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Protective role of Melissa sp extracte on cardiovascular system and impacts of imidacloprid in Wistar rats

Decant the jury :

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الملخص

الايميداكلو بريد المركب الأول من الفئة الجديدة من المبيدات الحشرية الكيميائية الأكثر استخداما في عالم الحقول والبستنة ، ونبتة الميليسا هذا النبات الطبي المعروف منذ حضارة اليونان القديمة ، وقد حاولنا في هذه الدراسة تقييم سمية هذا المبيد الحشري من ناحية، ومن ناحية أخرى تقييم التأثير الوقائي والتصحيحي لمستخلص نبتة الميليسا علىنموذج حيواني، هو فئران ويستار، تنقسم تجربتنا الى أربعة مجالات ومؤشرات الأيض، أظهرت نتائجنا مهمة للدراسة، دراسات تغيرات الوزن، دراسات مؤشرات الإجهاد التأكسدي ، المؤشرات البيوكيميائية إصافة خلاصة الميليسا لاحظنا دورها وبعد الوزن النسبي لقلب الجرذان واضطراب جل المعلمات المدروسة في أن ايميداكلو بريد يسبب بانخفاض تأثيرها التصحيحي

وبعد تحليلال النتائج أوضحت الدراسة الحالية أن التعرض لمبيد الإيميداكلوبريد لها تأثيرات سامة على المؤشرات المذكورة سابقا ،وبإضافة مستخلص نبتة الميليسا تحسنت معظمها.

الكلمات المفتاحية الا يميداكلوبريد نبتة الميليسا فئران ويستار

Abstract

Imidacloprid, one of the systemic insecticides and the first compound of the new class of chemical insecticides most used in the world in fields and in horticulture, and Melissa officinalis this medicinal plant known since ancient Greece In this study we are interested in the evaluation on the one hand of the toxicity of this insecticide, and on the other hand the evaluation of the preventive and corrective effect of the extract of Melissa officinal on an animal model; Wistar rats, Our experimentation is divided into four important areas of study, studies of weight changes, studies of oxidative stress parameters, biochemical and metabolic parameters, Our results show that imidacloprid causes a decrease in relative heart weight rats and disturbance in the parameters studied and after the addition of the extract of Melissa officinale we noticed its role and its corrective effect. The present study showed that the exposure to imidacloprid to toxic effects on the parameters mentioned previously, the supplementassions of Melissa officinale improved most of the studied parameters.

Keywords: Imidacloprid, Melissa officinalis, Rats Wistar ,.

Résumé

L’imidaclopride un des insecticides systémiques et le premier composé de la nouvelle classe d’insecticides chimiques les plus utilisés dans le monde dans les champs et en horticulture , et La Mélisse officinal cette plant medicinale connu depuis la Grèce antique Dans cette étude nous nous sommes intéressés à l’évaluation d’un coté de la toxicité de se insecticide , et d’un autre coté l’évaluation de l’effet préventif et correcteur de l'extrait de la *Melissa officinal* sur un modèle animal ; les rats Wistar, Notre expérimentation est divisé en quatre important axes d’études , études des changements de poid , études des paramètres de stress oxydatif , paramètre biochimique et métabolique , Nos résultats montrent que la l'imidaclopride provoque une diminution de poids relatif des cœur des rats et des perturbation dans les déférent paramètres étudié et après l'addition de l'extrait de *Melissa officinales* nous avons remarqué son rôle et son effet correcteur . La présente étude a montré que l’exposition au l'imidacloprid à des effets toxiques au niveau des paramètres mentionnés précédemment , la supplémentassions *de Melissa officinales* a amélioré la plupart des paramètres étudiés .

Mots clés: L’imidaclopride, *Melissa officinalis*, Rats *Wistar*,.

Thanks

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The work presented in this thesis was carried out in the Toxicology Laboratory, Department of Biology, University of Tébessa. At the end of this work, we thank Allah, the good merciful God for helping us to carry out this work.

I would particularly like to express my deepest thanks and our deep gratitude to our supervisor Mr. Rouabhi Rachid, professor at the University of Tébessa, and the dean of the faculty of science.

Who did me the honor of ensuring the direction of this work, and who knew how to be patient, and for his availability and numerous advices in all cases and all fields, thus allowing me to carry out this study takes place in the best possible conditions. Thank you sir for his presence, for his patience, his precious advice, monitoring, guidance and for his confidence.

We would like to thank the members of the jury, our president (Amamra R). And our examiner (Gasmisalim). Who does us the honor to judge this work of memory

Dedication

We dedicate this work … To our God ALLAH the Almighty for all the will and courage he has given us for the completion of this memory , he has been and will always be at us side to succeed in completing any work . and me islem I dedicate this work also To my beautiful babies in my family… Yousef , ChaymaTesnim , and Soulayman My sunshine and my joy of life. Thanks for the kisses, hugs, laughs, games and guiliguilis ... To my parents, Mom, Dad, You have always helped, supported and encouraged me. Without you, nothing would have been possible To my brothers, Yassin, and Bilel and Zaki, and my beautiful sisters, Hanene ,Saliha and Rourourou Thank you for putting up with me in difficult times. I wish you a happy life. To my friends in my life Nour, Djamila,Asma,and Naima for their encouragement .

Abreviation Table

Ml	Microliter
µmol	Micromoles
%	Percentage
AD	Alzheimer Disease
AOA	antioxidant Activity
ANOVA	Analysis of variance
ASAT	Aspartate transaminase
Ca2+	calcium ion
CAT	Catalase
CHOL	Cholesterol
CVS	cardiovascular Systme
DW	distilled water
EDTA	Ethylène-Diamine Tétraacétique
EO	Essential oil
GPx	Glutathione peroxidase
GSH	Reduced glutathione

GSH	Glutathione peroxidase
GST	Glutathione S-transferase
HBP	high blood pressure
HF	heart failure
L V	left ventricles
LPO	Lipid Peroxidation
MDA	Malondialdehyde Acid
Mg	Milligram

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Introduction

Ago the last two decades, the industrial revolution and technological development in the field of agriculture has considerably complicated environmental problems. The demographic explosion of the world population and their food and health needs are the main reasons leading to a dramatic increase in the production and use of pesticides likely to cause the appearance of several diseases (Gasmi. S, 2017)

Contamination by pesticides, which in particular induces over time, harmful effects on the health of populations. However, these substances have the capacity to enter the body through a multiplicity of entry routes (skin, ingestion, inhalation). farmers and people handling pesticides represent the most exposed population and run the risk of these products through food and the environment (Mahboubi.Y, 2013)

Several studies have indicated that the toxicity of pesticides may be associated with increased production of ROS (reactive oxygen species) which are the direct cause of various pathological conditions such as diabetes, Alzheimer's disease, rheumatism and cardiovascular diseases (Yurt B and Celik I, 2011), Thus the chemical industry is always looking for molecules which are all effective against target organisms and less harmful towards non-targeted organisms.

Pesticides also called phytosanitary products, Pesticides are classified according to their origin or their chemical structure, in mineral or organic insecticides, or natural or synthetic insecticides. 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). Inside habitats, they are also used to destroy several plagues such as flies, mosquitoes and cockroaches

In fact, neonicotinoid is considered to have excellent activity against insects capable of causing agonistic effects by binding to the nonsynaptic acetylcholine receptor receptors (RnAch), thus causing excitation, abnormal paralysis and death. pests (Gasmi. S, 2017)

However, the few studies on this subject carried out before did not allow a better understanding of the relationship between neonicotinoids for example and the etiology of certain diseases such as metabolic and neurodegenerative diseases, reproductive dysfunction, neurodevelopmental alterations and cancer (Gasmi. S, 2017)

Imidacloprid is a neonicotinoid insectiside which was widely used worldwide

(Jeschke PR et al., 2011) Generally, several experimental studies have confirmed the implications of imidacloprid in the appearance of tissue damage, gastrointestinal wanderings, neurological symptoms and even the death of the animal. , structural alterations at the level of different parenchyma: pulmonary, hepatic, thyroid, renal, these alterations are associated with hormonal and biochemical disturbances (Mahboubi.Y, 2013)

In recent years, interest in natural antioxidants, in relation to their therapeutic properties, has increased considerably. Antioxidants play an important role in health. Scientific research in various specialties has been developed for the extraction, identification and quantification of these compounds from medicinal plants (Laouar, A. 2018). For a long time, the use of plants in medicine and very old. man seeks to cure several diseases. Among the natural substances derived from plants, we find phenolic compounds, belonging to the class of so-called secondary metabolism compounds, manifest a spectrum of pharmacological properties such as:

antibacterial, anti-allergic, anti-inflammatory , anti-carcinogenic and cardioprotective and vasodilatory (LadohYemeda, C. et al., 2014)

In Africa, medicinal plants are widely used for the prevention and treatment of various diseases (Sangare, M. Et al., 2012), lemon balm, a honey, condiment and medicinal plant, has been known since ancient times for its properties. sedatives and as condiments. Highly prized in Arabic medicine, lemon balm already enjoyed a certain reputation as a stimulant and antispasmodic, lemon balm was a sovereign herb for most ailments and restored strength and health to those who were sick or tired. For traditional use, it delights the heart, helps digestion, strengthens the weakened heart, soothes palpitations and melancholic mood. (Nathalie, R. 2001).

The aim of this work is to assess the toxic effects of imidacloprid on the cardiovascular system using an animal model, the Wistar rat raised in the animal facility of the Department of Biology of the University of Tébessa and the effect opposite of Melissa officinalis. The objectives of this work are: -Determine the toxic effect of imidacloprid on the cardiovascular system -Determine some oxidative stress parameters: (MDA, GSH, GST, GPx, CAT).

-Determine Effects of imidacloprid and Melissa officinalis on the structural and functional integrity of the mitochondria

-Determine some cardiac parameters: (TGO, Cholesterol, Triglyceride,)

-determine Effects of imidacloprid and Melissa officinalis on parameters of overall growth - Determine the protective effect of Melissa officinalis on the toxicity of imidacloprid

Bibliograpc

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Chaptr I: pesticide and oxidative stress

1 . Pesticide Generals

Before the use of plant protection products, crop systems were designed to ensure the best trade-off between plant health risk and crop production potential. However, yield losses from agricultural production due to disease, pests and weeds could reach significant proportions. After the Second World War, pesticides allowed the development of agriculture and contributed to the increase in yields and the regulation of agricultural production. In addition, some experts believe that the increase in agricultural land yields has helped to limit deforestation and thus preserve 50% of the current forest area in 50 years. The use of plant protection products has also limited or eradicated a number of highly lethal parasitic diseases **(Meirhi, 2008) .**

1.1. Definition of pesticides

The word "pesticide" comes from the association of the Latin word "pestis" which means animal, insect, plant or pest (virus, bacterium, fungus etc.) likely to be harmful to man and his environment and the suffix "cide" (from the Latin verb caedo, caedere) which means killing . The term pesticide includes both phytopharmaceuticals for agricultural use and biocides formerly known as non-agricultural pesticides **.** which also refer to an active substance or preparation one or more active substances . The active substance (formerly called active) is the substance or microorganism that destroys or prevents the pest for the crop from settling or developing, and allows the class of pesticides to be found neonicotinoids **(Abdaouiand al , 2017)** .

1.2. Pesticide exhibition modes

Recent sanitary crises require better understanding and articulating links between agriculture, environment and public health. Because The risk of pesticides for man is located in the interface of these three areas. (**Merhi , 2008)**

Professional exhibition. The professional exhibition concerns people handling products, at the time of preparation, application and cleaning of treatment equipment **(Zeljezic and al , 2006) . Non-professional exhibition**. The entire population may be exposed to pesticides through residues of these compounds in its environment (water, air, suspended particles, dust) and its diet **(Baril and al , 2012) .**

and we can say that the term pesticides is a generic term that brings together Herbicides Fungicides Biocides and insecticid , And Since the pesticide used in our study is a type of Insecticide , We will address it in particular and not others **.**

2 . Nionicotinoide

The first molecules belonging to the neonicotinoid family were synthesized in the 1970s. The term "neonicotinoid" was proposed by Japanese researcher Izuru Yamamoto to differentiate this family from the ancient "nicotinoids", i.e. nicotine-containing plants, used as insecticides since the 18th century, Power Insecticide of the first molecules tested was however very weak, so research was continued to identify active chemical groups and synthesize molecules with better activity. Nithiazine was one of the first molecules of interest with satisfactory insecticide activity, systemic distribution in plants, and low toxicity in vertebrates .

But it was rapidly degraded by hydrolysis or photolysis, making it impossible for agricultural use. Approximately 2,000 molecules were tested before the discovery of imidaclopride by Shinzo Kagabu and its release in 1991 by Bayer CropScience. The family then expanded to include seven commercialized compounds: imidaclopride, thiaclopride, clothianidine, thiamethoxame, acetamipride, nitenpyram, and ditenfuran, sulfoxaflor **(Ivert , 2016)** .

And in the following addresses we will detail the information about Imidacloprid .

2.1. Imidaclopride

Imidacloprid is the first compound of the new class of chemical insecticides. It is found in many commercial formulations, Admire®, Confidor®, Gaucho®, Minister®, Provado® and Marathon®. Imidaclopride is one of the most widely used systemic insecticides in the world in fields and in horticulture. It is often used as a seed treatment, especially for corn, sunflower and rapeseed. 29 Leaf treatments for sucking insects including leafhoppers, aphids, thrips, white flies, or soil treatment against termites, grass and soil insects, some beetles including moles, it is used on rice, corn, potato, vegetables, sugar beet, fruits, cotton, hops and grass. Imidaclopride is classified by the U.S. S. Environmental Protection Agencyin the class of toxicity II and class III agent, and must be marked with the signa the "warn" or "attention" .**Al sayeda , 2007) .**

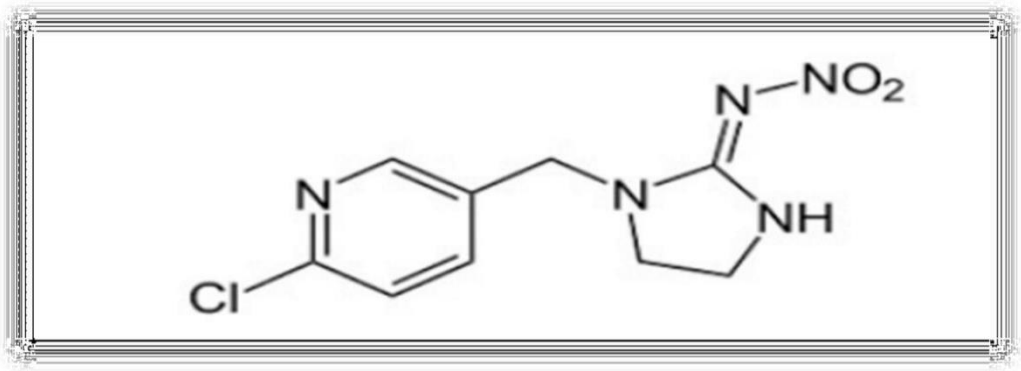


Figure 01 .Structure chimique de l'imidaclopride**(Jargot and al , 2015)**

Figure 02 .label of imidacloprid **(Jargot and al , 2015)**

Tableau 01 . chemical and physics Properties of imidacloprid**(Belhadef and al , 2015) .**

- Properties * Values

Molecular weight [g/mol]	255.7
hydro solubility [mg /L]	613 à 20°C (eau déminéralisée. pH 5.5) 607 à 20°C et pH 4 601à20°c et Ph 9
Henry's Constant [Pa.m3 /mol]	1.7 .10-10à 20°C
Octanol-water sharing coefficient log (log kow)	0.57 à21C° (demineralized water)
organic Coefficient adsorption (carbone) (koc) [L /kg]	225 (109-411)

2.2. Toxicological Effects

2.2.1 . Acute Toxicity

Imidaclopride is moderately toxic, oral LD50 is 450 mg/kg body weight in rats and 131 mg/kg in mice . LD50 in rats by skin is 5,000 mg/kg after 24 hours. It is considered nonirritant for the eyes and skin (rabbits), and has no effect on guinea pigs **.**

30 Regarding the toxicity of acute inhalation in rats, the airborne LC50 of imidaclopride is 69 mg/m3 of air in the form of aerosol, and 5323 mg/m3 of air in the form of dust. These values represent the concentrations at which possible airborne poisoning symptoms are maximized . Although no human poisoning has been reported in the literature, the expected signs and symptoms of poisoning are similar to the signs and symptoms caused by nicotine such as fatigue, contractures, cramps and muscle weakness, including those needed for breathing (**Al sayeda , 2007**) **.**

2 .2.2 Chronic toxicity

In rats , the thyroid is particularly sensitive to imidaclopride. Lesions of this gland are caused by doses starting at 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 retinat atrophy in females. (**Alsayeda , 2007**) **.**

2.2.3 Mutagenic effects

Imidaclopride is weakly mutagenic. Out of 23 laboratory mutagenicity tests, only two showed positive effects: changes in human lymphocyte chromosomes and genotoxicity on Chinese hamster ovary cells (**Al sayeda , 2007**) **.**

2.2.4 Neurotoxin effect of imidacloprid

The impact of nicotinics has been reported by **,** in the expensive wistar rat after the oral administration of 20mg / kg / day of imidacloprid. Their results showed the inhibition of the specific activity of acetylcholinesterase (AChE). Similar results have been reported by **(Rodrigues and al ., 2010) .**

2.2.5 effect on antioxidant activity

The work of Lonare and al., (2014) showed the establishment of a detoxification system in rats after the administration of a neonicotinoid imidaclipride orally at two doses 45 and 90 mg / kg. In fact, an increase in the activity of two detoxifying enzymes, GSH and GST, was recorded compared to the controls (Table). **(Lonare and al., 2014) .**

2.2.6 . Effect on the thyroid gland

The impact of neonicoinoids in rats remains unclear. The work of (Saadi et al., 2014) has shown the toxicity of a neonicotinoid imidacloprid in wistar rats, after the administration of two doses at 3.67 mg / kg / day and 5.18 mg / kg / day for 30 days orally. The results obtained revealed the quantitative assay of thyroid hormones FT3 and FT4 (measured by the ELISA technique). The results obtained shown in Table 3 reveal no significant deference in FT3 and FT4 between the control series and the treated series. **(Saadiand al., 2014) .**

2.2.7. Effect on reproduction

The experiments of (Nabiuni et al., 2015) carried out on the wistar rat, under laboratory conditions have shown the impact of a neonicotinoid imidacloprid on reproduction. The study was continued for 20 days before the administration of imidacloprid by ingestion in food. In order to study the toxicity of this pesticide on the reproduction process, a blood sample was taken to measure the following hormones, testosterone and progesterone

The results of this experiment confirm a significant decrease in the level of testosterone and progesterone compared to the controls (Table 4) **(Nabiuni and al., 2015) .**

2.2.8 .Teratogen Effects

A study of developmental toxicity in gastric tube-fed rats up to 100 mg/kg/day on gestation days 6 to 16 resulted in a NOEL of 30 mg/kg/day (based on skeletal abnormalities observed at the highest dose of 100 mg/kg/day) . A developmental toxicity study in rabbits fed by gastric tube with doses of imidaclopride during days 6 to 19 ofgestation, resulted in a NOEL of 24 mg/kg/day based on decreased body weight and skeletal abnormalities observed at 72 mg/kg/day (the highest dose) **(Al sayeda , 2007) .**

2.2.9. Carcinogenic Effects

There are no carcinogenic effects in a two-year study of carcinogenicity in rats fed up to 1,800 ppm of imidaclopride**(USA, 1995) .**

3 . Oxidative stress:

3.1. Definition

Oxidative stress is an imbalance between the defense systems between pro-oxidants and antioxidants **(Favier, 1997) .**

This imbalance between the defense systems and the production of free radicals leads to biochemical damage to the cells of the organism and due to their molecular consequences **(Gueye , 2007) .**

3. 2.The consequences of oxidative stress

The excessive production of free radicals causes direct damage to biological molecules (oxidation of DNA, proteins, lipids, carbohydrates), but also secondary damage due to the cytotoxic and mutagenic character of the metabolites released, in particular during oxidation. lipids. The body can also react against these abnormal compounds by the production of antibodies, which unfortunately can also be autoantibodies, creating a third wave of chemical attack **(Favier, 2003) .**

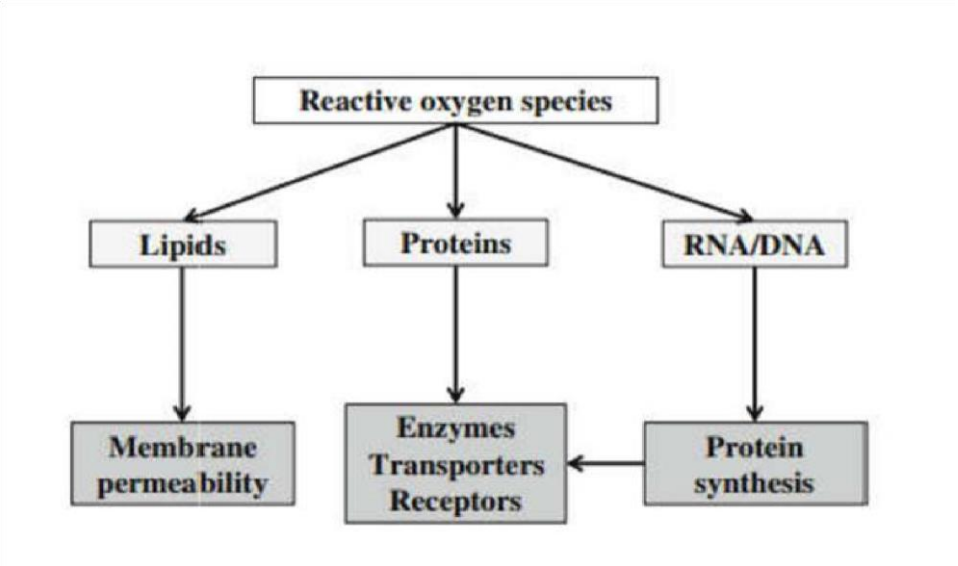


Figure 03: Targets of oxidative stress **(Bosoi and rose , 2012) .**

3.3 . Free radicals

A free radical is a molecule or a molecular fragment which contains an electron (or more) unpaired on its external electronic layer. (Justine et al., 2005) This considerably increases its reactivity by the necessity of combining with another electron to reach stability according to an oxidation phenomenon **(Bonnefont-Rousselot and al , 2003) .**

3.4. Origin of free radicals

Free radicals can be of endogenous origin, are produced by various physiological mechanisms in order to destroy bacteria within phagocytic cells (macrophages, polynuclear) or to regulate lethal cellular functions such as programmed cell death or apoptosis **(Ghezil and al , 2016) .** When they are of exogenous origin, free radicals are produced mainly in air pollutants (N, NO2), radiation products (X-ray, UV light), organic solvents, anesthetics, pesticides, drugs, and xenobiotics. **(Tessier and al ., 1994) .**

3.5 . Antioxidant defenses

Our organism is equipped with a whole complex system of enzymatic and non-enzymatic antioxidant defenses, located in the intra- and extracellular compartments **(Mette, 2001) .** An antioxidant is a substance which, when present at low concentrations compared to that of an oxidizable substrate, delays or prevents the oxidation of this substrate **(Halliwell , 1996) .** According to **(Valko et al , 2006)** A good antioxidant should :

• Acting particularly on free radicals

• Chelate transition metals

• Act at a physiological concentration at a relevant level

• interact with other antioxidants to regenerate and restore their original function

The body has a wide range of endogenous antioxidants in the form of enzymatic or nonenzymatic systems **(Berger and al , 2001)**

3.6 antioxidant system

3.6.1. Enzyme antioxidant system

The enzymatic antioxidant system consists of the interaction of 3 main enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT)

3.6.1.1 . Superoxide dismutase (SOD)

It is a metalloenzyme that catalyzes the disproportionation of O2. - in H2O2 and O2. Its frontline intervention in the elimination of RLOs gives it a decisive role in controlling cellular redox status and in aerobic survival **(Charlotte , 2012) .**

There are three classes of SOD, with different genes, unique protein structures and distinct subcellular locations. CuZnSOD (SOD1) is a cytosolic enzyme, MnSOD (SOD2) is mitochondrial and CuZnSOD extracellular (SOD3) **(Messner , 2012) .**

3.6.1.2. Glutathione peroxidase (GPx):

Glutathione peroxidase (GPx) is an antioxidant enzyme containing selenium that effectively reduces H2O2 and lipid peroxides to water and lipid alcohols, respectively, and oxidizes glutathione to glutathione disulfide. ; The oxidized glutathione will be regenerated thanks to the intervention of glutathione reductase which acts by oxidation of NADPH, H + formed mainly by the pentose phosphate pathway.

It should be noted that GPx can also catalyze other hydroperoxides of lipid origin and that there are 5 isoforms of different tissue locations. The most abundant is isoform 1 (GPx1) present at the cytoplasmic and mitochondrial level, and expressed in most cells **(Charlotte , 2012) .**

3.6.1.3 . Catalase (CAT)

Catalase is a tetrameric heme protein that detoxifies H2O2 into oxygen and water. It is a metalloprotein oxidoreductase enzyme that effectively removes H2O2 when it is present in high concentrations **(Thimraj , 2018) .**

2H2O2 O2 + 2H2O

This enzyme is particularly concentrated in the liver and Erythrocytes, while it is found in small quantities in the Skeletal Streak muscles, the heart and the brain **(Tessier, 1995) .**

3.6.1.2.Glutathione S-transferase (GST)

Transferases have exhibited various activities and participate in several types of reactions. Most of these enzymes can catalyze the conjugation of reduced glutathione (GSH) with compounds containing an electrophilic center by the formation of a thioether bond between the sulfur atom of the GSH and the substrate. In addition to conjugation reactions, a number of GST isoenzymes have other GSH-dependent catalytic activities, including reduction of organic hydroperoxides and isomerization of various unsaturated compounds **(Dzoyem, 2014) .**

3.6.2. Non-enzymatic antioxidant systems

Antioxidants are redox agents which react (scavenger effect) with oxidants and either stop or slow down the oxidation processes antioxidants oxidize to stable derivatives, or persist for a certain time in radical form **(Benaissa, 2012) .**

3.6.2.1 . Glutathione (GSH):

Glutathione (γ-glutatamylcysteinylglycine, GSH), a tripeptide, acts as an antioxidant by directly trapping free radicals by donating a hydrogen atom **(Fanucchi, 2014)**

In addition to neutralizing free radicals, Glutathione regenerates important antioxidants such as vitamins C and E, and maintains the redox state of the sulfhydryl protein groups essential to DNA, glutathione is also suggested to chelate the transition metals, thus reducing their toxic capacity **(Drisko, 2018) .**

3.6.2.2. Vitamin E

Vitamin E is a lipophilic antioxidant belonging to the tocopherol family (α, β, δ, γ). (Dominique, 2012), with significant antioxidant properties, offering protection against oxidative stress and inhibiting the peroxidation of fatty acids from membrane phospholipids **(Dhawan, 2014)** found in asparagus, avocados, nuts, olives and vegetable oils **(Scully, 2014) .**

3.6.2.3 . Vitamin C

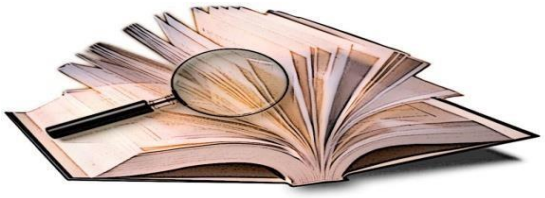
Vitamin C is, above all, an excellent trap for ROS. There are many functions: contribution to the proper functioning of the immune system, involvement in the synthesis of collagen and red blood cells as well as in the metabolism mechanisms of iron **(Haleng, 2007)** , Its main food sources are fruits (citrus, kiwi, cherries, melon) and vegetables (tomatoes, green vegetables, broccoli and cabbage) **(Jean, 2002) .**

3.7 . Use of antioxidants

Antioxidants have a variety of uses in industry. Antioxidants as preservatives: are used to delay the oxidation of an organic substance. (Ananya, 2012). These antioxidants can also be used in the dyeing industry to avoid the oxidation of sulfur dyes or tank dyes during dyeing and in the chemical industry to avoid the hardening of rubber or in metallurgy to protect metals from l 'oxidation **(Kahina, 2011) .**

3.8 . Diseases linked to oxidative stress

By revealing abnormal biological molecules and by overexpressing certain genes, oxidative stress will be the main initial cause of several diseases: cancer, cataracts, amyotrophic lateral sclerosis, acute respiratory distress syndrome, pulmonary edema, accelerated aging. Oxidative stress is one of the factors potentiating the appearance of multifactorial diseases such as diabetes, Alzheimer's disease, rheumatism and cardiovascular diseases**(Ghezil and al , 2016) .**



Chaptr II :Melissa officinalis

1. Reminders on phytotherapy

erbal therapy since the dawn of time and over the centuries man has used plants to feed but also to treat it self and most knowledge of medicinal plants is passed down from generation to generation . Chemists and in the 19th century, managed to isolate the active ingredients of certain plants: quinine of quinquina, morphine from opium, etc. Continuing their research, and at the beginning of the 20th century they also succeeded in making synthetic molecules. (**PasdeloupGrenez , 2018) .**

Also in many human societies, medicine by plants is developing, whether from written or oral traditions. Even today, some populations are treated exclusively by plants (South America, Africa, Asia...). Unfortunately, this knowledge, accumulated over the centuries, is gradually being lost for several reasons (loss of associated biodiversity, abandonment of traditions by younger generations...) (**Chassard , 2016) .**

Herbal medicine has many advantages which would explain the return to its use for example and economically, finds that herbal products are, much cheaper than conventional medicine products (especially herbal teas) also and in terms of public health herbal medicine does not generate drug dependence requiring withdrawal at the end of treatment and at ecological and environmental level we find that Drugs from the chemical industry, which accumulate in the environment potentially toxic drug substances on the contrary plants are taken from nature and returned to it after metabolism in the body. (**PasdeloupGrenez , 2018) .**

2. The Melissa

The lemon balm (which means "bee" in Greek) reminds us that its nectar is eagerly sought after by bees. it is also commonly called lemongrass or lemongrass, although the real lemongrass (Cymbopogon nardus) is an Asian grass. The English named it lemon-balm and the Germans Zitronenmelisse or Melissenkrau (**Ronat , 2001) .**

Figure 04: Melissa officinal**is (Hans, 2007) .**

2-1 History of Melissa

Medicinal plant is called any plant containing one or more active ingredients capable of preventing, relieving or curing diseases. The history of these plants is associated with the evolution of civilizations. In all regions of the world, also the history of peoples shows that these plants have always occupied an important place in medicine and the Egyptians constituted the first legislation of medicinal plants in "Pharmacopea" whose name derives from the word Egyptian "Farmake" (which heals) , and today, despite the profusion of purely chemical medicines, plants still win new victories (**Bounihi , 2015) .**

And We know La Mélisse from ancient Greece. The leaves of this plant were used by Theophraste (372 - 287 BC) and Hippocrates (460-377 BC) (**Wichtl and Anton, 1999) .**

Ancient Greece. At the time, its benefits were already recognized to calm anxious people and soothe nervous disorders. The Arabs used it as an antispasmodic and the Europeans as a calming digestive and antiviral treatment. In today's world, lemon balm is used in herbal medicine to treat anxiety and nervous disorders as well as gastrointestinal problems. It is also used for its stimulating effect on brain function and its effectiveness in the treatment of insomnia (**Youla, 2017)** .

2-2 Botanical Description

Lemon balm is a perennial herbaceous plant 40 to 80 cm. It has many square-sectioned stems that have broad leaves that appear from June to September and give off a lemon-like smell. The leaves of the lemon balm are harvested just before flowering in May-June, or in September during a second harvest (IESV 2015)**The underground part.**

It consists of underground stems, rowing, root-bearing and producing adventist buds that allow the plant to perpetuate and multiply.

The stem.

It is erect, quadrangular - typical character of the Lamiaceae-, more or less hairy; little branched at the base, it becomes strongly in the high parts at flowering. The branches at the top bear flowers and are well developed, while they are short and uns blooming in the lower part.

The sheet.

The leaves of the lemon balm are simple, opposite, oval, sometimes slightly cordiform, petioles, widely toothed saw, reticulated, measuring 5 to 8 cm by 4 to 5 cm. The upper face, bright dark green, is rough to the touch because it is covered with fine and short white tctor hairs. The ribs, protruding on the much paler and hairless underside, form a network between the branches from which the limb is raised, giving the underside a characteristic embossed appearance. The leaves of the axillary twigs are smaller

The flower

Flowering takes place from June to September. The type of inflorescence is the cyme. White, pink, briefly pedunculated, the flowers are grouped by three or six in unilateral axillary verticals, spaced along the stem and inserted into the underhead of the upper and central leaves.

The fruit It is a tetrakee 1.5 to 2 mm wide, consisting of four small ovoid akenes and smooth dark brown color. They stay long at the bottom of the parched chalice (**Ronat , 2001) .**

2.3. Habitat

The lemon balm is a perennial tufted shrub sub-shrub. It is spontaneous in woods, roadsides, along hedges and preferably in damp and shady places. It is also a cultivated plant.

Ronat , 2001. In the wild are found in North America, southern Europe and Asia Minor in Africa and Argentina. (**Wichtl and Anton , 1999) .**

2.4 .Cultivation and harvest

After the second year of cultivation, one can get the first normal harvest (**Wichtl and Anton, 1999)** . In the first year, the producer can only have 25% of the normal yield. Leaves and stems are collected before flowering, i.e. late June to early July. A second harvest can be done in late August to early September. In the past, pick-up was done with sickles, but today mechanical harvesters are used, especially when the harvest area is large. The drying of the plant is done directly after the end of the harvest (**Adimi, 2016)** .

2.5. Classification

The botanical classification places Melissa officinalis in :

Reign : Plantae

Under reign :Tracheobionta

Division: Spermatophyta

Branch: Spermaphytes

Under Branch: Angiosperms

Class :Dicotyledones true

Subclass :Asterials

Order :Lamiales Family : Lamiaceae

Kind . Melissa

Species ,. Melissa officinalis (**Bounihi , 2016) .**

2.6 .Used parts of the plant

Fresh or dried aerial parts, leaves and flowering luminaries are used

Figure 05: dried aerial parts of lemon balm **(Iserin,2001) .**

2 .7 . Composition of the Officinal Melissa

The chemical composition of aromatic plants medicinal and medicinal issues is complex. Scientific research defines natural plant substances as chemical compounds found in many families and plant species (**Firn, 2004) .**

According to various studies and scientific research on this plant, many substances have been identified for officinal lemon balm, in this part we will present the chemical compounds inside the plant of the official lemon balm, La officinal mix consists of two types of compositions: non-volatile organic nostotmsmocpolyphenol. And Volatile Compounds essential oils

2.7.1 .Non-volatile

Non-volatile Lemon balm leaves possess Flavonoids, Triterpenes, Phenol Acids, and tannins (**Iserin, 2001) .**

2.7.1.1-Flavonoids

Flavonoids in the broadest sense are almost universal pigments of plants. Almost always water soluble, they are responsible for the coloring of flowers, fruits and sometimes leaves (**Ronat , 2001) .** The termflavonoid refers to a very wide range of natural compounds belonging to the polyphenol family (**Seyoum and al, 2006**) . They probably intervene to protect plants from herbivores and control the transport of auxins (**Judd and al, 2002) .**

flavonoids have in common the C6-C3-C6 structure of the diphenylpropane **,** the three carbons serving as junction between the two benzene nuclei rated A and B generally form an oxygenated heterocycle C **(De Rijke and al., 2006).**

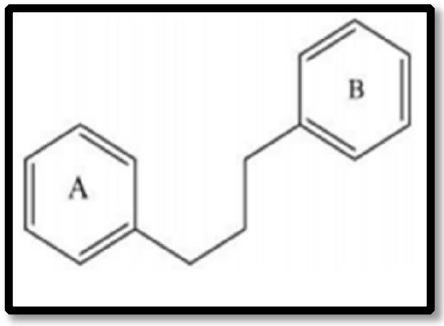


Figure06 . basic structure of flavonoïds**(De Rijke and al, 2006).**

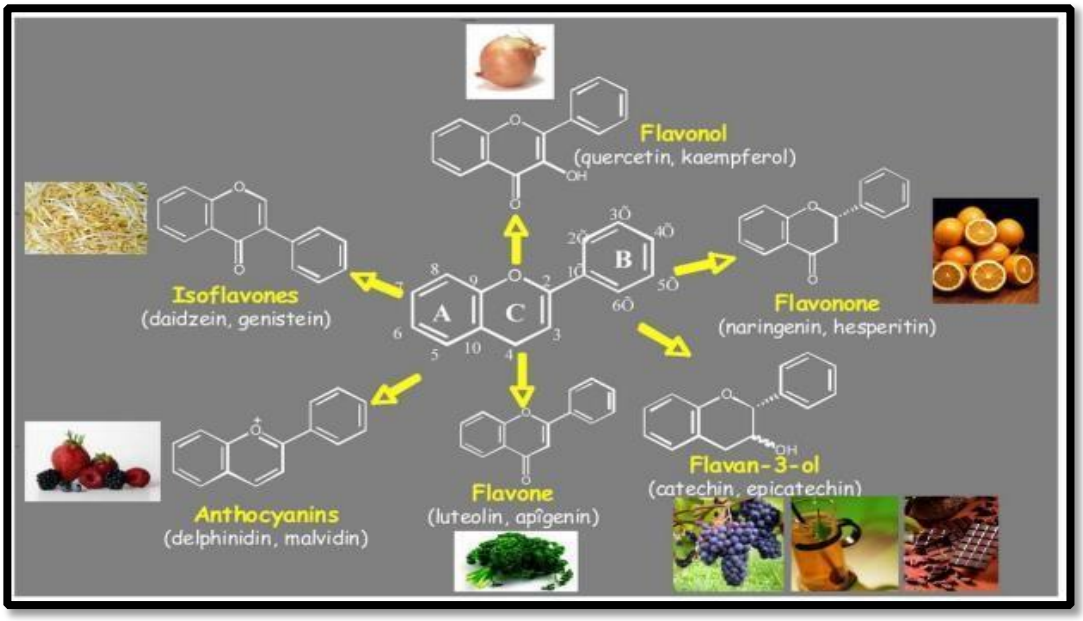


Figure07 . Structure of flavonoïds (aglycones) and position of the main substitutes **(Stoclet and Schini-Kerth, 2011) .**

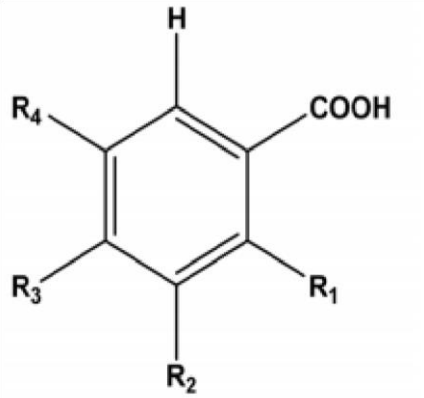
2.7.1.2 Phenolic acids

They are composed of a single phenolic nucleus and at least one carboxylic function (**Frédéric, 2011) .** These organic compounds have at least one function carboxylic and a phenolic hydroxyl **(Bruneton, 2009)** .

A-hydroxybenzoïc acids

They have a basic structure C6 - C1.et is derived from benzoic acid; these hydroxybenzoic acids

are very common, both in free form and in the form of esters or heterosides **(Bruneton, 2015) .**

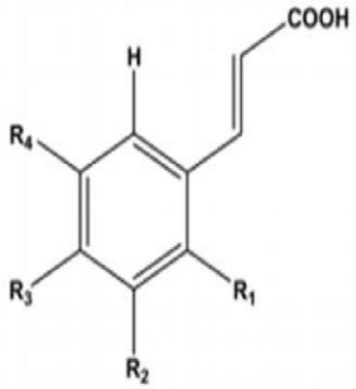


Nom	R_1	R_2	R_3	R_4
Acide benzoïque	H	H	H	H
Acide p-hydroxybenzoïque	H	H	OH	H
Acide vanillique	H	OCH_3	OH	H
Acide gallique	H	OH	OH	OH
Acide protocatéchique	H	OH	OH	H
Acide syringique	H	OCH_3	OH	OCH_3
Acide gentisique	OH	H	H	OH
Acide vératrique	H	OCH_3	OCH_3	H
Acide salicylique	OH	H	H	H

Figure 8. chimical Structure of hydroxybenzoïc acids **(Stalikas, 2007).**

B-Hydroxycinnamic acids

Are derived from cinnamic acid, whose basic structure is C6-C3, they include p-coumaric acid, o-coumaric acid, m-coumaric acid, colic acid, ferulic acid and sinapic acid **(Laribi , 2015) .**



Nom	R_1	R_2	R_3	R_4
Acide cinnamique	H	H	H	H
Acide o-coumarique	OH	H	H	H
Acide m-coumarique	H	OH	H	H
Acide p-coumarique	H	H	OH	H
Acide férulique	H	OCH_3	OH	H
Acide sinapique	H	OCH_3	OH	OCH_3
Acide caféique	H	OH	OH	H

Figure 9. chemical structures of hydroxycinnamic acids **(Stalikas, 2007) .**

2.7.1.3 Triterpenic Acids

Treterpenes are highly lipophilic molecules, specific to the plant world **(Bruneton, 2009)**. Are compositions in C 30, in the lemon balm, they are represented by ursilic acid and oleanolic acid **(Tessier, 1994) .** These are two isomers widely prevalent in the plant world **(Liu, 1995) .**

2.7.1.4 . Other compounds

Lemon balm leaves possess 8 to 10%water, 10 to 12% mineral materials, vitamin B1 and B2 .4% catenic tannins, succinic acid and a principle **(Paris and Moyse, 1971)** . They have properties on cardiac dethism**(Thoby, 2009) .**

2.7.2 . Volatile compounds an oil essential (essence volatile oils)is a liquid concentrated in odorous product, usually complex compositions containing the volatile principles contained in plants,Essential oil accounts for an average of only 0.08% of dry lemon balm leaves **(Bruneton, 2009) .** The essential oil, pale yellow in colour, is rich in volatile terpenic compounds which give it a slight

lemony smell **(Bahtiyarca and Cosge, 2006) .**The chemical components of different aromatic

These are complex and variable constituent components that belong mainly to two large families of chemical compounds: the group of terpenic**.**and some sesquiterpenes

2.7.2.1Terpenoids

Terpenoids are highly lipophilic compounds made up of isoprenic units (C5H8). They are mainly represented by monoterpenes and sesquiterpenes. Some of these products are highly volatile **(Bruneton, 2009) .A.Monoterpenes**

Are derived from the coupling of two isoprenic units (C5H8). They are widely represented in the lamiaceae and lauraceae, they met about 60 of the composition of the essential oil of lemon balm **(Gazengel and Orecchioni, 1999) .**

- **Monoterpenic hydrocarbons**The lemon balm contains two hydrocarbons
- monoterpenic: cis-ocimene and trans-ocimene**(Bruneton,2009) .**
- **Monoterpenic Alcohols**

The essential oil of lemon balm contains acyclic monoterpenic alcohols such as citronellol, geraniol**,**linaloland nerolpresent up to 5% **(Teuscher and al., 2005)** . **Monoterpenic Aldehydes**:

Monoterpenicadehydes are generally acyclic **(Bruneton,2009) .**They contribute to the aroma of the essential oil of lemon balm. They account for 50-97% of the latter **(Teuscher and al., 2005) .**-

Citral (neral - geranial)

- Citronellal

2.7.2.2 Sesquiterpenes

The essential oil of lemon balm contains sesquiterpenes are made up of units of the C15 terpenes. They account for 30% of the income. **(Teuscher et al., 2005)** .

We also find ather compounds , like , cyclohexanone , d . limonene , linalool **(Jafani and Sani ., 2016)**

2.8. Uses of lemon balm

Traditional uses: This plant is used to soothe teeth and soothe beats as well as in cases of gastrointestinal disorders and abdominal pain. It has a beneficial effect on morale. (**Iserin , 2001)**

Relaxing plant: Melissa is a calming plant in cases of anxiety nervousness, irritability and mild depression AND regulates nerve impulses. It soothes the heartbeat of nervous origin and it decreases the emotional. Mélisse is recommended when anxiety causes digestive disorders such as bloating, indigestion, colic, nausea and acidity (**Iserin, 2001) .**

Hormonal plant: Mélisse calms hyperexcitability due to thyroid problems **(Iserin, 2001) .**

Herpes: lemon balm eliminates rashes due to the virus and reduces its frequency (**Iserin , 2001)**

Culinary uses: In the kitchen, young lemon balm shoots can be added in quantity to meats, fish, fruit and vegetable salads that they perfume remarkably with their lemony and aromatic note.

Other uses: Melissa is used to treat insect bites, cuts and fever (**Leilazade, 2018**) .

2.9. Melissa dosage

2.9.1 Internal Use

Nervousand digestivesdisorders

Dye (1:5 in 45% ethanol): Take 2 ml to 6 ml, 3 times a day.

Infusion: Infuse 1.5 g to 4.5 g of dried aerial parts in 150 ml of boiling water and take 1 to 3 times a day.

(**Leilazade, 2018**) .

2.9, 2 External use

Insomnia, nervousness, restlessness

A gentle arm massage is done with a few drops of essential oil. You can also mix 10 drops of essential oil with a little liquid soap and pour into a hot bath (**Leilazade, 2018) .Labial Herpes**

Creams are dosed with 1% watery lemon balm extract and used twice a day until the lesions disappear(**Leilazade, 2018) .**

. Minor Injuries, Neuralgia 2 to 4 times a day on the affected area of a mixture of 5 drops of essential oil with 1 teaspoon of olive oil (**Iserin, 2001**) .

3. Pharmacological Properties of Melissa Compounds

In herbal medicine, officinal lemon balm is used for its properties:

3.1 Anti-oxidant properties

Some extracts have a marked antioxidant activity, which is explained by the presence of acid rosmarinic in water extracts **(Teuscher and al, 2005) .**Oxidative stress plays an important role in certain pathologies such as cardiovascular disease, cancer, Antioxidants are protective agents of cells, and Antioxidants fight against oxidative stress caused by chemical agents or, radiation so they protect cells. Some are endogenous synthesized by cells. Others are exogenous brought by food **(Nadji, 2010) .**

Thanks to its antioxidant properties that give cellular protection to neurons Mélisse essential oil can be associated with the treatment of Alzheimer's disease **(Bahtiyarca, 2006) .**

2.3 Effect on the cardiovascular tetsys

sdioocomlt are known for their protective effect on cardiovascular health by modifying several pathological processes which are involved in the onset of cardiovascular disease. Certain flavonoids have a positive effect in atherosclerosis and stable forms of cardiovascular disease by decreasing the oxidation of LDL by inhibiting LOX, reducing oxidative stress and reducing mcidissismoc**(Duchnowicz and al., 2012).** Flavonoids also have an interest in the treatment of arrhythmia and high blood pressure, especially through a decrease in oxidative stress. In the prevention of myocardial infarction, flavonoids would act by inhibition of platelet aggregation and a decrease in ROS **(Mladinka and al., 2010) .** **3.3 Anti-ulcetic**science in experiments made on rats, quercetin and naringenin have shown an important role in reducing ulcer and the protection of gastric cells. Other studies have established a close relationship between the anti-ulcer properties of quercetin, naringenine, rutine and kaempluerol, and the production of PAF (Platelet Activating Factor) which is a potential ulcer dieum. a reduction of gastrointestinal damage is probably due to the flawing inhibition by these flavonoids

(Izzo, 1996).

3.4Antiviral properties

Polyphenols are antiviral,they fight more especially the herpes virus which produces whitish **(Iserin ,.2001) .**

3.5 The central nervous system

Antidepressant effect: A watery extract of Melissa in mice has been shown to exhibit activity related to antidepressant activity in humans with imipramine therapy **(Doros, 2011) .** The Emamghoreishi (2009) study shows that 25 mg/kg of meliasse water extract administered

intraperitoneally reduces mouse immobility by 46%, increases escape attempts by 170% without altering the number of times the mouse swims **(Emamghoreishi and Talebienfor, 2009)**

.Hyperthyroidism

Lemon balm balances the functioning of the thyroid **(Iserin , 2001)**,Some previous symptoms obviously suggest hyperthyroidism: heart palpitations, fears, sweating, and hypertension (exist in about 1/3 cases hyperthyroidism) etc. The cause of chronic anxiety may indeed be due to underlying hyperthyroidism

Anti-inflammatory activity

Flavonoids are likely to reduce the release of histamine from basophils and mastocytes. Quercetin has an anti-inflammatory effect by inhibiting some synthetic enzymes such as nendooyeoycity**(Gonzalez and al., 2007).** K tsrle oi KtsuioidmcysmtysmcyAiystiyuodseumnysmcysryunysmciclursmcyociddyuomnmcidissisoueuyins moctmstotyleesitsonesytiit .dytyynytsKtsuioidmcymcimemsylsiytynuysmoc oi imtsismcyiorclsiisiddsiytysodynr**(Park and al , 2008)**

Chaptr III: Cardiovascular system of rat

1 . Cardiovascular system of rat

Rats are docile and easy-to-handle animals and are similar to human organs, they are the most commonly used species for animal experimentation. Both rats and mice account for more than 90% of the mammals used in biomedical research, and in order of importance the rat ranks second just behind the mouse. To give an order of ideas, in the United States, more than four million rats are used in the laboratory each year **(Descat, 2002).** The rat body is composed of three parts: the head, the trunk, and the limbs. A rat heart is made up of four cavities, two atria and two ventricles; the cardiovascular system performs a variety of functions; respiratory gas movement nutrients and waste. The rat is the first mammal to be domesticated for biological experimentation (**Rao and al, 1990)**

2. Definition

The rat cardiovascular system (CVS) consists of two parallel networks (the vascular system) the venous network that carries blood from the tissues to the heart and the arterial network that carries blood from the heart to tissues, heart, and Fluid (blood) The cardiovascular system ensures the flow of blood to all organs of the body **(Bradley and Calvert, 2009).**

3 . Function

It has the function of transporting substances between different parts of the body and intercellular communication, while eliminating their waste **(Dee unglaub, 2007).**So it carries oxygen, nutrients, hormones and many other substances essential to the body's homeostasis to cells, and rids them of their waste. The force that propels blood inside the body is provided by the pumping action of the heart **(Marieb, 2008).**

4 .Cardiovascular Anatomy

4.1. The general vascular system

THE General structure of the rat's circulatory system is almost identical to that of humans; pulmonary circulation carries blood through the lungs for oxygenation, then returns to the heart, systemic circulation circulates blood through the body after it leaves the heart (**https:/www.biologycorner.com/worksheets/rat_circuratory.html).**

4.2. .The heart

In the rat body,The central organ of circulation; The heart is a muscle and a hollow organ, The heart of a rat is relatively small, but extremely strong. Its average weight is 0.3g in adult rat of 300g weighs **(Bailly and Duprat, 1990).** An adult has a heart rate of 250 to 450 beats per 4 minute**(Akingbemi and Aire, 1994).** It is located between the two lungs, it has four cavities, two ventricles and two atria , and it is pumping blood to the arteries.

4.2.1. anatomyofHeart

4.2.1.1 . Heart cavity

The rat's heart consists of four chambers: left ventricles (VG) and right and left and right atria, right and left atria are distinguished by dark structures in the shape of an ear, the atria are located in the upper part of the heart,the ventricles is the starting point of blood circulation, it is located in the lower part of the heart the ventricles are separated by a partition called the interventricular septum, and the atria by inter-ear septum; The atrette and ventricleleft are communicated to each other by the mitral orifice (MI), the atrium and the right ventricle are communicated to each other by the tricuspid orifice (TRI)

(https:/www.passeportsante.net/fr/partier-corps/Fiche.aspx ?doc=cœur).

4.2.1.2 Heart Valves

Four valves give the blood one-way circulation. Each atrium communicates with the corresponding ventricle via a valve: the tricuspid valves on the right and the mitral valve on the left, the other two valves are located between the ventricles and the corresponding artery: aortic

valve and pulmonary valve
(https:/www.passeportsante.net/fr/partiercorps/Fiche.aspx ?doc=cœur).

4.2.1.3 Heart wall

The wall of the heart consists of three tunics, from the outside to the inside; pericardium, myocardium and endocardium. The pericardium, which is the outer tunic of the wall, is composed of a delicate connective tissue that makes the texture of the outer face of the heart smooth and slippery. The myocardium is the heart muscle tissue; it constitutes the bulk of the mass of the heart and is responsible for the pumping action provided by the heart. Endocardium is a fine end-end covering layer of connective tissue **(Tortora and Derrickson, 2007).**

4.2.1.4. Blood vessels

Blood vessels are the routes of transporting blood and metabolites Arterial circulation is ensured by the arteries divide the blood flow from the aorta to different tissues the capillary is a gas exchange site, nutrients, hormones between the arteries and venous that are at birth parallel to the arteries that carries blood from the tissues to the heart

4.2.2. Fonction of heart

ensures blood circulation through rhythmic contraction and with the respiratory system it allows oxygenation of the blood and the elimination of carbon dioxide The right heart propels poor blood into the pulmonary circulation in oxygen from the peripheral venous network The left heart propels the oxygenated blood through the pulmonary filter towards the peripheral arterial network

4.3. Blood

4.3.1. Definition of fluid tissue

Circulating in vessels consisting of cells floating in a yellow liquid substance, plasma, It has the function of transporting the O2, and nutrients, messengers to the tissues of the body to ensure their survival and functionality.

4.3.1.1 . Red blood cells

It is an anucleated cell in the shape of a biconcave disc flattened in its center. These cells are very easy to recognize on a smear, but it is important to observe them carefully to recognize variations in size, shape, color, structure and distribution. In photonic microscopy the erythrocyte appears as a pink grey disc, smaller than a human red blood cell with a diameter of 5.5 to 7 m. These extreme values are to be modulated according to age, young rats 2 to 4 weeks having larger erythrocytes adults over 8 weeks of time **(Hasegawa and Furuhama, 1998).**

4.3.1.2 . Platelets or Thrombocytes

Thrombocytes are small anucleated fragments of cytoplasm from megakaryocytes. They vary greatly in size in circulating blood but always range from 1 to 4 m and their volume is about 5 fl**(Groner and al,1986).** Their color is purple with some more pink azure granules. Their role being primarily to ensure the function of platelet aggregation in clotting, it is common to find them aggregated or in clusters on a blood smear because they were activated during the puncture or storage of the sample **(Descat, 2002).**

4.3.1.3 . White blood cells or leukocytes

Leukocytes are the largest of the blood cells. Morphologically one can differentiate several types of leukocytes:

Monocyte: It is a cell of the macrophagic lineage that measures 12 to 20 m in diameter. This is therefore the largest of the white blood cells **(Bailly andDuprat , 1990).**

Lymphocytes: The population mostly represented on a smear is that of small lymphocytes. They are 6 to 8 m in diameter and are characterized by a round core with very dense chromatin and a thin band of light blue cytoplasm. Larger lymphocytes are then observed in smaller quantities. As the name suggests, their diameter is larger (12 to 15 m) than that of small lymphocytes, which makes it possible to differentiate them **(Descat, 2002).**

Neutrophil granulocyte: It measures from 9 to 14 m. The almost colorless cytoplasm has a dusty appearance due to small pale pink neutrophil granulations that are less visible and less abundant than in humans **(Descat, 2002).**

Eosinophilic Granulocyte: Its diameter is 12 to 19 m. Its nucleus is annular, The cytoplasm is well colored because it contains many specific orange granulations and larger than neutrophil granulations **(Descat, 2002).**

5. Cardiovascular physiology

The rat has a closed circulatory system, deoxygenated blood flows into the right atrium through the tricuspid valve into the right ventricle, the blood is pumped from the right ventricle through the pulmonary semilunar valve into the pulmonary trunk that divides into right and left pulmonary arteries heading for the lungs .once in the lungs, blood is oxygenated and then returns to the left atrium through pulmonary veins. Blood is now in the left atrium and passes through the bicuspid valve into the left ventricle. the aorta pumps blood to the rest of the body

(https:/www.biologycorner.com/worksheets/rat_circulatory.html)

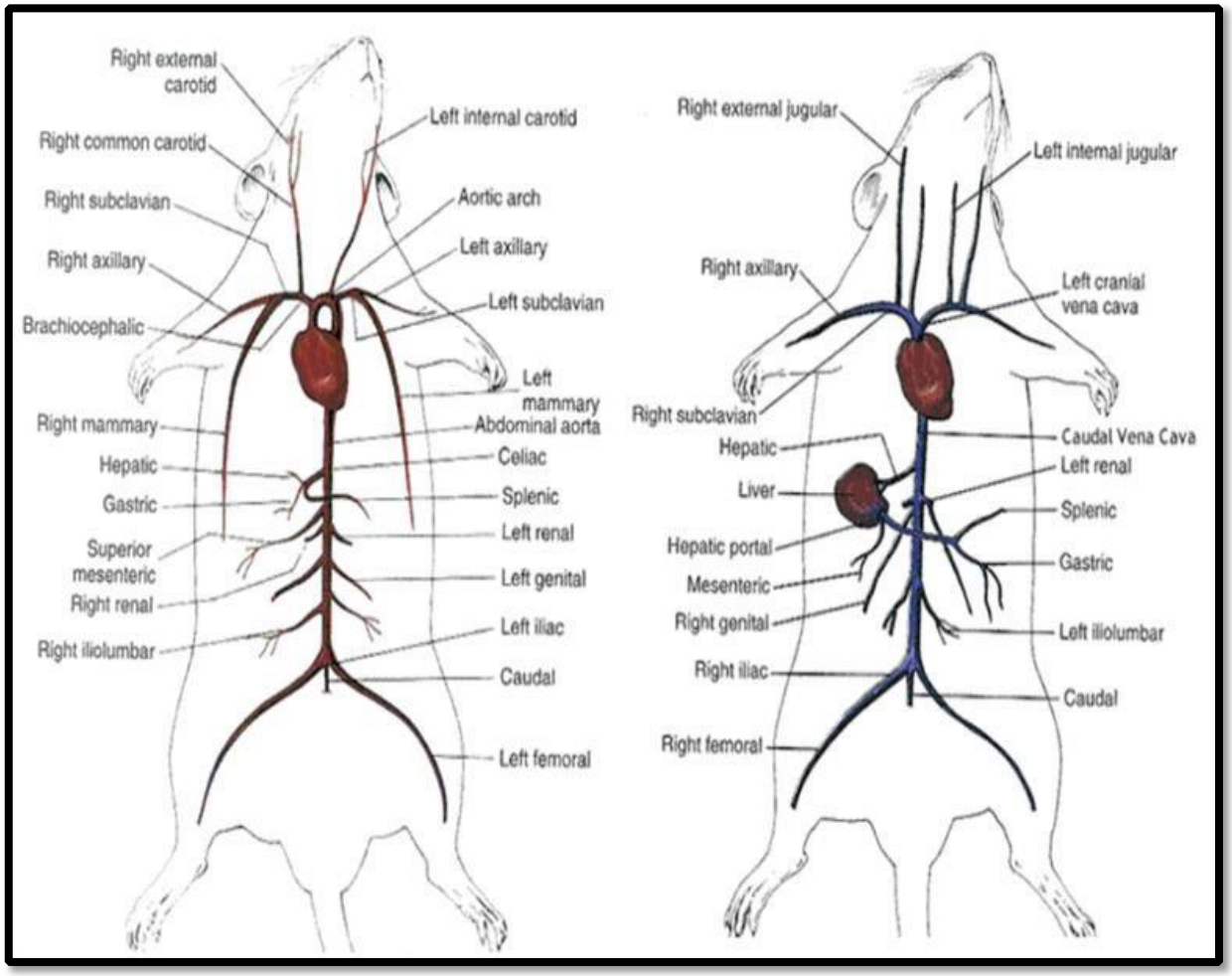
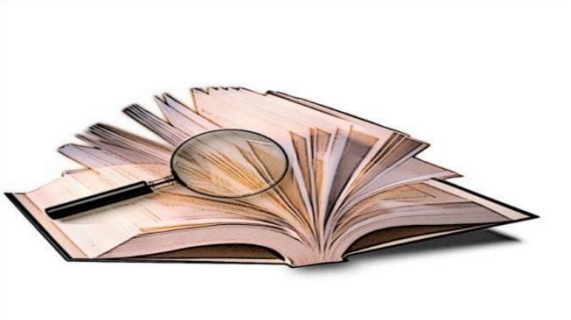


Figure 10 . transport of oxygenated and deoxygenated blood in the rat body

(https:/www.biologycorner.com/worksheets/rat_circulatory.html).

Practical part



Materials and methods

Materials and methods

1..Material and method

1.1. Material

1.1.1. Biological material

Our work is based on the line of male white rats Rattus rattus of the Wistar strain, we used 20 male rats, coming from the Pasteur Institute of Algiers (Center d'ElevagesElKouba, Algiers), six weeks old and having a weight body between 190 and 290 g They are mammals of the order rodents, widely used in various fields of research.

And the classification places white rats in :The
branch : Vertebrates.

Class : Mammals.

Ordre : Rodents .

under- ordre : Myomorphs.

Family :Muridae . under famille :Muridae**(Descat , 2002).**

Figure 11 : Male rats of the Wistar strain **(Personal photo)**

1.1.2. Chemical products

In this work, we used an insecticide which belongs to the family of neonicotinoid "Imidacloprid " in powder form and which has been diluted with distilled water, and also lemon balm plant in the form of extract and which has been diluted with distilled water

Figure12: Chemicalsproducts **(Personal photo)**

1.2. Methodology

1.2.1. Caring for animals

The animals are placed in special polyethylene cages for rats, which are lined with a litter made of wood shavings. It is changed twice a week until the end of the experiment followed by feeding and the water is filled in bottles.

Figure 13: Raising conditions for rats **(Personal photo).**

The dose of each treatment is calculated according to the weight of the rats of each batch , Imidacloprid and lemon balm are used in liquid form and given to rats by mouth , water in sufficient quantity daily and once a day

Figure 14: treating for rats (Personal photo).

The Raising was subjected to an adaptation period of approximately 90 days, under the conditions of the animal house, the temperature of which is kept constant (25 to 30 ° C) , The cages are cleaned and the litter is changed once every two days until the end of the experiment

1.2.2.Subdivision and treatment

After 90 days of adaptation, each rat was force-fed 1 time per day for 20 days.

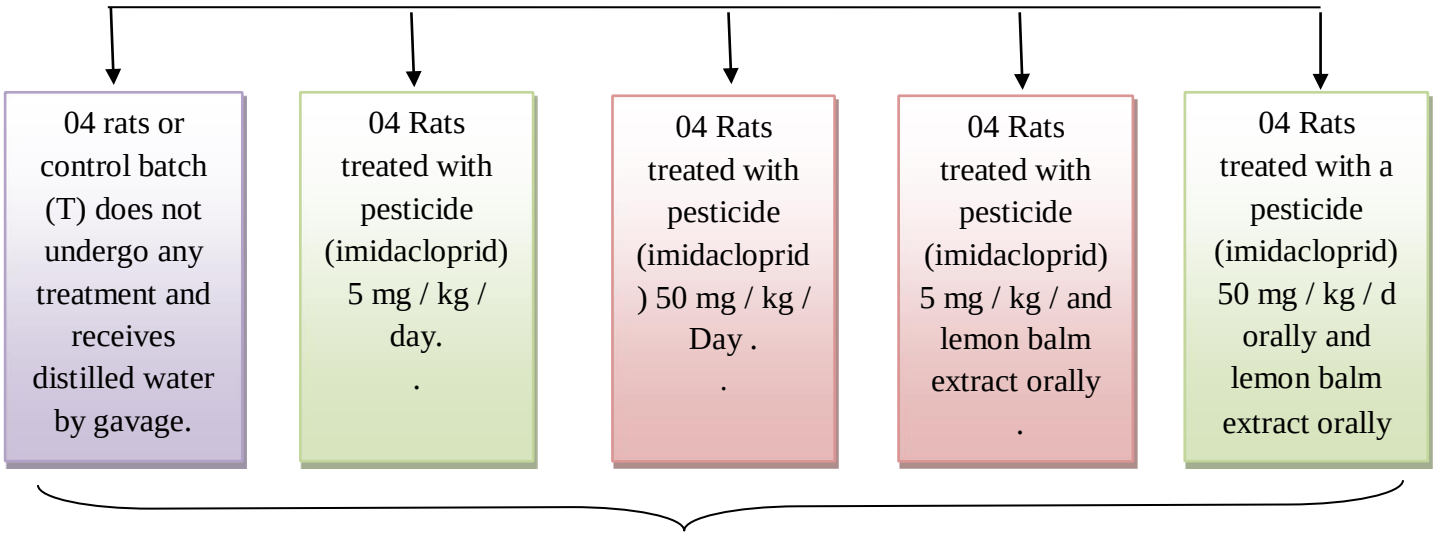
Thus, 05 batches of rats are distributed as follows:

- **Control batch**: 4 control batch (T) does not undergo any treatment and receives distilled water by gavage.

Lot A:4 rats treated with pesticide (imidacloprid) 5 mg / kg / day orally.

Lot B: 4 rats treated with pesticide (imidacloprid) 50 mg / kg / day oraly. **lot C**: 4 rats treated with pesticide (imidacloprid) 5 mg / kg / D + lemon balm extract per route

lot D: 4 rats treated with a pesticide (imidacloprid) 50 mg / kg / day and lemon balm extract orally .



After 20 days of treatment

90 days of adaptation

1.2.3 Sacrifice and removal

1.2.3.1 . Blood sample

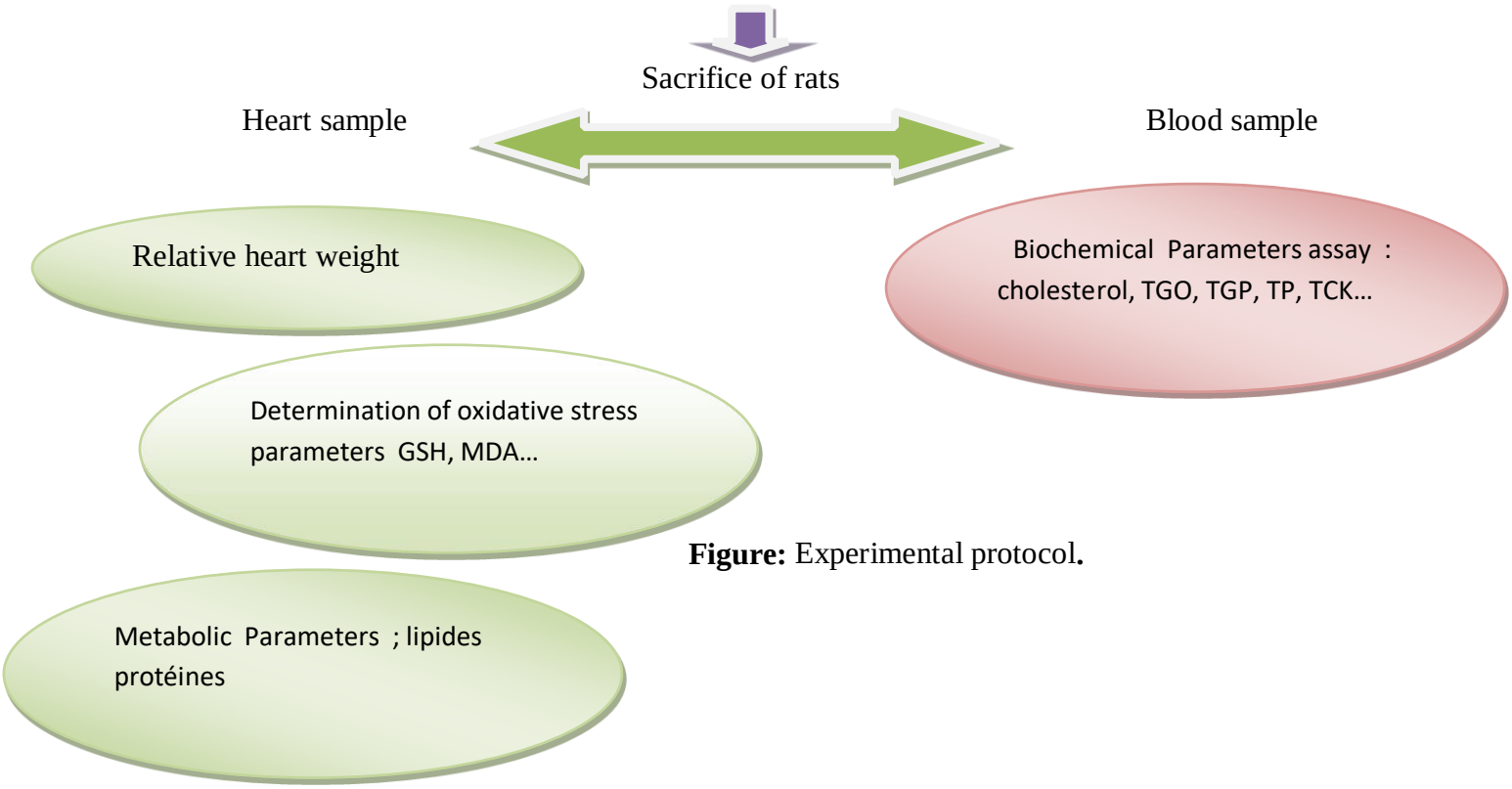
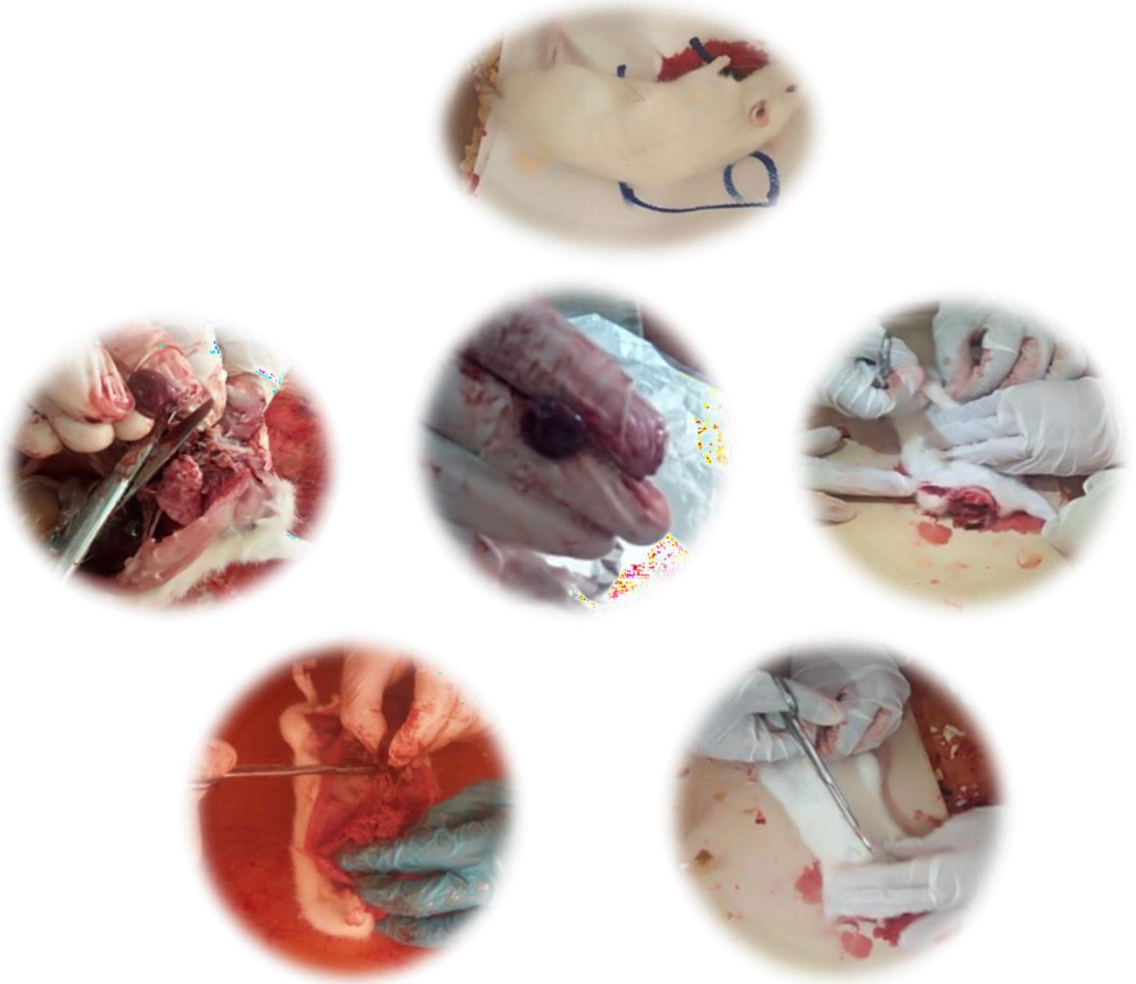


Figure: Experimental protocol**.**

After 20 days of treatment, the 5 groups are sacrificed after 12 hours of fasting (by decapitation), the blood is immediately collected in two labeled polyethylene tubes. One contains the dehydrated trisodium citrate anticoagulant, the citrate tube is gently inverted at least 5 times to avoid the formation of microcoagulants, and the other being dry, the latter is centrifuged at 5000 rpm for 15 minutes, The samples obtained were stored in the freezer at – 20 ° C until the biochemical parameters were analyzed.

1.2.3.2. Organ harvesting

The heart was quickly removed after dissection, rinse with physiological water prepared as follows: 0.9 g of NaCl with 100 ml of distilled water to remove the blood and dry with absorbent paper, and weighed and then stored by two methods : half in the freezer for the determination of the parameters of oxidative stress GSH, GST, GPx MDA and CAT. and the second half was fixed in Formol in order to carry out histological sections .



Estimated relative hearts weight

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

RHW (g/100g of WH) = WH / TW **x 100**

WH: weight of hearts (g). TW: total rat weight (g). RHW: relative heart weight (g)

Figure 15: hearts taken after sacrifice (personal photo)

1.3. Evaluation of biochemical parameters

1.3.1. Cholesterol dosage:

According to the Spinreact data sheet

• Principle:

The cholesterol present in the sample gives a link to a colored compound, according to the following reaction :

CHE

Cholestérol ester + H2O ⟶ Cholestérol+ Acides gras

CHOD

Cholestérol + O2 ⟹ Cholestérol+ H2O2

POD

2H2O2 + Phenol + 4- Aminophenazone ⟹ Quinoneimine + 4 H2O

The intensity of the color formed is proportional to the concentration of cholesterol present in the test sample. Read the optical density at 505 nm.

1.3.2. Dosage Transaminase glutamate oxaloacetate (TGO):

According to the Spinreact data sheet.

• Principle:

Aspartate aminotransferase (AST) also called glutamate oxaloacetate (GOT) catalyzes the reversible transfer of an amino group from aspartate to a-ketoglutarate forming glutamate and oxaloacetate. Oxaloacetate is reduced to malate by malate dehydrogenase (MDH) and NADH, H + (Murry, 1984).

ASAT

Aspartate + α –cétoglutarate glutamate oxaloacétate + oxaloacétateOxaloacétate + NADH+ H + MDH Malate + NAD Calculation :

ΔA/min X 1750 = U/L de TGO

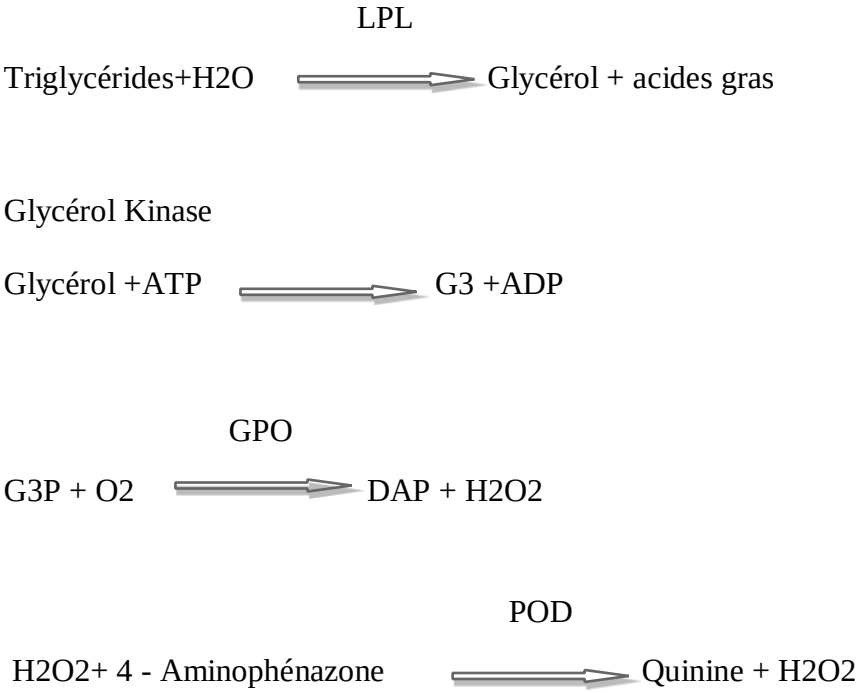
U / L: international unit / L is the amount of enzyme that transforms 1 µmol of substrate per minute

1.3.3. Determination of TRG triglycerides:

According to the Spinreact data sheet.

• Principle:

Triglycerides are determined according to the following reactions:



Incubate for 5 min at 37 ° C or 10 min at room temperature read the optical densities at 505nm. (Young and Pestaner, 1975; Fossati and Prencipe, 1977)

1.4. . Evaluation of oxidative stress parameters

1.4.1. Evaluation of non-enzymatic biomarkers

Homogenate preparation

One gram of hearts from rats of the different groups studied was used. After grinding and homogenization of the tissues in TBS (50 mM Tris, 150 mM NaCL, pH 7.4), the cell suspension was centrifuged (10,000 rpm, 4 ° C, 15 min), then the supernatant obtained is aliquoted in eppendorf tubes then stored at -20 ° C while waiting to perform the assays of the parameters of oxidative stress.

1.4.1.1. Glutathione Assessment (GSH)

• The principle

The assay for glutathione is carried out according to the Weckber& Cory method (1988). The principle of this assay is based on the measurement of the optical absorbance of 2-nitro-5mercapturic acid which results from the reduction of the acid. 5.5 dithio-bis-2-nitrobenzoic acid (DTNB) by the groups (-SH) of glutathione. For this we carry out a deproteinization in order to keep only the groupings (-SH) specific for glutathione

• Procedure

The experimental procedure for assaying glutathione is as follows:

• take 200 mg of tissue to which we add 0.8 ml of EDTA solution (Ethylene Acid Diamine Tetra Acetic) at 0.2M

• Take 0.8 ml of the homogenate.

• 0.2 ml of the salicylic acid solution (0.25%).

• Shake and leave for 15 minutes in an ice bath.

• Centrifuge at 1000 rpm for 5 min.

• Take 0.5 ml of the supernatant

• Add 1 ml of Tris buffer, pH 9.6.

• Mix and add 0.025 ml of 5.5 dithio-bis-2-nitrobenzoic acid (DTNB) to 0.01 M.

Leave for 5 min at room temperature and read the optical densities at 412 nm, against a blank containing distilled water replacing the amount of supernatant.

• Calculation

The concentration of glutathione is obtained by the following formula:

Calculation

The concentration of glutathione is obtained by the following formula:

GSH (nmol GSH/ mg protéine) = DO x 1 x 1.525

13100 x0.8 x 0.5x mg protein

- ✓ D0 : Optical density
- ✓ 1 : Total volume of solutions used in deproteinization (0.8 ml homogenate + 0.2 ml salicylic
- ✓ 1.525 : Total volume of the solutions used in the GSH assay at the supernatant level (0.5 ml supernatant + 1 ml Tris + 0.025 ml DTNB).

  13100 : Absorbance coefficient of the -SH group at 412 nm.

  0.8 : Volume of the homogenate.

  0.5: Volume of the supernatant.
- ✓

1.4.1.2. Assessment of MDA malondialdehyde

•Principle

MDA is one of the end products formed during the breakdown of polyunsaturated fatty acids (PUFA) mediated by free radicals. In our study, the levels of cardiac MDA were evaluated according to the method of Esterbauer et al, (1992). The assay is based on the condensation of MDA in an acidic and hot medium with thiobarbituric acid, to form a pink pigment absorbing at 530 nm,

• Procedure

-Take 375 µl of supernatant,

- Add a volume of 150µl of the TBS solution (50mM tris, NaCl (150mM; pH 7.4)) and 375µl of

TCA-BHT solution (TCA 20%, BHT 1%) -

Centrifuge for 10 minutes at 1000 rpm.

-Take a volume of 400µl of the supernatant

-Add 80µl of 0.6M HCL and 320µl of the tris-TBA solution (tris 26mM, TBA120mM).

- Mix and incubate in a water bath at a temperature of 80 ° C for 10 minutes.

-read the optical density at 530 nm against a blank containing distilled water replacing the amount of supernatant.

• Calculation

$$[\text{MDA}]\text{nmol/ml de plasma} = (DO \times 10^{6})/(\varepsilon \times L \times Fd)$$

DO : Optical density at 530 nm.

E : MDA molar extinction coefficient = 1.56 105 M cm.

L : Length of the optical path = 0.779 cm.

Fd : Dilution factor: Fd = 0.2083.

1.4.2. Enzyme biomarkers

1.4.2.1. Glutathione peroxidase (GPx) assessment

• Principle

The MDA assay is carried out according to the method of Flohe and 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

GPx

$H_2O_2 + 2GSH \quad GSSG + 2H_2O$

Procedure

The experimental procedure for assaying glutathione peroxidase is as follows:

• Take 0.2 ml of supernatant.

- recover in a tube containing 0.4ml of 0.1mM GSH and 0.2ml of 0.067M phosphate buffer, pH 7.8.
- Mix and incubate in a water bath at a temperature of 25 ° C for 5 minutes.
- add 0.2ml of H2O2 (1.3mM) to initiate the reaction.
- After 10 minutes add 1ml of 1% TCA (tri chloro-acetic acid)
- Shake and leave for 30 minutes in an ice bath. and centrifuge for 10 min at 3000 rpm
- A volume of 0.48 ml of supernatant is placed in a tank to which 2.2 ml of 0.32M Na2HPO4 is added with 0.32ml of 1mM DNTB. Mix well and read after 5 minutes

The absorbance reading is carried out for one minute and each 15sec at a wavelength of 412 nm against a blank containing GSH replacing the amount of supernatant.

- Calculation

The determination of the enzymatic activity of GSH-Px is obtained by the following formula:

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

- ✓ Échantillon OD sample : Optical density of the sample.
- ✓ DO standard : Optical density of the standard.
- ✓ 0.04: Concentration of substrate (GSH).

1.4.2.2. Assessment of glutathione S-Transferase (GST) activity

- **Principle**

The measurement of glutathione S-Transferase (GST) activity is determined according to the method of Habig et al. (1974), It is based on the conjugation reaction between GST and a substrate, CDNB (1-Chloro2, 4-dinitrobenzene) in a cofactor glutathione

(GST), the conjugation leads to the formation of a new molecule; 1-S-Glutathionyle 24Di nitrobenzene for measuring GST activity. The value of the optical density measured is directly proportional to the amount of conjugate formed itself linked to the intensity of the GST activity.

• Procedure

The experimental procedure for assaying glutathione S-Transferase is as follows:

- take 100 mg of tissue to which 1 ml of phosphate buffer (0.1M, pH6) is added,

- centrifuge at 14,000 rpm for 30 min

react 200 µl of the supernatant with 1.2 ml of the CDNB mixture (1 mM), -The reading of the absorbances 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.

• Calculate

The concentration of GST is obtained by the following formula:

$$\text{GST}(\mu\text{mol/min/mg de protéine}) = \frac{A \times v}{e \times d \times v} / \text{mg de protéine}$$

A : Absorbance.

V: Total volume of the tank = 1400 µl [200 µl of supernatant + 1200 µl of the mixture CDNB (1mm) / GSH (5mm) / sodium phosphate buffer (0.1 M, ph 6)].

E : Extinction coefficient = 9.6 mm-1cm-1.

D :tank thickness = 1 cm.

V : Volume of the supernatant = 200 µl.

Mg of protein: Concentration of proteins in the sample.

1.4.2.3. Catalase assay (CAT)

• The principle

Determination of catalase activity (CAT) The activity of catalase is determined according to the method of (Cakmak and Horst, 1991) The principle is based on the disappearance of H2O2 at 25 ° C by the presence of catalase.

• Procedure

- 100 µl of the crude enzymatic extract + 2850 µl of phosphate buffer (50 mM, PH = 7.2) are taken.
- 50 µl of hydrogen peroxideH2O2 is added to 0.3 ml.

-The reaction is triggered by the addition of hydrogen peroxide. Catalase activity is expressed in nmol / min / mg of proteins. The reaction is monitored by continuous reading for 15 minutes for 1 minute at a wavelength at 240 nm.

• calculation

$$L'activitéde\ CAT\ (MH_2O_2/min/m\ protiènes) = \frac{\Delta\ DO.VT}{\varepsilon.L.X.Ve}$$

• $\Delta \boldsymbol{DO}$: Variation of the optical density per minute as a function of time

• $\boldsymbol{\varepsilon}$: H2O2 extinction coefficient (3900 µM-1 .Cm-1. L)

• L: width of the cell or length of the optical path (1cm)

• X: quantity of proteins in mg / ml

• VT: total volume of the reaction mixture in ml

• Ve: volume of the enzyme extract in ml

1.5. Evaluation of metaboliaue parameters

1.5.1. Protein dosage:

The proteins are dosed according to the Bradford method (1976) and the extraction is according to Shibko et al, (1967) weighed 0.5 g of heart which will be cut and macerated in 5 ml of trichloroacetic acid TCA (20%) after grinding and filtration, a first centrifugation at 5000 rpm is carried out for 10 min then the supernatant is poured and the pellet is kept in the same tube, 1 ml of the Ether / Chloroform mixture is then added thereto (1 / 1), and after a second centrifugation (5000 rpm for 10 min), the supernatant 2 containing the lipids and the pellet 2 containing the proteins is obtained. The protein concentration is determined according to the method of Bradford (1976),

• Procedure

We proceeded to the following stages:

-one 1ml of distilled water is added to the base 2

- after shaking the tubes by agitator,

-only take 200 µl of aliquot using a micropipette, add 4 ml of BBC -

let stand for 5 minutes.

-then we read at a wavelength 595 nm.

1.5.2. Lipid assays

The lipids are dosed according to the Goldsworthy et al. Method (1972), 100 µl of the aliquot supernatant is taken, 1 ml of sulfuric acid is added and the tubes are stirred in a water bath at 100 ° C. for 10 min.

After cooling, take again with a micropipette 200 µl of aliquot dosage, add 2.5 ml of the 85% sulfophospho-vanillinic mixture, the whole is left for 30 minutes in the dark, then proceed with the reading at a wavelength of 530 nm.

1.6. permeability and mitochondrial swelling

The mitochondria are extracted according to the method described by Rustin et al (1994) .It is a purification by differential centrifugation, the hearts are quickly removed to make the preparation

• **Procedure**

Prepare the solution

- buffer (TSE, 4 ° C, ph7, 2) by (10mM Tris = 1.576g in 1L + 250Mm sucrose = 87.07 g in 1L + 0.1Mm EDTA = 0.037g in 1L)
- buffer (TS, 20 ° C, ph7, 2) by (250mM sucrose = 87.07g in 1L + 50mM Tris = 7.88g in 1L)

The experimental procedure for Mitochondrial Isolation and Swelling is as follows:

- take 300 mg of tissue + 3.5 ml of TSE, centrifuge at 10,000 rpm for 30 min
- the pellet obtained centrifuge 2nd time at 10,000 rpm for 30 min
- Take the supernatant from the second centrifugation at 14,000 rpm for 30 min
-the pellet obtained recovered in 1 ml of TSE and centrifuge at 14,000 rpm for 30 min
-the pellet of this last centrifugation recovered in 1 ml of TS and centrifuge at 14,000 rpm for 10 min
-the final pellet consisting of mitochondria recovered in 500 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 (Gasmi S, 2018)

• Assessment of swelling, permeability

According to the method of Crystal et al 1996, we carried out the estimation of 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 of 450nm wave for 3 minutes and every 30sec

1.7. Statistical analyzes

The results obtained were expressed by the average of four repetitions (mean ± standard deviation), and to better visualize using the Excel 2007 office to represent these results in the

form of graphs and histograms. The statistical analysis was carried out using Minitab® 19 software. The significance of the difference between the control batch and the treated batches is checked using anova on way sample test , and the comparison result as follows

- 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 P: Significance threshold

Results

2.1. Effect of Imidacloprid and Melissa Officinalis Extract on Weight

2.1.1 . Effect of Imidacloprid and Melissa Officinalis Extract on Relative Heart Weight of heeart of rats

For monitoring changes in relative heeart weights period we observed a non-significant decrease ($p > 0.05$) in the batch treated with imidacloprid at a dose (5 mg / kg / day), and a very highly significant decrease ($P < 0.001$) in the batch treated with the same pesticide in deferent dose (50mg / kg / day),

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

Table02 : variation of relative heeart weights in different experimental groups .

Experimental groups	control rats04	04Rats treated with pesticide imidacloprid 5 mg / kg /day	4Rats treated with pesticide imidacloprid 50 mg / kg /day	04 Rats treated with pesticid imidacloprid 5 mg / kg /day and melissa extract	04 Rats treated with pesticide imidacloprid 5 mg / kg /day and melissa extract
Relative weight	0,28±0,01	0,28±0,01	0,1825±0,0075	0,315±0,01	0,2275±0,008

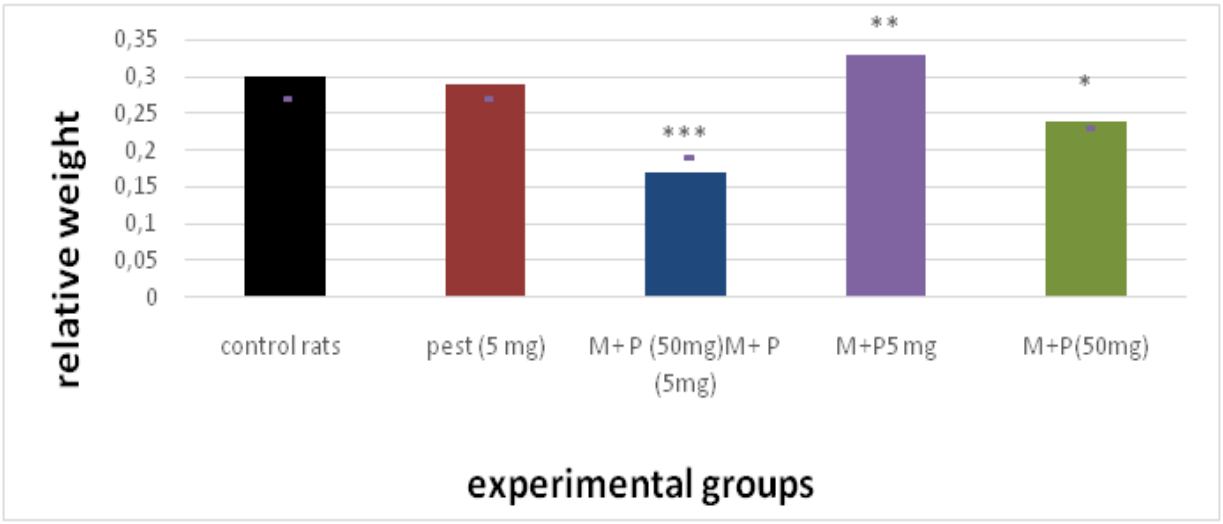


Figure16: . Evaluation of relativeheeart weights in rats treated with imidacloprid and Melissa Officinalis extract compared to controls rats .

2.1.2. Effect of Imidacloprid and Melissa Officinalis Extract on Weight gain of rats This figure represents the variation in weight gain in rats treated with imidacloprid and Melissa Officinalis extract compared to the controls.

In our work, no significant variation was observed in the batch treated with imidacloprid at a dose (5 mg / kg / day), we observed a very highly significant decrease (P <0.001) in the batch treated with the same pesticide at a dose (50mg / kg / day),

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

Table03 : variation of weight gain in different experimental groups .

Experimental groups	control rats04	04Rats treated with pesticide imidacloprid 5 mg / kg /day	4Rats treated with pesticide imidacloprid 50 mg / kg /day	04 Rats treated with pesticid imidacloprid 5 mg / kg /day and melissa extract	04 Rats treated with pesticide imidacloprid 5 mg / kg /day and melissa extract
Weight gain	21,25±1,375	8,5±2,25	2±0,5	10±1,5	5,25±0,875

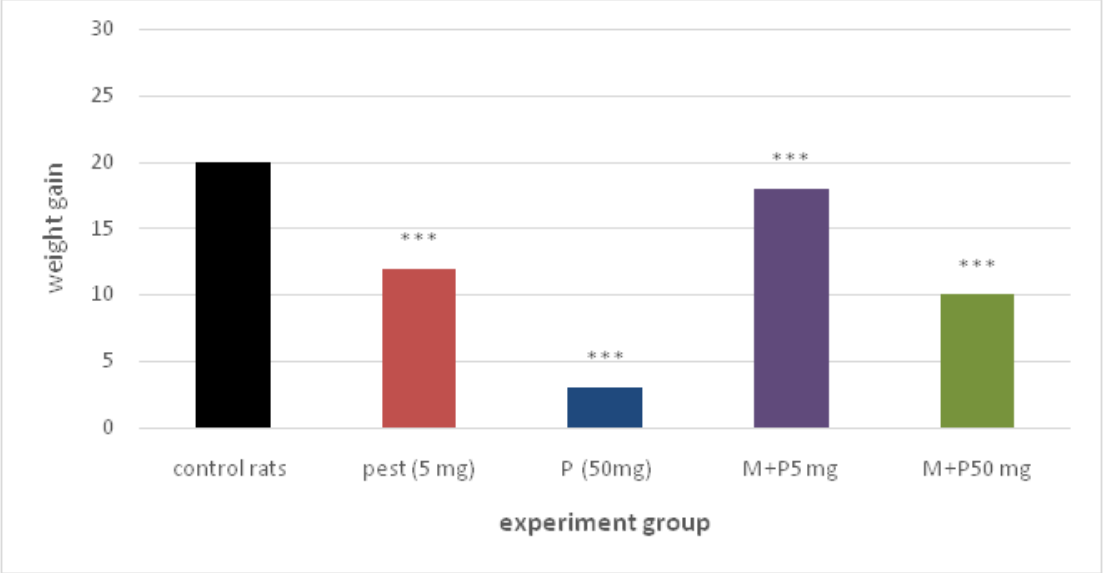


Figure17: . Evaluation of Weight gain in rats treated with imidacloprid and Melissa Officinalis extract compared to controls rats .

2.2 . Effect of imidacloprid and Melissa Officinalis extract on biochemical parameters

2.2.1. Effect of Imidacloprid and Melissa Officinalis Extract on Cholesterol

This figure represents the variation of Cholesterol levels in rats treated with imidacloprid and Melissa Officinalis extract compared to controls rats .

In our work we observed a non-significant increase ($p > 0.05$) in the batch treated with imidacloprid at a dose (5 mg / kg / day), and a very highly significant increase ($P < 0.001$) in the batch treated with the same dose pesticide (50mg / kg / day) and in the batch treated by the 50 mg / kg dose combination of imidacloprid and Melissa Officinalis extract On the other hand, no significant variation was observed in the batch treated by the combination of dose 5 mg / kg of imidacloprid and the extract of Melissa Officinalis **Table04 :** variation in Cholesterol levels different experimental groups .

Experimental groups **Cholestrol (g/l)**	control rats04	04Rats treated with pesticide imidacloprid 5 mg / kg /day	4Rats treated with pesticide imidacloprid 50 mg / kg /day	04 Rats treated with pesticid imidacloprid 5 mg / kg /day and melissa extract	04 Rats treated with pesticide imidacloprid 5 mg / kg /day and melissa extract
	0,432±0,012	**0,447±0,032**	**0,707±0,022**	**0,417±0,012**	**0,597±0,017**

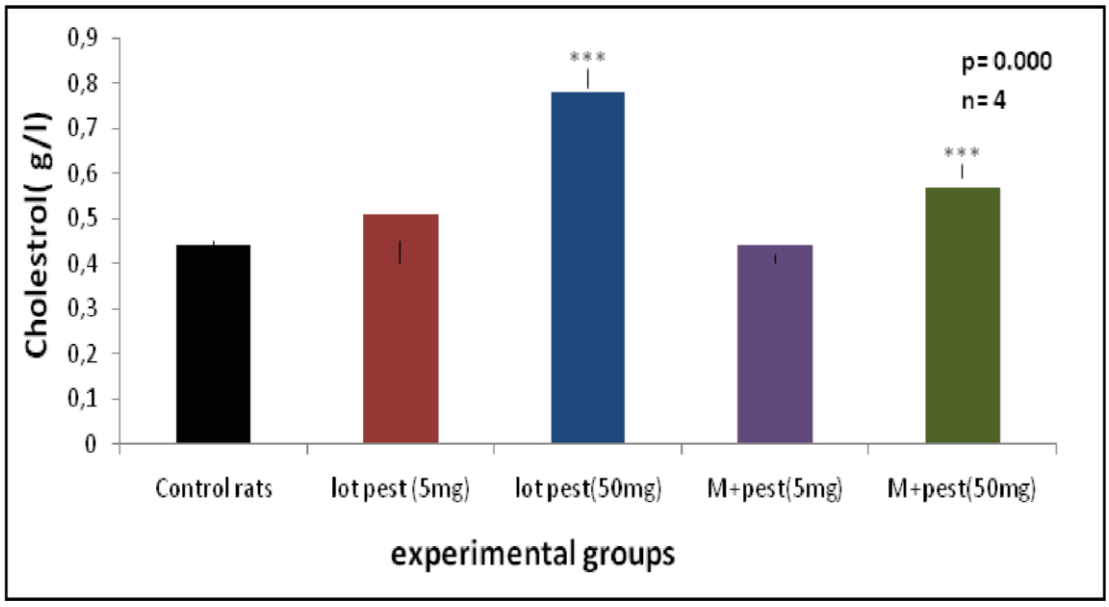


Figure18: . the variation of Cholesterol levels in rats treated with imidacloprid and Melissa Officinalis extract compared to controls.

2.2. 2Effect of imidacloprid and Melissa Officinalis extract on triglyceride

This figure represents the variation of triglyceride in rats treated with imidacloprid and Melissa Officinalis extract compared to controls.

In our work, no significant variation was observed in the batch treated with imidacloprid at a dose (5 mg / kg / day), we observed a very highly significant increase (P <0001) in the batch treated with the same pesticide at a dose (50 mg / kg / day), and in the batch treated by the dose combination 50 mg / kg of imidacloprid and extract of Melissa Officinalis Also, a highly significant decrease (0.01> P> 0.001) is observed in the batch treated by the combination of dose 5 mg / kg of imidacloprid and the extract of Melissa Officinalis, **Table 05:** variation of triglyceride levels in different experimental groups .

Experimental groups	control rats04	04Rats treated with pesticide imidaclopri d 5 mg / kg /day	4Rats treated with pesticide imidaclopri d 50 mg / kg /day	04 Rats treated with pesticid imidaclopri d 5 mg / kg /day and melissa extract	04 Rats treated with pesticide imidaclopri d 5 mg / kg /day and melissa extract
TRG (g/l)	**0,73±0,005**	**0,75±0,01**	**0,982±0,012**	**0,575±0,03**	**0,882±0,007**

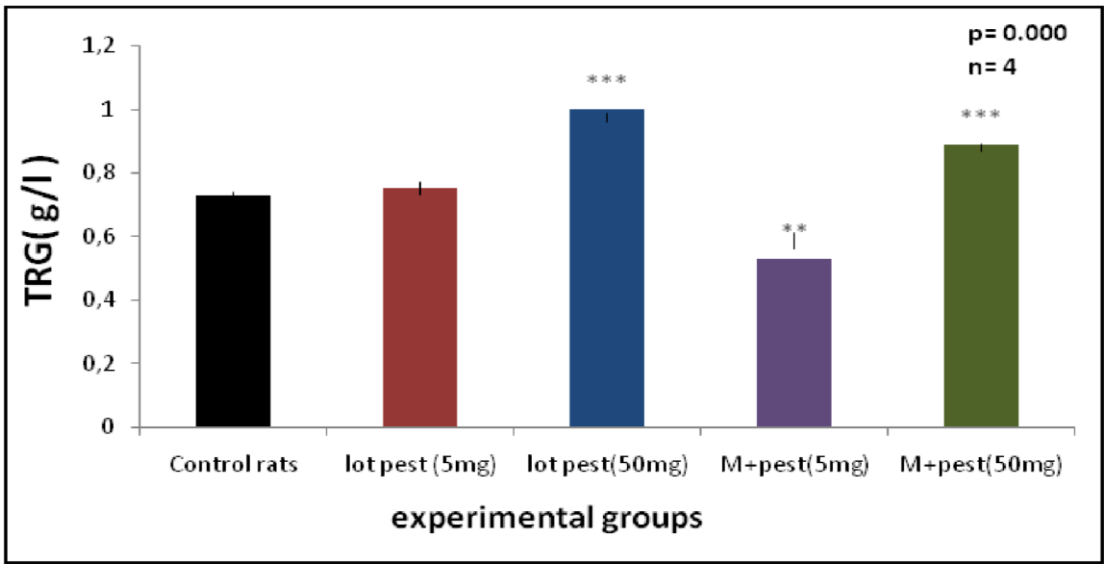


Figure19: . the variation of triglyceride in rats treated with imidacloprid and Melissa Officinalis extract compared to controls rats .

2.2.3.Effect of imidacloprid and extract of Melissa Officinales on the enzymatic activity of Transaminase glutamate oxaloacetate ASAT

This figure represents the variation of ASAT in rats treated with imidacloprid and the extract of Melissa Officinalis compared to the controls.

In our work, no significant variation was observed in the batch treated with imidacloprid at a dose (5 mg / kg / day), we observed a very highly significant increase (P <0001) in the batch treated with the same pesticide at a dose (50mg / kg / day)

Also, a highly significant decrease (0.01> P> 0.001) was observed in the batch treated with the 5mg / kg dose combination of imidacloprid and Melissa Officinalis extract, and a highly significant increase (0.01> P> 0.001) in the batch treated with the 50 mg / kg dose combination of imidacloprid and extract of Melissa Officinales**Table 06 :**variation of ASAT in different experimental groups .

Experimental groups	control rats04	04Rats treated with pesticide imidaclo prid 5 mg / kg /day	4Rats treated with pesticide imidaclo prid 50 mg / kg /day	04 Rats treated with pesticid imidaclo prid 5 mg / kg /day and melissa extract	04 Rats treated with pesticide imidaclo prid 5 mg / kg /day and melissa extract
ASAT UL/L	**67,75±2,875**	**69,5±4**	**133,25±9,25**	**54,5±3,25**	**85,5±3**

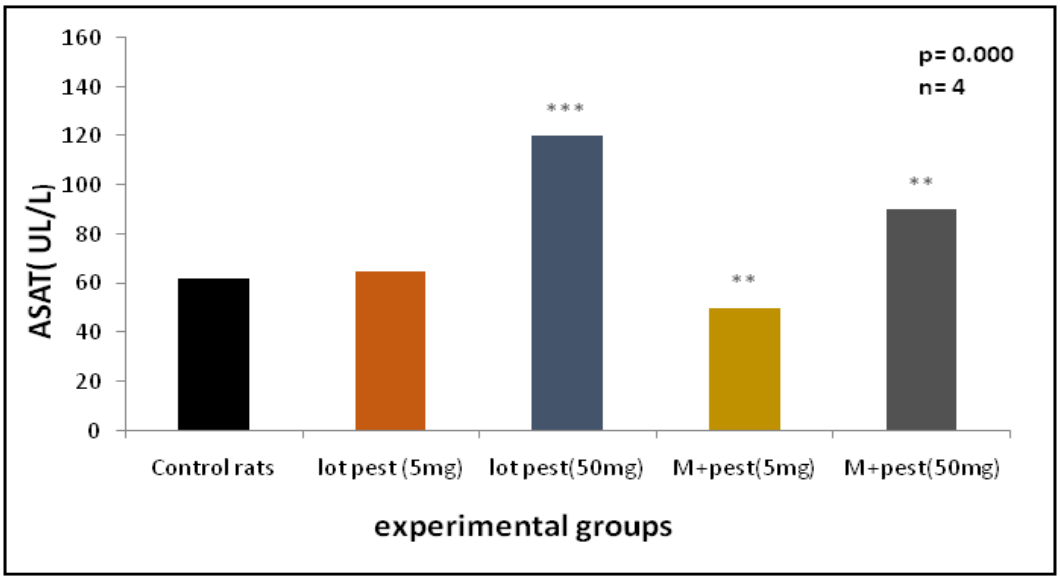


Figure20: . variation of ASAT in rats treated with imidacloprid and the extract of Melissa Officinalis compared to the control rats .

2.3. Effect of Imidacloprid and Melissa Officinalis Extract on the oxidative stress parameters in heart

2.3.1. Effect on non enzymatique parameters

2.3.2 Effect on Malondialdehyde MDA levels

This figure represents the variation in MDA levels in rats treated with imidacloprid and Melissa Officinalis extract compared to controls.

In our work we observe a non-significant increase (p> 0.05) in the batch treated with imidacloprid at a dose (5 mg / kg / day), and a highly significant increase (0.01> P> 0.001) in the batch treated with the same pesticide at a dose (50mg / kg / day)

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

Table 07: variation of MDA in different experimental groups

Experimental groups	control rats04	04Rats treated with pesticide imidaclopri d 5 mg / kg /day	4Rats treated with pesticide imidaclopri d 50 mg / kg /day	04 Rats treated with pesticid imidaclopri d 5 mg / kg /day and melissa extract	04 Rats treated with pesticide imidaclopri d 5 mg / kg /day and melissa extract
MDA(µmol /mg de protéine)	**0,543±0,092**	**0,539±0,016**	**2,426±3,018**	**0,532±0,211**	**0,726±0,138**

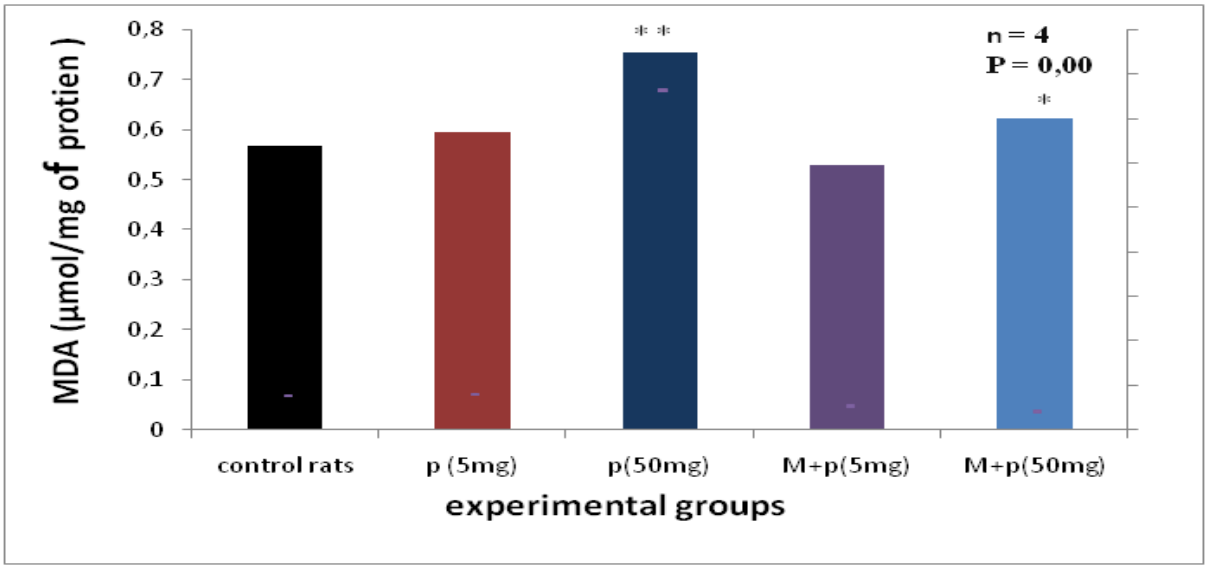


Figure21: . variation in Malondialdehyde MDA levels in rats treated with imidacloprid and Melissa Officinalis extract compared to controls rats .

2.3.3 Effect on Reduced Glutathione GSH levels

This figure represents the variation in GSH levels in rats treated with imidacloprid and Melissa Officinalis extract compared to controls.

In our work we observe a non-significant increase (p> 0.05) in the batch treated with imidacloprid at a dose (5 mg / kg / day), and a highly significant increase (0.01> P> 0.001) in the batch treated with the same pesticide at a dose (50mg / kg / day)

Also, a non-significant decrease (p> 0.05) is observed in the batch treated by the combination of dose 5 mg / kg of imidacloprid and the extract of Melissa Officinalis, and significant increase (0.05>P>0 , 01) in the batch treated by the 50 mg / kg dose combination of imidacloprid and extract of Melissa Officinales**Table08** **:** variation in GSH levels in different experimental groups .

Experimental groups	control rats04	04Rats treated with pesticide imidaclop rid 5 mg / kg /day	4Rats treated with pesticide imidaclop rid 50 mg / kg /day	04 Rats treated with pesticid imidaclop rid 5 mg / kg /day and melissa extract	04 Rats treated with pesticide imidaclop rid 5 mg / kg /day and melissa extract
GSH(µmol / mg de protéine)	**5,91 1E-06±1,97 E-07**	**5,3 E-06±2,21E -07**	**2,99E-06±4,42E-07**	**6,01E-06±3,25E -07**	**6,41E-06±2,42E 07**

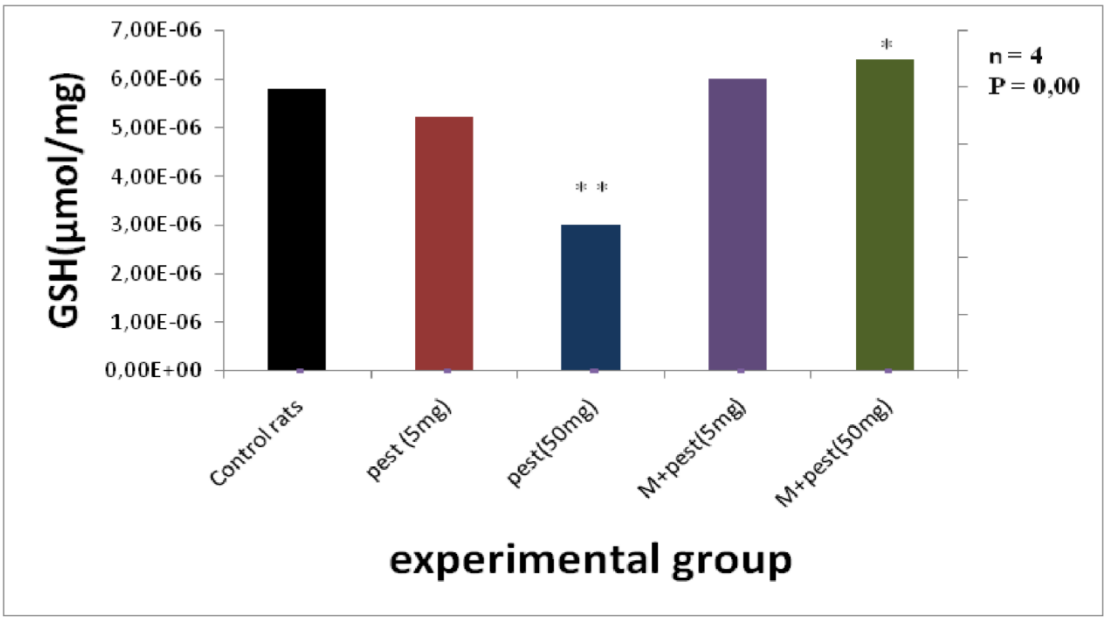


Figure22: . the variation in GSH levels in rats treated with imidacloprid and Melissa Officinalis extract compared to controls.

2.3.4 .Effect on non enzymatique parameters

2.3.5.Effect on Glutathione peroxidase (GPx) levels

This figure represents the variation in GPx levels in rats treated with imidacloprid and Melissa Officinalis extract compared to controls.

In our work, no significant variation was observed in the batch treated with imidacloprid at a dose (5 mg / kg / day), we observed a significant decrease (0.05> P> 0.01) in the batch treated with same pesticide dose (50mg / kg / day)

Also, a non-significant decrease (p> 0.05) is observed in the batch treated by the combination of dose 5 mg / kg of imidacloprid and the extract of Melissa Officinalis, and significant decrease (0.05> P>0 , 01) in the batch treated by the dose combination 50 mg / kg / day of imidacloprid and the extract of Melissa Officinales**Table09**: variation in GPx levels in different experimental groups

Experimental groups	control rats04	04Rats treated with pesticide imidacloprid 5 mg / kg /day	4Rats treated with pesticide imidacloprid 50 mg / kg /day	04 Rats treated with pesticid imidacloprid 5 mg / kg /day and melissa extract	04 Rats treated with pesticide imidacloprid 5 mg / kg /day and melissa extract
GPx(µmol /mg de protéine/min)	**1,2463±0,090**	**1,093±0,064**	**0,616±0,0198**	**1,041±0,0425**	**0,816±0,006**

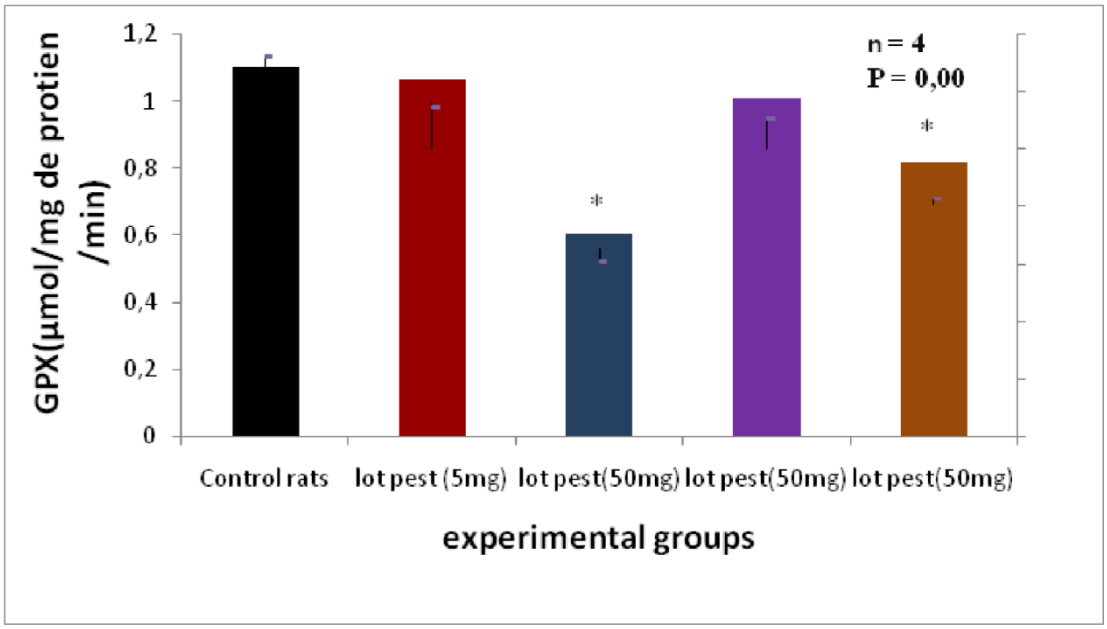


Figure23:. variation in GPx levels in rats treated with imidacloprid and Melissa Officinalis extract compared to controls rats .

2.3.6.Effect on glutathion S-Transférase (GST) levels

This figure represents the variation in GST levels in rats treated with imidacloprid and Melissa Officinalis extract compared to controls.

In our work we observe a significant increase (0.05> P> 0.01) in the batch treated with imidacloprid at a dose (5 mg / kg / day), and in the batch treated by the dose combination 50 mg / kg of imidacloprid and extract of Melissa Officinales, and very highly significant increase (P <0.001) in the batch treated with the same pesticide at a dose (50 mg / kg / day)

Also, no significant variation was observed in the batch treated by the combination of the 5 mg / kg dose of imidacloprid and the extract of Melissa Officinalis.

Table 10: variation in GST levels in different experimental groups

Experimental groups	control rats04	04Rats treated with pesticide imidacloprid 5 mg / kg /day	4Rats treated with pesticide imidacloprid 50 mg / kg /day	04 Rats treated with pesticid imidacloprid 5 mg / kg /day and melissa extract	04 Rats treated with pesticide imidacloprid 5 mg / kg /day and melissa extract
GST(µmol/mg/min	**0,116±0,002**	**0,18±0,242**	**0,604±0,007**	**0,123±0,000**	**0,165±0,002**

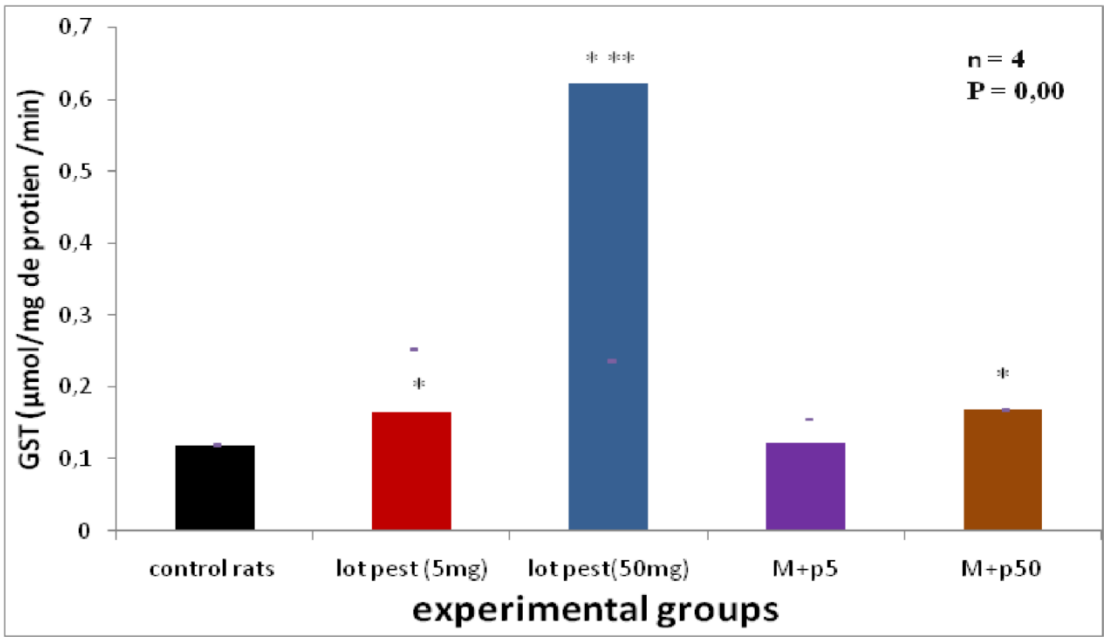


Figure24: . variation in GST levels in rats treated with imidacloprid and Melissa Officinalis extract compared to control rats .

2.3.7 Effect on catalas levels

This figure represents the variation in catalas levels in rats treated with imidacloprid and Melissa Officinalis extract compared to controls.

In our work, no significant variation was observed in the batch treated with imidacloprid at a dose (5 mg / kg / day), we observed a highly significant increase (0.01> P> 0.001) in the batch treated with the same pesticide in deferent dose (50mg / kg / day)

And , there was a significant decrease (0.05> P> 0.01) in the batch treated with the 5mg / kg dose combination of imidacloprid and Melissa Officinalis extract, also with the 50 mg / kg / day dose combination of imidacloprid and extract of Melissa Officinales**Table 11:** variation in catalase levels in different experimental groups .

Experimental groups	control rats04	04Rats treated with pesticide imidacloprid 5 mg / kg /day	4Rats treated with pesticide imidacloprid 50 mg / kg /day	04 Rats treated with pesticid imidacloprid 5 mg / kg /day and melissaextract	04 Rats treated with pesticide imidacloprid 5 mg / kg /day and melissa extract
Catalas(µmol mg of protien/ min)	**1,623±0,092**	**1, 391±0,016**	**0,426±3,018**	**1,732±0,211**	**1,696±0,138**

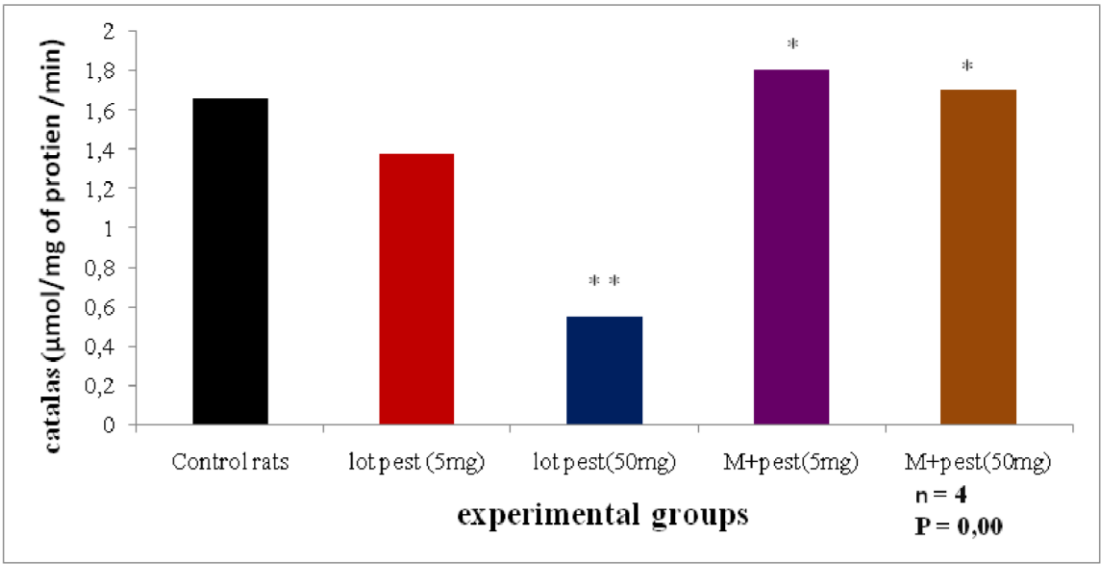


Figure25: . variation in GPx levels in rats treated with imidacloprid and Melissa Officinalis extract compared to controls rats .

2.4 Effect of imidacloprid and Melissa Officinalis extract on metabolic parameters

2.4.1. Effect of Imidacloprid and Melissa Officinalis Extract on Protein level

This figure represents the variation of Protein level in the rats treated with imidacloprid and the extract of Melissa Officinalis compared to the controls.

In our work, no significant variation was observed in the batch treated with imidacloprid at a dose (5 mg / kg / day), we observed a highly significant increase (0.01> P> 0.001) in the batch treated with the same dose pesticide (50mg / kg / day)

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

Table 12: variation of Protein level in different experimental groups .

Experimental groups	control rats04	04Rats treated with pesticide imidaclopr id 5 mg / kg /day	4Rats treated with pesticide imidaclopr id 50 mg / kg /day	04 Rats treated with pesticid imidaclopr id 5 mg / kg /day and melissa extract	04 Rats treated with pesticide imidaclopr id 5 mg / kg /day and melissa extract
Protien mg /g	**0,943±0,092**	**0,999±0,016**	**1,826±3,018**	**0,732±0,211**	**1,426±0,138**

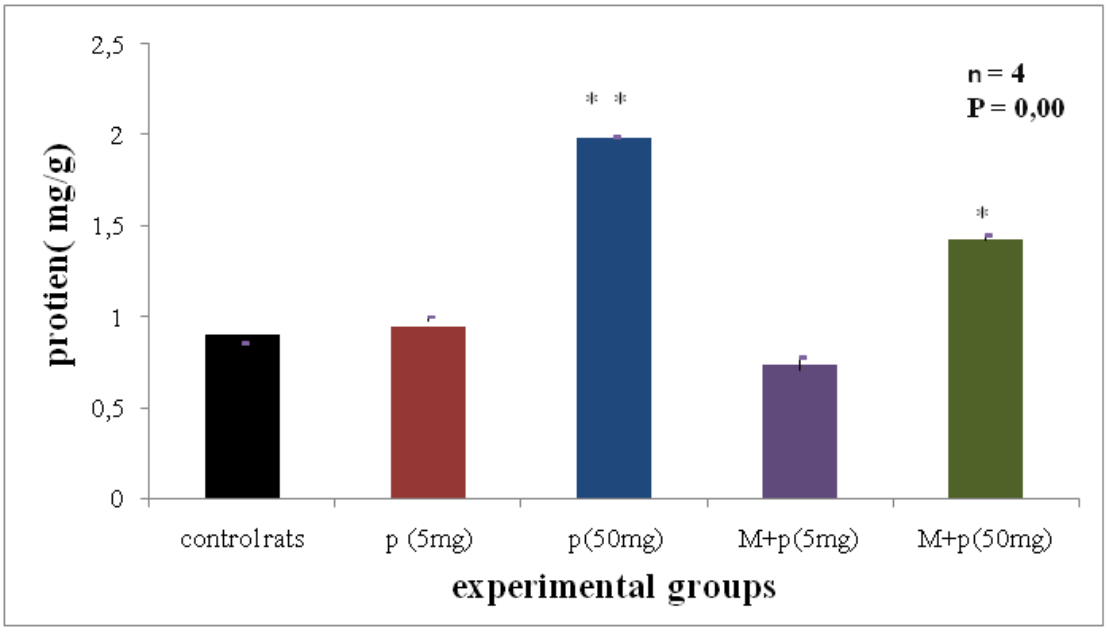


Figure26: . variation of protien in rats treated with imidacloprid and the extract of Melissa Officinalis compared to the control rats .

2.4.2. Effect of Imidacloprid and Melissa Officinalis Extract on lipids levels

This figure represents the variation of lipids level in the rats treated with imidacloprid and the extract of Melissa Officinalis compared to the controls.

In our work, no significant variation was observed in the batch treated with imidacloprid at a dose (5 mg / kg / day), we observed a highly significant decrease (0.01> P> 0.001) in the batch treated with the same dose pesticide (50mg / kg / day)

Also, a non-significant increase (p> 0.05) was observed in the batch treated by the combination of dose 5 mg / kg of imidacloprid and the extract of Melissa Officinalis, and a significant decrease (0.05> P> 0.01) in the batch treated with the 50 mg / kg / day dose combination of imidacloprid and extract of Melissa Officinales**Table 13:** variation of lipids level in different experimental groups .

Experimental groups	control rats04	04Rats treated with pesticide imidacloprid 5 mg / kg /day	4Rats treated with pesticide imidaclopri d 50 mg / kg /day	04 Rats treated with pesticid imidacloprid 5 mg / kg /day and melissa extract	04 Rats treated with pesticide imidacloprid 5 mg / kg /day and melissa extract
Lipides	**278,016±0,948**	**260,193±5,401**	**167,390±2,465**	**298,182±8,565**	**296,572±152,096**

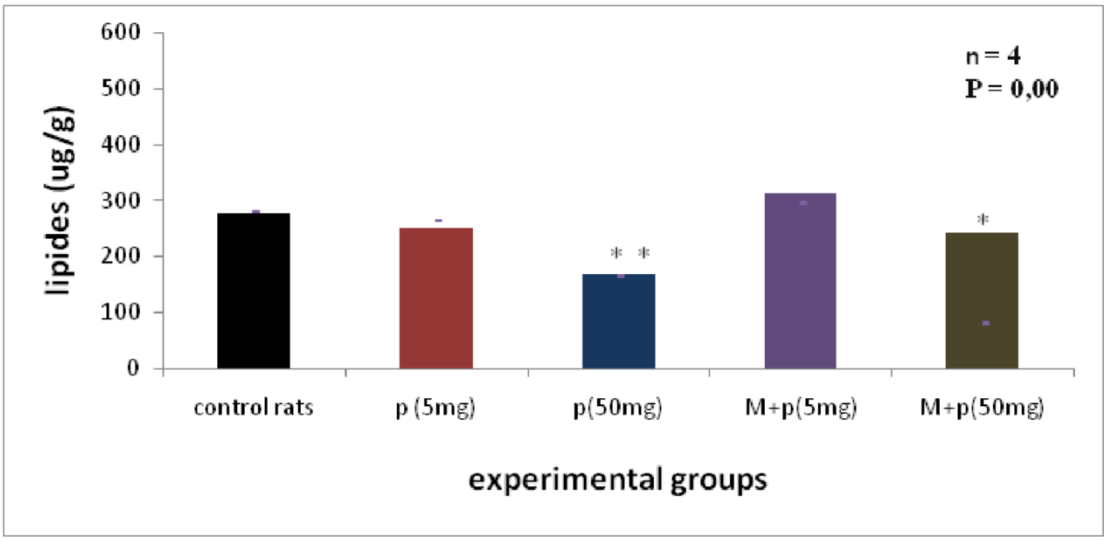


Figure27: . variation of lipids in rats treated with imidacloprid and the extract of Melissa Officinalis compared to the control rats .

2.5. Effect of Imidacloprid and Melissa Officinalis Extract on mitochondria

2.5.1.Effect of Imidacloprid and Melissa Officinalis Extract on mitochondrial swelling levels

This figure represents the variation of mitochondrial swelling in rats treated with imidacloprid and Melissa Officinalis extract compared to controls.

In our work, no significant difference was observed in the batch treated with the imidacloprid dose (5 mg / kg / day), and in the batch treated by the dose combination 5 mg / kg of the imidacloprid and the extract of Melissa Officinalis. we observe a very highly significant increase ($P < 0.001$) in the batch treated with the same pesticide at a dose (50 mg / kg / day).

Also, a significant increase ($0.01 > P > 0.001$) was observed in the batch treated with the 50 mg / kg dose combination of imidacloprid and Melissa Officinalis extract.

Table14 : variation of mitochondrial swelling levels in different experimental groups .

Experimental groups	control rats04	04Rats treated with pesticide imidacloprid 5 mg / kg /day	4Rats treated with pesticide imidacloprid 50 mg / kg /day	04 Rats treated with pesticid imidacloprid 5 mg / kg /day and melissa extract	04 Rats treated with pesticide imidacloprid 5 mg / kg /day and melissa extract
Swililing	0,141±0,0034	0,156±0,000	0,750±0,0140	0,1499±**000**	0,335±0,012

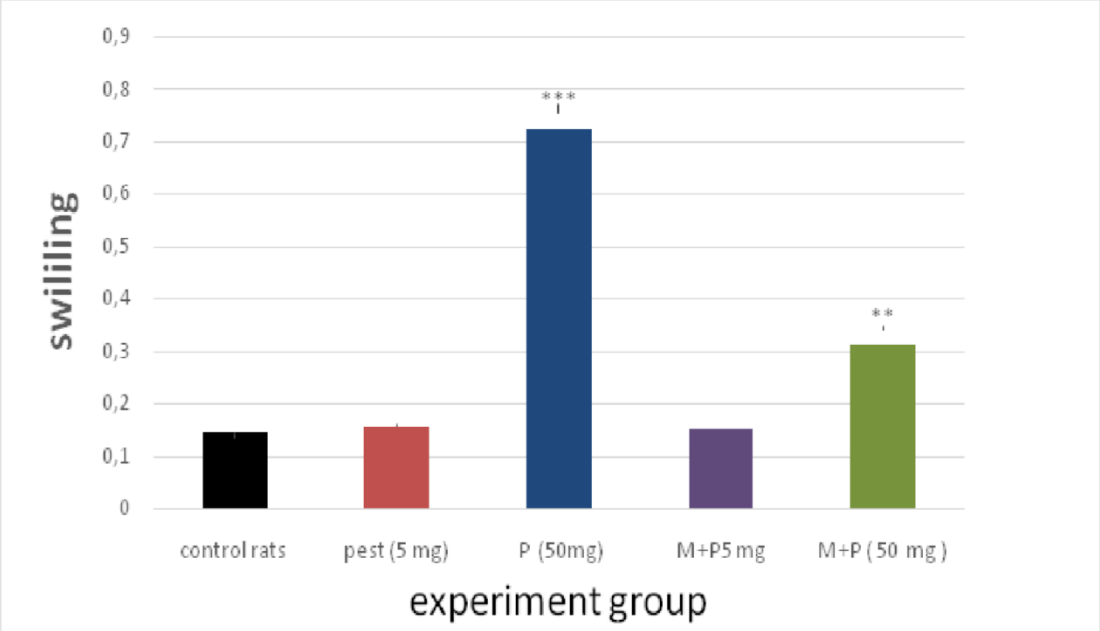


Figure28 . Evaluation of of mitochondrial membrane swililing in rats treated with imidacloprid and Melissa Officinalis extract compared to controls rats .

2.5.2. variation mitochondrial membrane permeability

This figure represents the variation of mitochondrial membrane permeability in rats treated with imidacloprid and the extract of Melissa Officinalis compared to controls.

In our work we observe very highly significant (P <0.001) mitochondrial permeability in the batch treated with imidacloprid at a dose (50 mg / kg / day),

However, no significant difference was observed in the other lots.

Table15 :variation of mitochondrial membrane permeability in different experimental groups .

Experimental groups	control rats04	04Rats treated with pesticide imidacloprid 5 mg / kg /day	4Rats treated with pesticide imidacloprid 50 mg / kg /day	04 Rats treated with pesticid imidacloprid 5 mg / kg /day and melissa extract	04 Rats treated with pesticide imidacloprid 5 mg / kg /day and melissa extract
Permeability	0,06±0,01	0,07±0,02	0,14±0,04	0,06±0,01	0,08±0,00

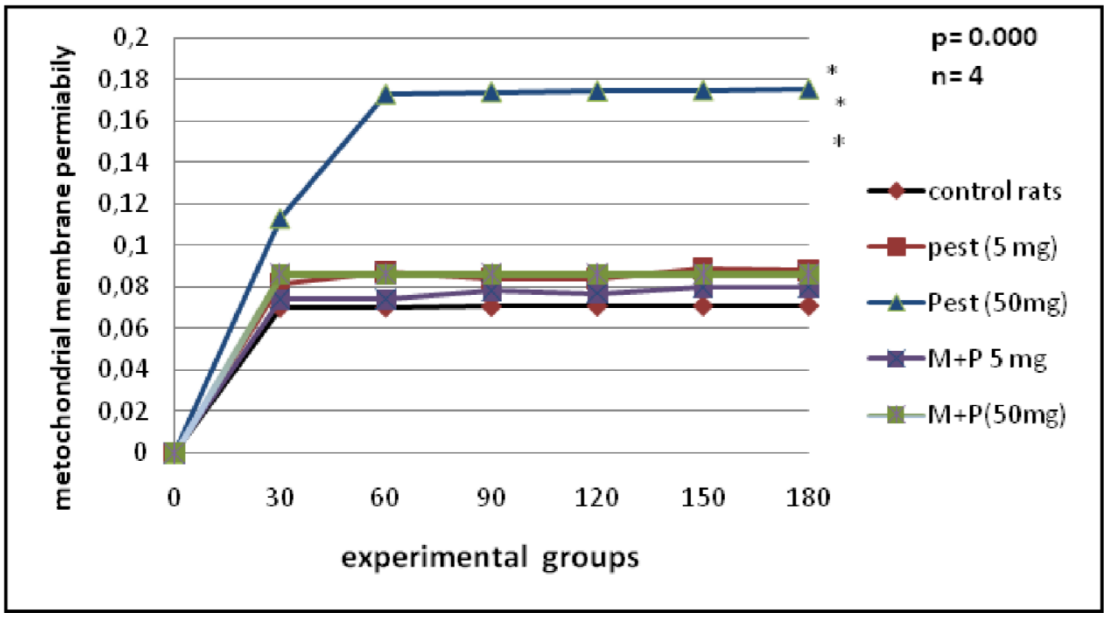
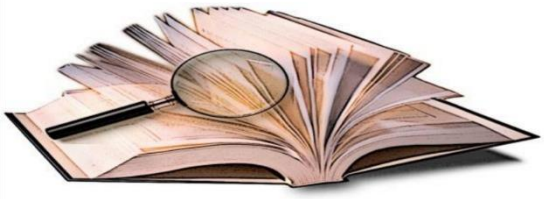


Figure29:. Evaluation of of mitochondrial membrane permeability in rats treated with imidacloprid and Melissa Officinalis extract compared to controls rats .



Discussion

Discussion

Since ancient times mankind has used various plants that exist in their habitats, in order to treat and cure all kinds of diseases; In recent years, attention has focused on one of the biological activities of medicinal plants which is antioxidant activity due to the role it plays in preventing chronic diseases by combating oxidative stress **(LAouar, A.2018)** Oxidative stress is one of the main mechanisms of toxicity associated with a range of xenobiotics in the environment, among which are pesticides and phytosanitary products **(Gasmi, S. 2018)**

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

The experimental presence focuses on a new generation neonicotinoid insecticide, namely imidacloprid

Through this study in adult male rats, we seek the protective role of the extract of Melissa Officinalis against the effects of the oral toxicity of two doses of imidacloprid on the cardiovascular in the wistar rat.

1. **The protective effect of Melissa Officinalis against imidacloprid-induced toxicity in rats on overall growth 1.1. Effect on weight gain**

In fact, no clinical sign of toxicity on weight gain was observed in male rats treated orally with imidacloprid at a dose of 5 mg / kg / day **(Bharadwaj, S. 2010)**

Treatment of rats with imidacloprid at a dose of 50 mg / kg / day for 21 days causes a decrease in weight gain. This decrease can be translated by the disruption of cell metabolism under the effect of oxidative stress caused by the pesticide ROS, (Gasmi, S. 2017), This result suggests that imidacloprid at a dose of 20 mg / kg / day has harmful effects on the body growth of experimental animals **(Bharadwaj, S. 2010)**

Furthermore, the treatment of rats poisoned with imidacloprid by the oral administration of the extract Melissa Officinalis 10 (mg / kg / day) resulted in an increase in weight gain. This could be the consequence of its antioxidant power by normalizing intracellular redox homeostasis and the restoration of the animals' psychic state **(Ronat N. 2001).**

1.2. **Effect on relative weight**

The treatment of rats with imidacloprid with the dose 50 mg / kg / day for 21 days causes a decrease in relative heart weight This decrease in relative weight is interpreted by a degradation of the heart cells caused by the increase in.

free unions by the administration of imidacloprid **(Carole, B. 2008)** Furthermore, the use of lemon balm has shown an improvement in the relative weight of the heart. This could probably be explained by the reduction in the accumulation of free radicals induced by the antioxidants present in lemon balm **(Ronat N. 2001).** decrease is improved by the administration of lemon balm thanks to its digestive, antioxidant and anti inflammatory property**. (Iserin P.2001)**

2. **Heart biochemical parameters discussions**

Our study made it possible to specify the values of the 5 parameters (Cholesterol, triglyceride, TGO, TP and TCK) and to consult their relationship with cardiovascular diseases.

In view of the results obtained, it appears that imidacloprid at a dose of 50 mg / kg / day causes disturbances in the biochemical parameters. On the contrary that imidacloprid at a dose of 5 mg / kg / day, In fact, a very highly significant increase ($P \leq 0.001$) was recorded in the enzymatic activity of ASAT, cholesterol, and TRG in rats exposed to l 'imidacloprid compared to controls. The increase in the activity of these enzymes in the blood can be practically due to tissue damage due to the increase in membrane permeability from which the membrane enzymes leak from the tissues to the blood **(Beaudeux and Durand, 2011).**

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Indeed, the toxicity of imidacloprid is mainly due to the appearance of free radicals which induce lipid peroxidation leading to the destruction of cell membranes **(Sangare et al. 2012).**

Our results are consistent with those (Bharadwaj, S. 2010) no clinical signs of toxicity were observed in male rats treated orally with imidacloprid at a dose of 5 mg / kg / day, but induced toxicological effects at 20 mg / kg / day in treated male rats

So triglyceride is a main risk factor which agrees with the study by Bruckert et al (1992) which shows that triglyceride appears as an important cardiovascular risk factor and with Gardner et al (1996) and with the study by **(Castelli et al. 1998)** which found that hypercholesterolemia is a major cardiovascular risk factor.

The trends in declining enzyme levels (ASAT, cholesterol and TRG) in rats treated with Melissa Officinali should be taken into account because the richness in phenolic compounds of this extract militates in favor of its use as cardio-protective substances with antitoxic activity. . Also, this decrease is an indicator of the regeneration of the process of repairing damage to the heart tissue due to imidacloprid and According to Cassettari de Carvalho and colleagues (2011), phenolic compounds protect biological systems by various mechanisms. **(Laouar, A. 2018)**

3. Effect of imidacloprid on the oxidative stress parameters of rat cardiovascular system and the protective effect of Melissa Officinalis extract

Furthermore, oxidative stress is a major consequence of myocardial ischemia, in particular by activation of endothelial xanthine oxidase; the EROs trained are directly implicated in post-infarction arrhythmias **(Baudin, 2006)**

3.1. Effect on MDA

The appreciation of the importance of oxidative stress in many pathologies makes it necessary to use different dosages, the most used of which is that of MDA, one of the end products formed during the decomposition of polyunsaturated fatty acids mediated by free radicals **(Laouar, A. 2013**). Our results show an increase in the level of malondialdehyde (MDA) in rats treated with imidacloprid at a dose of 50 mg / kg / day. This confirms oxidative damage such as the peroxidation of membrane lipids, due to the production of peroxyl radicals, The oxidation of unsaturated fatty acids of membrane phospholipids is capable of deforming the structure of the membranes and consequently causing its permeability and death. cell **(Kebièche et al., 2011).** especially in association with cardiovascular disease **(McCullough P et al., 2008)**. These latest results are in agreement with the work of **(Anja , M and Irena, B K. 2018)** who used the same pesticide at a dose of 8 mg / kg / day

The addition of the extract of Melissa officinalis at a dose of 10 mg / kg / day neutralizes these effects significantly), this result has the same meaning as the results of the study by **(Fetoui et al.2009)**,. who administered Vitamin C, and the study by **(Bolkent et al. 2005).**

A study carried out by Ikizler M et al, in 2007 proved that the perfusion of the heart with a solution containing quercetin (composed of lemon balm) for 30 minutes and more strongly

by an oral treatment with quercetin for 1 week reduced the levels of malondialdehyde in heart tissue after reperfusion by inhibiting the production of EROs in the heart.

3.2. Effect on (CAT and GPx)

Our studies have shown a significant decrease (p> 0.001) in the enzyme activity of enzymes in batches treated with imidacloprid at the dose (50mg / kg / day).

Our results are confirmed by several works, **(Anja, M and Irena, B K. 2018)** and the study **(Bardwa,, S. 2010)** shows that dose 5 mg / kg / day for 90 days has not produced changes in CAT and GPx .however dose 20 mg / kg / day for 90 days has produced sinificant changes CAT and GPx.

On the other hand, treatment of rats with 10 mg / kg / day of the extract of Melissa officinalis improves the decrease in the activity of this oxidative stress enzyme. Because the treatment of rats with this plant has antioxidant activity thanks to their capacity to sweep free radicals.

This enzymatic inhibition is corrected after the addition of the extract of Melissa officinalis, at a dose of (10 mg / kg / day), this result affirmed by the study by **Oloyede et al. (2011)**, And the study by **Bolkent et al. (2005)**. Which demonstrated for the first time an in vivo antioxidant efficacy effect of the aqueous extract of Melissa officinale against oxidative stress

3.3. Effect on GSH and GST

Glutathione is a thiol compound found in all cells of the body and plays a detoxification role. **(Romão et al., 2006)**

We also observe a significant decrease (p> 0.001) in GSH levels in the batches treated with imidacloprid at a dose of 50 mg / kg / day). Our results are confirmed by several studies, **(Anja, M and Irena, B K. 2018)** and the study **(Bardwa, S. 2010)** shows that the dose 10 mg / kg / day for 90 days has not produced changes in GSH. however dose 20 mg / kg / day for 90 days has produced sinificant changes in GSH

Indeed, this drop in GSH can be explained by its participation in the detoxification reactions of free radicals; the latter interacts directly with a strong affinity for thiol groups (-SH) of the GSH. glutathione can also interact with the free radicals generated by this metalloid **(Whanger, 1973)**, the xenobiotic inhibits glutathione synthetase, and glutathione reductase **(James et al., 2006)**, so little GSH is produced.

After the addition of the extract of Melissa officinalis, at a dose of (10 mg / kg / day) we observe an increase in the level of GSH which confirmed by the study by **Oloyede et al. (2011),** and the study by **Bolkent et al. (2005).**

Indeed has a scavenger effect, that is to say instead of the free radicals produced by imidacloprid neutralized by GSH, they will rather be captured by phenolic compounds by increasing the level of glutathione in the heart

The S-transferase glutathione (GST) enzyme plays an important role in the detoxification of xenobiotics and/or in the protection against harmful metabolites generated after the degradation of macromolecules following exposure to oxidative stress (Hayes et al, 1995). The results for glutathione S-transferase (GST) activity indicate an increase in the specific activity of this enzyme in rats treated with imidacloprid at a dose of 50mg/kg this result is supported by the study of (Mikoic A, BrcicKaraconji I .2018) increase is considered to be one of the fundamental indicators informing about cellular damage caused by SAR (Gasmi,S.2018) While mice treated with formalized lemon balm dose 10mg/kg shows a decrease

4. Effects of imidacloprid and lemon balm extract on the structural and functional integrity of the mitochondria

Mitochondria are essential for the production of cellular energy necessary for the maintenance of cellular function. Mitochondrial damage contributes to a decrease in ATP production. Alterations in one of the mitochondrial complexes cause the production of free radicals depolarization of the mitochondrial membrane which allows the influx of calcium into the cell and the triggering of the pathways of cell death **(Gasmi, S. 2018)**

Indeed, the exposure of rats to imidacloprid showed a significant increase in permeability and mitochondrial swelling in the heart This state of effects is the result of the action of ROS produced by the pesticides studied.

The increase in mitochondrial swelling is due to the disruption of the dependent pore voltages under the effect of ROS causing massive entry of water and Ca2 + and Na + ions via the mitochondrial membranes **(Gasmi, S. 2018),** Mitochondrial swelling then allows the externalization of several proteins such as cytochrome-c, from the mitochondria to the cytosol allowing cell death **(Zhang and Darley, 2000)**

The impermeability of the membrane is essential for oxidative phosphorization and therefore for cell survival, oxidative stress, can cause permeabilization of the mitochondrial

membranes **(Long, R.2014)** In addition, the treatment of rats poisoned with imidacloprid by oral administration of the extract Melissa Officinalis 10 (mg / kg / day) improves the increase in membrane permeability Because the treatment of rats with this plant has antioxidant activity thanks to their capacity to scan free radicals

Antioxidant is an ERO trapper like phenolic, and These results are in agreement with several recent works having studied the chemoprotective potential of quercetin (compound found in lemon balm) in the case of exposure to pesticides **(Gasmi, S. 2018)**

4.1. Discusion of Lipid Peroxidation

The results of our work show a decrease in the batch treated with the same pesticide at a dose (50 mg / kg / day) compared to the control, this reduction agrees with **Bantu (2013)** who explained that the decrease in the content of lipids results from the use of lipids to meet the energy demand in stress due to pesticides

And according to the work of **Mohany et al (2011)** reported that the oral exposure of the rat to imidacloprid causes lipid peroxidation and generates oxidative stress,

Polyunsaturated fatty acids such as linoleic acids are the preferred targets of oxygenated free radicals, This leads to a chain reaction of lipid peroxidation, which modifies the fluidity and permeability of the membrane **(Gasmi, S. 2018)** p 36

On the other hand, a treatment of rats with 10 mg / kg / day of the extract of Melissa officinalis improves the lipid decrease in oxidative stress. Because the treatment of rats with this plant has antioxidant activity thanks to their capacity to sweep free radicals.

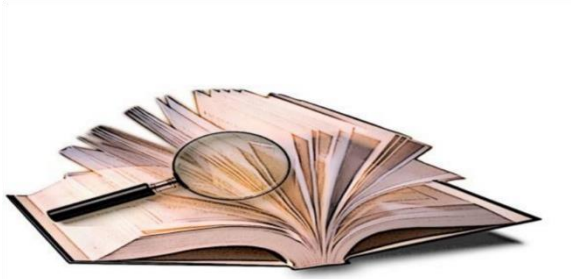
4.2 Protein Oxidation Discussion

The results of our work show an increase in the level of cardiac protein in rats treated with imidacloprid compared to the control, this increase could be linked to oxidized proteins which in turn form aggregates which accumulate in cells and in the extracellular compartment.

Oxygenated free radicals induce modifications in the primary, secondary and tertiary structures of proteins by the formation of carbonylated protein derivatives via several mechanisms including the fragmentation and oxidation of amino acids **(Gasmi, S. 2018)** p 36

On the other hand, a treatment of rats with 10 mg / kg / day of the extract of Melissa officinalis improves the lipid decrease

For the antioxidant role, vitamin C from mellisse ensures the protection and maintenance of proteins, lipids, enzymes and other antioxidants in their normal form by reducing metal ions and trapping free radicals **(Adimi. 2018)**



Conclusion et perspective

The objective of the present study was to evaluate the toxicity of pesticide which it is imidacloprid in the Wistar rat which causes a disturbance of the parameters of oxidative stress also of biochemical and metabolic parameters which differ according to the dose of administration and the opposite effect of the extract of Melissa officinale on this toxicity which has a detoxification role in the light of the results obtained, it can be concluded that the gavage of imidacloprid orally at a dose of 05 mg / kg / day and (50mg / kg / day of the body weight in the adult male rats induced disturbances on the parameters of oxidative stress also on the biochemical and metabolic parameters of the rats, we found that there is a disturbance in the Level of the parameters to evaluate conclude as follows:

The study of the relative weight showed a significant decrease between the rats treated and the witnesses

- A change in antioxidant status with an increase in liver content in MDA and GST levels and a decrease in the rate of GSH and the activity of CAT and laGPx
- A disturbance in biochemical parameters expressed by an increase in the cholestrol and TRG and ASAT levels while TCK and TP decreased in rats treated with imidacloprid
- A disturbance in metabolic parameters expressed by an increase in protein levels while lipids decreased in rats treated with imidacloprid

- A change in metochondrial status with an increase in swelling and metochondrrial permeability

- a correction of the disturbances caused after the addition of the extract of melissaoffisinalis.

Our results therefore confirm the toxicity of imidacloprid and the opposite effect of extract of Melissa officinale on this toxicity.

In perspective it is necessary to develop your research by extending the duration exposure in order to know if the disturbances could lead to the appearance of pathologies and do histological studies to properly study the effects.

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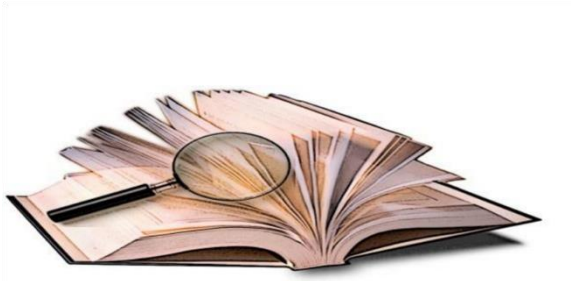

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