil **(Appendices).**

3.1.2. Proportionings of proteins

The method used for proportioning of proteins is that of **(Bradford, 1976)** who utilis **E** the BSA (Serum standard Bovine) comme Albumin, on the same sample used to proportion the lipids, one recovers the base resulting from the second centrifugation to which one added 1ml *NacOh* (0.1N) and one agitates energetiquement for the dissolution of proteins. Afterwards, one takes, by means of a micropipette, a volume of 100µl to which one ajoute you 4ml of reagent BBC (Blue Shining of Coumassie) (orthophosphoric 50mg BBC +*50ml* of acid with 85% and one supplements with 500ml with distilled water). This a blue color develops and one passes directly leséchantillons for reading with a wavelength 595nm. The calculation of the concentrations is done by the equation deduced from the range of calibration carried out starting from an albumin solution of serum of boeuf **(Appendices)**.

3.2. Parameters of the oxidizing stress

3.2.1. Proportioning of the activity of glutathion (GSH)

The proportioning of the glutathion is carried out according to the method of (**Weckbeker and Cory, 1988)**. The principle of this proportioning rests to the measure of the absorptance of *acid 2 - nitro-5-mercapturic*, this last results from the reduction of the *acid 5,5`-dithio-(a)-2-nitrobenzoique* (DTNB) by the groupings (- HS) of the glutathion. Once prepared, the sample (cytosol/matrice) must undergo a deproteinisation by the acid sulfosalycilic (0.25%) in order to protect the grouping-HS from the glutathion.

Briefly, the samples (fabric 200mg) are put individually in the presence of 8ml solution of EDTA (Acid Ethylene Diamine Tétra Acétique) at 0. 2M. The mixture put in ice floes is crushed using a porcelain rammer. L ' homogenate is then déprotéinisé by taking 0.8ml of this last to which one adds 0.2ml of a sulfosalycilic acid solution (SSA) to 0.25%. The mixture is vortexé and left during 15min in a bath of ice, and is centrifuged then during 5min with 1000 rpm. 0.5ml of survives is taken to which 1ml of plug sorting is added HCL+EDTA(0.02M), pH 9.6. To the mixture is added 0.025 ml of the acid 5,5` - dithio-(a)-2-nitrobenzoique (DTNB) to dissolved 0.01M in absolute methanol. The mixture is let rest during 5min at ambient temperature and the absorptance (A) is measured with 412nm.

The concentration of the glutathion is obtained by the following formula:

$$\text{GSH (nmol GSH/ mg protéine)} = \frac{DO \times 1 \times 1.525}{13100 \times 0.8 \times 0.5 \times mg}$$

DO: Optical density

1: Total volume of the solutions used in deproteinisation (0.8 ml homogenate + 0.2 ml of the salicylic acid).

1.525: Total volume of the solutions used in the proportioning of the GSH on the level of supernatant (0.5 ml surviving + 1 ml Sorting + 0.025 ml DTNB).

13100: Coefficient of absorptance of grouping –HS to 412 nm.

0.8: Volume of the homogenate after deproteinisation found in 1 ml.

0.5: Volume of the supernatant found in one 1.525 ml.

Concentration of GSH is measured compared to 1mg protein. It is for that this proportioning must be accompanies by proportioning by proteins.

3.2.2. Dosage of the activity of glutathion S-Transferase (GST)

The measurement of activity of glutathion S - Transferase (GST) is given according to the method **(Habig and *al.*, 1974)**, It is based on the reaction of conjugation between the GST and a substrate, the CDNB (1-Chloro2,4-di nitrobenzine) In cofactor glutathio N (GST), the conjugation entraine formation of a novelle molecule; 1-S-Glutathionyle 2-4Di nitrobenzine allowing to measure the activity of GST.

The value of the measured optical density directly proportional to the quantity of combined is formed it even related to the intensity of activity GST.

The samples sonthomogeneized in 1ml of plug phosphates (0.1M, pH6). The homogenate is centrifuged to 14000 rpm during 30 min and the recovered supernatant will be useful like source of enzymes. Proportioning consists in making react 200 µl supernatant with 1.2ml melange CDNB (1mM), GSH (5mM) [20.26mg CDNB, 153.65mg GSH, 1ml ethanol, 100ml plug phosphates (0.1M, pH 6)]. The reading of the absorptances is carried out during one minute and each 15 seconds with a wavelength of 340 Nm against a white containing 200 µl water distilled replace T the quantity of supernatant.

The concentration of the GST is obtained by the following formula:

$$\text{GST (nmol GST/min/mg protéine)} = \frac{\text{(DO échant/min-DO blanc/min)}}{\text{9.6 x mg de protéine}}$$

DO echantillon–DO blanc: average OD of samples per minute - average of OD of whites per minute.

ε: Coefficient of molecular extinction of

C-DNB, εC-DNB = 9.6 mM-1.cm-1

3.2.3. Proportioning of activity of glutathion peroxidase (GPx)

Enzymatic activity of GPx is measured by the method of **(Flohe and Gunzler, 1984)**, by using H_2O_2 as substrate. A volume of 0.2ml of cytosol/matrice is recovered in a tube containing 0.4ml GSH 0.1mM and 0.2ml of plug phosphates 0.067M, pH 7,8. The mixture is incubated with the Marie bath with 25°C during 05min. 0.2 ml of HO_2 1.3mM is added to initiate the reaction. After 10min 1ml of TCA 1% (acid chloro-acetic sorting) is added with an aim of stopping the reaction and the mixture is put in the ice during 30min and centrifuged during 10min 3000t/mn has. A volume of 0.48 ml of supernatant is placed in a tank to which one adds 2.2ml Na_2HPO_4 0.32M with 0.32ml of DNTB 1mM. This mixture formed a coloured compound and its optical density is measured with 412nm each 30seconde during 05min.

Determination (calculates) activity of GPx is made way according to:

- GSH Activity consumed /min/gr de protein.
- white = 0.04 micro mole of reduced GSH → DOb.
- Extract = 0.04 micro mole of reduced GSH → DOe.

So the concentration of reduced GSH wich will dioxydzed (disatteared) = DOe-DOb

X= (*D*Oe−*D*Ob) × 0.04/D0 b = at quantity of GSH reduced GSH disatteared (oxidized) in 0.2 extract in 1ml.

GPx activity = the amount of reduced oxidized reduced GSH X **5** / [protein].

3.2.4. Proportioning of enzymatic CAT activity (CAT)

The proportioning spectrophotometric of the activity Ca talase (CAT) is carried out according to the method of **(Cakmak and Horst, 1991)**. Decrease of absorptance is recorded during troisminutes by a spectrophotometer for a wavelength of 240nm and a coefficientd' molar linear extinction ε = 39400 $\mu M^{-1} cm^{-1} L$ for a final volume of 3ml, the reactional mixture contains: 100 µl of the enzymatic extract gross, peroxide 50µl of hydrog ene H_2O_2 to 0.3% and 2850 µl of plug phosphates (50mM, pH 7,2). The calibration of the apparatus sefait in the absence of the enzymatic extract. The reaction is started by the addition of hydrogen peroxide.

The activity of catalase is calculated according to the law according to:

$$Act = \frac{\Delta A.Vt}{\varepsilon.\Delta t.L.Ve.p}$$

Act: Enzymatic activity in μmol/min/mg of Proteins.

ε: Coefficient of linear extinction molar in. μM^{-1}. cm^{-1}

ΔA: Slope of the straight regression line (variation of the optical density according to time)**.**

Vt: Total volume of the reactional mixture in ml.

Ve: Volume of the enzymatic extract in ml.

L: Width of the tank of measurement in cm.

P: Content of Mg proteins.

T: Reading time in min.

To the wavelength 520 Nm are due.

3.2.5. Dosage of (malondialdehyde) MDA

Proportioning of MDA is carried out according to method of **(Esterbauer and *al.*, 1992).** Principle of this proportioning is based on the condensation of MDA in acid medium and hot with the acid thiobarbituric, to form a pink pigment. A quantity of 375 μl of supernatant is Pr raised in a dry tube, to which is added a volume of 150 μl of solution TBS (sorting 50mM, NaCl (150mM; pH7.4) and 375μl of the solution Tca-bht (TCA 20%, BHT 1%), the mixture is Vortexé and centrifuged with 1000t/min during 10min. A volume of 400μl is taken supernatant to which one adds 80 μl HCl 0.6M and 320μl of the solution sorting-TBA (sorting 26mM, TBA120mM). In end, the mixture is vortexé and is then incubated with the Marie bath with 80°C during 10minutes. Reading of the optical density of the samples is measured by spectrophotometry with 530 Nm.

The concentration of MDA is calculated according to the law of Beer-Lambert (C = E.C.L):

$$[C]\ (nmol/mg\ protéine) = \frac{DO.10^6}{\varepsilon.L.X.Fd}$$

C: Concentration in protein nmol/mg

DO: Optical density read with 530 Nm.

ε: Molar coefficient of extinction of the MDA = $1.56105 M^{-1} c m^{-1}$.

L: Length of the optical way = 0.779 cm.

X: Concentration of the protein extract (mg/ml).

Fd: Factor of dilution: **Fd** = 0.2083.

4. Statistical studies

Results obtained were expressed by the average of five repetitions (± standard deviation), and to better visualize the results obtained the graphical representation chosen is that of the histograms using the Excel 2013 office. Statistical analysis was carried out using the Office Excel version 2010, and the minitab® 18 each measured parameter was the subject of an analysis of variance with $\alpha \leq 0.05$ (TEST T) to a criterion (treatment), for significant analyzes of the treatment factor (Dunette and Tukey), and for the comparisons of the means. Which is used to compare between two samples (control and treated) if $P \leq \alpha$ / $\alpha = 0.05$ there are significant differences between the means and we reject the equality hypothesis.

- The value found by the calculation of the test can affirm that the populations are different with a risk of error p such as:

- $p > 0,05$ = 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 significant ***

Chapter 02 : Results

2. Results

In this experimental work, we proceeded to the evaluation of body weights; in the determination of some biochemical parameters of liver in wistar rat, after treatment with *imidacloprid* at the rate of two doses (5.50 mg) and their mixture with officinal Melissa Extract for 21 days.

Throughout the treatment period, no change in behavior or signs of toxicity was noted in rats exposed to *imidacloprid*.

2.1. Effects of *Imidacloprid* and their mixture with *Melissa* extract on overall growth parameters of rats

Results of evaluation of the growth parameters in terms of the weight gain and the relative weight of the liver during the 21 days of treatment of the different groups of rats with two doses of Imidacloprid (5 mg, 50 mg) and their mixture with Melissa extract.

2.1.1. Weight gain (GP):

Figure 14 illustrates weight gain evolution in the control rats treated with *Imidacloprid* at (5 and 50 mg), and their mixture.

Results of the weight gain assessment show a significant reduction ($p \leq 0.001$) in weight gain in the lots treated with IMI 5mg and IMI 50mg in comparison with the control lot.

On the other hand, there is an improvement in weight gain each time Melissa extract is combined with pesticides IMI.

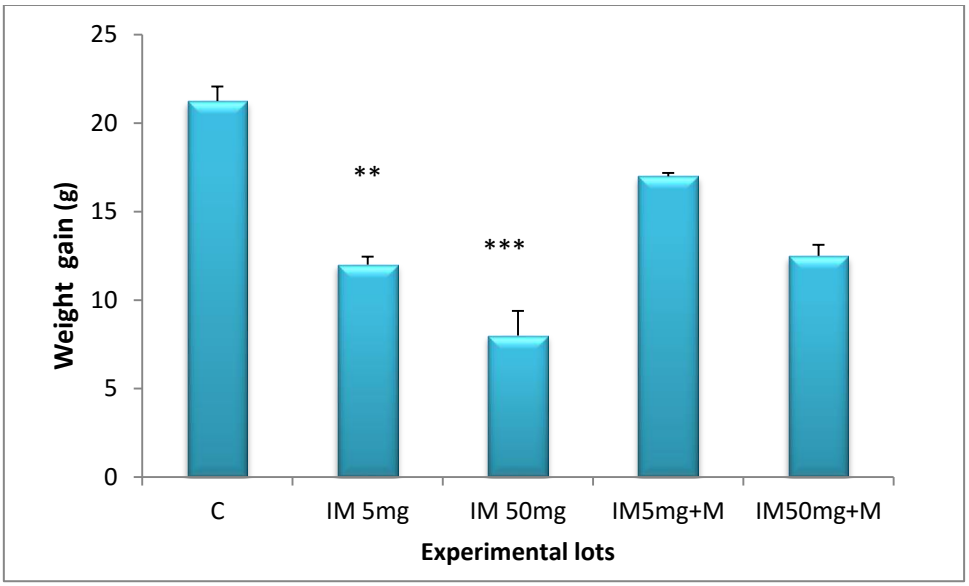


Figure 14. Evolution of body weight gain (GP) in control rats treated after 21 Relative weight of days of treatment with *Imidaclopride* and their *Melissa* extract.

2.1.2. Relative liver weight:

Figure 15 illustrates the variations in relative liver weight in the presence of *Imidacloprid*, and their associations with *Melissa* extract.

Results obtained following the evaluation of the PR show a non-significant increase (P> 0.05) in the relative weight of the liver in the group treated with IM5mg and 50mg and the mixture at 264g body weight in comparison with the control group.

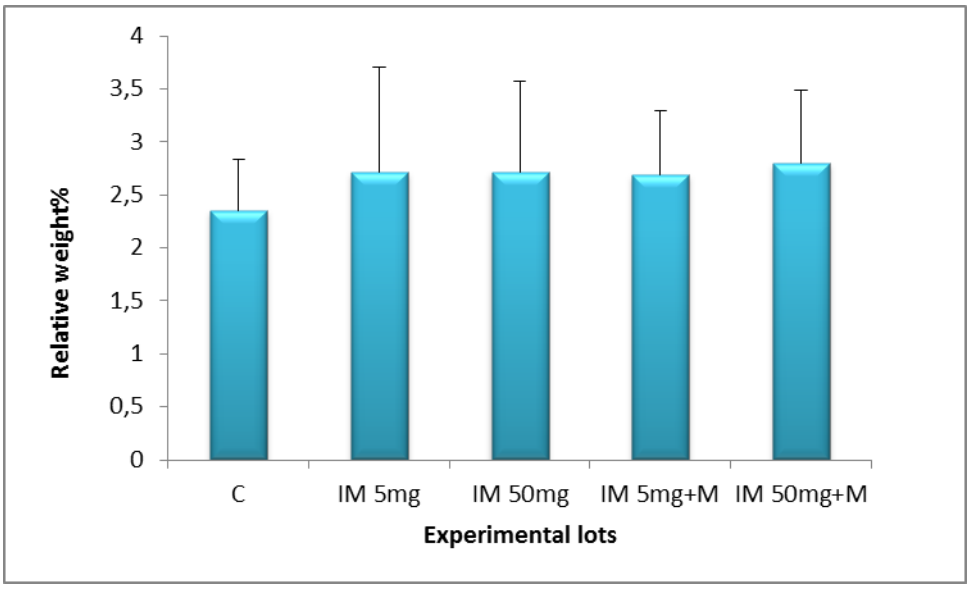


Figure15. Evolution of the relative weight of the Liver in rats treated for 21 days with *Imidaclopride* (5mg, 50mg) and their mixture *Melissa* extract.

2.2. Effect of Imidacloprid (5, 50mg) and Melissa extract on biochemical parameters in liver in rats

2.2.1. Effect of Imidacloprid and their mixture on lipid levels

Variation in liver lipid content in control rats treated with two increasing concentrations of *Imidacloprid* and *Melissa* extract are shown in Figure 16.

We note that the lipid level in the batches treated with IM decreased in a very highly significant compared to the control batches but after the addition of Melissa extract we notice a significant increase compared to rats treated.

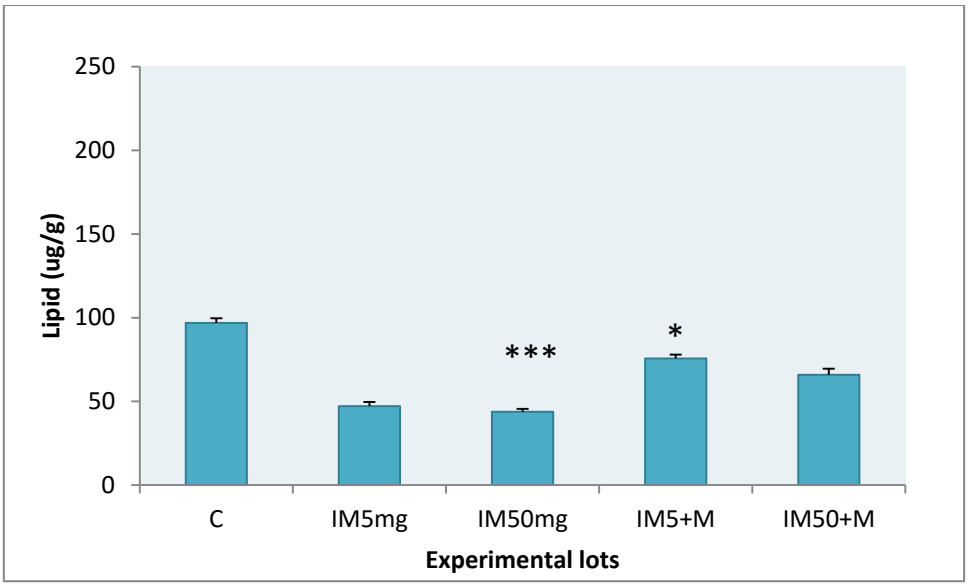


Figure 16 : Variation in liver lipid content in control rats treated with *IMI* (5mg, 50mg) and their mixture *Melissa* extract.

2.2.2. Effect of *Imidacloprid* and their mixture on protein levels

Variation in liver protein content in control rats treated with two increasing concentrations of *Imidacloprid* and *Melisa* extract are shown in Figure 17.

Results presented in figure 17 show that the hepatic protein level of the batches treated with *IMI* 5mg increase in a very highly significant manner compared to the control, this increase is neutralized after the addition of *Melissa* extract in a significant manner compared to the witnesses.

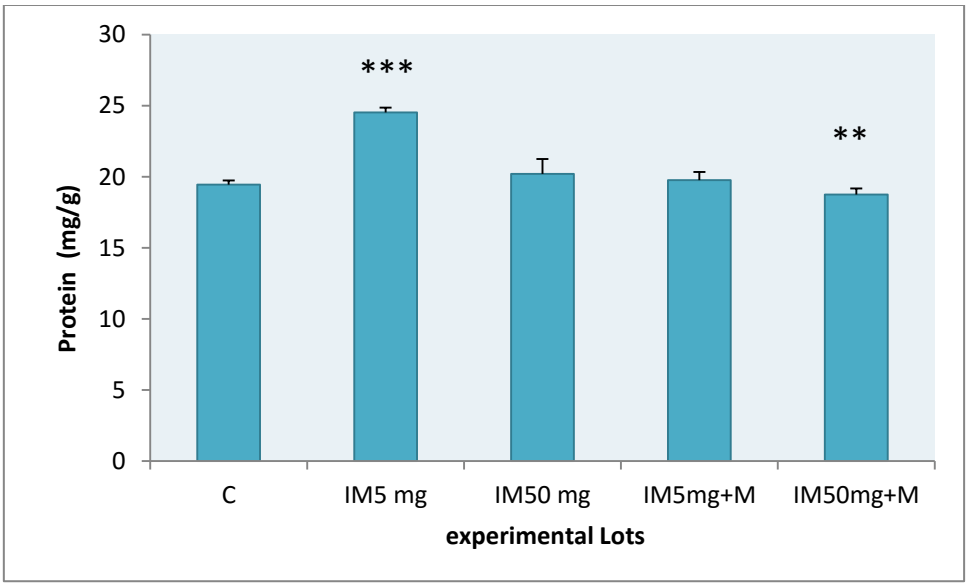


Figure 17: Variation in liver protein levels in control and treated rats with IMI (5mg, 50mg) and their mixture with Melissa extract.

2.3. Effect of *Imidacloprid* and *Meliss* Extract on the Parameters of Oxidative Stress in the Liver in Rats

2.3.1. Effect of *Imidacloprid* on and their mixture on the level of Malondialdehyde

MDA

According to the results presented in figure 18. A very highly significant increase in the hepatic MDA level is observed in rats treated with Imidacloprid, this increase is regulated after the addition of the Melissa extract compared to the controls.

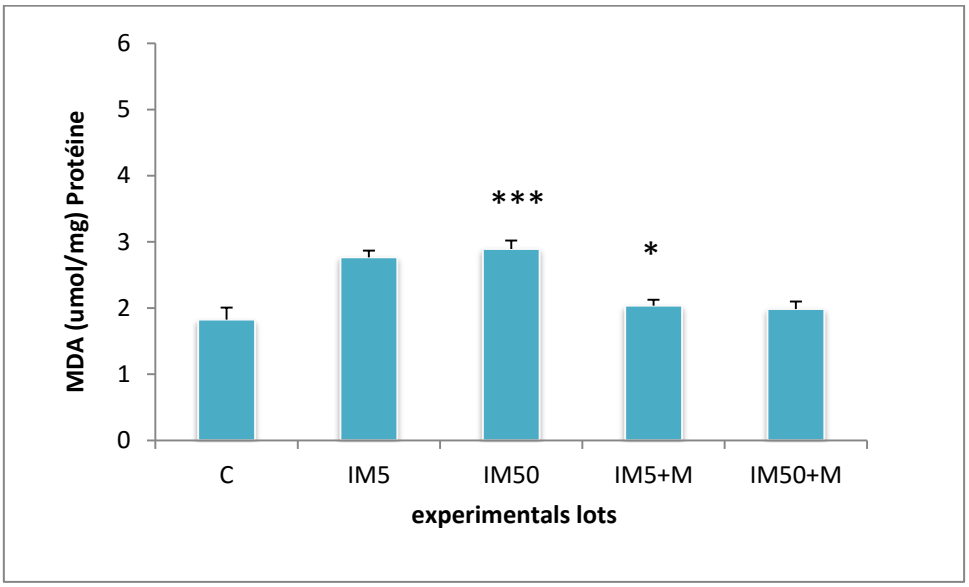


Figure 18: Variation in the level of hepatic MDA (µmol / mg of protein) in the control and treated rats after 21 days with Imidaclopride and their mixture Melissa extract.

2.3.2. Effect of Imidacloprid and their mixture on GSH activity (Glutathione reduced)

Variation in GSH levels in the treated and control rats is presented in figure 19.

Results obtained show a very highly significant reduction in the GSH level in the batches treated Imidacloprid (5, 50 mg) compared to the controls, this reduction is neutralized after the addition of Melissa extract in a highly significant manner.

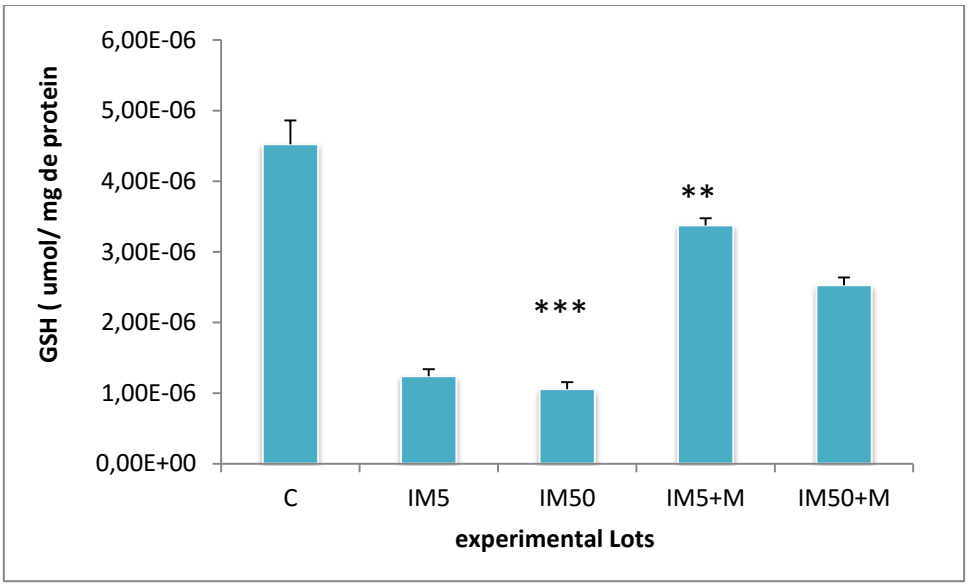


Figure 19 : Variation in the level of GSH (µmol / mg protein) in the liver in control and treated rats after 21 days of treatment with Imidacloprid and their mixture Melissa extract.

2.3.3. Effect of Imidacloprid and their mixture on the activity GST activity (Glutathione –S-transferase)

Figure 21 shows the variation of enzymatic activity of GST in the liver in the control and treated rats.

The administration of the pesticides studied for 21 days in rats, induces an increase in the enzyme activity of glutathione S-transferase (GST) in the liver compared to control rats.

While recovery has been recorded by significantly neutralizing GST activity in rats receiving the more Melissa extract IMI combinations.

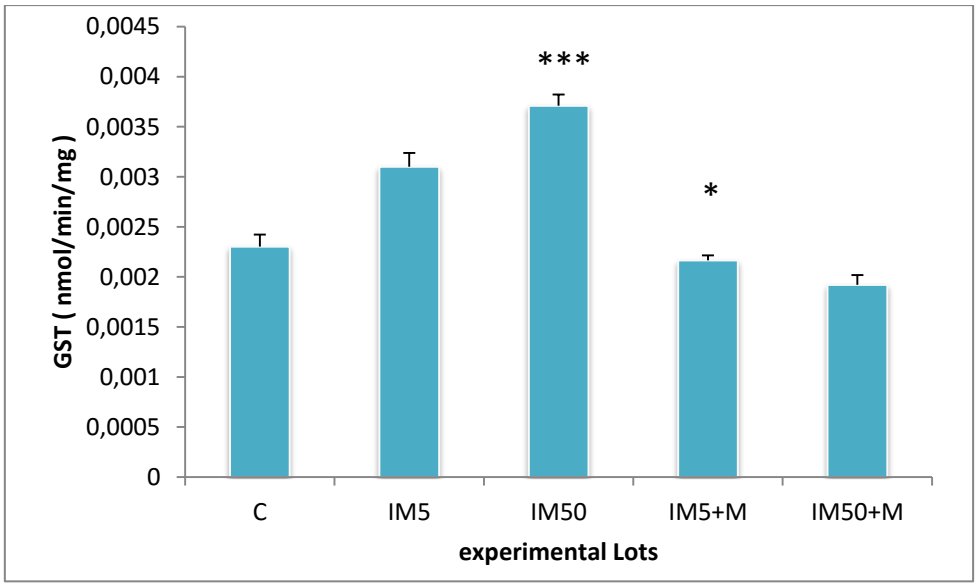


Figure 20: Enzymatic activity of hepatic GST in control and treated rats after 21 days of treatment with Imidacloprid (5mg/50mg) and their mixture with Melissa extract.

2.3.4. Effect of *imidacloprid* and their mixture on GPX activity

FIG. 21 shows the variation in the enzymatic activity of GPx in the liver of control and treated rats with two increasing concentrations of Imidacloprid individually and in combination with Melissa extract.

Over a period of 21 days of treatment, Our results show a highly significant decrease ($p \leq 0.005$) compared to the control of the hepatic GPx enzyme activity in rats treated with Imidacloprid (5 , 50 mg), this decrease is neutralized after addition of Melissa extract.

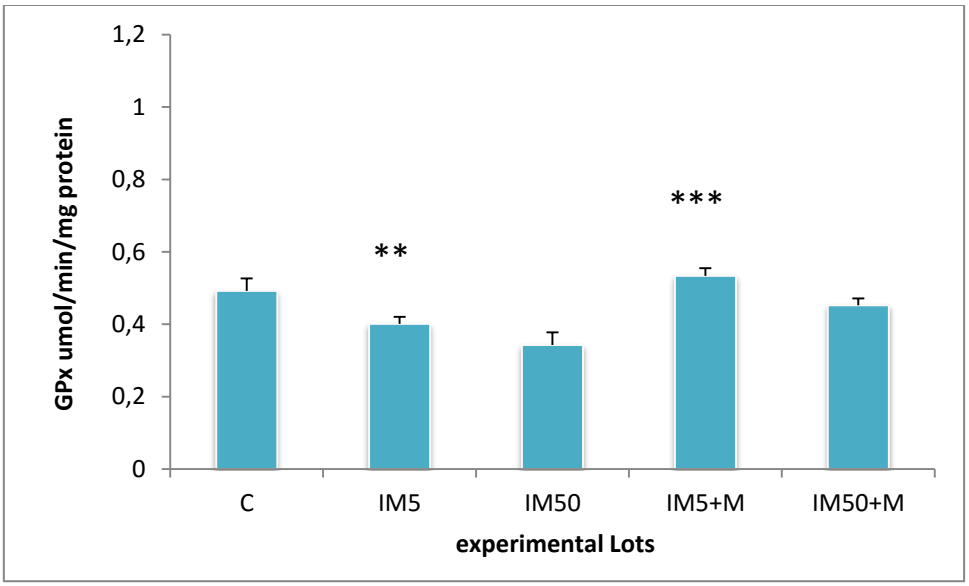


Figure 21 : Enzymatic activity of hepatic GPX (µmol / min / mg protein) in control and treated rats after 21 days of treatment with imidaclopride and their mixture Melissa extract.

2.3.5. Effet of Imidaclopride and their mixture on the CAT activity

Evolution of catalase activity in the control batches and treated with two increasing concentrations of Imidacloprid, their mixtures are illustrated in figure 22.

Our results reveal a very highly increased ($p \leq 0.005$) enzymatic activity of catalase in the batches treated with two concentrations of Imidacloprid, this increase is neutralized after the addition of Melissa extract.

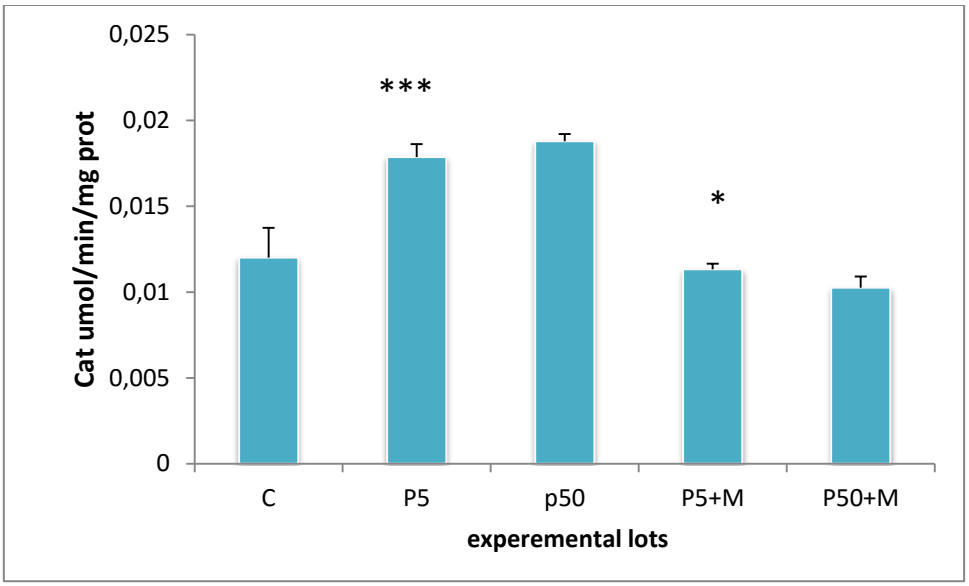


Figure 22: Variation in the enzymatic activity of Catalase (µmol / min / mg of protein) in control rats treated after 21 days of treatment with IMI and their mixture Melissa extract.

2.4. Mitochondrial swelling and patency assessment

2.4.1. Effect of Imidacloprid and Melissa Extract on Mitochondrial Swelling

Results of the 21-day follow-up concerning the effect of Imidacloprid and its combination with Melissa extract on mitochondrial swelling are reported in Figure (23).

Results obtained show a very highly significant increase in the IMI batch (5.50 mg). On the other hand, this increase is reduced for the lots treated with the addition of Melissa extract in a highly significant way compared to the lots treated only with Imidacloprid.

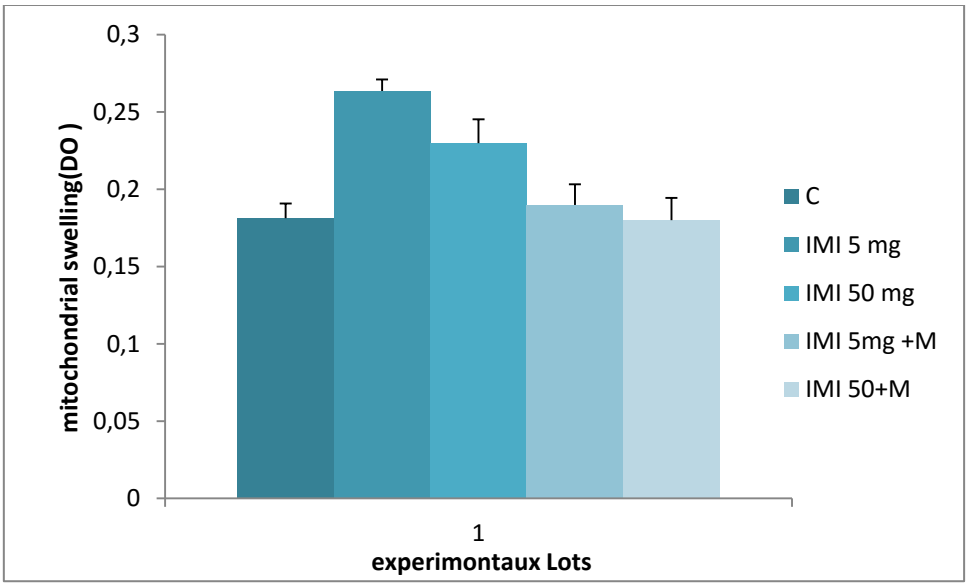


Figure 23 : Variation in mitochondrial swelling of liver cells in treated and control rats for 21 days of treatment with Imidiacloprid (5 /50mg) and their mixture Melissa extract.

2.4.2. Effect of Imidacloprid and Melissa Extract on Mitochondrial Permeability

Results concerning the effect of Imidacloprid and their combination with Melissa Extract on mitochondrial permeability during 21 days are represented in the figure (24).

Results show a very highly significant increase in mitochondrial permeability in the liver in the batches treated with Imidacloprid (5, 50 mg) compared to the control.

This increase neutralized after addition Melissa extract.

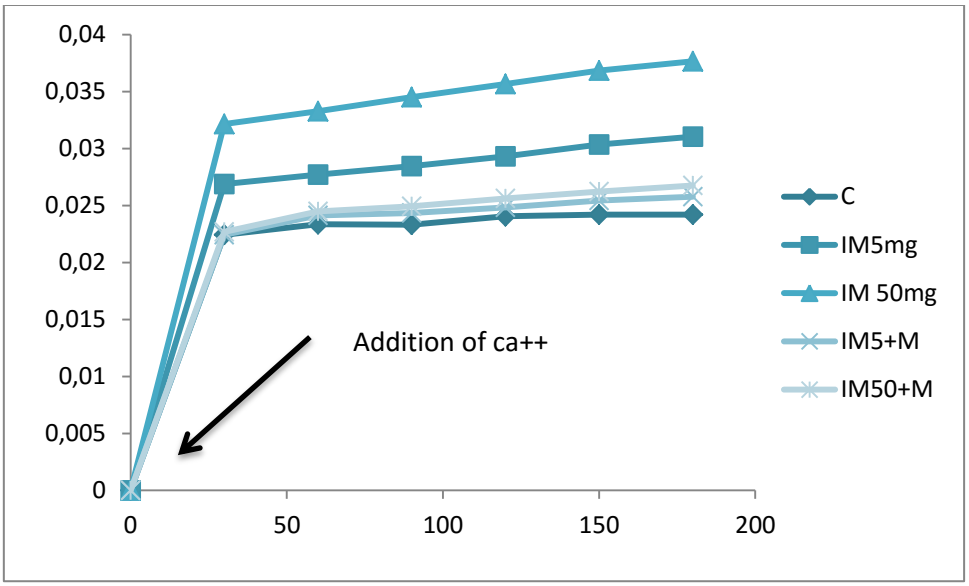


Figure 24 : Variation in mitochondrial permeability in the liver in rats treated with Imidacloprid and their Mixture Melissa extract for 21 days of treatment.

Chapter 03: Discussion

Discussion

Significant increases in risk for several pathologies have been highlighted in connection with exposure to pesticides and / or according to the categories of use (insecticides, herbicides, fungicides) or with exposure to certain chemical families and / or specific active substances **(Gérin and *al.*, 2003)**.

Insecticides are of concern because their mechanisms of action, directed against insects, can also disrupt human metabolism. They can cause serious neurological effects followed by sequelae or even death **(Gérin and *al.*, 2003)**.

Several studies show the impact of Neonicotinoid insecticides on health. One conducted on rats suggests that Neonicotinoids may adversely affect human health, especially brain development, liver and kidney function **(Kimura-Kuroda and *al.*, 2012)**.

The present experiment focuses on a new generation *Neonicotinoid* insecticide, namely *Imidacloprid*.

Through this study in Wistar rats, we are looking for the effects of oral toxicity of two increasing doses (5 and 50 mg / kg / days) on liver function biochemical parameters. And to reduce the effect of Imidacloprid and escape stress oxidative we introduced Melissa extract as an antidote.

Under our experimental conditions, no change in behavior was reported in all rats during the experiment. However, the percentage of mortality in rats is zero in all batches.

1. Effect of *Imidacloprid* on Relative Weight of Liver

Based on the results, we have seen a significant increase. The increase in relative weight may be due to the tissue enlargement of this organ caused by these xenobiotics (metal and pesticide), these results are in agreement with the work of (**Misra and *al.*, 1990; Novelli and *al.*, 1998).** This is explained, on the one hand, by the tissue hypertrophy of this organ caused by these pollutants and on the other hand, by the intense accumulation of *Imidacloprid* in this target organ. This result is in agreement with the work of (**Djabali, 2008)**. In addition, pollutants can lead to cell death by apoptosis of certain cell lines, due to the accumulation of toxic lipid derivatives such as ceramides. **(Wang and *al.*, 2000; Rudolf and Cervinka,**

2010). This liver abnormality is a phenomenon reported by many authors as a result of chemical aggression **(Huang and *al.*, 2012).**

2. Effect of *imidacloprid* on metabolit

- ✓ **Lipids**

Our results reveal a decrease in hepatic lipids in rats treated with *Imidacloprid* compared to controls, this decrease can be explained by the degradation of hepatic lipids by the activation of lipid peroxidation, this pathway stimulated by free radicals in oxidative stress caused by *Imidacloprid*.

The addition of *Melissa* extract caused a very highly significant neutralization of the lipid level which can be explained by cell regeneration to fight against *Imidacloprid*.

A disturbance in lipid levels. Several experimental studies interested in the effects of pesticides on the lipid profile. The administration of pesticide induces in the rat hepatotoxicity and a modification of lipid profile which is manifested by an increase in the level of Cholesterol and plasma Triglycerides, and according to **(Bouziane, 2002)** the alteration in the lipid profile may be due to a change in the activity of enzymes which play a role in lipid metabolism**.**

- ✓ **Proteins**

In our work we have shown an increase in the protein level in rats treated with *Imidacloprid*. These results are in line with those of **(Peccini and *al*,. 1994 ; Masaya and *al*., 2002)** who have shown a significant increase in the total protein level under the effect of chemical stress in different biological models (tadpoles, ciliated protists, rabbits)**.** Also these results are confirmed by **(Redhouane-Salah, 2004) (Rouabhi and *al.*, 2006)** which have shown a correlation between the disruption of the total protein level and toxicity by xenobiotics. This effect is explained on the one hand by the induction of the synthesis of stress proteins in relation to the phenomenon of bio activation / biotransformation and on the other hand by the lipid peroxidation generated by ROS.

However, this increase is neutralized after the addition of *Melissa* extract (the Imidacloprid / Melissa combination) significantly compared to the controls.

3. Effect of *imidacloprid* and meliss extract on parameters of oxidative stress in the liver in rats

Oxidative stress is conventionally defined as an imbalance in the balance between antioxidant defense systems and the production of ROS, in favor of the latter. **(Favier, 2003)** The liver is the main organ for the elimination of toxic waste from the body, because it contains an enzyme system involved in the detoxification process, which neutralizes all toxic substances **(Lecluyse and *al*., 2012) .**

Biochemical and enzymatic parameters in organisms exposed to toxic contaminants have been used as biomarkers and can be an important diagnostic tool for assessing exposure and the effects of xenobiotics **(Forbes and *al*., 1997).**

- ✓ **Malondialdehyde (MDA)**

According to the results obtained in our study, we marked a significant increase in the level of hepatic MDA in all the rats treated. Our results confirm those of **(Zaouani, 2010)** which demonstrated an increase in MDA levels in rats treated with pesticides.

The observed increase in the level of MDA, a key marker of oxidative stress and lipid peroxidation, is in agreement with several studies in animal models which have found an increase in MDA in the blood **(Baynes, 1991)**. The increase in the concentration of MDA suggests an increase in reactive oxygen species (ROS) **(Kakkar and *al.*, 1997)** which attack polyunsaturated fatty acids of the cell membrane and cause lipid peroxidation **(Battacharya and *al*., 1997)**.

- ✓ **Effect on GSH activity**

Glutathione is the major non-enzymatic antioxidant in animal cells: it is the most abundant sulfur reducing compound in the intracellular compartment, involved in metabolism, transport processes and the protection of cells against the toxic effects of compounds endogenous and exogenous, including reactive oxygen species and heavy metals **(Dickinson and Forman, 2002).**

The results obtained show a very highly significant reduction in the level of GSH in the batches treated with Imidacloprid compared to the controls. This result was confirmed by **Vadde and Rama, 2008 .**

In addition, the level of GSH is reduced due to the high level of superoxide and the free radicals GSH is converted into GSSG (oxidized glutathione) **(Bedwal, 1983; Loven and *al.*, 1986)**.

GSH protects cells from oxidative stress generated by environmental chemicals such as pesticides **(Che-Mendoza and *al.*, 2009).** It catalyzes the conjugation of various electrophilic substrates of the thiol group of GSH, producing less toxic forms **(Mansour; Mossa, 2009).** The decrease in GST activity observed in the present study could by far contribute to increased lipid peroxidation, represented by increased levels of MDA (**Zaidi and Banu, 2004)**. Additionally, this decrease reflects insufficient GSH levels **(Garg and *al.*, 2008 ; 2009).**

- ✓ **Effect on GST activity (GST)**

Regarding glutathione S-transferase (GST), enzymes catalyzing the conjugation of glutathione (has a nucleophilic group -SH) to a wide variety of compounds (carriers of electrophilic groups) and also involved in the transport and elimination of reactive compounds which perform other antioxidant functions, GST activity has also been widely used as a stress biomarker **(Fitzpatrick and *al.*, 1997).**

Our results are shown that, the exposure of rats to Imidacloprid (5, 50mg) sa induces a very highly significant increase in GST activity. The increase in GST activity is highly correlated with the decrease in the level of GSH, and a form of defense which translates the detoxification of xenobiotics in the body to allow its elimination, and a physiological response to compensate for the alterations which are due to free radicals and which suggests that the xenobiotic conjugated by GSH is catalyzed by GST for reduce its toxic effect, these results are confirmed by studies of **(Ognjanovićet and *al.*, 2008)**.

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

- ✓ **Effect on GPx activity**

Our results show a very highly significant decrease in GPx activity in treated rats. The fall in the level of reduced glutathione is consistent with the decrease in the enzymatic activity of glutathione peroxidase GPx (the first enzyme in the redox cycle of glutathione). This is probably due to the appearance of a large amount of peroxides under the influence of

xenobitics. The reduced ability of glutathione to reduce hydroperoxides formed during the metabolism of alphamethrin, under the action of glutathione peroxidase, leads to the massive oxidation of glutathione to oxidized glutathione, leading to an imbalance in the GSH / GSSG ratio.

The decrease in the activity of this enzyme (GPx) shows that the liver cells can probably contain a high concentration of H_2O_2 and organic hydro peroxides **(Little and O'Brien, 1968).**

- ✓ **Effect on CAT activity**

Our results show a very highly significant increase in CAT activity in the liver in rats treated with *Imidacloprid* (5,50 mg) compared to the control group.

Our results show a very highly significant increase in CAT activity in the liver in rats treated with *Imidacloprid* (5.50 mg) compared to the control group **(Cakmaket Horst, 1991)**. It consists of a transformation of hydrogen peroxide (H_2O_2) into water and molecular oxygen (O2). However, the production of hydrogen peroxide is induced by the presence of exogenous compounds in the body. So **(Azzouz, 2012)** highlighted a strong induction of CAT activity in *Parameciumtetraurelia*, treated with two pesticides: *AmistarXtra* and *Glyphosate*.

our work confirms those cited above since we have also highlighted an increase in Catalase activity in treated rats.

- The use of extracts of officinal Melissa as a protective molecule against the deleterious effects of pesticides has significantly improved the redox status in the liver, which confirms its virtues against oxidative stress by indirectly strengthening the cellular oxidizing systems or by directly scavenging the ROS.

4. Subcellular study

Mitochondria are essential for the production of cellular energy necessary for the maintenance of cellular function **(Clayton; Doda, 2001; David and *al.*, 2014 ; Lin and Beal, 2006)**. Mitochondrial Damage Contributes to Decreased ATP Production **(Cassarino and Bennettjr, 1999; Datta and Kaviraj, 2003)**. Alterations in one of the mitochondrial complexes cause the production of free radicals, which leads to depolarization of the mitochondrial membrane and subsequent activation of voltage-gated receptors, which allows

the influx of calcium into the cell and triggering pathways of cell death **(Lin and Beal, 2006 ; Romero and *al.*, 2012 ; Rodriguez and *al*., 2016).**

In our study, the analysis of the results obtained shows an increase in mitochondrial swelling in rats treated with IMI (5, 50mg) compared to the controls, is due to the disturbance of the giant pore voltages dependent under the effect of ROS causing l 'Entrance

mass of water and Ca2 + and Na + ions via mitochondrial membranes **(Romero and *al*., 2012; Morris and Berk, 2015 ; Henine and *al.*, 2016 ; Taib and *al*., 2016).** Cellular apoptosis is the final result of an attack by ROS, it is characterized morphologically by a dilation of the endoplasmic reticulum then, after increase in the mitochondrial volume and swelling of the cell, there is a rupture of the membranes **(Ben-Haiand, 2014).**

In our work, we observed the effects of *Imidacloprid* (5, 50mg) and their mixtures Melissa extract on the mitochondrial permeability isolated from Rats, **(Kon and *al*., 2004)** explain that the transition mechanism of mitochondrial permeability could be one of the fundamental mechanisms involved in alterations of the respiratory chain in conditions of drug intoxication.

Conclusion and perspective

Conclusion and perspectives

Pesticides, toxic chemicals, pose a real public health problem, both for users and for the population. Pesticides can also be very harmful, and they are suspected of posing a risk to human health and the environment by accumulating in ecosystems.

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 / days) on body weight and liver function in Wistar rats, we have shown that the doses tested, sespecially 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 liver 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 the levels of GSH and MDA, the activity of CAT, GPx, GST.
- Regarding effect of *Imidacloprid* on the mitochondria shows a toxic effect through the induction of permeability and mitochondrial swelling.
- The force-feeding of *Melissa* extract at a dose of 10 mg / kg / day for 20 days to rats treated with Imidacloprid restored all values to normal, which reflects the protective effect of *Melissa* extract on liver function.

In perspective, it would be interesting to develop this research from an operational point of view by deepening knowledge on:

- Investigation of the effect of this dose on the other vital organs.
- extend the duration 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.
- extend the duration 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.

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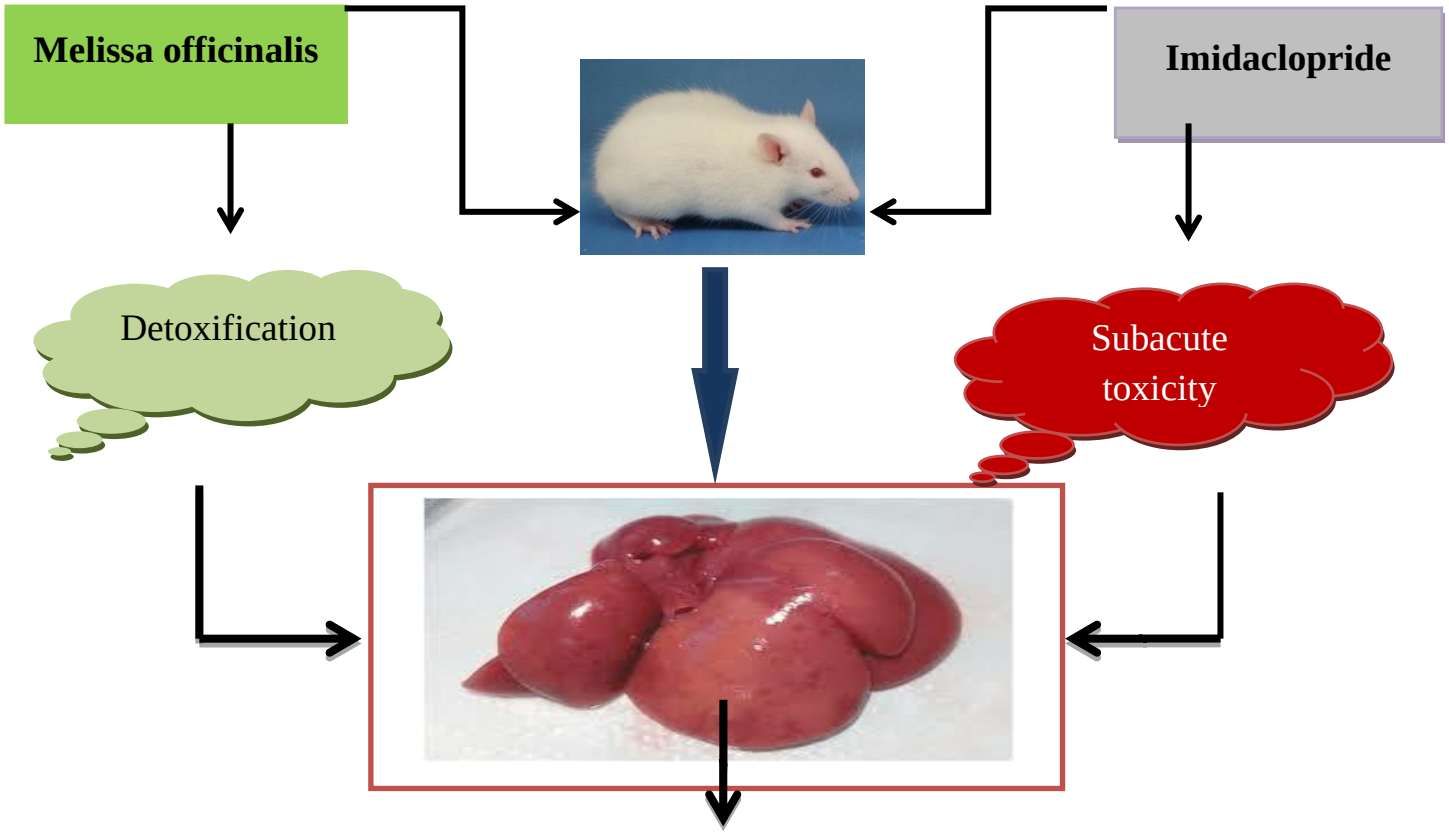
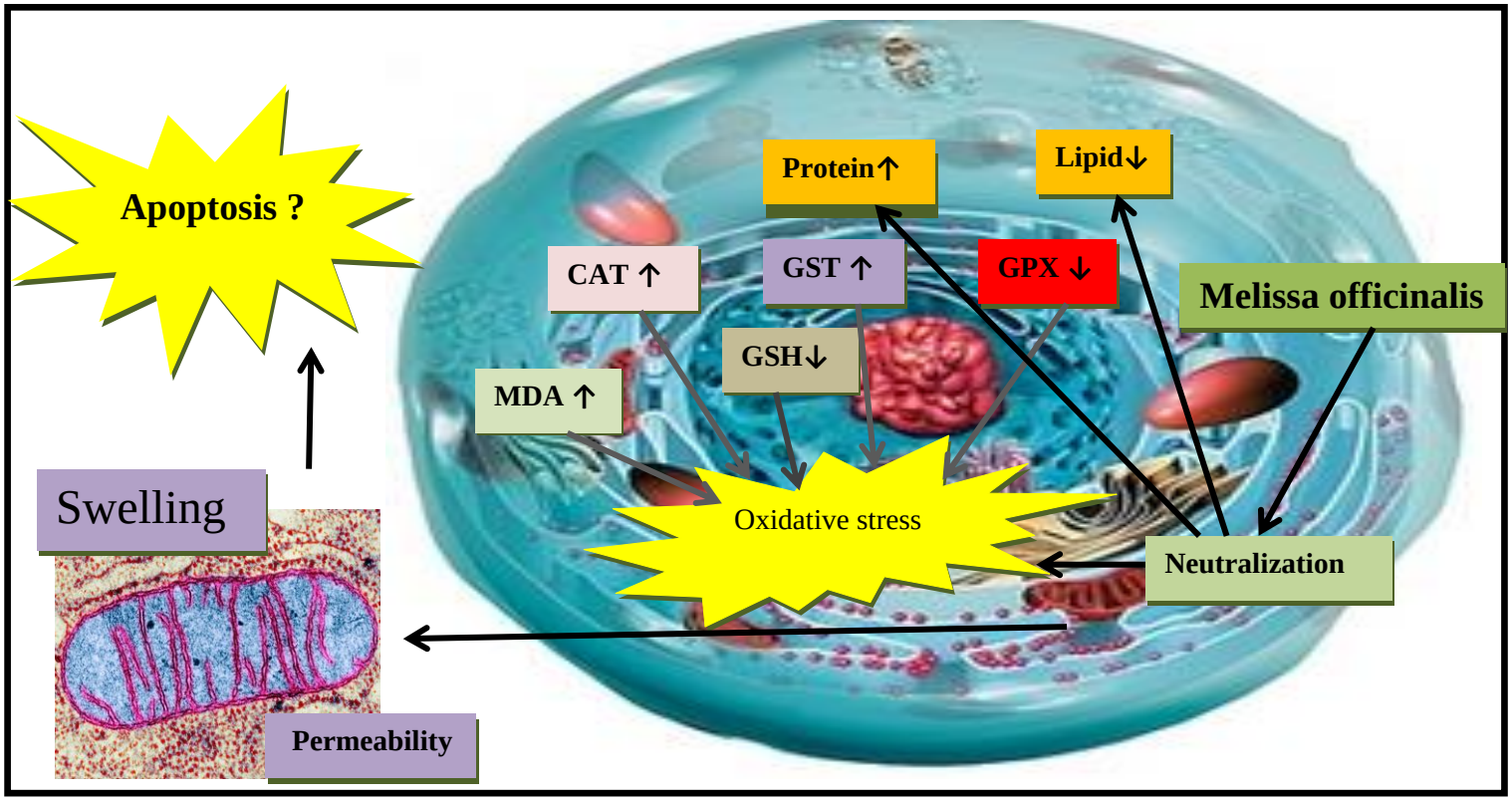
Hypothesis


Melissa officinalis
Imidaclopride
Detoxification
Subacute toxicity
Hepatocyte


Apoptosis ?
Swelling
Permeability
Protein↑
Lipid↓
CAT ↑
GST ↑
GPX ↓
GSH↓
MDA ↑
Oxidative stress
Neutralization
Melissa officinalis

Annex

ANNEXES

1. Calibration curve for lipid determination:

Tubes	1	2	3	4	5	6
Lipid stock solution (µl)	0	20	40	60	80	100
Ether solvent/ chloroform (µl)	100	80	60	40	20	0
SPV reagent (ml)	2.5	2.2	2.5	2.5	2.5	2.5

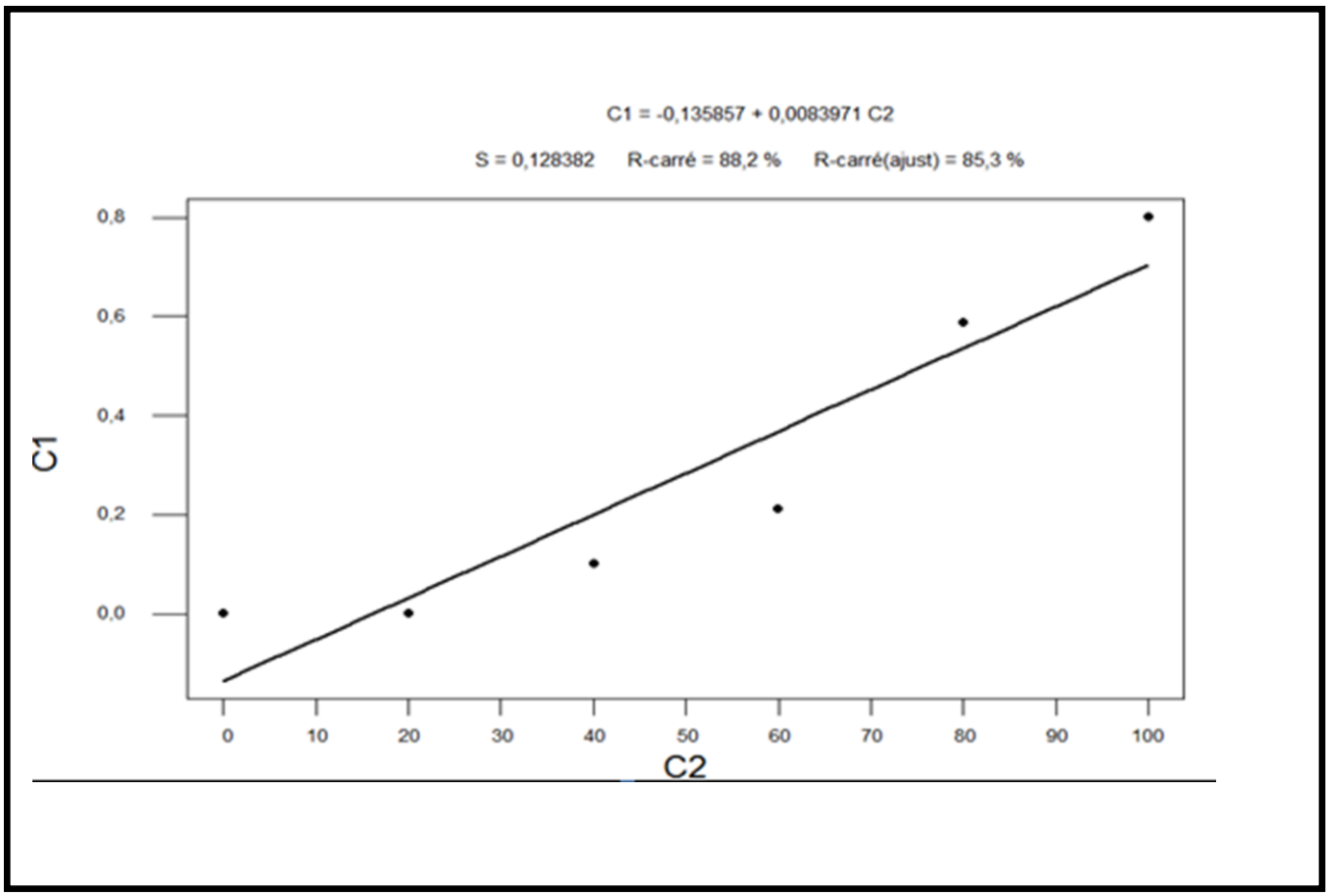


Table / Figure (A). Creation of calibration curve for the determination of lipids

The equation: Y=0.0282857+0.0019843X

2. Calibration curve for protein determination:

Tubes	1	2	3	4	5	6
Albumin stock solution (µl)	0	20	40	60	80	100
Distilled water (µl)	100	80	60	40	20	0
BBC reagent (ml)	4	4	4	4	4	4

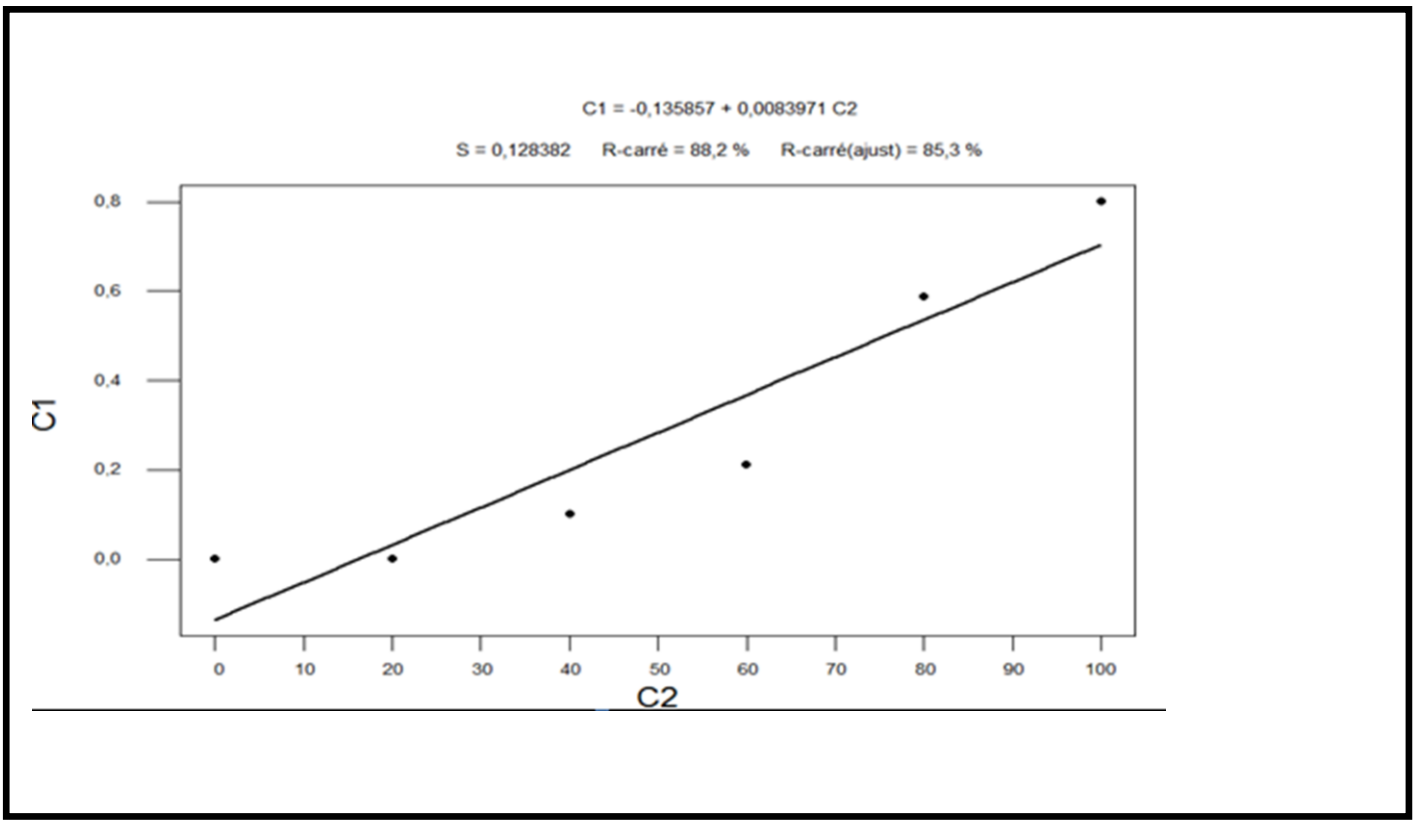


Table / Figure (B). Calibration curve for protein assay

The equation: Y=0.135857+0.0083971X

3. Material used in the different stages of the study:

3.1. Large laboratory equipment and devices:

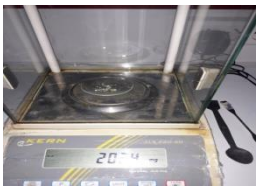
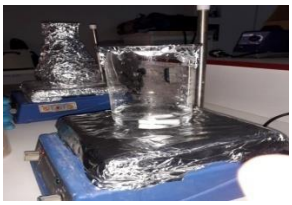
			
- Precision scale.	- Centrifuge.	- Water bath (MEMMERT)	- Magnetic agitator.
			- Fridge. - Ice bath.
- Spectrophotometer.	- Sand bath	- Vortex.	

Figure: Large laboratory equipment and devices.

3.2. *Small laboratory equipment:*

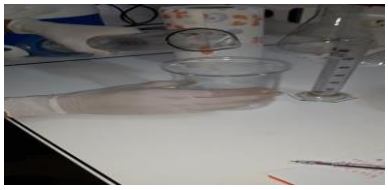
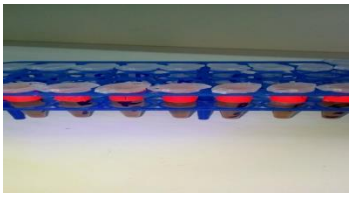
		
- Test tubes	- Mortars	- Glassware
		- Wash bottle. - Spatula. -Magnetic bar. -Magnetic rod extractor. - Cuvette for spectrophotometer (plastic and glass).
- Micropipettes	- Epindorphic tubes	

Figure: Small laboratory equipment.

4. Chemical material:

- Distilled water.
- TCA (Trichloro acetic).
- Anthrone.
- Sulfuric acid.
- Orthophosphoric acid.
- Vanillin.
- BBC (Brilliant Coomassie Blue).
- Ether.
- Chloroform.
- TS.
- TSE.
- Salmon phosphate.
- SSA (Sulfosalicylic acid).
- Tris.
- HCl.
- NaOH.
- EDTA (Ethylene diamine tetracetic acid).
- DTNB (5-5'-dithio-bis-2-nitrobenzoic acid).
- NaCl.
- TBA.
- GSH.
- CDNB (1-Chloro2, 4 di nitrobenzene).
- H2O2.
- Phosphate.