الجمهورية الجزائرية الديمقراطية الشعبية
People's Democratic Republic Of Algeria
وزارة التعليم العالي و البحث العلمي
Ministry Of Higher Education And Scientific Research
University of Badji Mokhtar – Annaba جامعة باجي مختار-عنابة

FACULTY OF SCIENCES

DEPARTMENT OF BIOLOGY

LABORATORY OF ANIMAL ECOPHYSIOLOGY

A thesis submitted for the fulfillment of Doctoral

in Biological Sciences

Specialty: Animal Biology

Entitled

Study of some pesticides on reproduction in laboratory animals

Presented by : ATI Samia

Supervisor : KHELILI Kamel (Prof, University Badji Mokhtar-Annaba)

Member of the jury:

ABDENNOUR Cherif	Prof	Chairman	University Badji Mokhtar-Annaba
KHELILI Kamel	Prof	Supervisor	University Badji Mokhtar-Annaba
HAMDI Leila	MCA	Examiner	University Badji Mokhtar-Annaba
DJABALI Nacira	Prof	Examiner	University Chadli Bendjedid-Taref
SLIMANI Souheila	Prof	Examiner	University 20 Aout 1955-Skikda
LAZLI Amel	Prof	Examiner	University Chadli Bendjedid -Taref

2023/2024

Thanks

I thank God for all his blessings,

and I thank my parents

my family for supporting me throughout this journey

I also extend my thanks to the chair of the discussion committee ***Cherif Abdennour***, ***Hamdi Leila***, ***Djebaili Nacira***, ***Lazli Amel***, ***Slimani Souheila*** *and the supervising professor* ***Khelil Kamel***.

do not forget everyone who contributed to my education, even if just a letter.

Thank you all.

Dedication

I thank God almighty first and foremost, for the great favor He has bestowed upon me.

I dedicate this thesis to those who have great favor upon me, my ***beloved parents*** *never cease to support me for all their efforts. Everyone who advised me or guided me in preparing this research and linking me to the required references and sources at whatever stage I went through., I say to all my friends (Dr Randa, Dr Abdelkader Soumya, Raed, Raouf, Amel, Noura, Samia, Mohcen, Hichem…)*

Thank you so much

List of figures

List of tables

Table of contents

Chapter 3: Results

Chapter 4: Discussion

Abstract

The aim of this study is to investigate the impact of an insecticide (Diflubenzuron, DFB), a herbicide (Glyphostae, GLY), and a fungicide (Aroxystrobine, AZO) on reproduction, plasma biochemistry, blood components, organs' histology and oxidative stress of domestic male rabbit*Cuniculus lepus*. Adult animals weighting 1700.54 ± 113.15 g,were divided into 4 groups; the control,the DFB(sub-groups D1: 250,D2: 500,D3: 750 mg/kg bw), the Gly (sub-groups D1:200, D2: 300, D3: 400 mg/kg bw), and the AZO (sub-groups D1:100,D2: 200,D3: 300 mg/kg bw),and then administrateddaily for 6 days/week by gavage for four weeks. The obtained results have shown a significant decrease in the total body weight of groups treated with the three pesticides, compared to the control. The spermogramme has indicated a significant decrease in most reproductive parameters (concentration, speed, motility, and vitality), as well as in the plasma testosterone level of all doses used from the three pesticides, compared to the control. Plasma cholesterol and triglycerides levels were increased significantly, in particular in the high doses of DFB and AZO groups, but decreased in the GLY groups compared to the control, while that of glucose was increased significantly in the DFB and GLY groups, however it decreased in the AZO groups. There was a significant decrease in plasma albumin concentration of DFB (D1, D2, and D3), GLY (D2, D3), and AZO (D3) treated rabbits, accompanied with a significant elevation of plasma creatinine and urea concentrations in almost the higher doses of DFB, GLY and AZO groups. Concerning the blood components, red blood cell count was significantly reduced, alongside hemoglobin concentration in all doses of the three pesticides, whereas those of white blood cell counts were elevated only in the highest doses. The oxidative stress parameters were affected by the three pesticides, as indicated by the significant decrease of hepatic (D2, D3) and testicular (D1, D2, D3) glutathione concentration. The histological study revealed certain alterations in hepatic tissuesdemonstrated by congestionofportalbloodvessels with inflammation, and alterations in the testicular seminiferous tubules of rabbits supplied with higher doses of the different pesticides. In conclusion, DFB, GLY, and AZO supplemented to male rabbits during one month have distorted most biological parameters, especially with the medium and the higher doses.

Keywords : *Pesticides, Reproduction,- Histology, Cuniculus lepus, oxidative stress, blood.*

الملخص

الهدف من هذا البحث هو دراسة تأثير المبيد الحشري (الدفلوبانزيرون) ومبيد الأعشاب (غليفوسات) ومبيد الفطريات (ازوكسيستروبين) على التكاثر والكيمياء الحيوية للبلازما ومكونات الدم والإجهاد التأكسدي، اضافة الى أنسجة الكبد والخصية عند ذكور الأرانب المحلية البالغة. قسمت الحيوانات (1700.54 ± 113.15غ) إلى 4 مجموعات هي مجموعة الشاهد، مجموعة الدفلوبانزيرون (ج1 : 250 ، ج2 : 500 ، ج3 : 750 ملغ/كلغ من وزن الجسم)، مجموعة الغليفوسات (ج1 : 200 ، ج2 : 300 ، ج3 : 400 ملغ/كلغ من وزن الجسم)، ومجموعة الازوكسيستروبين (ج1 : 100 ، ج2 : 200 ، ج3 : 300 ملغ/كلغ من وزن الجسم)، حيث اعطيت جرعات يومية لمدة 6 أيام/أسبوع خلال أربعة أسابيع. أظهرت النتائج المتحصل عليها انخفاضا معنويا في وزن الجسم الكلي للمجموعات المعاملة بالمبيدات الثلاثة مقارنة بجموعة الشاهد. كما أظهرت النتائج انخفاضاً معنوياً في معظم مؤشرات التكاثر الخاصة بالحيوانات المنوية (التركيز، السرعة، الحركة، والحيوية)، وكذلك في مستوى هرمون التستوستيرون البلازمي لجميع الجرعات المستخدمة من المبيدات الثلاثة، مقارنة بجموعة الشاهد. كما لوحظ ارتفاعا في مستويات الكوليسترول والدهون الثلاثية البلازمية بشكل ملحوظ، خاصة في الجرعات العالية لمجموعتي الدفلوبانزيرون و الازوكسيستروبين ، بينما انخفضت في مجموعات الغليفوسات مقارنة بجموعة الشاهد ، في حين ارتفعت مستويات الجلوكوز بشكل ملحوظ في مجموعات الدفلوبانزيرون و الغليفوسات. أما في مجموعات الازوكسيستروبين فكان هناك انخفاض معنوي في تركيز الألبومين البلازمي للأرانب المعالجة بالمبيد (ج1 و ج2 ج3) ، بينما انخفض في الغليفوسات في (ج2، ج3)أما الازوكسيستروبين فانخفض في المجموعة (ج3)، مصحوبًا بارتفاع معنوي كبير في تركيزات الكرياتينين واليوريا البلازمي في جميع المجموعات المعالجة بالمبيدات الثلاثة مقارنة بالمجموعة الشاهد. وفيما يتعلق بمكونات الدم، فقد انخفض عدد كريات الدم الحمراء بشكل ملحوظ، إلى جانب تركيز الهيموجلوبين في جميع جرعات المبيدات الثلاثة، في حين لم يرتفع عدد كريات الدم البيضاء إلا في الجرعات الأعلى في المجموعات المعاملة بالمبيدات الثلاثة، في حين تأثرت مؤشرات الإجهاد التأكسدي في المجموعات المعاملة بالمبيدات الثلاثة مقانة بالمجموعة الشاهد، و ذلك من خلال الانخفاض المعنوي في تركيز الجلوتاثيون الكبدي (ج2، ج3)، والخصية (ج1 ،ج2، ج3). كما أظهرت الدراسة النسيجية تلف في أنسجة الكبد تمثلت في احتقان الأوعية الدموية البابية مع الالتهاب، وتغيرات في الأنابيب المنوية للخصية لدى الأرانب التي تلقت جرعات أعلى من المبيدات المختلفة. في الختام، فإن إضافة مبيدات الدفلوبانزيرون والغليفوسات والازوكسيستروبين لذكور الأرانب المحلية خلال شهر واحد قد أحدثت اضطرابا في معظم المعايير البيولوجية، وخاصة مع الجرعات المتوسطة والعالية.

الكلمات المفتاحية: مبيدات حشرية، تكاثر، نسيج، إجهاد تأكسدي، دم

Résumé

Le but de cette étude est d'étudier l'impact d'un insecticide (Diflubenzuron, DFB), d'un herbicide (Glyphostae, GLY) et d'un fongicide (Aroxystrobine, AZO) sur la reproduction, la biochimie du plasma, les composants sanguins, l'histologie des organes et le stress oxydatif. du lapin domestique Cuniculus lepus. Les animaux adultes pesant 1 700,54 ± 113,15 g ont été divisés en 4 groupes ; le témoin, le DFB (sous-groupes D1 : 250, D2 : 500, D3 : 750 mg/kg pc), les Gly (sous-groupes D1 : 200, D2 : 300, D3 : 400 mg/kg pc), et l'AZO (sous-groupes D1 : 100, D2 : 200, D3 : 300 mg/kg pc), puis administré quotidiennement pendant 6 jours/semaine par gavage pendant quatre semaines. Les résultats obtenus ont montré une diminution significative du poids corporel total des groupes traités avec les trois pesticides, par rapport au groupe témoin. Le spermogramme a indiqué une diminution significative de la plupart des paramètres de reproduction (concentration, vitesse, motilité et vitalité), ainsi que du taux plasmatique de testostérone de toutes les doses utilisées des trois pesticides, par rapport au contrôle. Les taux plasmatiques de cholestérol et de triglycérides ont augmenté de manière significative, en particulier dans les groupes DFB et AZO à fortes doses, mais ont diminué dans les groupes GLY par rapport au contrôle, tandis que ceux du glucose ont augmenté de manière significative dans les groupes DFB et GLY, mais ont diminué dans les groupes DFB et GLY. les groupes AZO. Il y a eu une diminution significative de la concentration plasmatique d'albumine chez les lapins traités au DFB (D1, D2 et D3), au GLY (D2, D3) et à l'AZO (D3), accompagnée d'une élévation significative des concentrations plasmatiques de créatinine et d'urée dans les concentrations plasmatiques les plus élevées. doses des groupes DFB, GLY et AZO dans tout les groups traité au pesticide comparé au temoin. Concernant les composants sanguins, le nombre de globules rouges était significativement réduit, ainsi que la concentration d'hémoglobine à toutes les doses des trois pesticides, alors que ceux des globules blancs n'étaient élevés qu'aux doses les plus élevées. Les paramètres du stress oxydatif ont été affectés par les trois pesticides, comme l'indique la diminution significative de la concentration hépatique (D2, D3) et testiculaire (D1, D2, D3) de glutathion. L'étude histologique a révélé certaines altérations des tissus hépatiques démontrées par une congestion des vaisseaux sanguins portes avec inflammation et des altérations des tubes séminifères testiculaires de lapins recevant des doses plus élevées des différents pesticides. En conclusion, les DFB, GLY et AZO supplémentés à des lapins mâles pendant un mois du traitement la plupart des paramètres biologiques, notamment aux doses moyennes et élevées.

Mots clés : Pesticides, Reproduction, Histologie, *Cuniculus lepus*, stress oxydatif, sang

Introduction

The development of organic chemistry that began in the1940s, introduced a new era of synthetic pesticides that became the largest spectrum of industrial chemicals used in modern society. Today, we count more than 900 active ingredients (Ware & Whitacre, 2004) which enter in the composition of thousands of pesticides products, mainly used in agriculture; to control insects, fungi, weeds and other pests. However, even if pesticides' use is an essential tool in increasing productivity, enhancing quality, protecting livestock, and fighting vector diseases; there are now evidences that these products do create risk to man and his environment. Each year, pesticides contribute to an estimated 26 million human poisonings and 220,000 deaths worldwide (Pimentel & Pimentel, 2008).

Without pesticides, food production would be lower, and larger cultivated farm areas cannot produce the same amount of food, which would impact the world food supply and poison wildlife significantly. More frequent cultivation of the fields would increase soil loss due to erosion.Without pesticides, agricultural production would decrease, food prices would rise, farmers would be less competitive in global markets, and countries exports would drop, leading to many job losses (Delaplane , 2014).

Despite their benefits, pesticides can be hazardous to both humans and the environment. Because some pesticides can persist in the environment, they can remain there for years. Environmental contamination or occupational use can expose the general population to pesticides residues, including physical and biological degradation products present in the air, water, and food (Mostafalou *et al.*, 2013).

In Algeria, pesticides poisoning came into second position after drugs in the causes of acute intoxications (Mokrani, 2005). Although chronic effects of pesticides exposure on human health are less readily identifiable, wealth research conducted in this context (Krieger et al., 2010; WHO, 2008; MDRGF2008; Sténuit & Van Hammée, 2008; Cox ,2006; Pretty, 2005; Sanborn et al., 2004; Solomon *et al.*, 2000) found a significant association between pesticides exposure and many health problems includin cancer, neurological damage, reproductive and developmental disorders, immunotoxicity, endocrine disruption, renal and cardio-vascular diseases.

The objectives of this study are to evaluate the toxicity of Diflubenzuron (insecticide), Glyphostae (herbicide), and Aroxystrobine (fungicide) beings widely used by farmers, within the country, on adult male domestic rabbits *Cuniculus lepus,* with a focus on their repro-toxicity, histopathology, and biochemical and physiological disturbances of various doses (low, medium, and high).

To do so, biological indices have been studied as follows:

- The reproductive paremeters represented by sperm concentration, speed, motility, and vitality, and the plasma testosterone level.
- The biochemical and the blood components parameters representing the hepatic, the renal, the pancreatic, and the bone marrow functions.
- The stress oxidative parameters indicating how the stressed main organs cope with the subchronic toxicity.
- The histological study of two important organs of the body, one for detoxification, and the other for reproduction.

Chapter 1:Literature review

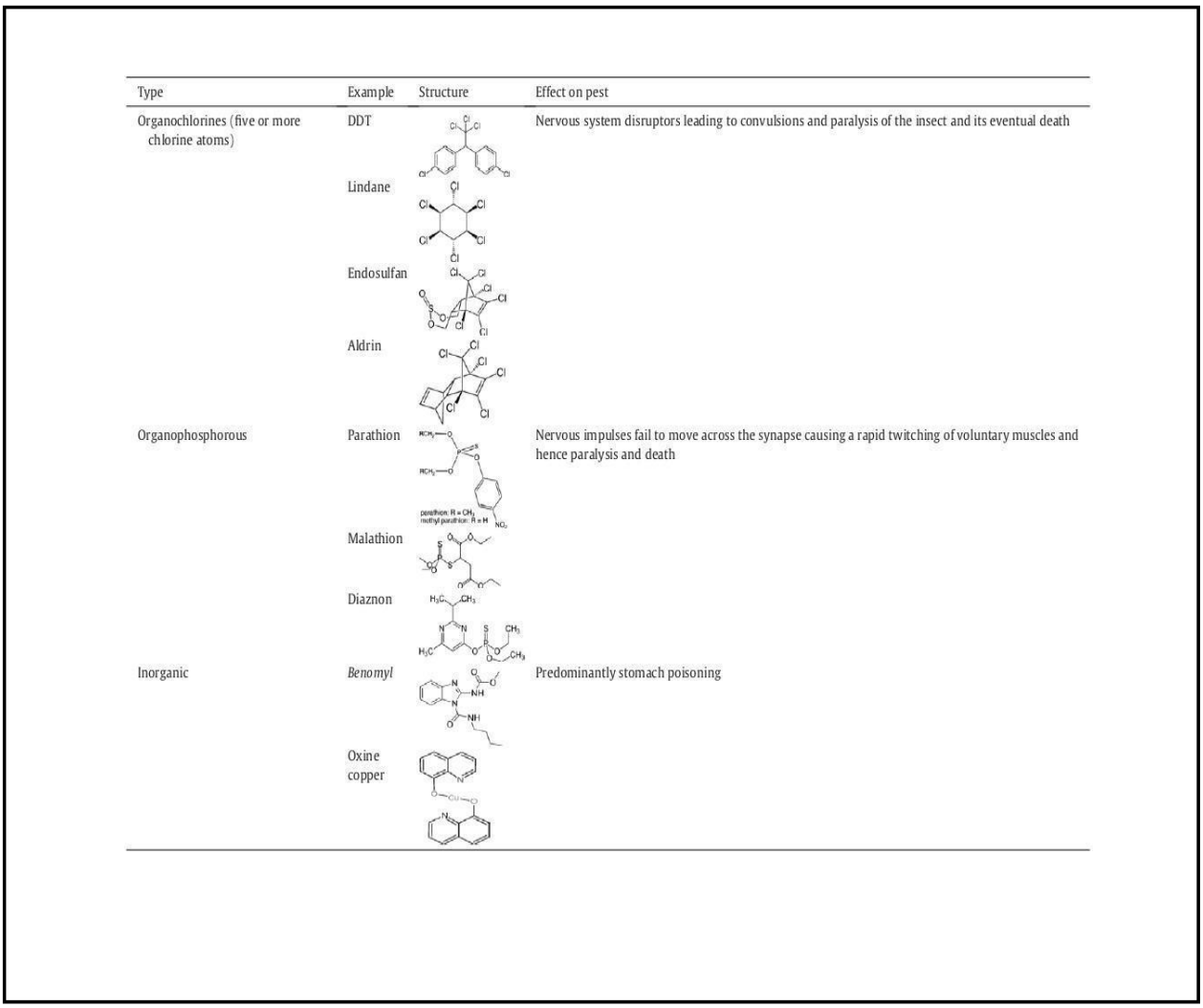
1 Pesticides historical uses

Farmers may come into contact with a variety of potentially hazardous pesticides, notably organic phosphorus (OP) compounds (Mostafalou & Abdollahi, 2013a,b). Pesticides are a substance used to prevent, eradicate, repel, or minimize any kind of pest. They are also capable of acting as plant regulators and defoliants (USEPA, 2014). Pest control chemicals have long been utilized. 4500 years ago, Sumerians used sulfur compounds to manage insects and mites. Pyrethrum, a pesticide made from the dried flowers of Chrysanthemum cineraria folium, has been used as an insecticide for more than two thousand years. Weeds have been controlled using salt or sea water. Until the 1940s, inorganic compounds including sodium chlorate and sulfuric acid, as well as organic chemicals obtained from natural sources, were commonly used in controlling pests 2010 (Unsworth). Throughout World War II (1939-1945), pesticide development raised due to the need to increase food supply and identify potential chemical warfare agents (Gupta 2007). As a result, synthetic pesticides such as DDT, aldrin, dieldrin, endrin, parathion, and 2,4- D experienced a significant increase in the 1940s. Pesticide use in agriculture was regarded as beneficial in the 1950s, and there was no worry about the potential dangers of these chemicals to the environment and human health (Unsworth, 2010). The target pests are only reached by less than 1% of the entire amount of pesticides used for weed and pest management. Spray drift, off-target deposition, run-off, and photodegradation are ways that a lot of pesticides are lost, and these processes can have a negative impact on specific species, societies, or the entire ecosystem, in addition to having an adverse effect on people (Hernández et al. 2013). Another important consideration is that low amounts of many chemicals may not have immediate, observable impacts on organisms, but they may still cause long-term harm such as genetic defects and physiological changes that shorten life expectancy (Poletta *et al.*, 2009).

Pesticides can be classified in a variety of ways, notably based on the pests they manage. Insecticides fight growth of insects or their existence, herbicides combat plants, weeds, and grasses, rodenticides combat rats and other rodents, avicides combat bird populations, fungicides tackle fungi, and nematodes (EPA 2004). The worldwide pesticide market can be categorized according to pesticide type as follows: 42.48% herbicides, 25.57% insecticides, 24.19% fungicides, and 7.76% other kinds of pesticides (Matthews *et al*., 2008).

Pesticides can be grouped on their chemical structure to Organophosphorus (chlorpyrifos anddiazinon), carbamates (carbarylandaldicarb), organochlorines (DDT and aldrin), pyrethrins and pyrethroids (cyfluthrin and cypermethrin), benzoic acids (dicamba), triazines

(atrazineandsimazine), phenoxyacetic derivatives (2,4- D), dipyridyl derivatives (diquatand paraquat), glycine derivatives (glyphosate), and dithiocarbamates (manebandziram) molecules (EPA, 2004). Pesticides with identical chemical structures have equivalent toxicity mechanisms and physicochemical features, as well as similar fate and transport qualities (Fig 1).



Type	Example	Structure	Effect on pest
Organochlorines (five or more chlorine atoms)	DDT		Nervous system disruptors leading to convulsions and paralysis of the insect and its eventual death
	Lindane		
	Endosulfan		
	Aldrin		
Organophosphorous	Parathion		Nervous impulses fail to move across the synapse causing a rapid twitching of voluntary muscles and hence paralysis and death
	Malathion		
	Diaznon		
Inorganic	*Benomyl*		Predominantly stomach poisoning
	Oxine copper		

***Figure 1**: Classification of pesticides based on chemical structure (Mariana et al., 2015).*

2 The pesticides environmental dynamic

Generally, pesticides generate toxicological and eco-toxicological consequences that can harm the environment quality (Mamy et al., 2008). It was estimated that during a treatment, 80 to 90% of the used pesticides' quantities do not reach the aimed pest, they drift towards the different elements of the environment as that of, which constitutes the direct support of farming, in addition to water and air that are directly impacted by contact with these molecules, then indirectly by the surrounding environment that are affected by these phytosanitary

products due to climatic factors; such as rain and percolation, or after evaporation in air (Bolognesi & Merlo, 2011). As a result, living organisms of the soil, water, and air, are affected with different ways (Huggenberger *et al.*, 1973) (Fig 2).

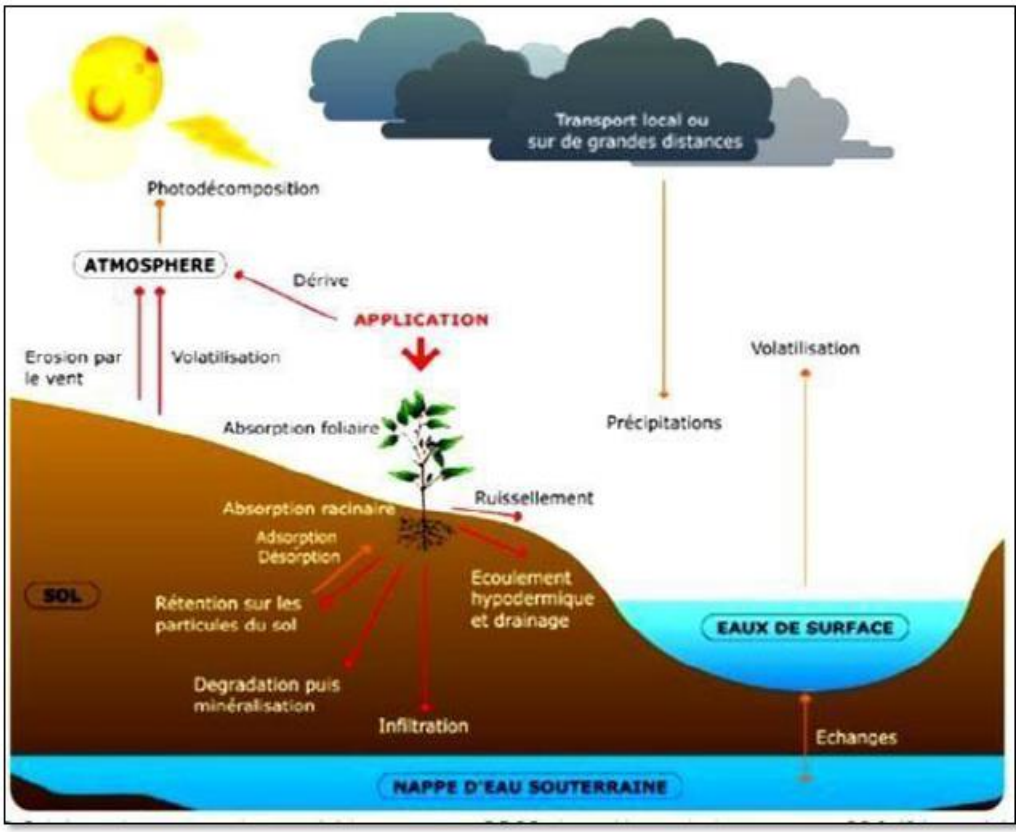


Figure 2: *Process of pesticides' diffusion in the environment (Atmo, 2017).*

3 Routes of pesticide exposure to human

Pesticide exposure can come directly from occupational, agricultural, and home usage, while they can also be transferred indirectly through diet. Moreover, the general population may be exposed to pesticides due to their application around major roads, etc. The main routes of human exposure to pesticides are through the food chain, air, water, soil, flora, and fauna (Anderson& Meade, 2014). Pesticides penetrate the body of an individual through a variety of ways: dermal, mouth, eye, and respiratory. As would be generally expected, the danger of pesticide contamination usually increases by increasing dose periods, in addition to the chemical structure (Meenakshi *et al.*, 2012) (Fig 3).

3.1 Dermal exposure

Dermal exposure is one of the most common and effective routes through which pesticide applicators are exposed to pesticides (Anderson & Meade, 2014). Dermal

absorption may occur as a result of a splash, spill, or spray drift, when mixing, loading, disposing, and/or cleaning of pesticides (Salvatore *et al.*, 2008). Absorption may also result from exposure to large amounts of residue. Pesticide formulations vary broadly in physicochemical properties and in their capacity to be absorbed through the skin (Beard *et al.*, 2014), which can be influenced by the amount and duration of exposure, the presence of other materials on the skin, temperature and humidity, and the use of personal protective equipment (Macfarlane *et al.*, 2013).

3.2 Oral exposure

Oral exposure of a pesticide usually arises by accident due to carelessness reasons (Damalas & Eleftherohorinos, 2011). The most frequent cases of accidental oral exposure were reported to occur when pesticides were transferred from their original labeled container to an unlabeled bottle or food container (Gilden *et al.*, 2010). There are many cases in which people have been poisoned by drinking pesticides kept in soft drink bottles or after drinking water stored in pesticide-contaminated bottles (USEPA, 2007).

3.3 Eye exposure

The potential for chemical injury is high for tissues of the eye. Some pesticides were reported to be absorbed by the eyes in sufficient quantities to cause serious or even fatal illness (Gilden et al., 2010). Granular pesticides pose a particular hazard to the eyes depending on the size and weight of individual particles (Jaga & Dharmani, 2006).

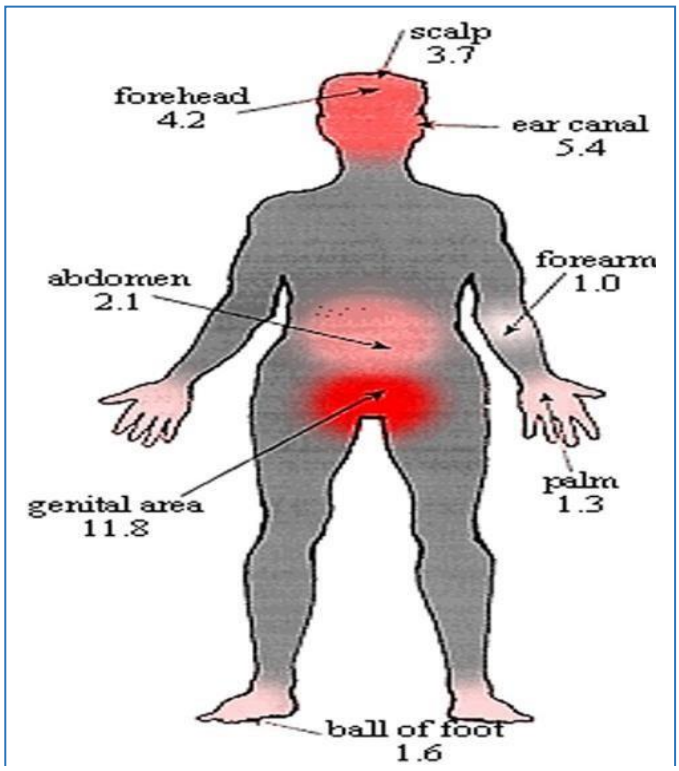


***Figure 3**: Dermal exposure to pesticide on different parts of the body (MOA, 2015).*

4 Pesticides Metabolism

As part of the requirement for registration, a detailed study is made of the behavior of the chemical as it is absorbed, transported, changed and excreted. It is important to know if there is a tendency for a chemical to store or be bound in a particular tissue, because this may be an indication of where it is acting. Many chemicals are changed by the liver and other organs into more soluble forms to facilitate excretion. However, for certain chemicals, that conversion intended as a detoxication mechanism results in a small amount of very reactive intermediate products that may be carcinogenic. It is necessary to learn whether and to what extent these kinds of reactions occur. It is also possible that products of these reactions exert toxic effects of their own, which should be distinguished from the activity of the parent compound. This is a characteristic of some of the organophosphate insecticides, which are changed to a more potent form after entering the body (Frank, 2003). The bio-transformation of pesticides takes place mainly in liver going through three consecutive phases (Fig 4).

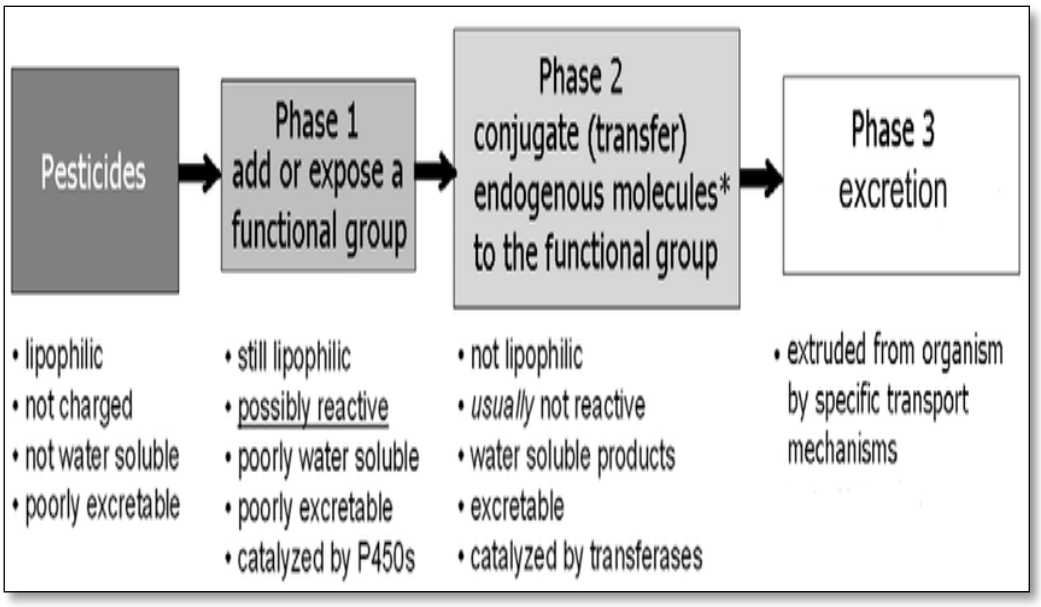


Figure 4: *Biotransformation of pesticides (Volodymyr, 2018).*

5 Pesticides as Endocrine distruptors

A variety of pesticides, are classified as endocrine disruptors (EDCs), which have been proven to interfere with the production, release, transport, metabolism, action, or elimination of hormones that are essential to maintaining homeostasis and regulating process of development (Bolognesi & Merlo, 2011; Khan & Law, 2005). EDCs in fish may lead male

fish to convert into female fish. Outward symptoms of developmental disturbance include decreased fertility or even sterility in adults, as well as poorer hatching rates and offspring survivability (Ewing, 1999; Goodbred *et al.*, 1997). Pesticides, as exogenous hormone agonists/antagonists, can interfere with endogenous hormone activity. Agonists can bind to hormone-binding proteins, while antagonists can displace endogenous hormones (Tollefsen, 2002). Some toxicants also disrupt the synthesis of hormone receptors (Scott & Sloman, 2004). The ultimate goal of reproduction is birth, and its success is dependent on both male and female reproductive systems (Gupta, 2011). Reduced reproductive capability may be one of the most detrimental impacts of persistent pollutants (e.g., pesticides) emitted by people (van der Oost *et al.*, 2003). The prevalence of these substances in the environment has becomes a worldwide problem. Pesticides, as sex hormone antagonists, can disrupt sexual development and other critical processes (Ewing, 1999; Gupta, 2011).

6 Herbicides

Herbicides, sometimes called «weed killers», are substances that are in charge of destroying or slowing down the bad herb's growth, named «weeds». They are distinguished between each other in terms of their penetration track in vegetables, and in terms of their movement inside the plant (Cirad, 2000). Acting on different processes of the plants' development growth, they disrupt the functionality of:

-The plant's physiology: the photo synthesis or the membrane permeability;

-The growth, the cellular division, the elongation, etc ;

-The cellular components biosynthesis: lipids, carotenoid pigments, amino acids, etc. (Batsch, 2011).

Glyphosate is among the most used herbicide in Algeria.

6.1 Glyphosate

Glyphosate (N-[phosphonomethylglycine]glycine), a widely applied non-selective systemic herbicide, is bit by bit becoming the most controversial pesticide product ever produced. The molecule has a very simple, still rather unique zwitterionic chemical structure (Figure 1): unlike most other pesticides it is highly soluble in water and, depending on the soil properties; it may be very strongly bound to soil matrices (Acquavella *et al.*, 2004) (Fig 5).

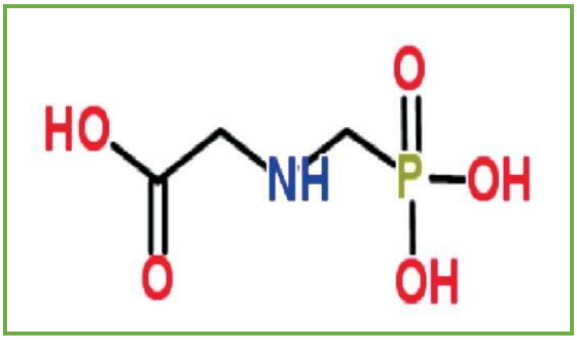


Figure 5: *Chemical structure of glyphosate (Tamas &Peter , 2016) .*

6.2 Mode of action of Glyphosate

Glyphosate kills the plant by interfering with the biosynthesis of key aromatic amino acids necessary for development. It inhibits the shikimate pathway enzyme 5-enolpyruvylshikimate-3-phosphate synthase. The Shikimate path is a metabolic system found in plants that is involved in the biosynthesis of aromatic amino acids (Gill *et al.*, 2017). 5-Enolpyruvylshikimate-3-phosphate synthase is responsible for chorismate biosynthesis. (Williams *et al.*, 2000) Chorismate is an intermediary in the synthesis of aromatic amino acids (tryptophan, phenylalanine, and tyrosine). Glyphosate is an antagonist of phosphoenolpyruvate, which is an active substrate for 5-enolpyruvylshikimate-3-phosphate synthase. Scarcity of the enzyme results in a lack of aromatic amino acids, which disrupts many metabolic processes in the plant and, as a result, causes the plant to die (Tu *et al*., 2001) (Fig 6).

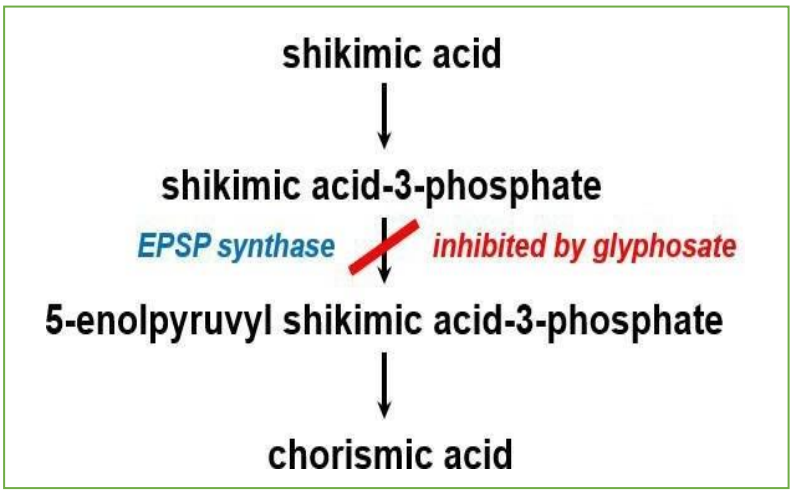


Figure 6: *Mechanism of action of Glyphosate (de Maria et al., 2006).*

6.3 Physiochemical characteristics of Glyphosate

Physico-chemical characteristics of glyphosate is summarised in table 1.

Table 1: Physiochemical characteristics of glyphosate (Environmental Health Criteria monograph, 1994).

Usage	**Glyphosate**
Commercial name	Glygosate 48%
Chemical name	N-(Phosphonomethylglycine)glycine
Chemical formula	C3H8NO5P
Molar mass	169.073 g·mol−1
Solubility in water	1.01 g/100 mL (20 °C)
Appearance	White gystalline powder

6.4 Glyphosate Metabolism

When glyphosate is consumed or inhaled, three protons that are positively charged are immediately released, or two protons and one potassium atom in the case of the potassium salt formulation. We hypothesize that the additional positive charges reduce the pH in the mouth, stomach, and intestines, resulting in greater acidity and contributing to the metabolic acidosis seen with glyphosate poisoning. The chyme becomes increasingly acidic in the stomach, causing overstimulation of the pancreas and raising the pH as it moves from the stomach to the small intestine. This is most likely owing to the metabolic acidosis described for significant amounts of glyphosate consumed in suicide attempts (Lee *et al*., 2000; Mahendrakar *et al*., 2014) (Fig 7).

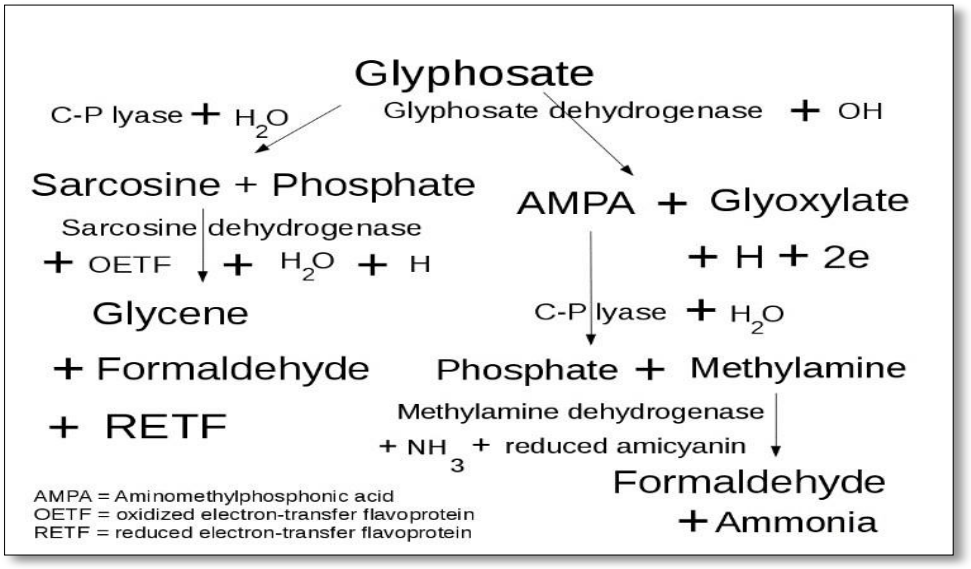


Figure 7: *Metabolic pathways for glyphosate (Nancy et al., 2016).*

After releasing the three protons, the glyphosate molecule is left with three negative-charged oxygen atoms that are very reactive. To create stable metallic compounds, they will selectively bind to any accessible metal, including Zn, Ca, Mg, Cu, Co, Fe, Cr, and Al (Caetano *et al.*, 2012). This feature has been connected to chronic renal disease among Sri Lankan agricultural laborers, ultimately led to formaldehyde. In animal tissue, there are no less than six distinct enzymes able to catalyze the conversion of formaldehyde to formic acid: aldehyde dehydrogenase, xanthine oxidase, glyceraldehyde-3-phosphate dehydrogenase, catalase, peroxidase, and aldehyde oxidase (Cooper & Kini, 1962). Formic acid is widely recognized as a contributor to metabolic acidosis and mitochondrial dysfunction (McMartin, 1980; Kruse, 1992).

6.5 Glyphosate and human health concerns

Academic researchers have found that glyphosates may be: endocrine disruptors. (Benachour&Seralin,2008; Romano *et al.*, 2010) carcinogenic, (Belle *et al*, 2007 ; IARC, 2015) capable of increasing the risk of spontaneous abortions(Arbuckle, 2001) associated with congenital malformations,100 and harmful to human nervous systems (Anadon *et al.*, 2008; Antoniou *et al.*, 2001). Other scientists worry about its toxicity to fetuses (Figure 8). Antoniou (2011), the International Agency for Research on Cancer the International Agency for Research on Cancer (IARC) determined that glyphosate was a possible human carcinogen, based on limited evidence of increased risk of non-Hodgkin lymphoma in humans, sufficient proof in animals (kidney, pancreas and skin cancers), and evidence of a carcinogenic mechanism involving genotoxicity and oxidative stress. (Bell *et al.*, 2007; IRAC, 2015).

Epidemiologic study suggests that there is a high correlation between the increasing use of glyphosates and several human health problems including hypertension, heart attacks, diabetes, obesity, Alzheimer, senile dementia, Parkinson, multiple sclerosis, autism, inflammatory diseases of the digestive system, intestinal infections, kidney pathologies, as well as cancers of the thyroid, liver, bladder, pancreas, kidney and leukemia. (Swanson *et al.*, 2014). These correlations alone do not prove that glyphosate induces these effects, but another study confirmed a metabolic pathway explaining the potential role of glyphosate in these pathologies.

6.6 Glyphosate as an endocrine disruptor

Glyphosate has been applied in many areas and causes serious pollution to soil and nearby ecosystems, and eventually increases the risk to human (Williams *et al.*, 2000). Exposure to glyphosate has been associated with many adverse effects on male reproductive system in both humans and rodents (de Brito Rodrigues *et al.*, 2016; Williams *et al.*, 2016b).

In mammals, glyphosate could alter sperm characteristics including sperm production, and even fetal development (Chan & Mahler, 1992; Gasnier *et al.*, 2009). Glyphosate causes libido and decreases ejaculate volume and sperm concentration in New Zealand rabbits, possibly due to its direct cytotoxic effect on spermatogenesis or indirectly on the hypothalamic-pituitary-testicular axis (Richard *et al.*, 2005). The reported male toxic effects and risk assessment include reduced ejaculate volume, testosterone concentration, sperm production of the seminiferous epithelium, sperm concentration, sperm morphology, sperm motility and sperm aberration rate.

However, other researchers have found contradictory results, in which there was no definitive evidence that glyphosate adversely impact reproductive function (Williams *et al.*, 2000). Athor reproductive toxicity studies in rodents have not reported any adverse effects, in which there was no difference in sperm vitality, motility, and sperm concentration, only an increase of abnormal sperm.

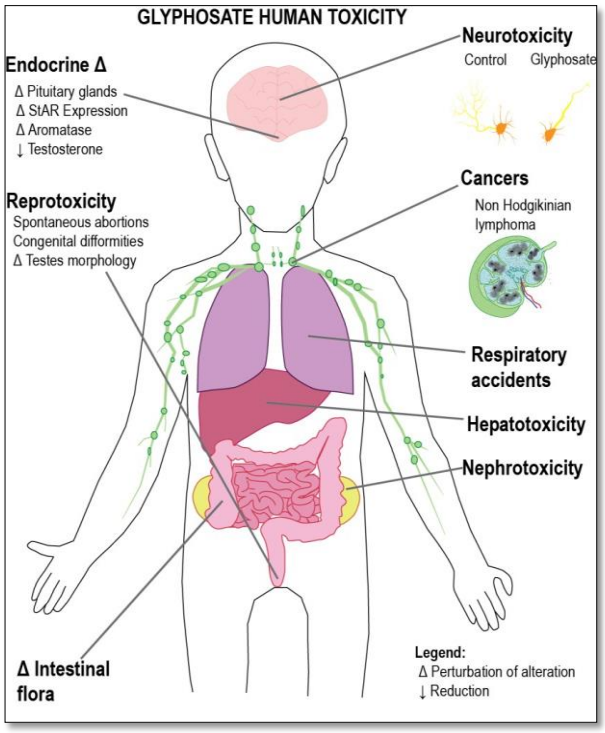


Figure 8: *Glyphosate human toxicity (Samsel & Seneff, 2013).*

7 Insecticides

Insecticides are biocides that are intended for the destruction of insects; in order to ensure the crop protection. Being widely used in agriculture to eliminate pests, they are also present in the domestic environment in the form of specialties against lice, veterinary drugs, household insecticides, gardening products, or even xylo protectors (Testud & Grillet, 2007). Being considered as neurotoxic products, their actions on the nervous system appear through blocking the spread of nerve impulses at the level of neurons and synapses, both at the level of the central and peripheral nervous system (Calvet *et al*., 2005). Some insecticides act by disturbing the insect reproduction's physiology (moulting disruptors), while others inhibit the chitin production, a major constitutive element of insects' exoskeleton (Batsch, 2011). Insecticides canal target larvae and insects' eggs.

7.1 Diflubenzurone

Diflubenzuron was first registered as a pesticide in 1976. It is an insecticide of benzoylurea (paterson, 2004), it is used in forest management and on field crops to selectively control insect pests, particularly boll weevils and gypsy moths. It is widely used as a larvicide for control of mosquito larvae. Among IGRs, chitin synthesis inhibitors diflubenzuron (DFB) is widely used against pest insects in forestry. DFB is very potent for mosquito control (Soltani & Rehimi, 2001; Rehimi, 2004) (Fig 9).

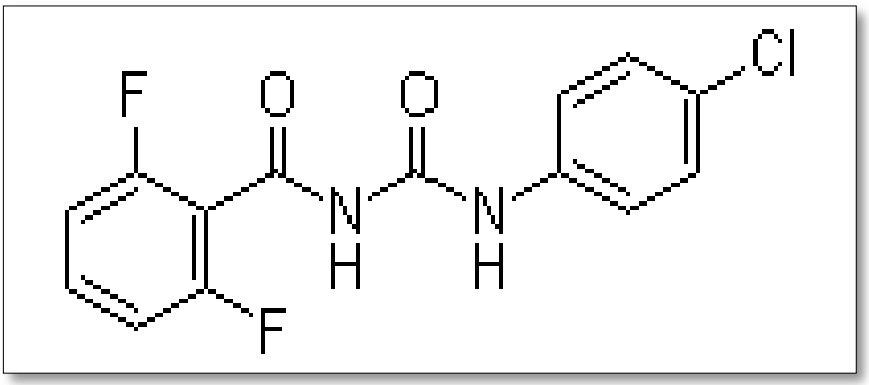


Figure 9:*Molecular structure of diflubenzuron (Maduenho et al., 2008).*

7.2 Physiochemical characteristics of Diflubenzuron

Physico-chemical characteristics are summarised in table 2.

Table 2: Physiochemical characteristics of diflibenzuron (WHO, 1995).

Usage	Deflubenzuron
Common name	Diflubenzuron
commercial name	Dimilin 25 WP
Chemical name	1-(4-chlorophenyl)-3-(2,6diflubenzoyl)urea
Chemical formula	C14H9CLF2NOO2
Molar mass	310.7 g mol-1
Solubility in water	0.08 mg at 25 and at PH 7
Appearance	White gystalline and solid

7.3 Mode of action of Diflubenzurone

Diflubenzuron inhibits chitin deposition in arthropods and is effective either as a stomach or contact insecticide. (Mabury & Crosby, 1996). Chitin is a polymer (repeating series of connected chemical subunits) of a glucose-based molecule and comprises a substantial proportion of the exoskeleton (outer-shell) of arthropods. Consequently, the inhibition of chitin synthesis disrupts the growth and development of pests. (DeCleraq *et al*., 1995a;b; Baishya & Hazarika, 1996; Griffith et al., 1996; Wright *et al*., 1996).

7.4 Diflubenzuron metabolism

Metabolites that are formed in vivo by an animal after diflubenzuron has been absorbed and metabolites that are formed in the environment through the degradation of diflubenzuron in environmental media – i.e., soil, air, and water. The in vivo metabolism of diflubenzuron has been reviewed by WHO (1996, 2001). In the environment as well as in sheep, eggs, and chickens, the major route of metabolism involves cleavage of the pure do bridge with the formation of 2,6-difluorobenzoic acid and 4- chlorophenyl urea. The latter compound is then metabolized to 4-chloroaniline. The formation of 4-chloroaniline is important to the human health risk assessment because this compound is classified as a carcinogen. The other pathway for the metabolism of diflubenzuron predominates in rats and cows and involves hydroxylation rather than cleavage of the ureido bridge. Hydroxylation of the aromatic rings involves the addition of a hydrogen-oxygen or hydroxy (OH) group to one of the rings. Hydroxylation increases the water solubility of

aromatic compounds. Particularly when followed by conjugation with other water-soluble compounds in the body, such as sugars or amino acids, hydroxylation greatly facilitates the elimination of the compound in the urine or bile. As detailed further by WHO (2001), the ureido bridge may also be cleaved in rats but 4-chloroaniline does not appear to be a major metabolite. No information has been located on the metabolism of diflubenzuron in humans (Fig 10).

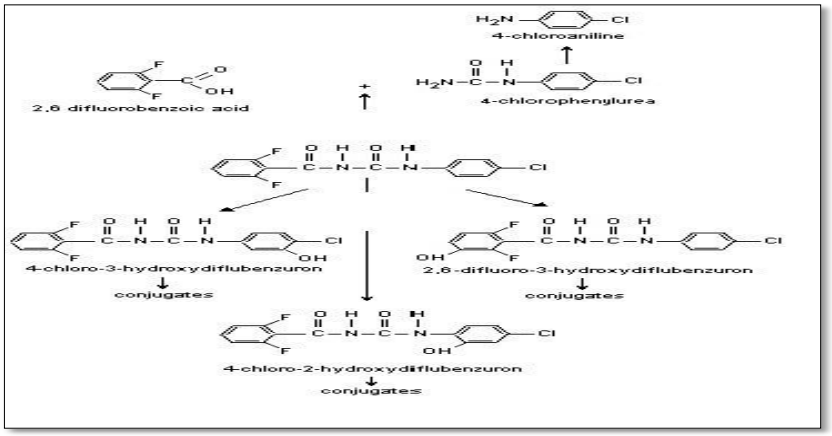


Figure 10: *Metabolism of diflubenzuron (WHO, 1996).*

7.5 Diflubenzuron as endocrine disruptor

The endocrine system participates in the control of metabolism and body composition, growth and development, reproduction, and many of the numerous physiological adjustments needed to maintain constancy of the internal environment (homeostasis). The endocrine system consists of endocrine glands, hormones, and hormone receptors. Endocrine glands are specialized tissues that produce and export (secrete) hormones to the bloodstream and other tissues. The major endocrine glands in the body include the adrenal, hypothalamus, pancreas parathyroid, pituitary, thyroid, ovary, and testis. Hormones are also produced in the gastrointestinal tract, kidney, liver, and placenta. Hormones are chemicals produced in endocrine glands that bind to hormone receptors in target tissues. Binding of a hormone to its receptor results in a process known as post receptor activation which gives rise to a hormone response in the target tissue, usually an adjustment in metabolism or growth of the target tissue. Examples include the release of the hormone testosterone from the male testis, or estrogen from the female ovary, which acts on receptors in various tissues to stimulate growth of sexual organs and development of male and female sexual characteristics. The target of a hormone can also be an endocrine gland, in which case, receptor binding may stimulate or inhibit hormone production and secretion (Dave, 2001).

Diflubenzuron has been tested for its ability to cause birth defects (i.e., teratogenicity) as well as its ability to cause reproductive and developmental impairment. Teratogenicity studies typically entail gavage administration to pregnant rats or rabbits on specific days of gestation. Two such studies were conducted on diflubenzuron: one in rats (Kavanagh , 1988a) and one in rabbits (Kavanagh ,1988a).

8 Fungicides

Fungicides are substances that are exclusively designed to eliminate or limitate the development of fungi. Their effect can be qualified as «preventive» when its action is located before the parasite's penetration in the plant tissues, as «curative» when it intervenes on filaments that are already well installed in the tissues; before the appearance of the first symptoms, or as «eradicating» when it intervenes on filaments that are already well installed in the tissues; with the appearance of the disease's first signs (Azzouz, 2012). The mode of action can be observed on one single site, or on many targets, by using multi-sites fungicide (Batsch, 2011). Most of used fungicides have only one site of action to stop or alter the proper functioning of a reaction; that is necessary for the fungi's survival, which generates the death of the cell. However, if these cells mutate at the level of the fungicide's unique site of action, the product can become inactive because it will no longer recognize its target, which results in a pathogenic resistance to fungicide. Multi-sites fungicides are, in this case, allies of choice since the acquisition of a resistance by the pathogen must go through the mutation of many targets, a mutation which has not yet arrived (Aprifel, 2004).

8.1 Azoxystrobin

Azoxystrobin operates as a systematic, broad-spectrum fungicide in the methoxy acrylate category, that originate from strobilurins found in nature. it's widely used in agriculture in about 50 countries to protect crops from fungal diseases for rice, wheat, beans, and grape etc. (Bartlett et al., 2001) (Fig 11).

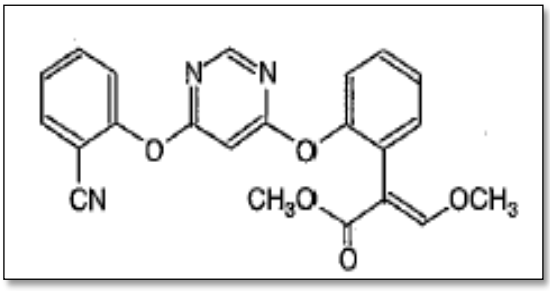


Figure 11:*Chemical structure of Azoxystrobin (Tang et al., 2016).*

8.2 Mode of Action of Azoxystrobin

Azoxystrobin and other strobilurins inhibit mitochondrial respiration by blocking electron transport. They bind at the quinol outer binding site of the cytochrome b-c1 complex, where ubiquinone (coenzyme Q10) would normally bind when carrying electrons to that protein. Thus, ATP production is sttopped (Sauter**,** 1995**;** Wong &Wilcox, 2001) (Fig 12).

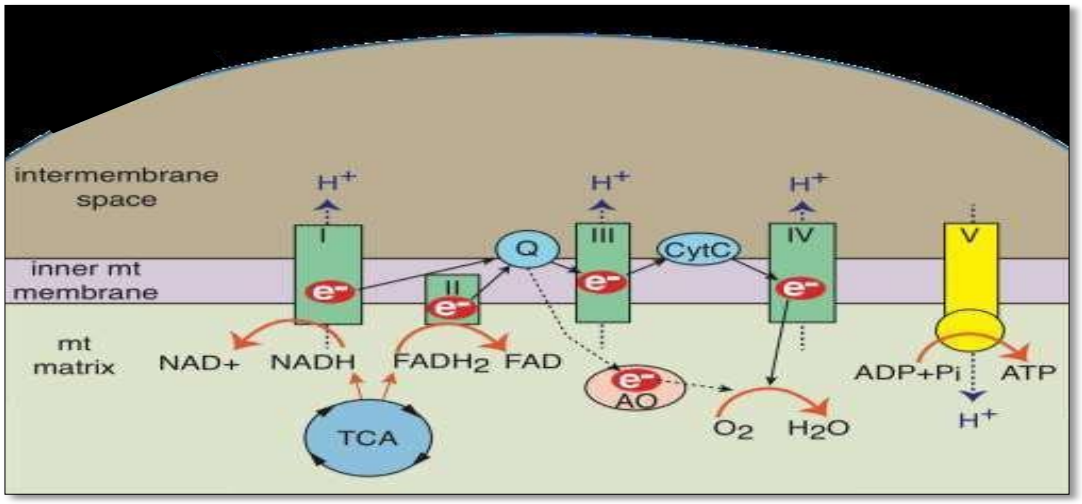


Figure 12:*Mode of action of azoxystrobilin in membrane of mitochondron (Wood & Hollomon, 2003).*

8.3 Physiochemical characteristics of Azoxystrobin

Physico-chemical characteristics ofare summarised in table 3.

Table 3: Physiochemical characteristics of azoxystrobin. (Pesticide list,2003).

Usage	**Azoxystrobin**
commercial name	Azoxystrobin
Chemical name	Methyl (E)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate
Chemical formula	C22H17N3O5
Molar mass	403.4 g/mol
Solubility in water	6.7 mg/l (20 °C)
Appearance	White crystalline solid

8.4 Azoxystrobin Metabolism

The proposed metabolic pathway for azoxystrobin in goats and hens (poultry) is shown in Figure13. The extensive metabolism of azoxystrobin in the lactating goat includes to following proposed mechanisms:

- Cleavage of the ether linkage between the phenyl acrylate ring and the pyrimidinyl ring to give Compound 28.
- Cleavage of the ether linkage between the cyanophenyl ring and the pyrimidinyl ring to give Compounds 13 and 3.
- Hydrolysis of the ester group or oxidative o-dealkylation to give Compound 2, which is further metabolized by cleavage of the ether linkage between the cyanophenyl ring and the pyrimidinyl ring to give Compound 10 (Compound 10 could also result from o- demethylation of Compound 3, and Compound 2 also undergoes o-demethylation to give Compound 20).
- Hydroxylation of the phenyl acrylate ring (hydroxyl position undetermined) to give Compound L1, followed by conjugation with glucuronic acid to give asolubel compound (Fig 13).

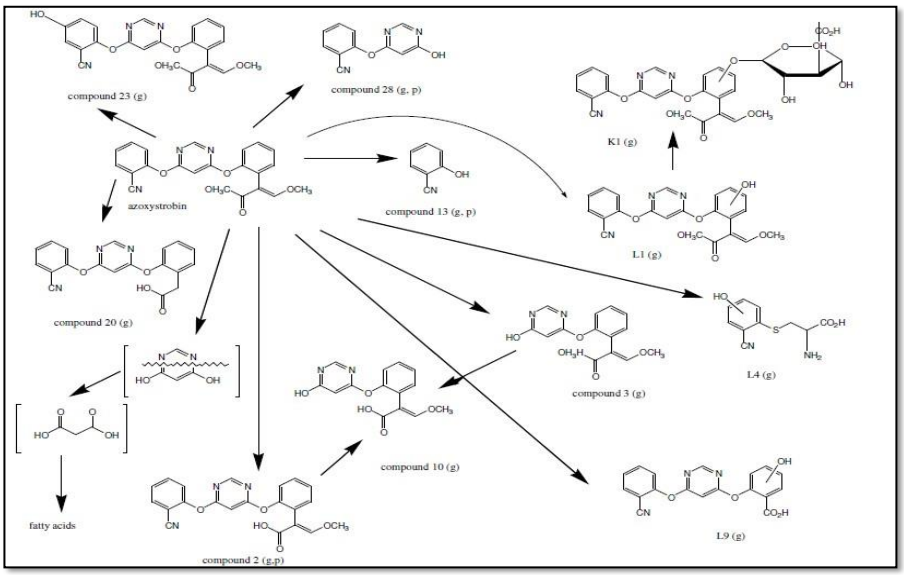


Figure 13:*Proposed metabolic pathway of azoxystrobin in goats (g) and poultry (p).*

Chapter 2:

Materials and methods

1 Biological Materials

In this study, male domestic rabbits *Cuniculus lepus* were obtained in April from Annaba region, with an age ranging from 06 to 07 months, their average weight was 1700,54 ±113.15 g. Animals were reared in the breeding house of the biology department inside metallic cages with dimensions of 53×60×50cm in natural conditions of temperature, humidity, ventilation and photpperiod. Animals were fed on a mixture consisting of wheat, corn, barley, as well as fresh green plants, and supplied with water on a daily and regular basis. Animals were left for 15 days to cope with experimental conditions.

2 Pesticide administrations

Pesticide were supplied to animals in the morning by gavage a rate of 1 ml/rabbit/6 days/week during 4 weeks, and the control group was also placed in the same experimental conditions (Fig 13). Male rabbits were divided as follows:

2.1 Treatment with Diflubenzuron insecticide

Group 1: It was used as a control group (n=5) (C).

Group 2: It comprised of three equal sub-groups of 5 animals each, that received three different doses of **DFB** by gavage (D1: 250 mg/kg body weight, D2: 500 mg/kg b.w, and D3: 750 mg/Kg b.w).

2.2 Treatment with Glyphosate herbicide

Group 1: It was used as a control group (n=5) (C).

(C) **Group 2:** It consisted of three equal sub-groups of 5 animals each, that received three different doses of**GLY**by gavage (D1: 200 mg Gly/kg b.w., D2: 300 mgGly/kg b.w., and D3: 400 mg Gly/Kg b.w).

2.3 Treatment with Azoxystrobin fungicide

Group 1: It was used as a control group (n=5) (C).

(C) **Group 2:** It consisted of three equal sub-groups of 5 animals each, that received three different doses of **AZO** by gavage (D1: 100 mg AZO/kg b.w., D2: 200 mg AZO/kg b.w, and D3: 300 mg AZO/Kg b.w.) (Fig 14).

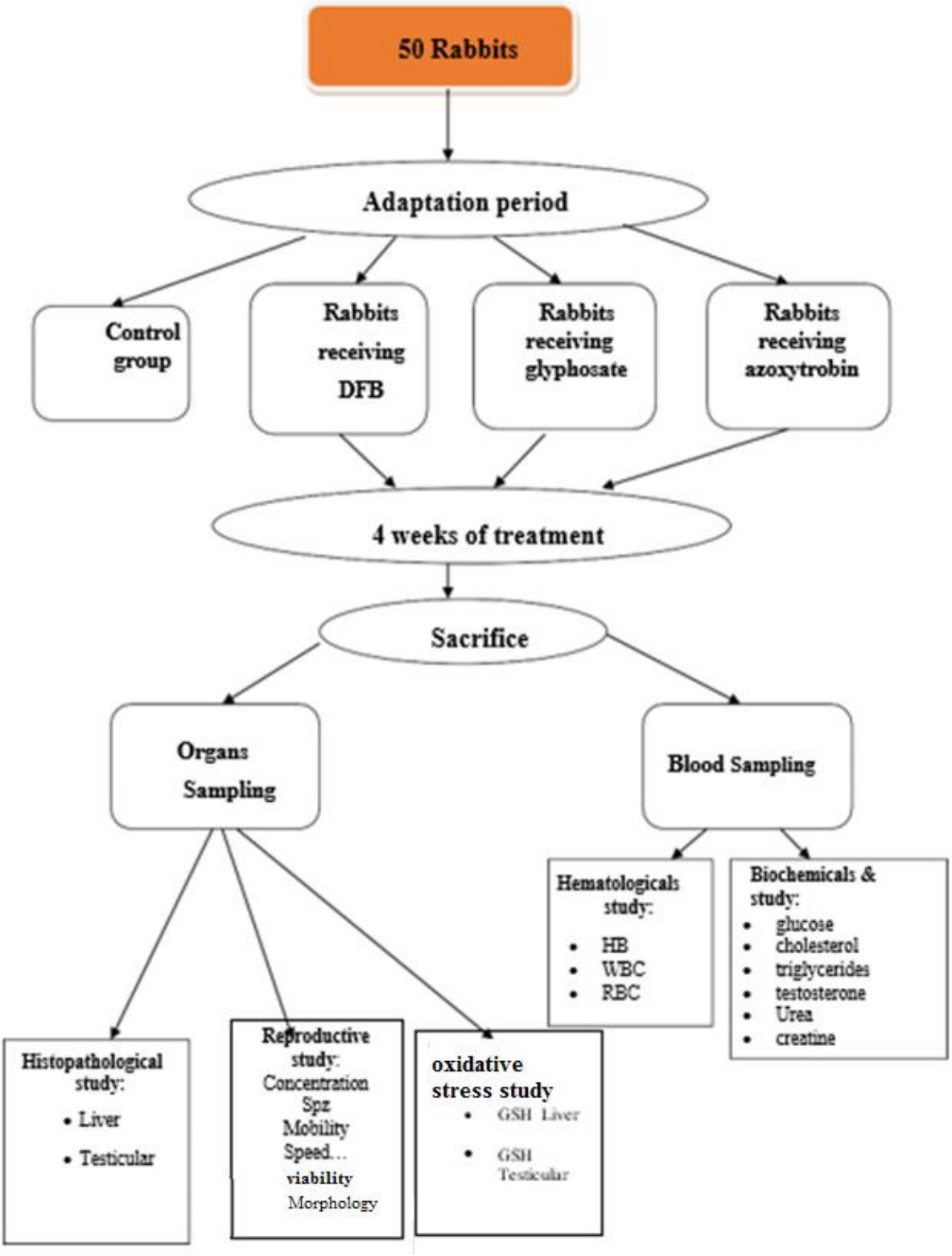


Figure 14: *The experimental protocol scheme.*

3 Samples collection

3.1 Blood

Blood was obtained immediately after the sacrifice and it divided into different test tubes as the following:

Tubes containing EDTA: A fraction of blood was used for blood analysis; the other fraction was centrifuged at3000 rpm for 15 minutes, in which the plasma was frozen at -20°C in order to be used in the biochemical analysis.

Dry tubes: Serum was obtained from this fraction to estimate the concentration of testosterone.

3.2 Organs' collection

After sacrifice, the animals dissection was carried out to remove the liver, testicles, and the epididymis; which then weighed, and portions of the liver and testicles were kept inside aluminum paper and placed in the freezer (-23 °C) in order to estimate glutathione level, and the other portions of the liver, and the right testicules were placed in the alcohol-based Bouin solution for the histological study.

3.3 Sampling of sperm liquid

An incision was made at the level of the epididymis' head zone, and a white milky fluid which is the epididymis seminal fluid was released, where a drop of 0.1 µl of semen was taken and placed in 49 µl of the physiological liquid NaCl (0.9%), in order to study some biological indicators of sperm; concentration, velocity, movement ratio, percentage of sperm vitality.

4 Estimation of reproductive parameters

Biological parameters were obtained by carrying out a spermogramme according to OMS (1993).

4.1 Sperm concentration

In order to inhibit animal movement, 50g of sodium carbonate were dissolved in 250 ml distilled water, and then 20 ml of the solution was taken, and 20 ml of 30% concentration formol was added, then 49 µl of the mixture were taken, and 0.1 µl of semen were added, then dropped into the Mallassez cell and covered with a lamella.

– The number of sperm was counted in 05 fields (05 large squares) in x400 magnification.

The concentration of sperm is estimated by the following correlation: N/(n×v×d) = (ml/610x spz) C.

C: The concentration of sperm

S: Sperm

D: Mitigation factor= 50

V: The size of the Mallassez cell

n: The number of sperm calculated in 05 large squares of the Mallassez cell

N: Number of squares = 100 squares.

4.2 The estimation of sperm movement ratio (mcm/s)

a drop of sperm was added to the Nageotte slide and then covered with a coverslip.

The observation was done by light microscope at x100 and x400 x magnification.

The Nageotte slide has parallel horizontal lines with a distance of 0.5 micrometer between each parallel line. The principle of this process is to estimate the time at which a sperm will cross two parallel lines successively, using a Chronometer, and then the speed was calculated according to the following correlation = Sp = S/ T(mcm/s).

Sp: Speed (Mcm/s).

D: Distance between two parallel lines (micrometer).

T: Time (second). The crossing time of at least 10 sperm was calculated, and then the average spee peed was estimated.

4.3 Sperm percentage estimation.

The principle of this experiment was that normal healthy cells do not allow the passage of biological colorant (Eosine at a 1%), unlike dead cells which allows the colorant to cross the plasma membrane.

a-The method of detection: A drop of sperm was placed in a normal slide and a drop of Eosine was added. After mixing the sample, which can spread out over the entire surface of the slice, left until drying and then placed on top of a screen, observed at x100 and then x400, and then the ratio of live sperm to dead was calculated.

b-TEST- HOS(Test hypo-osmotic) : This test was based on the study of the extent to which the sperm plasma membrane is resistant to low osmotic pressure, this environment is causing morphological changes at the level of sperm flagellum (Fig 15).

The solution used:

Exactly 0.735 g sodium citrates (Na_3 C6H5O7, $2H_2O$) and 1,351 g fructose were dissolved in 100 ml distilled water, and then they were frozen at (-20°C).

After thawing, the solution was well shaken before use.

Operation mode :

1ml solution was placed into the eppendorf tube for 50 minutes in the incubator at 37°C.

After that, 0.1 ml of sperm was added, and mixed well, and then left in the incubator below 37°C for 30 min.

The observations with the light microscope at 100x100, then at 400x400 magnification were realized.

The proportion of sperm that showed formal changes in the level of flagellum (live coal) and normal sperm (dead) was calculated (OMS, 1993; Jeyendran *et al*., 1984).

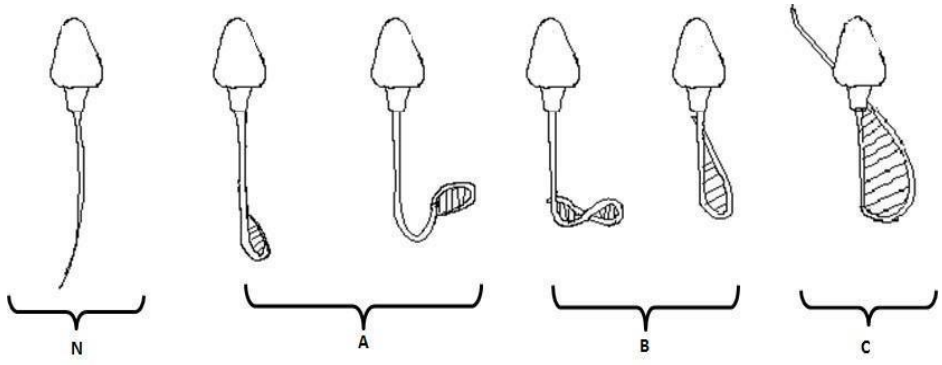
Observed changes

N: There is no formal change

A: Little change at the flagellum level

B: A significant change at the level of the flagellum

C: A significant change to flagellum level



N: absence of formal change; A: little change in flagellum level; B: a significant change in the flagellum level; C: a significant change in flagellum level.

Figure 15:*Diagram showing the formal changes of sperm under low osmotic pressure (OMS, 1993).*

4.4 Estimation of serum testosterone

4.4.1 The principle

The concentration of testosterone was calibrated by a technique that involves the use of the Test vidas, which allows the estimation of serum testosterone by using the ELISA method, whereby the calibration was based on the combination of the immune enzyme method eventually supported by the estimation of the amount of light radiation of the complex (Antigen generator -antibody) that is proportional to the concentration of testosterone (litwak, 1992).

4.4.2 The substances used

Quantity	Symbol	Compounds
1×12×8 ml	MTP	Plate Fragile Separation strips Anti-testosterone antigen generator for mice serum
1×25 ml	ENZCONJ	Operational accompaniment enzymes containing testosterone associated with HRP, fixatives
1×7×1 ml	CAL A – G	Operational G-a calibrator 0; 0.02; 0.5; 1.0; 2.0; 6.0; 16 nano/ml containing testosterone hormone, human serum, fixative
2×1 ml	CONTROL 1 + 2	Operational witness 1+2 containing testosterone hormone, human serum, fixatives
1×12 ml	TMB SUBS	Operational base solution TMB containing, TMB, Tampon, fixatives.
1×12 ml	TMB STOP	An operational TMB suspensive solution :1m H2SO4.
1×100 ml	WASHBUFCONC	A concentrated washing Solution for (x10)
2×	FOIL	Adhesive paper

4.4.3 Operating principle

- Lay 25 µl of the standard, the control, and samples at the designated rooms in the plate.
- Lay 200 µl of associated enzymes in all rooms.
- Cover the plate with adhesive paper, then mix well for 10 seconds.

  Incubate the samples in the incubator for 60 minutes, at (18–25°C).
    - Removal of the adhesive paper, pull out of the used solution in the incubator, wash the plate 3x with 300 ml diluted solution, and then distillate the plate by inverting it over absorbent papers.

Lay 100 µl of TMB solution in each room.

The plate was placed in the incubator below a temperature (18-25°C).

We stop the TMB reaction by placing 10 µl of TMB inhibitor solution, in each room, the contents are briefly mixed by shaking delicately the plate. Then the color will be changed from blue to yellow.

10 minutes after laying a TMB suspense solution, the reading should take place along the wave 450 nm (600-650 nm).

4.4.4 Reading results

The contents were briefly mixed by shaking gently a plate. Then the color was changed from blue to yellow.

Optical density (OD) was drawn; the concentration of samples was read from the standard curve.

Sample results were multiplied by the dilution factor, as follows:

Testosterone (nano g/mole) x3.47= nano mole/L.

5 Estimation of blood components

The study was conducted by an automated counting device called Erma Inc., a device that calculates cell electrical frequencies and is able to give 18 blood component indicators for this study.

6 Estimation of plasma parameters

6.1 Estimation of plasma glucose

Glucose concentration in plasma blood was estimated by the enzymatic method (Trinder,1969; Kaplan, 1984a).

6.1.1 Principle

Glucose was oxidized by the Glucose oxydase (GOD) enzyme to provide gluconic and hydrogen peroxide (H2O2).The product H2O2 reacts to the peroxydase (Pod) enzyme with the presence of 4-aminophenazone.

A pink-colored Quinoneimine compound produces the amount of this compound that is proportional to the amount of glucose (Spain).

$$\beta - D - glucose + O_2 + H2O \xrightarrow{GOD} Acide\ gluconique + H2O$$

$$H_2O_2 + Phénol - 4 - Aminophénazone \xrightarrow{POD} Quinoméimene + H2O2$$

6.1.2 The detector used:

The first detector	Tris pH 7,4 Phénol	92 Mmol/L 0.3 Mmol/L
The second detector(enzymes)	Glucose oxydase (GOD) Peroxydase (POD) 4-Aminophenazone (4-AP)	15000 Mmol/L 1000 Mmol/L 2.6 Mmol/L
The standard solution	Glucose	100 mg/dL

6.1.3 Preparation method

The content of the detector bottle 2 should be dissolved into detector 1, the resulting solution is the one we use to calibrate the glucose and remains for a month at (2°C-8°C) as well as 7 days at the laboratory temperature.

6.1.4 Calibration method

- Detector solution (white).
- Sample: Plasma blood
- Standard solution: 1g/L glucose

These solutions were placed in the tubes in the following manner:

	White	Standard	Sample
The standard	-	10 µL	-
The sample	-	-	10 µL
The detector	1 ml	1 ml	1 ml

The tubes were well shacked, then left for 30 minutes at the laboratory temperature.

The light intensity of the samples versus white along the light wave 505nm (409- 505nm) was estimated using a spectroscope.

Glucose concentration was calculated by the following correlation:

(1=n)n x(for light sample density)/(for standard light density)= glucose concentration (g/l).

6.2 Estimation of plasma cholesterol

Cholesterol concentration wa0s estimated by colorful enzyme method (Trinder, 1969)

6.2.1 The principle

The concentration of cholesterol was calculated after the enzyme rehydration and then oxidized by cholesterol oxydase, the H_2O_2compound then was formed, The latter was converted by the Peroxyted's hydrogené enzyme into quinoneimine, which takes pink color, the amount of this compound is proportional to the amount of cholesterol. Enzyme reactions are performed according to the following equations Kit Spinreact (Spain):

Esters de cholesterol +H2O cholestérol estérase Cholesterol + Acide gras

Cholesterol + O2 $\xrightarrow{\text{Cholestérol oxydase}}$ Cholest - 4 - en - 3 - one + H2O2

2H2O2 + Phenol + 4 - Aminophenazone $\xrightarrow{\text{POD}}$ + 4H2O2

6.2.2 The detectors used

Detector 1	PIPES pH 6,9 Phenol	90 Mmol/L 26 Mmol/L
Detector 2 nzymes	Cholesterol estérase (CHE) Cholesterol oxydase (CHOD) Peroxydase (POD) 4-Aminophénazone (4-AP)	1250 U/ L 300 U / L 300 U / L 034 Mmol/L
Standard solution	Cholesterol	2 g/ L

6.2.3 Calibration method

- Detector solution (white).

Plasma blood sample- the standard solution (2g/L).

	White	Standard	Sample
Standard	-	10 µL	-
Sample	-	-	10 µL
Detector	1 ml	1 ml	1 ml

The tubes must be shaked for 10 minutes at the lab temperature.

After 10 minutes, the light density of the samples is read versus the white along a 505nm (500-505nm) light wave using the spectroscope.

The concentration is calculated with the following correlation:

Cholesterol concentration (g/l) = (the sample light density)/(the standard light density) x n(n =2).

6.3 Estimation of plasma triglycerides

6.3.1 The detector used

Concentration is estimated according to the following interactions:

Triglycerides $\xrightarrow{\text{Lipoproteine lipase}}$ glycerol + acide gras

Glycérol – ATP $\xrightarrow{\text{glycérolkinase.Mg}}$ glycerol - 3 - Phosphate – ADP

Glycerol - 3 - phosphate + O_2 $\xrightarrow{\text{Glycérol - 3 - Phosphate oxydase}}$

H_2O_2 – dihydroycetone -P H_2O_2 + amino - antipyrin – chloro - 4 - phénol

$\xrightarrow{\text{proxydase}}$ Quinomeimene rose + H_2O_2

First detector	Tompon pipes pH 7,5 p-Chlorophenol	5 Mmol/L 2 Mmol/L
Second detector	Lipoproteine lipase (LPL) Glycerol kinase (GK) Glycerol-3 oxydase (GPO)	150000 U/L 800 U/L 4000 Mmol/L
	Peroxydase (POD) 4-Aminophenazone(4-AP) ATP	400 U/L 037 Mmol/L 033 Mmol/L
Standard solution	triglycerides	2 g / L

6.3.2 Preparation method

The content of the detector bottle 2 must be dissolved in detector 1, the solution obtained is used to estimate the concentration of Triglyceride; among its properties is that it remains stable for 60 weeks at (2°C-8°C) or for one week at the laboratory temperature.

6.3.3 Calibration method

- Detector solution (white)
- Specimen: Plasma blood standard solution

We spill these solutions into the tubes in the following way:

	White	Standard	Sample
Standard	-	10 µL	-
Sample	-	-	10 µL
Detector	1 ml	1 ml	1 ml

- The tubes was shacked and left for 10 minutes in the lab temperature.
- Then the light density of the samples was read versus the blanc in the spectroscope device along a light wave 505 nm (490-505 nm).

6.3.4 Concentration calculation method

Glycerates concentration (g/L) =(Sample density light/ Standard density light) n(n× = 2

6.4 6.3. Estimation of plasma total proteins

This study was conducted through Kit spinreact, Spain (Biuret et al., 1974) .

6.4.1 The principle

This method relies on the reaction of proteins' peptide bonds to the copper peculiarities in the middle of a core and the formation of purple-colored complex that is sensitive to the intensity of their color density.

$$\text{Proteins} + Cu^{+2}\ ^{\text{pHalcalin}}\ \text{Cu – protein complex}$$

6.4.2 The used detectors

Detector 1 Piore detector	Sodium iodique Na-K-trtrate Potassium iodique copper sulfate	100 Mmol/L 15 Mmol/L 5 Mmol/L 19 Mmol/L
Detector 2 White	Sodium iodique Na-K-trtrate	10 Mmol/L 16 Mmol/L
The standard	Protein	60 g/L

6.4.3 Calibration method

We spill these solutions into the tubes in the following way:

	White	Standard	Sample
Distilled water	0.02 ml	-	-
standard	-	0.02ml	-
Sample	-	-	0.02ml
detector	1 ml	1 ml	1 ml

The tube was well shacked and left in a bathroom with a temperature of 37°C. For 15 minutes. The concentration of samples was read against white at 540 nm.

(6=n)n x(light-density sample)/(light Photodensity) = protein concentration (g/l).

6.5 Estimation of plasma Albumin

6.5.1 The detectors used

The detector	Succinate buffer, Ph 4.20 Green bromocresol Brige 35	87 mmol/L 0.2 mmol/L 7.35 mmol/L
Sample	Bovine albumin	5 g/dl

6.5.2 The titration method

	white	**Standard**	**samples**
Standard (ml)	5	5	5
Sample (mcL)	-	20	-
Detector (ml)	-	-	20

6.5.3 Estimation of plasma urea

The urea concentration was estimated according to the enzymatic colorimetric method (Kit spinreact, Spain) as the following interactions:

$$\text{Urea} + H_2O \xrightarrow{Urease} (NH_4^+)2 + CO_2$$

$$NH_4^- + \text{Salicylate} + NaCLO \xrightarrow{Nitroprusside} \text{Indophenol}$$

The Indophenol takes the green color, and the colorimetric density was directly proportional to the concentration ratio (Kaplan, 1984b).

6.5.4 The used detectors

The detector 1 **Buffer**	Phosphate pH 6, 7 EDTA Sodium salicylate Sodium nitroprusside	50 mmol/L 02 mmol/L 400 mmol/L 10 mmol/L
The detector 2 **NaClO**	Sodium hypochlorite (NaClO) Sodium hydroxide	140 mmol/L 150 mmol/L
The detector 3 **(Enzymes)**	Urease	3000 unity/L
Standard solution	Aqueous urea	50 mg/dL

The detector 3 dissolved in the reagent 1, and then mixed well.

6.5.5 The preparation method

	White	**Standard**	**Sample**
Standard (ml)	**1.00**	**1.00**	**1.00**
sample (mcL)	**-**	**10**	**-**
detector (mcL)	**-**	**-**	**10**

The mixture is well mixed, and then was put it in the incubator for 10 minutes at a temperature of 15 to 20 °C.

	white	**Standard**	**sample**
standard 2 (ml)	1.0	1.0	1.0

The mixture was mixed,and thenit was put it in the incubator at a temperature of 15-25°C for 30 min.

The optical density of the titer was read versus the white at 580 nm.

The Urea concentration was calculated according to the following equation:

$$\text{:The Urea concentration (mg/dl)} = \frac{\textit{the optical density of the sample}}{\textit{the optical density of the titer}} \text{X n (n =}$$

6.6 6.6. Estimation of plasma creatinine

6.6.1 The detectors used

Detector 1	Picric acid	17.5 mmol/L
Detector 2	Sodium hydroxide	0.29 mmol/L
Detector 3	Creatinine	2 mg/L

The detector 1 content is mixed with the reagent 2. The resulted reagent remains steady for 10 days in the laboratory's temperature.

6.6.2 The titration method

	White	Standard	Sample
Standard (ml)	1.0	1.0	1.0
Sample (mcL)	-	100	-
Detector (mcL)	-	-	100

The tube was mixed, and then left for 30 seconds, after that the optical density A1 was obtained. After 80 seconds, the optical density of A2 was obtained.

$$\text{The creatinine concentration (mg/dl)} = \frac{\Delta A \text{ sample} - \Delta A \text{ the white}}{\Delta A \text{ the titer} - \Delta A \text{ the white}} \text{ X n (n=2)}$$

$$\text{XA} = A_2 - A_1$$

6.7 Estimation of hepatic and testicular GSH

6.7.1 The principle:

The glutathione concentration was estimated by the Weckbeker & Cory method (1988), to measure the optical density of 2-Nitro-5-marcapturique resulting from the reduction of dithio-bis-2-nitrobenresque-'5.5 by SH aggregates-) for glutathione and for the removal of proteins from a mixture, and maintenance of sulfosalicylique.

6.7.2 Preparation method:

Structured solution (C4H11NO3) tris 0.4 mol, contains 0.02 mol of EDTA:
Dissolution of 4.8456g of tris + 0.74444 g of EDTA in 100 ml distilled water, and then adjusting pH at 9.6 by adding HCl or NaOH.
EDTA solution (002, mol): dissolution of 1.8714 g of powder in 250 ml distilled water.
Sulfosalicylique solution (0.25g) solution: dissolution of 0.25g of powder in 100 ml distilled water.
DNTB solution (0.01 mol): dissolution of 99 mg of powder in 25 ml of distilled water.

6.7.3 Calibration method:

Preparation of the sample mixture

200 mg of tissue (liver, testis) was taken and placed in a test tube and 0.02 mol of EDTA was added, and then homogenized for 3 minutes for liver and 5 minutes for testicle. Tubes containing the samples were placed in an ice-filled container to avoid overheating. The obtained texture was centrifuged at 5000 rpm for 15 min.

The resulting (floating) extract was used to measure the concentration of protein and glutathione.

6.8 Estimation of protein in the tissue:

The concentration of total proteins was measured according to the Bradford method (1976), which uses Brilliant Blue Coomassie (BBC) as a detector by the following stages:

- Add 5 ml of BBC to 0.05 ml of floating extract and mix well;
- After 5 minutes, the light density was read under a 595 nm for the white solution containing distilled water in place of the extract;
- Estimate the concentration of proteins in samples based on the standard curve.

6.8.1 The standard curve preparation:

- A solution of bovine serum albumin BSA (1 mg/ml)wasprepared using distilled water.
- Different concentrations of this solution were used (20, 40, 60, 80, 100 µL). The volume in each tube was completed to 100 µL with distilled water.
- 4 ml of BBC blue Coomassie was added to all tubes.
- Tubes were well mixed.
- The light intensity of these solutions was read at 595 nm versus a white that contains 100 µL of distilled water + 4 ml of a Coomassie blue solution.

6.8.2 Method of protein removal

- 0.8 ml of sample was added to 0,2 ml Sulfosalicylic acid (0.25%), mix well and then leave it for 15 minutes in the freezer, place the sample in the centrifuge at 1000 rpm for 05 minutes.
- The resulting (floating) extract is used to measure glutathione concentration.

- 0,5 ml of floating solution is taken, and 1 ml of Tris (0.4 mol), containing 0.02 mol of EDTA (PH=6), is added to a mixture of 0.025 ml DTNB solution (0.01 mol) dissolved in methanol. Leave this mixture for 05 minutes at room temperature for interaction and color stability.
- The light intensity of the samples is read at 412 nano versus a white solution containing distilled water.

6.8.3 How to calculate concentration:

$$\frac{1{,}525 \times 1 \times DO}{protéine\ mg/0.5 \times 0.8 \times 13100} = (protéine\ mg/nm)GSH$$

Where:

Do: the light density of the sample.

1: Total volume used to remove proteins (0.2+0.8).

1.525: Volume of used solutions + floating solution (0.5+1+0.025). **13100**: The absorption constant of SH aggregates at 412 nano-mole.

0.8: The volume of the mixture used.

0.5: The volume of the solution floating from the mixture.

7 Histological study

The study was carried out for both the liver and testicle at Pathological Anatomy Laboratory in the Hospital of Ibn Rochd, through the method of (Martodja & Martodja, 1967; GABE, 1968). Portions of testicle and liver were saved in Bouin solution in order to preserve the tissue from damage (keep the shape as close as possible to the normal condition) and also to facilitate the procedure of the segmentation.

7.1 Preparation of Bouin solution

Formol 26 ml, acetic acid 7 ml, 1% concentration of Picric acid solution. 45 mL (1g of picric powder+ 99 mL of 95° alcohol).

7.2 Histological processes

The process of removing water and storage in the paraffin was used by an automatic device which allows samples to pass through 07 consecutive baths: 03 baths of ethanol

with upward concentrations (70 90-100), 2 bath of Xylene and 2 baths of paraffin for 24 hours.

Samples were placed in templates known as Barres de Leukrat 5 micrometer segments are realized using a microtome Reiechert-2030Jung type.

The sections from the slides are labeled with a 0.2% gelatinous water strip. The slides were set on a heated plate and then dry at 70°C for 1 or 2 minutes.

Before coloring, the paraffin is removed by passing through two 10-minute Xylene bath, then two 10-minute ethanol baths

- The labyrinth of samples is restored on a water bath

Hematoxyline for 10 minutes.

- Wash with water.
- The slides immersion in the Eosin solution at 2 % concentration for 5 minutes.
- Wash again by water.
- Set the slides in a bathroom of alcohol for 1 minute.
- To outline the segment, place the slides in two pesticides bath for two minutes.
- Finally, the sections were placed between a slide and a jacket, examined under the microscope by magnifying (x10) and then (x40), and then taken directly.

7.3 Statistical study

The results obtained in figures and tables were presented as average ± standard deviation (± SD).

Statistical analysis of results was carried out by loading single-parameter variance AVI to compare all sub-groups with the control. Student t-test was also appliedby using 13-Minitab.

Chapter 03: Results

1. Total body weight

Results are shown in fig 15. Total body weight of Diflubenzuron DFB treated rabbits was significantly lower in the three doses ($p \leq 0.05$) after 2 weeks, while there was a significant decrease of totals body weightat 500mg /kg in the third weeks of treatment, whereas the highest dose caused significant decrease in the fourth weekscompared to the control.

Concerningthe result of glyphosate,there was significant decrease in the medium and the high doses compared to the control. In the second and the third weeks, total body weight was decreased with all doses in comparison to the control. Contrary, a considerable reduction of total body weight with the dose 400mg /kg in the fourth weeks was recorded.

In the groups treated with azoxystrobin, results indicated that no effect of on total body weight during the first and the second weeks, but there was a significant variation after the third week of treatment with the medium and the high doses. Within the past week, a significant decrease with doses 200mg/kg and 300mg/kg ($p \leq 0.05$) was recorded, while the highest dose caused a significant reduction (fig 16).

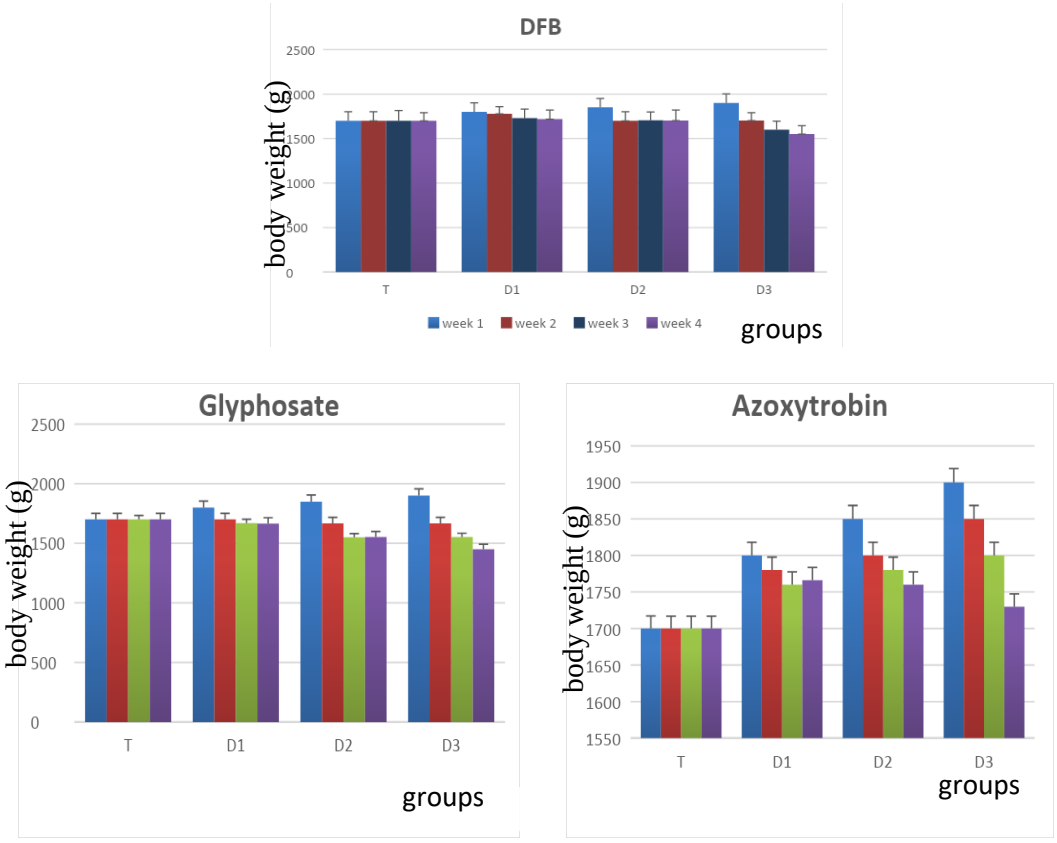


Figure 16: *Variation of total body weight during the period of treatment of male rabbits ($\bar{X}$ ±SD, n = 5).*

2. Reproductive parameters

2. 1 Sperm Concentration

The effects of Diflubenzuron on sperms concentration of the male rabbits are presented in figure (17).

Results showed a significant reduction in sperm count of rabbits exposed to three different doses (250mg/kg, 500mg /kg and 750mg/kg when compared to control, in which sperms counts were decreased with the increasing dose.

Similarly, results of glyphosate indicated a significant decrease in sperm concentration in the three doses when compared to the control group, in which sperms counts were reduced with the increasing dose.

Concerning azoxystrobin, results indicated no significant variation in sperms concentration of male rabbits at low dose (100mg/kg), but there was a significant decrease in medium and high dose compared to the control.

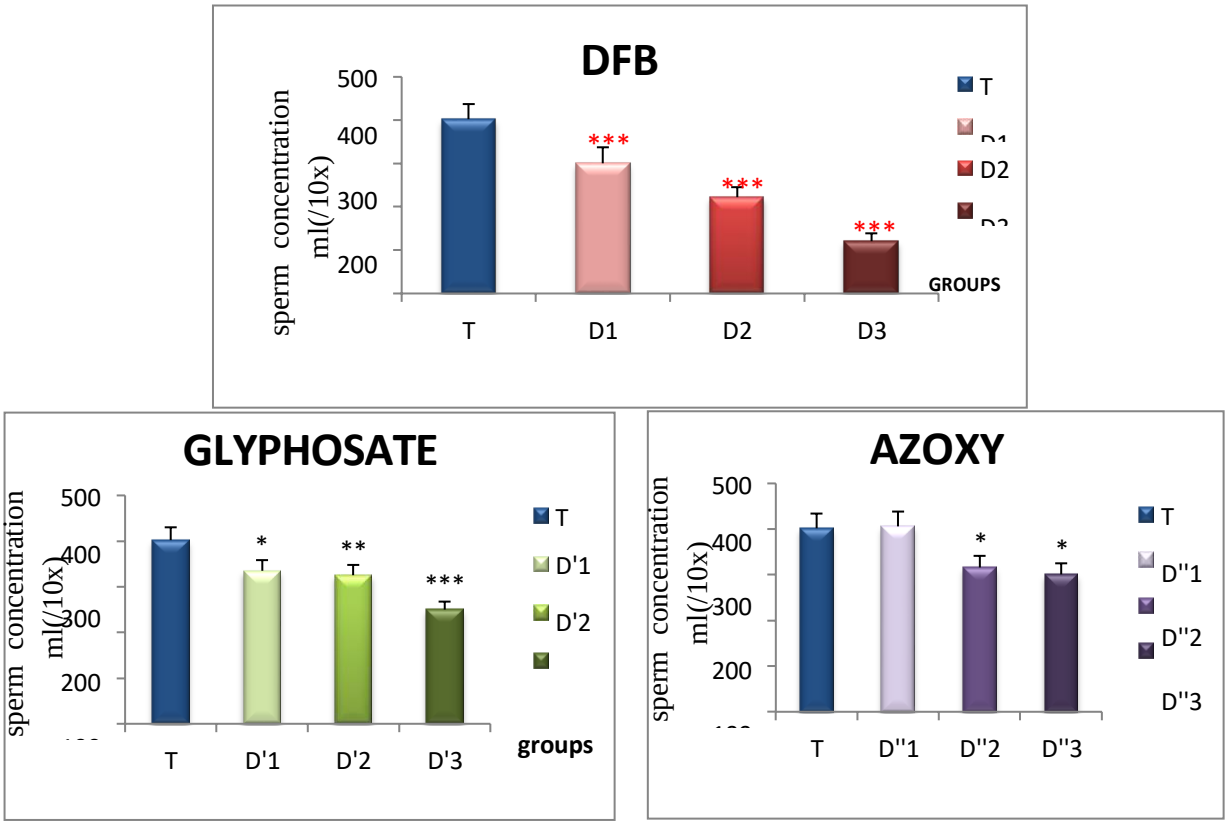


Figure 17:*Effect of pesticides on sperms counts (x106/ml) in male rabbits ($\bar{X}$±SD, n = 5)*

2. 2 Sperms motility

Resultsindicated that sperm motility was decreased in groups receiving DFB at various concentrations (250 mg/kg, 500 mg/kg and 750 mg/kg) compared to the control (Fig 17).

Concerning the progressive sperm motility, glyphosate has decreased it with increasing doses compared to the control (Fig 18).

Azoxystrobin had a significant decrease on sperm motility at all doses after one month of treatment compared to control.

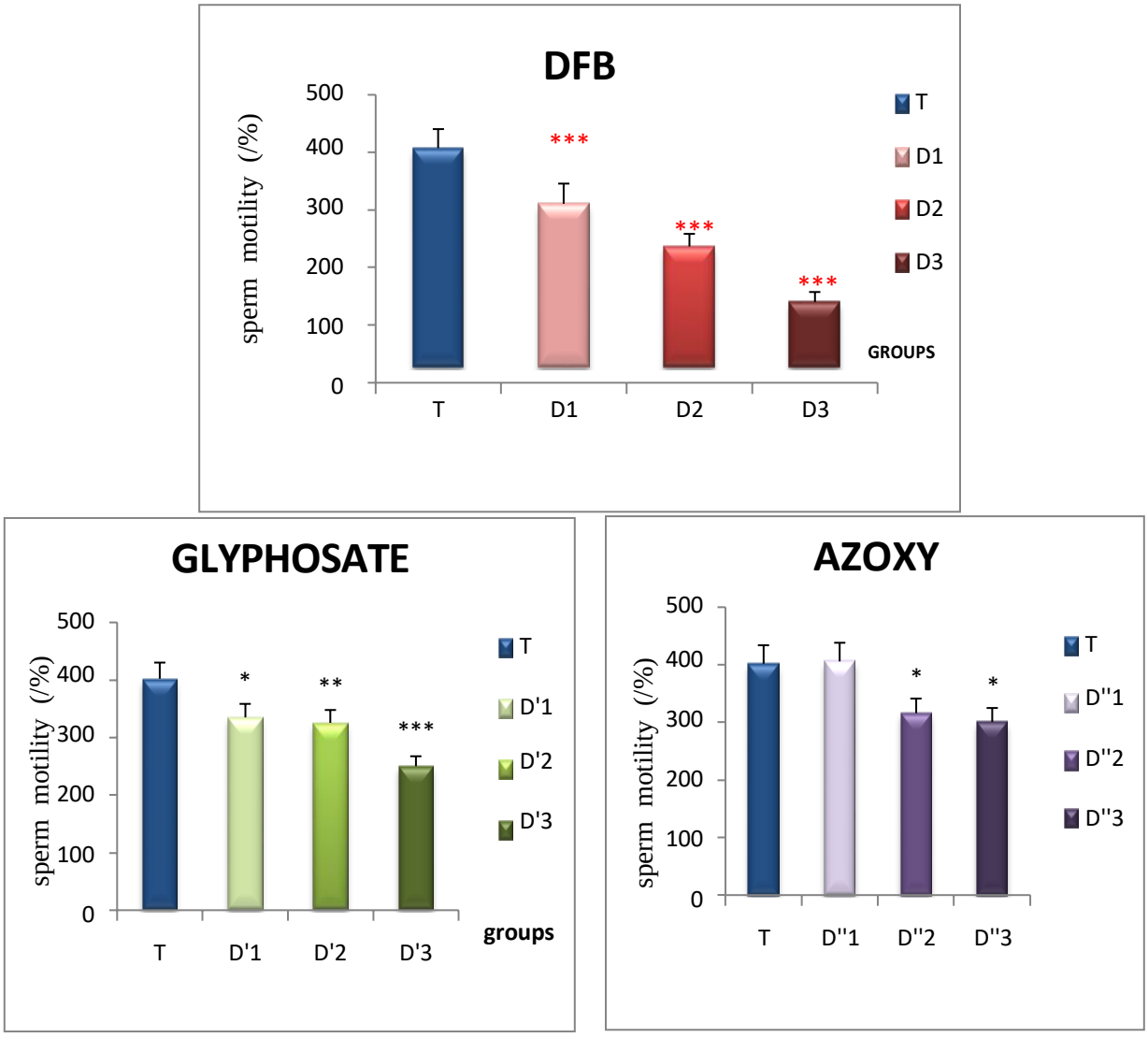


***Figure 18**:Effect of pesticides on sperms motility (%) of male rabbits ($\bar{X}$±SD, n = 5).*

2. 3 **Sperm speed**

Results reported in figure 19 showed that sperms speed was decreased in groups receiving DFB at all doses (250 mg/kg, 500 mg/kg and 750 mg/kg).

In groups treated of glyphosate, results indicated a significant decrease in sperm speed with the three doses.

Azoxystrobin had no significant effect at low dose, but the other doses had caused a significant reduction after one month of treatment (Fig 19).

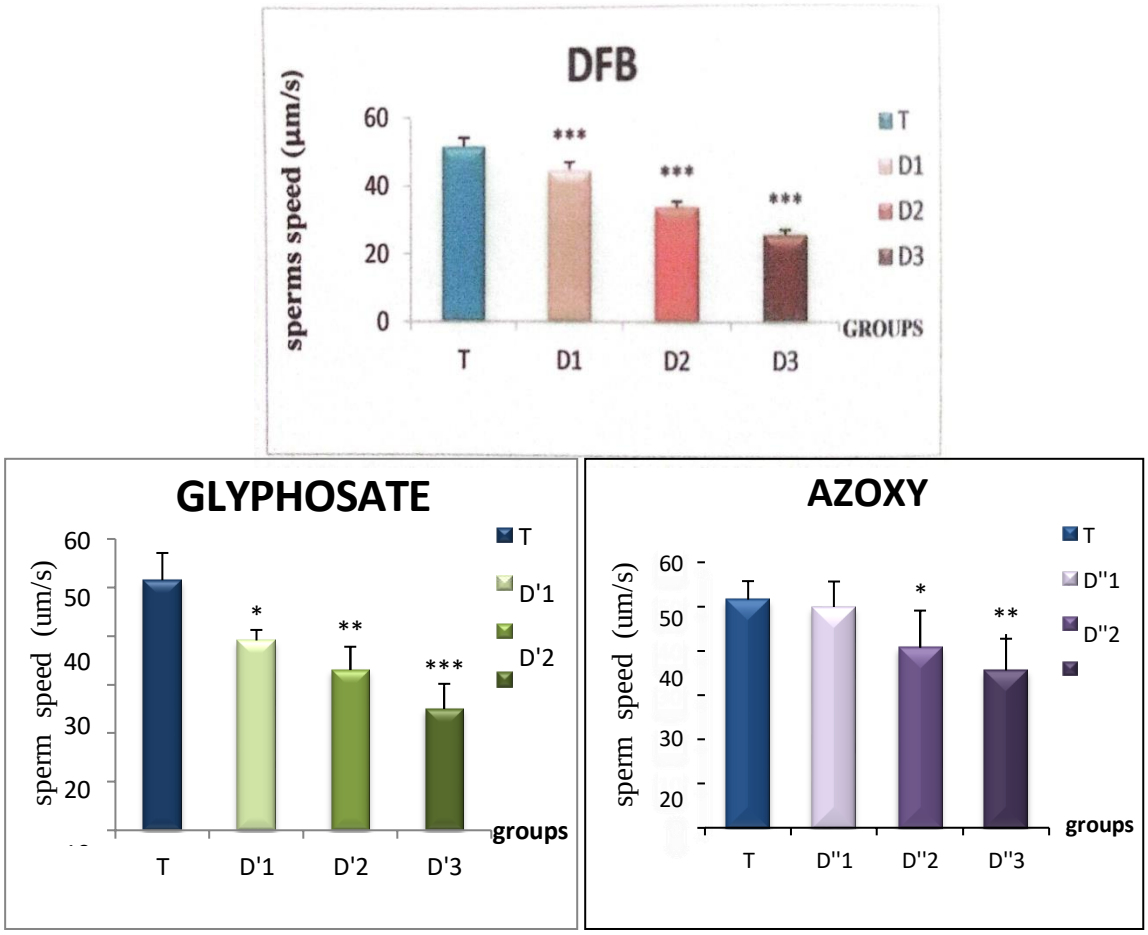


Figure 19:*Effect of pesticides on sperms speed (µm/s) of male rabbits ($\bar{X}$±SD, n = 5).*

2. 4 Sperm viability

The percentage of alive sperms decreased significantly in all three groups treated with the three doses of diflubenzuron (Fig 20).

In the group of glyphosate, the percentage of alive sperms reported a significant decrease with increasing the dose of pesticide during the whole period of the experiment in comparison with the control (Fig 20).

The percentage of sperm viability was decreased significantly in the rabbits received azoxystrobin with medium and high dose only (Fig 20).

Concerning dead sperms, diflubenzuron has raised the percentage in all treated groups.

In animals treated with glyphosate, it was observed a significant increase in dead sperms with increasing the doses compared to the control (Fig 20).

For azoxystribin treatment, results indicated a significant increase in the second and the third group only.

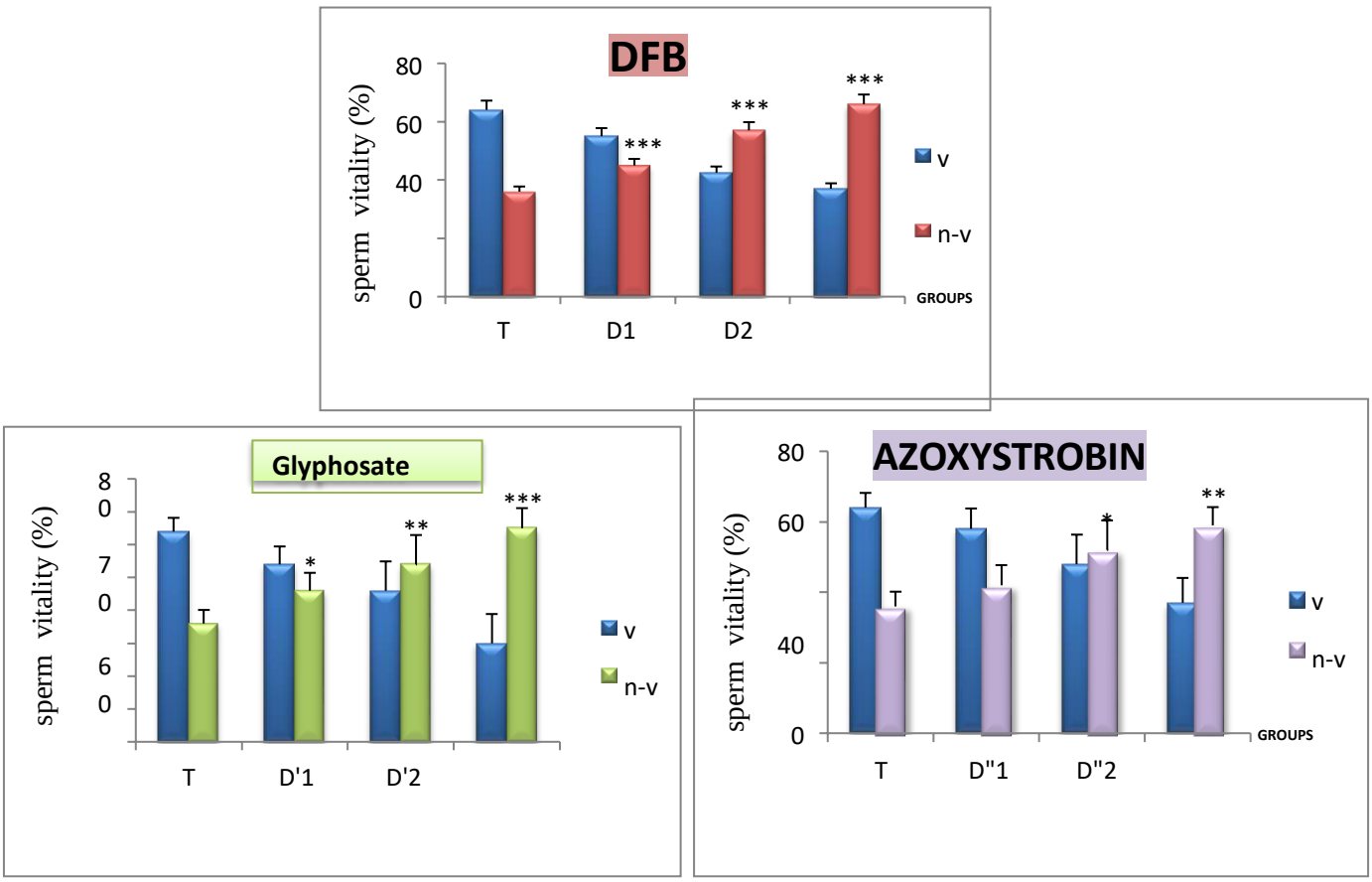


Figure 20: Effect of pesticides on sperm viability (%) of male rabbits ($\bar{X}$±SD, n = 5).

2. 5 Sperm morphology

DBF treatment has showed significant increase in sperm morphology at all doses 250, 500, 750mg/ kg compared to the control (Table 4).

Table 4: Effect of DFB (250 mg /kg, 500 and 750 mg /kg) on sperms morphology on male rabbits

Parameter	A	B	C	N	V
T	18±1.22	21±1.23	22±1.64	38±3.13	62±2.28
D1	15±2.45***	19±3.56**	18±4.56***	55±1.45***	43±2.34***
D2	13±1.22***	18±2.14***	16±3.21***	82±3.65***	25±1.45***
D3	12±1.54***	17±3.21***	15±2.34***	95±2.45***	15±1.34***

(M±SD, N=5) *p≤0.5; **p≤0.01;***p≤0.00

N: absence of formal change; A: little change in flagellum level; B: a significant change in the flagellum level; C: a significant change in flagellum level.

The effect of glyphosate on sperm morphology of male rabbits after 4 weeks of treatmenthas indicated a significant increase at300 and 400 mg/kg (Table 5).

Table 5: Effect of glyphosate (200 mg /kg, 300 and 400 mg /kg) on sperms morphology of male rabbits.

Parameter	A	B	C	N	V
T	18±1.22	20±1.23	22±1.64	38±3.13	57±2.28
D'1	17±1.45	18±2.56*	20±3.43*	66±3.52***	45±2.98***
D'2	14±2.65**	16±3.22**	18±2.13***	75±2.46***	43±3.65***
D'3	12±2.76**	13±3.12***	16±2.54***	82±1.34***	38±0.45***

(M±SD, N=5) *p≤0.05; **p≤0.01;***p≤0.001

N: absence of formal change; A: little change in flagellum level; B: a significant change in the flagellum level; C: a significant change in flagellum level.

As can be seen in the table 6, azoxystrobin have reduced the number of normal sperms in comparison to the control category. Generally, the findings revealed a significant increase in abnormal sperms morphology.

Table 6: Effect of azoxystrobin (100 mg /kg, 200 and 300 mg /kg) on sperms morphology of male domestic rabbits.

parameter	A	B	C	N	V
T	18±1.22	20±1.23	22±1.64	25±3.13	33±2.28
D''1	17±1.45*	19±3.21	20±3.12	66±2.31***	55±1.76**
D''2	15±3.22**	17±2.13*	19±4.21**	75±3.65***	52±3.10***
D''3	13±2.34***	14±3.12**	17±2.34**	82±4.76***	47±2.34***

(M±SD, N=5) *p≤0.05; **p≤0.01;***p≤0.001.

N: absence of formal change; A: little change in flagellum level; B: a significant change in the flagellum level; C: a significant change in flagellum level.

2. 6 **Testosterone**

A significant decrease in plasma testosterone concentration of rabbits orally given DFB at dose at 250mg/kg, 500mg/kg, and 750 mg/kg after one month treatment was found in comparison to the control.

Concerning the results of glyphosate, the level of testosterone was significantly reduced at the doses of (200mg /kg), 300mg/kg and 400mg/kg compared to the control.

Similarly, results revealed an important reduction in testosterone level with the increasing doses of azoxystrobin (Fig 21).

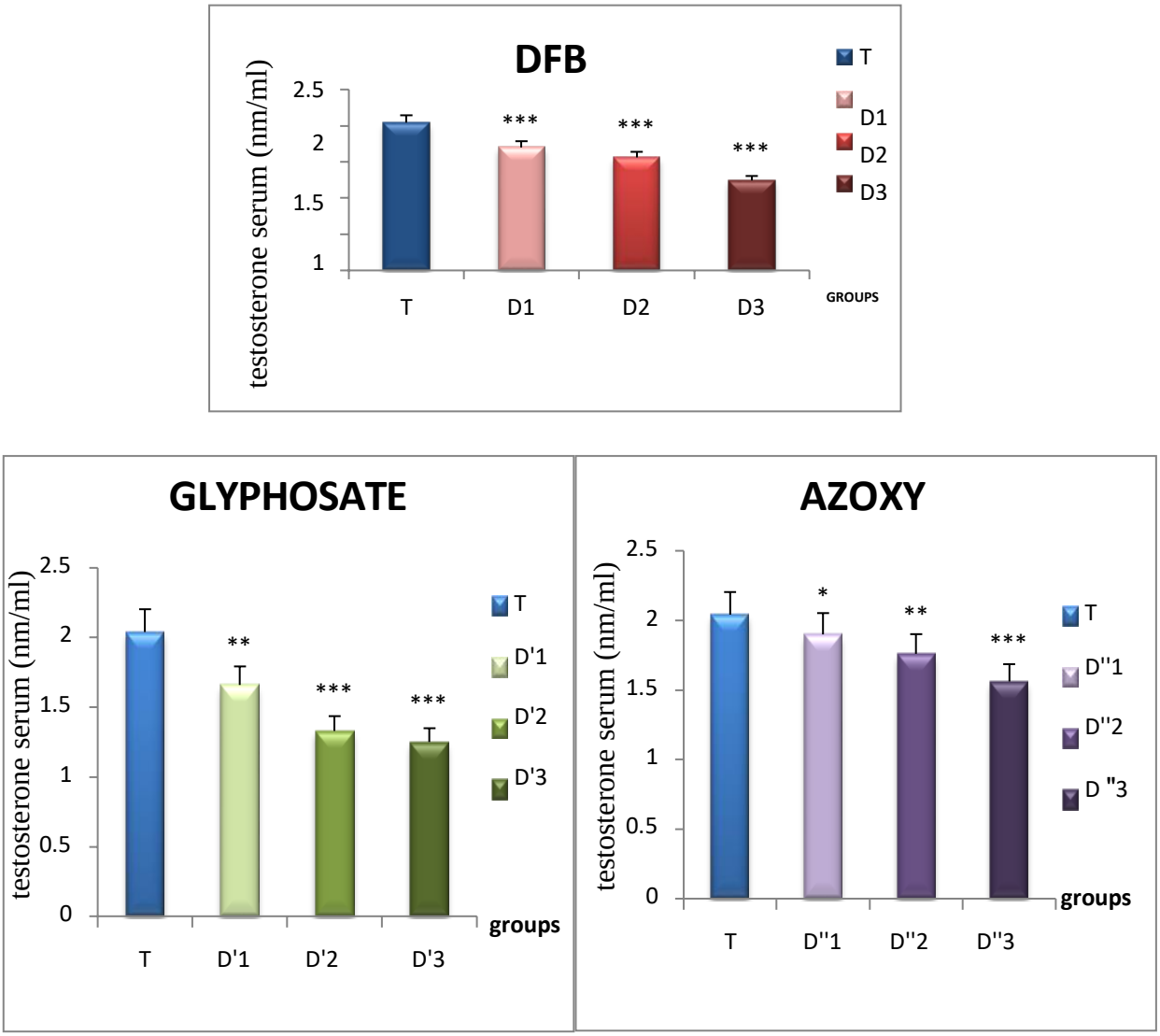


Figure 21: *Effect of pesticides on Testosterone plasma on male rabbits ($\bar{X}$±SD, n = 5).*

3. Biochemical parameters

3.1 Cholesterol

A significant increase in plasma cholesterol level at low dose (250mg/kg) was noticed, with a significant increase in medium and high dose of rabbits treated with DFB (Fig 22).

Glyphosate showed an increase decrease in the second (300mg/kg) as well as in the third group (Fig 22).

On the other hand, a significant decrease increase of cholesterol level in azoxystrobin treated rabbits at medium (200mg/kg) and high dose (300mg/kg) was recorded compared to the control.

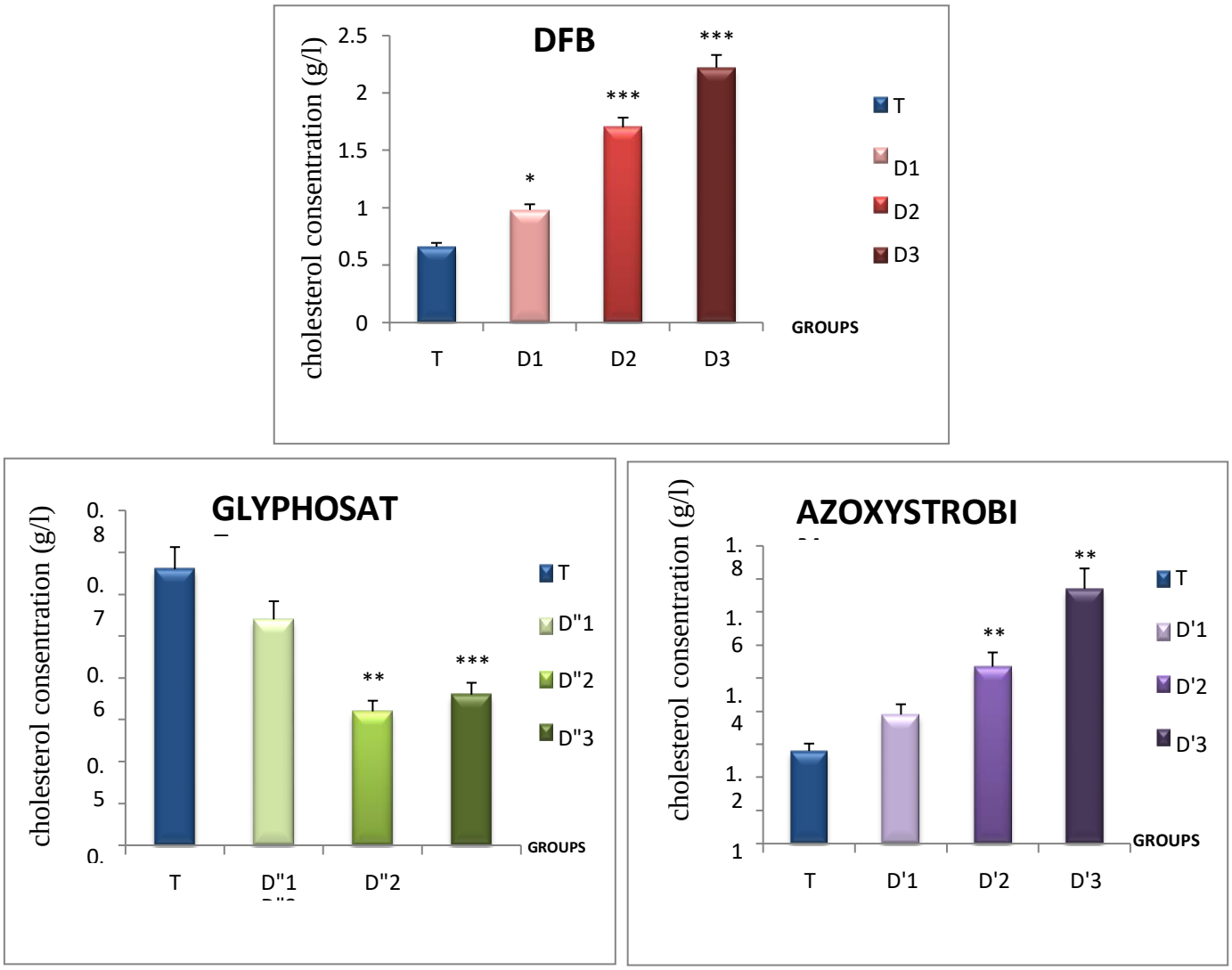


Figure 22:*Effect of pesticides on Cholesterol concentration of male rabbits ($\bar{X}$±SD, n = 5).*

3.2 Triglycerides

The present results showed an important rise in plasma triglycerides' level at low (250mg/kg), medium (500mg/kg) and high dose (750mg/kg) in group treated with DFB (fig 23).

In the group receiving glyphosate, a significant decrease at 200 mg/kg, 300mg/kg and 400mg/kg was observed compared to the control.

Concerning the group of rabbits treated with azoxystrobin, results indicated a significant decrease in triglycerides concentration at all doses (fig 23)

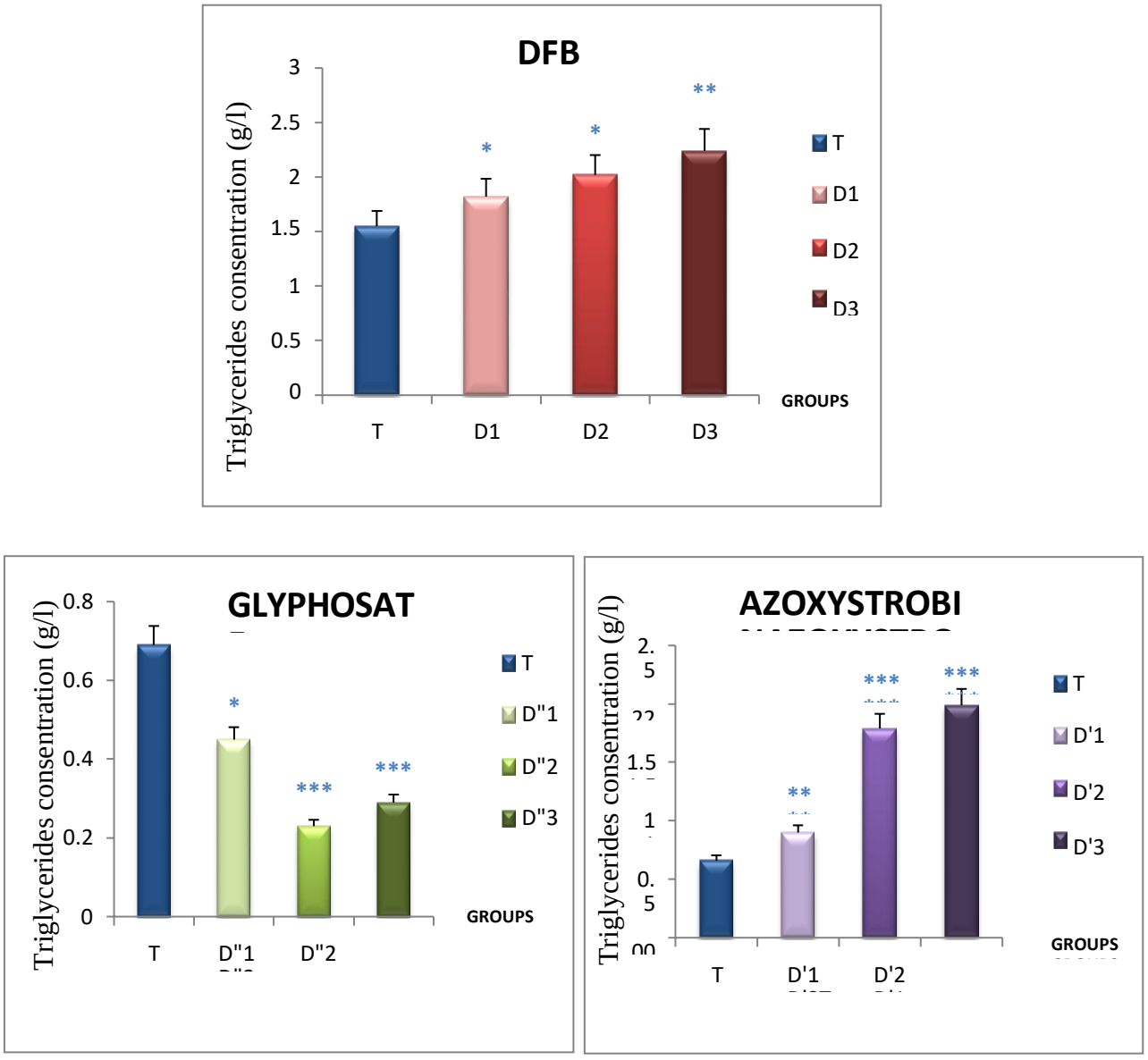


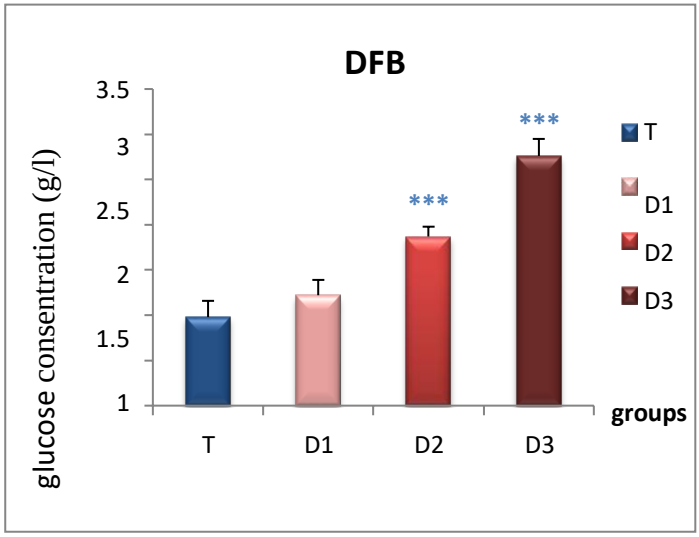
Figure 23:*Effect of pesticides on Triglycerides' concentration of male rabbits ($\bar{X}$±SD, n = 5).*

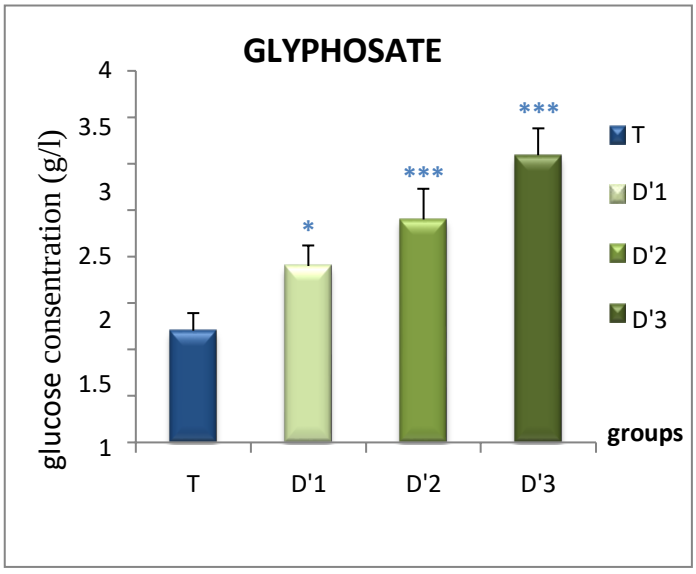
3.3 Glucose

Results recorded a significant increase in plasma glucose concentration for the medium (500mg/kg) and the high dose (750mg/kg) compared to the control (fig 24).

For glyphosate, results showed a significant increase in glucose concentration of treated rabbits at low dose (200mg/kg), as well as at medium (300mg/kg) and high dose (400mg/kg) (fig 24).

Concerning the group treated with azoxystrobin, a significant decrease in glucose level after 4 weeks treatment of male rabbits at medium, and high dose compared to the control (fig 24).





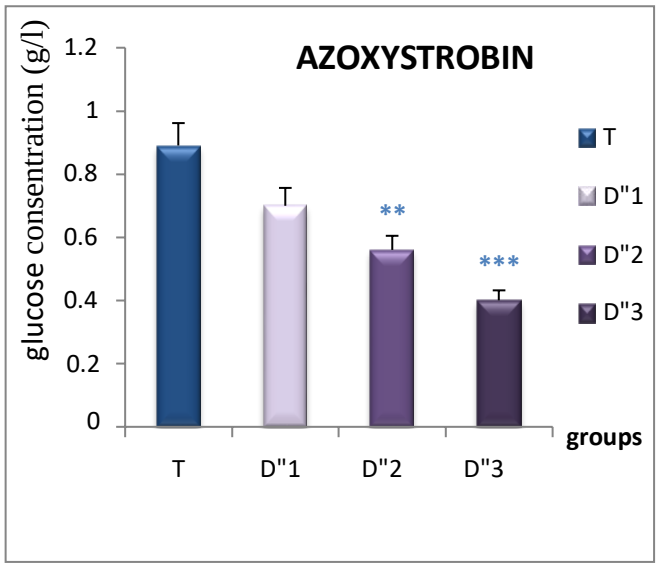


Figure 24:*Effect of pesticides on glucose concentration in male rabbits ($\bar{X}$±SD, n = 5).*

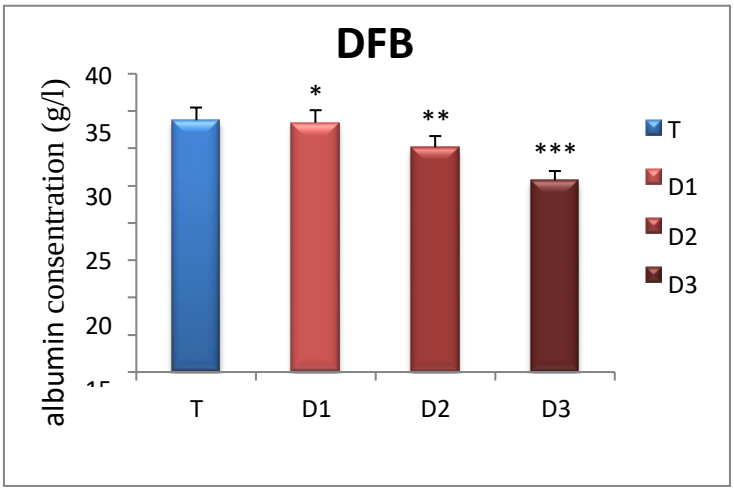
3. 4 **Albumin**

The mean concentration of plasma albumin in control and pesticides-treated male domestic rabbits was showed in (fig 25).

DDFB intake resulted in progressive significant decrease in albumin concentration with increasing doses.

For Glyphosate, the significant decrease of albumin concentration was only observed in the medium and the high doses compared to control.

For azoxystrobin, results indicated a significant decrease of albumin level in male rabbits exposed to the high dose only (300mg/kg).



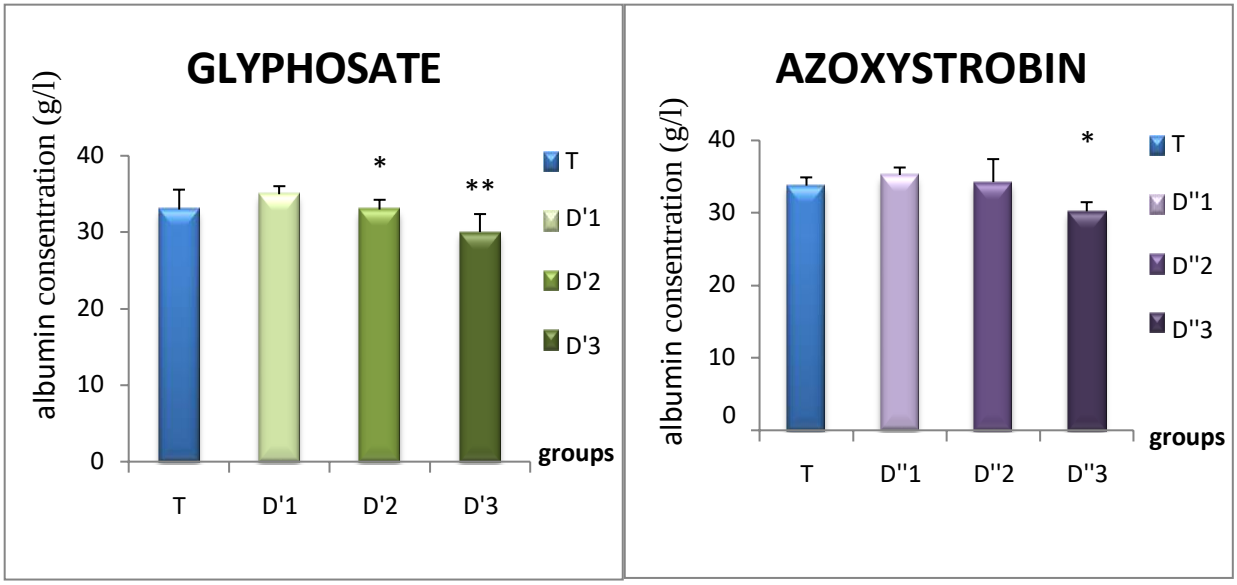


Figure 25:*Effect of pesticides on albumin concentration (g/l) of male rabbits ($\bar{X}$ ±SD, n = 5).*

3.5 Creatinine

The experimental trials for oral doses of Diflubenzuron after 4 weeks of administration in male domestic rabbits revealed that creatinine concentration significantly increased at 250,500 and 750 mg/kg ($p \leq 0.001$) compared to the control (Fig 26).

Glyphosate has significantly increased albumin concentration at the medium (200mg/kg at $p \leq 0.01$) and the high dose (400mg/kg).

Concerning the oral administration of Azoxystrobin, it caused a significant increase in creatinine concentration after administrating the three doses compared to the control (Fig 26).

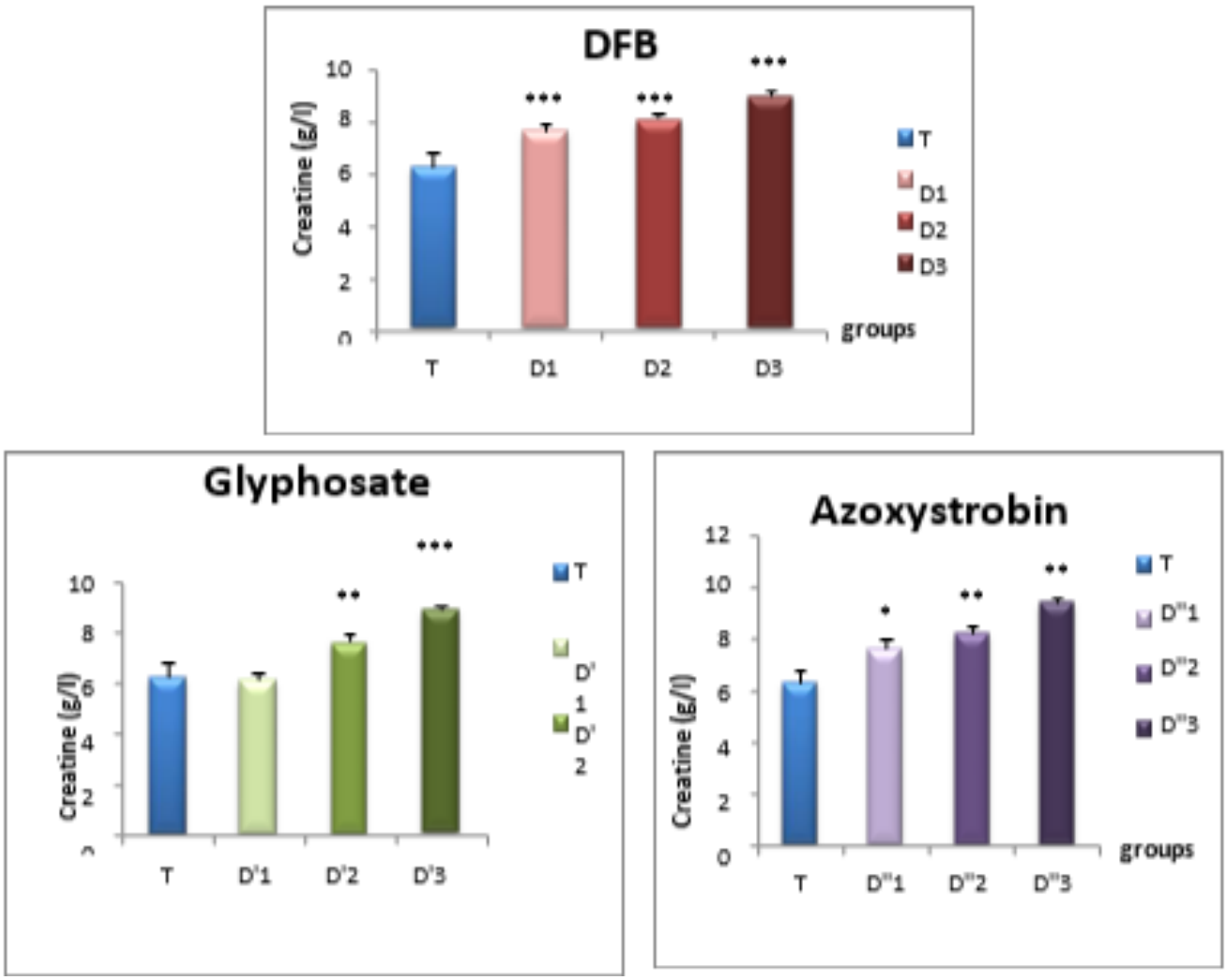


Figure 26:*Effect of pesticides on creatinine level (g/l) of male rabbits ($\bar{X}$ ±SD, n = 5).*

3. 6 Urea

Plasma urea concentration has increased significantly when male rabbits treated with the 3 doses (250, 500 and 750mg/kg) of DFB compared to the control (Fig 27).

For glyphosate treated rabbits, a significant increase of urea was only observedat 300 mg/kg and 400 mg/kg(Fig 27).

Results of serum urea concentration in the groups received azoxystrobin was significant increased when using the three doses compared to the control (Fig 27).

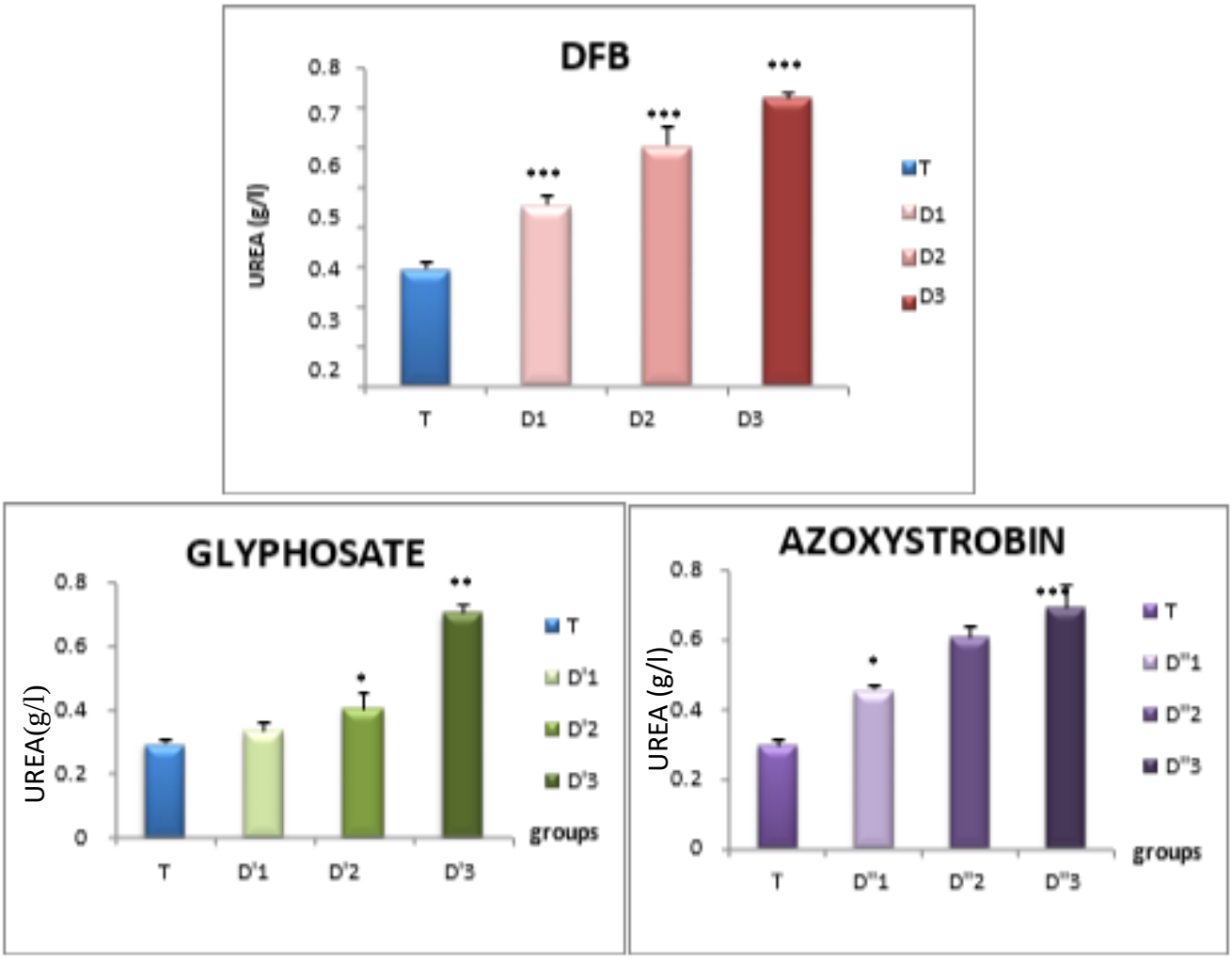


Figure 27: *Effect of pesticides on Urea concentration (g/l) of male rabbits ($\bar{X}$ ±SD, n = 5).*

4. Blood components

4.1. Red blood cells

Results presented in figure 28 indicated that DFB decreased RBC count significantly. The RBC count in rabbits treated with glyphosate showed also significant decrease with increasing dose (Fig 28).

In the groups receiving Azoxystrobin, a significant decrease of RBC was seen at 200 and 300mg/kg and at 300 mg/kg compared to the control.

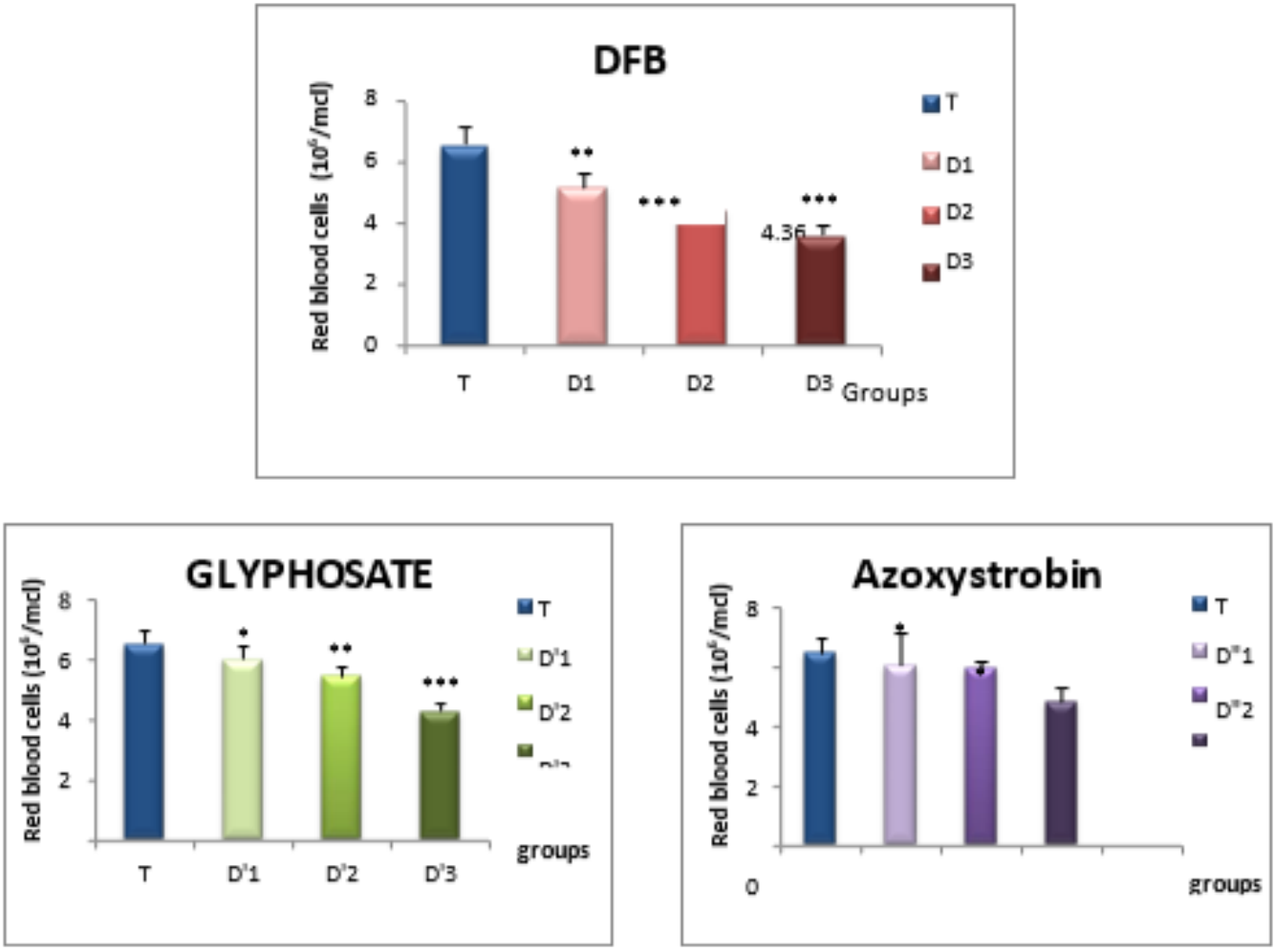


Figure 28:*Effect of pesticides on red blood cell counts (x106⁄mcl) of male rabbits ($\bar{X}$±SD, n = 5).*

4.2. Hemoglobin

Hemoglobin concentration of the treated rabbits with the three different doses of DFB was significantly decreased compared to the control.

Results in fig 29 showed a significant decrease of Hb of male rabbits exposed to all doses of glyphosate compared to control.

The same results were observed in the groups received Azoxystrobin (fig 29).

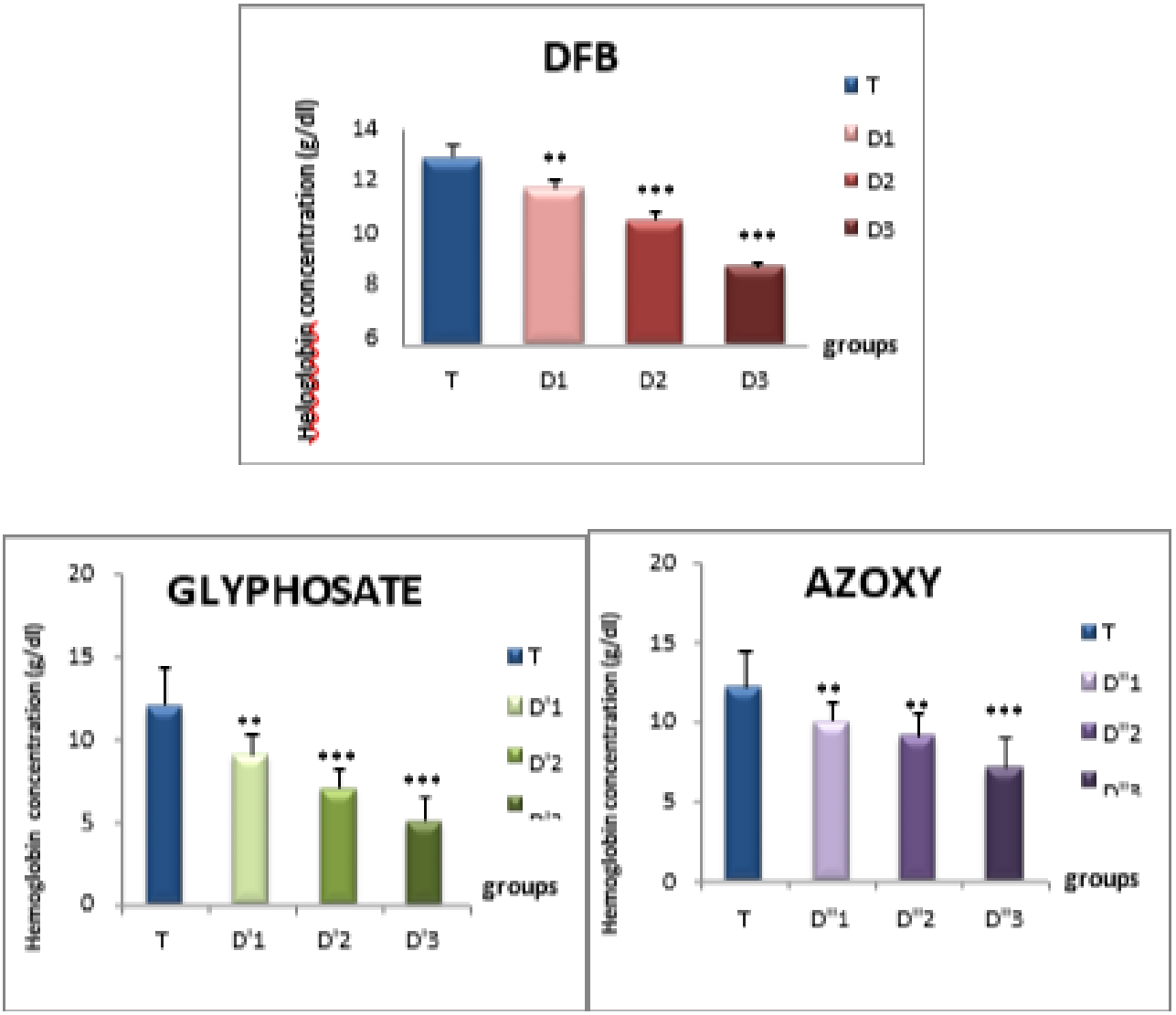


Figure 29:*Effect of pesticides on Hb concentration (g/dl) of male rabbits ($\bar{X}$±SD, n = 5).*

4.3. White blood cells

There was a significant elevation in WBC count in rabbits supplied with the highest dose of DFB and glyphosate compared to the control (Fig 30).

Concerning the group of rabbits treated with Azoxystrobin, the findings revealed an important rise of WBC counts ($p \leq 0.05$) through the medium (200mg/kg) and the high dose only (fig 30).

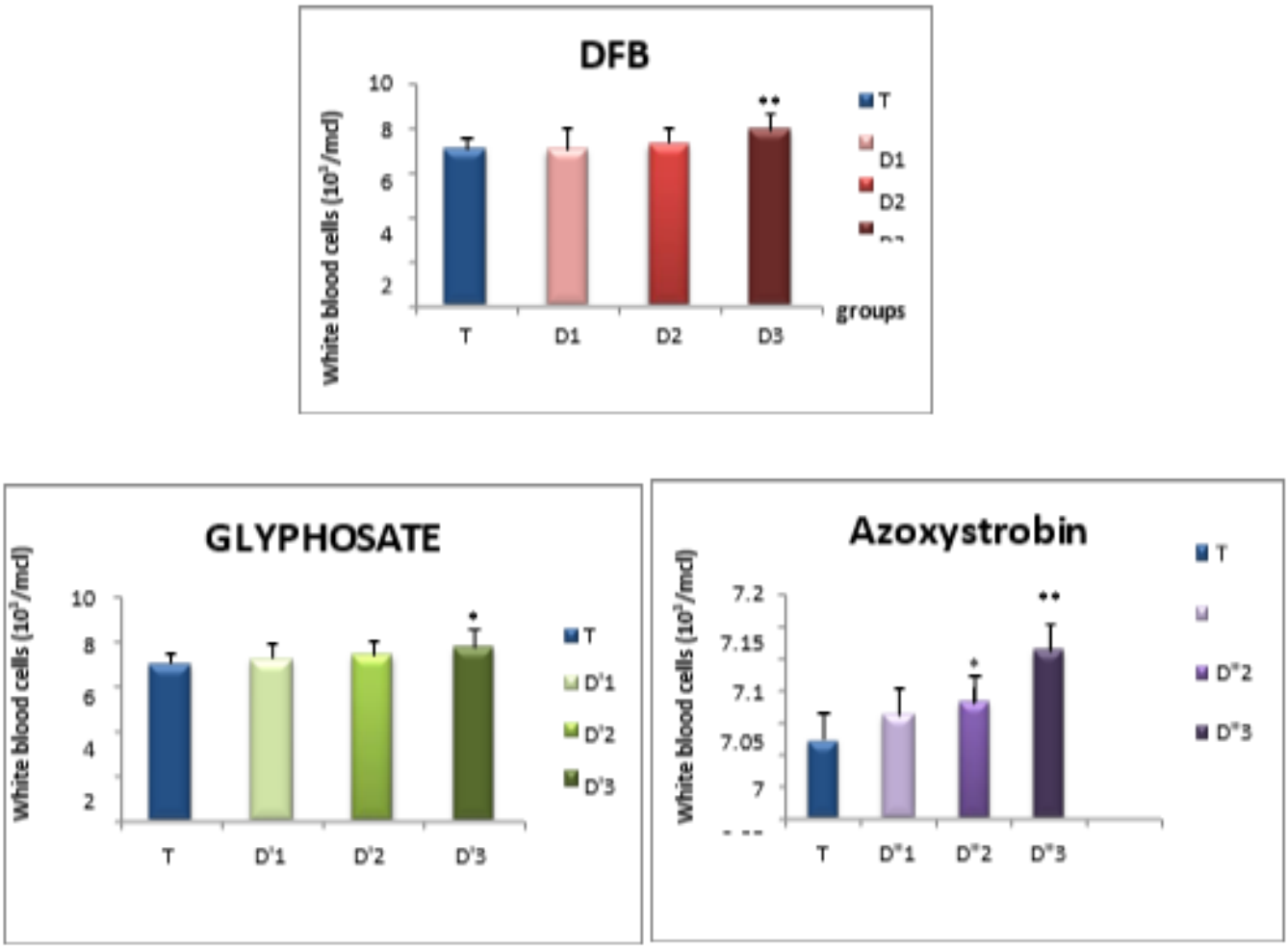


Figure 30: *Effect of pesticides on WBC (x103/mcl) of male rabbits ($\bar{X}$±SD, n = 5).*

5. Stress oxydatif parameters

5. 1. Liver Glutathione

Glutathione levels of male rabbits treated with DFB were presented in Fig 31. The results revealed a significant decrease of glutathione level concerning low, medium and high doses after four-week treatment.

Findings revealed considerable reduction of glutathione levels in rabbits given glyphosate and azoxystrobin at only the medium and the high doses compared to the control (fig 31).

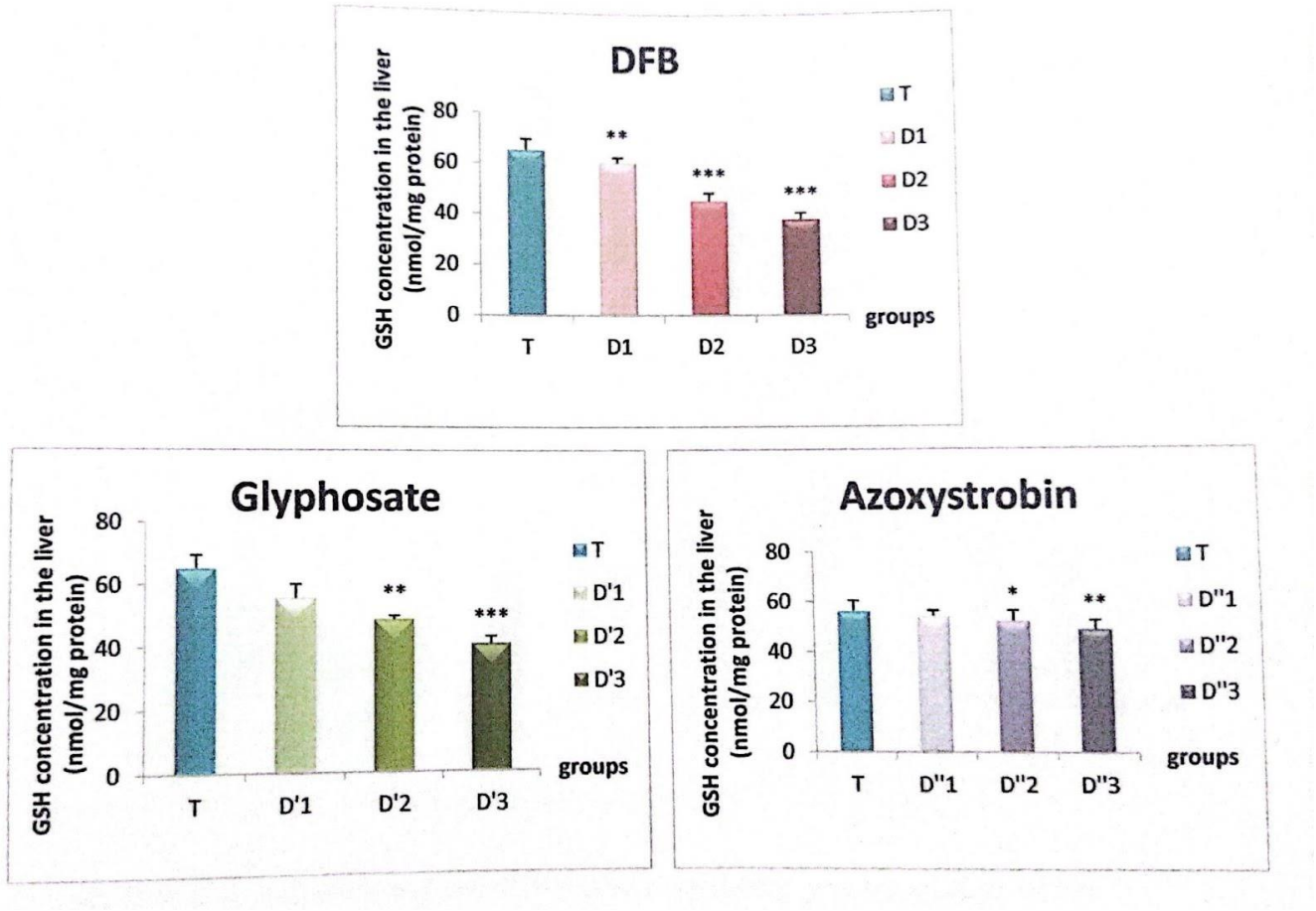


Figure 31: *Effect of pesticides on hepatic GSH (nmol/mg protein) in male rabbits ($\bar{X}$±SD, n =5).*

5.2. Testicular Glutathione

In the Diflubenzuron treated rabbits, the value of testicular GSH showed a significant elevation for all doses (250mg/kg, 500mg/kg and 750mg/gk) after one month exposure (Fig 32).

In the groups treated with glyhosate, a significant decreases at the 3 doses (200, 300 and 400mg/kg) was observed compared to control group.

Concerning azoxystrobin, treated rabbits had a significant decrease of GSH level with increasing doses (fig 32).

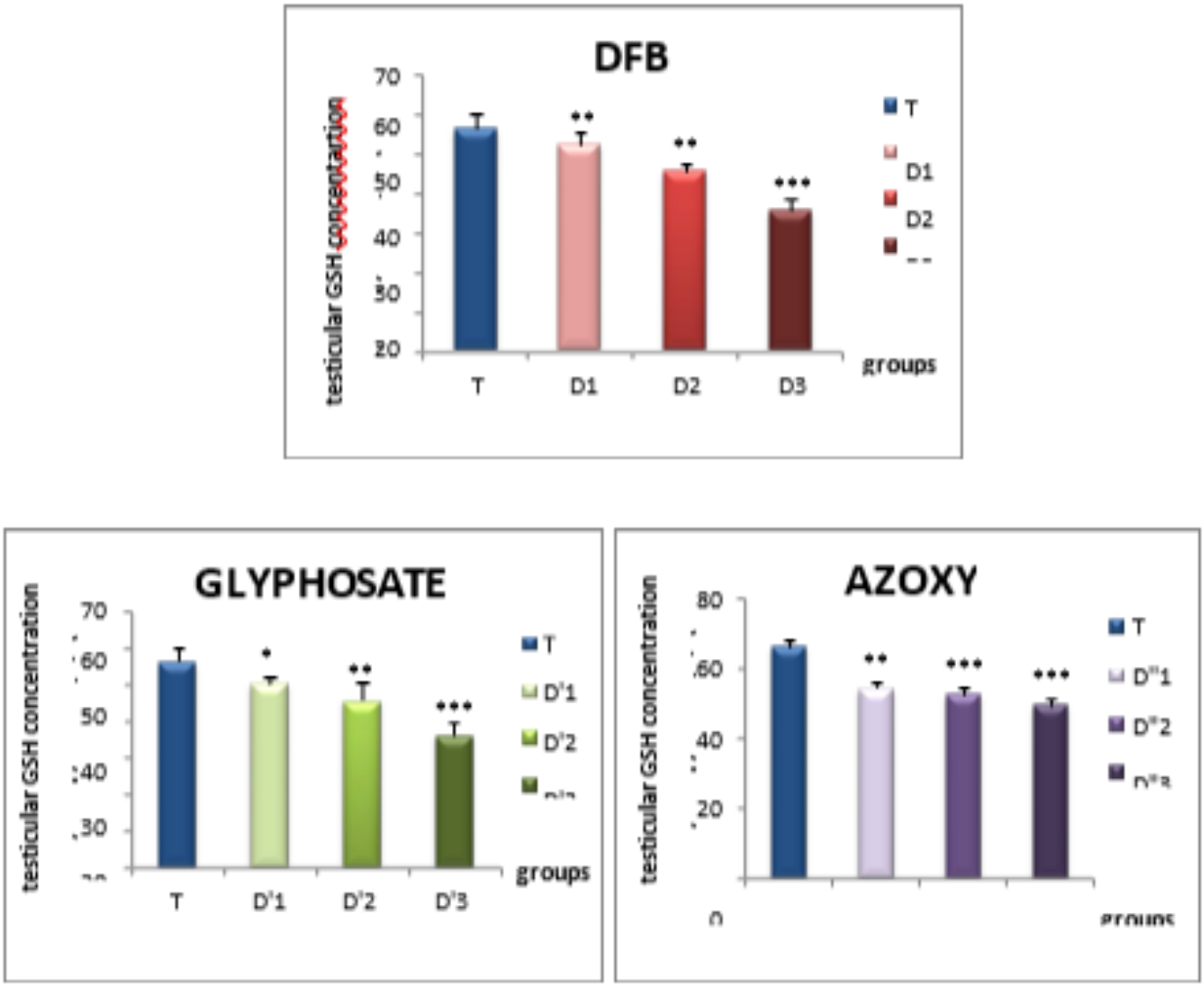


Figure 32:*Effect of pesticides on testicular GSH (nmol/mgprotein) of male rabbits* ($\bar{X}$ *±SD, n = 5).*

6. Histological study

6.1. Testicules

Compared to control group (T), which all tissue components are normal, the histopathological examination of testicular rabbits treated with DFB showed fission in seminiferous tubules, and separation of germ cells from the basal membrane in low and middle doses (250, 500mg/kg bw), with the occurrence of abnormal intracellular space between seminiferous tubules in the higher dose of 750mg/kg bw (Fig 33).

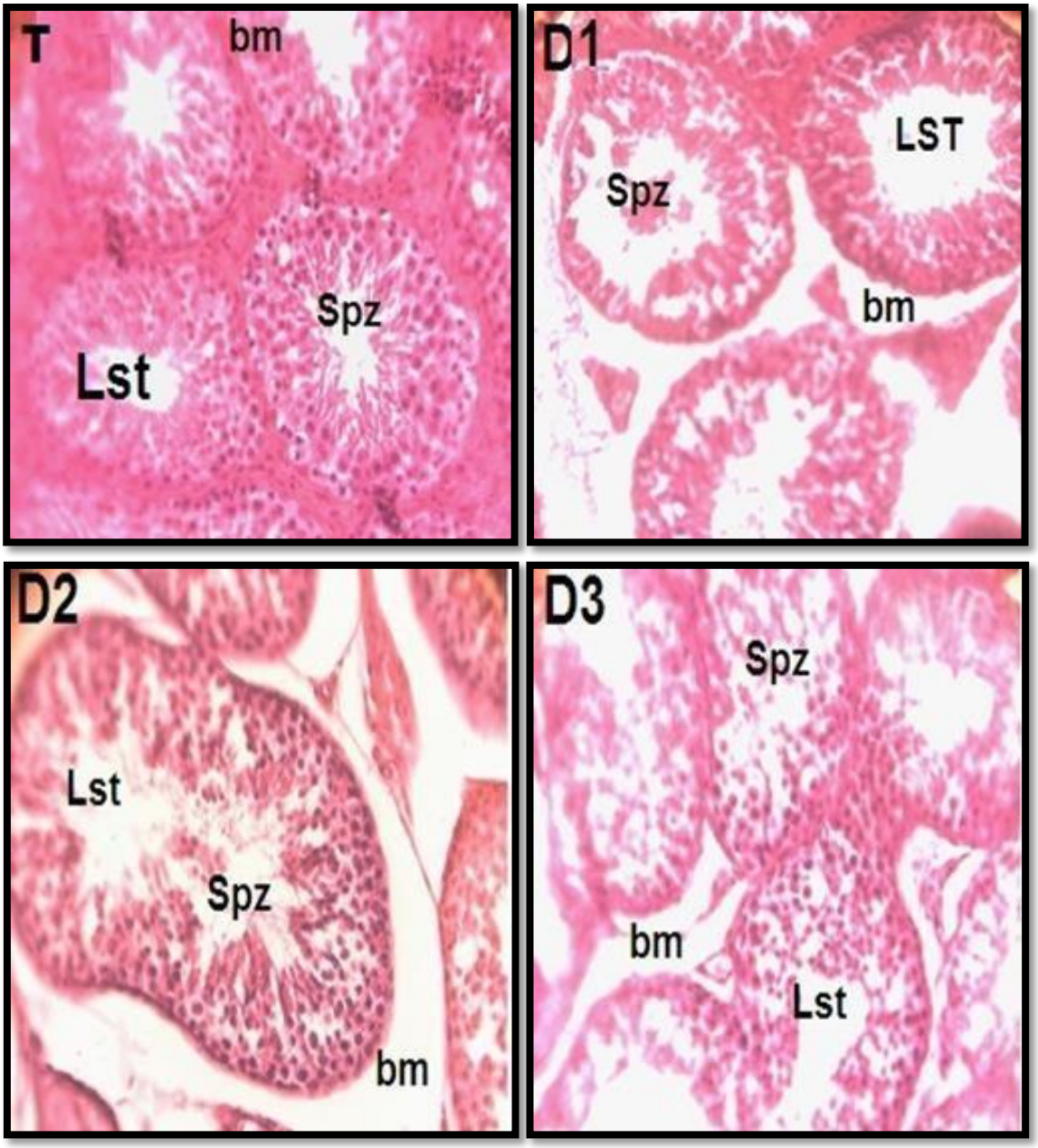
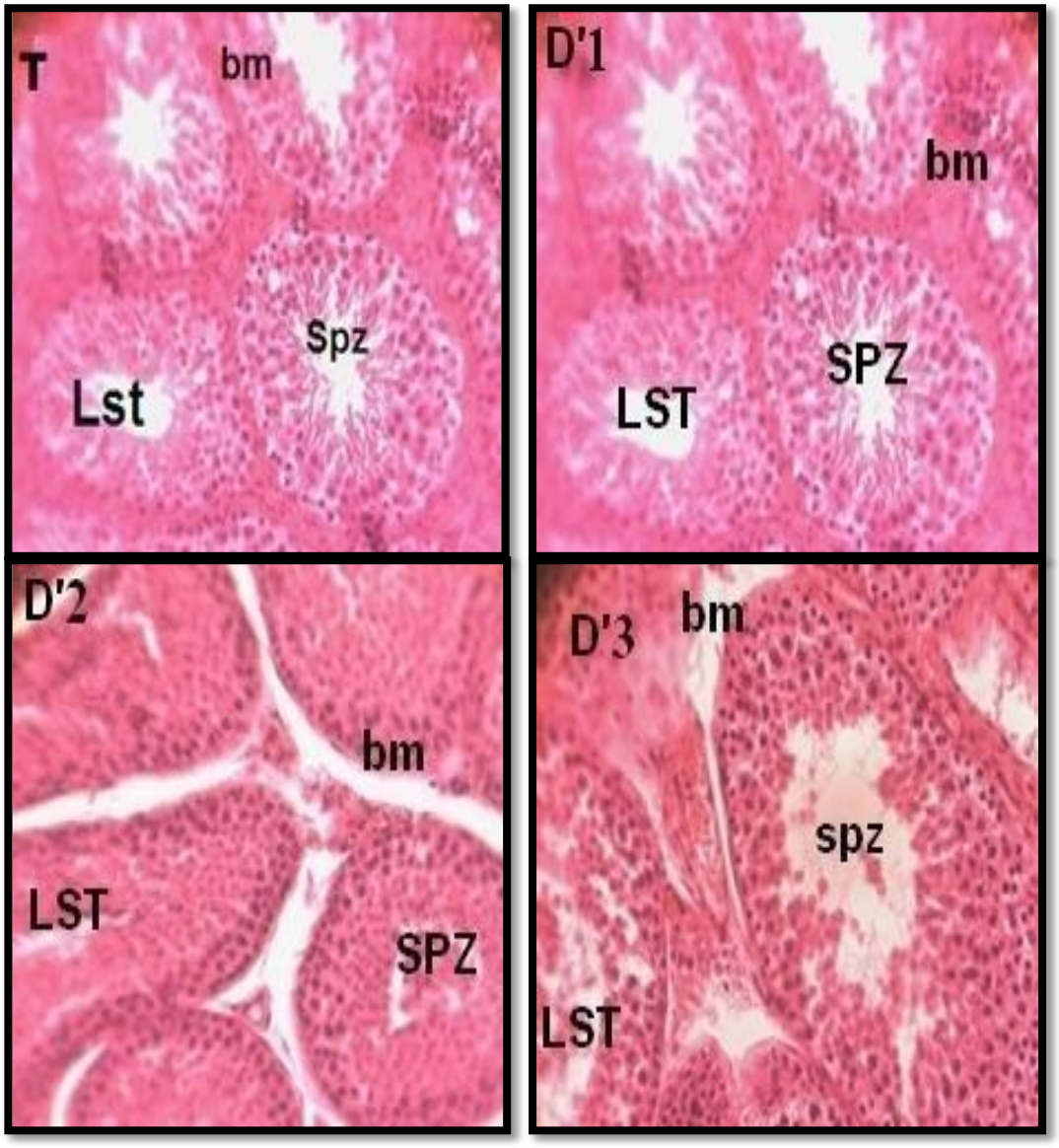


Figure 33: *Testicular histology of rabbits from the control and groups treated with DFB(x400).*

Spz*: permatozoa;* ***Lst****: lumenofseminiferoustubules;* ***bm****: basalmembrane*

The histological architecture of rabbit testes have been modified by glyphosate at 200 and 300mg/kg bw, whereas that of 400 mg/Kg bw has widen the intracellular space between seminiferous tubules and made the lymen more empty of spermatozoids (Fig 34).



.

Figure 34: *Testicular histology of rabbits from control and groups treated with Glyphosate (x400).*

Spz*: permatozoa;* ***Lst****: lumen of seminiferous tubules;* ***bm****: basal membrane.*

The AZO fungicide at low and middle doses of 100 and 200 mg/kg bw has altered the testicular histology at the seminiferous tubules.The highest dose of 400 mg/Kg bw has made wide deteriorations on this reproductive organ by separating the the meniniferous tubules within the rabbit testes (Fig 35).

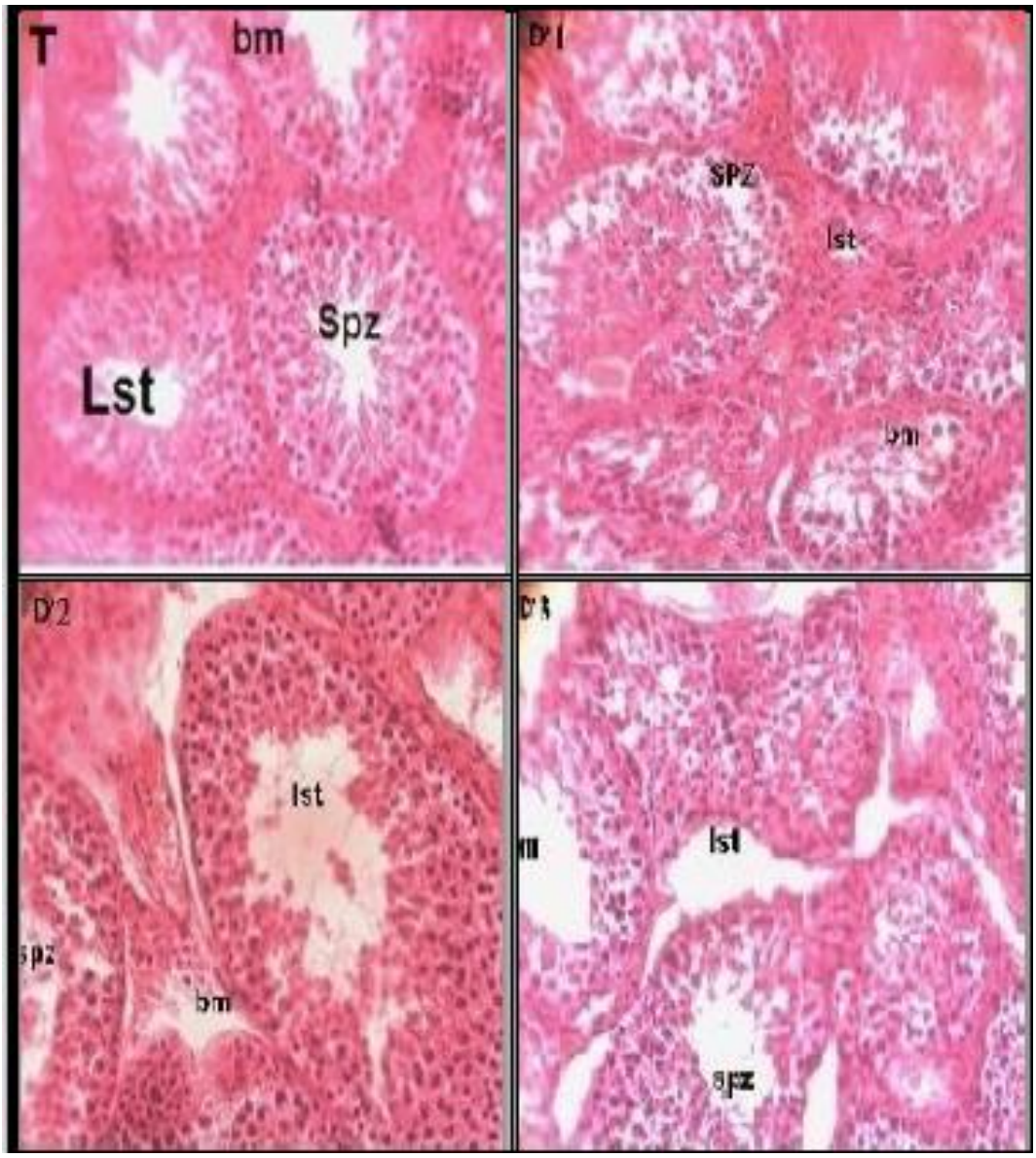


Figure 35: *Testicular histology of rabbits from control and groups treated with AZO (x400).*

*Spz: **Spz**: permatozoa; **Lst**: lumen ofseminiferous tubules; **bm**: basal membrane*

6.2. Liver

Histopathological examination of liver specimens taken from control group showed normal histological structure of the central vein and the surrounding hepatocytes of the parenchyma.

High dose of pesticides (750mg/kg of Diflubenzuron, 400mg/kg of glyphosate, and 300mg /kg of azoxistrobin) demonstrated the presence of congestion in portal blood vessel and portal infiltration with inflammatory cells. (fig36).

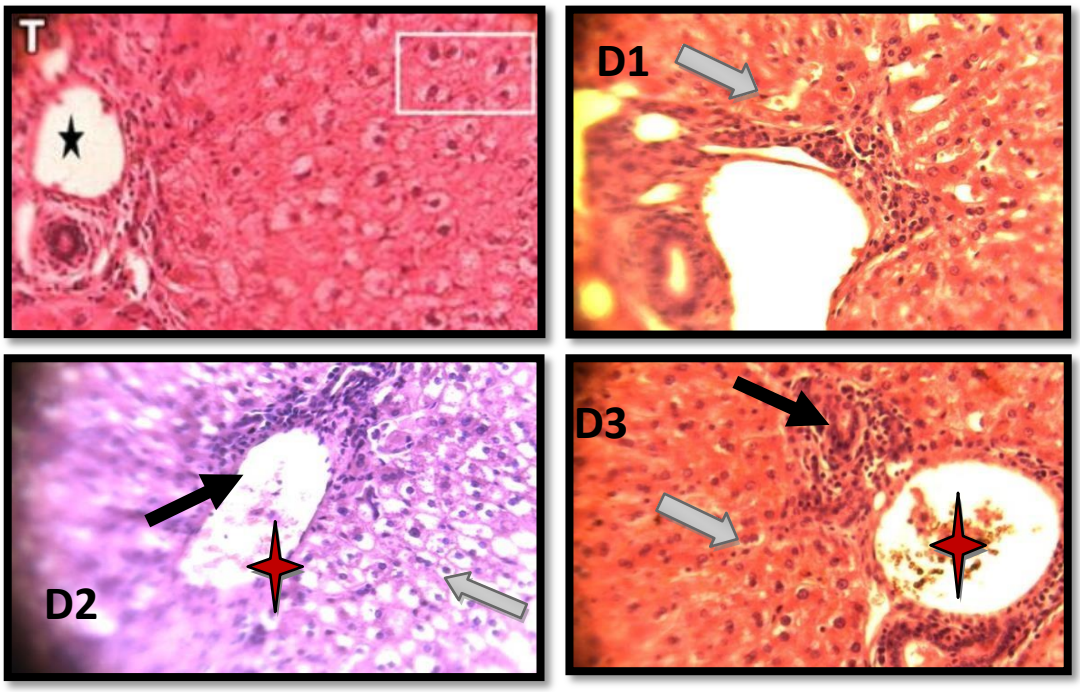
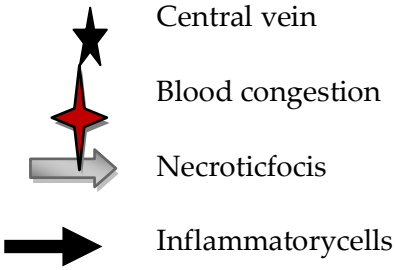


Figure 36: *Liver histology of male rabbits from the control and groups treated with DFB (x400).*



Central vein

Blood congestion

Necroticfocis

Inflammatorycells

The histological study revealed certain alterations in hepatic tissues demonstrated by congestion of portal blood with inflammation in three doses of glyphosate herbicide (200mg/kg, 300mg/kg, 400mg/kg) compared to the control group (fig 37).

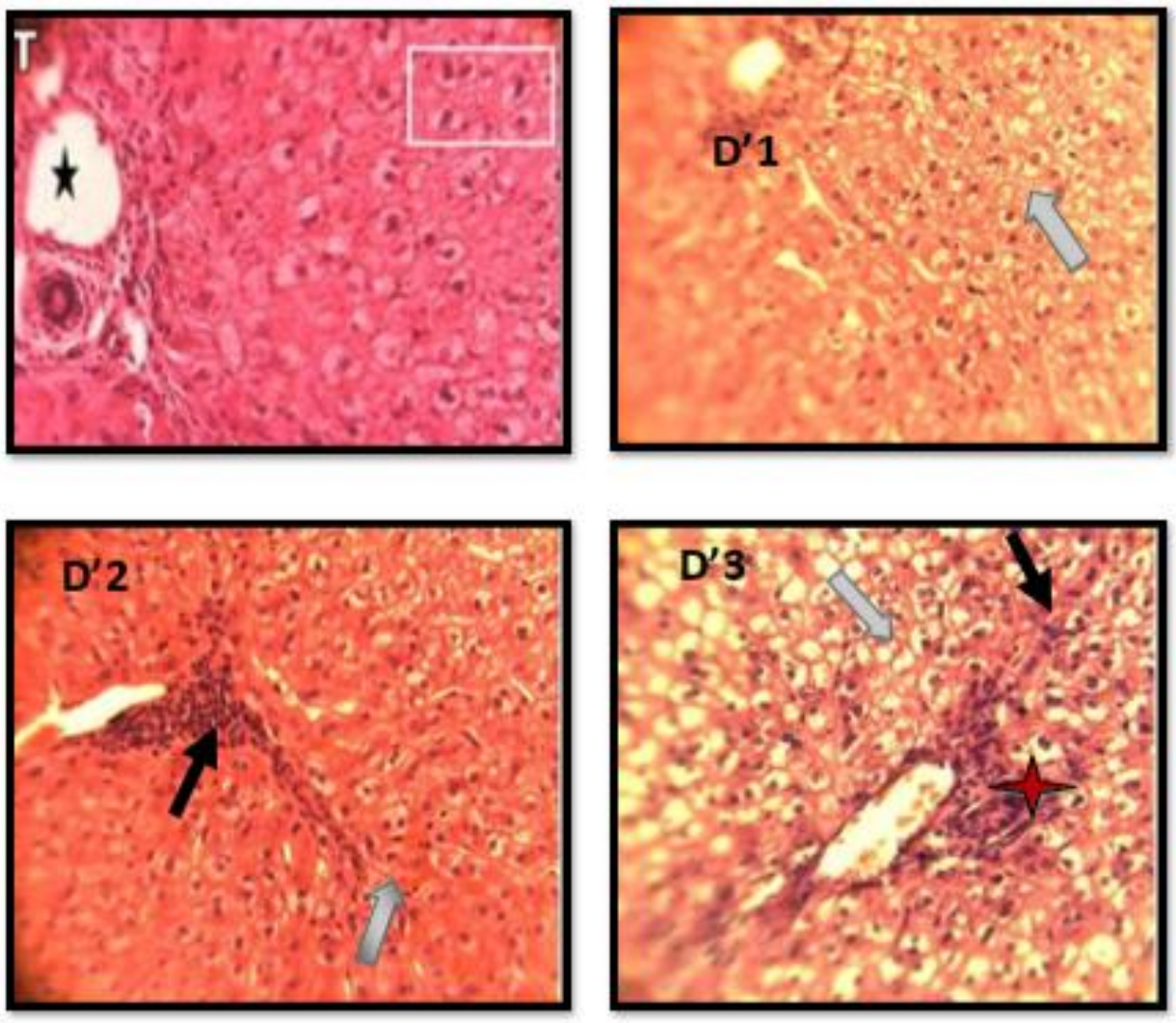


Figure 37: *Liver histology of male rabbits from the control and groups treated with Glyphosate (x400).*

Centralvein

Bloodcongestion

Necroticfocis

Inflammatorycell

Concerning liver histological study, our results revealed that ayzoxystrobin fongicide induced certain modificacations indicated by cells inflammation in the treated groups compared to the the control (fig 38).

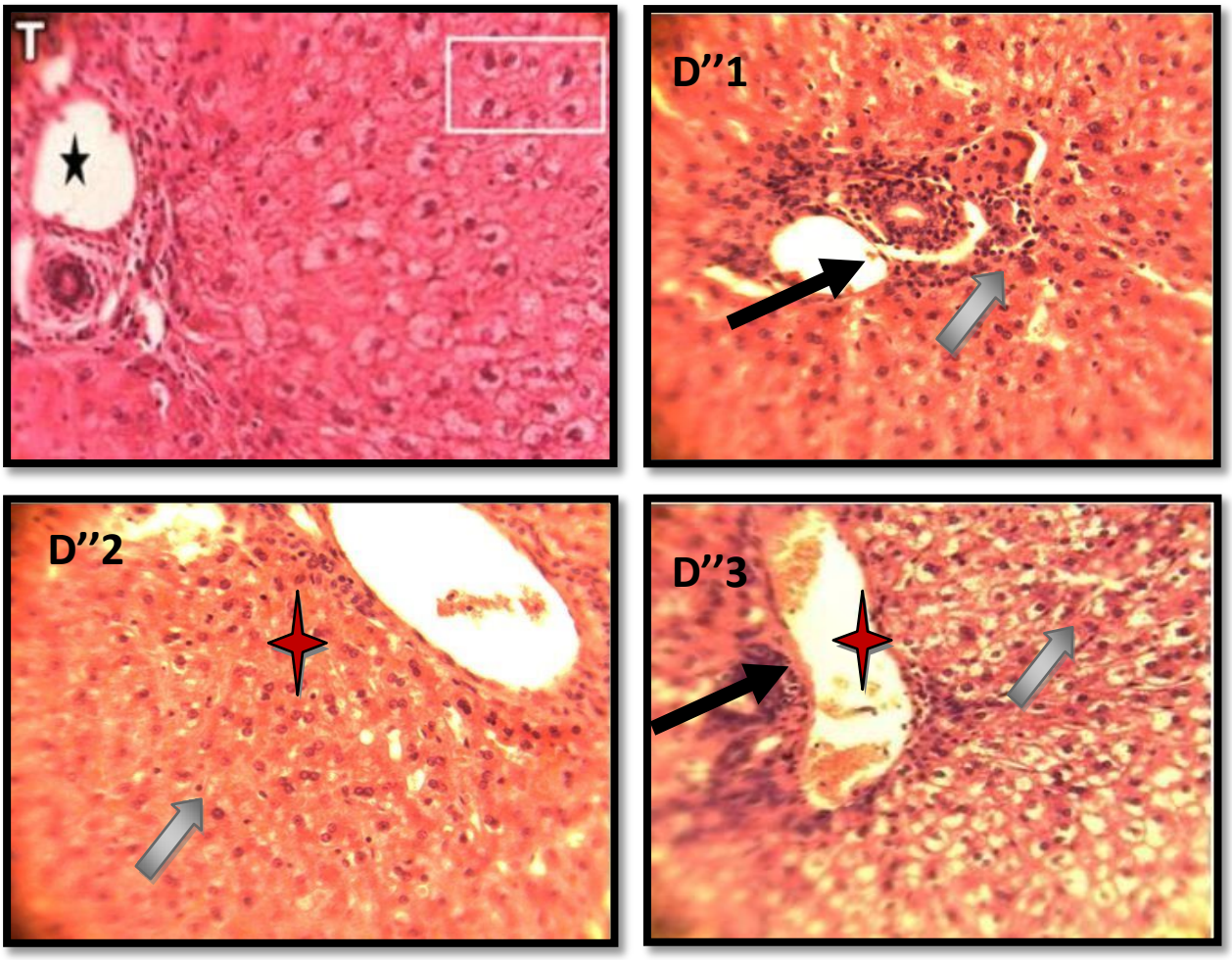
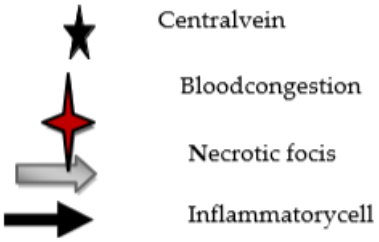


Figure 38: *Liver histology of male rabbits from the control and groups treated with Azoxystrobin (x400).*



Chapter 4: Discussion

In the present study, total body weight was significantly reduced in every group treated with pesticide; this may be attributed to a decreased food intake. Thus, less food intake is due to the loss of appetite, which could be responsible for the reduced body weight in groups treated with pesticides. There was gradient decrease in food and water consumption, as this may be indicative of pesticides induced toxicity hampering the body basal metabolism.

Similarly,reduced food consumption was observed in rats and mice, which had received high doses of triadimefon, CBZ and acetamiprid(Koprucu *et al*., 2010; Hocine *et al*.,2016).Also, weight loss in the treated groups may be a result of the combination of oxidative stress and adrenal mediated stress caused by inhibition of cholesterol ester hydrolase, which could lead to less food intake,and then decreased body weight.Wissem (2011) reported that the body weight of animals receiving 250 mg/kg of an organophosphate was decreased.Our studies were similar to Chevier (2000), who suggested that insecticides could be released from adipose tissues, along with mobilizing of lipids leading to weight loss. Besides, similar results were found by Patil (2018) after treating male wistar rats with Carbendazim at 100,200 and 400mk/kg. Considering the exposure to chemicals could interfere with the growth pattern of an animal and in the interpretation of possible reproductive impacts (Clegg *et al.*, 2001), results indicate that the reproductive effects caused by difenoconazole are related to its direct action. Goetz *et al.* (2006) have nor observed significant differences in food consumption after the oral exposure of rats to myclobutanil and propiconazole fungicides at 100 and 500 ppm, from the gestational period until postnatal day 120. Meanwhile, animals exposed to 1800 ppm of triadimefon had a reduced food intake rate from the first week of exposure (Goetz *et al*., 2006). The reduction in body weight in response to cypermethrin intake may be a result of oxidative stress and/or to an increase in the degradation of lipids and proteins (Adjrah *et al*., 2013).This finding is supported by the observed significant decrease in protein content in cypermethrin-treated rabbits.Thus, our results agreed with Ali (2015)who treatedrabbits with different doses of cypermethrin.

The findings of the present study revealed that the oral gavaging of three different pesticides (diflubenzuron, glyphosate and azoxystrobin) over 4 weeks, decreased rabbit spermatozoid concentration, speed quality, motility and vitality. Besides, concentration, and durability of the examining sperm density and viability in the tail of epididymiswere notably reduced in the three groups treated with pesticides. We can explain, through these results, that the pesticides infiltrate the blood circulation and directly lead to the production of infertile sperm by damaging the testicular tissues; or provoking mutagenic effects (Leaser *et al*.,

1998). Pesticides'exposure affectsseminal vesicle and prostate, which contribute respectively to the production of 60% and 30% of the seminal volume, reducing then the seminal volume (Yucra *et al*, 2006). In the same line, pesticides generate disorders in the normal differentiation of sperm chromosomes (Waem *et al*., 2009). In our study, we observed a decrease in the sperm parameters of groups treated with DFB. Najai (2013) reported that raisingpesticide doses can decrease the number and progressive motility of sperm, in addition to the number of dead or immotile sperm. Various studies reported that pesticides might reduce the level of sperms steroid hormones, by increasing catabolism that is directly affect testicular tissue, and directly and indirectly affect the endocrine system (Fattahi *et al.,* 2009). Our work is similar to other studies as that of Yousef *et al*,. (2003), who reported impairments of reproductive system in rats exposedcypermethrin.Similar effects were observed by Issam (2011) after subcutaneous PM insecticide treatment. Geroge (2018) proved the toxicity of glyphosate herbicide on human sperm mobility and ADN fragmentation by affecting the mitochondrial function.Our results were in line with Eman (2019), in which mancozeb fungicide led to the reduction in the reproductive parameters of male rabbits. However, Sperm motility depends on the integrity of the middle piece and tail for producing energy. Therefore, any factor interfering in the assembling of tail structure protein components and/or modifying the concentration/function of ATP synthesis can lead to decreased sperm motility.

In this study, there were an apparent decrease of serum testosterone concentration of rabbits exposed to the three types of pesticides and in all doses. It was found that the exposure to GBH led to disruption of normal testicular architecture, coupled with a decrease of testosterone secretion as well as abnormal sperms properties (Owagboriaye *et al.,* 2017). The decrease in testosterone level after exposure to Roundup® has been observed in previous studies (Romano et al., 2010; Dallegrave *et al.,* 2007), but not with glyphosate alone. The disturbances in hormonal regulation during prenatal and neonatal periods may induce adverse effects on the male reproductive system (Palanza *et al*., 2016). In fact, exposure of rats to 50 to 450 mg/kg/day of GBH (Roundup®) during pregnancy and lactation affects serum testosterone levels and spermatozoa parameters (Dallegrave *et al.,* 2007; Romano *et al*. 2012) and caused changes in sexual behavior of the male offspring (Romano et al., 2012). Unlike many studies that focused on GBHs, recent studies showed that glyphosate did not affect male reproduction in rat (Dai *et al.,* 2016) or decrease the total sperm production at the high dose of 500 mg/kg/day without affecting testosterone level (Johansson *et al*., 2018). However, some studies support the hypothesis that glyphosate affects sperm parameters in fish (Lopes *et al.,*

2014; Uren Webster et al. 2014; Gonçalves *et al*., 2018). In humans, data from existing epidemiological studies does not support the idea that glyphosate is toxic to reproduction and human development (de Araujo *et al*., 2016). However, a recent study associatesd exposure to glyphosate to shortened gestational length (Parvez *et al*., 2018). As glyphosate didn't affect the expression of the Cyp19a1 transcript, we suggest that the decrease could not be explained by the alteration of the activity of the aromatase enzyme, encoded by Cyp19a1, which transforms testosterone into estradiol. However, in studies using human placental and embryonic cells, it has been shown that the treatment with low concentrations of GBHs led to changes in aromatase activity and mRNA level (Richard *et al*., 2005; Benachour *et al*., 2007). The toxicity of glyphosate may be mediated via its effects on endocrine regulation of the hypothalamic-pituitary axis (Yousef *et al.,* 1995). On the other hand, Uren Webster et al., (2014 hypothesized that glyphosate disrupts the steroid biosynthetic pathway by causing oxidative stress.Some pesticides are endocrine disrupting agents causing hormonal imbalance and disruption in male fertility. As they work on the synthesis, storage, release and bonding of hormones, the latter are affected, leading to change in their levels and functions (Tabb & Blumberg, 2006; Souheilo *et al*., 2011). As well as, they disturb the normal functioning of reproductive hormone and indirectly decrease sperm production, differentiation and spermatogenesis (Whorton, 1994; Melissa, 2008). We observed that glyphosate exposure resulted to a significant decrease in the spermatozoa numbers leading to a decrease of epididymis weights. This decrease in the spermatozoa counts demonstrates that exposure to glyphosate or a GBH could reduce male fertility.

Azoxystrobin treatment of the present study established a significant decline in sperm parameters after, which can be explained by reducing the percent of the live sperm cell and the viability of the epididymal sperm. Joshi *et al* (2005) noted that Wistar rats orally subjected to mancozeb was at the dosage level of 500 mg/kg/day for 30 days produced a vital decrease in testicular sperm cell density, and the sperm motility in cauda epididymis. Ananthan (2016) said that the effect of mancozeb at the dose of 300 mg/kg body weight for 60 days induced a significant decrease in the epididymal sperm counts, motility, and viability. It is notable that epididymis is the main organ for the maturation of sperm and is androgen-dependent in regard to its structure and performance (Rao &Sharma, 2001).

Fungicide treatment also brought an essential loss of sperm reserves in the rat cauda epididymis (Kempinas *et al*., 1998). Moreover, Khan and Sinha (1996) revealed that intake of mancozeb at 1000 mg/kg b.w for 35 days to males of 6–8 weeks old Swiss albino mice made

a significant decrease in sperm count, combined with a labeled increase in the frequency of abnormal head sperm. After 30 days mancozeb subjection in mice, there was inhibition of spermatocyte maturation (Ksheerasagar & Kaliwal, 2003). In addition, the number of the sperm was diminished and the intertitular blood vessels were dilated and engorged with blood. Moreover, Ksheerasagar & Kaliwal (2010) said that the influence of mancozeb on testicles and accessory sex organs pointed out two principal impacts on the male reproductive system. Study of Dong *et al*. (2018) evaluated the reproductive impact of difenoconazole exposure on male fish Oryzias melastigma, in which they observed inhibited testicular development and a significant decrease of spermatozoa that was consistent with the reduction of fertilization success in the F1 generation. Nevertheless, studies of exposure of rats to propiconazole (Costa *et al.,* 2015) and tebuconazole (Yang *et al*. 2018) showed no change in testicular sperm counts. In addition, a decrease of spermatozoa was observed in the epididymis in all groups exposed to difenoconazole. Yang *et al.* (2018) also reported a decrease in epididymal sperm count after exposure to tebuconazole.

Numerous factors contribute to the reduction of testosterone by inhibiting steroids, a defect in production of androgen, or degeneration of leydig cells, therefore, the level of testosterone is could be reduced (Taib *et al*., 2014), probably by causing a disorder in hormone metabolism or inhibitingits receptors and affecting the process of gene transcription (Perry *et al*., 2011), or inhibitingthe p450 hepatic enzymes function that metabolizes testosterone, estradiol and estrone (Scott *et al*., 2010).Exposure to GBH during rat puberty induced changes in the progression of puberty, testicular morphology, and reduced testosterone production (Romano et al., 2010). In adult rats, GBH exposure leads to disruption of normal testicular architecture, coupled with a decrease of testosterone secretion as well as abnormal sperms properties (Owagboriaye *et al*., 2017). The toxicity of GBHs is for now attributed to the surfactants and adjuvants that are added during commercial preparations (Mesnage *et al.,* 2013; Johansson *et al*. 2018). On the other hand, recent investigations showed that glyphosate has not affectedrat male reproduction (Dai *et al*., 2016) or decreasedtotal sperm production at the high dose of 500 mg/kg/day without affecting testosterone level (Johansson *et al.,* 2018). The decrease in testosterone level after exposure to Roundup® has been observed in previous studies (Romano *et al.,* 2010, Dallegrave *et al*., 2007) but not with glyphosate alone. As glyphosate didn't affect the expression of the Cyp19a1 transcript here, we suggest that the decrease could not be explained by the alteration of the activity of the aromatase enzyme, encoded by Cyp19a1, which transforms testosterone

into estradiol. However, in studies using human placental and embryonic cells, it has been shown that the treatment with low concentrations of GBHs leads to changes in aromatase activity and mRNA level (Richard *et al.*, 2005; Benachour *et al.*, 2007). The toxicity of glyphosate may be mediated via its effects on endocrine regulation of the hypothalamic-pituitary axis (Yousef *et al.*, 1995). On the other hand, Uren Webster et al. hypothesized that glyphosate disrupts the steroid biosynthetic pathway by causing oxidative stress (Uren Webster *et al*., 2014). Our results are alike the finding ofArish (2017) that used permethrin and observed its effects on testicles parameters of male rats, and sa that of observed by Parinya (2018) in farmersexposed to pesticides, which led toa decrease in testosterone level.

In this work, Hemoglobin level and red blood counts were gradually decreased and directly related to the increasing dose ofpesticides and time of exposure; however, leukocytes count was significantly increased which may be linked to the oxidative stress(El-Desoky *et al*., 2012).According to the study of Gholami *et al*.,(2017), glyphosate and their metabolites caused hemolysis, hemoglobin oxidation and provoked a rise inRBC with increasing dose. These results agree with those of Basir (2011) who observed a decrease in RBC counts, as well as hemoglobin and hematocrit values at a high dose when the adult male dogswere fed capsules containing thiophanate methyl fungicide. Other studies have showed similar findings (Wael, 2012) after treating male mice with metalaxyl fungicide.Our results are similar to that found by Sanam(2019) who reportedthat hematologic parameters have been decreased after treating rabbits *Oryctolagus cuniculus* that received 50, 100, and 150mg/kg of glyphosate fungicide.Other studies showed the decrease in the RBC counts, hemoglobin levels, which were possibly due to inhibited RBC production, decrease in the number of matured RBC released into blood stream and shortened life span of circulating RBC (Dorucu & Girgin,2002). This agrees with Farzeen & Razia (2021) that pesticides poisoning increasedRBC destruction as a result of impaired biosynthesis of haem in the bone marrow resulting in lower levels of hemoglobin and hematocrit. Moreover, Riaz(2017)reported that a diminution of Hb level was seen in male rabbits after cypermethrin exposure, and they suggested that this pesticideinduced modifications in hematological indices.Similarly, the reduction in hemoglobin level may be due either to an increase in the hemoglobin destruction rate or a decrease in the hemoglobin synthesis (Dorucu & Girgin,2002). Moreover, anemia could have been observed because of RBC destruction, induced by pesticides.Thus, the reduced number of RBChas led to lower hemoglobin and hematocrit levels (Zorriehzahra,2010).

Our results showed that the natural immunity system of the rabbits was activated, attempting to cope with the injury caused by pesticides. Yousef,(2003) suggested that Cypermethrin given to male rabbits at 24 mg/kg bwmay provoked a systemic damage of the rabbit's immune system due to acute stress and infections induced by toxicity, which is in line with that of Bigoniyaa (2015) throughthe activation of the animal's defense mechanism against toxic invasion. These results agree also with Nunta(2020) when treated rabbits with organochlorine pesticides and that of Asieh (2018) after treating male wistar rats with a dose of 10 and 25mg /kg bw of diazinon.

Concerning biochemical parameters, our results showed a significant increase in glucose levels in rabbits treated with diflubenzuron and glyphosate as rabbits need an extra production of energy to combat such induced stress generated by pesticides, by affecting the lymbic system which activates the hypothalamus to produce corticolibirine(CRH) the latter stimulate the pituitary gland to release ACTH, which is an activator of adrenaline glands for the production and secretion of cortisol in blood to elevate blood sugar levels (Maryam *et al*.,2013); this result agrees with that of Assia (2020) and Ambali (2011), where the increase in blood glucose level in rabbits may induce hyperglycemia through reduced insulin secretion and hepatic glucogenesis, but stimulate glycogenolysis pathway resulting in disease conditions such as diabetes mellitus (Mossa& Abbassy,2012). This finding is in parallel with that reported by Paul *et al*., (2009) and Veerappan *et al*., (2012). Therefore, glucose homeostasis was affected by cypermethrin administration. This insecticide induces hyperglycemia which involve one or more mechanisms; 1) reduction in insulin secretion as a result of the destructive action of cypermethrin on the beta cells in the pancreas (Kalender *et al.*, 2005 and Eraslan *et al*., 2008), 2) impairment in hepatic function due to oxidative stress, which reduce liver ability to glycogenesis (Abdou *et al*., 2012 and Bhatti *et al.*, 2014), and 3) stimulation of hepatic gluconeogenesis and glycogenolysis (Veerappan et al., 2012; Bhanu and Deepak (2015). Similar finding by El Demerdach (2003) and Menna (2004) after treating animals with cypermethrin insecticide.

Cholesterol levels in this study were increased in rabbits treated with diflubenzuron and glyphosate. Other researches proved that pesticides caused a decreased lipoprotein lipase LPL activity in adipose tissue, and an increase in the total plasma cholesterol levels. Plasma triglycerides levels were reported to be increased in rats treated with carbendazim, which led

to an increase in lipogenesis and accelerated the acetyl-coA, which is known to be the precursor of cholesterol biosynthesis. Our results were in line with that of Wael (2012) after treating mice with metalaxyl fungicide.On the other hand, we found a reduction in plasma glucose, triglycerides, cholesterol and protein levels of rabbits treated with fungicide, where these results are similar to that of Baligar & Kaliwal, (2001), who reported that mancozeb fungicide at 600, 700 and 800 mg/kg/day induced a significant decrease in the level of rat glycogen and protein contents.

Concerning azoxystrobin, th present study is in agreement with other investigations that documented that OPs provoke an increase in the level of total lipid mainly cholesterol and triglycerides (Abdou and Mazoudy, 2010; Al-Attar, 2015; Al-Attar *et al*., 2017). The pathoetiology of observed hypercholesterolemia could be associated with hepatocytes damage due to oxidative stress mediated cell membrane breakdown that gave rise to leak out of cholesterol to the blood.The increased total triglycerides can be explained by enhanced adipocyte lipolysis due to pesticides–induced insulin resistance as well as inhibition of plasma lipoprotein lipase and hepatic lipase (Abdou & El-Mazoudy,2010; Chatterjeeand, 2011; Bishop *et al*., 2013; Al-Attar *et al.*, 2017).

GSH level has been decreased in the actual work atthe low doses of the three pesticides studied. The GSH might play an antioxidant safeguarding role of the sperm cell membrane, through it oxidation to GSSG by glutathione reductase (Biswas & Rahman, 2008). changes in the levels of different glutathione forms (either reduced GSH or oxidized GSSG) may indicate a shift in the prooxidant-antioxidant balance, which often takes place under pesticide-induced stress conditions (Maher, 2005; Lushchak, 2012; Atamaniuk *et al*., 2013). Generally, exposure to pesticides caused a reduction in GSH level (El Shennawy, 2009; Shorma *et al*., 2005).

Our results were in parallel to that of Larsen (2012) after treating rabbits with diazinon insecticide, which led to a decrease in glutathione concentration.Similar findings obtained by Mehmet (2018) showed that pesticides decreased GSH level in human blood. Our work confirmed the results of Eman (2019) after treating male rabbits with mancozeneb at a dose 100kg/mg. The level of GSH has been associated with many adverse effects on male reproductive system in both human and rodents. It is capable of preventing damage to important cellular component caused by reactive oxygen species such as free radicals, peroxides and lipids (Pompella *et al.*, 2003).

Urea and creatinine concentrations werenotably increased in the 4th weeks of the experiment. Such findings are in agreement with that reported in other studies (Saxena &Saxena, 2010; Sankar *et al*., 2012; Sakr & Albarakai, 2014). Urea is formed by the liver as an end product of protein breakdown and it is one marker of the kidney function (Debra Manzella, 2008 and Tawfik & Al-Badr, 2012). The increase in serum urea observed in the present study may be due to impairment in its synthesis as a result of damaged hepatic cells, disturbance in protein metabolism and a decrease in its renal filtration rate. The decrease in the serum protein level observed in the present study may support this explanation. Creatinine is break-down product of creatine phosphate in muscles, and is usually produced at a fairly constant rate by the body. Creatinine is chiefly filtered out of the blood by the kidneys and has been found to be a fairly reliable indicator of kidney function (Tawfik & Al- Badr, 2012). As the kidneys become impaired for any reason, for example in case of cypermethrin poisoning, the blood creatinine level may rise due to poor renal clearance. Thus, rises in blood creatinine level was observed with damage to functioning nephrons and apparently impair renal function (Zama et al., 2007 and Ambali *et al*., 2010).

As indicated in the present results, significant decreases in the levels of total albumin during the 4th weeks of treatment by the three different pesticideswere found. Similar results were reported in other studies after the oral administration of different doses of cypermethrin (Lakkawar *et al*., 2006; Ahmad *et al*., 2011; Veerappan *et al*., 2012 and Mhya *et al*., 2014). The reduction in serum protein levelsmayattribute to alterations in protein and free amino acid metabolism and their hepatic synthesis. Also, the protein level suppression may be due to loss of protein either by reduction in protein synthesis or increased proteolytic activity or degradation (Ncibi *et al*., 2008 and Shin and Moon, 2010). In addition, the observed decrease in serum proteins could be attributed in part to the damaging effect of pesticides like cypermethrin on liver cells.

Through the histological study, the results showed analteration in the hepatocytes as well as in the size and shape of the somniferous tubules, with blood congestion and necrosis in all rabbits received pesticides with the different doses. Many studies reported that liver tissue is the primary target of pesticides such as organophosphorus, causing detriment to both liver structure and functions. Ezzi (2016) suggested that chlorpyrifos had a dose–dependent effect on central vein and portal triad of rat liver.Acute high dose of dizine caused hypertophil and swilling of hepatocytes and vascuolisation of cytoplasm (Beydilli *et al*.,2015). Such study

is in the same line for OPs toxicity. Studies have reported the presence of hyalinization, vacuolisation, nucleus necrosis,and hepatocellular oedema after exposure to low dose of trichlorfon (Li *et al*.,2017). The testicular sections of rabbits treated with the three pesticides have obvious structural changes after four weeks, where the rupture appeared on the level of sperm tubules. In addition, the lumens of the tubules wereempty of sperm. Pesticides reduced the level of lipids and proteins at the cellular membrane level of Sertoli cells, and the size and shape of the somniferous tubules were modified. These findings agreed with Li(2014) using cypermethrin, where vacuolizationswere appeared as intercellular spaces, may be because of the death of germinal lineage cells and lack of elements of the somniferous cells.Triazophos is another pesticide associated with liver damage in experimental female albino rats (Charma, 2014), in which a variety of histopathological modifications,such as infiltration vacuolization, and necrosis. Our results on testicular histology are in parallel with that of Heba (2016) who treated rats with Cypermethrinat 50 and 70mg/kg, and that of Ghada(2017) with various degrees of harmful changes in the histological structure of testis and liver of male rabbit exposed to organophosphorus pesticides, and that of Somaryyeh (2017) about organophosphorus on liver tissues. The disturbance of the male reproductive function can also be observed if there are changes in testicular morphology. In rats, exposure to Roundup® can provoke the appearance of vacuoles and the degeneration of the seminiferous epithelium (Dallegrave *et al*.,2007); there are also changes in the epithelial height and luminal diameter of seminiferous tubules (Romano *et al.*, 2010, Romano *et al*., 2012).

Conclusions and perspectives

Conclusion and perspectives

In this project, the effect of sub-chronic oral exposure of insecticide (Diflubenzuron, DFB), a herbicide (Glyphostae, GLY), and a fungicide (Aroxystrobine, AZO) on reproduction, plasma biochemistry, blood components, and also the organs' oxidative stress and histology of the domestic rabbit Cuniculus lepus male rabbits were investigated.

Results obtained have shown that the three pesticides used caused harmful effects on spermatozoan concentration, speed, vitality and morphology, and on testosterone biosynthesis.

The study demonstrated that the insecticide DFB has increased plasma cholesterol, triglycerides, glucose, urea, creatinine, and WBC levels, but it decreased RBC, Hb, hepatic and testicular glutathione, and albumin.

However, the herbicide GLY given to rabbits has altered many parameters as that observed in the decrease of cholesterol, triglycerides, albumin, RBC, Hb, aand testicular and hepatic glutathione, accompanied with an elevation in glucose, urea, creatinine, and WBC levels.

The fungicide AZO also has made notable modifications by increasing the levels of cholesterol, triglycerides, urea, creatinine, WBC, whereas it decreased the levels of glucose, albumin, RBC, Hb, and hepatic and testicular GSH.

Concerning histology, all pesticides have made histo-pathological injuries to both liver and testicules.

In conclusion, the pesticides used at different doses have clearly induced reprototoxicity, hepatotoxicity, hematotoxicity, and nephrotoxicity to male rabbits.

In future, our scientific perspective will focus on the following:

- The mode of action of pesticides at the testicular molecular levels,
- The toxicological effects of pesticides as a mixture on the reproductive functions.
- The natural detoxification of pesticides by using local products.

References

References

A Del Pino J, Martinez M, CabalerroV, Ares I,Nieto I, Martinez-Marranaga M. Neurotoxicological effects of the herbicide glyphosate. Toxicol Letters, 2008; (180S): S164.

Abdou HM & El Mazoudy RH. Oxidative damage, hyperlipidemia and histological alterations of cardiac and skeletal muscles induced by different doses of diazinon in female rats, *Journal of Hazardous* Materials, 2010 ;182 (1–3) : 273–278.

Abdou HM, Hussien HM & Yousef MI. Deleterious effects ofcypermethrin on rat liver and kidney: protective role of sesame oil. Journal of Environmental Science and Health B 47 ,2012; (4): 306-314.

Acquavella J, Alexander B, Mandel J, Gustin C, Baker B,Chapman P, **Bleeke M**. Glyphosate biomonitoring for farmers and their families: results from the farm family exposure study. Environ. Health Perspect, 2004 ;**112** :321-326. activity and haematological indices in rats: the protective role of vitamin.

Ahmad L, Khan A& Khan MZ. Cypermethrin Induced Biochemicaland Hepato-renal Pathological Changes in Rabbits. International Journal ofAgriculture and Biology ,2011;**13** (6): 865-872.

Ambali SF, Abubakar AT, Shittu M, Yaqub LS, Anafi SB& AbdullahiA. Chlorpyrifos-induced alteration of hematological parameters inWistar rats: Ameliorative effect of Zinc, ournal of Environmental Toxicology , 2010;**4**: 55-66.

Ananthan G,. Spermatotoxic effect of mancozeb in adult rats. Int. J. Sci. Res. 5, 2016; (11), 48–69.

Anderson SE,Meade BJ. Potential health effects associatedwith dermal exposure to occupational chemicals. Environ. Health Insights,2014 **;8**: 51–62.

Antoniou M, Habib M, Howard C, Jenning R, Leifert C,Onofre Nodari R Robinson C,Fagan J. Roundup and birth defects - Is the public being kept in the dark? Earth Open Source: 2011; p 52.

Arbuckle T,Lin Z, Mery L, An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population. Environ Health Perspect, **2001** ; (109) : 851-57.

Asieh Kb, Mohammad H and Amir M J. Protective effects of glycyrrhizin on sub-chronic diazinon-induced biochemical,hematological alterations and oxidative stress indices in male Wistar rats , 2018;Drug and Chemical Toxicology.

Azzouz Z. Etude des Effets Toxiques d'un Fongicide (Amistar Xtra) et d'un Herbicide (Glyphosate) sur la Biologie et le Comportement de tetraurelia Paramecium Thèse de Doctorat en Biologie Animale, Spécialité : Toxicologie Cellulaire, UniversitéVolume Badji Mokhtar, Annaba, 2012 ;p. 14, 15.

References

Baishya RL& Hazarika L. Effect of methoprene and diflubenzuron on water, lipid, protein and chitin content of Dicladispa armigera (Coleoptera: chrysomelidae). Entomon, 1996 ;**21**(1): 7-11.

Baligar P N & Kaliwal B B ."Induction of gonadal toxicity to femalerat after cronic exposure to mancozeb" Ind. Health, 2001;vol.39, no.3, pp:235- 243.

Bartlett D, Clough J, Godwin J, Hall A, Hamer M. Parr-Dobrzanski B. The strobilurin fungicides. Pestic. Sci, 2002; 58: 649–662.

Basir A, Khan A, Mustafa R, Khan M Z, Rizvi F, Mahmood F, Yousaf A "Toxicopathological effects of lambda-cyhalothrin in female rabbits (Oryctolagus cuniculus)".Hum Exp Toxicol, 2011; vol.30 no.7, pp:591-602.

Batsch D.L'impact des Pesticides sur la Santé Humaine, Thèse de Doctorat, Diplôme d'Etat de Docteur en Pharmacie, Université Henri Poincaré - Nancy 1, Faculté de Pharmacie, 2011 ;p14,15.

Beard, J.D., Umbach, D.M., Hoppin, J.A., et al . Pesticide exposure and depression among male private pesticide applicators in the agricultural health study. EHP , 2014; **122** (9): 984–991.

Belle R, Le Bouffant R, Morales J, Cosson B, CormierP, Mulner-Lorillon O. L'embryon d'oursin, le point de surveillance de l'ADN endommagee de la division cellulaire et les mecanismes a l'origine de la cancerisation. J. Soc Biol, 2007 **;201** (3) :317-327.

Belpoggi F, Soffritti M, Guarino M, Lambertini L, Cevolani D, Maltoni C. Results of Long-Term Experimental Studies on the Carcinogenicity of Ethylene-bis- Dithiocarbamate (Mancozeb) in Rats. Annals of the New York Academy of Sciences,2006, **982**:123-136.

Benachour N, Sipahutar H, Moslemi S, Gasnier C, Travert C, Séralini GE. Time- and dosedependent effects of roundup on human embryonic and placental cells. Arch Environ Contam Toxicol,2007;53(1):126–133. doi:10.1007/s00244-006-0154-8.

Benachour N,Seralin G.-E. Glyphosate Formulations Induce Apoptosis and Necrosis in Human Umbilical, Embryonic, and Placental Cells. Chem Res Toxicol, **2008 ; 22** (1) : 97-105.

Beydilli H,Yilmaz N,Cetin ES,Topal Y,Celic OI,Sahin C,Topal H,Cigeric IH,Sozen H.Evaluation of protective effect of silibin against diazinon induced hepatotoxicity and free radical damage in liver .Iran Red Crescent Med J, 2015 ;17(4) .

Bhanu AP& Deepak M. Impact of cypermethrin on biochemical aspects of clinical importance in the blood of freshwater fish Cyprinus carpio. Journal of Entomology and Zoology Studies,2015; 3 (1): 126-128.

Bhatti G K, Sidhu IP S, Saini N K, Puar S K, Singh G& Bhatti J S. Ameliorative role of melatonin against cypermethrin induced hepatotoxicity and impaired antioxidant defense system in Wistar rats. Journal Of Environmental Science,2014;8 (1): 39-48.

References

Bigoniyaa P, Sahu T, & Tiwari V. Haematological and biochemical effects of sub-chronic artesunate exposure in rats. Toxicology Reports, 2015 ;2: 280-288.

Biswas SK, Rahman I. Environmental toxicity, redox signaling and lung inflammation: the role of glutathione. Mol. Aspect. Med,2009;30: 60–76.

Bolognesi C. Genotoxicity of pesticides: a review of human biomonitoring studies. Mutat Res,2003.; 543: 251-72.

Bradford NM,TC Caetano MS, Ramalho, Botrel DF, da Cunha EFF and de Mello WC. A rapid and sensitive method for the quantitation of microgram quantiles.

Calvet r. Barriuso E. Bedos C. Benoit P. Chary M P. Coquet Y ,Campbell AW. Glyphosate: its effects on humans. Alternatives Therapies in Health and Medicine, 2014; **20**: 9-11.

Cassault-Meyer E, Gress S, Seralini G. Galeraud-Denis I. An acute exposurecancer incidence in the agricultural health study cohort. Enviro, 2014.

Chan P, Mahler J. NTP technical report on the toxicity studies of glyphosate (CAS No. 1071-83-6) administered In dosed feed to F344/N rats and B6C3F1 mice. Toxic. Rep. Ser. 16, 1992.

Chan PC, Mahler JF. NTP technical report on the toxicity studies of glyphosate (CAS no 1071-83-6) administered in dosed feed to F344/N rats and B6C3F1 mice. Toxicity Reports Series, 1992; 16: 1-D3.changes. Toxicology, 2005 ;211 (3) : 197–206.

Chevrier J, Dewailly EÂ, Ayotte P, MaurieÁge P, DespreÂs J-P and Tremblay A.Body weight loss increases plasma and adipose tissue concentrations of potentially toxic pollutants in obese individuals International Journal of Obesity ,2000; 24: 1272-1278.

ClamanP. Men at risk: occupation and male infertility. Fertil. Steril. . 2004, 81; 57–60,19-26

clinical correlation. Ed. Wiley & Sons,1992**;** 901-925.

Cooper JR &Kini MM. Biochemical aspects of methanol poisoning. Biochem Pharmacol, 1962;**11**:405-416.copper tolerance: an example of how “omics” research can accelerate discovery of new woodcorrelations. Vol. Lipids and lipoproteins. Philadelphia, 2013.

Costa NO, Vieira ML, Sgarioni VP. Evaluation of the reproductive toxicity of fungicide propiconazole in male rats. Toxicol ,2015;335:55–61.https://doi.org/10.1016/j.tox.2015.06.011.

Costa NO, Vieira ML, Sgarioni VP. Evalution of the reproductive toxicity of fungicide prppiconazole in male rats. Toxicol ,2015;335:55–61.https://doi.org/10.1016/j.tox.2015.06.011.

Coullery R,Ferrari M, Rosso S, Neuronal development and axon growth are altered by glyphosate through a WNT non-canonical signaling pathway.Cox C. Ten Reasons not to Use Pesticides J Pestic. Reform ,2006 ; 26(2):10-12.

Dai P, Hu P, Tang J, Li Y, Li C. Effect of glyphosate on reproductive organs in male rat. Acta Histochem,2016 ;118(5):519–526. doi:10.1016/j.acthis.2016.05.009.

References

Dallegrave E, Mantese FD, Oliveira RT, Andrade AJM, Dalsenter PR, Langeloh A. Preand postnatal toxicity of the commercial glyphosate formulation in Wistar rats. Arch Toxicol,2007;81(9):665–673. doi:10.1007/s00204-006-0170-5.

Dallegrave E, Mantese FD, Oliveira RT, Andrade AJM, Dalsenter PR, Langeloh A. Preand postnatal toxicity of the commercial glyphosate formulation in Wistar rats. Arch Toxicol,2007;81(9):665–673. doi:10.1007/s00204-006-0170-5.

Damalas CA, Eleftherohorinos GE. Pesticide exposure, safety issues, and risk assessment indicators. Int. J. Environ. Res. Public Health ,2011; 8 (5): 1402–1419.

Davies JE, Barquet A, Freed VH, Haque R, Morgade C, Sonneborn RE, Vaclavek. C Human pesticide poisonings by a fat soluble organophosphate insecticide. Archives of Environmental Health,1975; **30**: 608-613.

de Araujo JSA, Delgado IF, Paumgartten FJR. Glyphosate and adverse pregnancy outcomes, a systematic review of observational studies. BMC Public Health,2016;16:472.doi:10.1186/s12889-016-3153-3.

De Clercq D, Vinuela E, Smagghe G, Degheele D. Transport and kinetics of diflubenzuron and pyriproxyfen in the beet armyworm, Spodoptera exigua, and its predator Podisus maculiventris. Entomologia Experimentalis et Applicata, 1995a ;**76** (2): 189-194.

De Clercq P, De Cock A, Tirry L, Vinuela E, Degheele D. Toxicity of diflubenzuron and pyriproxyfen to the predatory bug Podisus maculiventris. Entomologia Experimentalis et Applicata, 1995b ;**74**(1): 17-22.

de Souza JS, Kizys MML, da Conceição RR, Glebocki G, Romano RM, Ortiga-Carvalho TM, Giannocco G, da Silva IDCG, Dias da Silva MR, Romano MA, et al. Perinatal exposure to glyphosate-based herbicide alters the thyrotrophic axis and causes thyroid hormone homeostasis imbalance in male rats. Toxicology,2017; 377:25–37.doi:10.1016/j.tox.2016.11.005.

Debra Manzella RN. Kidney disease in diabetes, 2008;http://diabetes.about.com/od/preventingcomplications/p/kidneydisease.htm.

Delaplane K S. Pesticide Usage in the United States: History, Benefits, Risks, and Trends. The University of Georgia, Athens, Georgia1996;http://ipm.ncsu.edu/safety/factsheets/pestuse.pdf (Accessed 20 August 2014).).Detoxification, Regulatory Toxicology and Pharmacology, 2019 ;105 :86-98.

Dong X, Zhang L, ChenM, Yang Z, Zuo Z,Wang C. Exposure to difenoconazole inhibits reproductive ability in male marine medaka(Oryzias melastigma). J Environ Sci ,2018;63:126–132. https://doi.org/10. 1016/j.jes.2017.05.030.

Dorucu M & Girgin A. The Effect of Cypermethrin on some Haematological Parameters of Cyprinus carpio. Aquaculture International, 2002 ;9 (2): 183-187.

References

Ecobichon DJ. Carbamate insecticides. In: Krieger R (ed). Handbook of Pesticide Toxicology. San Diego, CA: Academic Press,2001; p.1087-1106.

El-Demerdash FM, Yousef MI& Al-Salhen K S. Protective effects of isoflavone on some biochemical parameters affected by cypermethrin in male rabbits. Author information ,2003;38 (3): 365-78.

El-Demerdash FM, Yousef MI& Al-Salhen K S. Protective effectsof isoflavone on some biochemical parameters affected by cypermethrin inmale rabbits. Author information, 2003;38 (3): 365-78.

El-Desoky G, Abdelreheem M, Abdulaziz AO, ALOthmanZ, Mahmoud M, Yusuf K. Potential hepatoprotective effects of vitamin E and selenium on hepatotoxicity induced by malathion in rats, Afr J Pharm Pharmacol, 2012; 6:806−13.https://doi.org/10.5897/AJPP11.628.

El-Medany AH, Hagar HH. Effect of fluconazole on the fertility of male rabbits. Arzneimittelforschung, 2002;52:636–640. https://doi.org/10.1055/s-0031-1299943.

El-Shenawy N S. Oxidative stress responses of ratsexposed to Roundup and its active ingredient glyphosate.Environ. Toxicol. Pharmacol, 2009 ;28: 379–385.

Eman E. Elsharkawya, Mahmoud Abd El-Nassera, Aliaa A Bakheet . Mancozeb impaired male fertility in rabbits with trials of glutathione Environnementaux des Pesticides, Exemple du désherbage des cultures résistantes ou non au glyphosate, Innovations Agronomiques, Livre,2008; p. **3** : 121-143.

EPA., Environmental Protection Agency. Overview of the Ecological Risk Assessment Processin the Office of Pesticide Programs,U.S., Environmental Protection Agency http://www.epa.gov/espp/consultation/ecorisk-overview.pdf (Acessed 13 August 2014).

Eraslan G, Kanbur M, Silici S, Altinordulu S. & Karabacak M. Effecs of Cypermethrin on Some Biochemical Changes in Rats: The Protective Role of Protective Role of Propolis. Faculty of Veterinary Medicine, 2008;57 (5): 453-460.

Ewald G..Chronic measures of toxicant-induced effects on fish. Ann Zool Fennici, 1995;**32**:311-6.

Ewing RD. Diminishing returns: Salmon decline and pesticides. Funded by the Oregon pesticide education network, biotech research and consulting. Oregon: Oregon Pesticide Education Network ,1999.

Ezz L,Belhaji Salah I,Haous Z,Sakly A ,Grissa I,Chakroun S,Kerkeni E,Hassine M ,Mehdi M,Ben Cheikh H .Histopatholigical and genotoxic effects of chlorpyrifos in rats .Environ Sci Pollut Res Int ,2016 ;23 :4859-67 .

Farzeen S & Razia I. Impact of cypermethrin on blood profile, tissue redox parameters and the observation of histopathological changes in the liver of Rabbit (Oryctolagus cuniculus), Appl. Biol Pure, 2021; 11(2):465-479.

References

Favier A. Le Stress Oxydant, Intérêt Conceptuel et Expérimental dans la Compréhension des Mécanismes des Maladies et Potentiel Thérapeutique, l'Actualité Chimique,2003 **;** 109 – 115.

Fenik J, Tankiewicz M, Biziuk M. Properties and determination of pesticides in fruits and vegetables. Trends in Analytical Chemistry ,2011; **30**(6): 814-826.

Frank N. Dost. Cf. Acknowledgements. Pesticide testing for registration toxicity environmental behaviour and epidemiology, 2003; ISBN 0-7726-4981-2.

Gasnier C, Dumont C, Benachour N, Clair E , Chagnon M, Seralini, G.E. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines Toxicology ,2009 ;262 : 184–191.

George A, Katerina K, Georgia L, Christina M, Mara S, Konstantinos D, Alexandros D .The Effect of Glyphosate on Human Sperm Motility and Sperm DNA Fragmentation, Int.J.Environ. Res. Public Health ,2018;p6-8.

George A, Katerina K, Georgia L, Christina M,Mara S, Konstantinos D and Alexandros D. The Effect of Glyphosate on Human Sperm Motility and Sperm DNA Fragmentation.intrnational journal of environmental research and pulic health . Int.j.Envoron .Res.Public Health,2018 ; 15(1117) :8–5.

George j &shukla Y. Emptying of intracrllular calcium pool and oxidative stress imbalace are associated with the glyphosate-induced proliferation in human skin kerationocytes HaCaTcells , 2018 .

Ghada H,Abdel-Rahmen ,Abeer E,El-Metwally,Hanaa A,El-Hallawany , Amal HAli .Protecting role of selenium on the cytotocic effect of profenofos on Rabbit fertility , Alexandaria Journal of veterinary Sciences,2017;54(1):79-96.

Gholami M, Khayat ZK, Anbari K, Obidavi Z, Varzi A,Boroujeni MB, Alipour M, Niapoor A, Gharravi AM. Quercetin ameliorates peripheral nerve ischemia-reperfusion injury through the NF-kappa B pathway, Anat Sci Int. 2017; 92:330–37.

Gilden RC, Huffling K, Sattler B. Pesticides and health risks.,J.Obstet.Gynecol.Neonatal.Nurs, 2010; 39 (1): 103–110.

Goetz AK, Ren H, Schmid JE, Blystone CR, Thillainadarajah I, Best DS,Nichols HP, Strader LF, Wolf DC, Narotsky MG, Rockett JC, Dix DJ. Disruption of testosterone homeostasis as a mode of actionfor the reproductive toxicity of triazole fungicides in the male

Gonçalves BB, Nascimento NF, Santos MP, Bertolini RM, Yasui GS, Giaquinto PC. Low concentrations of glyphosate-based herbicide cause complete loss of sperm motility of yellowtail tetra fish Astyanax lacustris. J Fish Biol,2018 ;92(4):1218–1224.doi:10.1111/jfb.13571.

References

Goodbred SL, Gilliom RJ, Gross TS, Denslow NP, Bryant WL, Schoeb TR.**Griffith M, Barrows E, Perry S**. Effects of aerial application of diflubenzuron on emergence and flight of adult aquatic insects. J Econ Entomol,1996; **89**: 442-446.

Grisolia C, de Oliveira D, de Oliveira G. Ecotoxicological assessment of glyphosate-based herbicides: effects on different organisms. Environ. Toxicol. Chem. 36, 1755–1763. Williams, G.M., Berry, C., Burns, M., de Camargo, J.L., Greim, H., 2016b. Glyphosate rodent carcinogenicity bioassay expert panel review. Crit. Rev. Toxicol, 2017 ;**46** : 44–55.

Grover P, Danadevi K, Mahboob, M, Rozati, R, Banu, BS, Rahman MF. Evaluation of genetic damage in workers employed in pesticide production utilizing the Comet assay.Mutagenesis **,** 2003; 18 (2): 201–205.

Gupta PK. Herbicides and fungicides. In: Gupta RC(ed): Reproductive and developmental toxicology ,2011; (pp503-21).

Gupta PK. Toxicity of herbicides. In: GUPTA, R. C. (Ed) Veterinary toxicology: Basic and clinic principles. USA: Elsevier,2007; p.567-586.

Guyton K, LoomisZ, Grosse D, El Ghissassi Y, Benbrahim-Tallaa, GuhaL N, Scoccianti C, Mattock H,Straif K. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate (International Agency for Research on Cancer). Lancet Oncology ,2015; S1470-2045, (15).

Hauser R, Sokol R. Science linking environmental contaminant exposureswith fertility and reproductive health impacts in the adult male. Fertil. Steril, 2008 ;89, e59–e65.Health Perspect ,2010;118:1117–25.

Heba El-Sayed M, Samia A, Abd El-Baset, Asmaa A, Kattaia A, Rania A. Zidan& Mona M. A. Efficacy of naringenin against permethrin-induced testicular toxicity in rats. International journal of Experminatal pathology, 2016;97,37-49.

Hernández AF, Parrón T, Tsatsakis AM, Requena M, Alarcón R, López-Guarnido O..Toxic effects of pesticide mixtures at a molecular level: Their relevance to human Health. Toxicology, 2013;**307**: 136-145.

Heydens WF, Lamb IC, Wilson AGE, Chloroacetanilides. In: Krieger R (ed). Handbook of Pesticide Toxicology. San Diego, CA: Academic Press, 2001; p. 1543–1558.

Ho IK, Fernando JC, Sivam SP, Hoskins B. Roles of Dopamine and GABA in neurotoxicity of organophosphorous cholinesterase inhibitors. Proceedings of the Western Pharmacoly Society,1984;. **27**: 177-180.

Hocine L, Merzouk H, Merzouk SA & Ghorzi H. The Effects of Alpha Cypermethrin Exposure on Biochemical and Redox Parameters in Pregnant Rats and their Newborns. Pestic Biochem and Phys ,2016;134: 49-54.

Hocine L, Merzouk H, Merzouk SA & Ghorzi H. The Effects of AlphaCypermethrin Exposure on Biochemical and Redox Parameters in Pregnant Rats and their Newborns. Pestic Biochem and Phys, 2016 ;134: 49-54.

References

Hori Y, Tanaka T, Fujisawa M, Shimada Koxicokinetics of DL-glufosinate enantiomer in human BASTA poisoning. Biological and Pharmaceutical Bulletin, 2003; **26**:540-543.

Husak VV, Mosiichuk NM, Maksymiv IV, Sluchyk IY, Storey JM, Storey KB, et al. Histopathological and biochemical changes in goldfish kidney due to exposure to the herbicide Sencor may be related to induction of oxidative stress. Aquat Toxicol, 2014; **155**: 181-9.

IARC. Report of the Advisory Group to Recommend Priorities for IARC Monographs during; Internal Report 14/002; International Agency for Research on Cancer: Lyon, France,2015-2019 ;60p.

IARCGlyphosate. Internal Report 14/002; International Agency for Research on Cancer: Lyon, France, 2015; p 60.

Inoue Y, Onodera M, Fujita T, Fujino Y, Kikuchi S, Endo S..Factors associated with severe effects following acute glufosinate poisoning. Clinical Toxicology,2013; 51: 846-849.

INRASciences & Impact, R4P (Réseau de Réflexion et de Recherches sur les Résistances aux Pesticides), ANSES (Agence National de Sécurité Sanitaire Alimentation, Environnement, Travail), Fongicides Agricoles : Modes d'Action, Mécanismes de Résistance et Exemples par Filière, Colloque Résistance aux Pesticides à Montréal, Site web : https://irda.blob.core.windows.net.

Issam C, Zohra H, Monia Z. et al.Effects of dermal subchronicexposure of pubescent male rats to permethrin (PRMT) on the histological structures of genital tract, testosterone and lipoperoxidation. Exp. Toxicol. Pathol, 2011; 63:393–400.

Jaga, K, Dharmani C. toxicity from pesticide exposure: a recent review. Environ. Health Prev.Med, 2006; 11 (3), 102–107.

Jansons M, Pugajeva I, Bartkevičs V. Occurrence of glyphosate in beer from the Latvian market. Food Addit Contam Part Chem Anal Control Expo Risk Assess, 2018;35(9):1767–1775.doi:10.1080/19440049.2018.1469051.

Joshi SC, Gulati N, Gajraj A. Evaluation of toxic impacts of mancozeb on testisin rats. Asian J. Exp,2005; Sci. 19, 73–83.

K. Larsena b R, Najlea A,Lifschitzb , Virkel G .Effects of sub-lethal exposure of rats to the herbicideglyphosate in drinking water: Glutathione transferaseenzyme activities, levels of reduced glutathioneand lipid peroxidation in liver, kidneys and small intestine .Environmental toxicology pharmacology, 2 0 1 2 ; 3 4 :811–818.

Kalender S, Ogutcu A, Uzunhisaracikli M, Acikzoz F, Durak D, Ulusoy Y & Kalender Y.Diazonin induced hepatotoxicity and protective effect of vitamins E on some biochemical indices and ultra-structural changes.Toxicology ,2005;21: 197-206.

Kalender S, Ogutcu A, Uzunhisarcikli M, Acikgoz F, Durak D, Ulusoy Y& Kalender Y. Diazinon–induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes. Toxicology Aug 1, 2005;211 (3): 197-206.

References

Karalliedde, L., Feldman, S., Henry, J., Marrs, T (editors). Organophosphates and healthو London: Imperial College Press, 2001.

Kempinas WD1, Suarez JD, Roberts NL, Strader L, Ferrell, J, Goldman JM,Klinefelter GR. Rat epididymal sperm quantity, quality, and transit time after guanethidine-induced sympathectomy. Biol. Reprod,1998 ;59 (4), 890–896.

Khalifa FK, Khalil FA, Barakat HA. &Hassan MM. ProtectiveRole of Wheat Germ and Grape Seed Oils in Chlorpyrifos-Induced OxidativeStress, Biochemical and Histological Alterations in Liver of Rats. AustralianJournal of Basic and Applied Sciences ,2011;**5** (10): 54-66.

Khan ZM, Law FCP. Adverse effects of pesticides and related chemicals on enzyme and hormone systems of fish, amphibians and reptiles: A review. Proc Pakistan Acad Sci**,** 2005; 42:315-23.

Kidd S, Eskenazi B, Wyrobek A. Effects of male age on semen quality and fertility: a review of the literature. Fertility and sterility, 2001;75(2): 237-48.

Klaassen, C.D &Watkins, J.B (Editors), Casarett & Doull's ,(1999). Toxicology The basic science of poisons (fifth edition). New York: McGraw-Hill Companies.

Knutson RD, Taylor CR, Penson JB, Smith EG. Economic impacts of reduced chemical useو Knutson and Associates, College Station, Texas,1990.

Koprucu K, Yonar SM & Seker E. Effects of cypermethrin on antioxidant status, oxidative stress biomarkers, behavior, and mortality in the freshwater mussel Unio elongatulus eucirrus. Fish Sci, 2010 ;76(6): 1007-1013.

Koprucu K, Yonar SM & Seker E. Effects of cypermethrin on antioxidant status, oxidative stress biomarkers, behavior, and mortality in the freshwater mussel Unio elongatulus eucirrus. Fish Sci ,2010;76(6): 1007-1013.

Krieger R, Doull J, Hemmen JV, Hodgson E, Maibach H, Reiter L, Ritter L, Ross J, Slikker W, Vega H. Hayes Handbook of Pesticide Toxicology, Third Edition, 2010 ; Volume 1, Academic Press .

Ksheerasagar RL, Kaliwal BB. Temporal effects of mancozeb on testes, accessory reproductive organs and biochemical constituents in albino mice. Environ.Toxicol. Pharmacol,2003;15: 9–17.

Ksheerasagar RL, Kaliwal BB.Effect of mancozeb on thyroid, testis, accessory reproductive organs and biochemical constituents in albino mice. Recent Res. Sci.Technol,2010 ; 2: 7–17.

Kwiatkowska M, Nowacha-Krukowska H, Bukowska B. The effects of glyphosate, its metabolites and impurities on erythrocyte acetylcholinesterase activity. Environmental Toxicology and Pharmacology,2014; **37**: 1101-1108.

Kwiatkowska M,Huras B& Bukowska B.The effect of metabolites and impurities of glypgosate on human erythrocytes (in vitro).Pesticide Biochem and Phisiol,2014 ;109 :34-43.

References

Lakkawar AW, Chattopadhyay SK, Somvanshi R& Sharma AK. Experimental cypermethrin toxicity in young rabbits-a hematological andbiochemical study. Praxis veterinaria ,2006;**54**: 195-202.

Larsen SB, Giwercman A, Spano M, Bonde JP. A longitudinal study of semen quality in pesticide spraying Danish farmers. The ASCLEPIOS Study Group. Reprod Toxicol,1998; 12:581–589.

Lee HL, Chen KW, Chi CH, Huang JJ and Tsai LM. Clinical presentations and prognostic factors of a glyphosate-surfactant herbicide intoxication: a review of 131 cases. Acad Emerg Med, 2000;7(8):906-10.

Li B,Ma Y,Zang YH.Oxidative sterss and hepatotoxity in the frog ,Rana chensinesis,when exposed to low doses of trichlorfon ,J Environ Sci HealthB,2017 ;52 :476-82 .

Li T,Chiang JY.Bile acid signaling in metabolic disease and drug therapy .Pharmacol Rev ,2014 ;66 : 948-83.

Lifeng T, Shoulin W, Junmin J et al. Effects of fenvalerate exposure on semen quality among occupational workers. Contraception ,2006;73: 92–96.

Litwack G. Biochemistry of hormones: steroid hormones, text book of biochemistry with

Lopes FM, Varela Junior AS, Corcini CD, da Silva AC, Guazzelli VG, Tavares G, da Rosa CE. Effect of glyphosate on the sperm quality of zebrafish Danio rerio. Aquat Toxicol Amst Neth, 2014 ;155:322–326. doi:10.1016/j.aquatox.2014.07.006.

Lushchak VI. Glutathione homeostasis and functions:potential targets for medical interventions. J AminoAcids. 2012;2012:736-837.

Maduenho L & Martinez C. Acute effects of diflubenzuron on the freshwater fish Prochilodus lineatus**. Comparative Biochemistry and Physiology, Part C**, 2008 ;**148:** 265–272.

Mahendrakar K, Venkategowda PM, Rao SM and Mutkule DP. Glyphosate surfactant herbicide poisoning and management. Indian J Crit Care Med, 2014;8(5):328–330.

Maher P. The effects of stress and aging on glutathione metabolism. Ageing Res Rev, 2005;4:288-314.

Mamy L, Barriuso E, Gabrielle B, Marrs TC, Maynard RL .Evaluer les Risque Neurotransmission system as targets for toxicants: a review. Cell Biology and Toxicology ,2013; 29: 381-396.

Manna S, Bhattacharyya D, Basak DK& Mandal T K. Single oral dose toxicity study of α-cypermethrin in rats. Indian Journal of Pharmacology,2004;36 (1): 25-28.

Matthews G, Bateman R, Miller P. Book Pesticide application methods. John Wiley & Sons. Available http://onlinelibrary from. wiley,2008.

References

Matviishyn TM, Kubrak OI, Husak VV, Storey KB, Lushchak VI,Mayhew DA, Taylor GD, Smith SH, Banas DA . Twenty four month combined chronic oral toxicity and oncogencity study in rats utilizing atrazine technical grade. Lab Impact of Pesticides on Environmental and Human Health http://dx.doi.org/10.5772/59710 225 Study No.: 410-1102, Accession No.262714-262727, American Biogenics Corp., Decatur, 1986; p. 2-6.

McMartin KE, Ambre JJ and Tephly TR. Methanol poisoning in human subjects. Role for formic acid accumulation in the metabolic acidosis. Am J Med,1980;**68**(3):414-8.

MDRGF. Mouvement pour le Droit et le Respect des Générations Futures, 2008, Dossier pesticides.http://www.mdrgf.org/archive.html.

Meenakshi, Sharon P, Bhawana M, Anita S, VK G. A short review on how pesticides affect human health. Int. J. Ayurvedic Herbal Medic,2012; **5** :935–946.

Mehmet E D, Serap D, Mehmet D, Yasemin T. IN VITROeffects of some pesticides on glutathione –s transferase activity. Fresenius Environmental Bulletin,2018; 26:8023-8029.

Melissa J.Effects of environmental and occupational pesticide exposure on human sperm.Human Reproduction Update ,2008; 14(3): 233–242.

Mesnage R, Defarge N, Spiroux de Vendômois J, Séralini GE. Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc,2015; 84:133–153. doi:10.1016/j.fct.2015.08.012.

Mhya DH, Umar IA. & Onyike E. Anti-peroxidative and Biochemical Protective Activity of Khaya Senegalensis Stem Bark Extract on Rats Fed Pesticide infused feed. International Journal of Pharma Sciences and Research ,2014;**5** (7): 385-390.

Ministry of Agriculture (MOA). British Columbia, Canada. (Available at),2015**;** http://www.agf.gov.bc.ca/pesticides/b_2.html2.

Mokrani K. Favorisées par la commercialisation anarchique des produits à usage domestique, les intoxications par pesticides, un problème de santé publique. J. La Tribune (Algeria), editionof 20 November 2005.

Mossa AH & Abbassy MA . Adverse Haematological and Biochemical Effects of Certain Formulated Insecticides in Male Rats. Journal of Environmental Toxicology, 2012 ;6(4) : 160 - 168.

Mostafalou S, Abdollahi M. Environmental pollution by mercury andrelated health concerns: renotice of a silent threat. Arh. Hig. Rada Toksikol. , 2013b; **64**:179–181.

Mostafalou S, Abdollahi M. Pesticides and human chronic diseases: Evidences, mechanisms, and perspectives. Toxicology and Applied Pharmacology,2013; **268**:157-177.

Mostafalou S, Abdollahi M.. Current concerns on genotoxicity of pesticides'.J. Pharmacol,2012a;**8**:473–474.

References

MostafalouS, Abdollahi M. Current concerns on genotoxicity of pesticides. Int. J. Pharmacol, 2012c; 8 (6): 473–474.

Najafi Gh, Hobenaghi R, Hoshyari A, Moghadaszadeh, Ghorbanzadeh B. The effect of atrazine on spermic parameters and fertility potential in mature rats. Arak Medical University Journal, 2013; 15(69): 85-94.

Nancy L. Swanson1 PhD, Judy Hoy2 BS and Stephanie Seneff3 PhD. Evidence that glyphosate is a causative agent in chronic sub-clinical metabolic acidosis and mitochondrial dysfunction ,2016; international journal of human and nutrition functional medcine.International Journal of Human Nutrition and Functional.

Ncibi S, Ben Othman M, Akacha A, Krifi MN & Zourgui L. Opuntia ficus indica extract protects against chlorpyrifos-induced damage onmice liver. Food and Chemical Toxicology ,2008;**46** (2): 797-802.NeuroToxicology, 2016**;**52:150-161.

Niimi AJ. Review of biochemical methods and other indicators to assess fish health in aquatic ecosystems containing toxic chemicals. J Great Lakes Res,1990;**16:**529-41.

Nuntah JN, Abolagba OJ, Igene JO, Usifoh SF, Omoti CE & Usifoh CO. Organochlorine Pesticides Quantification in Smoke-Dried Fish Samples and Extrapolative Toxicity Effect in Rabbit Samples. South Asian Res J Bio Appl Biosci; Vol-2 , 2020: 54-64.

OMS. Manuel de laboratoire de l'OMS analyse du sperme humain et de l'interaction des on proteins Utilising the Principe of protein–dye-binding. Anal Biochem,,oxygen receptor. Ann Clin Biochem, 1969; 6: 24-7.

Owagboriaye FO, Dedeke GA, Ademolu KO, Olujimi OO, Ashidi JS, Adeyinka AA. Reproductive toxicity of Roundup herbicide exposure in male albino rat. Exp Toxicol Pathol OffJ Ges Toxikol Pathol,2017;69(7):461–468. doi: 10.1016/j.etp.2017.04.007.

Palanza P, Nagel SC, Parmigiani S, vom Saal FS. Perinatal exposure to endocrine disruptors: sex, timing and behavioral endpoints. Curr Opin Behav Sci,2016; 7:69–75.doi: 10.1016/j.cobeha.2015.11.017.

Parinya P, Chandresh L, Dana B B, Tippawan Pr, John D. Meeker, Priya E S, Héctor M.Investigation of Associations Between Exposures to Pesticides and Testosterone Levels in Thai Farmers 4, Arch Environ Occup Health. 2018, 04; 73(4): 205–218.

Park JS, Kwak SJ, Gil HW, Kim SY, Hong SY. Glufosinate Herbicide Intoxication Causing Unconsciousness, Convulsion, and 6th Cranial Nerve Palsy. Journal of Korean Medical Science,2013;**28**: 1687-1689.

Parvez S, Gerona RR, Proctor C, Friesen M, Ashby JL, Reiter JL, Lui Z, Winchester PD. Glyphosate exposure in pregnancy and shortened gestational length: a prospective Indiana birth cohort study. Environ Health, 2018;17(1):23. doi:10.1186/s12940-018-0367-0.

References

Patil N V, Lonare M K, Sharma M, Lalhriatpuia P C, Saini1 SP S and Rampa S. Hemato-Biochemical Alterations Mediated by Carbendazim Exposure and Protective Effect of Quercetin in Male Rats.Toxicology International, 2018 ; 25(1) : 7-18.

Patterson M. Diflubenzuron. Analysis of risks to endangered and threatened salmon and steelhead. EPA, Environmental Field Branch. Office of Petsicide Programs., USA , 2004.

Paudyal BP. Organophosphorus poisoning. Journal of Nepal Medical Association ,2008; 47(172): 251-258. 224 .

Penney, D.G. (Editor). Carbon Monoxide Poisoning. Boca Raton: Taylor & Francis, CRC Press,2008.

Perry MJ, Venners S A, Chen X, Liu X, Tang G, Xing H et al. Organophos-phorous pesticide exposures and sperm quality. Reprod. Toxicol,2011 ;31 : 75–79.

Perry, M.J. Effects of environmental and occupational pesticide exposure onhuman sperm: a systematic review. Hum. Reprod. Update, 2008;**14**: 233.

Petrelli G, Mantovani A. Environmental risk factors and male fertility and reproduction. Contraception, 2002; 297–300.

Pimentel D, Pimentel M. Food Energy and Society.3d Ed. CRC Press: Taylor and Francis Group, LLC , (2008). P 402.

Poletta GL, Larriera A, Kleinsorge E, Mudry MD. (2009). Genotoxicity of the herbicide formulation Roundup® (glyphosate) in broad-snouted caiman (Caiman latirostris) evidenced by the Comet assay and the Micronucleus test. Mutation Research; **672**: 95-102.

Pretty J. The Pesticide Detox: towards a more sustainable agriculture. Earth **Solomon G, Ogunseitan OA, Kirsch J (2000**). Pesticides and Human Health: A Resource for Health Care Professionals. Physicians for Social Responsibility and Californians for Pesticide Reform.www.igc.org/cpr.can,2005.rat. Toxicol Sci ,2006;95:227–239. https://doi.org/10.1093/toxsci/kfl124.

Recio-Vega R, Ocampo-Gómez, G, Borja-AburtoVH, Moran-Martínez J, Cebrian-Garcia ME. Organophosphorus pesticide exposure decreases sperm quality: association between sperm parameters and urinary pesticide levels.J. Appl. Toxicol,2008; 28: 674–680.

Rehimi N. “Etude de La Reproduction Chez Culex pipiens pipiens. Aspects: Morphologique, Ethologique et Physiologique. Effets de Quelques Inhibiteurs du Déve- loppement sur Quelques Paramètres Biologiques,” Ph.D. Thesis, Annaba University, Algeria, 2004.

Riaz M & Yousafzai AM . Toxic Effects of Insecticides Malathion and Cypermethrin on Hematological Parameters in Blood of Male Rabbits ,2017.

Richard S, Moslemi S, Sipahutar H, Benachour N, Seralini G-E. Differential effects of glyphosate and roundup on human placental cells and aromatase. Environ Health Perspect,2005;113(6):716–720.

References

Richard S, Moslemi S, Sipahutar H, Benachour N, SeraliniG . Differential effects of glyphosate and roundup on human placental cells and aromatase. Environ Health Perspect,2005 ;**113** : 716–720.

Romano M, Bernardi M,Furtado P, Oliveira C . Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. Archives of Toxicology, 2010 ; **84** (4) :309-317.

Romano MA, Romano RM, Santos LD, Wisniewski P, Campos DA, de Souza PB, Viau P, Bernardi MM, Nunes MT, de Oliveira CA. Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression. Arch Toxicol,2012; 86(4):663–673.doi:10.1007/s00204-011-0788-9.

Romano RM, Romano MA, Bernardi MM, Furtado PV, Oliveira CA. Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. Arch Toxicol,2010; 84(4):309–317. doi:10.1007/s00204-009-0494-z.

Sakr SA. & Albarakai AY. Effect of cinnamon on cypermethrininducednephrotoxicity in albino rats. International Journal of AdvancedResearch ,2014;**2** (7): 578-586.

Salvatore AL, Bradman A, Castorina R, et al. Occupational behaviors and farmworkers' pesticide exposure: findings from a study in Monterey County, California. Am. J. Ind. Med, 2008; **51**: 782–794.

Samana N,Razia I,Aftab A,Muhammad FM,Muhammad J,Rachid Y,Muhammad S,AbidH,Thir A,Sayed UH,Abdul R.Effect of glyphosate on hematological and biochemical parameters of Rabbit (oryctolagus cuniculus).pur Appl.Biol, 2019;8(1) :78-92.

Sampogna RV,Cunard R. Roundup intoxication and a rationale for treatment. Clinical Nephrolpgy,2007; **68**(3): 190-6.

Samsel A, Seneff S. Glyphosate's suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: pathways to modern diseases. Entropy, **2013 ;**15 ;(4) : 1416-1463.

Samsel, A&Seneff S. Glyphosate's suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: pathways to modern diseases. Entropy, **2013;**15:(4): 1416-1463.

Sanborn M, Cole D, Kerr K, Vakil C, Bassil K, Sanin LH . Pesticides Literature Review.The Ontario College of Family Physicians, 2004 .

Sankar P, Telang AG& Manimaran A. Protective effect ofcurcumin on cypermethrin-induced oxidative stress in Wistar rats.Experimental and Toxicologic Pathology,2012;**64** (5):487-93.

Sauter H. Mitochondrial respiration as a target for antifungals: Lessons from research on strobilurins. Antifungal Agents, Discovery and Mode of Action, 1995;173–191.

References

Saxena P& Saxena AK. Cypermethrin Induced BiochemicalAlterations in the Blood of Albino Rats. Jordan Journal of Biological Sciences, 2010; **3** (3): 111-114.Sciences, 2017 ;24 (6) : 1162–1171.

Scott GR, Sloman KA. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquat Toxicol,2004;**68**:369-92.

Scott W, Connie M, Peter C. A review of pesticide exposure and cancer incidence in the agricultural health study cohort. Environ Health Perspect ,2010;118:1117–25.

Shadnia, S, Azizi, E, Hosseini, R, Khoie, S, Fouladdel, S, Pajoumand, A, Jalali, N, Abdollahi, M. Evaluation of oxidative stress and genotoxicity in organophosphorous insecticides formulators. Hum. Exp. Toxicol,2005;**24**: 439–445.

SharmaY, Bashir S , Irshad M, Nag T C, Dogra TD. Dimethoate-indiced effects on antioxidant status of liver and brain of rats following subchronic exposure. Toxicology ,2005 ;215 :173.

Shin MO& Moon JO. Effect of dietary supplementation of grapeskin and seeds on liver fibrosis induced by dimethyl nitrosamine in rats. Nutrition Research and Practice ,2010;**4** (5): 369-374.

.

Sogorb MA, Vilanova E . Enzymes involved in the detoxification of organophosphorus, carbamate and pyrethroid insecticides through hydrolysis. Toxicology Letters,2002; **128**: 215-228.

Soltani N. &Rehimi N. "Laboratory Evaluation of Andalin. A New Insecticide Interfering with Cuticle Deposition, against Culex pipiens," Journal Algérien de Medecine, Vol. 11, 2001, pp: 28-33.

Somayyah KM, Ahmed A, Hamid RR and Mohammad A. Adverse effects of organophosphorus pesticides on the liver : a brief summary of four decades of research. Arch Hig Rada Toksikol,2017;68:261-275.

Souheila S, Mohamed Salah B, Cherif A. Pesticide exposure and reproductive biomarkers among male farmers from north-east Algeria. Annals of Biological Research, 2011; 290-297. 93 10.

Souza, MS, Magnarelli GG, Rovedatti MG, Santa Cruz S, Pechen De D'AngeloA M. prenatal exposure to pesticides: analysis of human placental acetylcholinesterase, glutathione s-transferase and catalase as biomarkers of effect. Biomarkers, 2005 ;10(5) :376-389.

Sténuit J, Van Hammée ML. Aperçu sur l'épidémiologie des pesticides ,2008 .

TabbMM, Blumberg B. New modes of action for endocrine-disrupting chemicals. Mol.Endocrinol., 2006; 20: 475-482.

References

Taguig A,Boubsil S,Abdenour C,Boulakoud MS.Study of the subacute toxicity of a fungicide on rabbit (Batna region). J Adv Pharm Edu Res, 2020 ;10(2) :104-109.

Taib I, Budin S, Ghazali A, Jayusman P, Mohamed J. Fenitrothion alters sperm characteristics in rats: Ameliorating effects of palm oil tocotrienol-rich fraction. Exp. Anim 2014; 63(4): 383–393.

Tamas K& Peter S. On glyphosate; Scientific journal of the European Ecocyles Society ,2016.

Tawfik MS& Al-Badr N. Adverse Effects of Monosodium Glutamate on Liver and Kidney Functions in Adult Rats and Potential Protective Effect of Vitamins C and E. Food and Nutrition Sciences ,2012;**3**: 651-659.techniques. Harper and Row. 2nd Ed. 1974.

Tollefsen KE. Interaction of estrogen mimics, singly and in combination, with plasma sex steroid-binding proteins in rainbow trout (Oncorhynchus mykiss). Aquat Toxicol,2002;**56**:215-25.Toxin Rev, 2017; 36(1): 1–6.

Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJ, et al..Male reproductive health and environmental xenoestrogens. Environ Health Perspect, 104 1996 ,(4):741–803.

Toygar M, Aydin I, Agilli M, Aydin FN, Oztosun M, Gul H, Macit E, Karslioglu Y, Topal T, Uysal B, Honca M. The relation between oxidative stress, inflammation, and neopterin in the paraquat-induced lung toxicity. Human and Experimental Toxicity, 2014; In press. Ver volume ou colocar link.

Tsui MTK, Chu LM. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. Chemosphere, 2003;52(7):1189– 1197. doi:10.1016/S0045-6535(03)00306-0.

U.S. Environmental Protection Agency (USEPA Pesticides: Health and Safety. National Assessment of the Worker Protection Workshop #3. (Available at), 2007; http:// www2.epa.gov/pesticide-worker-safety Understanding the Inactivation Process of Organophosphorus Herbicides: A DFT Study of Glyphosate Metallic Complexes with Zn 2+, Ca 2+, Mg 2+, Cu 2+, Co 3+, Fe 3+, Cr 3+, and Al 3+. Int J of Quant Chem,2012;**112**:2752–2762.

Unsworth. History of pesticide use. International Union of pure and applied chemistry (IUPAC). http://agrochemicals. obi2Task=sobi2Details&catid=3&sobi2Id=31.

Uren Webster TM, Laing LV, Florance H, Santos EM. Effects of glyphosate and its formulation, roundup, on reproduction in zebrafish (Danio rerio). Environ Sci Technol,2014; 48(2):1271–1279. doi:10.1021/es404258h.

USEPA. United States of Environmental Protection Agency. About pesticides. U.S. EPA. http://www.epa.gov/pesticides/about/index.htm (Accessed 27 August 2014).

Veerappan M, Hwang I. & Pandurangan M. Effect of cypermethrin, carbendazim and their combination on male albino rat serum. International Journal of Clinical and Experimental Pathology ,2012;**93**: 361-369.

References

Volodymyr I. Lushchaka, Tetiana M. Matviishyna, Viktor V. Husaka, Janet M. Storeyb, Kenneth B. Storeyb.Pesticide toxicity: A mechanistic achapproa**.** EXCLI Journal 2018;**17:**1101-1136 .

Wael M. Al-Amoudi. Hematological and Biochemical Effects of Metalaxyl Fungicide on Mice Albino American Journal of Biochemistry, 2012; 2(5): 62-66.

Ware G, Whitacre D . The Pesticide Book. 6th Ed. Meister Pro Information Resources, (2004).

WHO. Pesticides: Children's Health and the Environment, (2008).

Whorton M. Male occupational reproductive hazards. In: ZENZ C. (Editor). Occupational medicine, 3th Edition. Mosby,1994; 2: 870-3. 12.

Williams GM, Kroes R, Munro IC. Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. Regulatory Toxicology and Pharmacology,2000; **31**: 117-65. 226 .

Wissem M, Aziza I, Hadj H, Aicha B, Aghleb B, Olivier T et al. Effect of Endocrine disruptor pesticides. Int. J. Environ. Res. PublicHealth, 2011; 8: 2265-2303.

Wong F, Wilcox W. Comparative physical modes of action of azoxystrobin, mancozeb, and metalaxyl against Plasmopara viticola (grapevine downy mildew). Plant Dis,2001; **85**(6) : 649–656.

Yang JD, Liu SH, Liao MH, Chen RM, Liu PY, Ueng TH. Effects of tebuconazole on cytochrome P450 enzymes, oxidative stress, and endocrine disruption in male rats. Environ Toxicol ,2018;33(899):907.https://doi.org/10.1002/tox.22575.

Yousef MI, Demerdash FM, Kamel KI & Salhen KS. Changes in some hematological and biochemical indices of rabbits induced by isoflavones and cypermethrin. Toxicology, 2003 ;189(3): 223-234.

Yousef MI, Salem MH, Ibrahim HZ, Helmi S, Seehy MA, Bertheussen K. Toxic effects of carbofuran and glyphosate on semen characteristics in rabbits. J Environ Sci Health B;1995;30(4):513–534. doi:10.1080/03601239509372951.

Yucra S, Rubio J, Gasco M, Gonzales C, Steenland K, Gonzales G F. Semenquality and reproductive sex hormone levels in Peruvian pesticide sprayers. Int.J. Occup. Environ. Health ,2006; 12 : 355–361.

Zama D, Meraihi Z, Tebibel S, Benayssa W, Benayache F, Benayache S,Vlietinck AJ. Chlorpyrifos-induced oxidative stress and tissue damagein the liver, kidney, brain and fetus in pregnant rats: The protective role of the~74~butanolic extract of Paronychia argentea L. Indian Journal of Pharmacology**,** 2007;**39** (3): 145-150.

Zeljezic D, Garaj-Vrhovac, V. Chromosomal aberration and single cell gel electrophoresis (Comet) assay in the longitudinal risk assessment of occupational exposure to pesticides. Mutagenesis, 2001;**16** (4): 359–363.

References

Zorriehzahra MJ, Hassan MD, Gholizadeh M & Saidi AA . Study of some hematological and biochemical parameters of rainbow trout (Oncorhynchus mykiss) fry in western part of Mazandaran province, Iran. Iran J Fish Sci, 2010