People „s Democratic Republic of Algeria

Ministry of Higher Education and Scientific Research

University of Larbi Tebessi –Tebessa-

Exact sciences and natural and life science faculty

Department of Applied Biology

Field :Natural and Life Sciences

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thesis

Presented with a view to obtaining the Master's degree

In: Biological sciences

Option:Toxicology

Entitled:

Impact Of imidacloprid On Nephrotic Function Of Wister Rats

By :

Ms. Guerti ahlem **Ms. Touahria soumaya.**

jury :

Me.Hamel.M	**Ass. prof**	**university of Tebessa**	**président**
M. Rouabhi R	**prof**	**university of Tebessa**	**supervisor**
M. Gasmi S	**Ass.prof**	**university of Tebessa**	**Examiner**

Graduation day : 27/6/2020

Abstract :

Pesticides are substances that meant to control pests includingall of the following :herbicide,insecticides which may include termiticide , neonicotinoide the most common of neonicotinoide is imidacloprid which is a systemique insecticide that acts as an insect neurotoxic. In this study we were interested to know his impact on nephrotic function of wistar rats by usine two defferent concentation 5 and 50 mg we used 12rats and devided them into 3 groups in each group we put 4 rats then we had treated the first group by concentration 5 mg and the second group with concentration50mg and we keep group as a control .our study is planned on many axes of experimentation :study of blood paramter bichemical paramters oxidative stress paramters . mitochondrial paramters .our result shown us at a concentration 50mg decrease in biochemical paramters , increase in blood paramters, increase in metochondrial paramters (swilling and permeability) decrease in GSH and MDAand GST level and increase in (CAT, GPX) levels and at a concentration 5 mg our result sown us no changed in all paramter compared to control group

Key words : oxidative stress, imidacloprid, blood paramters , biochemical paramters , mitochondrial paramters .

Resume :

Les pesticides sont des substances destinées à lutter contre les ravageurs, y compris tous les insectes suivants: herbicide, insecticides qui peuvent inclure des termiticides, néonicotinoïdes, le plus commun des néonicotinoïdes est l'imidaclopride: qui est un insecticide systémique qui agit comme un insecte neurotoxique. Dans cette étude, nous étions intéressés de connaître son impact sur la fonction néphrotique des rats Wistar par usine deux concentation différentielle 5 et 50 mg, nous avions utilisé 12 rats et devise en 3 group et répartissant s 4 rats dans chaque groupe et nous avions traité le premier groupe par concentration 5 mg et le deuxieme par concentration 50 mg et nous garder le groupe comme témoin. notre étude est planifiée sur de nombreux axes d'expérimentation: étude du paramètres sanguin, paramètres bichimique, paramètres du stress oxydant, paramètres mitochondriaux .notre résultat nous a montré une concentration de 50 mg diminution du paramètres biochimique,augmentation du paramétres sanguin, augmentation du paramétres mitochondriaux (gonflement et perméabilité) diminution du niveau GSH et MDA et GST et augmentation des niveaux (CAT, GPX) et à une concentration de 5 mg notre résultat ne nous a pas changé dans tous les paramètres par rapport au groupe témoin

Mot cles : stress oxidant,,imidaclopride, paramétres de sang , paramétres mitocondriaux , paramétres biochemique

:

ملخص

انَبُذاثيهٍاد حسخعًَم نَكبفحت اِفبث وي ٍضًَُهب َزكش يبُذاث انحششاث وي ٍاشهش أ َىاعهب االًَُذاكهبشَذ وهى يبُذ حششٌ عصبٍ فٍ دساسخُب هز اهخًَُب بًعشفت حبدٌُش عهً وضبئف انكهً حُث اسخعًَهب 21جشرا وقًُب بخقسًُهى انً 3يجًىعبث حُث قًُب بًعبنجت انًجًىعت االونً ة5يغ و1ة55 يغ وابقًُب عهً يجًىعت كشبهذ وفٍ كم يجًىعت وضعُب 4جشرا ٌ دساسخُب كبَج يقسًت نعذة يحبوس سئُسُت دساست يعبَُش انذو واالجهبد انخأكسذٌ و انًعبَُش انبُىكًًُبوَت)بشوحٍُ ونُبُذ(ويعبَُش انًُخىكىُذسٌ) االَخفبخ وانُفبرَت)انُخبئج انًحصم عهُهب اضهشثعُذ إسخعًَبل حشكُز 55يغ اسحفبع فٍ يعبيالث انذو وآَخفبض فٍ انًعبَُش انبُىكًًُبوَت)نُبُذ وبشوحٍُ (واسحفبع فٍ يعبَُش انًُخىكىُذسٌ) االَخفبخ وانُفبرَت (وآَضب اسحفبع فٍ يسخىي CAT و Gpxوآَخفبض فٍ يسخىيGSH و GST و MDAيب عُذ إسخعًَبل حشكُز mg5اضهشث َخبئجُب عذوو وجىد أ ٌ حغُُشاث ٌ جًُع انًعبيالث يقبسَت ببنفئشاٌ انشبهذة

انكهًبث انًفخبحُت: االجهبد انخأكسذٌ ,االًَُذاكهبشَذ ,-انًعبَُش انبُىكًًُبوَت, يعبَُش انذو, يعبَُش انًُخىكىُذسٌ

.

Acknowledgement :

First of all I would like to express my warmest thanks to the Professor Rouabhi Rachid who was for me an attentive Memory Director and available despite its many charges and without whom this brief would not have never seen the light of day. His competence, his scientific rigor and his foresight

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

-Mem Hamel M President of the jury

- Mr.Gasmi S the Examiner

- I would also like to address all my gratitude to Miss Henine Sara (PhD student) and Mem karima (technician of laboratory A) four their help and support

DEDICATION

To my dear parents, let them find here my gratitude for their supportThroughout my studies.

-To my lovely brother (guerti seif).

To all my frends that gave me the support througout the year

I dedicate this modest work.

AHLEM

IOIDACIDED:

I dedicate this work to my beautifaul mother and to my dear father and brothers, Ammar, Wahiba

-To my dear husband who supported me and gave me all the support and to my esteemed husband's family.

-To all my friends and all those who stood beside me and helped me with all they have.

-To my honorable professors in my entire educational career.

Soumaya

Abreviation list :

ACh ………………. Acetylcholine

AChE……… …….Acetylcholinesterase

ALT……………..Alanine Aminotransferase

ALT………………Alanine Aminotransferase

AP…………………Alkaline Phosphatase

AP1 ………………activator proteins1

ALS …………………. amyotrophic lateral sclerosis

CNS………………….Central Nervous System

CYP………………….Cytochrome P450

CAT …………. ……catalase.

CLT…………………… claddic lybrinth test

DPR ……….. …….Department of Pesticide Regulation

DHLA …………… dihydrolipoic acid

ERK……………….Extracellular Signal-Regulated Kinase

EC-SOD …………. extracellulaire superoxide dismitase.

FAO……… ……….Food and Agriculture Organisation

FIFRA …………….Federal Insecticide, Fungicide, and Rodenticide Act

GLDH……………..Glutamate Dehydrogenase

GWSS……… ……Glassy-Winged Sharpshooter

GR ……………… glutamate reductase.

GSTM1 ………….. glutathion –s-transferase-mu

GSTP1 …………… glutathion –s-transferase-p1

GPX1 …………….. glutathion peroxidase

GSH …………….. glutathion –s- transferase.

IMI ………………...imidacloprid

LC50………………..Median Lethal Concentration

LD50……….. ……Median Lethal Dose

LOEL…………… Lowest Observed Effect Level

MFO……………..Mixed Function Oxidases

MFO……………..Mixed Function Oxidases

MMAD…………….Aerodynamic Droplet Size

MTD ……………...maximum tolerated dose

nAChR……………. Nicotinic Acetylcholine Receptor

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Introduction

The neonicotinoids are a new major class of highly potent insecticides that are used for crop protection against piercing– sucking insects of cereals, vegetables, tea and cotton, and for flea control in cats and dogs.1 Since their introduction in the market in 1991, they have been increasingly used worldwide and have sometimes replaced organophosphate and carbamate insecticides due to their moderate toxicity to mammals(Benzidane *et al ;* 2011).

The first commercialized neonicotinoid was imidacloprid (IMI), followed by clothianidin (CTD). The insecticidal activity of neonicotinoids is primarily attributed to their action on nicotinic acetylcholine receptors (nAChRs) (Tomizawa and Casida, 2005). The neonicotinoid acute toxicity mechanism is due to their nicotinic agonist effects, acting on insect and mammal nAChRs(Tomizawa and Casida, 2003) On the other hand, several lines of evidence in the literature show that neonicotinoid insecticides present a higher selectivity for insect nAChRs than for mammalian ones(Oliveira *et al ;* 2011) However, these neonicotinoids are more selective towards mammal nAChRs when they are metabolized after their administration (Roberts and Hutson, 1999).

Imidacloprid, 1[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine, a chloronicotyl has been an extensively used insecticide for crop protection worldwide during the last decade due to its low soil persistence and high insecticidal activity at low application rates.9,10 Its sales are one of the fastest growing in the insecticide market globally due to its low selectivity for insects and relatively low risk to humans(Matsudad *et al* ; 2001).

Toxicological studies of imidacloprid are limited and the acceptable daily intake (ADI) has been previously reported as 0.006 mg per kg per day based mostly on unpublished reports (Soleski, 2001) A case of acute poisoning in humans was recently reported following the ingestion of a formulation containing 10% imidacloprid15 and few mortality reports due to ingestion have been published.16 Animal studies have confirmed the relatively low toxicity in vertebrate animals when compared to insects(Bal *et al* ; 2012).

It is moderately toxic and its acute oral LD50 is 450 mg kg-1 for rats and 150 mg kg-1 for mice(Tomlin and Tomlin, 1997) In male rats, the NOAEL dose of IMI is 14 mg per kg BW per day. However, acute ingestion of a 20% formulation of imidacloprid even following a large ingestion in patients of self poisoning is relatively safe(Mohamed *et al* ; 2009).

Pesticides are pollutants increasingly present in the surrounding environment, they are often persistent and can be bioaccumulated through biological chains such as soil–plant– food or water–aquatic organism–food**(** Preston, 2002**).**

chapter01 : wistar rats

definition

the wistar rat is an outbread albino rat. This breed was developed at the wistar institute in 1906 for in biological and medical research , and is notably the first rat developed to serve as a model organism at a time when laboratories primarily used the common house mouse(hist ,1993).

Figure 1 : wistar rat(Hist *et al* ;1993).

2-Comportement test

2-1-Classic Labyrinth Test for Neurobehavioral Evaluation in Wistar Rats

The Classic Labyrinth Test (CLT) is a simple way to evaluate behaviors in rodents such as learning ability, memory, and anxiety. The protocol presented here describes the procedure for use with rats, but the protocol can also be adapted for use in mice if a smaller device is used. In short, the CLTuses a square-shaped maze with a starting point and a stopping point. After the animal is trained, the animal is allowed to view and explore the labyrinth freely for 10 min. During this time, all of the animal's vertical and horizontal movements within the labyrinth are recorded. This is a very challenging task because it requires the animal to remember the quickest path between the starting points and the end. In cases where the labyrinth is designed so that the animal only needs to walk forward, it is quite easy for healthy rats, but for rats exposed to neuro-xenobiotics (drugs, pesticides) there will be disturbances in their path. Researchers use many different versions of this

test and the procedure for each version can vary significantly. Here, we present a working protocol that enables the detection of traces of some toxic substances that may be exposed to individuals over a long period and in very small amounts under specific conditions such as drugs, medicines and pesticides(Gasmi ;2018).

2-2-Use of the vsoc maze to study sociability and performence

Styding social behavior in mouse models empowers the understanding of the neurobiological mechanisms involved which are affected in neuropsychiatric disorders allowing the evaluation of therapeutic strategies behavioral methods available are time consuming and reducing thr length of behavioral session may render more manageable experiments and reduce animal stress. We validated a new reliable and sensitive methode to study two features of social behavior (sociability and performence for social novelty) in two strains of male wistar rats)in breed strain and out breed strain (Martinez-Torrez *et al* ; 2019).

Chapter02 : pesticid in general

1-introduction

As the Food and Agriculture Organisation of the United Nations (FAO) defined, pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest, including vectors of human or animal disease or weed which can cause harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs. Pesticides can be classified according to their **target**, their **mode or period of action**, or their **chemistry**.

They may be chemical substances, biological agents (such as viruses or bacteria), antimicrobials, disinfectants or devices used against any pest (Saravi and Shokrzadeh, 2011). Mainly, agricultural pesticides are divided into five categories based on the targeted pest and include: insecticides, herbicides, fungicides, rodenticides, and fumigants (Saravi and Shokrzadeh, 2011).

Recently, the European Food Safety Authority (EFSA) tried to replace the expression „pesticide" due to its negative implications, with the new term-„plant protective product" (PPP)

Pesticides are a highly diverse group of compounds and present one of the most important groups of chemical stressors in the environment (Hanazato *et al* ; 2005). As an alternative to first generation pesticides, modern pesticides have been designed to avoid some of the deleterious effects of these pesticides that had a long residence time in the environment and accumulated in the food chain (Meleiro Porto *et al* ; 2011). Newer classes of highly selective, systemic and single mode activity pesticides were introduced in the early 1990s and promised to address pest more specifically. Over the past twenty years, a class of systemic insecticides called **neonicotinoids** gained increasing interest in the agricultural sector. The term **_neonicotinoid pesticides'** comprises a group of several different **insecticides**, but it is usually used to indicate the four that are most widely applied: imidacloprid, thiacloprid, clothianidin and thiamethoxam. One of these representatives**, imidacloprid (IMI)**, was the main focus of this research.

-The use of **neonicotinoids** has been approved in the EU and they are currently in use. IMI, thiamethoxam, acetamiprid and thiacloprid are registered also on the Slovenian market. However, since May 2008, the insecticides thiamethoxam, clothianidin and IMI were banned for treatment of corn seeds (Zea mays), sugar beet seeds (Beta vulgaris) and oilseed rape seeds (Brassica napus) (The Official Gazette of the Republic of Slovenia No. 50/2008). Despite all the precautions taken in order to avoid the poisoning of bees, a large number of bee"s colonies died in the eastern part of

Slovenia during April 2011 (Pomor čebel v Pomurju dobiva nove razseznosti, 2011). Due to repetitive negative events, the Phytosanitary Administration of Slovenia banned completely clothianidin, IMI and thiamethoxam for any seed treatment, also other EU member states such as France, Germany and Italy imposed similar bans for IMI and clothianidin. Moreover, clothianidin is completely banned in Slovenia for any agricultural use (The Official Gazette of the Republic of Slovenia No. 31/2011). It was estimated that in Slovenia approximately 2,400 kg of neonicotinoids was sold in the year 2004 (Phytosanitary Administration of the Republic of Slovenia, personal communication, 2005; Žabar, 2012).

2-Neonicotinoids–Imidacloprid

2-1-Neonicotinoids

are a group of insecticides derived from nicotine isolated from the tobacco plant (Nicotiana tabaccum) which presents insecticidal activity and has been used extensively as natural insecticide. The developing road from nicotine to neonicotinoids was long and complicated. Neonicotinoids effective control of insect pests and helminthic parasites has been achieved by targeting invertebrate (insect) nAChRs (Matsuda *et al* ; 2005; Tomizawa and Casida, 2003; 2005). The major commercial insecticides targeting insect nAChRs were not derived from natural products but rather from the discovery of synthetic nitromethylene heterocyles (Kagabu, 1997). Introduction of the 6-chloro-3-pyridylmethyl and nitroimine moieties led to the development of the first type of nicotinic insecticide called imidacloprid (Kagabu, 1997).

2-2-Imidacloprid (IMI)

is one of the major representatives of the new generation of neonicotinoid insecticides. It was patented for the first time in 1985 by Bayer and was placed on the market in 1991. Today it has been made commercially available by Bayer AG and Nihon Tokushu Noyaku Seizo KK. It is a nicotine derived compound (neonicotinoid) with a large potential distribution due to its agonistic action on insect nAChRs and its selective toxicity to insects over vertebrates (Tomizawa and Casida, 2003). IMI [1-[(6-chloro-3 pyridynil) methyl]-*N*-nitro-2- imidazolidinimine] has the molecular formula($C9H10ClN5O$) , with a molecular weight of 255.7 g mol-1. In appearance, it consists of colourless crystals.

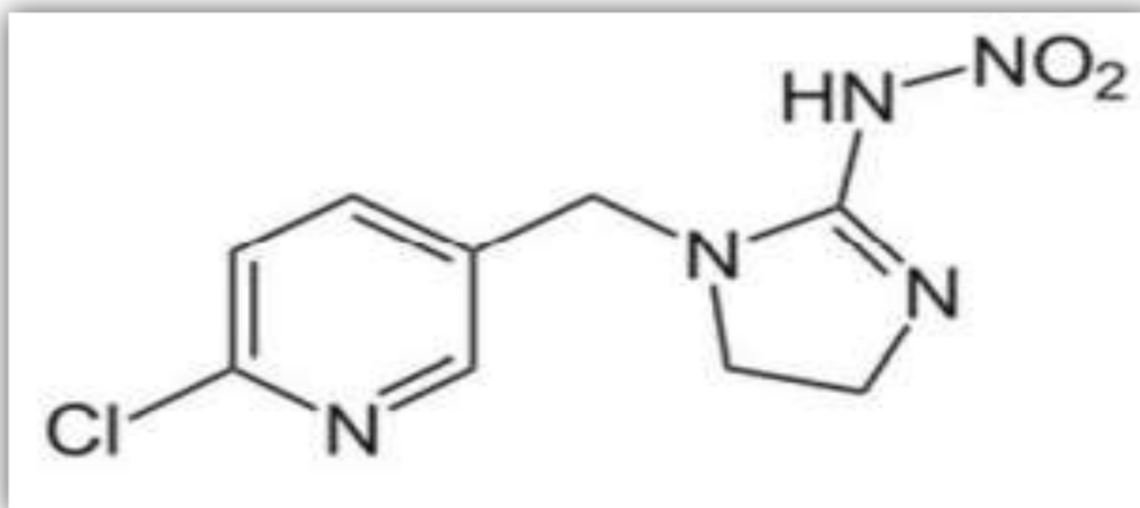


Figure 2: Chemical structure of imidacloprid(Tomizada and casida ;2015).

This component is a relatively new pest control substance, which is having the fastest growing sales worldwide (Tomizawa and Casida, 2005) and is generating increasing concern on its possible impacts on natural ecosystems (Matsuda *et al* ; 2001; Jemec *et al* ; 2007) pointed out remarkable data revealing the turnover toward insecticidal seed treatment. By the year 2005 seed coating developed into a €535 million market, with a 77 % share for neonicotinoid insecticides.

IMI"s physicochemical properties render it useful for a wide range of application techniques, including foliar, seed treatment, soil drench, and stem application (Millar and Denholm, 2007).

It is a versatile, broad-spectrum, systemic insecticide with activity against sucking insects (e.g. aphids, leafhoppers, whiteflies, and termites), several species of *Coleoptera*, *Diptera, Lepidoptera* and parasites on a different types of crops (Tomlin, 1994; Tomizawa and Casida, 2005). In addition, it is also applied as veterinary medicine against parasites and fleas in dogs and cats. Other important neonicotinoids are acetamiprid, clothianidin, dinotefuran, thiacloprid and thiameth

3-Commercial formulations of IMI

used for the control of different pest insects are available in a very large number (e.g. Admire, Merit, Confidor 200SL, Provado and Gaucho) (PMRA, 2005). Formulations of IMI are present as: a slurry or flowable concentrate for seed treatments, granule, wettable powder, soluble concentrate,

and suspension concentrate, water dispersible granules, and dustable powder (Tomlin, 2004).make a pure pesticide molecule applicable and highly functional, a. i. are combined with solvents or surfactants to accomplish an effective action of these formulations in agricultural applications. These supplementary substances often represent the highest proportion in pesticide marketed mixtures and include co-formulants (e.g. dimethyl sulfoxide (DMSO), *N*-methylpyrrolidone (NMP), propylene carbonate and other solvents) that could modify IMI‟s toxicity and bioavailability. Formulations of IMI include also other chemicals such as crystalline quartz silica and naphthalene chemicals which have also associated toxicological characteristics (Cox, 2001). Even a minor concern regarding the toxicity of these compounds and their possible synergistic effect with IMI or other ingredients should be continuously considered (Tobiassen *et al* ;. 2003; Surgan, 2005).

It is important to notice that the commercial formulations are the ones applied in the environment with relevant soil (50 g ha-1 to 320 g ha-1) and foliar concentrations (73 mg L-1 to 150 mg L-1). Recent evaluation of the data relative to different formulations noted high levels of IMI in leaves and in blossoms of treated plants, and increases in residue levels over time (CDPR, 2011). Data indicated that the use of these IMI formulations on annual basis may be at the end cumulative. Due to recent findings certain commercial products within the class of neonicotinoids (containing a. i. IMI) were placed under re-evaluation and need further studies (CDPR, 2011).

Even if IMI has been in use for a relatively short period of time compared to other common pesticides, it is considered to being used in the largest quantity worldwide of all insecticides (Cox, 2001; Ware and Whitacre, 2004). Comparative toxicity studies indicate IMI‟s adverse effects to some aquatic invertebrates and have noticed high species-specific response to IMI, which suggests that IMI toxicity data may not be generalised (Jemec *et al* ;. 2007). In addition, IMI can be applied at very low rates and during whole year

4-Physical and chemical properties

Table *1* : Physical and chemical properties of imidacloprid*(*Jemec *et al,*.2007)

Chemical name	**1-(6-chloro-3-pyridin-3-ylmethyl)-N-nitroimidazolidin-2- ylidenamine**
CAS Registry number	13826-41-3
Common name(s)	Imidacloprid
Physical appearance	light yellow powder
Molecular formula	C9H10ClN5O2
Molecular weight	255.66g/mol
Structural Formula	
Solubility	0.58 g/l water at 20°C. Soluble in acetone, acetonitrile, methylene chloride and dimethylformamide, DMSO
Melting point	120-134°C
Vapor pressure	1.5x10-9 mmHg at 20ºC
Henry's Constant	9.9 x10-13 atm m3g.mol-1 at 20ºC

5- Environmental Fate

In the environment, the principal routes of dissipation for imidacloprid are aqueous photolysis, microbial degradation and uptake by plants. Imidacloprid photodegraded rapidly (half-life of 4 hours) in water, compared to soil (half-life of 171 days). It was hydrolytically stable at pH 5 and 7, but hydrolyzed slowly in sterile alkaline solutions (half-life of 355 days). The half-life of the imidacloprid degradation in anaerobic soil was 27 days. Imidacloprid was persistent in aerobic soil under laboratory conditions (half-life of 188 to > 365 days). The presence of vegetation substantially increased the rate of imidacloprid degradation in the soil (half-life of 48 days). Studies on the imidacloprid mobility in soil revealed that imidacloprid residues leached into the 6-12 inch soil depth under field conditions. Imidacloprid is currently listed by the DPR as a potential ground water contaminant, based on its high solubility in water, mobility and persistence in soil. The low vapor pressure of imidacloprid indicates that its volatilization from soil and leaf surfaces may not be a major route of dissipation. Presently, information on imidacloprid residues in ground and surface water or in air samples in California is not available. The major degradation product of imidacloprid in the environment is desnitro-imidacloprid. Other products, which have been found in laboratory studies, included 5-hydroxy-imidacloprid, imidacloprid-urea, 6-chloronicotinic acid and carbon dioxide. (http://www.cdpr.ca.gov/docs/empm/pubs/fatememo/imid.pdf).

5-1-Hydrolysis

The hydrolysis of imidacloprid was investigated in sterile aqueous buffered solutions at 25ºC for 30 days (Yoshida, 1989). Pyridine-labeled 14C-imidacloprid (5 ppm) was hydrolytically stable at pH 5 and 7. It hydrolyzed very slowly at pH 9 with an estimated half-live of 355 days. In alkaline solutions the major metabolite (7% of the total radioactivity) could not be identified. The other hydrolysis product was imidacloprid urea (1.7% of the total radioactivity).

5-2-Photolysis or Photodegradation

The photodegradation of [pyridinyl-14C-methyl]imidacloprid was studied in sterile water, under the conditions of maximum hydrolytic stability (pH 7 at 23ºC). Imidacloprid (5.4 mg/l) was continuously irradiated with a sunlight-simulating xenon lamp. The half-life of the photodegradation was 57 min. Based on this half-life, the environmental half-life was estimated at about 4.2 hours. Similarly, imidacloprid was degraded quickly (~ 4h) under natural sunlight in the

greenhouse. The major photodegradation products were desnitro-imidacloprid (17.2%) and imidacloprid urea (10 % of the applied radioactivity, Anderson 1991). The photodegradation of [pyridinyl-14C]imidacloprid was investigated on sandy loam soil. [Pyridinyl-14C] imidacloprid was applied at a concentration of 48.5 mg/kg onto the soil layer. It was the continuously irradiated with a sunlight-simulating xenon lamp for 15 days at 25ºC. Imidacloprid degraded with a half-life of 38.9 days under the experimental conditions. The calculated environmental half-live was 171 days. The major photodegradate was 5-hydroxy imidacloprid (Yoshida 1990).

5-3- Microbial Degradation

The anaerobic metabolism of imidacloprid was investigated in microbially active water and the accompanying sediment, which were obtained from a pond. Imidacloprid was applied to the water at an application rate of 0.6 mg/l and incubated for 358 days. This dose rate was about 1.5 folhigher that the actual field use rate (0.5 lb a.i./acre). Under these conditions, [pyridinyl-14Cmethyl]-imidacloprid degraded with a half-life of 27 days. Desnitro-imidacloprid was identified as the only major metabolite. After 60 days, large amounts of this metabolite were bound to the sediment. At the end of the incubation period, imidacloprid degraded to less than 0.1 % in both, water and sediment. The final degradation product was carbon dioxide (Fritz and Hellpointer, 1991).

The aerobic metabolism of imidacloprid was studied on microbially active, sandy loam soil in the dark, at 20ºC. [Pyridinyl-14C-methyl]-imidacloprid was applied to the soil at a dose rate of 0.33 mg/kg soil. After 366 days of incubation, imidacloprid accounted for more than 60% of the applied radioactivity. The extrapolated half-life of its degradation was greater than 1 year. Seven metabolites were observed at the end of the incubation period, but they represented less than 2% of the applied parent compound. The degradation of imidacloprid in soil was proposed to be via denitrification, oxidation and cleavage of the dihydro-imidazole ring to yield 6-cloronicotinic acid and ultimately carbon dioxide (Anderson *et al.,* 1991).Additional aerobic metabolism studies of imidacloprid were conducted on microbially active, flooded sandy loam soil BBA 2.2, Hoefchen silt and Monheim 1 sandy loam in the dark at 20ºC. The application rates were 0.33 mg/kg (BBA 2.2 and Monheim 1 soils) and 0.36 mg/kg (Hoefchen silt) and the incubation period was 100 days. Under the experimental conditions, the radiolabeled imidacloprid degraded with estimated half-lives of approximately 188, 248 and 100 days, respectively (Anderson *et al.,* 1990; Anderson and Fritz, 1990; Anderson and Fritz, 1991). The effect of growing vegetation on the degradation of imidacloprid in soils was investigated in BBA 2.2 loamy soil. The soil was fertilized to maintain a

nutrient supply for the plants. [Pyridinyl-14C-methyl]-imidacloprid was applied at 0.23 mg a.i./kg soil and grass was then planted as vegetation. The samples were incubated in the greenhouse at 17-20ºC for 274 days. The half-life of imidacloprid degradation in samples with vegetation was 48 days, whereas in samples without vegetation the degradation was significantly slower (half-life 190 days). Under vegetation, the main metabolite was desnitro-imidacloprid, whereas 5-hydroxy-imidacloprid was the major degradate in the soil without growing vegetation. The grass absorbed about 10% of the applied radioactivity. Therefore, the accelerated degradation of imidacloprid could not be attributed solely to the uptake by the vegetation (Scholz, 1992).

5-4-Mobility and Field Dissipation

5-4-1- Soil

A soil adsorption/desorption study was carried out to characterize the mobility of imidacloprid in soil (Fritz, 1988). Aqueous solutions of [pyridinyl-14C-methyl]-imidacloprid were equilibrated for 48 h at 25ºC with four different soil types – sandy loam, slit loam, low-humus sandy soil and silty clay. The highest tested concentration of imidacloprid was approximately 290 mg/l. The soil to water ratio was 1:4. Based on the soil-carbon sorption constant (Koc), the mobility of imidacloprid could be classified as high in silt (Koc of 132) and medium in low-humus sandy soil, silty clay and sandy loam (Koc of 157, 212 and 256, respectively). In a subsequent study, aqueous solutions of [pyridinyl-14C-methyl]-imidacloprid were incubated at 25ºC with four different soil types – sand, loamy sand, slit loam and loam. The mobility of imidacloprid was classified as medium (Koc of 277-411; Williams et al, 1992).

A soil adsorption/desorption study was carried out to characterize the sorption properties of the major metabolite of imidacloprid, desnitro-imidacloprid. Aqueous solutions of [14C]-desnitroimidacloprid were equilibrated at 25ºC with four different soil types – sand, loamy sand, slit loam and loam. The highest tested concentration of desnitro-imidacloprid was approximately 250 ppm. The soil to water ratio was 1:3 for sand, loamy sand and 1:5 for slit loam. Desnitroimidacloprid had a stronger sorption affinity for soil than the parent compound. it can be classified as a medium mobility compound in sand and low mobility compound in loamy sand, slit loam and loam . Therefore, desnitro-imidacloprid is less likely to leach through the soil than the parent chemical Dyer *et al* ;.1992)

5-4-2-Ambient Air

Preliminary ambient air monitoring studies were conducted in Santa Clara, Imperial and Butte Counties, California Ground applications of imidacloprid foliar spray were used in residential properties, businesses, commercial parking lots, curbsides and public parks in Cupertino (Santa Clara county), Imperial Spa (Imperial County) and Chico (Butte County), A total of 8 air samples from the treated areas were analyzed at the time of application, 24 and 48 h post application. Information on the geographic relationship between the location of the monitoring and the application site was not provided. There were no imidacloprid detections in the air. The detection limit was 0.5 µg/sample.

5-4-3- Ground Water

The California Pesticide Contamination and Prevention Act (PCPA) of 1985 established a set of data requirements for identifying potential ground water contaminants. Pesticides with parameters exceeding Specific Numerical Values (SNVs) established by DPR, are considered to pose a risk to ground water (Kollman and Guo, 2000). The SNVs include: Solubility (SNV>3 ppm), Koc (SNV<1900 cm3/g), Hydrolysis (SNV>14 days), Aerobic Metabolism (SNV>610 days) and Anaerobic Metabolism (SNV>9 days). DPR identified imidacloprid as a potential ground water contaminant based on its high water solubility (514 ppm), low Koc (262 cm3/g), long hydrolysis half-life (t1/2=30 days), long aerobic soil metabolism (t1/2=997 days) and long anaerobic soil metabolism (t1/2=27 days). Recently, DPR developed analytical methods for detection of imidacloprid residues to perform actual screening for imidacloprid and its metabolites in the water of the California wells.

5-4-4- Surface Water

Preliminary surface water monitoring studies were conducted in Santa Clara, Imperial and Butte Counties, California. Imidacloprid was applied via soil injection or foliar spray in areas infested with GWSS (DPR 2001; 2002). A total of 11 surface water samples were collected at five creeks and at a fishpond. The location of the monitoring sites was generally indicated as upstream or downstream of the application area, however, more specific information was not provided. Imidacloprid residues were not detected in any of these samples. The detection limit was 0.05 ppb.

5-4-5- Field Dissipation

A series of field dissipation studies were performed at different sites (Georgia, Minnesota and California) with various soil types to evaluate the degradation and mobility of imidacloprid under actual field conditions. Imidacloprid formulation 240FS (23.3% a.i. liquid suspension) was applied to the soil at the highest recommended rate of 0.5 lb a.i./acre. Soil core samples were analyzed for imidacloprid immediately post-application through 18 months. Each core was sectioned into 6-inch segments. The half-life for imidacloprid dissipation in loamy sand and sandy loam was 12 days. Imidacloprid applied to a field planted with corn had a half-life of 7 days. Residues at or above the detection limit (10 ppb) were not detected below 0-6-inch soil depth (Rice *et al.*, 1991). Imidacloprid applied to a tomato plot dissipated with a half-life of 53 days. Residues above the detection limit (10 ppb) were detected below the 6-inch soil depth, indicating that the pesticide had leached into the 6-12 inch soil dept (Rice *et al* ;. 1991c). The half-life for imidacloprid dissipation in the turf grass was 61-107 days with no leaching below the 0-6 inch soil depth (Rice *et al,*. 1992).

6- Imidacloprid mode of action

IMI mode of action is based on the interference of the neurotransmission in the nicotinic cholinergic nervous system. IMI binds to nicotinic acetylcholine receptors (nAChRs) at the neuronal and neuromuscular junctions in insects and vertebrates. The nAChRs are members of the cys-loop ligand-gated ion channel (LGIC) superfamily consisting of five membrane proteins arranged around a central cation permeable pore (Sine and Engel, 2006) These ion channels rapidly transduce the actions of the excitatory chemical neurotransmitter acetylcholine (ACh) to membrane depolarisation at the level of synapses. The receptor is normally present in a closed state; however, after ACh binding, the complex channel opens a pore and becomes permeable for cations.

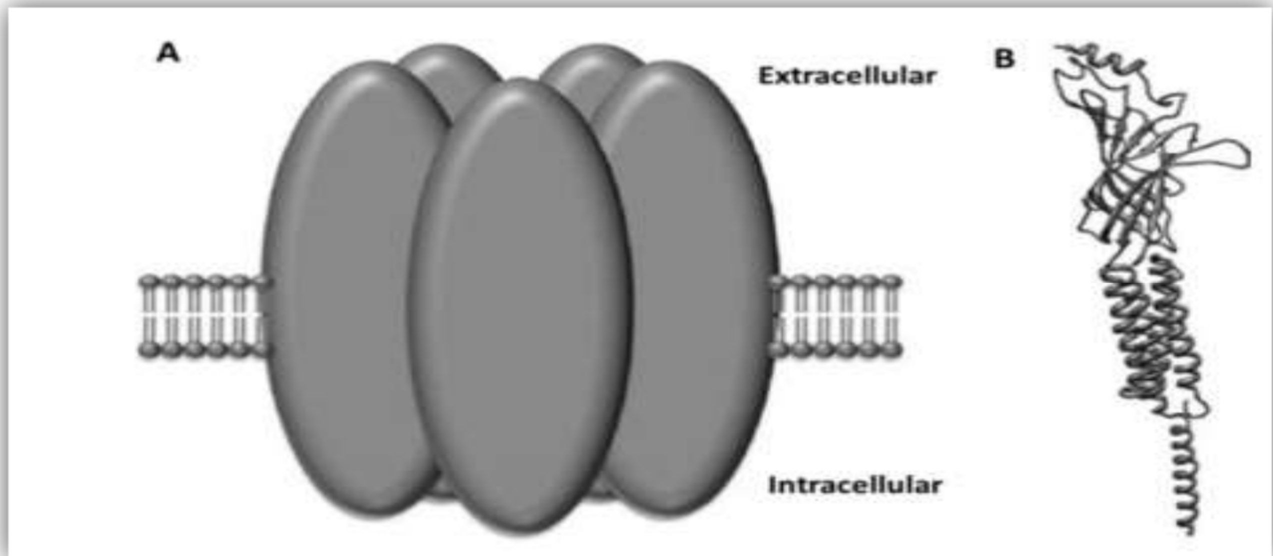


Figure 3: Nicotinic acetylcholine receptors (nAChR) and subunit structure. (A) Schematic representation of nAChR, presenting the pentameric arrangement of subunits around a centralcation-permeable pore. (B) Three dimensional structure of an individual nAChR subunit showing the structure of the polypeptide backbone (Millar, 2009**)**.

Similarly to the naturally occurring signal transmitting ACh, IMI stimulates certain nerve cells by acting on a receptor protein. IMI fits to the receptor that normally receives the molecule of ACh and irreversibly blocks postsynaptic nAChRs (Tennekes, 2010) . In contrast to ACh, which is quickly degraded by enzyme acethylcholinesterase (AChE), IMI is inactivated either very slowly or not at all (Cox, 2001). Prolonged activation of the nAChR by IMI causes desensitisation and blocking of the receptor and leads to paralysis and death.

6-1-Insect nAChRs

high affinity IMI binding sites in insect nAChRs were detected in a broad range of insects, including green peach aphid, glassy-winged sharpshooter, whitefly, cockroach,

migratory locust and fruit fly (Zhang *et al.,* 2000; Tomizawa and Casida, 2003). The 2-nitroimino-imidazolidine group plays a key role in the selectivity of insect nAChRs. Neonicotinoids are not protonated and instead of an easily protonated nitrogen they have an electronegative nitro or cyano pharmacophore. This electronegative pharmacophore is proposed to associate with a cationic subsite in the insect nAChRs (Tomizawa and Casida, 2005). In general, the potency of IMI for insect brain nAChRs is considerably higher than for mammalian channels. For example, the binding affinity of IMI to nAChRs of *Drosophila* sp. is 12 over 550-fold greater than the affinity to the mammalian receptors (Tomizawa, 2001).

6-2-Mammalian nAChRs :

structural studies suggested that the electron deficient nitrogen atom of the imidazolidine group in IMI molecule corresponds to the protonated form of nicotine and

interacts with the mammalian nicotinic receptors (Matsuda *et al*., 2000). The binding affinity and agonist potency of IMI have been reported for several vertebrate species. IMI showed a partial agonist activity with the recombinant chicken α4β2 and α7 receptors (Matsuda *et al*., 1998; 2000). IMI presented also agonist action on nAChRs in BC3H1 muscle cells and mouse N1E- 115 neuroblastoma (Zwart *et al ;*. 1994).

It is important to describe several additional effects that occur at the receptor and cellular level after exposure to IMI. It was demonstrated that IMI (after 3 days of exposure) up-regulates the α4β2 nAChR subtype in mouse M10 cell line (Tomizawa and Casida, 2000). This up-regulation is often associated with the receptor desensitisation (Pauly *et al* ;. 1996). At the cellular level, the immediate effect of nAChRs activation is an increase in the level of intracellular calcium and consequent membrane depolarisation which then activates a complex downstream signalling pathway (Berg and Conroy, 2002). One of the key components involved in the nAChR downstream signalling pathway is the extracellular signal-regulated kinase (ERK), also known as mitogen-activated protein kinase (MAPK). In the study of Tomizawa and Casida (2003) IMI activated the ERK cascade in mouse neuroblastoma N1E-115 cells after 30 min incubation. The IMI stimulation of α4β2 receptor was joined with the phosphorylation of ERK in a Ca2+ and protein kinase C (PKC)-dependent manner (Tomizawa and Casida, 2003). ERK pathway is a necessary intermediate in the signalling from the nAChR to expression of specific genes (Chang and Berg, 2001). Several human neuropathologies have been linked to genetic alterations of nAChRs genes (Lindstrom, 2002). These receptors are also involved at different levels in several neurodegenerative diseases such as Parkinson and Alzheimer�Ÿs. There is a growing number of facts (even if studies are not entirely consistent) which correlates long-term low-dose pesticide exposure along with the specific agricultural working conditions to a variety of disease conditions including cancers, reproductive health problems and a range of neurological disorders(Parrón*et al* ;. 2011).

7-Toxicology profil

7-1- Pharmacokinetics

7-1-1-Absorption

7-1-1-1- Oral Absorption

To investigate the oral absorption of imidacloprid, Wistar rats (5/sex/dose) received a single

intravenous (i.v.) dose of 1 mg/kg or single oral doses of 1 and 20 mg/kg of 14C-imidacloprid (methylene-labeled, 150.7 µCi/mg; Klein, 1987; Klein and Karl, 1990)These doses corresponded to 0.2% and 5% of the rat oral LD50 (424 mg/kg, Table 1) and did not cause toxic signs. In parallel experiments, rats were pre-loaded for 14 days with non-radiolabeled imidacloprid (1 mg/kg) and then received single oral doses of 1 mg/kg or 20 mg/kg 14Cimidacloprid. Additional tests were carried out to measure the radioactivity in the expired CO2 in 5 male rats, which received a single oral dose of 20 mg/kg 14C-imidacloprid. To characterize the bile excretion, five more rats were bile-fistulated, prior to receiving intraduodenally a single dose of 1 mg/kg 14C-imidacloprid (Klein, 1987). In the above studies, imidacloprid was labeled with 14C in the methylene moiety. Because nearly half of the identified metabolites did not contain the imidazolidine moiety, a comparative study was conducted, using imidacloprid labeled with 14C in the 4- and 5- position of the imidazolidine moiety (Klein and Brauner, 1991). In the later experiments, radiolabeled imidacloprid was given as a single oral dose to 5 male and female rats at 1 mg/kg; or to 5 male rats at 150 mg/kg. There were no major differences in the behavior of the total radioactivity between the methyleneand imidazolidine-radiolabeled imidacloprid. The absorption of orally administered 14C-imidacloprid was rapid as evidenced by the calculated lag-time of less than 2.5 min. The average half-life of absorption was estimated as about 35 min (for both sexes and all dose-groups). After oral administration of 1 mg/kg or 20 mg/kg [14C]- imidacloprid, the plasma concentrations reached maximum between 1.1 h and 2.5 h at; and at 4 h after 150 mg/kg (Klein, 1987; Klein and Brauner, 1991a). Approximately 90-98% of the administered radioactivity was recovered within 24 h. Adjusted for 100% mass balance, the average radioactivity recovered in the urine within 48 h was 78% (males) and 74% (females) at 1 mg/kg imidacloprid. At 20 mg/kg imidacloprid, these values were and 77% and 82% for males and females, respectively. The urinary recovery after 14 day-preloading was in the same range (74% for the males and 75% for the females (Klein and Karl, 1990). Adjusted for 100% recovery, the 48-hour radioactivity in the feces was 18-26% of the administered activity. The major urinary metabolites were 6-chloronicotinic acid and its glycine conjugate (WAK 3583), which represented about 30% of the recovered radioactivity (Klein and Karl, 1990;). Additional urinary metabolites included 5-OH-imidacloprid (WAK 4103, 15-18%), the parent compound (9-15%) and olefinic imidacloprid (NTN 35884, 8-13%). Metabolites identified in the feces included a glycine conjugate of 6-methylmercaptonicotinc acid, imidacloprid, olefinic imidacloprid and desnitro-imidacloprid (all at 2-3%).

7-1-1-2- Dermal and Inhalation Absorption

There were no pharmacokinetic studies to determine the rate and extent of imidacloprid absorption upon inhalation exposure or via the dermal route. In the absence of data for inhalation uptake, both the DPR and the USEPA assume a default of 100%. The dermal absorption could be estimated by comparing the oral and dermal LD50. The ratio between the oral and dermal LD50 for rats was 8.5% (see Tabl 1 and studies by Bomann, 1989b and Krotlinger, 1989). An oral developmental toxicity study and a 28-Day subchronic dermal toxicity study in rabbits were the only available studies to compare the oral and dermal thresholds. The developmental toxicity study established a maternal LOEL of 72 mg/kg/day (Becker and Biedermann, 1992; Section III.G.2. under DEVELOPMENTAL TOXICITY). A LOEL could not be determined from the 28- day dermal study in rabbits, because no toxicity was observed at the only tested dose of 1000 mg/kg/day (Flucke, 1990, Section III.C.4 under SUBCHRONIC TOXICITY). A dermal absorption of 7.3% could be calculated from the oral LOEL of 72 mg/kg/day and from a dermal NOEL of 1000. However, this dataset may not provide a reliable estimate of the dermal absorption, because a dermal toxicity threshold was not clearly defined in the 28-day dermal study.

7-1-2- Distribution (Tissues and Organs)

Analysis of the basic pharmacokinetic parameters revealed that imidacloprid was widely and rapidly distributed in the rat body. This was evidenced by (*i*) the large apparent distribution volume (Vc), which accounted for about 84% of the total body volume distribution volume and (*ii*) by the short half-life for distribution of the radioactivity after an i.v. administration (3 h). The same half-lives for distribution of the radioactivity (2.6-3.6 h) were calculated after single oral doses of 1 and 20 mg/kg or after multiple doses of 1 mg/kg. To quantify the radioactivity in the body, male rats were sacrificed at 1-48 h (5 animals/ time point) after oral administration of 20 mg/kg 14C-imidacloprid. Imidacloprid had a high ability to permeate tissues, as radioactivity was detected in all of the 13 tested tissues and organs. All tested organs contained the highest radioactivity at 40 min to 1.5 h after dosing. The highest concentrations were measured in the gastrointestinal tract, liver, kidney, lung and heart. The respective concentrations (P) 1 of the radioactivity in these tissues normalized to dose were 4 (GI), 1.3-1.7 (liver and kidney), 0.9 (lung) and 0.74 (heart). The brain, which is a presumed target for imidacloprid, was not among the tissues and organs analyzed for radioactivity. Overall, the results from the distribution studies indicated that there was no dependence on dose, sex or pretreatment.

The whole body autoradiography confirmed the findings from the quantitative pharmacokinetic studies. In these experiments, one male rat was injected intravenously with 20 mg/kg methylenelabeled 14C-imidacloprid and sacrificed 5 min later (Klein, 1987). Six more male rats received the same dose orally and were sacrificed in a period of 1-to 48 h. Sections from the fixed rats were then prepared for autoradiography. Five min after an i.v. injection, the concentration of the radioactivity in the blood was lower than in many of the organs such as liver, kidney, muscle and thyroid, thus indicating a fast turnover of imidacloprid. One hour after an oral administration, imidacloprid was readily absorbed from the gastrointestinal tract, as the radioactivity was found in nearly all organs and tissues. The high labeling of the liver and over the entire kidney was indicative of an ongoing biotransformation and renal excretion. Other tissues with increased radioactivity included the glandular organs (adrenal, thyroid and salivary glands) and connective tissues associated with the skin, walls of aorta and spinal cord. The intensive labeling over the kidney at 4 and 8 h was consistent with the high rate of renal excretion estimated in the quantitive pharmacokinetic study (about 60% at 8 h). Radioactivity was also found in the CNS, thus indicating that imidacloprid and its metabolites penetrated the blood-brain barrier. However, of all analyzed organs and tissues, the fatty tissues and the CNS had the lowest labeling.

7-1-3-Metabolism

Based on the profile of the metabolites, two major routes were proposed for the imidacloprid metabolism in the rat (Klein and Karl, 1990; Thyssen and Machmer, 1999). In the first route, imidacloprid undergoes oxidative cleavage to imidazolidine and 6-chloronicotinic acid. The imidazolidine is directly excreted via the urine. The nicotinic moiety is detoxified via glutathione conjugation to a derivative of mercapturic acid and then to mercaptonicotinic acid. The mercaptonicotinic acid is, in turn, conjugated with glycine to hippuric acid-conjugate for excretion. The second route involves hydroxylation in the imidazolidine ring, followed by elimination of water and formation of an unsaturated metabolite (olefinic imidacloprid NTN 35884). The metabolism in hens and goats was similar to that in rats (Klein and Brauner, 1991 Karl *et al* ;. 1991; Klein 1992). Studies with recombinant human isozymes of CYP450 have suggested that a single isozyme, CYP3A4, both oxidizes and reduces imidacloprid at the imidazolodine and nitroimine moieties (Schulz-Jander and Casida, 2002). A major metabolite upon incubation of imidacloprid with the human microsome-NADPH system was tentatively identified as a derivative of hydrazone (the =N-NO2 moiety becomes =N-NH2 or desnitro-imidacloprid). Based on these results, a yet unidentified

microsomal NADPH-nitro reductase was implicated to bioactivate imidacloprid in humans into the more toxic metabolite desnitro-imidacloprid (Schulz-Jander *et al* ;. 2002). In rats, there were no sex differences in the metabolic profile at the lower dose (1 mg/kg imidacloprid (Klein and Karl, 1990). However, male rats showed an increased ability to metabolize higher doses of imidacloprid (20 mg/kg), resulting in significantly lower amount of the parent compound and an increased level of the metabolite olefinic imidacloprid. The formation of other biotransformation products was similar in males and females. From a toxicological point of view, the formation of the metabolite desnitro-imidacloprid (NTN 33823) in rats is of particular interest, because of the following considerations: (*i*) this metabolite displayed a nicotinic-type action with a markedly higher toxicity to mammals than imidacloprid, (*ii*) desnitro-imidacloprid was identified as the major degradation product of imidacloprid in the environment (i.e. the major photodegradate and the major product of microbial and plant metabolism) and (*iii*) desnitro-imidacloprid was a major metabolite produced *in vitro* with human liver microsomes. In rats, desnitro-imidacloprid was identified only in the feces and represented a relatively small amount of the total recovered radioactivity (about 2-3%, Klein, 1987). In hens, desnitro-imidacloprid was detected in eggs, muscle and fat tissues (5-12%, Klein and Brauner 1990, 1991).

An additional biotransformation product of imidacloprid, the nitrosoimine metabolite WAK 3839, was identified in the urine of chronically fed rats and mice (1 year, 1800 ppm, Klein, 1990b). WAK 3839 represented about 9% of total urinary radioactivity. Since this metabolite was not found after a single dosage of up to 150 mg/kg, it was proposed that the reduction of the NO2-moiety of imidacloprid takes place only if the enzymes catalyzing other biotransformation reactions (e.g. oxidative cleavage to 6-chloronicotinic acid) are saturated by chronic “flooding” of the liver with imidacloprid. In rats, the acute toxicity of WAK 3839 was about 5-fold lower than that of imidacloprid (Kaoru, 1991).

The toxicity of the major metabolites of imidacloprid in rats, (6-chloronicotinic acid 4- or 5-OHimidacloprid and olefinic imidacloprid) has not been evaluated in mammals. Studies in invertebrates showed that the olefinic- and hydroxy- compounds had similar acute toxicity to the parent compound, whereas the 6-chloronicotinic acid did not act as a nicotinic agonist (Nauen, *et al.*, 2001). All of these compounds contain the 6-chloropyridinyl moiety and are included in the tolerances established for the imidacloprid residues.

7-1-4-Excretion

The half-lives for excretion of the radiolabeled imidacloprid were calculated in rats after a single i.v. dose of 1 mg/kg, after single oral doses of 1 and 20 mg/kg or after multiple doses of 1 mg/kg Klein, 1987). The excretion half-life values varied greatly (from 26 h to 118 h), but the variation was not dose-, sex-, or route-dependent. In allose groups, less than 1% of the radioactivity was left in the body after 48 h of dosing. The results from the whole body autoradiography confirmed that within 24-48 h the radioactivity was nearly eliminated from the body, with skin, nasal mucosa, liver, kidney and the thyroid being the only tissues with residual radioactivity. Altogether, these data indicated that imidacloprid did not significantly accumulate in the rat body.

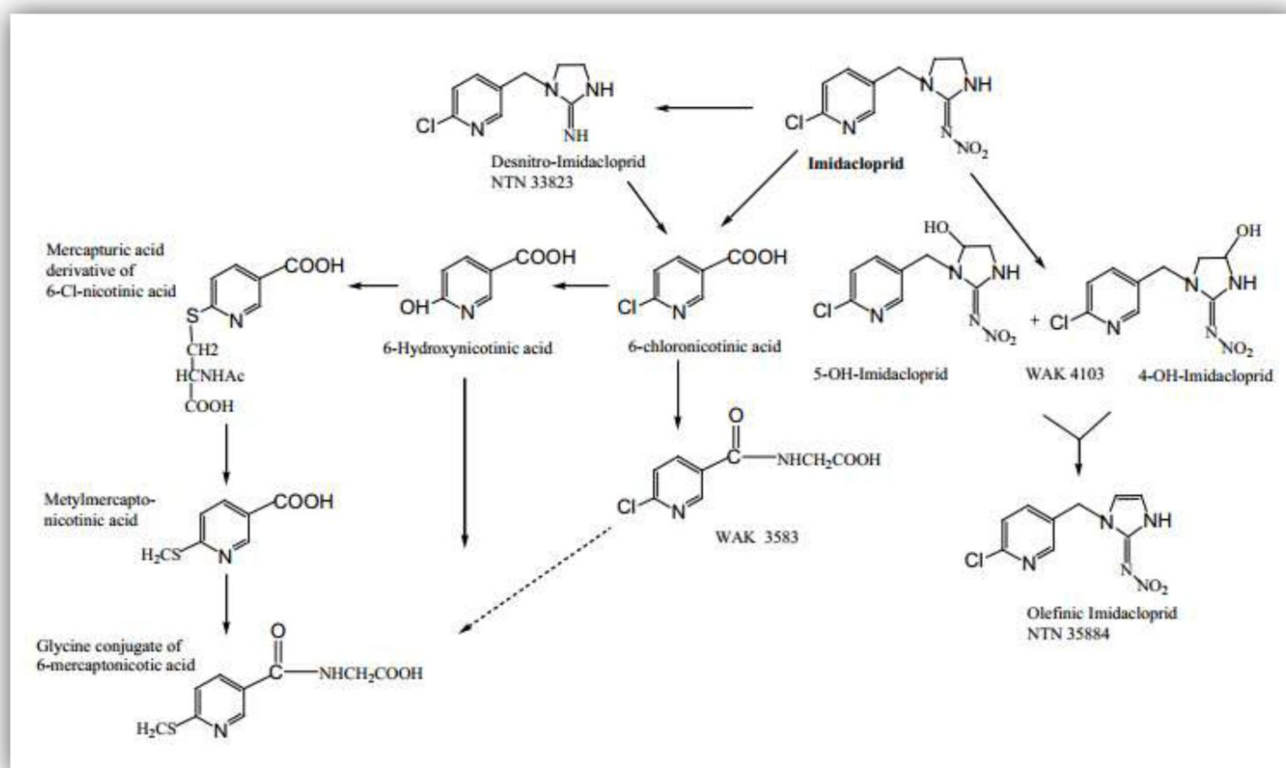


Figure 4. Biotransformation Pathways of Imidacloprid (Thyssen and Machmer, 1999).

chapter03 : **Anatomical and Physiological similarities of Kidney**

1-Introduction

The kidney is a vital organ of the mammalian body and becomes a foremost subject of medical research because many renal diseases in humans are incurable when the kidney is severely damaged (Fedorova *et al*,2008). It is also a primary target organ in preclinical studies, in which drug-induced nephrotoxicity is a recurrent finding in preclinical studies. In acute renal failure, the damaged tubular epithelium is repaired through repopulation and tubular function recovers in most cases. The tubular epithelium is especially sensitive to toxic compounds because of water and solute absorption and active transport systems, which result in the concentration of toxicants in the tubular cells (Pazhayattil and Shirali 2014).

The nephron is the function unit of kidney and greatly varies in its structure amongst different vertebrates; also the formation of nephrons shows a variable degree of differences among species. In birds, the kidney has two kinds of nephrons; one is reptilian type and small sized, without loops of Henle, and other is mammalian type large in size with long or intermediate length loops (Lentine *et al* ;.2017).

In contrast, the morphological process of podocytes development has been reported in the classic kidney of human, horse, goat, monkey, rabbit, cat, dog, chicken, medaka fish , rat and guinea pig indicating that, it would be a good model system for studying renal regeneretion.

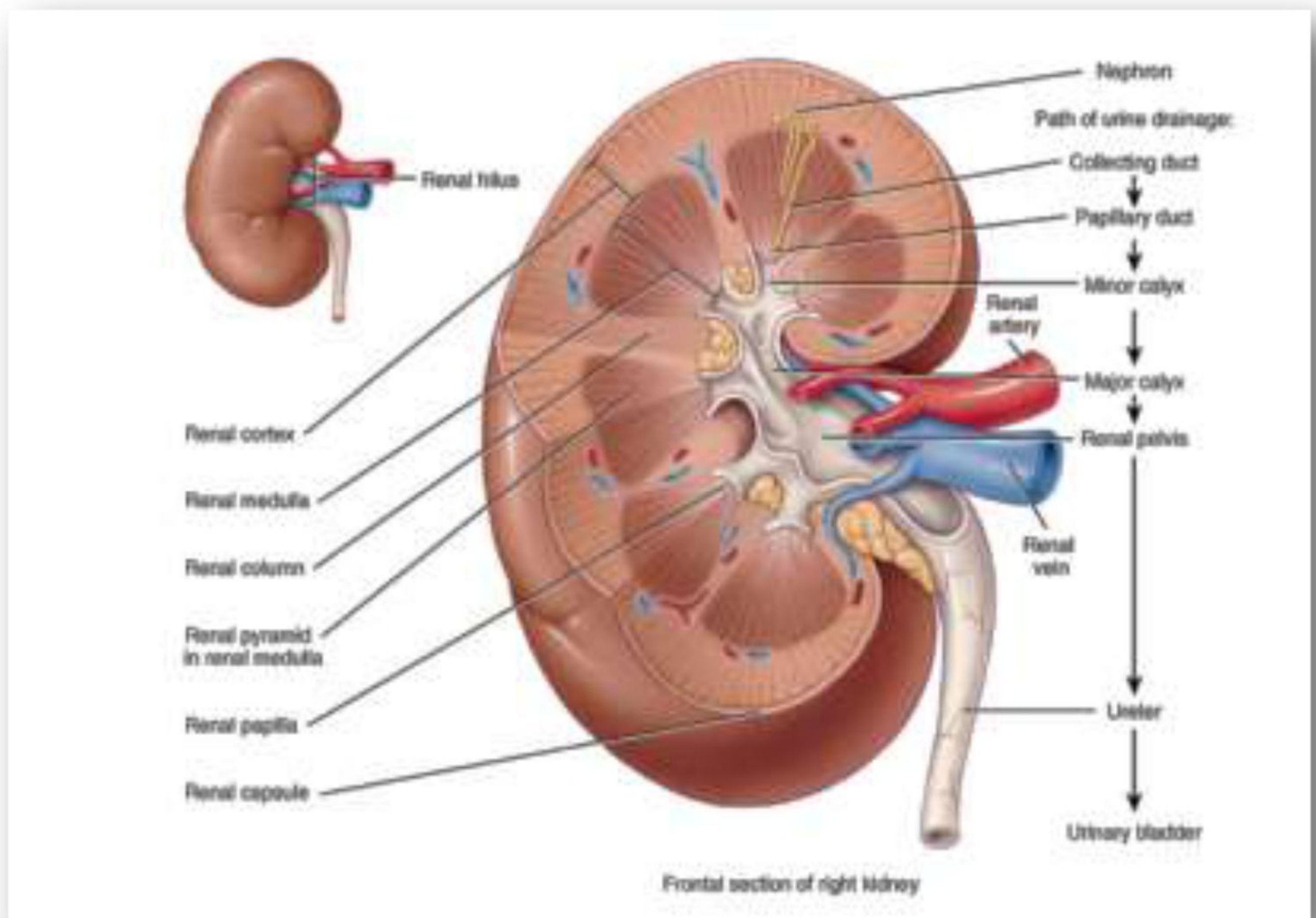


Figure 5: Kidney anatomy and pathway of urine drainage(lentine *et al*,.2017).

2-The physiological function of the kidney

Urine is the wastage of by-product formed through excess water and waste molecules during the metabolic process of renal system filtration. The primary purpose of the renal system is to regulate blood volume, maintain plasma osmolarity and removal of wastage *via* urine, which is mostly a convenient way to performs many body functions (Lentin *et al* ;.2017).

2-1-Urine formation occurs through following three processes:

-Filtration

-Reabsorption

-secret

2-1-1-Filtration

During filtration blood enters the afferent arteriole and flows into the glomerulus where filterable blood components, such as water and nitrogenous waste, moves towards the inside of the glomerulus, and non-filterable components, such as cells and serum albumins, exit *via* the efferent arteriole. These filterable components accumulate in the glomerulus to form the glomerular filtrate. On average, about 20% of the total blood pumped by the heart per minute enters into the kidneys to undergo filtration The remaining 80% of the blood flows throughout the body to facilitate tissue perfusion and proper exchange of gas(Lentin *et al* ;.2017).

2-1-2-Reabsorption

During reabsorption molecules and ions present in the blood will be reabsorbed into the circulatory system. The fluid passes through the components of the nephron (i.e. proximal/distal convoluted tubules, the loop of Henle and collecting duct) as water and ions are removed due to the change in fluid osmolarity (ion concentration). In the collecting tube, secretion will occur before the fluid leaves the ureter in the form of urine(Feherh ,2017) .

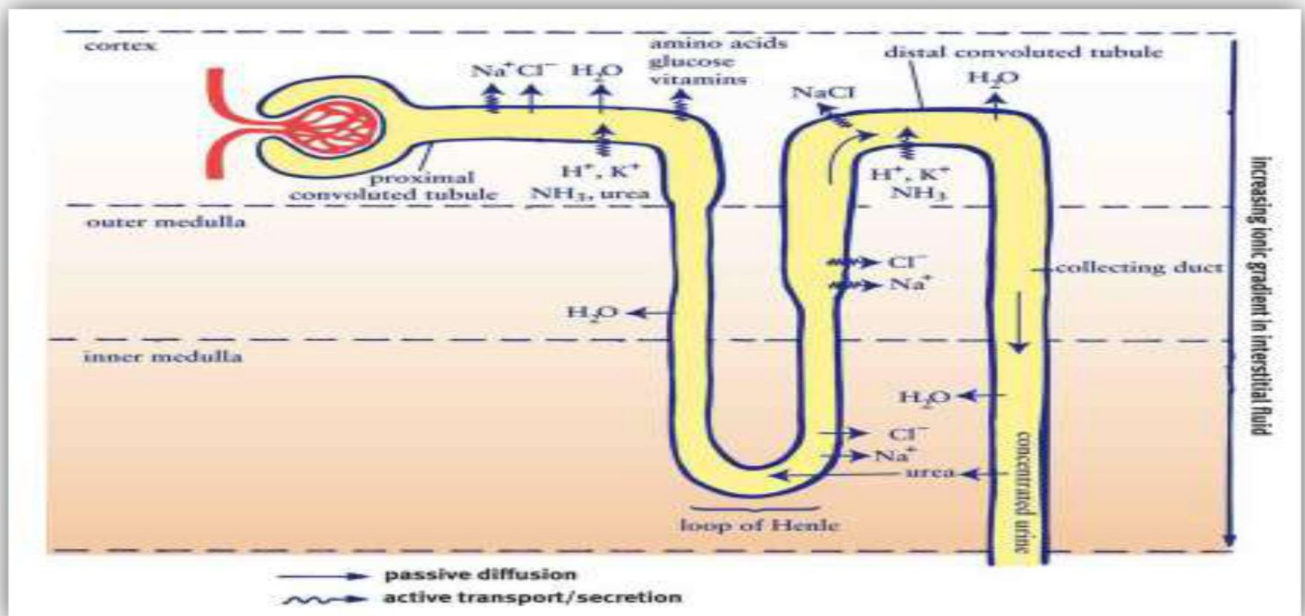


Figure 6: The physiological function of kidney and process of urine formation(Feher,2017).

2-1-3-Secretion

During secretion some substances such as hydrogen ions, creatinine and drugs will be removed from the blood through the peritubular capillary network into the collecting duct(Feher ;2017).

Pratical part :

1-Materials and methods

1-1-materials

1-1-1-**chimical product** : in these work we had used the imidacloprid which is systemic insect her chemical formula(C9H10CIN5O2) ,we brought it poudre than we mixed it with distilled water to get the imidacloprid solution .

1-1-2- animals

We brought the rats from pasteur institute which is located in algeria ; all the rate was male , young , sexuelle mature their life span is 0.5 years, weighed from (127 g to 243) .

1-2-Methods

1-2-1- Breeding conditions: During these period which is 3 monthwe put them in a certain temperature 25c and humidite 40-44 and we were able to feed them daily and and change there batches every week

1-2-3- dosage seletion : We had selected two defferent doses 5 mg /kg and 50 mg/kgOfimidaclopridandtreatedtheratsorally for 20 days , the select of this 2 doses based on study realized on the exploration of this insecticide is shows that the dose 5 mg is NOEL dose and the 50 mg is toxic dose

1-2-4- reparation and treatement of rats :

After preparation of imidacloprid we treated the rats :

GroupeA : treated by 5 mg

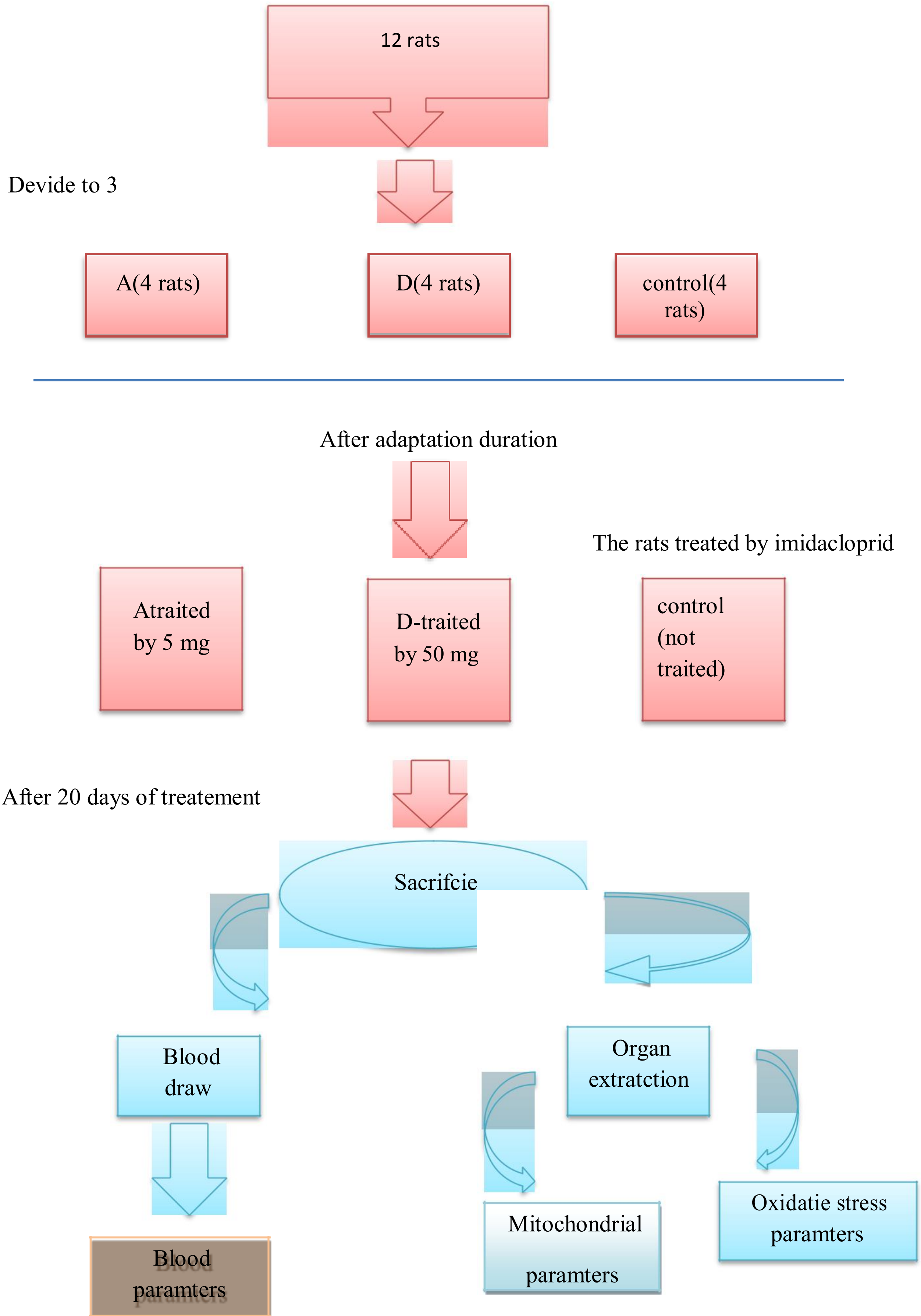
GroupeD : treated by 50 mg

Contrôl : did not treated

1-2-5-**the sacrifice** :

after the adaptation period (20 days) we had slaughtered the rats and drawed the blood after that we puted in dry tube for (blood paramters) and dissected the rats then we extracted the organes (for mitochondrial test and oxidative stress paramters)

2-Experimental part plan :



3-Parameter analyzed and methods:

3-1- Blood paramters:

3-1-1- urea determination :

The urea dosage was carried out by the enzymatic colorimetric urease method according to the technical sheet (Spinréact).

Urea is enzymatically hydrolyzed with ammonia (NH_4^+) and carbon dioxide (CO_2). The ammonia ions thus formed react with salicylate and hypochlorite (NaClO), in the presence of a nitroprusside catalyst, to form a green indophenol, according to the reactions below: Experimental protocol

- Add 10µl of sample to 1ml of reagent 1 [urease 30,000 U / L dissolved in a phosphate buffer pH6.7: 50mmol / l containing (EDTA: 2mmol / l, sodium salicylate: 400mmol / l, sodium nitroprusside: 10mmol / l)].

3-1-2--Creatinine determination :

according to the Spinréact technical sheet.PrincipleThe creatinine present in the sample reacts with picric acid in an alkaline medium to give a colored complex, measured in a defined time interval (Murray *et al*., 1984).

Experimental protocol

- Add 100µl of sample (serum) to 1ml of working reagent [V / V: R1: 17.5 mmol / l of picric acid + R2: 0.29 mmol / l of sodium hydroxide].

Reading

Read the absorbance (Al) after 30 seconds and after 90 seconds (A2) for the sample at a wavelength 492nm.

3-2- mitochondrial paramters :

3-2-1-Preparation of cytosolic samples:

one gram of renal tissue was homogenized in 2 ml of phosphatesaline buffer solution (PBS; pH 7.4). Then the homogenates were centrifuged at 3000 rpm for 15 min at 4 ° C and the resulting supernatant was used for the determination of levels of MDA, GSH and the enzymatic activity of GST, GPx, SOD and CAT.

3-2-2-Preparation of mitocondrial suspension:

The mitochondria are extracted according to the method described by(Rustin *et al* 1994), it is a purification by differential centrifugation. Briefly, after decapitation of the rats, kidneys are quickly removed and immersed in TSE buffer (10mM tris, 250mM sucrose, 0.1mM EDTA, pH 7.2 at 4C °). The renal tissue is cut finely and potérisés 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 allowing thus removing large cellular debris. The recovered pellet is centrifuged a second times at 10000rpm for 10min. The supernatants from the two centrifugations are recovered 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 base from the latter centrifugation is resuspended in 1 ml of TS buffer (250 mM sucrose, 50 mM tris, 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 assessment of structural integrity and functional of the mitochondria, the rest is stored at -80 ° C for further assays.

3-2-3- Preparation of the mitochondrial matrix:

The mitochondrial suspension is frozen-thawed 6 to 8 times with increased potization and repeated, to burst the mitochondria. After centrifugation for 10 min at 9600 rpm, the supernatant is used as a source of stress parameters (Lahouel *et al*;. 2015).

F. Assessment of mitochondrial swelling, permeability and respiration According to the method of (Crystal *et al* 1996), we carried out the estimation of the permeability mitochondrial based on the rate of Ca ++ ions crossing their membranes, this patency followed by an increase in the size of the mitochondria detected at a 540nm wavelength for 3 minutes and every 30sec. Breathing has been estimated using an Oxygraph (Hansatech®) according to the method described by (Rouabhi *et al* ;.2006; 2009)

3-3- Evaluation of oxidative stress parameters :

3-3-1-Glutathione (GSH) dosage :

The dosage of glutathione is carried out according to the method of (Weckbeker and Cory 1988). The principle of this assay is based on the measurement of the absorbance of 2-nitro-5- acid mercapturic, the latter resulting from the reduction of 5,5`-dithio-bis-2-nitrobenzoic acid (DTNB) by groups (-SH) of glutathione. Once prepared, the sample (cytosol / matrix) must undergo deproteinization with sulfosalycilic acid (0.25%) in order to protect the SH-groups from glutathione. Briefly; the samples (200mg of tissue) are placed individually in the presence of 8 ml

of EDTA solution (Ethylene Diamine Acid Tetra Acetic) at 0.2M. The mixture put in ice cubes is crushed using a pestle in porcelain. The homogenate is then deproteinized by taking 0.8ml of the latter to which add 0.2ml of a 0.25% sulfosalycilic acid (SSA) solution. The mixture is vortexed and left for 15 min in an ice bath, and then centrifuged for 5 min at 1000t / min. 0.5ml of the supernatant is removed to which is added 1ml of tris-HCL + EDTA buffer (0.02M), pH 9.6. To the mixture is added 0.025ml of 5,5`-dithio-bis-2-nitrobenzoic acid (DTNB) at 0.01M dissolved in absolute methanol. The mixture is left to stand for 5 min at room temperature and the absorbance (A) is measured at 412nm.

3-3-2-Determination of MDA :

The MDA assay is carried out according to the method of(Esterbauer *et al* 1992). The principle of this assay is based on the condensation of MDA in an acid medium and under hot conditions with the acid thiobarbiturate, to form a pink pigment. An amount of 375 µl of supernatant is taken in a dry tube, to which is added a volume of 150 µl of the TBS solution (sorted 50 mM, NaCl (150 mM; ph 7.4) and 375 µl of the TCA-BHT solution (TCA 20%, BHT 1%), the mixture is Vortexed and centrifuged at 1000 rpm for 10.The MDA assay is carried out according to the method of (Esterbauer *et al* 1992**)**. The principle of this assay is based on the condensation of MDA in an acid medium and under hot conditions with the acid thiobarbiturate, to form a pink pigment. An amount of 375 µl of supernatant is taken in a dry tube, to which is added a volume of 150 µl of the TBS solution (sorted 50 mM, NaCl (150 mM; ph 7.4) and 375 µl of the TCA-BHT solution (TCA 20%, BHT 1%), the mixture is Vortexed and centrifuged at 1000 rpm for 10 min. Nephrotoxicity of two pesticides (p5mg and p50mg) and the prevention of this toxicity by quercetin in rats taken from the supernatant to which 80 µl of 0.6M HCL and 320 µl of the tris-TBA solution are added (tris 26mM, TBA120mM). At the end, the mixture is vortexed and is then incubated in a water bath at 80 ° C for 10 minutes. The reading of the optical density of the samples is measured by spectrophotometry at 530 nm

3-3-3-Glutathione peroxidase (GPx) assay :

The enzymatic activity of GPx is measured by the method of(Flohe and Gunzler 1984**),** using

H_2O_2 as a substrate. A volume of 0.2ml of cytosol / matrix is recovered in a tube containing 0.4ml of 0.1mM GSH and 0.2ml of 0.067M phosphate buffer, ph 7.8. The mixture is incubated in a water bath at 25 ° C for 05 min. 0.2ml of H2m2 1.3mM is added to initiate the reaction. After 10min 1ml of TCA 1% (tri chloro-acetic acid) is added in order to stop the reaction and the mixture is put in ice for 30 min and centrifuged for 10 min at 3000 rpm. A volume of 0.48 ml of supernatant is placed in a tank to which 2.2ml of 0.32M Na_2HPO_4 is added with 0.32ml of 1mM DNTB. This mixture formed a colored compound and its optical density is measured at 412nm every 30sec for 05min.

3-3-4-Glutathione S-Transferase (GST) activity assay :

The measurement of glutathione S-Transferase (GST) activity is determined according to the methode(Habig *et al*1974), It is based on the conjugation reaction between GST and a substrate, CDNB (1-Chloro2,4-dinitrobenzene) into a cofactor glutathione (GST), conjugation leads to the formation of a new molecule; 1-S-Glutathionyle 2-4Di nitrobenzene to measure GST activity.The value of the measured optical density is directly proportional to the amount of conjugate formed itself related to the intensity of GST activity. The samples are homogenized in 1ml of phosphate buffer (0.1M, pH6). The homogenate is centrifuged at 14,000 rpm for 30 min and the recovered supernatant will serve as a source of enzymes. The assay consists in reacting 200 µl of the supernatant with 1.2 ml of the CDNB mixture (1 mM), GSH (5mM) [20.26mg CDNB, 153.65mg GSH, 1ml ethanol, 100ml phosphate buffer (0.1M, ph 6)]. The absorbance reading is carried out for one minute and each 15sec at a wavelength of 340 nm against a blank containing 200 µl of distilled water replacing the amount of supernatant.

3-3-5-Assay of the Catalase activity

The spectrophotometric assay of the catalase activity (CAT) is carried out according to the methode (Cakmak and Horst 1991**).** The decrease in absorbance is recorded for three minutes by a spectrophotometer for a wavelength of 240nm and a coefficient linear linear extinction $\varepsilon = 39400$ $\mu M^{-1}.cm^{-1}.L$ for a final volume of 3ml, the mixture reaction product contains: 100µl of crude enzyme extract, 50µl of hydrogen peroxide 0.3% H_2O_2 and 2850µl of phosphate buffer (50mM, ph 7.2). The calibration of the device is made in the absence of the enzyme extract. The reaction is triggered by the addition of water oxygenated.

3-4-Evaluation of biochemical parameter

3-4-1-fat assay : Tissue lipids are evaluated according to the method (Goldsworthy *et al.,* 1972), using 2 00µl ofhomogenate in 5ml of 20% trichloroacetic acid (TCA), crushed and filtered this

mixture ; and directly applied centrifugation at 5000 rpm for 10 min. The pellet is kept in a tube containing 1 ml of the Ether / Chlorophorme mixture, and after centrifuging this mixing at 5000 rpm for 10 min, 100 µl of the supernatant is removed, to which 1 ml is added sulfuric acid and after stirring the tubes in a water bath at 100 ° C for 10 minutes. After cooling, the sample is taken again using a 200 µl micropipette of the extract to which 2.5 ml of the 85% sulfophosphovanillin mixture is added (0.38 g vanillin + 195ml orthphosphoric acid + 55ml H2O) and leave this mixture 30min in the dark, reading at a wavelength of 530nm. The actual concentrations are calculated from the equation deduced from the calibration range carried out from a stock solution prepared in using sunflower oil.

3-4-2-Protein dosage

The method used for the determination of proteins is that of Bradford (1976) which uses the BSA as standard, on the same sample used to measure the lipids, the pellet from the second centrifugation to which 1 ml of NaOH (0.1N) has been added and the mixture is stirred energetically for the dissolution of proteins. Then we take, by means of a micropipette, a volume of 100µl to which 4ml of the BBC reagent (Brilliant Blue of Coumassie) (50mg BBC + 50ml of 85% orthophosphoric acid and make up to 500ml with distilled water). Thus a blue color develops and we pass directly samples for reading at 595nm wavelength. Calculation of concentrations is done by the equation deduced from the calibration range made from an albumin solution of beef serum

4-Statistical calculations :

Results were expressed as Mean ± SEM. Statistical significance , and to better visualize using the Excel 2013 office to represent these results in the form of graphs and histograms. Statistical analysis was performed using the Minitab® 19.1 software . was determined by one way analysis of variance anddunnet„stestandtuckey, 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

4-results

4-1-Relative kidney weight

kidney weight :	groups of rats treated		
	Control	concentration5mg(group A)	(concentration50mg Group D)
Left kidney weight (g /100g of BW)	0.3225±0.01707	0.3225±0.01707	0.385±0.0057**
Right kidney weight (g/100gof BW)	0.33±0.0141	0.33±0.0141	0.365±0.033***

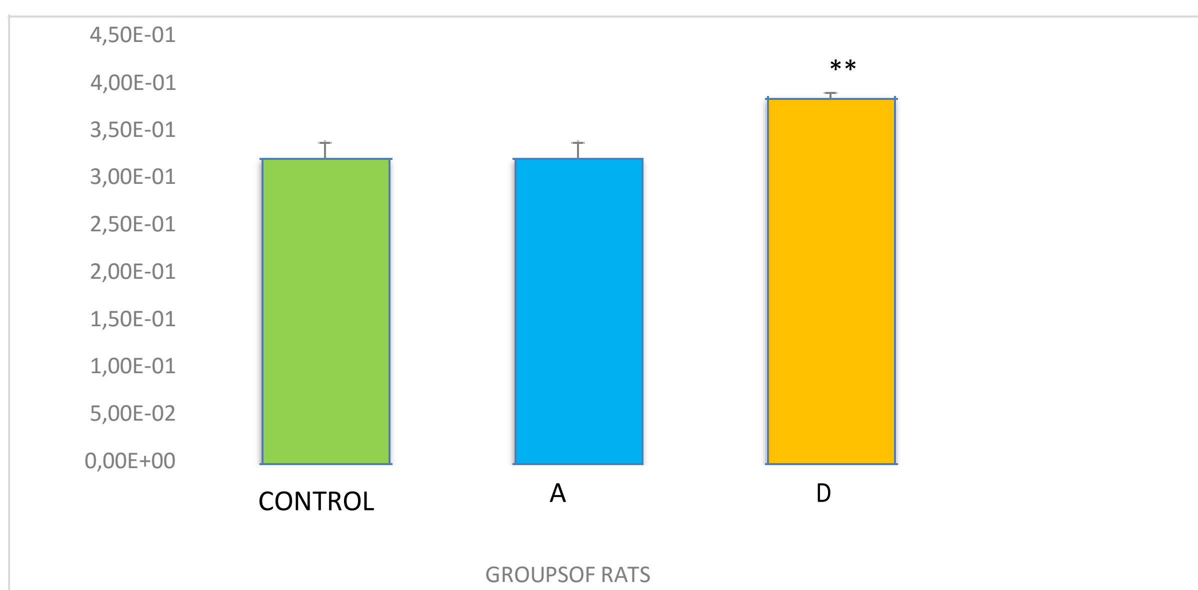


Figure 07 : Evaluation of relatif left kidney weigh(g/100gBW) in contrôl and treated group

BW : body weight

The results obtained of relatif left kidney weight shown us highly significant increase p≤0.01) of relatif left kidney weight in the groupe D that treated with concentration 50mg and non significative change of relatif left kidney weight 0.05<p in the groupe A that treated with concentration 5 mg

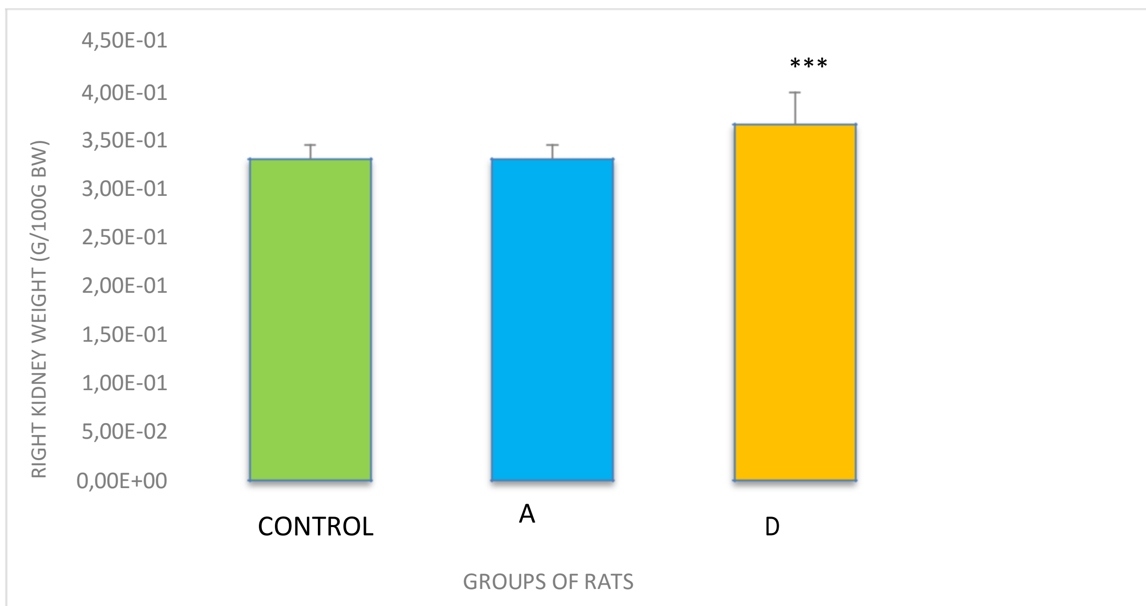


Figure 8 : Evaluation of right kidney weight(g/bw) in control and treated group

BW : body weight.The results obtained of relatif right kidney weight have shown us very highly significant increase $p<0.001$ of relatif right kidney weight in the groupe D that treated with concentration 50mg and non significant change$0.05<p$ of relatif right kidney weight in groupA that treated with concentration 5 mg when they both (A,D) compared to contrôl group.

4-2-impact of imidacloprid on oxidative stress parameter :

Parameters	groups of rats		
	Contrôl	concentration5mg(group A)	concentration50mg (groupD)
GSH(nmol/min/mgof p)	1.23^E-5±0.64^E-6	8.06^E-5±3.39^E-5 **	2.29^E-5±1.53^E-5 **
GPx (umol/min/mgof p)	5.37^E-12±2.75^E-12	5.37^E-12±2.75^E-12	9.61^E-12±1.31^E-12
GST(nmol/min/mgof p)	7.25E-07±2.8^E-07	7.25^E-7±2.8^E-7	2.4^E-7±1.03^E-7
MDA(nmol/min/mgof p)	1.43^E-5±0.98^E-06	1.69^E-5±0.98^E-06	9.18^E-6±5.78^E-5
CAT(umol/min/mgof p)	3.14^E-10±2.37^E-10	3.14^E-10±2.37^E-10	2.27^E-9±0.57^E-09

4-2-1- Glutathione (GSH)

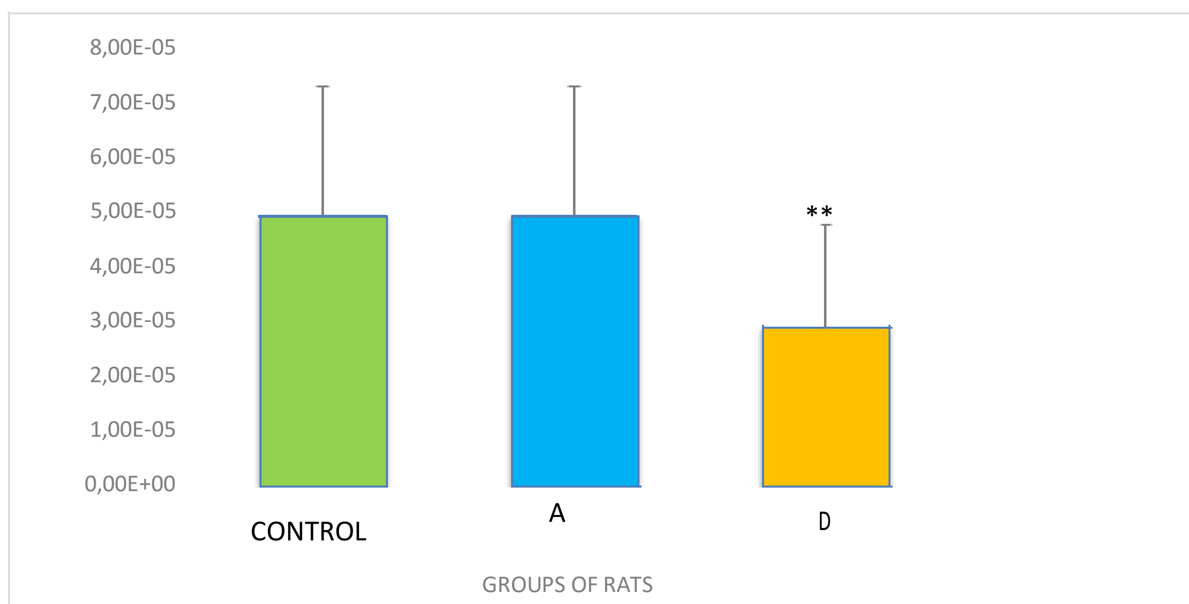


Figure 9: Evaluation of GSH level(nmol/min/mg of proteins) in treated and contrôl group for 20 days of treatement.

Statistical analysis of the results obtained after the evaluation of the cytosolic activity of GSH showed us highly significant decrease ($p \leq 0.01$) of GSH level in groupD that treated with concentration 50 mg and no significant change of GSH level in group A that treated with concentration 5 mg all compared to control group

4-2-2- GST

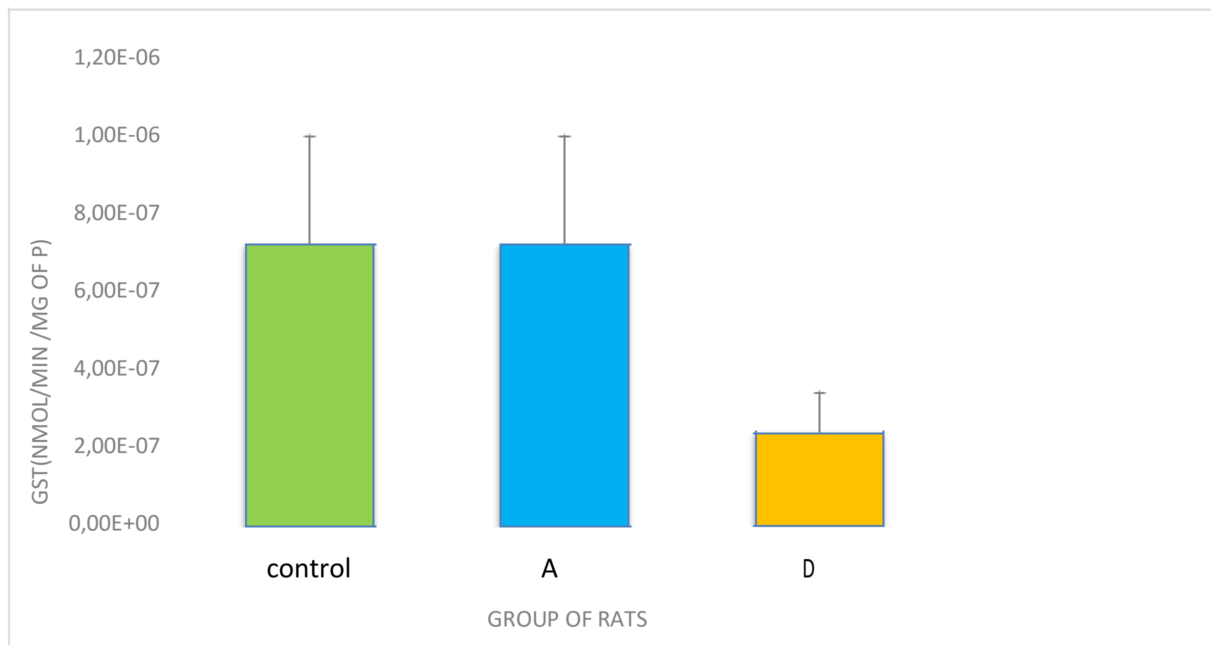


Figure 10 : evaluation of GST level(nmol/min/mg of p) in contrôl and treated group for 20 days of treatement.

Statistical analysis of the results obtained after the evaluation of the cytosolic activity of GST showun us non significative decrease ($P > 0.05$) of GST level in groupD that treated with concentration 50mg and non significant change of GST level in group Athat treated with concentration 5 mg all compared to control group

4-2-3-CAT :

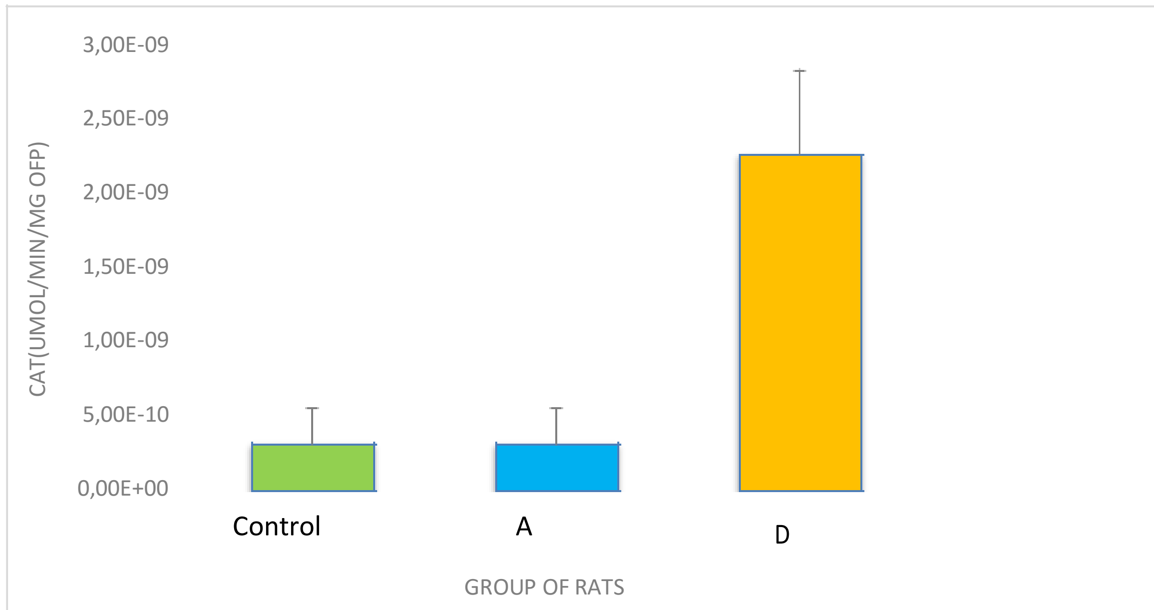


Figure11 :Evaluation of CATlevel (umol/min /mg of p)in control and treated group for20 days of treatement

The results of the evaluation of catalase activity in the cytosol of the kidney showun us non significant increase ($P > 0.05$) of CATlevel in the group D that treated with concentration 50 mg and non significant change of CAT level in the group A that treated with concentration 5 mg compared to control group.

4- 2-4-Gpx :

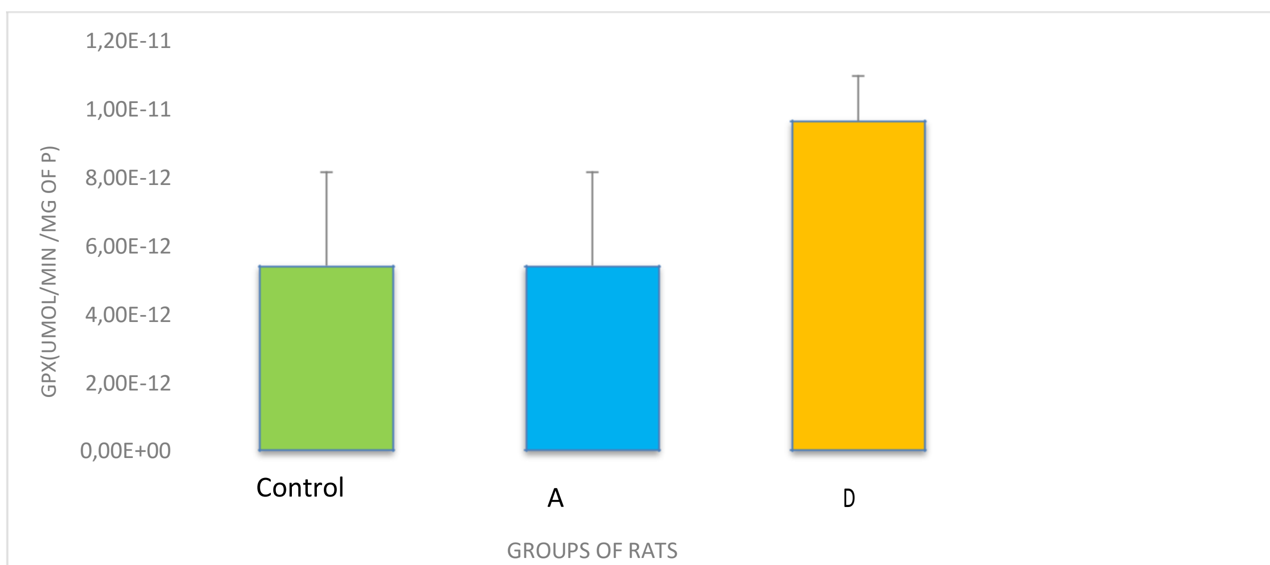


Figure13 : Evaluation ofGPX level(umol/min/mg of p) in contrôl and treated group for 20 days of treatemet

Statistical analysis of the results obtained after the evaluation of the cytosolic activity of GPx shown us non significative increase ($P > 0.05$) of GPX level in the group D that treated with concentration50mg and non significant chang of GPX level in group A that treated with concentration5 mg when they are all compared to the control group.

4-2-5-MDA :

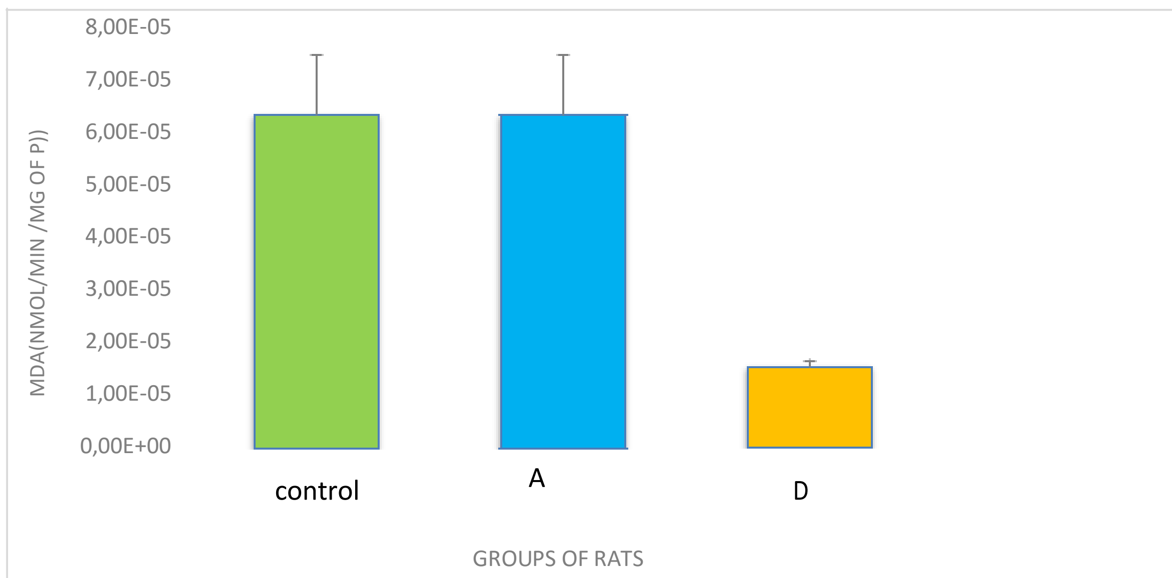


figure14 : Evaluation of MDAlevel (noml/min /mg of p) in control and treated group for 20 days of treatement.

Statistical analysis of the results obtained after the evaluation of the cytosolic activity of MDA show us non significative decrease ($P > 0.05$) ofMDA level in group D that treated with concentration 50mg and non significant change of MDAlevel in group A that treated with concentration 5 mg when they are all compared to the control group.

4-4--Effects of imidacloprid on biochemical parameters :

Parameters	groups of rats		
	Control	Concentration 5mg group A	Concentration 50mg group D
Protein(g/l)	9.715^{E}-05±1.26E-5	9.7175^{E}-5±1.26^{E}-05	7.56^{E}-5±4.01^{E}-5*
Fat(g/l)	7.2^{E}-06±2.55^{E}-06	7.2^{E}-6±2.55^{E}-6	5.29^{E}-6±2.18^{E}-5*

4-3-1-Proteins

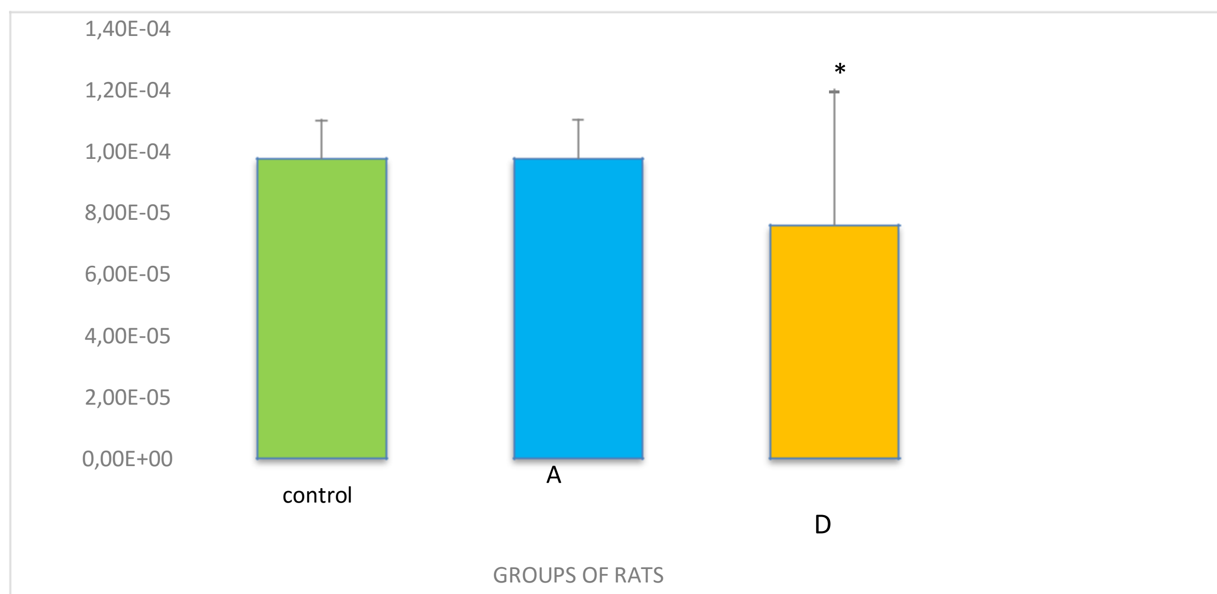


Figure 15 :Evaluation of protein level (g/l) in control and treated group for 20 days of treatement

Statistical analysis of the results obtained after the evaluation of the cytosolic activity of Proteins have show us a significative decrease0.05> P> 0.01 of protein level in group D that treated with concentration 50mg and non significative change of MDA level 0.05<p in group A that treated with concentration5mg when they are all .

4-4-2-fat:

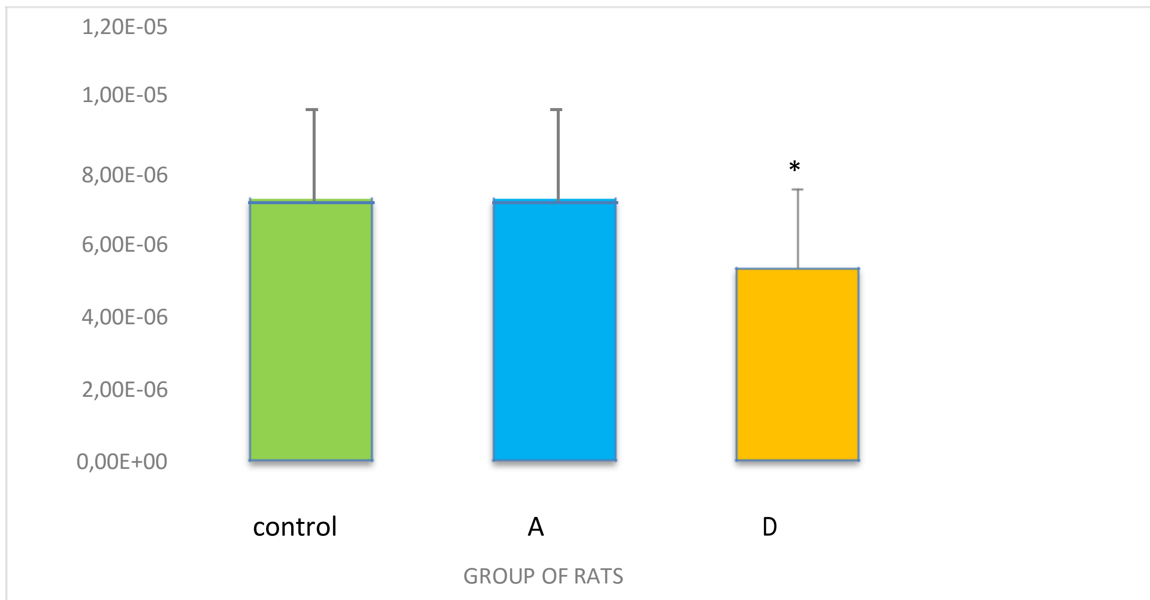


Figure 16 : Evaluation of fat level (g/l) in control and treated group for 20 days of treatement

Statistical analysis of the results obtained after the evaluation of the cytosolic activity of lipide (fat) have show us non significative change (P > 0.05) of lipide level in group A that treated with concentration 5mg and significative decrease 0.05> P> 0.01 of lipide level in groupD that treated with concentration 50mg when they are all compared to the control group.

4-5—impact of imidacloprid on blood paramtres :

Parameters	**groups of rats**		
	Contrôl	concentration5mg group A	concentration50mg group D
Uree(mmol/l)	8.4 E-06± 1.89 E-06	8.4 E-06±1.89 E-06	1.43 E-5±0.26 E-5**
Creatine(umol/l)	4.10 E-04±1.90 E-04	4.10 E-04±1.90 E-04	6.50 E-04±1.70 E-04**
Creatinine clearence(umol/l)	7.40 E-04±-0.50 E-04	7.40 E-04±0.50 E-04	3.88 E-04±0.80 E-04**

4-4-1-Cratinine :

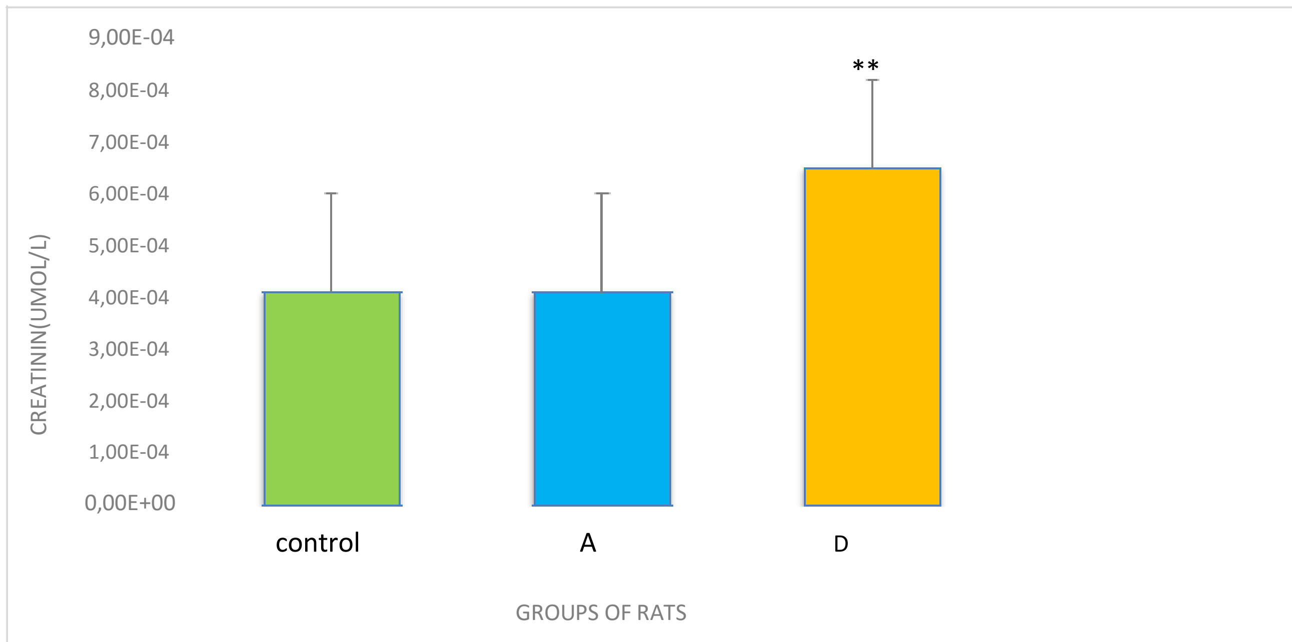


Figure 17 :Evaluation of creatinin level (umol/l) in control and treated group for 20 days of treatement

Statistical analysis of the results obtained after the evaluation of the cytosolic activity of creatinine have show us non significant change($P > 0.05$) of creatinin level in group A that treated with concentration 5mg and highly significant increase (**) $0.01> P> 0.001$ of creatinin level in groupeD that treated with concentration 50mg when they are all compared to control group

4-4-2-Urea

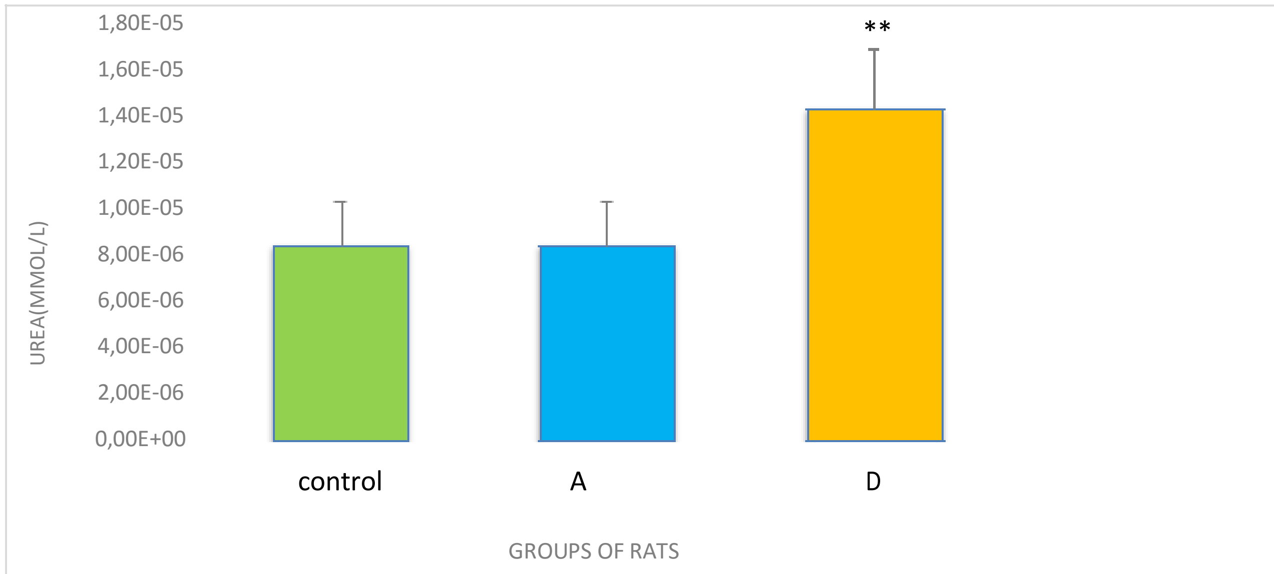


Figure18 : Evaluation ofurea level (mmol/l) in control and treated group for 20 days of treatement

Statistical analysis of the results obtained after the evaluation of the cytosolic activity of urea have show us non significant change ($P > 0.05$) of urea level in groupA that treated with concentration 5mg and highly significant increase(**) $0.01 > P > 0.001$ of urea level in groupDthat treated with concentration 50mg when they are all compared to the control group.

4-4-3-Creatinine clearence :

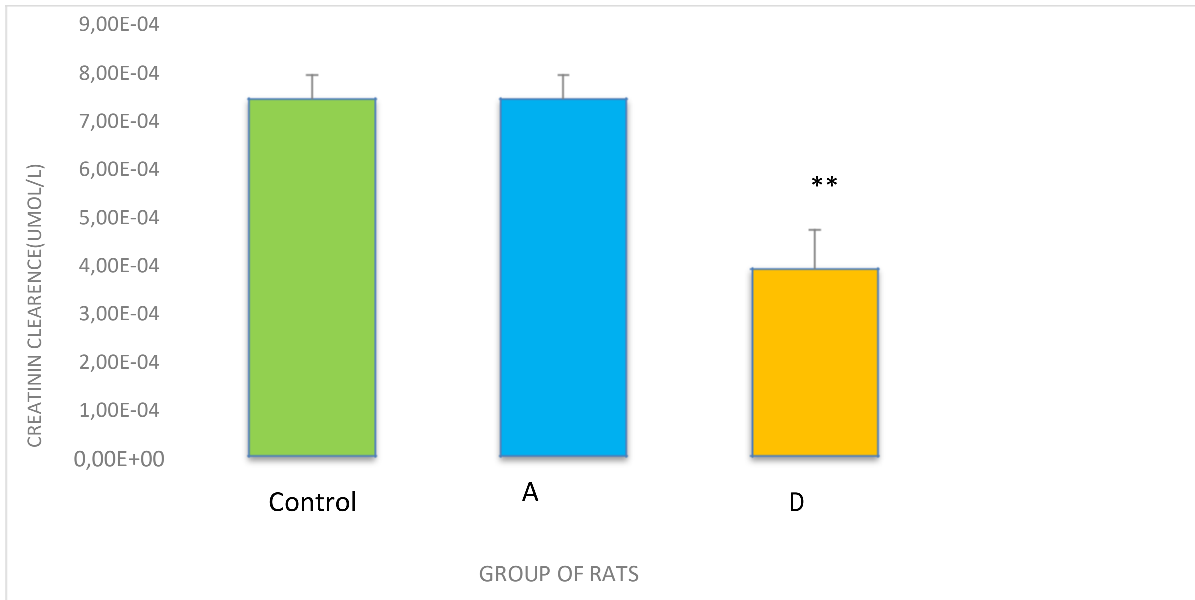


Figure 19 : Evaluation of creatinine clearence(umol/L) in trated and controle groupe for 20 days of treaement

Statistical analysis of the results obtained after the evaluation of the cytosolic activity of creatinin clearence have shown us non significant change($P > 0.05$) ocreatinin clearencef in group A that treated with concentration 5 mg and highly significant decrease of creatinin clearence in groupD(**) $0.01 > P > 0.001$ that treated with concentration 50 mg when they are all compared to the control group

4-5- Mitochondrial paramters :

4-5-1-impact of imidacloprid on mitochondrial swelling :

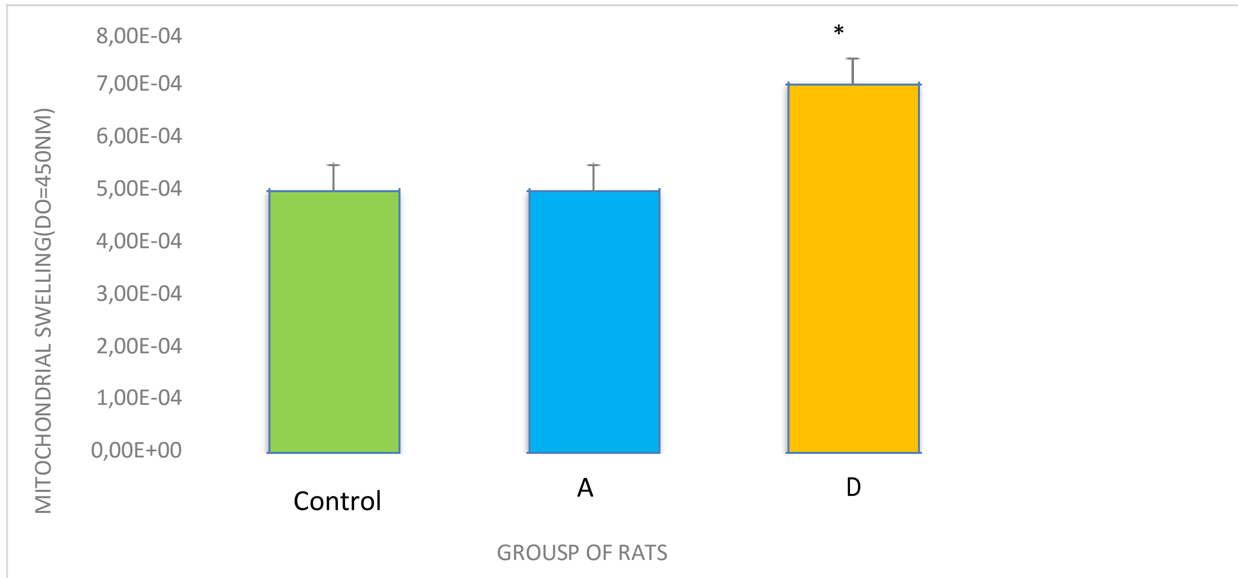


Figure20: Evaluation of mitocondrial swelling in treated and control groupes

Statistical analysis of the results obtained after the evaluation of metocondrial swelling have shown us non significant change of mitochondrial swilling in group A that treated with concentration 5mg(P > 0.05) and significant increase of mitocondrial swilling in groupD that treated with concentration 50 mg(*) 0.05> P> 0.01 when they are all compared with contrôl group

4-5-2-Effect of imidacloprid on mitochondrial permeability :

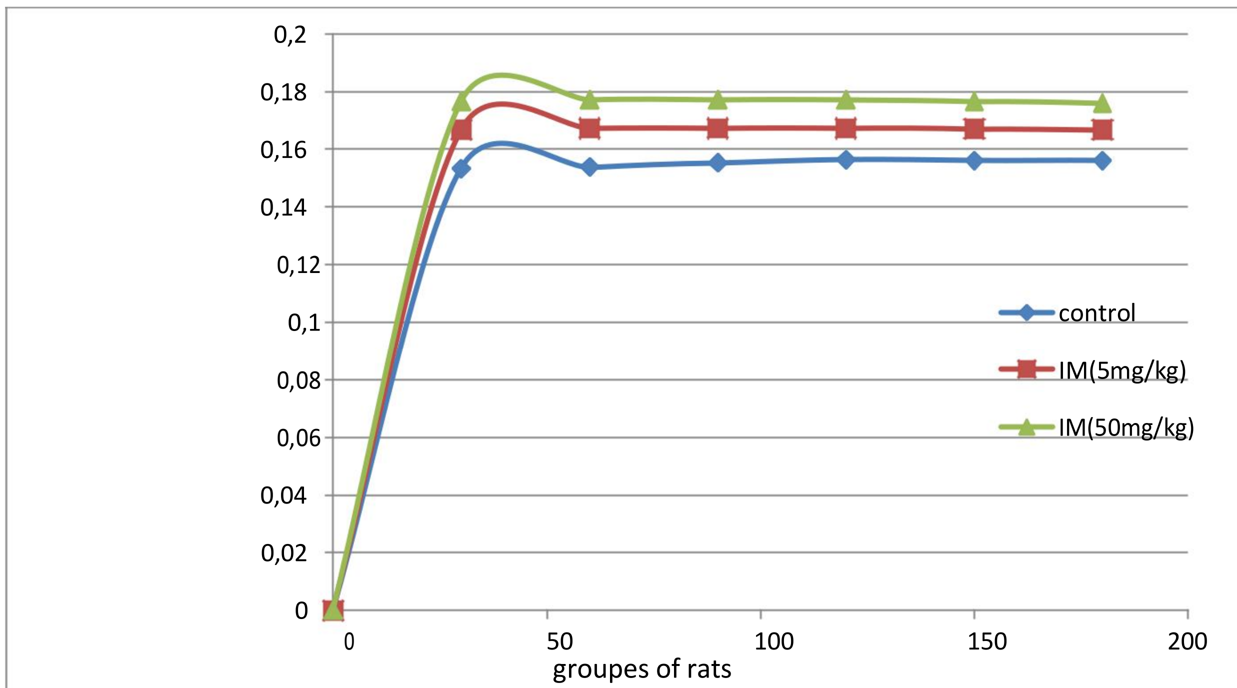


Figure 21 : Evaluation of metochondrial permeability in treated and control group

Statistical analysis of the results obtained after the evaluation of metocondrial perméability shown us significant increase(*) 0.05> P> 0.01 in group D that treated with concentration 50mg and non significant increase P > 0.05) in group A that treated with concentration 5 mg when they are all compared with contrôl group.

discussion

5- discussion:

The world of biological and medical sciences is invaded by a new concept, that of "oxidative stress", which is defined as an imbalance between the production of free radicals and their elimination by antioxidant defenses (Favier., 2003). The imidaclopride induces the formation of reactive oxygen species (superoxide radicals, hydrogen peroxide and hydroxyl radicals) (Duncan et al., 2012).oxidative stress can be reduced by several secondary metabolites isolated from plants that show antioxidant activity.

Impact of imidacloprid on kidney ‗s weight

As for kidney „s weigh on abtained highly significant increase in group that treated with concentration 50mg in the left kidney which explained by tissue enlargement of the left kidneys caused by imidacloprid .And non significant change in the group A that treated by concentration 5 mg because the concentration 5 mg considered NOEL dose. , And we have noticed also a very highly significant increase in the right kidney weights of rats $p<0.001$ that treated with concentration 50 mg / kg / day. This can be explained by the same reason which is tissue enlargement of the kidneys caused by imidcloprid . those results was in agremment with (Mithilesh Kumar Srivastava,2010)

5-1- Impact of imidacloprid on blood paramters : Creatinine and urea: are breakdown products of protein metabolism. They are eliminated by the kidneys, and generally used as an indicator of kidney functioncorrect. records us higly significant result $0.002 <p$ of groups D that treated by concentration 50 mg of imidacloprid compared to control group and no significative result$0.05<p$ in groupe treated by concentration 5 mg**,**our result showed an increase of serum level of creatinin and urea in rats treated by concentration 50mgand non changement in their level for rats that treated by concentration 5compared to control group our result is agree with(laxman prasad srivastava *et al* ;.2010) the result that we have allow to suggest that concentration 5mg is NAOEL concentration for kidney and have no impact on kidney „s function . and the concentration 50mg is toxic concentration for the kidney. Our result shown us highly significant decrese in creatinine clearence in group D that treated with concentration 50mg and this is due to poor ability of the kidneys to filter creatinine out of theblood and no significant change group A that treated with concentration 5 mg that due to the no impact of this concentration on the kidney

5-2-impact of imidacloprid on oxidative stress paramters :

The body permanently protects itself against the formation and the aggression ofoxidants through various defense mechanisms, both enzymatic and non-enzymatic(Mithilish *et al;*.2010). Among mainlyfind reduced glutathione (GSH):it is a powerful antioxidant, important for cell protection, -detoxification ofERO, conjugation and excretion of toxic molecules (shipra bhardawaj *et al*;.2010). It's adirect scavenger of free radicals, a co-substrate necessary for GPx activity and GST(shipra bhardawaj *et al* ;.2010).When treating animals with imidacloprid, the GSH levels are clearlyreduced following their action to neutralize free radicals generated by the imidacloprid (laxman prasad srivastava *et al*;.2010) this phenomenon also causes its depletion and facilitates the peroxidation of lipids and the oxidation of thiol groups of proteins (miyhilish kumar srivastava *et al*;. 2010). Imidaclopride interacts directly with a strong affinity for thiol groups (-SH) of the GSH(Szkudelski., 2001; Nermeen *et al*., 2010). So little GSH is produced. And an increase in oxidized glutathione (GSSG), and consequently a decrease in activityGSH-dependent enzymes. Furthermore, the treatment of rats by imidacloprid at a concentration of 5mgand 50 mg / kg / day improved GSH levels in the kidneysGutteridge., 1988; Huk *et al*;. 1998) this result is with agrement with (shipra Bhardwj *et al*;.2010) that had study the impact of imidacloprid on the detoxifiying enzyme GSH from Folsomia candida (collemba), The result suggest that the alteration of GSH level may be involved in the response of rats to exposure of imidacloprid and can be used as biomarkers to monitors the toxic effects of imidacloprid,GPx is a selenium dependent enzyme, it breaks down hydrogen peroxidein water and molecular oxygen by the oxidation of reduced glutathione (Ewis and Abdel-Rahman., 1995). In the experimental study, we found an insignificant result 0.05 <p of GPX level in group D that treated by 50 mg of imidacloprid compared to control group and we noticed non changement in GPXlevel in rats at a concentration 5mg that mean that the concentration 5 mg is NOEL and the concentration 50 mg stimiled the GPX activity . The same result was obtained by **(**upasana kapoor ;2010**)**,Glutathione S-transferase is an enzyme with an important role in the detoxification of xenobiotics and protection against harmful metabolites generated after degradation of macromolecules (Hayes and Pulford., 1995). In our work we obtained insignificant result0.05<p in group D that treated by concentration 50 mg and we noticed decrease in GST level in rats at a concentration 50mg and non changement in GST level at a concentration 5 mg that mean that the concentration 50 mg is toxic and 5 mg is NOEL for antioxidant enzyme GST . the same result was obtained by (Mithilish kumar srivastava;2010).Catalase (CAT) is a hemoprotein that catalyzes the reduction of peroxidesof hydrogen to H2O and oxygen and protects the tissues from hydroxyl radicals which are veryreagents (Sathishsekar and Subramanian., 2005). There has been an non significant result0.05<p in batches that treated by concentration 5mg and 50mg compared to control group

we had noticed an increase in CAT level of rats that treated by 50mg compared to control group and non changement in CAT level in group that treated by 5 mg compared to control group. These results are in agreement with those published by(laxman prasad Srivastava *et al* ;2010) What we found explain that the concentration 5 mg is NOEL and 50 mg stimiled the CAT activity.MDA, it is the product of the peroxidation of unsaturated fatty acids, used asindicator of oxidative damage (upasana kapoor *et al*;. 2010). Our research shows an no significative result in MDA level of rats treated by 5mg and 50mg.We had noticed decrease in MDA level of rats treated byconcentration 50mg compared to control group a,d non changement in MDA level in group A that treated with 5 mg , our resultare consistent with several studies such as that published by(shipra Bhardawaj *et al*;2010) What we found explain that the concentration 50 mg is toxic concentration and 5 mg is NOEL .

5-3-Impact of imidacloprid on biochemical paramters :

We obtained significant result in proteins 0.02<p of rats treated by concentration 50mg we noticed decrease in proteins level compared to control,group that back to that the proteins

degraded into amino acids then into urea and creatinine. This is confirmed by the decrease in the protéins level, therefore increased urea and creatinine in treated rats by imidacloprid.

Lipids have an essential role as a constituent of biological membranes (cholesterol, phospholipids, glycophospholipids).They make up the structure of certain hormones (steroid hormones) and certain vitamins.They also serve as an energy reserve in the form of triglycerides.

In adipocytes, lipids also provide thermal and mechanical protection.glycerides and in particular triglycerides which are molecules formed from a molecule of glycerol linked to three molecules of fatty acids by ester bonds.The breakdown of fatty acids, contained in certain lipids, also allows cells to produce ATP. This degradation occurs in the mitochondria, according to a set of reactions grouped under the term of Lynen helix (or β-oxidation of fatty acids).

Our result shown significant decrease in lipide level in group of rats that treated with concentration 50 mg of imidclopride compared to contrôl group this explained by exposure to

stress which lead to thedemolition of lipid to obtain energy. And shown us also no significant change in protein and lipide in groups of rats that treated with concentration 5 mg which explain that the 5 mg is NOEL (no observed dose) mean that it have no impact on proteins and lipdes. The same result was obtained by (Shipra Bhardwaj *et al*,.2010)

5-4- mitochondrial paramters :

Mitochondria are a part of eukaryotic cells. The main job of mitochondria is to perform cellular **respiration**. This means it takes in nutrients from the **cell**, breaks it down, and turns it

into **energy**. This **energy** is then in turn used by the **cell** to carry out various functions.The production of ATP by oxidative phosphorylation is the primary function of mitochondria. Mitochondria in higher eukaryotes also participate in cytosolic Ca^{2+} buffering, and the ATP production in mitochondrial can be mediated by intramitochondrial free Ca^{2+} concentration. Ca^{2+} retention capacity can be regarded as the capability of mitochondria to retain calcium in the mitochondrial matrix. Accumulated intracellular Ca^{2+} leads to the permeability of the inner mitochondrial membrane, termed the opening of mitochondrial permeability transition pore (mPTP), which leads to the leakage of molecules with a molecular weight less than 1.5 kDa. Ca^{2+}-triggered mitochondria swelling is used to indicate the mPTP opening. Here, we describe two assays to examine the Ca^{2+} retention capacity and Ca^{2+}-triggered mitochondrial swelling in isolated mitochondria. After certain amounts of Ca^{2+} are added, all steps can be completed in one day and recorded by a microplate reader. Thus, these two simple and effective assays can be adopted to assess the Ca^{2+}-related mitochondrial functions.(ZHANG *et al* , 2018) Our result shown non
significant changement in swilling of mitochondria for the group that treated by concentration 5mg of imidaclopridcompared to contrôl group . and shown also significative increase in swilling of mitochondria for group that treated by concentration 50mg of imidacloprid. Also we have
obtained increase in the permeability of metochondria with augmentation of imidacloprid concentration These is due to accumulation intracellular of calcuim as a result of oxidative stress what lead to opening of mPTPpore and leakage of molecules and ions from inner membrane causing mitocondrial swilling , the same result was obtained by (shipra bharwaj ;2010)

Conclusion and perspective :

This work allowed us to highlight the effect of imidacloprid on the evolution of relatif kidney weight, as well as the biochemical ,blood and mitochondrial parmaters in the light of the result obtaine we can conclude:

- The treatement of young male rats by concentration 50 mg causing perturbation in bochemical paramters , blood paramters , mitochondrial paramters and also oxidative stress paramters

-The treatement of young male rats by concentration5mg didn‟t cause any changement or perturbation compared to contrôl group

The imidacloprid is neurotoxic insecticide but have extreme impact on nephrotic function of wistar rats in the uses of high concentration as a perspective we suggest :

- The use of imidacloprid with low concentration
- Developpe this research by using more oxidative stress paramters like (SOD), and renal failure paramters (magnesuim , calcuim…)
- Developpe the aim of this work by using meduim concentration to see there impact .

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