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Neurobehavioral impacts and sociabilty and preference for social noverlty of wistar rats after treatment with neonicotinoide (Imidacloppride)

By

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ملخص

الهدف من هذا العمل هو دراسة تأثير المبيدات الحشرية التي تنتمي إلى عائلة نيونيكوتينويد على أدمغة الفئران .

حيث تطرقنا في هذا العمل لدراسة تأثير إيميداكلوبريد بجرعات مختلفة (5 و 50 ملغ / كلغ / يوم) على مستوى المستقبلات مثل البروتينات الكلية والكربوهيدرات والدهون ، وعلى معدل الأنشطة الإنزيمية GST ، GPx، CAT، وكان هناك أيضًا اهتمام بدراسة الميتوكوندريا ونفاذيتها عند هذه الفئران.

ولقد أظهرت نتائجنا أن إيميداكلوبريد يسبب زيادة في البروتينات وانخفاض في مستويات الكربوهيدرات والدهون. وفيما يتعلق بالأنشطة الإنزيمية ، فلقد سجلت نتائجنا انخفاض ملحوظ في GST - GPx- AchE وزيادة معتبرة في CAT اما بالنسبة للأنشطة الغير الإنزيمية فلقد أظهرت زيادة في مؤشر GSHو MDA في دماغ الفئران.

وأخيرًا ، تشير دراستنا إلى أن هناك تأثيرات جد ملحوظة في السلوك العصبي والمؤانسة الاجتماعية وتفضيل الحداثة الاجتماعية للفئران واستر بعد العلاج باستخدام نيونيكوتينويد (إيميداكلوبريد).

الكلمات الرئيسية: نيونيكوتينويد ، إيميداكلوبريد ، فئران واستر، السلوك العصبي ، المؤانسة الاجتماعية، تفضيل الحداثة الاجتماعية.

Abstract

The aim of this work is to study the effect of neonicotinoid insecticides on rat brains.

In this work we examined the effect of imidacloprid in different doses (5 and 50 mg / kg / day) on the level of receptors for brains such as total proteins, carbohydrates and fats, and on the rate of enzymatic activities GST, GPx, CAT, and there was also interest in studying mitochondria and its permeability in rat wistar.

Our results showed that imidacloprid causes an increase in proteins and a decrease in carbohydrate and fat levels. Regarding enzymatic activities, our results showed a noticeable decrease GST - GPx - AchE and a significant increase in CAT. As for non-enzymatic activities, it showed an increase in the index of GSH and MDA in the rat brain.

Finally, our study indicates that there are very noticeable effects on nervous behavior, socialisation, and social preference for rat wistar after treatment with neonicotinoid (imidacloprid).

Keywords: neonicotinoid, imidacloprid, wistar rats, Neurobehavioural, sociability social novelty.

Résumé

L'objectif de ce travail est d'étudier l'effet des insecticides néonicotinoïdes sur le cerveau des rats.

Dans ce travail, nous avons examiné l'effet de l'imidaclopride à différentes doses (5 et 50 mg / kg / jour) sur le niveau de récepteurs pour le cerveau tels que les protéines totales, les glucides et les graisses, et sur le taux d'activités enzymatiques GST GPx, CAT, et il y avait aussi un intérêt à étudier les mitochondries et sa perméabilité chez les rats wistar.

Nos résultats ont montré que l'imidaclopride provoque une augmentation des protéines et une diminution des taux de glucides et de lipides. En ce qui concerne les activités enzymatiques, nos résultats ont montré une diminution notable de GST - GPx - AchE et une augmentation significative de CAT. Quant aux activités non enzymatiques, elles ont montré une augmentation de l'indice de GSH et de MDA dans le cerveau du rat.

Enfin, notre étude indique qu'il existe des effets très perceptibles sur le comportement nerveux, la socialisation et la préférence sociale pour les rats wistar après un traitement avec des néonicotinoïdes (imidaclopride).

Mots-clés: néonicotinoïdes, imidaclopride, rats wistar. Neurocomportementaux, Nouveauté sociale

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To all those who contributed directly or indirectly in the realization of this work .

DEDICATION

At this particular time in my life, I want to dedicate this modest work: To my dear father **Mesbah khaled:** your encouragement your advice my pushed to go far i am very grateful for your love and that god almighty preserves your health and grants you a long life. To my adorable and dear Mum **Mayoufa Khaled:** a thousand dedications cannot express what i owe for your sacrifices your patience during my studies, your moral support. This work is only a simple testimony of my great and eternal love. May Almighty God Preserve Your health and grant you a long life.

To my sisters **Nariman, her small family, her husband Kamal, Wassila** her husband **Dihda , Ismahan** and **Mabrouka**.

I dedicate this work in particular my aunt **Bahya** to all the members of my extended family (***my brothers* Lazhar and Meftah, Azzouz** in **Habib** and **Kamal** and their children, and **my uncles**)

And to each family **Khaled**

For my close friends

To all those who contributed to the accomplishment of this work

Abreviations list

% **:** Percentage

$: Dollar

µg **:** Microgram

µl : Microliters

µmol **:** Micromole

µm **:** Micrometer = 10-6m

Ach **:** Acetylcholine

AchE **:** Acetylcholinesterase

ANOVA : Analysis of variance

ACT : Acetamipride

ACHR : Acetylcholine Receptors

BBC **:** Brilliant Blue of Coumassie

BCSG : Bayer Crop Science Group

BSA **:** Bovine Albumin Serum

° **C** : Celsius

Ca ++ : Calcium ++

Ca2 + : Calcium ion

C **:** Concentration

CAT **:** Catalase

CDNB : 1-chloro, 2.4-dinitrobenzene

Cu **:** Copper

Cm **:** Centimeter

C9H10CIN5O2: Imidaclopride

Cl : Chlor

DDT : Dichlorodiphenyltrichloroethane

DNA **:** Deoxyribonucleic acid

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

DO **:** Optical density

ERO : Reactive oxidizing species

EFSA **:** European Food Safety Agency

ECHA **:** European Chemical Agency

EDTA **:** Ethylene-Diamine-Tetra acetic Acid

Eg : example

Fd : Dilution factor

Fe **:** Iron

GPx **:** Glutathione peroxidases

GST : Glutathione-S-transferase

GSSG **:** Oxidized Glutathione

GSH : Glutathione

g : Gram

GR **:** Glutathione reeducates

H **:** hydrogen

H2 **:** Dehydrogenase

H20 **:** Water

HO **:** hydroxyl.

HO2 • **:** Radicalhydroperoxyle

HCl **:** hydrochloric acid

HOCl : Hypochloric acid

H2O2 **:** Hydrogen peroxide

H302 **:** Harmful substance if swallowed

i.p. : Intra peritoneal (intraperitoneal bite)

k **:** kelvin unit international thermodynamic temperature system

Kg **:** Kilogram

L : Length

LC50 **:** Lethal concentration 50

LD50 **:** Lethal dose 50

mAChRs **:** muscarinic-type acetylcholine receptors

MDA : Malondialdehyde

Mg **:** Milligrams

ml **:** Milliliter

min **:** Minute

M : Mole

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

Mg / ml **:** Milligram / milliliter

Mn : Manganese

N° : Number

nm : Nanometer

NO : Nitric oxide

NOAEL : No observed adverse effect level

NaoH : Sodium hydroxide

NO • **:** Nitric oxide

NO3 **:** Peroxynitrite

nAChR : nicotinic acetylcholine receptors

(nAChRs): post-synaptic nicotinic acetylcholine receptors

O2 **:** Oxygen

OH. : Hydroxyl radical

O2- : The superoxide anion

PAMP : Molecular Pattern Associated with a Pathogen

PPM **:** part of million

PPSE **:** Excitatory Post-synaptic Potential

RL **:** Free radical

ROS **:** Reactive oxygen

R˙ **:** Radical

ROO • : Pyroxyl radicals

ROOH **:** Hydroperoxydelipidique

RO • : alkoxylradical.

SN : Nervous system

SNC **:** Central Nervous System

SNP **:** Peripheral Nervous System

SOD **:** Super oxide-dusmitase

S : Second

TCA **:** Trichloroacetic acid

T : Witness

TBA : Thiobarbituric acid

TCA : Trichloroacetic.

TCP : trichloro- pyridinol

V : volume

T **:** Time

TBA : Thiobarbituric acid

Vt : Total volume

Ve **:** Volume of the enzyme extract

WD **:** Distilled Water

Zn **:** Zinc

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Introduction

Introduction

Pesticides also called phytosanitary products, describes a group of chemicals used for the prevention, control or elimination of living organisms deemed harmful. Pesticides are classified according to their chemical structure or their origin, in mineral or organic insecticides, or natural or synthetic insecticides. Four main groups are distinguished: non-organic insecticides (arsenicals and sulfur compounds), organic insecticides of plant origin (neonicotinoids, pyrethrum and rotenone) or synthetic (organochlorine, organophosphates, pyrethroids and carbamates) and growth regulators (inhibitor of chitin synthesis, inhibitor of ecdystéroides and inhibitor of juvenile hormone).

Among the pesticides common in the last decade, neonicotinoids which are widely used in agriculture against the ravages of insects **(Moriya et *al*., 1992).** It is used in particular to protect fruits, vines, vegetables, cereals, soybeans, cotton and ornamental plants (**Buckingham et *al*., 1997; Casida, 2010; Schaafsma et *al*., 2015**). Inside habitats, they are also used to destroy several plagues such as flies, mosquitoes and cockroaches **(kilani-Morakchi et *al*., 2006; Messiad et *al*., 2015).**

However, the intensive use of pesticides has led to serious environmental problems and risks to human health due to their high toxicity **(Soso et *al*., 2007; Grote et *al.*, 2008; Stefanidou et *al*., 2009; Holmes, 2010; Walker et *al*., 2011**). Numerous epidemiological studies suggest a correlation between the professional use of neonicotinoids and the appearance of certain pathologies in the populations concerned. Carcinogenic and neurotoxic or endocrine disruption effects**,** infertility problems. Or the weakened immune systems are more common in these populations, or high toxicity in non-target organisms such as bees **(Nauen et *al*., 2001; Schmuck et *al*., 2001; Suchail et *al*., 2001; Elhassani et *al*, 2008; Mommaerts et *al,* 2010)**

Thus, this manuscript is divided into three parts; the first is devoted to general information on neonicotinoids and imidacloprid, their impact and toxicity on the environment and non-target organisms. The second part is neurobehavioural and sociability studies and preference for the social novelty of wistar rats after a treatment with neonicotinoid (imidacloprid) at the end the practical part consists of studies carried out on the toxicity of neonicotinoids at the level of wistar rat brains by renowned researchers and whose the results are benchmarks in the field.

Chapter 01: Generality

I. Neonicotinoid

I.1. Historical

Since their discovery in the 1980s, neonicotinoid pesticides have become the most widely used class of insecticides, worldwide, with large-scale applications ranging from the protection of plants (crops, vegetables, fruits) and with many insect pests had developed resistance to organophosphates, carbamates and pyrethroids on the market **(Georghiou et Mellon, 1983; Denholm et *al*., 2002; Alyokhin et *al*., 2008).**

The discovery of imidacloprid by Shinzo Kagabu, and its subsequent market introduction in 1991, began the era of the neonicotinoid insecticide class (**Tomizawa et Casida, 2011**)

Imidacloprid was followed in 1999 by thiamethoxam and clothianidin, which is a metabolite of thiamethoxam (**Meredith et *al.*, 2002).**

Over the next two decades, neonicotinoids became the most widely used insecticides of the five major chemical classes (the others being organophosphates, carbamates, phenylpyrazoles, and pyrethroids) in the world market **(Jeschke et Nauen, 2008; Jeschke et *al.*, 2011; Casida et Durkin, 2013).**

In 1990, the global insecticide market was dominated by carbamates, organophosphates and pyrethroids. In 2008, a quarter of the insecticide market was represented by neonicotinoids, which increased by 27% in 2010 **(Casida et Durkin, 2013), a**nd almost 30% consisted of neonicotinoid and fipronil combined, the other classes having contracted. As a result **(Jeschke et *al*., 2011) .**In the same year, imidacloprid became the best-selling insecticide in the world and the second-largest pesticide sales glyphosate being the best-selling **(Pollack, 2011)** of registered uses for more than 140 crops in 120 countries **(Jeschke et *al*., 2011)** an herbicide, while fipronil is a powerful insecticide.

Neonicotinoids are now widely used for a wide variety of crops around the world

In 2009, the global neonicotinoid market was US $ 2.63 billion. Imidacloprid represented the largest proportion (41.5%) of it, and was Worth $ 1.09 billion, with - in descending order of the shared market - respectively: thiamethoxam, clothianidin, acetamiprid, thiacloprid, dinotefuran and nitenpyram for US $ values of 0.63; 0.44; 0.28; 0.11; 0.08 and 0.008 billion. During the 2003-2009 period, sales of individual neonicotinoid products (the only exception being nitenpyram) increased between 1.6 and 14.6 times, for a total increase in sales of all products by 2.45 times Neonicotinoids are of enormous economic importance worldwide, especially in the fight against pests that have already developed resistance to other classes of insecticides **(Jeschke et *al.,* 2011).**

I.2. Definition

Neonicotinoids are a family of insecticides that act on the central nervous system of insects. This type of product has been present on the French market since 1994. This denomination of neonicotinoids concerns the following active substances: thiamethoxam, imidacloprid, thiacloprid, acetamiprid, and clothianidin, which are for example up to 10,000 times more toxic than DDT for bees**. (Benayas et *al.,* 2009).**

Neonicotinoids are neurotoxins, synthetically similar to nicotine. They are widely applied on many arable, horticultural and ornamental crops, and are found in concentrations between 1 and 15 nanograms per gram (ng / g), sometimes up to 100 ng / g. When we put bee colonies or bumblebee nests on arable land anywhere in the developed world, the pollen and nectar stores they harvest usually contain a cocktail of several neonicotinoids and others pesticides, mainly fungicides. **(Goulson, 2012)**

Neonicotinoids are the only insecticides with 3 effective modes of application that can partly explain their success. These 3 modes are as follows:

- Treatments of the aerial parts of plants: IMI, ACT, thiacloprid, thiamethoxam, dinotefuran.
- Soil treatments: IMI, clothianidin
- Seed coating: IMI, thiamethoxam, clothianidin. **(Goulson, 2012)**

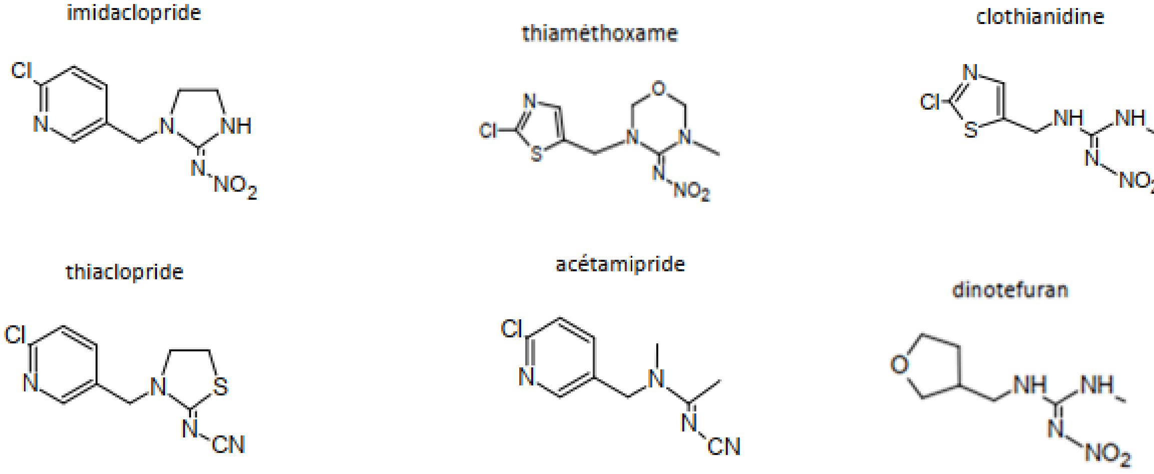


Figure 01: The chemical formulas of the types of neonicotinoids **(David et *al.*, 2007).**

I.3. Classification of neonicotinoids

Neonicotinoids have the distinction of being among the least persistent insecticides in the soil. This persistence in the environment can be accessed via the half-life of plant protection products, t1/2. This period is the time necessary for half of the concentration of a product in the soil to be degraded. While this 1/2 is several years for organochlorine insecticides such as DDT (5.5 years), it is only 2 to 3 months for pyrethroids and neonicotinoids **(Gupta et *al*., 2007; Hatcher et *al*., 2008)**; Not only are they not very persistent, neonicotinoids are effective at low doses. While to be effective, DDT had to be applied at the rate of 2,000 g per hectare, and 500 g per hectare for organophosphates such as chlorpyrifos, only 50 to 200 g per hectare of pyrethroids and 70 to 100 g of neonicotinoids1 per hectare are sufficient **(Calvet et *al*., 2005; Regnault-Roger et *al*., 2005)**. The most important factor in their success is the selectivity of neonicotinoids for insects of mammals. After pyrethroids, neonicotinoids are the insecticides with the strongest mammalian / insect selectivity factor (**Tomizawa et Casida, 2005**). This selectivity factor is calculated by establishing the ratio between the lethal dose 50 of mammals and the lethal dose 50 of insects for a given insecticide. The higher the selectivity factor, the more selective the insecticide is to insects over mammals. This selectivity for insects gives neonicotinoids a high level of "safety" in use compared to other classes of insecticides, which with their efficacy at low doses, is largely behind their success **(Elbert et *al*., 2008).** Pyrethroids also have these same characteristics, however only neonciotinoids have a systemic action offering long-term total protection from the seed stage to the adult stage of the plant **(Elbert et *al*., 2008).**

I.4 .Agricultural environment and use of neonicotinoid

The use of neonicotinoids covers four main areas: the protection of crop plants and ornamental plants against insects and herbivorous mites, pest control in urban areas to target harmful organisms such as cockroaches, ants, termites, wasps, flies, etc., veterinary applications (against fleas, ticks, etc. on pets and livestock, and fleas in stables), fish farming to control the rice weevil infestations in the rice-crayfish rotation **(Barbee et Stout, 2009 Chagnon et *al.*, 2014)**. Figures on the relative economic importance of these four fields of application are rare, but to give an indicative example, sales of imidacloprid 2010 from Bayer Crop Science (covering plant protection and use as a biocide) amounted to 597 million euros **(Bayer Crop Science, 2011),** while in 2010 the sales of Bayer Healthcare's imidacloprid (in veterinary applications) amounted to 408 million euros **(Bayer Healthcare, 2011)**. Overall,

the greatest use appears to be for the protection of crops, ornamental plants, arboriculture, horticultural plants, nurseries and forestry. **(Bayer Healthcare, 2011).**

In agriculture, horticulture, nursery and forestry neonicotinoids and fipronil can be applied in different ways such as spraying (foliar), coating seeds, pilling seeds, soil treatment, spreading granules, soaking the plants, chemigation, soaking (of the soil), by applications in the furrow, injection into the trunk of the trees, mixed with irrigation water, flooding the flower bulbs and applying paintbrush on the stems of fruit trees. Seed coating and soil applications account for approximately 60% of their uses worldwide **(Jeschke et *al.*, 2011)**

I. 5. Impacts on biodiversity

Most ecosystem soil services are biologically publicized and as pesticides can cause depletion or disruption of non-target biotic communities in soils, it follows that pesticides can pose risks to ecosystem processes and services, ground. The effects of pesticides in soils can range from acute and chronic toxicity, direct, in organisms, to many subtotal and indirect effects on behavior, functional roles, predator-prey relationships and network dynamics. Some or all of these can occur at the organism, population, or community level and, therefore, can impact soil biodiversity or ecosystem stability **(Edwards, 2002).** Soil biodiversity is linked to the ecological functions that support ecological services **(Benayas et *al.*, 2009)**, disturbances in biodiversity and ecological function due to pesticides could harm ecosystem services **(Goulson, 2013).**On soil biodiversity and their implications in the functioning of ecosystems have been demonstrated for other pesticides that affect microbes **(Johnsen et *al.*, 2011)**

I.6. Mode of action of neonicotinoids

Neonicotinoids act within the central nervous system by interfering with neuronal transmission. They bind to the nicotinic acetylcholine receptors (nAChR) in the postsynaptic neuron, thus acting as "false neurotransmitters" (agonists). This interference with the signal from the neurotransmitter acetylcholine is the cause of continuous activation of the receptor, which leads to neurotoxic symptoms. Neonicotinoids have a greater affinity for the receptors of insects to which they thus bind more strongly than for those of vertebrates, mammals or the like, so that their toxicity for mammals is lower than it is for insects and that the reversibility of intoxication is greater for vertebrates **(Tomizawa et Casida, 2005; Jeschke et *al.*, 2011)**

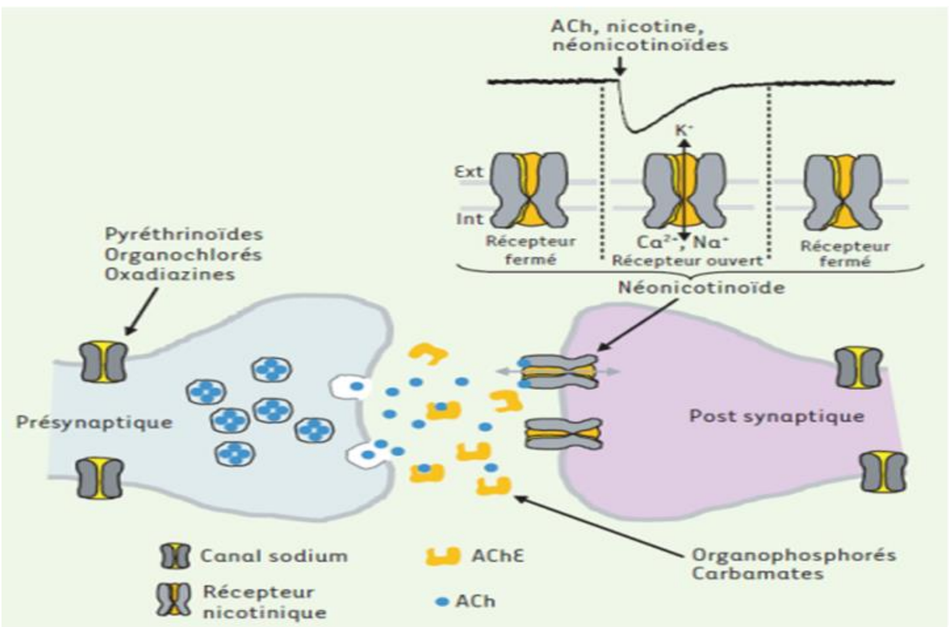


Figure 02: Mode of action of neonicotinoids **(Tomizawa et Casida, 2005).**

I.7. Neonicotinoid toxicity

There is no epidemiological study of toxicity from chronic exposure to neonicotinoids. This is surprising when we know that a third of the insecticides sold worldwide are neonicotinoids. Nevertheless, recent publications show the risks of chronic effects of neonicotinoids for human health, through studies on animals or on human cell cultures:

I.7.1. Neurological toxicity

A Japanese study has highlighted the impacts in mammals of acetamiprid and imidacloprid on the cerebellum, leading to morphological abnormalities in brain development and behavioral disorders. Following this publication, the European Food Safety Agency (EFSA) issued in 2013 a potential link between these two molecules and developmental neurotoxicity. In other words, she identified a potentially harmful effect on the development of neurons and brain structures in a fetus or young child. In addition, imidacloprid inhibits a liver enzyme which leads to the accumulation in the body of a neurotoxic substance, the ALA delta. **(Sauer, 2014)**

I.7.2 Endocrine disruption (thyroid and reproduction)

The Canadian Pest Management Regulatory Agency (PMRA) considers three neonicotinoids (clothianidin, acetamiprid and thiamethoxam) to be potential endocrine disruptors and suspected to affect reproductive effects in animals. Concerning thiacloprid, a French study and in evidence its action of endocrine disruptor on the thyroid in the rat and describes synergistic effects for the association thiacloprid-deltamethrin on the thyroid, association well known in the pesticide Protéus widely used in France on the rapeseed. Thiacloprid has recently been the subject of a proposal for a category 2 reprotoxic classification by ECHA (European Chemicals Agency) leading ANSES "to consider the substance as having endocrine disrupting effects" xi. The AGRITOX database of ANSES classifies this substance as risk 1B (substance presumed toxic for human reproduction). The same endocrine disrupting effects have been demonstrated for imidacloprid in mammals and birds. Other studies have shown the endocrine disrupting action of clothianidiin in rats upon prenatal exposure**. (Sauer, 2014)**

I.7.3 Human genotoxicity and cytotoxicity

An in vitro study has shown the cytotoxic effects of thiacloprid during cell division of human lymphocytes. Exposure of human lymphocytes to pesticides based on thiacloprid, clothianidin or imidacloprid has shown cytotoxic effects and DNA damage paving the way for carcinogenesis and mutagenesis. **(Sauer, 2014)**

I.7.4 Cocktail effects

In the presence of certain other pesticides the toxicity of neonicotinoids may be greatly increased. These synergistic properties are to be taken into account since many pesticide residues are present in the human body. The following were observed in particular:

1 / the synergistic effect of imidacloprid and mancozeb on the thyroid in mice

2 / the synergistic effects of the association thiacloprid and deltamethrine on the thyroid in rats (association found in the Protein widely used on French rapeseed)

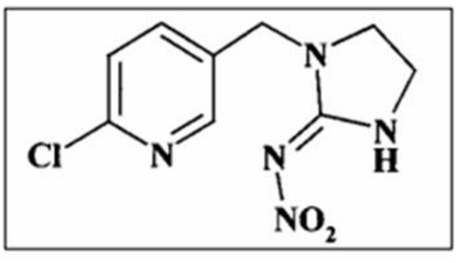
3 / In addition, strong cellular toxicity has been demonstrated on human cells of commercial formulations containing imidacloprid or acetamiprid combining the active substance with its adjuvants. This toxicity can be up to 1000 times greater than that of the active substance alone. **(Sauer, 2014)**

II. Imidacloprid

II.1. Definition

Imidacloprid is a neurotoxic insecticide that is part of the neonicotinoid family (Figure 03). It was the first neonicotinoid marketed in the world in 1991 by the Bayer Crop Science Group (BCSG). 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 addition, these insecticides have a high selectivity towards insects compared to mammals **(Tomizawa et Casida, 2003).**

Neonicotinoids currently dominate the insecticide market worldwide **(Sparks,. 2013).**Imidacloprid is the most widely marketed neonicotinoid (41.5% of the neonicotinoid market), making it the most widely used insecticide in the world **(Jeschke et *al*., 2011).**



$C_9H_{10}ClN_5O_2$

Figure 03: Structure of imidacloprid or 1- (6-chloro-3-pyridylmethyl) –Nnitroimidazolidine 2-ylideneamine **(Jeschke et al., 2011).**

Imidacloprid is currently used in the veterinary field as a flea and tick treatment, in the field of gardening or in domestic use for the elimination of termites or cockroaches. It is also used in the agricultural field (more than 140 crops) against mealybugs, thrips, sucking insects in arboriculture and in cereal crops. Insecticides can be used in three different application modes: treatment of aerial parts, treatment of soil or coating of seeds (systemic agent). The success of imidacloprid also lies in the fact that it is marketed according to the three known modes of application. **(Jeschke et al., 2011)**

II.2. Physical properties

Imidacloprid is in the form of a whitish, crystalline powder. As an active substance in plant protection products, imidacloprid must have purity equal to or greater than 970 g / kg (annex to EC regulation 1107/2009). Imidacloprid is non-volatile, very slightly soluble in

water (610 mg / L at 20 ° C), soluble in most solvents (dimethyl sulfoxide, dichloromethane, 2-propanol, acetone ...) and sparingly soluble in aliphatic solvents (eg: hexane). **(Jargot et *al.*, 2015)**

Tableaux 01: Physical properties of imidacloprid **(Jargot et *al.,* 2015)**

Nom Substance	Détails	
Imidacloprid	Formule	$C_9H_{10}ClN_5O_2$
	N° CAS	138261-41-3
	Etat Physique	solide
	Masse molaire	255,66
	Point de fusion	non applicable
	Point d'ébullition	144 °C
	Densité	1,54 à 23 °C
	Pression de vapeur	4.10^{-7} Pa à 25 °C
	Coefficient de partage n-octanol / eau (log Pow)	0,57 à 20 °C - pH 7

I.3. Chemical properties

Imidacloprid is a stable product under normal storage conditions. Hazardous decomposition products are formed in the event of fire, such as carbon or nitrogen oxides, hydrochloric acid or hydrogen cyanide. **(Jargot et *al.*, 2015)**

II.4. Imidacloprid exposure

Imidacloprid is a combustible liquefiable solid. It can in particular generate explosive atmospheres in the form of dust. To fight a fire in which the substance imidacloprid is involved or in the vicinity, the recommended extinguishing agents are preferably water sprayed with additive and anti-alcohol foams. Chemical powders or carbon dioxide should be avoided as they can resuspend the particles and generate atmospheres that can explode. Qualified responders will be equipped with self-contained, self-contained breathing apparatus and protective suits. **(Mnif et *al.*, 2011)**

II.5. the target of imidacloprid

Imidacloprid is a neurotoxicant that specifically acts on the nicotinic acetylcholine receptors (nAChRs) located in the nervous system of insects. These receptors are composed of five subunits which form a channel allowing the selective passage of Na +, Ca2 + and K + ions. Each subunit is composed of an extracellular C-terminal domain, four transmembrane domains (TM1-TM4) and an extracellular N-terminal domain which contains 6 distinct regions looping significant AF in the acetylcholine binding site. **(Talley et *al*., 2008)**

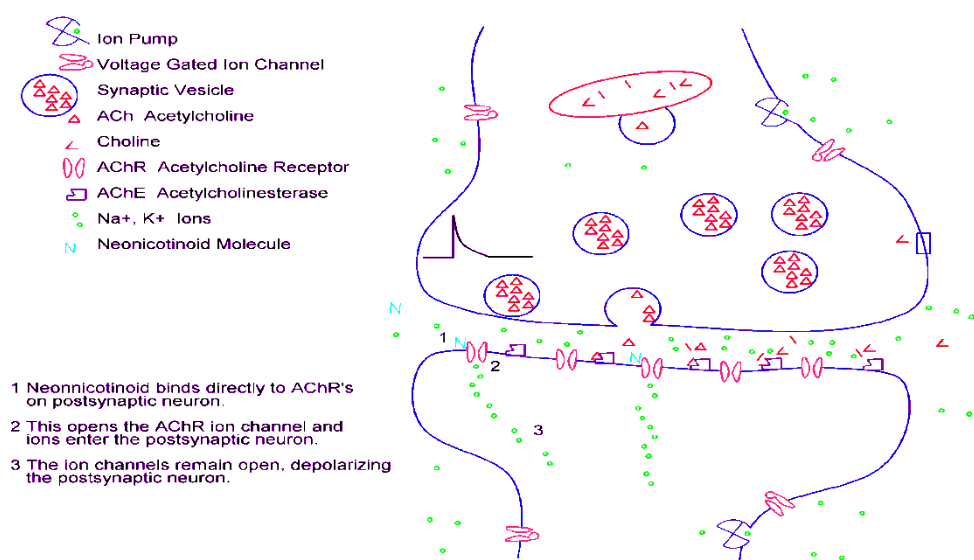


Figure04: Schematic representation of the target of imidacloprid **(From Thany et *al*., 2007).**

The activation of the presynaptic element is characterized by an action potential (PA) which, by propagating at the level of the synaptic button allows the activation of voltage-dependent calcium channels and the migration of acetylcholine vesicles (ACh) to the synaptic cleft. The release of ACh by the presynaptic neuron activates the post-synaptic nicotinic acetylcholine receptors (nAChRs). Activation of these nAChRs results in an outgoing flow of K + ions and an inward flow of majority Na + ions. This entry of sodium ions creates a depolarization of the membrane and thus generates an excitatory post-synaptic potential (PPSE), making it possible to transmit nerve information to the post-synaptic neuron. ACh can also bind to muscarinic-type acetylcholine receptors (mAChRs) that regulate the release of ACh. At the synaptic cleft, ACh is degraded to choline and acetate by acetylcholinesterase (AChE). Choline is recaptured by the presynaptic element to synthesize new ACh molecules

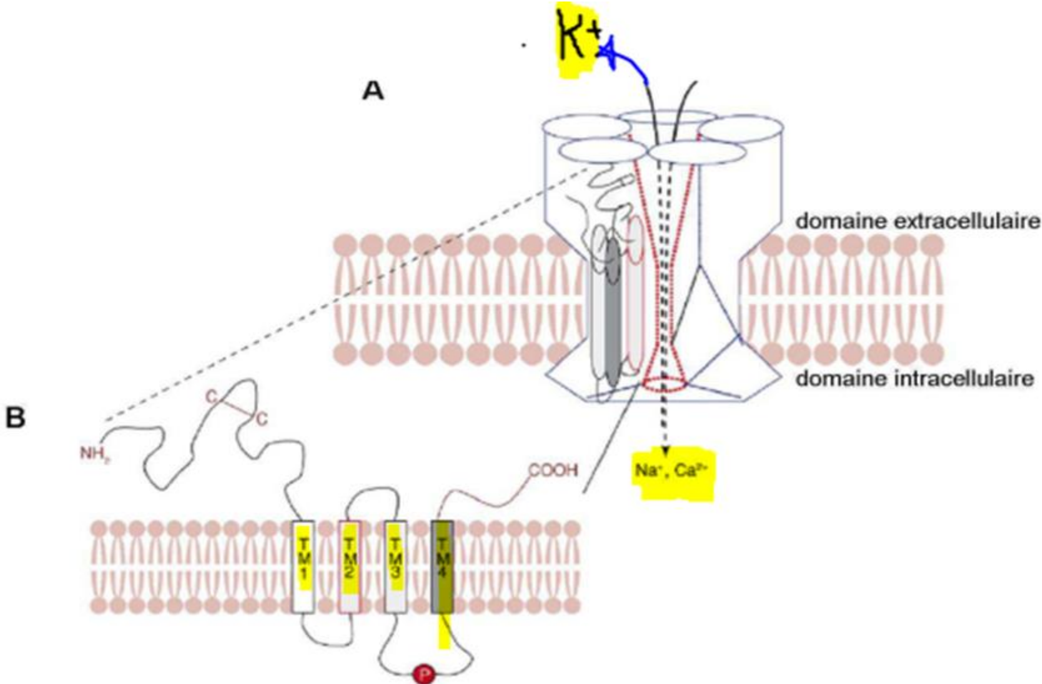


Figure05: Structure of the nicotinic acetylcholine receptor**,** -(A) Pentameric receptor forming a selective pore to Na +, Ca2 +, K + ions through the plasma membrane. -(B) Each subunit is made up of 4 transmembrane domains (TM1-TM4). The intracellular loop between the TM3 and TM4 domains presents a potential phosphorylation site **(Thany et *al*., 2007**).

II.6. Toxicokinetics and Metabolism

II.6.1. In animals

II.6.1.1. Absorption

After single administration by gavage of a dose of 1 or 20 mg / kg bw or after repeated administration by gavage of a dose of 1 mg / kg bw for 14 days in rats, imidacloprid is rapidly and widely absorbed. The maximum concentration is reached in 1 to 2 hours. The bioavailability, calculated on the basis of urinary and biliary excretion, is greater than 92% in 48 hours**. (Jargot et *al*., 2015)**

II.6.1.2. Distribution

Imidacloprid is rapidly and widely distributed throughout the body, primarily in the liver, kidneys, lungs and skin. It should be noted that the penetration of imidacloprid across the blood-brain barrier is relatively limited in the rat: among the organs studied, the brain has the lowest radioactivity levels 48 hours after oral or intravenous administration. **(Jargot et *al*., 2015).**

II.6.1.3. Metabolism

The metabolism of imidacloprid is very important: up to 90% of the administered dose is metabolized in the liver. At least 16 metabolites have been identified in rats. Two main biotransformation pathways have been demonstrated in rats: either oxidative cleavage between the methylene group and the cycleimidazolidine followed by conjugations, or hydroxylation of the imidazolidine cycle. **(Jargot et *al*., 2015)**

Figure06: Metabolism of imidacloprid (I): ethylene-bridge hydroxylation of the imidazolidine ring and elimination of water **(Robin, 2003)**

Figure07: Metabolism of imidacloprid (II): nitro-group reduction to nitrosimine and further loss of NO to form guanidine **(Robin, 2003)**

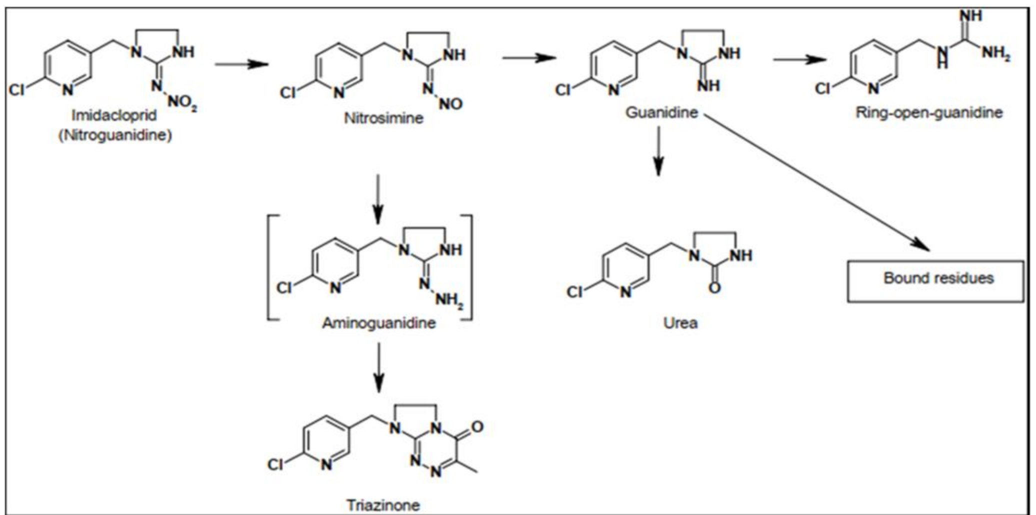


Figure08: Metabolism of imidacloprid (III): Oxidative cleavage of the methylene bridge to form 6-chloropicolyl alcohol and subsequent oxidation to 6-chloronicotinic acid **(Robin, 2003)**

II.6.1.4. Elimination

Excretion is rapid and almost complete: 48 hours after the administration of a low oral or intravenous dose (1 mg / kg bw) or a high oral dose (20 mg / kg bw), plus 95% of the administered dose is excreted: 73-75% in the urine and 20-25% in the feces. During intra-duodenal administration of a dose of 1 mg / kg bw in rats after cannulation of the bile duct, biliary excretion represents up to 36% of the administered dose, testifying to the existence of a enterohepatic cycle. No accumulation is observed. **(Jargot et *al*., 2015)**

II.6.2. In humans

No data is available in humans at the date of publication of this toxicological data sheet. **(Jargot et *al*., 2015)**

II.7. Human toxicity

Accidental exposures to imidacloprid-based preparations are most often of low to moderate severity during ingestion (minor digestive signs), inhalation (cough, dyspnea) and ocular projection (conjunctivitis, keratitis). Have been the cause of serious disorders (neurological, cardiovascular, respiratory and digestive), which can lead to death. The

published data concern the effects reported in humans during exposure to commercial preparations (phytosanitary products for professional use or amateur, biocidal products for domestic use, external antiparasitic drugs for animals). The presence of co-formulants in preparations is likely to significantly modify the toxicological profile observed in animals from studies carried out on the active substance alone**. (Jargot et *al*., 2015)**

II.8. Acute toxicity of imidacloprid

Tableaux 02: Acute toxicity of imidacloprid at Wistar Rats **(PMRA, 2016)**

Route of administration	Character	Toxicity
Oral use	LD50 (males) = 424 mg / kg bw; LD50 (females) = 450 to 475 mg / kg bw Clinical signs at ≥ 100 mg / kg bw (males) or 250 mg / kg bw (females): 9 mobility, apathy, respiratory problems, shaky gait, narrowed palpebral fissures, tremor and / or transient spasms (resolved after 2 to 6 days)	**HIGH TOXICITY**
Dermal	LD50 (males / females)> 5,000 mg / kg bw No clinical signs of toxicity or any abnormalities at necropsy; 9 intake of p.c. (females).	**LOW TOXICITY**
Inhalation	LC50 (males / females)> 0.069 mg / L (aerosol) LC50 (males / females)> 5.32 mg / L (powder) Clinical signs in rats exposed to the powder for 4 hours at a dose ≥ 2.58 mg / L: labored breathing, reduced mobility, horripilation; slight tremors 1 to 2 h after administration of the 5.32 mg / L dose; signs resolved on day 1	**LOW TOXICITY**

II.9. Chronic toxicity

In the rat, the thyroid is particularly sensitive to imidacloprid. Lesions of this gland are caused by doses from 17 mg / kg of body weight per day in males. Slightly higher doses, 25 mg / kg per day, reduced weight gain in females. At higher doses, 100mg / kg per day, the effects include retinal atrophy in females. **(PMRA, 2016)**

II.10. Teratogenic effects

A developmental toxicity study in rats fed a gastric tube up to 100 mg / kg / day on days 6 to 16 of gestation resulted in a NOEL of 30 mg / kg / day (based on skeletal abnormalities

observed at highest dose of 100 mg / kg / day) **(Pike et Reed, 1993)**. A developmental toxicity study in rabbits fed by gastric tube with doses of imidacloprid during days 6 to 19 of gestation, had as consequence a NOEL of 24 mg / kg / day based on the decrease in body weight and skeletal abnormalities observed at 72 mg / kg / day (the highest dose) **(PMRA, 2016)**

II.11. Carcinogenic effects

There is no carcinogenic effect in a two-year carcinogenicity study in rats fed up to 1,800 ppm imidacloprid **(PMRA, 2016)**

II.12. Human health and safety

The toxicological database submitted for imidacloprid is adequate to define the majority of the toxic effects that could result from exposure to this substance. In subchronic and chronic toxicity studies in laboratory animals, the main targets were the liver, kidneys, thyroid gland, eyes and nervous system

No evidence of carcinogenicity was noted in rats and mice after long-term exposure. In reproductive or developmental toxicity studies, no signs of increased sensitivity were observed in the young.

Exposure of persons who come into contact with treated surfaces is not expected to cause a risk of concern when imidacloprid is used according to the label directions. **(Seifert, 2005 ; Sheets, 2010 ; Terayama et *al*., 2016)**

III.1. Oxidative stress

Oxidative stress is defined as an imbalance between the production of free radicals and their destruction by antioxidant defense systems. Free radicals are molecules or atoms that have one or more unpaired electrons on their outer layer which makes it unstable **(Gardès-Albert et *al*., 2003)**, formed by the loss or gain of electrons at based on a non-radical compound **(Berger, 2006; Fontaine, 2007).** EROs have two sources of production, exogenous such as radiation products, drugs, pesticides and organic solvents **(Vergely et Rochette, 2005; Flora et *al*., 2008)**, and endogenous sources in particular reside in the mitochondria, via its respiratory chain **(Favier, 2003).** The production of these pro-oxidant species is normal at low concentration and is accompanied by an important physiological role. At high concentrations, their effects become deleterious for cells, tissues and various physiological functions. **(Berger, 2006; Fontaine, 2007)**

III.2. Oxidative stress biomarkers

To prevent or limit cell damage, organisms have developed antioxidant defense systems

III.2.1. the main non-enzymatic antioxidants

Are glutathione, vitamin E, vitamin C, carotenoids and uric acid. Glutathione (GSH in its reduced form) is particularly important because it is the substrate for several antioxidant enzymes (it occurs in the reduced GSH form and the oxidized form (GSSG.). The GSH / GSSG ratio is often used as marker of a cell's oxidation level. Maintaining a high level of glutathione is therefore essential to prevent possible Oxidative damage (**Halliwell et Gutteridge, 1999).**

III.2.1.1. Glutathione (GSH)

Reduced glutathione is a tripeptide characterized by the presence of a sulfidryl group, the latter is responsible for the reduction of free radicals (Gardès-Albert, and al., 2003), depending on the reaction:

$$GSH + OH^{\circ} \longrightarrow GS^{\circ} + H_2O \quad \& \quad GSH + R^{\circ} \longrightarrow GS^{\circ} + RH$$

Glutathione can also react with Fe3 + and Cu2 + ions and thus limit their participation in the generation of free radicals by the Fenton reaction:

$$GSH + Fe^{3+} \longrightarrow GS^{\bullet} + Fe^{2+} + H^{+}$$

$$GSH + Cu^{2+} \longrightarrow GS^{\bullet} + Cu^{+} + H^{+}$$

III.2.2. The main antioxidant enzymes

Are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione S-transferase (GST). These molecules will interrupt the radical reaction chain in molluscs **(Stegeman, 1992).**

- SOD is the first line of enzymatic defense, catalyzes the conversion of the superoxide anion (O2-.) Produced by the mitochondrial respiratory chain into hydrogen peroxide (H2O2) **(Badary et *al.,* 2003).**
- The (GPx) is the second line of enzymatic defense, prevents the formation of free radicals, it can on the one hand reduce H2O2 to H2O and on the other hand organic hydroperoxides (ROOH) to alcohol (ROH) **(Favier, 2003; Fontaine, 2007).**
- CAT is mainly present in peroxisomes, lysosomes and mitochondria. Neutralizes hydrogen peroxide (H2O2) in water and molecular oxygen **(Casetta et *al*., 2005).**

III.2.2.1. Superoxide dismutase (SOD)

Is the first line of enzyme defense, catalyzes the conversion of the superoxide anion (O2- °) produced by the mitochondrial respiratory chain in hydrogen peroxide (H2O2), of mammals find cytoplasmic SOD (Cu-Zn-SOD), mitochondrial SOD (MnSOD) **(Badary et *al*., 2003).** SOD is found in all regions of the brain. The intense increase in activity has been associated with manic and depressive events **(Andreazza et *al*., 2007).**

$$O_2^{-\circ} + 2H^{+} \xrightarrow{SOD\ (Cu/Zn)} H_2O_2 + O_2$$

III.2.2.2. Glutathione peroxidase (GPx)

This is the second line of enzyme defense, prevents the formation of free radicals, in mammals. It is a selenium enzyme found in the cytosol and mitochondria. It can reduce H2O2 to H2O on the one hand and organic hydroperoxides on the other hand (ROOH) in alcohol (ROH) **(Favier, 2003; Fontaine, 2007).**

$$2H_2O_2 + 2GSH \xrightarrow{GPx\ (Se)} GSSG + H_2O$$

$$ROOH + 2GSH \xrightarrow{GPx\ (Se)} ROH + GSSG + H_2O$$

III.2.2.3. Catalase (CAT)

Catalase CAT is present mainly in peroxisomes, lysosomes and mitochondria. Neutralizes hydrogen peroxide (H2O2) in water and molecular oxygen. Since CAT and GPx have low levels in the brain compared to the SOD level, this is why an oxidative effort created by a high rate of metabolism can favor neurodegenerative diseases**. (Casetta et *al*., 2005)**

$$2H_2O_2 \xrightarrow{CAT\ (Fe)} 2H_2O + O_2$$

Chapter 02: Practical part

1. Material and methods

1.1. Material

1.1.1. Biological material

The experiments were carried out at laboratory level at L'arbi Tébessi University in Tebessa, on 12 white male Rattus rattus rats of the Wistar strain, from the Pasteur Institute in Algiers. Rats used aged nine weeks and having a body weight between 170 and 300 g. They are mammals of the rodent order, widely used in scientific research

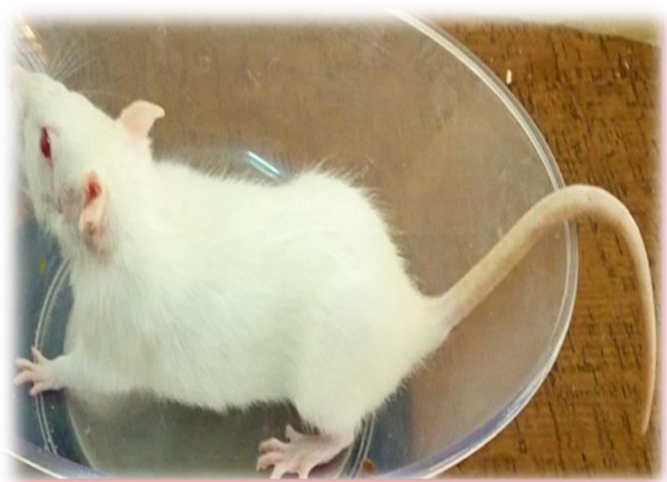


Figure 09: Animal material

1.1.2. Chemical material

In this work, we used imidacloprid which is part of the family of neonicotinoids for the treatment of rats in solution form with two (02) doses (5 and 50 mg)

1.2. Methodology

1.2.1. Raising rats

The rats were divided into (03) lots at the rate of (04) rats per lot. They were subjected to a 20 day adaptation period in the pet store in the Department of Biology, Faculty of Science, and University of Tebessa. The ambient temperature is 23 ± 2 ° C and a natural 12 / 12H photoperiod with a hygrometry of 60%. The rats are raised in polyethylene cages (04 rats

for each cage) which are lined with a litter made of wood shavings. The cages are cleaned and the litter is changed once every two days until the end of the experiment.

Figure10: Raising rats.

1.2.2. Preparation of the aqueous neonicotinoid solution

For the preparation of the solutions we put (0,24mg and 2,4mg) of powdered pesticide with 100 ml of distilled water in two deferential vials after shaking with an agitator. Solutions administered to rats orally

1.2.3. Weight measurement

The weight measurement is carried out on the rats always during the breeding period, during the treatment with a balance.

Figure11: Weight measurement.

1.2.4. Treatment of rats

After the adaptation period of all the rats (15 days), they were randomly divided into three groups of five (04) rats, the rats were treated for 20 days as follows:

- **Group 1**: the control group.
- **Group 2:** the rats were treated with neonicotinoid because of (5 mg / Kg) orally.
- **Group 3**: the rats were treated with neonicotinoid (50mg / Kg) orally.

Figure12: Treatment of rats

1.2.5. Neurobehavioural study

In this part which studies the neurobehavioral and social effects and prefernce for the social

Novelty of wistre rats after the exposure of neonicotinoids (imidacloprid). To achieve this objective, applied two tests, *classic labyrinth test and maze V soc test*

-Classic labyrinth test for memorizing wistre rats after exposure to neonicotinoids.

-Maze V soc test for the social novelty of wistre rats after the exposure of the pesticide

- **Classic Labyrinth Test**

This test is carried out in a square enclosure (120x120x35 cm) in pastic with several labyrinth passages of identical size (25x35 cm) of variable length depending on the crossing,

also comprising a departure area and another arrival area prepared for ***Dr: Gasmi Salim***. This labyrinth is placed on a table and followed by a video camera fixed on a vertical support and the camera on the PC to record the behavior of the rats the duration of the test is 20 min and between each test the labyrinth is cleaned with l ethanol 10 % **(Kulli Jaako-Movits et *al*., 2005).**

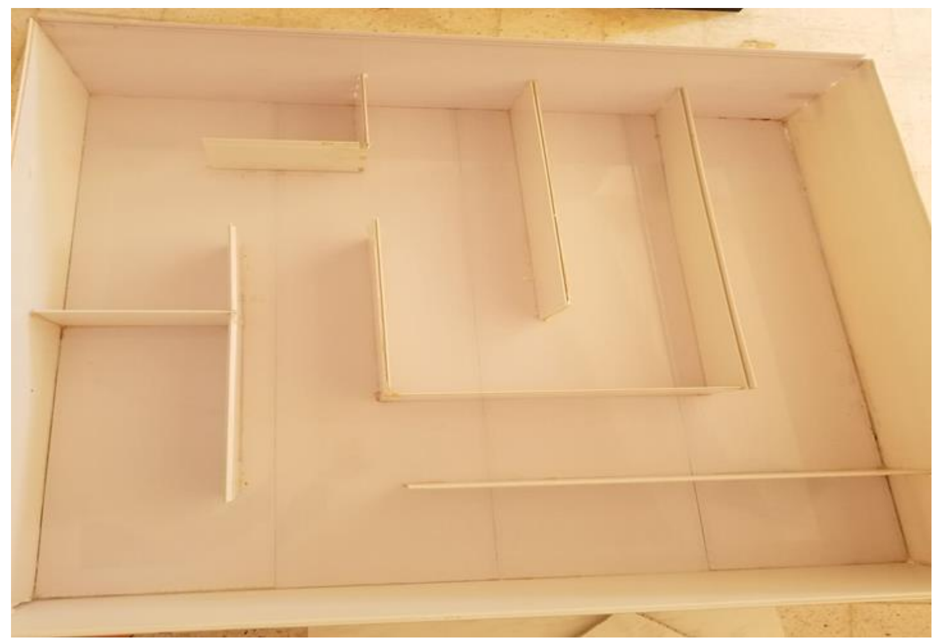


Figure13: Classic Labyrinth Test

Control rats usually show a short passage in the labyrinth between the starting point and the arrival one. This suggests that the exploration behavior will result in a higher time spent in the passageway centers, which will be considered as aversive or anxiety-provoking for the stressed animal; while the leak behavior is going to be observed when the animal will spend more time in the starting point or corners, which will be associated with a shelter. To assess the animals' level of anxiety, we measure the time spent in the different parts of the device. So the principle of the test is based on the fact that an animal that explores more time the corners or places of initial deposit is considered anxious.

We applied this test in two stages:

- ✓ 1st phase: before treatment as a control
- ✓ 2nd phase: during the treatment period after 5, 10 and 15 day

To assess the animals' level of anxiety, we measure the time spent in the different parts of the device. Thus, the principle of the test is based on the fact that an animal which explores more the corners or places of initial deposit is considered as anxious **(Gasmi, 2018**).

- ✓ **Maze V soc test**

We applied this test for the first time in our faculty with the aim of evaluating the neurobehavioral and social effects and preference for the social novelty of wistre rats after the exposure of neonicotinoids.

Studying social behavior in mouse models empowers the understading of the neurobiolgical Mechanisms involved, are affected in nouropsychiatric disorders, allowing the evluation

Social behaviors are important in numerous species to establish the networks and relationships that define social communities (**Berry et Bronson, 1992**).Among those behaviore, sociability is defined as the tendency to, seek out social interaction **(Caldwell, 2012).**Some neuropsychiatic disorders, such as autism spectrum disorders(ASD) display a maeked alteration in sociability combined with ather features **(American, Psychitic Assocaition, 2013).**This characteristic highlights meed for experimental behavioral setting in animal models to address the research on such complex mlti-factted desorders **(Caldwell, 2012)** .Mus musulus is a social specie showing reciprocal social interaction, sexual and parenting behavoirs territorial scent marking and aggressive behaviores **(Ricceri et *al*.,2007 ;Silverman et *al*., 2010)** .The wide repertoire of mouse behaviors makes this specie suitable for modeling human disorders characterized by disruption in social recognition and social behavior **(Crawley,2004 ;Yang et *al*., 2007).**Such rodent models wodels warrant the evaluation of potential therapeutic approaches for treatment **(Moy et *al*., 2004)** .Commonly mic conserve a charactristic pattem of social behaviore ,initiating social contact and approach when exposed to an unfamiar conspecific **(Moy et *al*., 2004)**

Several paradims have been described to measare social behavior in mouse modles **(Silverman et *al*., 2010).**Among those ,the most common is the crawley's sociability test also called three-chamber apparatus **(Chadman et *al*., 2008 ;Mc Farlan et *al* ., 2008 ;Moy et *al*., 2008**).Which allaws to study two different parameters :sociability and prefernce for social novelty .Sociability is defined as the tendency of the expermental mouse to spend more time exploring an unfamiliar mouse than exploring an object or an empty chamber Insted prefernce

for social novelty is defined as the propensity to spend more time exploring a new unfamiliar mouse than the now-familair mouse that was explored in the sociability phase **(Moy et *al.*, 2004 ;Yang et *al.*, 2011).**Due to the length of the different phase in the Crawly's sociability test ,the procedure is time-consuming and experimental variability is sometimes troublesome.

We have adapted a V-maze ,previously used successfully to evaluate novel object –recognition memory **(Puighermanal et *al.*, 2009 ;Busquets-Garcia et *al.*, 2011 et 2013 ;Navarro-Romero et *al.*, 2019),**to assess social behaviore .We found that the using this new maze (Vsoc maze) provides an advantageaus approach in time ,while maintaining accuracy,to reveal particular social phenotypes in tow mouse lines frequently used in biomedical research

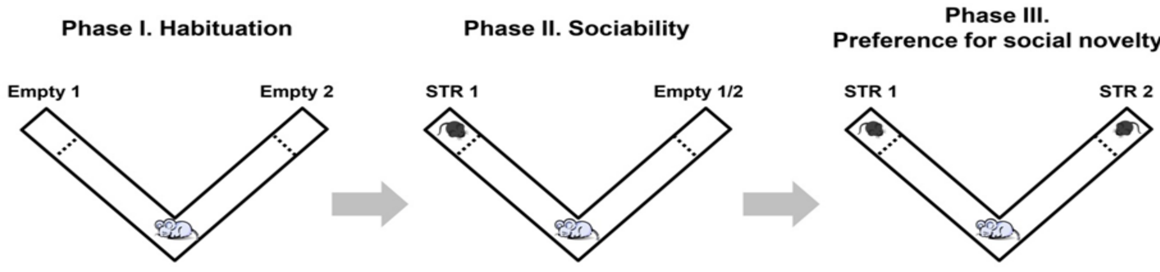


Figure14: The phase of maze Vsoc test (Moy and al,.2004)

- ✓ Tissue paper
- ✓ Experimental mice
- ✓ 30% ethanol (v/v)
- ✓ Video Camera (e.g ; Sony Handycam camcorder,HDR-CX240)
- ✓ Stopwatches (x2) (e.g ; sper, Sper Scientific, 810029

Labyrinth of Vsoc we used a modified version of the V-shaped maze **(Busquets-Garcia et *al.*, 2011)** Vsoc Figue. It consists of a drawing structure: the maze wall (height 25cm), made of wood (6 mm wide) and the maze cover, and made of transparent glass. The lanes in the V-soc maze are (90 cm long and 25 cm wide) internal procedures in the passages when the cover was inserted into the V-soc maze, and rooms were used to allocate strange mice events, the design was intentionally simple to enhance the exploratory activity of the experimental

mouse under analysis, and to facilitate animal handling Cleaning between sessions (**Martinez et *al*., 2019).** We applied this test during the treatment period after 5, 10 and 15 day .

Figure15: Maze Vsoc test

1.2.6. Sacrifice and taking of samples

A. Animal sacrifices

At the end of the treatment period, the rats are fasted overnight. They are sacrificed by decapitation

Figure16: Sacrifices of rats

B. Removal of certain organs

After the dissection, the brains taken from one part of the organ are used for the preparation of the homogenate and the other is kept to make histological sections. Organ homogenates are used for the determination of oxidative stress and biochemical parameters (protein, lipids, and carbohydrates, MDA, GSH, GPx, GST, and CAT)

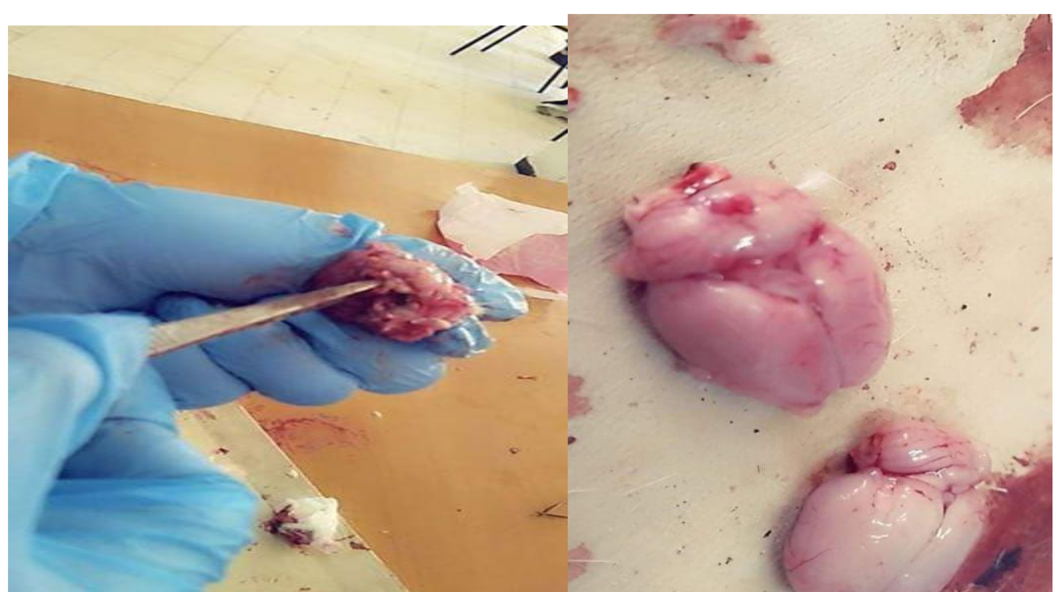


Figure17: Organ harvesting from rats

C. Preparation of mitochondrial suspensions

The extraction of mitochondria is done according to the method described by **(Rustin et *al*., 1994)**, it is a purification by differential centrifugation. Briefly, after decapitation of the rats, the brains are quickly removed and immersed in TSE buffer (10 mM tris, 250 mM sucrose, 0.1 mM EDTA, and pH 7.2 at 4 ° C.). The nervous tissues are finely cut and poterized in 3.5ml of TSE, which allows the destruction of cells and the release of mitochondria. The recovered homogenate is centrifuged at 10,000 rpm for 10 min, thus eliminating large cellular debris. The recovered pellet is centrifuged a second time at 10,000 rpm for 10 min. The supernatants from the two centrifugations are collected and centrifuged at 14000 rpm at 4 ° C. for 10 min. The pellet obtained is resuspended in 1 ml of TSE and centrifuged at 14000 rpm for 10 min. The pellet from this last centrifugation is resuspended in 1 ml of TS buffer (250 mM sucrose, 50 mM tris, and pH 7.2 at 20 ° C.) and centrifuged for 10 min at 14000 rpm. The final pellet consisting of mitochondria, and is recovered in 500 µl of TS buffer to obtain the fresh mitochondrial suspension, a fraction of which will be used

directly in the evaluation of the structural and functional integrity of the mitochondria, the rest is stored at -80 ° C. for following dosages. **(Gasmi, 2018)**

D. Assessment of mitochondrial swelling, permeability and respiration

According to the method of **(Krystal et *al.,* 1996),** we carried out the estimation of the mitochondrial permeability based on the rate of Ca ++ ions which cross their membranes, this permeability followed by an increase in the size of the mitochondria detected at a length 540nm wave for 3 minutes and every 30sec. Breathing was estimated using an Oxygraph (Hansatech®) according to the method described by **(Rouabhi et *al*., 2006; 2009)**

1.3. Methods of dosages

1.3.1. Determination of biochemical parameters

A. Protein dosage

The assay of the proteins is carried out according to the method of **(Bradford, 1976)** which uses the brilliant blue of coomassie (BBC) as reagent. This reveals the presence of proteins in the blue dyes. And (BSA) as standard. The calibration range was carried out from a stock solution of bovine serum albumin (BSA). (1 mg / ml) and the BBC (storage about 21 days at 4 ° C) which is prepared as follows:

- 100 mg of BBC + 50 ml of ethanol Agitation for two hour
- 100 ml of orthophosphoric acid are then added and the whole is made up to 1000 ml with distilled water;
- The protein assay was carried out in an aliquot fraction (100 ml).
- The absorbances were read in a spectrophotometer at a wavelength of 595 nm.

B. Carbohydrate dosage

The determination of carbohydrates was carried out according to the method of **(Douris et *al*., 2009)**. This method uses anthrone as reagent (150 mg of anthrone, 75 ml of sulfuric acid and 25 ml of distilled water) and a stock solution of glucose (1g / l) as standard, the method consists in:

- Add 4 ml of anthrone reagent to an aliquot fraction of 100 µl of the supernatant contained in a test tube of the different samples
- Heat the mixture in a water bath at 80 ° C for 10 min, a green color develops whose intensity is proportional to the amount of carbohydrate present in the sample
- The absorbance is read at a wavelength of 620 nm.

C. Lipid dosage

Total lipids were determined according to the method of **(Goldsworthy et *al.*, 1972)** using the sulfo-phospho-vanillin reagent (0.38 g of vanillin, 195 ml of 85% ortho-phosphoric acid and 55 ml of distilled water) and a stock solution lipids (2.5mg / ml) as standard.

- Added 1 ml of sulfuric acid (98%), after stirring, the tubes are heated in a water bath (100 ° C for 10 min); 200 µl of each tube are then withdrawn and 2.5 ml of reagent are added;
- The absorbances were read after 30 min of darkness at a wavelength of 530 nm.

1.3.2. Oxidative stress parameters

This includes enzymatic and non-enzymatic parameters.

1.3.2.1.Non-enzymatic biomarkers

- **Determination of Malondialdehyde (MDA)**

MDA is one of the end products formed during the decomposition of polyunsaturated fatty acids (PUFA) mediated by free radicals.

Principle

Malondialdehydes (MDA) are assayed according to the method of (**Flohe et Gunzler, 1984).** This method is based on the colorimetric measurement of the reaction between Thiobarbituric acid (TBA) and Malondialdehyde (MDA) in an acidic and hot medium (100 ° C) giving a reddish brown product whose intensity of coloring is measured at a wavelength of 530 nm.

Experimental protocol

- Prepare homogenates from 200 mg of organ with TP homogenization buffer (pH 7.4);
- Centrifuge at 3000 rpm for 10 min;

- Take 375 µl of supernatant;
- Add 150 µl of TBS buffer solution (50 mM Tris, 150 mM NaCl pH 7.4);
- Add 375 µl of TCA-BHT solution (20% TCA, 1% BHT);
- Shake and centrifuge at 1000 rpm for 10 min;
- Take 400 µl of supernatant;
- Add 80 µl of 0.6 M HCl;
- Add 320 µl of Tris-TBA solution (Tris 26 mM, TBA 120 mM);
- Mix and incubate in a water bath at a temperature of 80 ° C for 10 min;
- Read the optical density at λ = 530 nm.

The absorbance is directly proportional to the amount of MDA formed, thus giving an accurate assessment of the peroxidized lipids. The concentration of MDA is calculated according to the Beer-Lambert law (DO = E.C.L):

$$[c]\left(\frac{nmol}{mg} of Protein\right) = \frac{DO \times 10^6}{\varepsilon \times L \times X \times Fd}$$

- C: the concentration in nmole / mg of proteins.
- DO: optical density read at 530 nm.
- E: MDA molar extinction coefficient = 1.56.105 M- / cm.
- L: Length of the tank used (1cm).
- X: concentration of protein extract (mg / ml)
- F.d: Dilution factor (Fd = 0.2083).

Glutathione (GSH) dosage

The principle of this assay is based on the measurement of the optical density of 2-nitro-5-mercapturic acid. The latter results from the reduction of 5, 5'-dithio-2-nitrobenzoic acid (Ellman reagent or DTNB) by the groups (-SH) of glutathione. Once prepared, the homogenate must undergo deproteinization with 0.25% sulfosalicylic acid in order to protect the (-SH) groups from glutathione **(Weckbeker et Cory, 1988)**

Experimental protocol

Prepare the homogenates from 1 ml of culture with EDTA phosphate buffer (0.02M);

- Take 0.8ml of the homogenate to which add 0.2ml of a 0.25% sulfosalicylic acid solution (SSA);
- Stir the mixture and leave for 15 min in an ice bath;
- Centrifuge at the speed of 1000 rpm for 5 min;
- Take 0.5 of the supernatant;
- Add to the mixture: 1ml of Tris-EDTA buffer (0.02M EDTA, pH 9.6), 0.025ml of DTNB and 0.5ml of the supernatant.
- Let stand for 5 minutes at room temperature for color stabilization. The colorimetric reaction develops instantly;
- Measure the absorbances at 412 nm against the blank.

The concentration of glutathione is obtained after application of the following formula:

$$GSH\left[M\frac{GSH}{mg}GSH/mg des\ protiènes\right]\frac{DO \times 1 \times 1{,}525}{13100 \times 0{,}8 \times 0{,}5}$$

- DO: the optical density.
- 1: The total volume of the solutions used in deproteinization (0.8 ml of the homogenate + 0.2 ml of salicylic acid).
- 1.525: The total volume of the solutions used in the determination of GSH at the level of the supernatant (0.5 ml supernatant + 1 ml Tris-EDTA + 0.025 ml DTNB).
- 13100: Absorbance coefficient (concerning the grouping (–SH) at 412 nm).
- 0, 8: The volume of the homogenate.
- 0.5: The volume of the supernatant found in a 1.25 ml

The GSH concentration is measured relative to 1 mg of protein. This is why this dosage must be accompanied by the protein dosage.

1.3.2.2. Enzyme biomarkers

- **Glutathione peroxidase (GPx) activity assay**

The enzymatic activity of glutathione peroxidase (GPx) was measured by the method of (**Flohe et Gunzler., 1984**). This method is based on the reduction of hydrogen peroxide (H2O2) in the presence of reduced glutathione (GSH), the latter is transformed into (GSSG) under the influence of GPx according to the following reaction:

$$H_2O_2 + GSH \xrightarrow{GPx} GSSG + 2H_2O_2$$

Experimental protocol

- Prepare homogenates from 200 mg of organ with TP homogenization buffer (pH 7.4);
- Centrifuge at 3000 rpm for 10 min;
- Take 0.2 ml of supernatant;
- Add 0.4 ml of GSH (0.1 mM);
- Add 0.2 ml of TBS buffer solution (Tris 50 mM, 150 mM NaCl, pH 7.4);
- Incubate in a water bath at 25 ° C for 5 min;
- Add 0.2 ml of H_2O_2 (1.3 mM) to initiate the reaction, left to act for 10 min;
- Add 1 ml of TCA (1%) to stop the reaction;
- Put the mixture in an ice bath for 30 min;
- Centrifuge for 10 minutes at 3000 rpm;
- Take 0.48ml of supernatant;
- Add 2.2ml of TBS buffer solution;
- Add 0.32ml of DTNB (1mM);
- Mix and after 5 minutes read the optical densities at 412 nm.

The determination of the enzymatic activity of GSH-Px is done using the following formula:

$$\text{GPx}(\mu\text{mol. mg de protéine}) = \frac{(\text{DOe} - \text{DOb}).\,0{,}04}{\text{DOb}}$$

- DO: échantillon : Densité optique de l'échantillon.
- DO étalon: Densité optique de l'étalon.
- 0.04: Concentration de substrate (GSH).

Glutathione S-Transferase (GST) activity assay

Glutathione S-transferase belong to a family of essentially cytosolic multifunctional enzymes, involved in intracellular transport and biosynthesis, they catalyze conjugation reactions between an endogenous peptide, glutathione, and reactive molecules with electrophilic sites. The measurement of glutathione S-Transferase (GST) activity is determined according to method (**Habig and*al*., 1974)** it measures the kinetics of formation

between a model substrate, chlorodinitrobenzene (C-DNB) and glutathione; It is based on the conjugation reaction between GST and a substrate, CDNB (1-Chloro2, 4 di nitrobenzene) and in a cofactor glutathione (GST), conjugation leads to the formation of a new molecule.

Experimental protocol

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

$$\text{GST(nmol GST/min/mg protéine)} = \frac{(\text{DO échant/min} - \text{DO blanc/min})}{9{,}6 \times \text{mg de protéine}}$$

- ΔDO sample - ΔDO blank: average of the OD of the samples per minute - average of the OD of the whites per minute.
- ε: Molecular extinction coefficient of C-DNB, ε C-DNB = 9.6 mM-1.cm-1
- L: Optical path of the tank = 1cm

- **Catalase activity assay (CAT)**

The spectrophotometric determination of catalase activity (CAT) is carried out according to the method of (**Cakmak et Horst, 1991**). The decrease in absorbance is recorded for three minutes by a spectrophotometer for a wavelength of 240 nm and a linear linear extinction coefficient ε = 39400 µM-1.cm-1.L for a final volume of 3 ml, the reaction mixture contains: 100 µl of the crude enzyme extract, 50 µl of 0.3% hydrogen peroxide H2O2 and 2,850 µl of phosphate buffer (50 mM, pH 7.2). The device is calibrated in the absence of the enzyme extract. The reaction is triggered by the addition of hydrogen peroxide.

1.3.3. Neurotransmitter estimation

- **Determination of acetylcholinesterase (AChE)**

The most common AchE assay method and that of (Ellman et al., 1961), it consists in providing the enzyme (AchE), an artificial substrate, acetylthiocholine, whose AchE-catalyzed hydrolysis releases thiocholine and acetic acid. The amount of thiocholine obtained is proportional to the activity enzymatic revealed thanks to a colorimetric method involving DTNB (5-5- dithio-bis-2- nitrobensoic acid) which binds with thiocholine to form a yellow complex, TNB (5-thio-2-nitrobensoic acid) which is dosed.

The specific activity of AchE is determined according to the formula of (Ellman et al., 1961):

$$\textbf{AChE}\ (\mu M/min/mg\ de\ protéines) = \frac{\Delta D_0 / mn \times Vt}{1{,}36 \times 10^4 \times Vs} \Big/ mg\ de\ protéines$$

- Δ Do: slope of the regression line obtained after hydrolysis of the substrate in function of time.
- x 104 : DTNB molar extinction coefficient.
- Vt: total volume in the tank: 1.3 ml [0.1 ml supernatant + 0.1 ml DTNB + 1 ml tris buffer (0.1 M, pH 7) + 0.1 ml acetylthiocholine].
- Vs: volume of the supernatant in the tank: 0.1 ml.
- Mg of protein: quantity of protein expressed in mg.

1.4. Statistic study

The results obtained were expressed by the average of six repetitions (mean ± deviation type), and to better visualize using *the Excel 2018* office to represent these results in the form of graphs and histograms. Statistical analysis was performed using *the Minitab® software 18.1*. The significance of the difference between the control batch and the treated batches is verified using *the Dunette test* and the comparison result as following**:**

- 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.

Results

2. Results

2.1. Effect of imidacloprid treatment on growth parameters in rats

The results of the evaluation of growth parameters in terms of weight gain and relative weight during the 20 days of treatment with imidacloprid

2.1.1. Weight gain

The results of the weight gain assessment (fig.18) show a very highly significant decrease (p≤0.001) in weight gain in the lots treated with the two doses (5 and 50 mg / kg / day) of MI in comparison with the control lot.

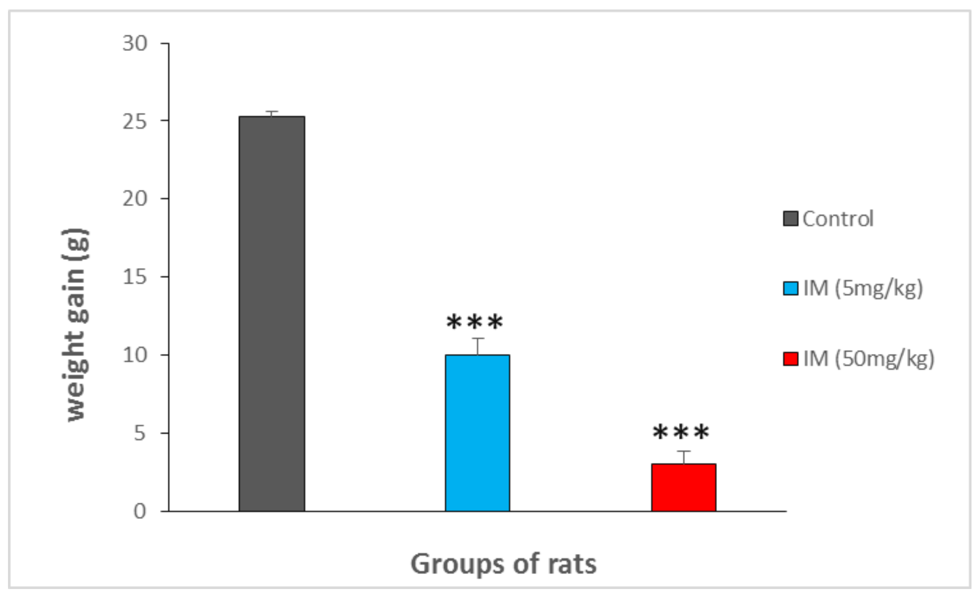


Figure18. Evolution of weight gain in control rats treated after 20 days of imidacloprid treatment (n = 04; p = 0.000)

2.1.2. Relative brain weight

The results obtained following the evaluation of the relative weight show a significant increase (0.05> P> 0.01) in the relative weight of the brain in the group treated with MI, (5 mg / kg / d) in comparison with the control group, by against the dose of 50 mg / kg / day of IM There is a very highly significant increase compared to the control group (fig. 19)

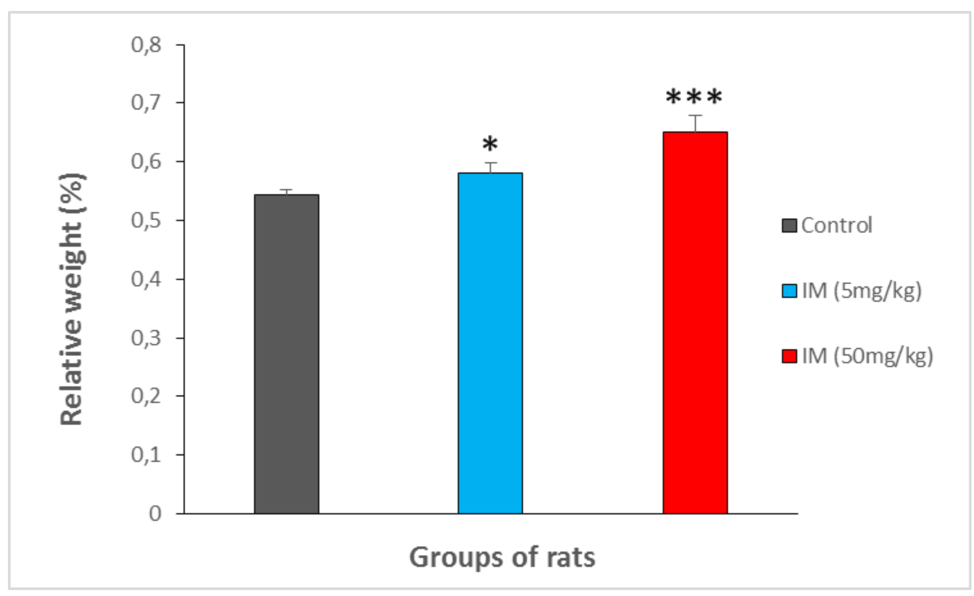


Figure 19: Evolution of the relative weight of the brain in rats treated for 20 days with Imidacloprid (n = 04, p = 0.000)

2.2. Effect of imidacloprid treatment on brain metabolites

This part aims to examine the effect of imidacloprid on the biochemical composition of rat brains, by determining the protein, lipid and carbohydrate levels after 20 days treatment.

2.2.1. Effect on brain protein levels

Our results also show a very highly significant increase in brain protein levels in rats treated with the 5 and 50 mg / kg / day dose of imidacloprid compared to the control group, (p = 0.002 between the treated). The results recorded are shown in Figures (20).

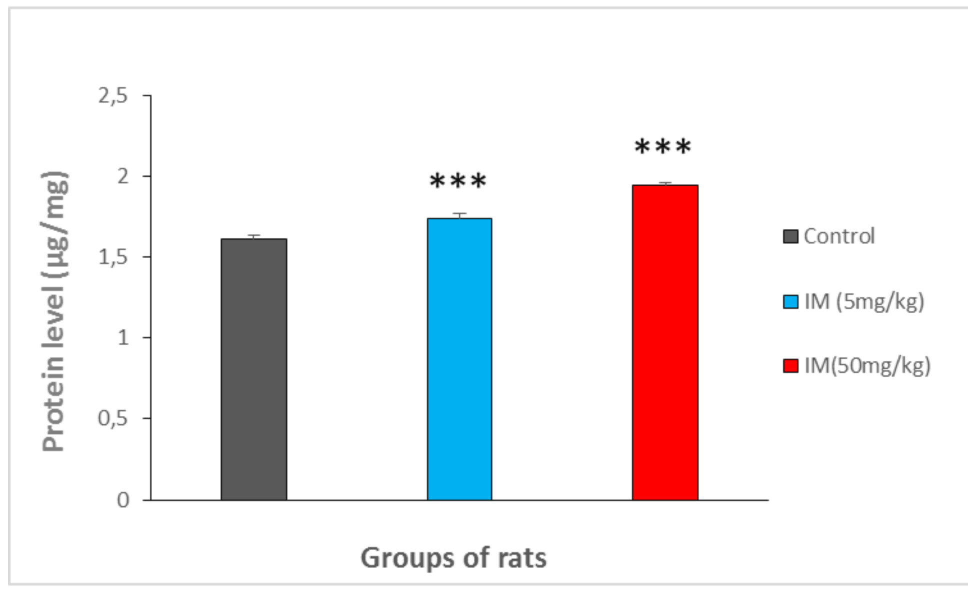


Figure 20: Variation in brain protein levels of control and treated rats after 20 days of treatment (n = 04, p = 0.0002).

2.2.2. Effect on the lipid level of the brain

Our results show a very highly significant decrease (P <0.001) in brain lipid levels in rats treated with imidacloprid compared to the control group.

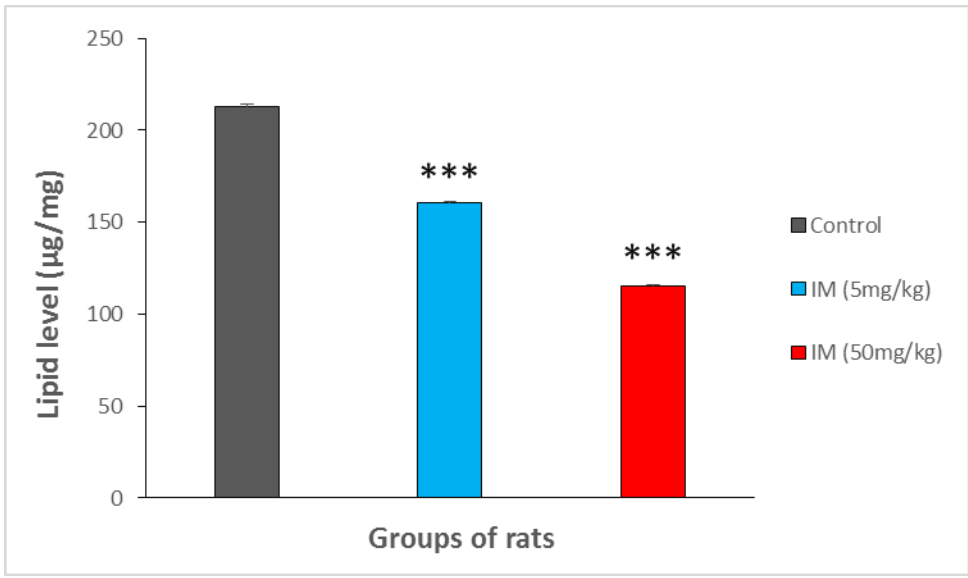


Figure 21: Variation in brain lipid levels of control and treated rats after 20 days of treatment (n = 04, p = 0.000).

2.2.3. Effect on the level of brain carbohydrates

Our results show a highly significant decrease (0.01> P> 0.001) in brain glucose levels in rats treated with the two doses (5 and 50 mg / kg / day) of imidacloprid compared to the control group.

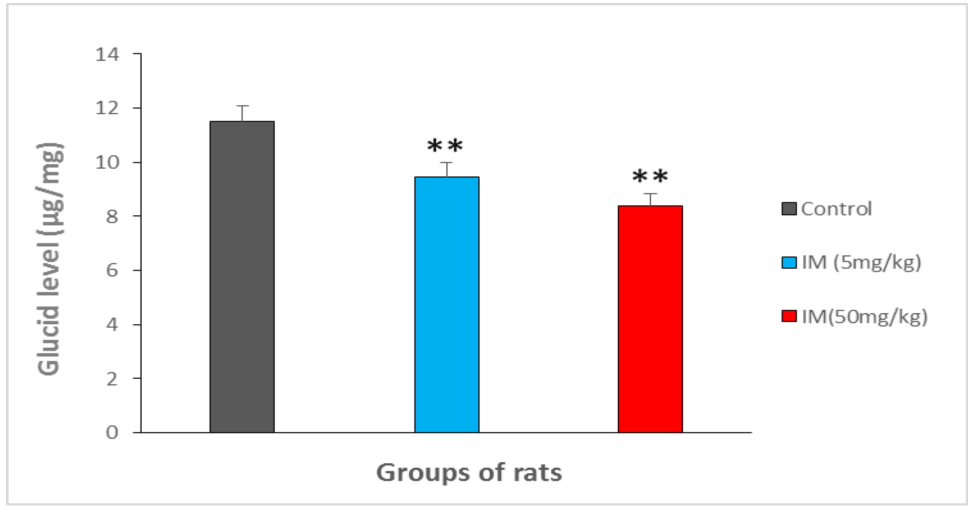


Figure 22: Variation in carbohydrate levels in control and treated rats after 20 days of treatment (n = 04, p = 0.00).

2.3. Treatment effect on non-enzymatic parameters

2.3.1. Treatment effect on the level of Malondialdehydes on brains

The results show a highly significant decrease (0.01> P> 0.001) in brain malondialdehyde levels in rats treated with imidacloprid compared to the control group.

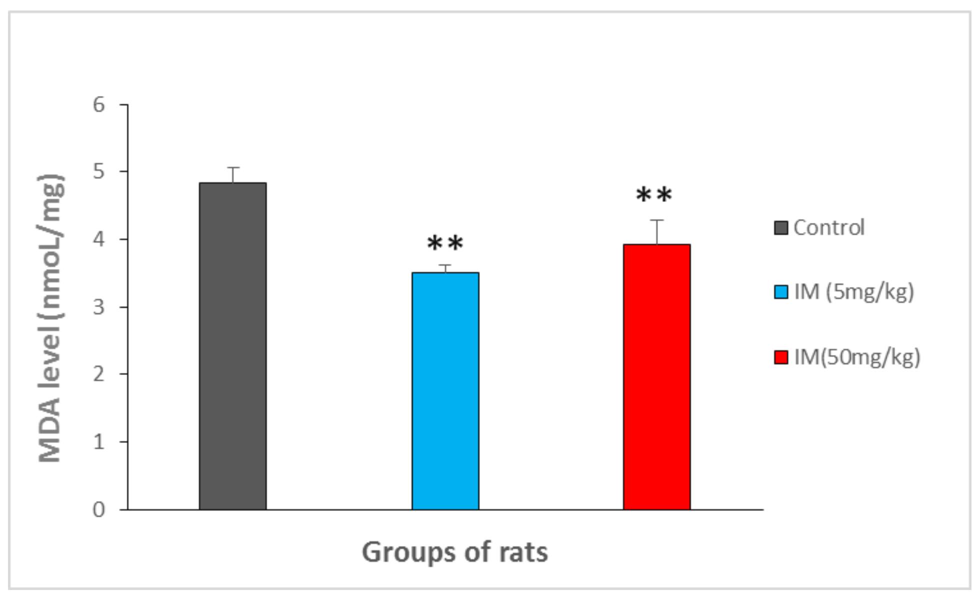


Figure 23-: Variation in MDA levels in the brains of control and treated rats after 20 days of treatment (n = 04, p = 0.00).

2.3.2. Effect of treatment on the level of GSH

The results obtained are shown graphically in Figures (24). A significant increase (0.05> P> 0.01) in GSH levels after treatment of rats with imidacloprid at a dose of 5 mg / kg / day and a very highly significant increase (P <0.001) for the second dose (50 mg / kg / day) versus witness report .

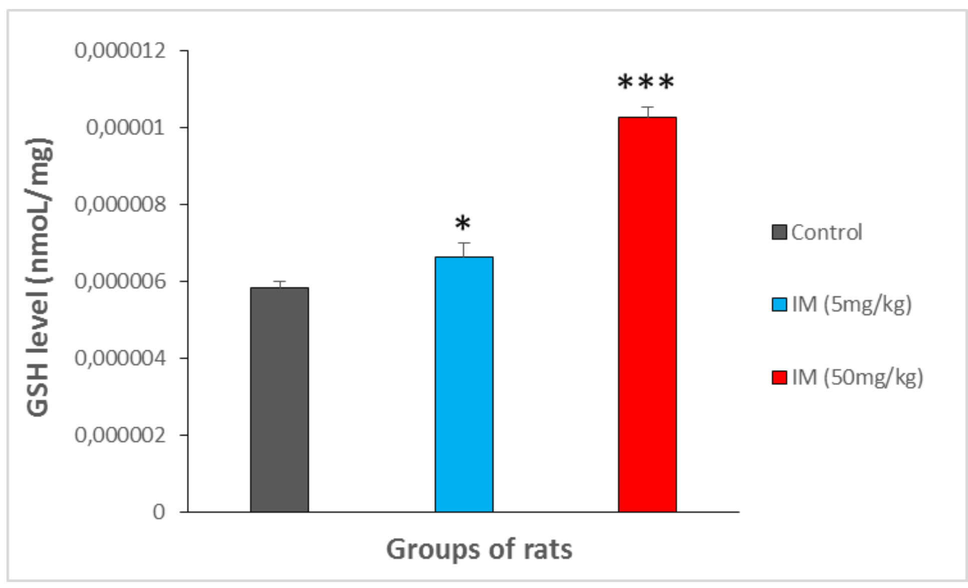


Figure 24: Variation in brain GSH levels in control and treated rats after 20 days of treatment (n = 04, p = 0.000).

2.4. Effect of imidacloprid treatment on enzyme parameters in the brains of rats.

2.4.1. Effect of imidacloprid treatment on glutathione S-transferase activities in rat brains.

The results obtained are shown in Figures (25). Administration of imidacloprid results in a highly significant decrease (0.01> P> 0.001) in the enzymatic activity of glutathione S-transferase compared to the control group.

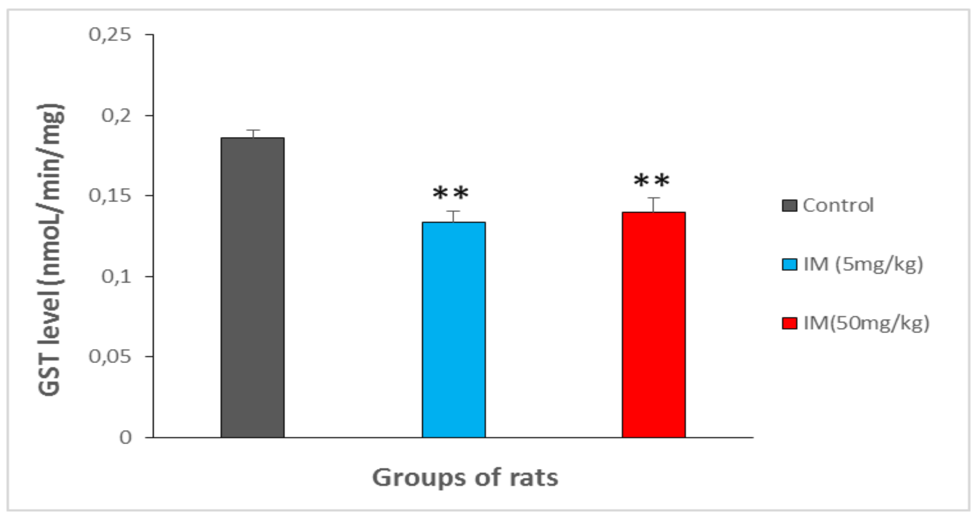


Figure 25: Variation in brain GST activity in control and treated rabbits after 90 days of treatment (n = 04, p = 0.00).

2.4.2. Effect of imidacloprid treatment on catalase activity in the brains of rats.

Our results show a highly significant increase (0.01> P> 0.001) in the catalase activity of rat brains, with dose (5mg / kg / day) and very highly significant increase (P <0.001) for the second dose (50mg / kg / day) compared to control

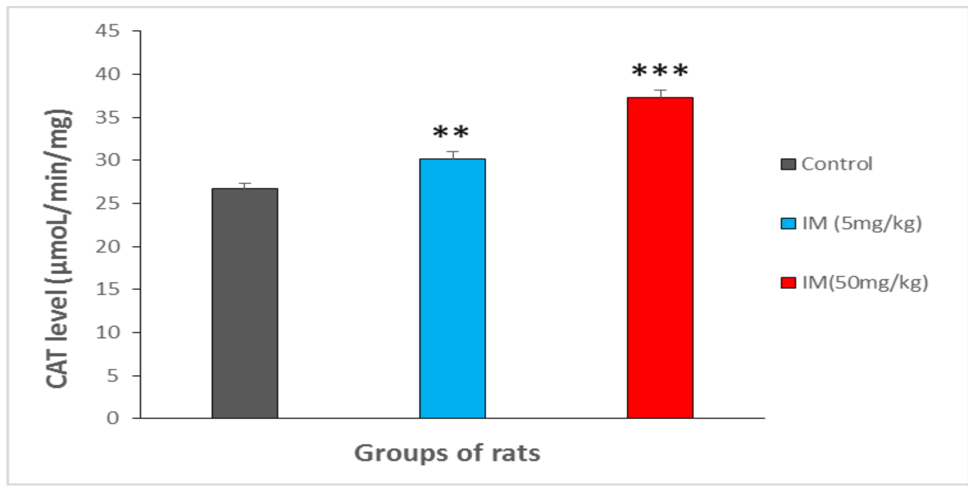


Figure 26: Variation in the CAT activity of the brain of control and treated rats after 20 days of treatment (n = 04, p = 0.000)

2.4.3. Effect of treatment with imidacloprid on the activity of glutathione peroxidase in the brains of rats.

Figures (27) illustrate variations in glutathione peroxidase (GPx) activity in the brains in the presence of imidacloprid and Se after treatment for 20 days. We note that the glutathione peroxidase (GPx) activity decreased in the very highly significant phase (P <0.001) in the treated rats compared to that of the controls.

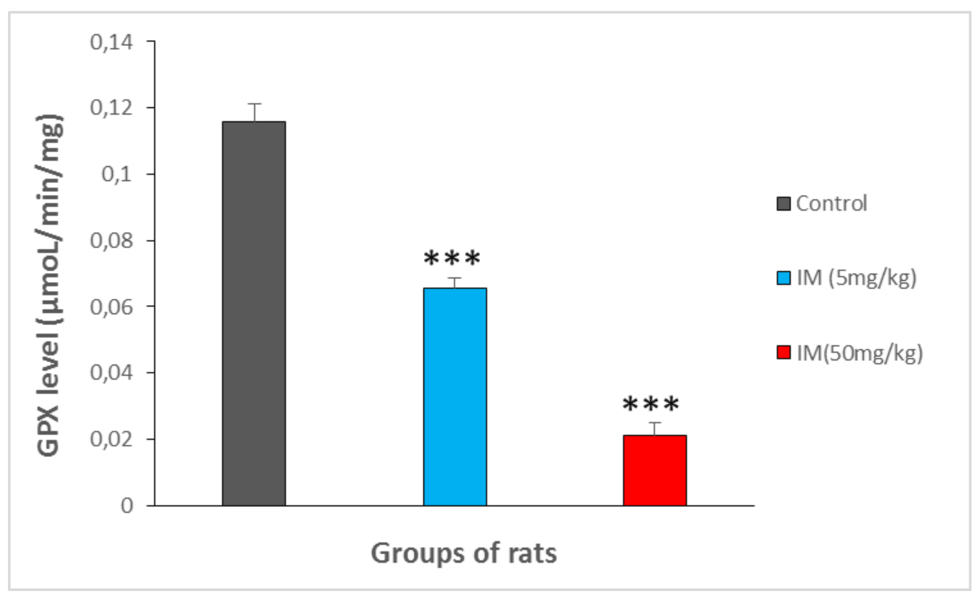


Figure 27: Variation in the GPx activity of the brain of control and treated rats after 20 days of treatment (n = 04, p = 0.000).

2.5. Effect of imidacloprid treatment on the activity of acetyl choline-esterase in the brain of rats

AchE activity was calculated for each sample by applying Ellman's formula.

The results obtained are shown graphically in Figures (28). We note that the activity of AchE was significantly decreased at a dose of 5 mg / kg / day and a highly significant decrease (0.01> P> 0.001) by the dose 50 mg / kg / day in the treated rats compared to that of the controls, In the brains of rats.

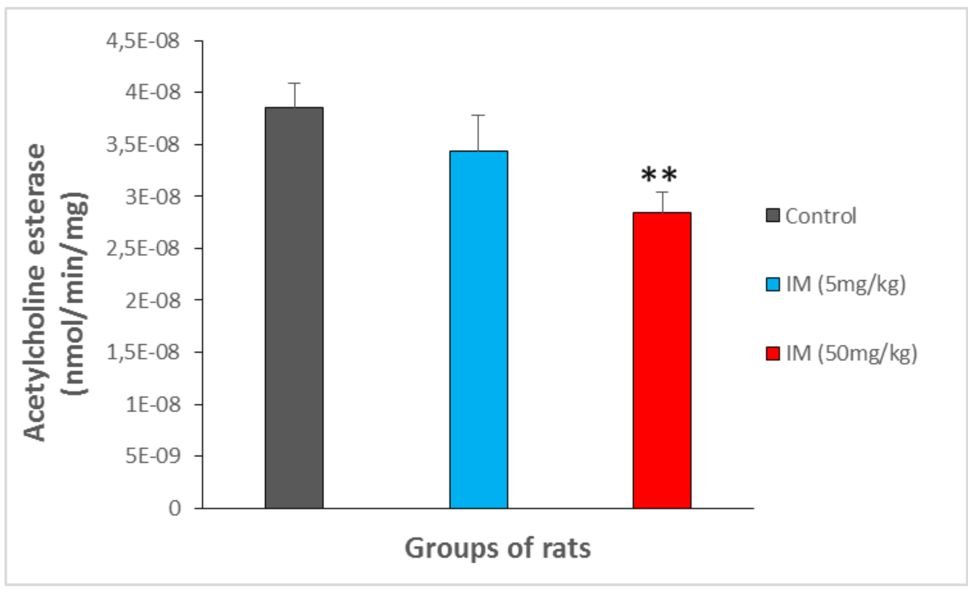


Figure 28: Variation in the AchE activity of the brain of control and treated rats after 20 days of treatment (n = 04, p = 0.002).

2.6.1. Effect on mitochondrial swelling.

Measuring the optical density at 540 nm provides information on the sizes of the mitochondria (The size of the mitochondria is proportional to the optical density at 540 nm). There is an increase in absorbance in rats treated with imidacloprid compared to controls. The results obtained are considered in (Figures 29).

ANOVA reveals a very highly significant increase in the sizes of brain mitochondria treated with the 50 mg / kg / day dose, and a highly significant increase in those of brain, compared to control.

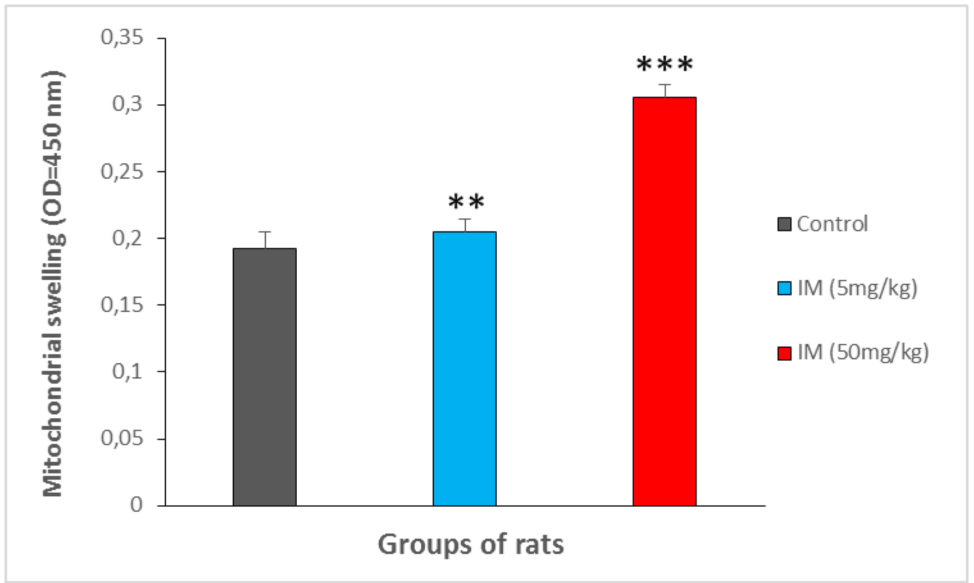


Figure 29: Variation in the mitochondria sizes of the control and treated rats after 20 days of treatment (n = 04, p = 0.000).

2.6.2. Effect on mitochondrial permeability

Figure (30) highlights the increase in mitochondrial permeability in the brains of control rats, treated with imidacloprid after treatment for 20 days.

After the addition of calcium we notice a non-significant increase (p> 0.05) in the size of the mitochondria in rats treated with 5 mg / kg / day and a very highly significant increase (P <0.001) in that treated with the dose. 50 mg / kg / day compared to controls, which means high permeability.

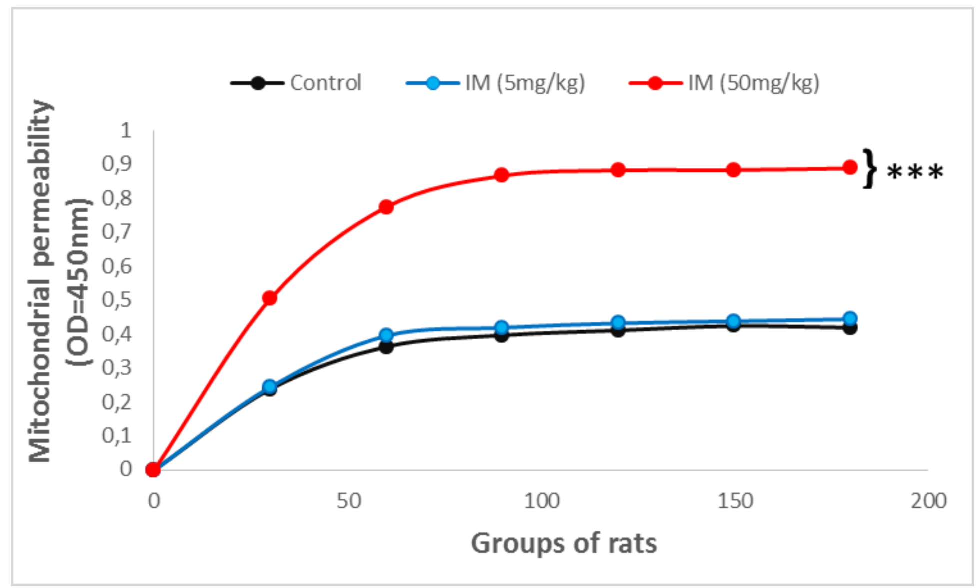
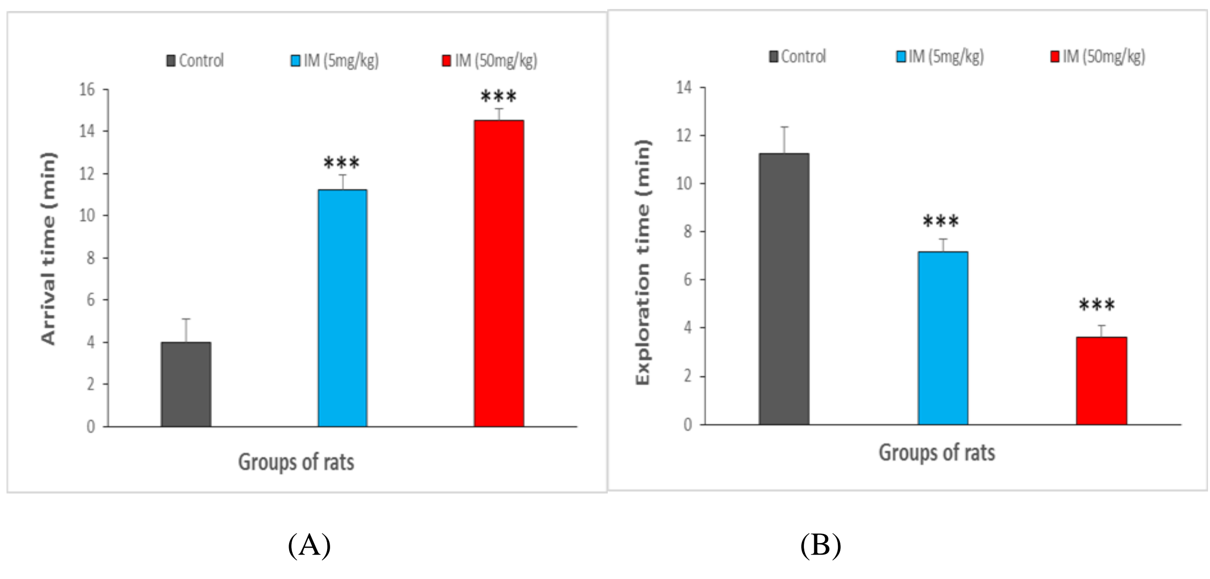


Figure 30: Variation in permeability of brain mitochondria in control and treated rats after 20 days of treatment (n = 04, p = 0.000).

2.7. Effects of imidacloprid pesticides on behavioral behavior in rats

2.7.1. Classic Labyrinth Test

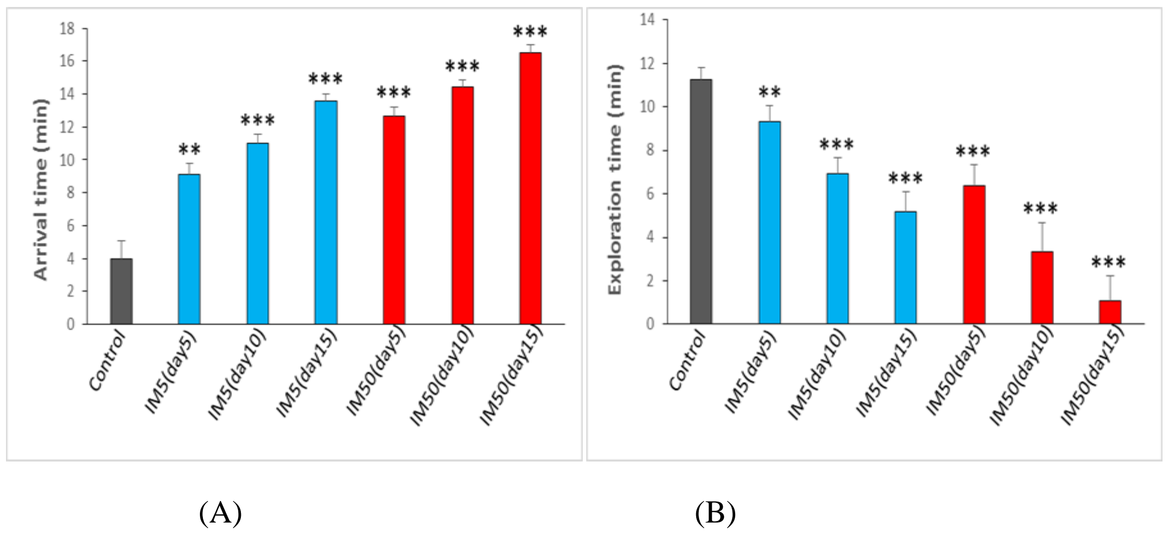
The results obtained following the examination of this test on animals are illustrated in Figure 31. The statistical analysis of these results showed a very highly significant increase ($p \leq 0.001$) in the time of arrival (fig. 31- A) and a very highly significant reduction in exploration time (fig. 31-B) they are compared to the controls.



(A) (B)

Figure 31: Variation in Classic Labyrinth test data in groups of rats treated with imidacloprid. (A): Time to reach the final point in the labyrinth (B): Time to explore the middle of the labyrinth.

-the detail the results of the classic labyrinth test, by 5.10 and 15 days. in rats treated with imidacloprid compared to control rats shows in (Figure 32)



(A) (B)

Figure 32: Variation of the Classic Labyrinth test data in time-dependent groups of rats: (A): Time to reach the final point in the labyrinth (B): Time to explore the middle of the labyrinth.

2.7.2.. Maze Vsoc test

The following figures represent the results of the Maze Vsoc test, where:

- Figure (33-A) shows the movement of rats at two empty maze after treatment with pesticides compared to controls Habituation phase.

- The figure (33.B) represents phase II sociability the results obtained from this test show a very significant decrease in shame in stranges 1 and very significant increase in empty 2 in rats treated with IMI compared to controls

- The figure (33-C) preference and socail novelty is a very hoarse significant increase in stranger 1, and a very significant decrease in stranger 2 in rats treated with imidacloprid compared to control rats

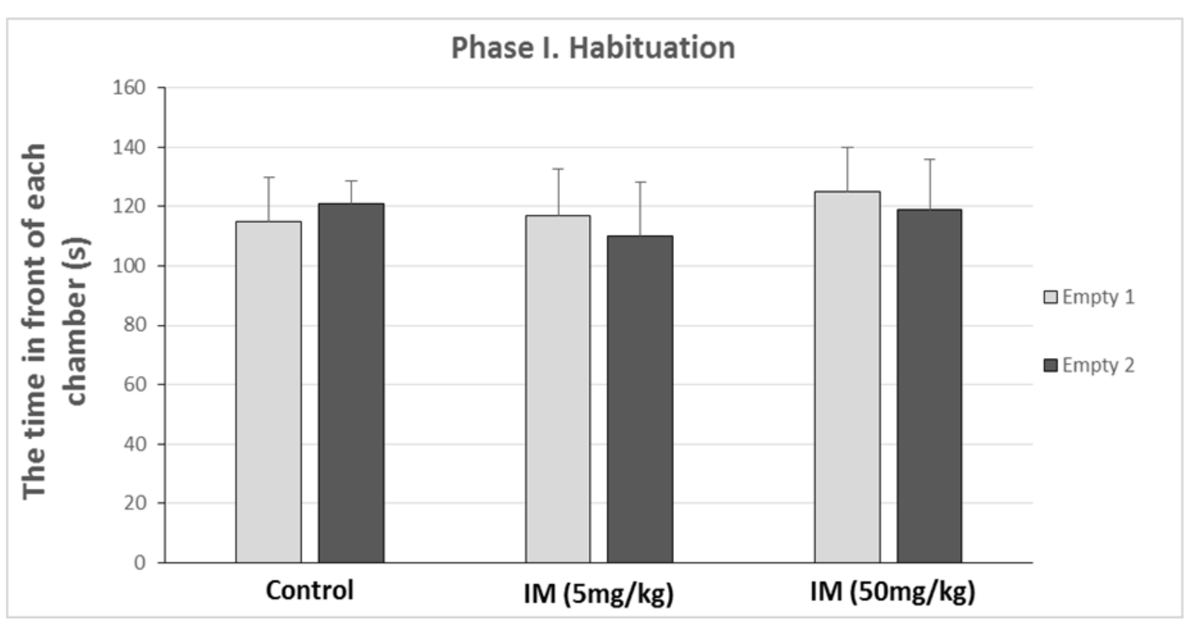


Figure (33-A): Variation in phase I data from the maze Vsoc test in groups of rats treated with imidacloprid. . (n = 04, p = 0.000).

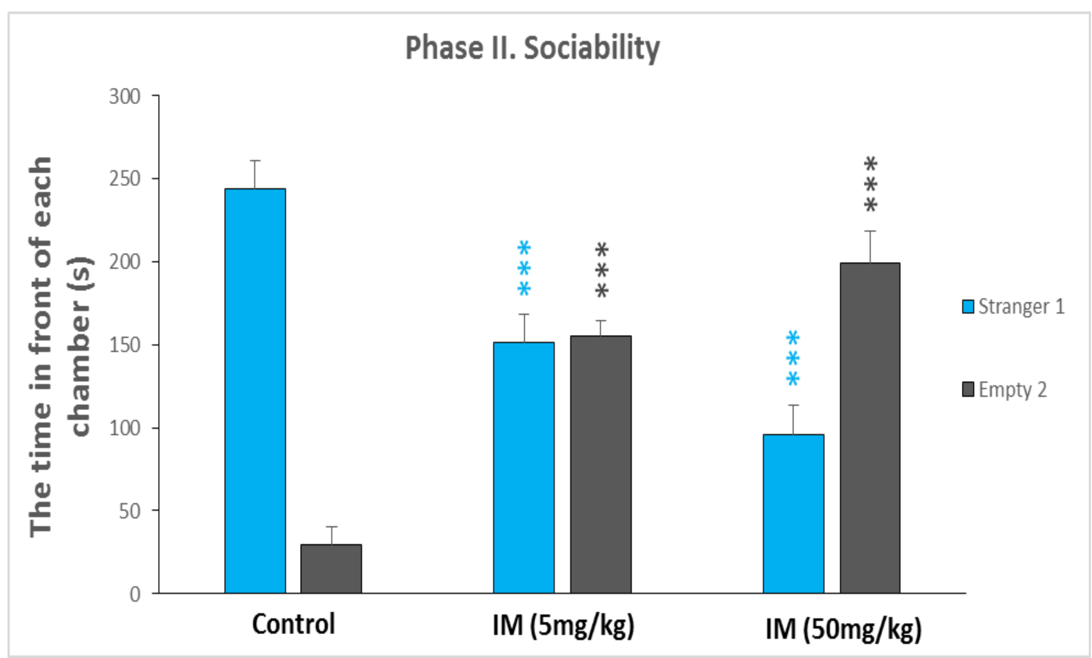


Figure (33-B): Variation in phase II data (sociability) of the maze Vsoc test in groups of rats treated with imidacloprid. . (n = 04, p = 0.000).

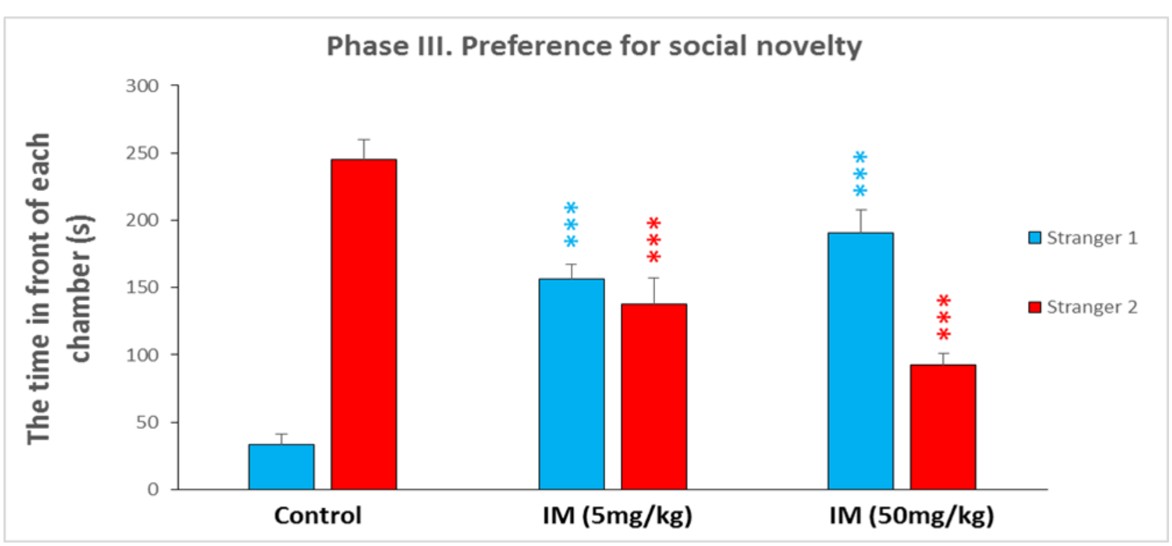
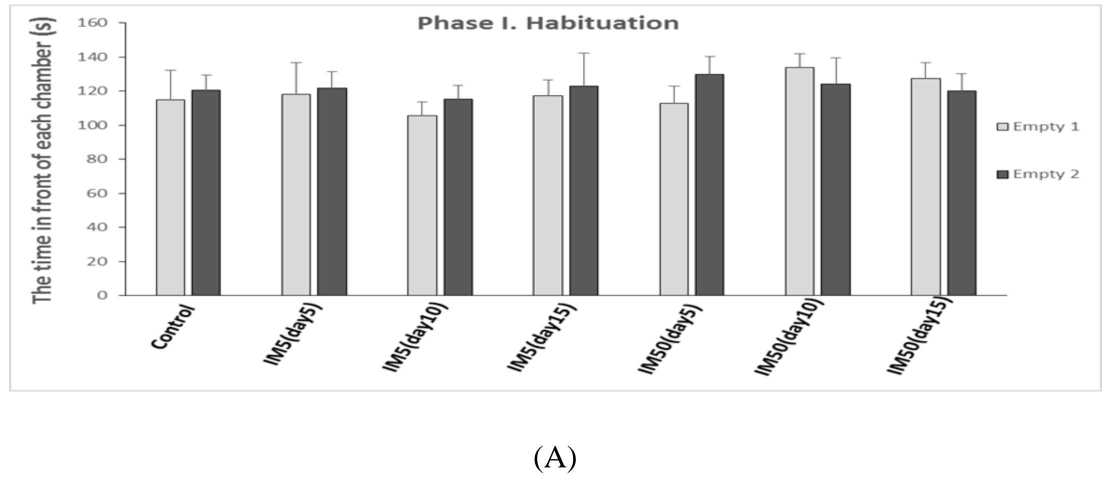
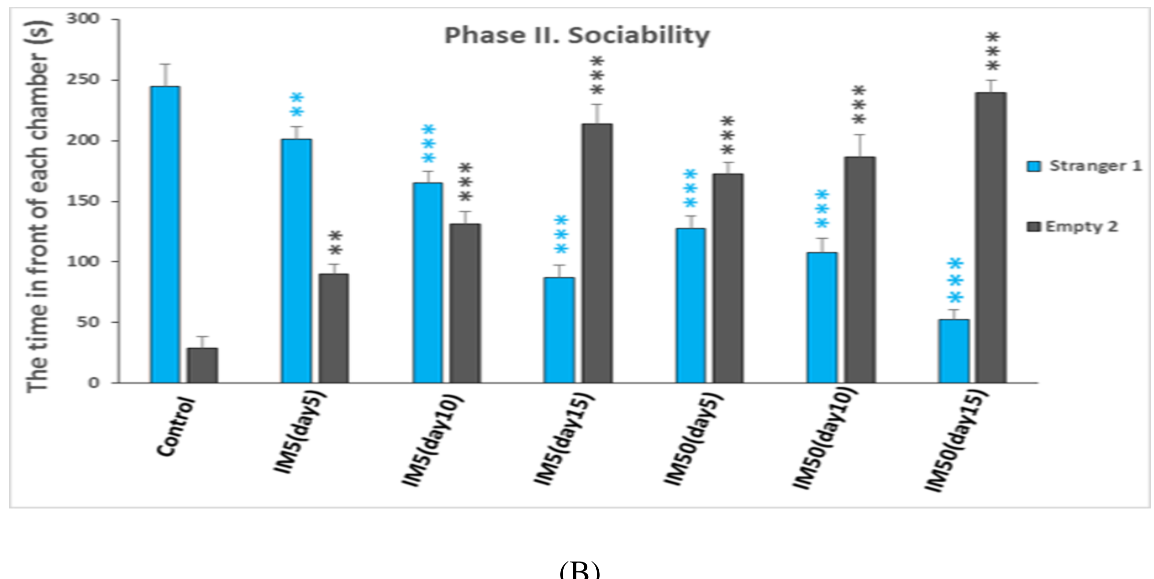


Figure (33-C): Variation in phase III data (preference for socail novelty) of the maze Vsoc test in groups of rats treated with imidacloprid. (N = 04, p = 0.000).

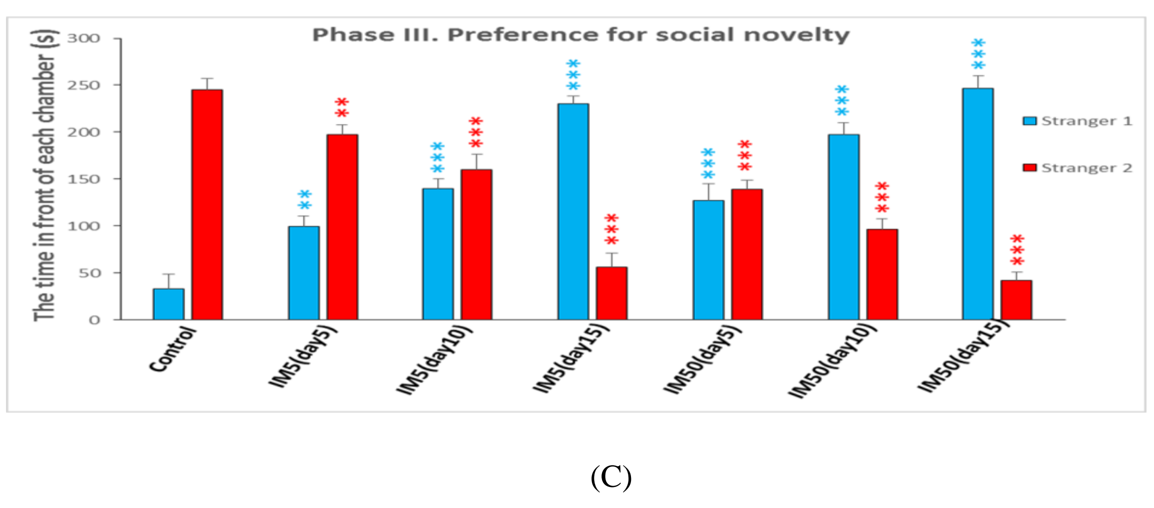
-the detail the results of the maze Vsoc test, by 5.10 and 15 days. In rats treated with imidacloprid compared to control rat's shows in (Figure 34)



(A)



(B)



(C)

Figure 34: Variation of maze Vsoc test data in groups of rats treated with time relievers: (A): phase I Housing, (B): phase II Sociability, (C) Preference for social novelty.

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Discussion

3. Discussion

We tried in the present work to assess the effects of imidacloprid on enzymatic and biochemical parameters in the brains of wistre rats as a biological model, so we looked for neurobehavioral effects and sociability and preference for social novelty. Wistre rats after treatment with neonicotinoid (imidacloprid) for a better understanding of the mechanisms and causes of toxicity; the results obtained are analyzed and discussed based on several studies and works reported in the monograph.

Neonicotinoids are insecticides commonly applied in the coating of maize and soya seeds to control crop pests **(Jeschke et *al*., 2016; Schaafsma et *al*., 2016)**. Their extensive use in agriculture coupled with a high leaching potential represents a high risk for non-targeted organisms such as pollinators **(Creswell, 2011)** and aquatic biota **(Pisa et *al*., 2014)**. NEOCs such as imidacloprid (lMI) and thiamethoxam (TMX) are in fact frequently detected in surface waters **(Hladik et *al*., 2014; Main et *al*., 2014; Schaafsma et al., 2015; Giroux, 2018)** at concentrations that could be considered toxic for the aquatic life. Both sublethal (growth, biodiversity and behavior) and lethal effects have been observed in aquatic invertebrates exposed in controlled conditions to environmental relevant concentrations of NEOCs, suggesting that they represent a threat also for non-target organisms **(Gibbons et *al*., 2015; Pisa et *al*., 2014; van der Sluijs et *al*., 2015; Morrissey et *al*., 2015).**

3.1. Effect of neonicotinoids on antioxidant activity.

The detoxification system involving detoxification enzymes remains the only means of studying the toxicity of xenobiotic. The enzymes involved are mainly divided into: phase I enzymes, called functionalization enzymes (oxid²ases, reeducates, hydrolases) used to unmask the electrophilic or nucleophilic elements of the xenobiotic. Phase II enzymes, known as conjugation enzymes, which bind endogenous hydrophilic derivatives (glutathione, glycoside, phosphate or glycuronic acid) to the functional groups revealed in phase I, these conjugation enzymes are transferases, the best known of which are glutathione- S transferase (GSTs).

The work of **(Loaner et *al*., 2014)** showed the establishment of a detoxification system in rats after the administration of a neonicotinoid imidacloprid orally at two doses 5 and 50 mg / kg / day. In fact, an increase in the activity of two detoxification enzymes GSH and a decrease in the enzymatic activity of glutathione S-transferase compared to the control group.

3.2. Neurotoxic effect of neonicotinoids.

The impact of nicotinic has been reported by **(Bhardwaj et *al*., 2010)** in the expensive wistar rat after the oral administration of 5mg / kg / day of imidacloprid. Their results showed the inhibition of the specific activity of acetylcholinesterase (AChE). Similar results have been reported by **(Rodrigues et *al*., 2010)**. **(Banerjee, et *al*., 2014)** have demonstrated the alteration of this key nervous system enzyme after administration of the pendimethalin compound to rats. However, the intraperitoneal injection of imidacloprid at a dose of 50 mg / kg / day for 20 days causes the rat to increase in the specific activity of this enzyme, thereby inducing alteration of the nervous system and muscular tetany **(Abou-Donia et *al*., 2008).** Recent work by **(Lonare et *al.*, 2014)** reported a significant decrease in acetylcholinesterase in the plasma and brain of rats treated orally with imidacloprid.

3.3. Effects on the rate of metabolites

3.3.1 Effects on protein levels

The significant increase in the level of proteins in a dose-dependent manner to acetamiprid can be explained by the synthesis of enzymes and peptides defending against the homeostatic imbalance of oxidative stress; by inducing the synthesis of stress proteins in relation to the phenomenon of bio activation / biotransformation on the one hand and on the other hand by lipid peroxidation generated by free radicals **(Anadn et *al.*, 1991; Benbouzib, 2012; Rouabhi et *al.,* 2015)** .In addition, during stress, animals synthesize stress proteins. Thus, the observed increase in protein level could be explained by an increase in the protein synthesis of snails in response to such a stimulus **(Radwan et Mohamed., 2013)**. Our results are in good agreement with those reported by **(Radwan et Mohamed., 2013)** who found a significant increase in total protein in wistre rats after exposure to 0.2 LD50 of imidacloprid for 1, 3 and 7 days. In addition, our results are consistent with work carried out on male rats of the Wistar treated with a neonicotinoid insecticide. Acetamiprid **(Chakroun et *al*., 2016)**. Other studies have also shown a significant increase in total protein after exposure to imidacloprid-polluted soil in earthworms for one week. An increase in total protein content was also detected in juveniles of P. scaber, after two weeks of feeding on 5 mg imidacloprid / g dry food **(Drobne et *al.*, 2008).** The same effect has been shown by the work of **(Bourbia, 2013)** after the application of commercial insecticides based on thiamethoxam with doses of 0, 25, 50, 100 and 200 mg / L on wistre rats after six weeks of treatment.

3.3.2 Effects on lipid levels

The dose-dependent decrease in lipid levels after exposure of rats to acetamiprid may be due to chemical stress caused by the test compound. Indeed, results reported that the harmful effect of chemical compounds could be attributed to the increased formation of malondialdehyde (MDA); the result of the activation of lipid peroxidation (degradation of polyunsaturated fatty acids) by frees radicals **(Dixit et *al*., 2001; Alpha Jalloh et *al*., 2009**). Or may be due to reduced lipid synthesis or lipase activity involved in lipid oxidation. According to **(Kemajl Bislimi et *al*., 2015)** the decrease in lipid levels in treated snails is the consequence of oxygenated free radicals which are the cause of lipid degradation through β-oxidation. Our results are consistent with the results obtained in wistre rats at sublethal doses of imidacloprid after 1, 3 and 7 days of treatment **(Radwan et Mohamed. 2013)**. The same effect was shown by the work of **(Bourbia, 2013)** after the application of commercial insecticides based on thiamethoxam with doses of 0, 25, 50, 100 and 200 mg / L on wistre rats after six weeks of treatment **(Kemajl Bislimi et *al*., 2015**), who found a decrease in the lipid level in snails after studying the effect of industrial pollution on certain biochemical parameters in garden snails rats.

3.4. Effects on the rate of biomarkers of oxidative stress

3.4.1. Effect of imidacloprid on glutathione (GSH)

One of the major detoxification reactions ensuring the protection of the organism living in a polluted environment is the conjugation with the Thiel group (-SH) of glutathione. GSH is a tripeptide well known to be an element of the first line of defense against oxidative stress and therefore considered an essential compound which maintains cellular integrity thanks to its reducing property and its active participation in cell metabolism, of which it protects cell membranes against damage caused by free radicals **(Sies et Akerboom, 1984; Martinez-Alvarez et *al*., 2005; Lam, 2009; Sauer, 2014; Aoun et Tiranti., 2016)** .Some important roles of glutathione are the reduction or inactivation of EROs by the formation of glutathione disulfide (GSSG) and the conjugation of reduced glutathione (GSH) for the elimination of xenobiotic **(Di-Monte et Lavasani., 2002; Arora et *al*., 2016; Rjeibi., 2016).** GSH is registered in our study in rats after exposure to imidacloprid. In addition, GSH is an antioxidant whose reduction should contribute to the protection of the animal from the effect of the test compound. Indeed, the possible attachment of this insecticide to the Thiel groups of GSH would possibly prevent cell damage. In this study, the observed increase in lipid

peroxidation and a concomitant decrease in the level of GSH, which suggests that the increase peroxidation can be a consequence of the depletion of GSH, or can be interpreted by an indirect inhibition of GSH by their bond with the oxidative molecules produced during the metabolism of pesticides after exposure on the one hand and on the other hand, by their use by GST in conjugation reactions with the insecticides tested **(Birsen Aydin, 2011).** In agreement with our results, the decrease in the GSH level was also observed in the hepatopancreas of two adults and wistre rats and their juveniles treated with the insecticide thiamethoxam at doses of 200 and 400 mg / L after 96 hours of exposure **(Douafer, 2015)** .In addition, the decrease in GSH levels was also observed in Hepatopancreas. treated with pesticides: carbofuran and parquet **(Salama et *al*., 2005)** and in the same species exposed to heavy metals **(Abdel-Halim et *al*., 2013; Nowaskowa et *al*. 2012)** study the anti-oxidative defense system in H. aspersa exposed to different metals (Zn, Fe, Cd, Pb and Mg) and the reduction of GSH is proportional to the amount of metals accumulated in the hepatopancreas. Also, the in vivo evaluation of biomarker of oxidative stress in the digestive gland of Theba pisana exposed to sublethal doses of copper-based pesticide, shows a significant drop in the level of GSH **(El-Gundy et *al*., 2009).** Thus our result is similar to the results in male mice treated with doses of 14.976 mg / kg) of imidacloprid **(Kawther et *al.*, 2010)**. In addition, **(Ramazan Bal et *al*., 2012)** studied the effects of imidacloprid on GSH levels in the reproductive organs of male rats after exposure to 20 mg / kg / day for 60 days, and found the same results. **(Birsen Aydin, 2011)** has reported a significant decrease in GSH levels in the lymphoid organs of rats after exposure to 112.5 and 22.5 mg / kg / day for 30 days of thiacloprid.

3.4.2. Effect of imidacloprid on the level of malondialdehyde (MDA)

MDA is considered to be a biomarker of oxidative stress in general and of lipid peroxidation in particular **(Grana et *al*., 2012)**. It is a major oxidant produced from peroxidized polyunsaturated fatty acid **(Zhang et *al*., 2004)**. MDA is the result of the attack of polyunsaturated lipids by ERO under certain stress conditions, in particular with organic contaminants (PAHs) , PCBs, pesticides) and inorganic (metals). In several studies, MDA has been used as a lipid peroxidation biomarker in snails **(Grana et *al*., 2012).** In the current study, a decrease in the level of MDA in hepatopancreas. This clearly shows that imidacloprid is capable of promoting the generation of ERO, which results in lipid peroxidation in the liver tissues and suggesting their deleterious effects in these tissues through the generation of free radicals **(Mona K Galal et *al*., 2014; Kapoor et *al.,* 2010., Kamal et Hashem, 2012)** .In our

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

3.5. Effects of pesticides and quercetin on animal neurobehavioural

Numerous research reports have shown that exposure to stress has a complex effect on learning, locomotive activity, memory and mental development **(Roosendaal et *al*., 2009; Cazakoff et *al*., 2010; Schwabe et *al*., 2012)**. A behavioral disturbance was observed following the evaluation of the various parameters linked to neurobehavioural biology by applying the various tests in rats exposed to pesticides in order to evaluate their locomotors, emotional, exploratory, memorization and learning activity. The results of the Open-field (OF) test, classic labyrinth test (CL), Morris pool test (MWM) and sucrose test which showed behavioral changes such as increased anxiety, fear, nervousness, short-term memory loss, and inability to think. These due to the gradual increase in mitochondrial ROS **(Shuichi et *al*., 2012)**. This result is consistent with the previous results produced by several studies **(Griffith, 1991; Jorgensen et Mouritsen, 2000; Kelley et *al*., 2011; Kim et *al*., 2005; Morris, 1984; Tsai et *al*., 2010).** In this study, we found that the immobility time and the swimming time of the rats treated with DM and AC changed compared to the control rats, demonstrating also a decrease in a highly significant way the locomotors activity of these rats. The labyrinth test is one of the most important behavioral models for assessing anxiety, memorization and learning. The increase in the number of tours, the time of arrival and the time spent in the

midst of the cage are considered to be the most representative indicators of anxiolytic activity. The increase in arrival time at the end point in the classic labyrinth test in rats treated with the two insecticides alone or in mixture may be the result of a loss of tissue mass and therefore of neurodegeneration affecting the areas principles of memorization and learning by these pesticides (**Mani et *al*., 2014**). This neurodegeneration causes a state of insufficient memory and learning capacity in rats infected with these

Conclusion

Conclusion

The present work, which we have carried out, is devoted to the study of the effect of neonicotinoids in increasing doses during different treatment periods on metabolites at levels such as carbohydrates, lipids and proteins, on stress parameters. Oxidative either non-enzymatic such as GSH and MDA or enzymatic (GPx, GST, CAT), and on the activity of Acetylcholine-esterase in wistre rats. Our objective was to know if Neurobehavioral effects and sociability and preference for the social novelty of wistar rats after a treatment with neonicotinoid (imidacloprid).

In the light of our results we can conclude that:

-administration of imidacloprid by the oral route in wistre rats induces a disturbance at the level of the metabolites at the levels of brains, by an increase in protein levels, and a decrease in carbohydrate and lipid levels compared to the control batch.

-a better characterization of the neurotoxicity of imidacloprid through the assay of GSH, MDA, GPx, CAT, GST at brain level which is explained by a depletion of the glutathione detoxification system, an increase in GST activity, CAT and a decrease in the activity of GPx and acetylcholine esterase, also a decrease in the lipid peroxidation marker (MDA).

-Study neurobehavioural and sociability and preference for the social novelty of wistar rats.

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ANNEXES

Annexes

1. 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

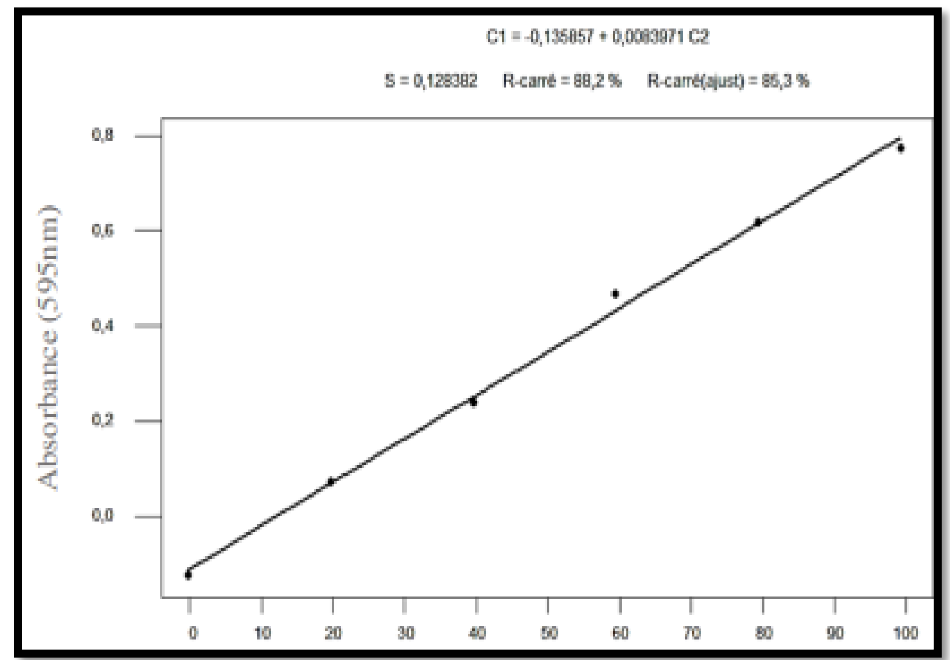

Table / Figure (A). Realization of calibration curve for the determination of proteins

The equation: $Y = 0.135857 + 0.0083971X$

2. Calibration curve for lipid determination

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

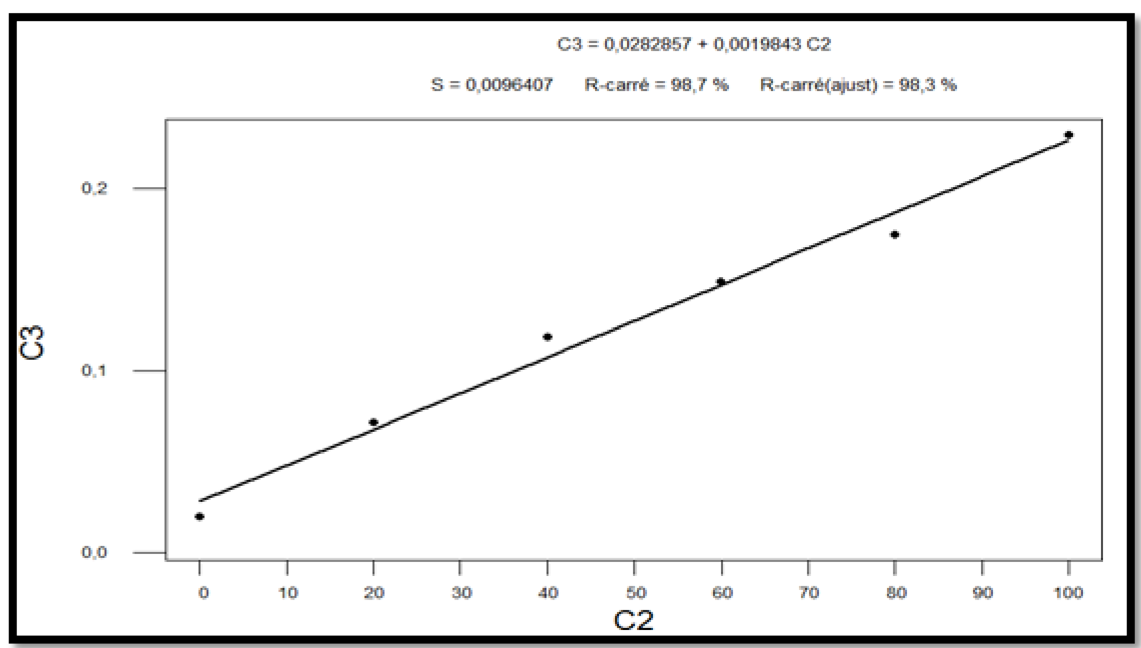

Table / Figure (B). Creation of a calibration curve for the determination of lipids

The equation: $Y = 0.0282857 + 0.0019843X$

3. Calibration curve for carbohydrate dosing

Tubes	1	2	3	4	5	6
Stock solution (glucose) (µl)	0	20	40	60	80	100
Distilled water (µl)	100	80	60	40	20	0
Anthrone reagent (ml)	4	4	4	4	4	4

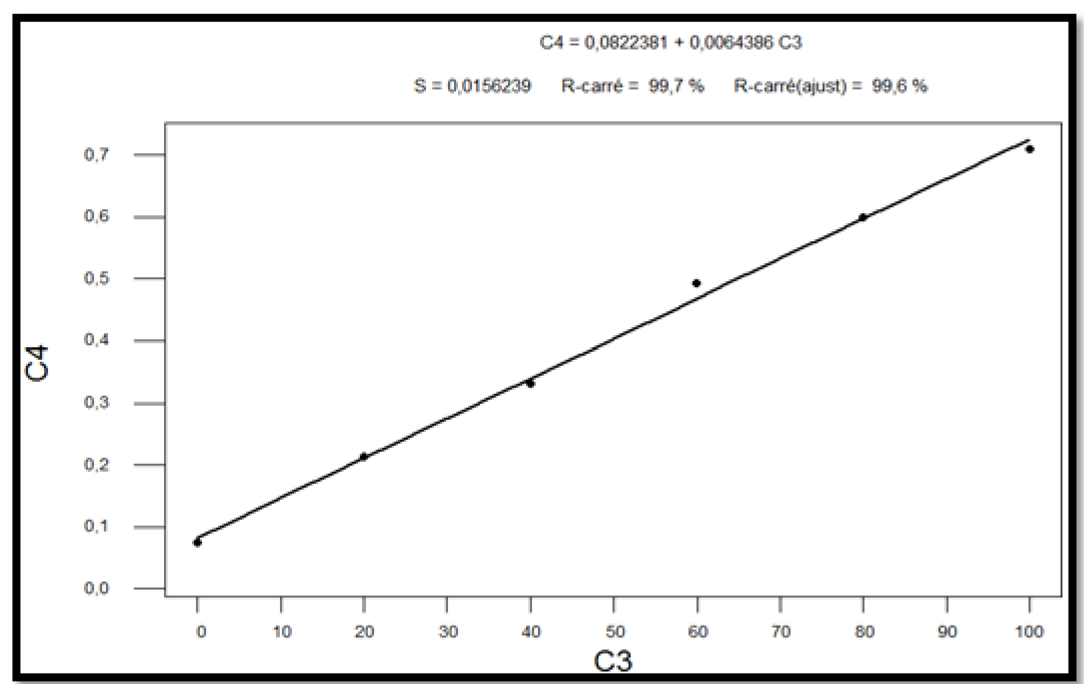

Table / Figure (C). Carrying out a calibration curve for carbohydrate dosing

The equation: $Y=0.0822381+0.0064386X$

Material used in the different stages of the study

4.1 .Large laboratory equipment and devices

- Bain Mari (MEMMERT).
- Centrifuge (SELECTA).
- Fridge
- Precision balance (KERN).
- Magnetic stirrer (WITEG).
- Spectrophotometer (UV mini 1240, SHIMADZU).
- Vortex (THERMOS).

4.2.Small laboratory equipment

- Beakers
- Cuvette for spectrophotometry (plastic and glass).
- End caps.
- Glassware
- Magnetic rod extractor.
- Magnetic bar.
- Mortars
- test tubes
- Micropipettes
- epindorphic tubes
- Pear.
- 100µl and 1000µl micropipettes.
- Graduated pipettes.
- Pasteur pipettes.
- Dry glass and plastic tubes.
- Racks for different types of tubes.
- Wash bottle.
- Spatula.
- Magnetic bar
- Magnetic rod extractor.
- End caps.
- Cuvette for spectrophotometry (plastic and glass).
- Aluminium foil.
- Toilet paper
- Beakers
- Graduated cylinders.

5. Chemical equipment

- Distilled water.
- TCA (Trichloro acetic).
- Anthrone.
- Sulfuric acid.
- Orthophosphoric acid (85%).
- Vanillin.
- BBC (Brilliant Coomassie Blue).
- Ether.
- Chloroform.
- Ethanol (95%).
- BSA (Beef serum albumin).
- Glucose.
- Sunflower oil.
- Sodium phosphate dibasic.
- SSA (sulfosalicylic acid).
- Sodium phosphate monobasic.
- Tris.
- HCl.
- NaOH.
- Absolute methanol.
- EDTA (Ethylene Diamine tetra etic acid).
- DTNB (5-5'-dithio-bis-2-nitrobenzoic acid).
- NaCl
- TBA
- BHT
- GSH
- CDNB (1-Chloro2, 4 di nitrobenzene)
- H2O2
- Phosphate
- Calcium Ca + 2