Faculty of Veterinary Medicine

Assuit University

Oxidative Stress and Genotoxicity of Nano Lambda-Cyhalothrin in albino rats

Thesis Submitted by

Aliaa Ahmad Bakheet

M.Sc. of Veterinary Medicine 2017

For the degree of

PhD

In Forensic Medicine and Toxicology

Under supervision of

Prof. Dr. Mahmoud Abd El-Nasser Aly

Professor of Forensic Medicine and Toxicology

Faculty of Veterinary Medicine

Assuit University

Prof. Dr. Eman Ezz Eldawela Elsharkawy

Professor of Forensic Medicine and Toxicology

Faculty of Veterinary Medicine

Assuit University

Prof. Dr. Doha Yahia Ahmed Ahmed

Professor and Head of Department of Forensic Medicine and Toxicology

Faculty of Veterinary Medicine

Assuit University

Prof. Dr. Manal Mohamed Sayed

Chief Researcher of Toxicology

Animal Health Research Institute Assuit

2022

﴿ اللّهُ لاَ إِلَهَ إِلاَّ هُوَ الْحَيُّ الْقَيُّومُ لاَ تَأْخُذُهُ سِنَةٌ وَلاَ نَوْمٌ لَّهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الأَرْضِ مَن ذَا الَّذِي يَشْفَعُ عِنْدَهُ إِلاَّ بِإِذْنِهِ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ وَلاَ يُحِيطُونَ بِشَيْءٍ مِّنْ عِلْمِهِ إِلاَّ بِمَا شَاء وَسِعَ كُرْسِيُّهُ السَّمَاوَاتِ وَالأَرْضَ وَلاَ يَؤُودُهُ حِفْظُهُمَا وَهُوَ الْعَلِيُّ الْعَظِيم ۝٢٥٥ ﴾

[سوره البقرة الايه ٢٥٥]

List of abbreviations

Abbreviation	Full word
LD_{50}	Median Lethal Dose
2,4-D	2,4 dichlorophenoxyacetic acid
2D	Two-dimensional
3-PBA	3-phenoxybenzoic acid
Ag	Silver
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
b.wt.	Body Weight
C. pipiens	*Culex pipiens*
CAS	Chemical Abstracts Service
CAT	Catalase
CFMP	3-(2-chloro-3,3,3-trifluoroprop-1-enyl) 2,2 dimethylcyclopropane carboxylic acid
CN	C≡N functional *group,* known as *cyano group*
Comet	Single-Cell Gel Electrophoresis
Cu	Copper
CXC chemokine	A subfamily of the chemokine
CYP450s	Cytochromes P450

DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic acid
ECF	The extracellular fluid
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
FT-IR	Fourier Transform Infrared Spectroscopy
G6PDH	Glucose-6- phosphate dehydrogenase
GSH-Px	Glutathione peroxidase
GSR	Glutathione reductase
GSH	Reduced L-glutathione
GSTs	Glutathione S-transferases
H&E	Haematoxylin and Eosin
HGB	Haemoglobin concentration
HCT	Haematocrit
HSP-70	Heat shock protein
IFN-γ	Interferon –γ
IgM	Immunoglobulin M
ILs	Interleukins
in vivo	Within the living organisms or cells
LC	Lambda-cyhalothrin

LCH	Lambda-cyhalothrin (λ cyhalothrin)
LC-MCs	Lambda-cyhalothrin –microcapsule
LCN	Lambda-cyhalothrin Nano particles
LGT	Low melting Agarose
LPO	Lipid peroxidation
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Main corpuscular volume
MDA	Malondialdehyde
Milli-Q Water	Water purified using a Millipore Milli-Q lab water system
MPV	Mean platelets volume
mRNA	Messenger Ribonucleic Acid
MSNs	Mesoporous silica Nano-pesticides
mV	Millivolts
Nano	Unit prefix meaning "one billionth ", denotes a factor of 10^{-9}
NC	Nanocarrier
NF-κB,	Nuclear factor kappa B
NMs	Nano-materials
NO	Naitric Oxide

NPs	Nano-pesticides
O. niloticus	*Oreochromis niloticus*
$0H^-$	Hydroxyl radicals
OTM	Olive tail moment
PCO	Protein carbonyl
PDI	Poly Dispersity Index
PLT	Platelets count
POD	Total peroxidase
PUFA	Polyunsaturated fatty acids
RBCs	Red blood cells count
RDW	Red blood cells distribution width
RNDP	Responsive nanoscale delivery platform
ROS	Reactive oxygen species
rpm	Revolutions Per Minute
S.E	Standard error
SCA	Structural chromosomal aberration
SOD	Superoxide dismutase
TAC	Total antioxidant capacity
TM	Tail moment
TBARS	Thiobarbituric acid reactive substances
TEM	Transmission Electron Microscopy

tGSH	Total intracellular glutathione
Ti	Titanium
TLC	Total leukocyte count
TLR-7	Toll-like receptors
TNF α	Tumor necrosis factor alpha
US EPA	Enviromental protection agency
UV	*Ultraviolet* radiation
WBCs	Total white blood cells count
WHO	World Health Organization
WTP	Willingness-to-pay
µg/g	Micrograms Per Gram
µg/L	Micrograms Per Liter
ng/mL	Nano Gram Per Mili Liter
µm	Micro metter
nm	Nano metter
Pg	Picograms or 10^{-12} grams
g/dL	Grams/deciliter
fL	Femtoliters or 10^{-15}Liter

List of Tables

List of Figures

List of photomicrographs

Contents

Content

Acknowledgement

Acknowledgement

First of all, my great thanks to **ALLAH** for all the things I have.

I believe that my humble words not sufficient to express my thanks and indebtedness to my professor, ***Prof. Dr. Mahmoud Abd El-Nasser Aly***, Professor of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Assuit University for, his kind endless help, creative and overflowing knowledge and advice, keen interest in directing and supervision, perfect guidness in this work and any attempt to define my indebtedness to him will be far from complete.

I am greatly honored to express my deepest gratitude and thank to ***Prof. Dr. Eman Ezz Eldawela Elsharkawy*** Professor of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Assuit University for, her constructive supervision, follow up, moral help, continuous support and creative advice, she spent a lot of time in helping me, she gave me a big aid and support in this work.

I am greatly honored to express my deepest gratitude and thank to ***Prof. Dr. Doha Yahia Ahmed*** Professor and Head of Forensic Medicine and Toxicology department, Faculty of Veterinary Medicine, Assuit University for, her constructive supervision, continuous support and creative advice.

I express my thanks to ***Prof.Dr.Manal Mohamed Sayed***, Chief Researcher, Animal Health Research Institute, for, her advice and help.

I express my thanks to ***Prof. Dr. Sary Khalil Abdel-Ghaffar***, Professor of Pathology and Clinical pathology department, Faculty of Veterinary Medicine, Assuit University for, moral help, continuous support and creative advice.

I express my thanks to ***Prof. Dr. Gamal S Zayed***, Professor of Pharmaceutics and Pharmaceutical Technology, department of Pharmaceutics and Industrial Pharmacy, Faculty of pharmacy, Alazhar University, Assiut branch, for creative advice. He spent a lot of time in helping me in the preparation of nano form of lambda cyhalothrin, he gave me a big aid and support in this work.

Finally I wish to thank all staff members and colleagues in the Forensic Medicine and Toxicology department, Faculty of Veterinary Medicine, Assuit University for, their continuous encouragement.

Dedication

I dedicate this work to

My great Mother and Father for their support, encouragement and helping to find my steps in life. Thanking them is never enough.

My husband, for his support.
Finally, I would like to dedicate this work to my lovely son and daughter Amr and Yasmin.

Introduction

Biotic and abiotic environmental challenges represented the highly need to protect the agriculture, plants and crops against the potential hazards. Thus, nano-formulation of pesticides has been developed to replace the classical ones **(Agathokleous et al., 2020).**

Recent advances in applications of engineered nano-materials (NMs) in agriculture have attracted a research interest to develop novel nano-pesticides. In this regards, the use of nano-pesticides can improve the pesticide efficacy, controlled release, and delivery to the targeted pathogens. Consequently, the usage of nano-pesticides can increase the safety and security of the global food production **(He et al., 2019)**.

It has been suggested that nano-agrochemicals may be superior to conventional products and great expectations are placed in the applications of nanotechnologies in this sector. However, no systematic comparison has yet been carried out **(Kah, et al., 2018).** Conventional pesticides have offered several problems regarding their formulations and usages, such as low solubility of active ingredients in water, non-selectivity, and uncontrolled release **(Kah and Hofmann, 2014; Sarlak et al., 2014)**. It has been reported that about 0.1% of pesticides are delivered to the target, while 99.9% are empty in the surrounding environment **(Özkara et al., 2016)**. This poor delivery efficiency results in several hazardous effects, such as water and soil pollution, increased pest, pathogen resistance, and loss of biodiversity **(Côa et al., 2020).**

Comparisons of nano-formulations have pure active ingredients are necessary to elucidate the mechanisms by which nano-formulations may change the behavior of that ingredient. Moreover, comparing with conventional formulations is equally important to evaluate performances

and competitiveness against existing products. Comparisons considering well-selected non-nano analogues, and using protocols that are adapted to the particulate nature of nano-agrochemicals are urgently needed **(Kah, et al., 2018)**.

Considering that the nano-pesticides have smaller size, stronger penetrating power to cells, and easier access to targeted microorganisms than conventional pesticides, that has to put forward the following doubts: Compared with common pesticides, are nano-pesticides scattered in the environment more likely to enter non-targeted organisms and have unpredictable consequences for the non-targeted organisms? This question was asked by **Al-Mubaddel et al. (2017).**

The nano-formulations aiming to increase the solubility of an active ingredients and they are likely to affect the fate of these ingredients. Therefore, more experiments performed under realistic conditions are required to evaluate whether these effects will have a significant impact on the distribution, transport, and degradation processes of given active ingredients. A key question relates to the stability of nano-formulations following application, investigation of the toxicological effect, environmental behavior, and pharmacokinetics of nanoparticles, besides studying the interaction mechanism between nanoparticles and plants, and evaluating their potential impact on the quality and safety of agricultural products can provide a theoretical basis for the development of nanopesticides and the sustainable implementation of nanotechnology in agriculture **(Elsharkawy, 2020).**

Nano-pesticides can extend the lifetime of traditional pesticides, increasing their availability, and changing the exposure dynamic in the environment. It was reported that NPs can cross the cell biological barriers leading to structure damages and cell death. There are reports that confirm the potential of nano-pesticides to be accumulated and transferred through food

chain, causing long-term effects to the ecosystem. Furthermore, nanoparticles can suffer environmental transformations, which can impact on their physicochemical properties, transport, fate, and toxicological profile **(Côa et al., 2020).**

Implementing environmentally friendly practices have been becoming increasingly essential to success in today's nanotechnology businesses. However, the understanding of the environmental impacts of nano-pesticides is still unknown that makes the field of research and development of nano-materials an inspiring sector to determine the toxicological relationships that these materials can present to the human health and the environment **(Adisa et al., 2019)**

Therefore, the objective of this study was carried out to fulfill the following points:

1. To apply a simple technique for preparation and characterization of the novel nano emulsion of Lambda Cyhalothrin insecticide compound.
2. To detect and evaluate the novel pesticide properties as larvicidal efficacy against *C. pipiens* larvae of nano Lambda Cyhalothrin insecticide versus the conventional compound.
3. To evaluate the oxidative stress, lipid peroxidation and immune-toxicity (TNF α inflammatory mediator and cytokine) of nano and conventional insecticide Lambda Cyhalothrin on male rat as a lab animal model for non-target organism.
4. To evaluate geno-toxicity using Single-Cell Gel Electrophoresis (Comet) assay for assessing levels of DNA damage of nano and conventional Lambda Cyhalothrin insecticide exposure on male rat.

5. To declare the histopathological profile of different organs including liver, kidney, brain and testicles after the exposure to nano and conventional Lambda Cyhalothrin insecticide.

Review of Literature

Growing demand of food

The growing world population and nature's limited ability to produce food are at the root of growing food security concerns around the world **(Kidane and Kejela, 2021)**. Responding to the growing demand for food is, without a doubt, the most fundamental issue and the most striking demographic and environmental challenge. There is a general concern that the size of the population may someday exceed the global food supply **(Jambo et al., 2021)**. Food insecurity may result from the unavailability of food, insufficient purchasing power **(Elbushra, and Ahmed, 2020; Khanna, 2020)**, improper distribution and improper use of food **(Anghinoni et al., 2021)**, natural catastrophes **(Chen et al., 2021; Pakravan-Charvadeh et al., 2021),** political violence, and geopolitical factors contribute to a disproportionate distribution of food globally **(Nabuuma et al., 2021)**.

According to the Food and Agriculture Organization of the United Nations, pests and diseases have caused a 30% loss in world food production, especially in developing countries **(Rodrigues et al., 2017)**. Therefore, a large number of pesticides and fertilizers need to be used to ensure food production. However, due to rain washing, dust drifting, photo-oxide degradation, and other reasons, 90% of available pesticides cannot be used to kill target organisms since most of the active ingredients flow into the soil or rivers and accumulate, leading to the resistance of target organisms and to the loss of biodiversity **(Lowry et al., 2019).** Therefore, there is an urgent need to develop new agricultural chemicals and find the right balance between environmental protection, high efficiency, and low cost (**Zhao et al., 2022**). The use of interdisciplinary programs to promote the development of sustainable agriculture is one of the current research

hotspots, with nanotechnology emerging as a feasible method to achieve this goal **(Savary et al., 2019).**

New era of nanotechnology

Nanotechnology was named the fifth revolutionary technology of the present century, with wide application prospects in various fields **(Chhipa, 2016; Saratale et al., 2018)**. Along with its continuous development over the last decades, nanotechnology has exhibited outstanding application prospects in the field of agriculture. In this context, nanopesticides have been designed, produced and experimentally used in the field **(Wang et al., 2021)**. Nanotechnology can provide a novel platform to achieve a dynamic balance between agricultural production and environmental sustainability **(Chhipa and Joshi, 2016; Li et al., 2019).** Nanoscience and nanotechnology can harness the extraordinary properties of materials at the nanoscale (<100 nm) to make an important contribution in such innovations **(Kah et al., 2019; Adisa et al., 2019)**. Nanopesticides are currently an area of intense interest in nanotechnology and agriculture and food communities, as reflected by several reviews on this topic during the last five years **(Kah et al., 2018; Kah et al., 2019; Camara et al., 2019; Adisa et al., 2019; Singh et al., 2021)**. Nanopesticides can be roughly classified into two categories: (a) nanosized active ingredient (AI) and (b) molecular active ingredient nanocarrier (NC) complexes **(Bilal et al., 2020; Shan et al., 2020; Xu et al., 2020).**

Benefits of nanopesticides

Nanotechnology offers new opportunities to facilitate development of novel AIs and reuse existing chemistries through nanoformulations (for example, using a nanocarrier (NC) delivery system) that enable new pesticide functionalities, such as slow release of AI, increased stability, enhanced penetration (through cell membranes) and a greater efficacy of the AI in controlling the target organisms **(Kah et al., 2018; Camara et**

al., 2019) often with a view to reducing application rates through greater efficacy and/or targeted delivery. In addition, the slow-release (nanoencapsulated) and nanocomposite formulations of metal oxides have been found to be more potent in disease control than conventional formulations **(Adisa et al., 2019)**. Double-stranded RNA loaded on designer, non-toxic, degradable, layered double hydroxide clay nanosheets not only offered greater stability to the AI (RNA) against plant viruses, but also resulted in reduced wash-off in rain and enhanced uptake and transfer inside the sprayed plant **(Mitter et al., 2017)**. Nanopesticides (for example, with NCs or novel AIs) are in an advanced state of research, development and testing and are likely to be presented for regulatory evaluation. Indeed, some nanopesticides are already commercially available **(Kah et al., 2019).** There is currently no internationally accepted definition of nanopesticides, and thus regulatory agencies may adopt different size ranges and different limits for the fraction of nanosized particles **(US EPA 2017; Miernicki et al., 2019). Kah et al. (2021)** stated that use an operational definition of a nanopesticide as a plant protection product in which a nanomaterial is used to enhance the functionality, increase the utility and/or alter the risk profile of a conventional AI or is presented as a new AI. This perspective does not cover materials that are called 'biocides' in the EU, and which include substances used in livestock breeding, food packaging and household kitchen or canteen settings. Some current nanopesticide formulations have sizes larger than the 1–100 nm nanoscale size range, similar to the situation with nanomedicines **(Etheridge et al., 2013; Boverhof et al., 2015).**

Nanopesticides types

Wang et al. (2022) identified two major types of nanopesticides.

Type 1are metal-based (for example, Ag, Cu and Ti) nanopesticides and Type 2 include materials in which the AIs are encapsulated by nanocarriers (for example, polymers, clays and zinc nanoparticles (NPs).

Type 1 nanopesticides, Ag-, Ti- and Cu-based nanomaterials (NMs) are the most common analytes These NMs have strong antimicrobial activity rendered by adhesion, dissolution (for example, Ag+ and Cu2+ ions), cytotoxicity and oxidative stress (reactive oxygen species, ROS), and genotoxicity-induced cell death. These nanopesticides can control plant pathogens, including bacteria and fungi.

Type 2 nanopesticides focus more on the (RNDP) Responsive nanopesticides: 'responsive nanopesticides' is a 'composite' term that represents nanoscale bactericides, insecticides, fungicides, herbicides and nematicides that have an RNDP of AIs. Upon biotic and abiotic stimuli or stresses (for example, heat, drought and salinity), the RNDP enables the responsive delivery of AIs to a desired target area within a required time and dose in a controlled, targeted and synchronized manner to combat agricultural pests. The platform is primarily composed of nanocarriers and AIs, RNDP showing potential to meet sustainable agriculture goals. Most nanocarriers are biocompatible, cost-effective and stimuli-responsive, and are categorized into two major groups: polymer- and clay-based. Chitosan, cellulose and polylactide are common natural polymers for making nanocapsules, nanospheres, nano(hydro)gels and nanomicelles for AIs. Mesoporous silica NPs (MSNs) and montmorillonite are typical clay-based NMs with demonstrated high AI encapsulation capacity. Other emerging nanocarriers include advanced nanocomposites and two-dimensional (2D) NMs with large specific surface areas that facilitate AI loading. For Type 2 nanopesticides, most AIs are conventional pesticides, such as the insecticides avermectin and essential oils, as well as the herbicides atrazine, 2,4 dichlorophenoxyacetic acid (2,4-D) and glyphosate.

Nanopesticides manufacturing

For manufacturing nanosuspensions, there are two converse methods "bottom-up" and the "top-down" techniques **(Jassim and Rajab, 2018)**.

(1) Bottom-up methodology, antisolvent precipitation is an effective way to prepare micro or nano-sized drug particles. In this precipitation method, first, the drug was dissolved in the solvent, and then, the solution containing drugs was quickly added into the anti -solvent. Crystal precipitation occurs under the condition of drug concentration super saturation. To ensure better stability of the nanosuspension; the used stabilizer should have enough affinity for the particle surface, and have a high diffusivity that can quickly cover the generated surface. Besides that, the quantity of stabilizer should be able to completely cover the surface of particles **(Krishna and Prabhakar, 2011).** All-Trans retinoic acid nanosuspensions were prepared with a precipitation method. The use of simple and low-cost equipment and also benefit for higher saturation solubility is the advantage for precipitation technique compared to other methods of nanosuspension preparation. Precipitation technique is not applicable to drugs that are poorly soluble in aqueous and nonaqueous media. In this technique, the drug needs to be soluble in at least one solvent, which is miscible with non-solvent **(Zhang et al., 2006)**.

(2) Top-down technologies

(a) Media milling (nanocrystals or nanosystems).

In this method, the nanosuspensions are produced using high-shear media mills or pearl mills. The media mill consists of a milling chamber, a milling shaft, and a recirculation chamber. The milling medium is framed of glass, zirconium oxide, or highly cross-linked polystyrene resin. The milling chamber is charged with the milling media, water, drug, and stabilizer, and the milling media or pearls are then rotated at a very high shear rate. The milling process is performed under controlled temperatures. The high energy and shear forces generated as a result of the impaction of the milling media. The drug provided the energy input to break the microparticulate drug into nanosized particles. The uni-modal distribution profile and mean

diameter of <200 require a time profile of 30–60 min. Once the formulation and the process are optimized, very slight batch-to-batch variation is observed in the quality of the dispersion.

(b) High pressure homogenization (Disso Cubes). The main principle is high pressure, i.e., 100–1500 bars. By this pressure, we can easily convert the micron size particle into nanosize particle. Moreover, it initially needs the micron range particle, i.e., <25 μm, so that we have to get the sample from the jet mill because using a jet mill, we can reduce the particle size up to <25 μm. Moreover, we can use this equipment for both batch and continuous operations. Capacity is also 40 mL– 1000 L. Here, first, we have to convert the particles into presuspension form (after jet milling) **(Sutradhar et al., 2013)**. **Yang et al. (2010)** prepared nanosuspension and microsuspension by high-pressure homogenization. Their crystalline state was evaluated by differential scanning calorimetry and powder X-ray diffraction. Both evaluations indicated that the lattice energy of drug particles decreased with the decrease of particle size. **Sun et al. (2012**) showed that particle size reduction could increase the solubility and in vitro dissolution rate.

The smaller the particle size, the higher the dissolution rate.

(c) Emulsion. These emulsions are also useful for the preparation of nanosuspensions. The drugs which were insoluble in volatile organic solvents or partially soluble in water are prepared by this method. Initially, organic solvents, such as methylene chloride and chloroform, were used. However, environmental hazards and human safety concerns about residual solvents have limited their use in routine manufacturing processes. Relatively safer solvents such as ethyl acetate and ethyl formats can still be considered for use **(Rabinow, 2004).**

(d) Microemulsion. Microemulsions are thermodynamically stable and isotropically transparent dispersions of two immiscible liquids, such as

oil and water stabilized by an interfacial film of surfactant and co-surfactant. The drug can be either loaded into the internal phase, or the pre-formed microemulsion can be saturated with the drug by intimate mixing. Suitable dilution of the microemulsion yields the drug nanosuspension. An example of this technique is the griseofulvin nanosuspension, which is prepared by the microemulsion technique using water, butyl lactate, lecithin, and the sodium salt of aurodeoxycholate **(Rabinow, 2004).**

Nanopesticides hazards and toxicity

In addition, the global market for pesticides is estimated to grow from US$75 billion in 2013 to US$90 billion by 2023 **(Hofmann et al., 2020).** Nanopesticides have the potential to result in multibillion-dollar benefits **(Gilbertson et al., 2020).** Nevertheless, only a few commercial products among synthesized nanopesticides have been commercialized (e.g., Kocide 3000 (Dupont), and AZteroid FC (Vive Crop Protection), as a result of three major barriers to technology readiness and implementation (i.e., efficient delivery at the field scale, regulation and safety concerns, and consumer acceptance) **(Hofmann et al., 2020).** Currently, research on nanopesticides has been mainly focused on their performance **(Kah et al., 2018),** mechanisms **(Chatterjee et al., 2014),** environmental fate **(Su et al., 2019)**, and ecosystem implications **(Klaine et al., 2012; Pandey et al., 2018).** However, huge knowledge gaps exist related to nano-governance and socio-economic aspects, particularly in public perceptions and willingness-to-pay (WTP) for nanopesticides, which are essential to promote the usage and market share of nanopesticides **(Kah et al., 2018).**

A large number of studies demonstrated the potential benefits of nanopesticides compared with conventional pesticides, including strong stability, superior bioavailability and high efficacy. Also, the release of active ingredients can be a controllable process in the laboratory through

encapsulating active ingredients into nanocarriers, suggesting that nanopesticides have the potential to deliver active ingredients to the targeted organisms efficiently. However, a coin has two sides; the enhanced stability of active ingredients implies persistence, and good dispersion indicates a high possibility of interactions between nanopesticides and non-target organisms. It is of importance to balance between the potential benefits and the environmental risks when nanopesticides developers design and fabricate novel nanopesticides **(Xu et al., 2022).**

Nanopesticides have obvious pesticidal activity and as such can exhibit toxicity toward non-target organisms. The observed adverse impacts and toxicity vary depending on, nanopesticide properties, species of non-target organisms, exposure route, concentration, duration and environmental matrices. Nanopesticides can cause negative impacts on plants, microcrustaceans and soil microbiota at the physiological, metabolic and genetic levels. These include structural changes of plant tissues, reduction of chlorophyll content, alteration of the antioxidant defense system, metabolic reprogramming and genetic over-regulation **(Zhao et al., 2018).**

Huang et al. (2022) indicate that the dynamic behavior of nano and microsized lambda cyhalothrin (LC)-microcapsule (LC-MCs) in water bodies and their toxicological risk against three life stages of zebrafish and found that the release and sedimentation velocity of LCMCs in aquatic environments, as well as toxicity to fish, were effectively controlled by particle size. When the size distribution of LC-MCs ranged from 3 μm to 27 μm, the capsules released slowly and sank quickly to the bottom of the water, which ultimately would induce long-term and chronic ecotoxicological risk to benthic organisms. However, LC-MCs with diameters between 100 nm and 3 μm could be evenly distributed throughout the water bodies and rapidly release their active components,

thus posing homogeneous and acute hazards to multiple aquatic organisms. The evaluation demonstrated that LC-MCs with larger particle sizes could significantly reduce their toxicity to adults, larvae and embryos of zebrafish, whereas exposure to nanosized MCs results in comparable toxicity or teratogenicity in larvae and embryos compared to EC exposure. Additionally, due to differences among their living habits, absorption pathways, accumulation and transport efficiency, the tested fish adults, larvae and embryos possessed dramatically different toxic sensitivities to LC-MCs.

The fate of nanopesticides in the environment

The environmental behavior, impacts of nanopesticides and their interactions with biological systems are complex and might be different from traditional formulations. As a consequence, there is a doubt if nanopesticides can be evaluated and classified in the same way as traditional pesticides within current ecotoxicity protocols and regulatory guidelines **(Handy et al., 2012; Kah et al., 2018).** An example it is the open issue concerning what dose metric better represents the complex conditions found in the nananoecotoxicology studies. While in the case of organic and inorganic substances, the mass concentration is linearly related to the number concentration and the data are expressed in terms of mass/volume or mass/mass, in the case of nanoparticles the use of particle number concentration-based metrics may be more recommended. In summary, the impacts of nanopesticides on non-target organisms are mediated by nano-bio-eco interactions that occur into the environment. These complex interactions are governed by biotic and abiotic factors, such as biological, chemical, and physical transformations, which modify the physicochemical properties and colloidal behavior of the nanoparticles **(He et al., 2018).** The transformations of NMs involve oxidation-reduction reactions that can be catalyzed by sunlight action (photooxidation and

photoreduction), leading to the NMs dissolution and/or sulfidation. In addition, NMs can be physically transformed by homoaggregation and heteroaggregation processes that disturb their colloidal stability, reducing their surface area and reactivity. Thus, these phenomena can induce the NMs sedimentation, extending their persistence in the environment. The surface of NMs can be coated with naturally occurring biomacromolecules and/or geomacromolecules, such as proteins, lipids, and humic substances. This coating, known as biomolecule corona, results in a new biological identity which will govern the nanoparticle-organism interactions **(Markiewicz et al., 2018).** Furthermore, organic and inorganic ligands (i.e., environmental pre-existing contaminants) can either be attached on the NMs or only combined with them, leading to joint and unexpected toxicity effects. All these transformation processes will impact on the nanopesticides environmental behavior, fate, and toxicity, making it difficult to predict the toxic effects of such substances **(Deng et al., 2017)**. Since nanopesticides may be transformed into the environment, it is also recommended monitoring the long-term effects of these substances. According to **(Mancini et al., 2019)**, this knowledge is crucial for understanding the real impacts on nontarget populations and also providing feedback for review of licensing conditions in a post-approval context. This assessment would propitiate meaningful information, not only about the chronic effects of nanopesticides, but also regarding toxicodynamic and toxicokinetic responses, besides the unexpected synergistic interactions between nanopesticides with other classical pollutants. Due to the intrinsic properties of NMs, it has been a challenge to develop standardized procedures to accurately measure the NMs damage on non-target organisms. Technical modifications have been performed in standard ecotoxicity protocols (i.e., developed for studying classical pollutants) to adapt for the nanomaterials reality **(Kleiven and Oughton, 2015; Petersen**

et al., 2015). Adaptations of culturing media have also been strategically conducted to improve the colloidal stability of NMs during the ecotoxicity assays **(Brinke et al., 2011),** and also to better reflect the soil natural conditions **(Tyne et al., 2013).** It is clear from the above discussion that an environmental risk assessment is mandatory for nanopesticides development lined up with environmental, health, and safety issues. Likewise, the harmonization of methodologies in nanoecotoxity assays is fundamentally necessary **(Kah, 2015; Baun et al., 2017).**

The indiscriminate use of nanopesticides can result in undesirable effects on terrestrial and aquatic non-target organisms. Nanopesticides can extend the lifetime of traditional pesticides, increasing their availability, and changing the exposure dynamic in the environment. Some researchers have found that NPs can cross the cell biological barriers leading to structure damages and cell death. There are reports that confirm the potential of nanopesticides to be accumulated and transferred through food chain, causing long-term effects to the ecosystem. Furthermore, nanoparticles can suffer environmental transformations, which can impact on their physicochemical properties, transport, fate, and toxicological profile **(Côa et al., 2020).**

Lambda Cyhalothrin

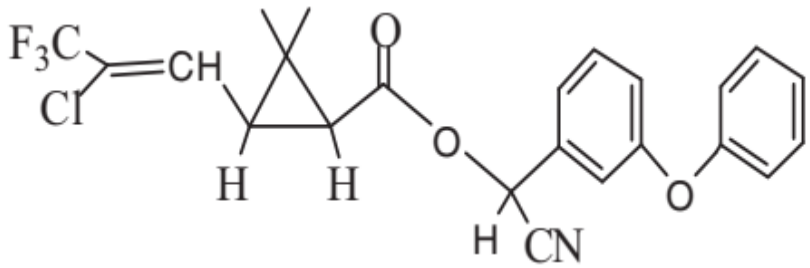


Figure 1: chemical structure of ʎ-cyhalothrin

Physical and chemical properties

Pyrethroids represent more than a quarter of global insecticide use and are integrated in agricultural, residential and public health programs to control pests **(Aznar-Alemany and Eljarrat, 2020).** Pyrethroids are derived synthetically from pyrethrins, which are extracted from the flower of a plant, *Chrysanthemum cinerariaefolium* (**Ullah et al., 2015**). As a class, the synthetic pyrethroids continue to be one of the most widely-used insecticides worldwide. They are used in agriculture to control pest infestations of row crops, forestry, horticulture, and livestock. Pyrethroids are also employed in medicines to treat scabies, louse, and flea infestations. Moreover, these versatile compounds continue to have a vital role in public health practices such as the control of Plasmodium parasites and the Zika virus, which can be transmitted into a human host by insect vectors. Thus the importance of pyrethroids in food production, economic development, and public health practice is clear. Nevertheless, it is also important to be vigilant of any environmental health impacts that might emerge from exposures to these compounds. Furthermore, the widespread and sometimes improper use of pyrethroid insecticides has also resulted in the evolution of resistance in many mosquito and pest species **(Lv et al., 2016).** Thus continued responsible stewardship of these economically and medically important compounds is needed to maximize their benefits and minimize untoward effects on the environment and human health **(Ross and Carr, 2019).**

Among several pyrethroid insecticides, Lambda-cyhalothrin (LCH) (λ cyhalothrin) is widely used for controlling the pest population of several vegetables, cereals, cotton, etc. **(Chatterjee et al., 2021)**. LCH, a synthetic Pyrethroids insecticide, has been extensively used in the last two decades in many developing countries, to control agricultural pests and insects of

veterinary as well as human concern **(Fetoui et al., 2010).** Agriculturally, it is applied on cotton, cereals, various vegetables and fruits with applications made to control aphids, Colorado beetles and lepidopteran larvae, and was found in milk, the blood of dairy cows and cattle meat **(Khashan, 2016).**

The mode of action

Voltage-gated sodium channels are the principal targets for the neurotoxic effects of pyrethroids on insects, while voltage-gated calcium channels and voltage-gated chloride channels are secondary targets. Pyrethroids affect sodium ion channels in the nervous system of insects, increase the opening time, ultimately stimulate nerve cells and cause paralysis **(Mukherjee et al., 2010)**. Calcium signals regulate a variety of neural development processes **(Soderlund, 2012)**,while chloride channels stabilize membrane resting potential, regulate cell volume and transepithelial transport **(Stamou et al., 2013).** Additionally, voltage-gated calcium channels and chloride channels can explain most of the special symptoms of poisoning caused by type II pyrethroids **(Frank et al., 2018).**

Toxicokinetics and metabolism

Pyrethroids have a high oral absorption rate in mammals (mouse, rat, etc.), but a low dermal penetration rate. It is well known that when the direct oral dose of the mammal is below the safe level (<1/10 LD50), the pyrethroid and its metabolites do not substantially accumulate in any particular tissue or organ after systemic absorption **(Kaneko, 2010).** Besides, the metabolic pathways consist of two main processes. The phase I reactions are oxidation, hydrolysis, and the phase II reactions are the production of hydrophilic and lipophilic (**Kaneko, 2011**). In general, the acid and alcohol moieties of the pyrethroid can be quickly and completely excreted within a few days after exposure. However, the carbon in the CN group of

pyrethroids have a certain degree of bioaccumulation in the skin and stomach, probably due to their diffusion into the extracellular fluid (ECF) and binding with serum albumin, resulting in endogenous thiocyanate. Because pyrethroids are rapidly metabolized and metabolites are also easily excreted, they do not cause severe bioaccumulation in mammals (**Kaneko, 2010; Kaneko, 2011**). However, pyrethroids have moderate potential to bioretention in aquatic organisms (**Corcellas et al., 2012**). When mammals such as humans ingest aquatic organisms that have been contaminated with pyrethroids, pyrethroids are transferred from aquatic organisms to mammals **(Corcellas et al., 2015; Muggelberg et al., 2017**). Above a safe dose, the mammal may develop symptoms of poisoning or even death. Also, the pyrethroids accumulate in different generations. When the mother is exposed to pyrethroids, the baby may also develop pyrethroid accumulation after taking breast milk (**Corcellas et al., 2012**).

Lambda-cyhalothrin is metabolized by cleavage of the ester linkage to form cyclopropane -carboxylic acids and the corresponding phenoxybenzoic acids or alcohols. In most cases the parent compound is the principal constituent of the residue. Studies of lambda-cyhalothrin metabolism in ruminants and poultry have been reviewed. Lambda-cyhalothrin is the major component of the residue in animals, except in kidney and liver, where, in addition to the plant metabolites (**US EPA, 2007**).

Because of their large use, several *in vitro* metabolism studies and in vivo experiments in mammalians have been carried out for different pyrethroids to clarify their common metabolic pathways **(Abe et al., 2017).** The main metabolic pathways were found to be oxidations of both acid and alcohol moieties, ester cleavage, and conjugation reactions **(Wang et al., 2016).** The metabolism of lambda-cyhalothrin, which is among the most widely applied pyrethroid in agriculture, has been documented to occur by

hydrolysis catalyzed by esterases **(Anadón et al., 2006).** It is absorbed dermally, as it is fat soluble, or by inhalation. In case of food or drink contamination, people can absorb LCH through the gastrointestinal tract. It is metabolized in the liver. The metabolites cis-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropane carboxylic acid (CFMP) and 3-phenoxybenzoic acid (3-PBA) are excreted with urine **(Khemiri et al., 2018)**. The 3-PBA is a common metabolite of pyrethroids, and it can cross the blood–brain barrier. It can be also accumulated in the brain **(Kuder and Gundala, 2018).** Urinary LCH metabolite concentrations are significantly higher in rural inhabitants than in the cities **(Wielgomas et al., 2013; Wielgomas and Piskunowicz, 2013)**. Glutathione-S transferases (GSTs) have a function in LCH metabolism. LCH inhibits GSTs in a competitive mechanism in the livers of non-target organisms causing oxidative stress **(Özaslan et al., 2018).**

Toxicodynamics

In spite of claims of low mammalian toxicity of pyrethroid, several investigations reported the toxicological evidence of pyrethroid among various species of animals **(Khan et al., 2012**) Pyrethroids are easily absorbed through gastrointestinal and respiratory tract due to their lipophilic nature and also make them easier to be stored in the lipid rich internal tissues like body fat, skin, liver, kidney, central and peripheral nervous systems. Hemato-biochemical studies are important for the analysis of the functional status of animals to suspected toxic agents. It may act as a strong evidence against toxicity of contaminated pyrethroid insecticides. Recent reports have clarified that exposure to pyrethroid leads to a significant modification in hematological findings **(Khan et al., 2009).** Although pyrethroid insecticides are not persistent in the environment, their frequent use contributes to maintain background levels in the human body

as shown by measurements of metabolites in urine and blood samples collected from various populations **(Choi et al., 2017; Li et al., 2020; Valcke et al., 2020)**. In addition to this background exposure, agricultural workers are also exposed in the workplace, making their levels of exposure higher than those of the general population **(Ratelle et al., 2016).**

The main enzymes involved in the biotransformation of pyrethroids, in particular CYP450s (CYP1A1, 1A2, 2A1, 2B1, 2B2, 2E1, 3A1, 3A2, 3A4, 3A5, 4A1, 2C8, 2C9, 2C19), are also implicated in the biotransformation of several other pesticides used concomitantly in agriculture **(Martinez et al., 2018).**

Toxically LCH forms a high level of free radicals, which induce oxidative stress. Exposure to toxic chemicals and environmental pollutants damage the histoarchitecture of gonads (**Nnamonu et al., 2019).** It interacts with the normal functioning of the nervous system by altering the opening and closing of calcium and chloride channels and the voltage-gated sodium channels, resulting in hyperactivity, uncoordinated behavior, paralysis and eventually death **(Song et al., 2021). Chakroborty et al. (2019)** declared that renal toxicity was measured by a significant decrease in renal index, reduction in kidney protein and an increase in serum protein in lambda-cyhalothrin intoxicated rats. At the same time, lambda-cyhalothrin induced a significant renal oxidative stress demonstrated by elevated renal malondialdehyde content and oxidized glutathione level accompanied by a reduction in reduced glutathione and antioxidant enzymes in rats. The data reported on the genotoxicity of synthetic pyrethroids, including LCH, are rather controversial, however it was reported that LCH has a clastogenic/genotoxic potential as measured by the bone marrow SCA and MN tests in Wistar rats **(Çelik et al., 2003)**. In animals, lambda-cyhalothrin caused alteration in metabolic and physiological processes. For instance,

Fetoui et al., (2010) reported the toxic effects of lambda-cyhalothrin on the rat kidney. **Fetoui et al. (2009)** reported that biochemical and histopathological changes in the liver of rats exposed to Lambda-cyhalothrin**.** It was reported that LCH induced cytotoxic oxidative stress by altering antioxidant defense mechanisms and increasing lipid peroxidation via generation of reactive oxygen species (ROS) in different organs of rabbits and rats (**El-Demerdash, 2007; Abdallah et al., 2012)**.

The exposure of rats to LCH led to hepatotoxicity and severe injury of renal structure due to its toxicity **(Wang et al., 2014).** It was also demonstrated that renal activities, tissue malondialdehyde (MDA), histopathology, protein carbonyl levels, reduced glutathione levels and AO enzyme activities were altered significantly by lambda-cyhalothrin **(Fetoui et al., 2010**). LCH increased the production of reactive oxygen species and DNA damaged levels, leading to harmful immune effects **(Zick et al., 2008).** Equally, other results indicated that LCH caused significant rise in the kidney, brain and liver weight and plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Nevertheless, the plasma content of bilirubin, urea, creatinine, and glucose were significantly raised. On the contrary, plasma total protein and albumin was reduced **(Abdel-Mobdy and Abdel-Rahim, 2015).** Immunotoxicity of lambda-cyhalothrin was conducted in wistar albino rats by using parameter for humoral immunity such as haemagglutination test and total globulin estimation whereas for cellular immunity intradermal tuberculin test and in-vivo/in-vitro splenocytes proliferation test **(Bhoopendra and Nitesh, 2014). Abbassy et al. (2014**) demonstrated that exposure to LCH induced oxidative stress; lipid peroxidation and reduced CYP450 activity in the plasma of LCH- treated male rats. **Al-Amoudi, (2018)** showed that LCH affected the thyroid function and structure by disrupting the normal levels of antioxidant enzymes, thyroid hormones level and by causing an

abnormal histological arrangement and DNA damage in the thyroid gland. **Khalil et al. (2020**) showed that LCH exposure caused a significant lowering in growth performance, hematological and immunological indices. Moreover, LCH disrupted the oxidant/antioxidant status and dysregulated the expression of stress and immune-related genes, downregulating the mRNA transcript level of Immunoglobulin M heavy chain (IgM), Interferon (IFN-γ), CXC-chemokine, and Toll-like receptors (TLR-7) in the spleen. In liver tissue, the heat shock protein (HSP-70) expression was upregulated, while that of cytochrome CYP450 1A (CYP 1A) was downregulated. LCH affects the O. niloticus immune response through the negative transcriptional influence on genes linked to immunity and induction of oxidative injury of the immune organs. **Ezenwosu et al. (2021)** has demonstrated that lambda cyhalothrin affected various antioxidants activities as SOD, MDA, GSH-Px, GSR, CAT and gonad function as the testes photomicrographs showed necrotic conditions in the spermatogenic cells with nuclear pyknosis and cytoplasmic swelling while that of the ovary displayed vacuolations, flabby oocytes, and degenerated ovaries changes.

Materials and Methods

Chemicals

Lamdada λ-cyhalothrin technical grade (LC)

IUPAC name: [(*R*)-cyano-(3-phenoxyphenyl)methyl] (1*S*,3*S*)-3-[(*Z*)-2-chloro-3,3,3 trifluoroprop-1-enyl]-2,2-dimethylcyclopropane-1carboxylate, 97.8% purity (CAS number 91465-08-6) purchased from Kafr El Zayat Pesticides and Chemicals Co. (Kafr El-Zayat, Gharbia, Egypt). Tween 80 and methanol are pure analytical grade chemicals purchased from Sigma–Aldrich.

Lambda-cyhalothrin Nano particles (LCN) were prepared by specific nano-technical unit at Faculty of pharmacy, Al- Azhar University, Assuit. The primary particle size is from 70.3:77.53 nm.

Kits for biochemical evaluation

Rat TNF-α (Tumor Necrosis Factor- Alpha) was assayed using Elabscience Biotechnology Co., Ltd., Houston, Texas, USA. ELISA Kit. Superoxide dismutase (SOD) was assayed using colorimetric spectrophotometer kits of Elabscience Biotechnology Co., Ltd., Houston, Texas, USA. Malondialdhyde (MDA) was assayed using colorimetric kits according to the manufacturer's protocol (Biodiagnostic Company, Cairo, Egypt).

Comet assay material; buffer (NaCl, Na2EDTA, pH 7.5), 1% GP-42 normal agarose , Low melting (LGT) agarose , Na4EDTA, Trizma, 0.1% sodium lauryl sulfate (SDS), 10% dimethyl sulfoxide, and Triton X-100) (NacalaiTesqe, Inc., Kyoto, Japan) NaOH, Tris buffer, Ethidium bromide (Wako Pure Chemical Industries, Ltd., Japan).

Animals

Target insect pests

Larvae of field strain (*Culex pipien)* were collected from faculty of science, zoology department, Entomological Research Laboratory, Assiut University, Egypt. The larvae were recognized as *Culex pipien* strains. The larvae were acclimatized under suitable temperature and humidity for a period of 24 h. The larvae were fed with glucose and yeast mixture. Twenty larvae of *C. pipiens* were placed in 250 ml sterile beaker containing 200 ml of water **(WHO, 2005).**

Non target animals

A total number of 72 healthy adult Sprague–Dawley male rats aged 8–10 weeks with an average body weight of 150–200g were used in this study. Rats were purchased from the Experimental Animal Center, Faculty of Medicine, Assiut University, Egypt. The animals were kept in plastic cages and allowed to adapt for a week before treatment. Animal facilities were operated under controlled temperature (24–26 °C) and a 12-h light–dark cycle. Rats were fed on standard food pellets, and tap water was supplied *ad libitum*. The design of the experiment was in agreement with the ethical rules prescribed by the Faculty of Veterinary Medicine, Assiut University.

Preparation of Lambda-cyhalothrin nano - emulsion (LCN)

LCN was successfully prepared by solvent evaporation technique according to **Knieke et al. (2014)** and **Pan et al. (2015)**. The process includes the following key stages: (1) 100 mg lambda-cyhalothrin was dispersed in 40 Ml of methanol. (2) 1ml tween was dispersed in deionized water, and added drop wise to lambda-cyhalothrin solution. (3) The mixture was emulsified in a high shearing machine (NANOJ H10, ATS, Shanghai China) at 1500 rpm for 5 min. (4) Subsequently, the emulsion

was cooled to ambient temperature with stirring at 600 rpm on a magnetic stirrer. Finally, the solution was sonicated for 10 minutes and filtered with a nano-filter and LCN was obtained.

Characterization of the prepared Lambda-cyhalothrin nano-emulsion (LCN)

1-Transmission Electron Microscopy (TEM)

The transmission electron microscopy (HR-TEM) images were carried out in the unit of Electron Microscopy, Assuit University. The morphology of the nano lambda-cyhalothrin was characterized by TEM (HT7700, Hitachi Ltd., Tokyo, Japan) with 80 kV accelerating voltage. 2 µL diluted solution (25 µg/mL) were placed onto a carbon-coated copper grid and were dried at room temperature for TEM measurement.

2-Particle size and polydispersity index (PDI) assay

The samples were dispersed in deionized water for analysis. The mean particle size, 90% diameter percentile (D90) and polydispersity index (PDI) of the nano particles were determined by dynamic light scattering (DLS) (Zetasizer Nano ZS90, Malvern, UK) at 25°C. PDI less than 0.3 meant a fairly narrow size distribution and good dispersion. All data were measured in triplicate. The Zeta potential of the nano-emulsion was documented by the electrophoretic mobility procedure in the Zetasizer device in which the samples were diluted four-fold with milli-Q water and values were represented in millivolts (mV) obtained from two cycles with an average of 20 scans**.**

3-UV-Visible Absorption Spectroscopy

UV-visible absorption spectra were recorded on a Perklin–Elmer lambda 40 spectrophotometer using 1 Cm matched quartz cell over a wavelength range of 200 to 1000 nm**.** Aliquots (3 mL) of the suspension were

measured to determine the surface plasmon resonance absorption maxima with distilled water as a reference**.**

4-Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR used for molecule distribution. Solutions of lambda-cyhalothrin nano-emulsion and conventional lambda-cyhalothrin on infrared spectrometers (Gasco FT-IR Japan). Spectra were measured in transmission mode with a resolution of $4cm^1$. The scanning processed from 400-4000 cm^{-1} scans per sample on a Perkin Elmer Spectrum RX1 apparatus. The data were represented in infrared transmittance percentage.

5- Larvicidal activity bioassays

Twenty larvae of *C. pipiens* were placed in 250 ml sterile beaker containing 200 ml of water. Conventional lambda cyhalothrin and nano lambda cyhalothrin were added separately to the beaker containing larvae at different concentrations of 0.1, 0.05, 0.01, 0.001 **(WHO, 2005)**. A control set was also kept. All the samples were maintained in room temperature. The larvicidal effects of both formulations were tested by obtaining the mortality rate after 24, 48, and 72 hours of the exposure time. Dead larvae were recognized when they were immobile. The test was done in three replicates. The mortality data in both treatments with LC and LCN were obtained after 24, 48, and 72 hours of exposure periods. The dead larvae were recognized when they were stunted and failed to reach the surface water (**Macêdo et al., 1997).**

Experimental animal design

Animals were divided randomly into four groups of eighteen animals each. The conventional form of LC was dissolved in corn oil, while the LCN was dissolved in distilled water.

Conventional-treated rats: 18 rats were given 1/20 of LD50 (79 mg/kg b.wt) dissolved in 1 ml corn oil as a vehicle, orally twice weekly. The LD50 for lambda-cyhalothrin has been determined as 79 mg/kg for male rats **(Kidd and James, 1991).**

Lambda-cyhalothrin nano- treated rats: 18 rats were given nano λ cyhalothrin which equal **1/80** of LD50 **(Kidd and James, 1991)** orally, twice weekly**.**

Control group [1,2]: 36 rats kept as control and divided into two sup- group have18 rat each. First and second sup-groups administered 1 ml corn oil and 1 ml distilled water, respectively, orally, twice weekly.

Collection of samples

Three randomly selected rats from each group were euthanized under diethyl ether anesthesia after 24 h of last exposure to LC and LCN. Samples were collected after 2, 4, 6, 8, 10 and 12 weeks post-exposure. Blood samples were collected from the descending aorta, and serum samples were harvested and kept at - 20 °C until analysis. Whole blood with and without anticoagulant for haematological studies. Serum samples prepared for biochemical assay. The blood and liver specimens were immediately collected and prepared for comet assay. Liver, kidneys, brain and testis samples were collected and kept in 10% neutral buffered formalin for histopathological examination.

Adopted methods:

1. Body weight and absolute and relative organs weight

The total body weight and the absolute organs weight of rats from different experimental groups were obtained before euthanization and

autopsy. And also, the relative organs weight of liver, kidney, brain and tested was calculated.

2. Haematological analysis

The blood sample with EDTA was used for haematological analysis. Haematological examination was performed by Medonic Vet. A haematologic analyser (Medonic CA 620, Sweden) directly after the blood samples were received by the research laboratory and within one to two hours of collection. Haematological variables measured were total red blood cells count (RBCs), haemoglobin concentration (HGB), red blood cells distribution width (RDW), haematocrit (HCT), main corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets count (PLT), mean platelets volume (MPV), total white blood cells count (WBCs), lymphocytes count and per cent, neutrophils count and per cent, monocytes count and per cent.

3-Oxidative damage Indicators

Superoxide dismutase (SOD) level

SOD activity was detected by measuring the inhibition of autoxidation of epinephrine at pH 10.2 at 30 °C as defined by (Okado-Matsumoto and Fridovich, 2001).

4- Lipid peroxidation

Determination of serum malondialdehyde (MDA) level

Determination of serum MDA was done using colorimetric kit supplied by Bio-diagnostics (Dokki, Giza, Egypt). This assay is based on the reaction of MDA with chromogenic reagent at 45 °C to yield a stable chromophore with absorbance at 534 nm, which is measured using UVspectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd., Korea). The rate of lipid

peroxidation was expressed as nanomoles of reactive substance formed per min per milligram of protein **(Grotto et al., 2009).**

5-Determination of concentration of tumor necrosis factor-alpha (TNF-α)

TNF-α level in the serum was determined by enzyme-linked immunosorbent assay (ELISA) using rat TNF-α immunoassay kit according to the recommendations of the manufacturer Elabscience Biotechnology Co., Ltd**.,** USA**.**

6-Comet assay (single cell gel electrophoresis)

Sample preparation

Liver homogenization

The liver specimens (0.5 g) were minced separately, suspended in chilled homogenizing buffer (0.075 M NaCl, 0.024 M Na2EDTA, pH 7.5), and then homogenized gently using bench top homogenizer (PRO Scientific, USA) surrounded by ice at 800 rpm. To obtain the nuclei, the homogenate was then centrifuged at 1500 rpm for 10 min at 0 °C, and the precipitate was re-suspended in chilled homogenizing buffer and allowed to settle for 1–2 min.

Blood samples

Whole blood samples freshly obtained were used directly.

Comet assay procedure

DNA damage in the blood and liver was detected using comet assay according to the methods of **Sasaki et al. (1997)** as follows:

Slide preparation stage fully frosted slides (Matsunami Glass Ind, Japan) were layered twice with 100 µL of 1% GP-42 normal agarose (NacalaiTesqe, Inc., Kyoto, Japan). An amount of 75 µL of nuclear suspension (supernatant) was mixed with 75 µL of 2% low melting (LGT) agarose (NacalaiTesqe, Inc., Kyoto, Japan) at 40 °C, and the mixture was

layered on the slide using a cover slide. Finally, 100 µL of agarose GP-42 was layered on the surface, covered with another slide and allowed to gel.

Lysing stage the slides were placed immediately into a chilled lysing solution (2.5 M NaCl, 100 mM Na4EDTA, 10 mM Trizma, 0.1% sodium lauryl sulfate (SDS), 10% dimethyl sulfoxide, and Triton X-100) and kept at 4 °C in the dark for 60 min.

Unwinding and electrophoresis stage the slides were placed on a horizontal gel electrophoresis platform (Cleaver Scientific Ltd., UK) and covered with chilled alkaline solution (300 mM NaOH and 1 mM Na2 EDTA, pH 13) in the dark at 0 °C for 10 min, electrophoresis was conducted at 0 °C in the dark for 15 min at 25 V and approximately 300 mA, and then the slides were rinsed with 400 mM Tris buffer (Wako Pure Chemical Industries, Ltd., Japan) with pH 7.5 for 7 min to neutralize the excess alkali. The neutralized slides were kept in ethanol for 5 min and then allowed to dry at room temperature and then stained with 50 µL (20 µg/mL) ethidium bromide (Wako Pure Chemical Industries, Ltd.) just before microscopical examination.

Slides examination the nuclei on the slides were examined at a 200-fold magnification using a fluorescence microscope (Olympus BX-43, Japan) equipped with a green filter. The image of the cells was captured using digital camera. At least 50 nuclei per slide were analyzed using Comet Assay Software Project (CASP) to measure the diameter of the head, % of DNA in the tail and the length of tail of the comet to obtain DNA migration. Tail moment (TM) and Olive tail moment (OTM) were used as accurate indicators of DNA damage;

$$\text{Tail moment} = \text{Tail length} \times \%\text{Tail DNA}/100$$

$$\text{Olive Tail Moment} = (\text{Tail mean} - \text{Head mean}) \times \%\text{Tail DNA}/100$$

7- Histopathological investigation

Fresh specimens from liver, kidneys, brain and testes of rats from all experimental groups were collected and fixed in 10% neutral buffered formalin. The tissues were dehydrated in a graded alcohol series, cleared with methyl benzoate, embedded in paraffin wax, sectioned at 4 µ thickness and stained with hematoxylin and eosin, histopathological examination by light microscopy (Olympus CX31, Japan) and photographed using digital camera (Olympus, Camedia C-5060, Japan) (**Bancroft and Gamble., 2008**).

8-Statistical analysis:

a. **Statistical analysis of larvicidal bioassay**

Means of data values of larval mortality and standard error were calculated for each concentration of LCN and conventional lambda cyhalothrin. The Student's *t*-test was applied to investigate the significance between the different concentrations of LC and LCN and the mortality rate after 24, 48, and 72 hours of exposure. Results were considered to be statistically significant at $p < .05$.

b. **Statistical analysis of laboratory animals**

The values are expressed as mean ± standard error (S.E) for three animals in each time. Differences between groups were assessed by one-way analysis of variance (ANOVA). Tukey – Dennett test was performed for inter-group comparisons using IBM SPSS Statistics 2010, version19 (IBM SPSS Statistics, Inc., USA). Significance at *P*-values <0.01 and <0.05 have been given respective symbols in the figures.

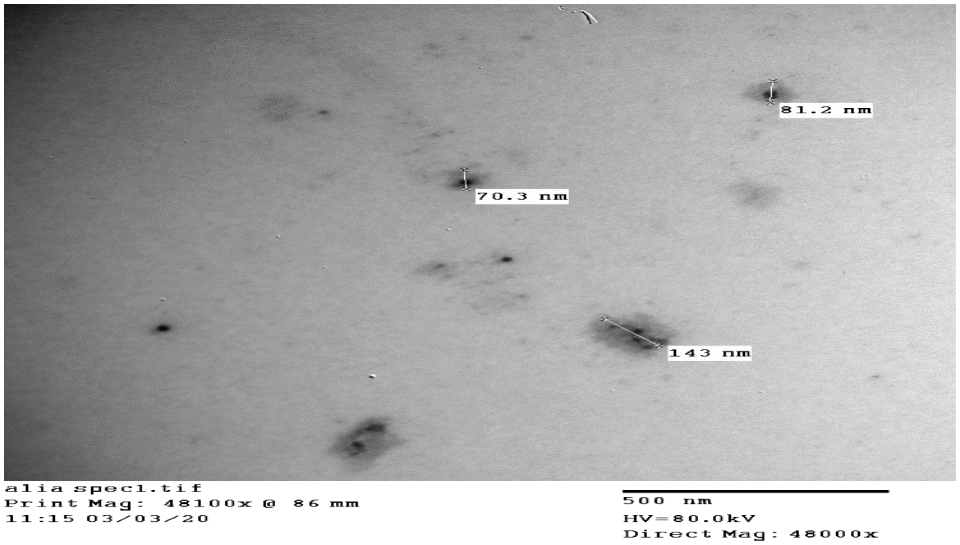
RESULTS

A. Lambda-Cyhalothrin Nano-emulsion Characterization

1-TEM Analysis

The TEM image revealed that the LCN morphology is nearly spherical and has an average size of 70.3 nm in diameter (Figure 1).

Figure 1: TEM image of LCN revealed an average size of 70.3 nm in diameter.



2- Dynamic Light Scattering for Zeta Potential and Particle Size

The average dynamic nano-size by DLS. Zetasizer reached 77.51 nm in diameter. The prepared LCN showed a good stability condition of nano-solution as the poly-disparity index (PDI) is lower than 0.5 (Figure 2). Zeta potential of LCN showed a negative surface charge value (-17. 8 mV) which was sufficiently high to avoid LCN aggregation. This value represents a stable and dispersed suspension of LCN and there is no tendency to form aggregates in a short period of time (Figure 3).

Figure 2: The average dynamic nano-size of LCN by DLS-Zetasizer and PDI.

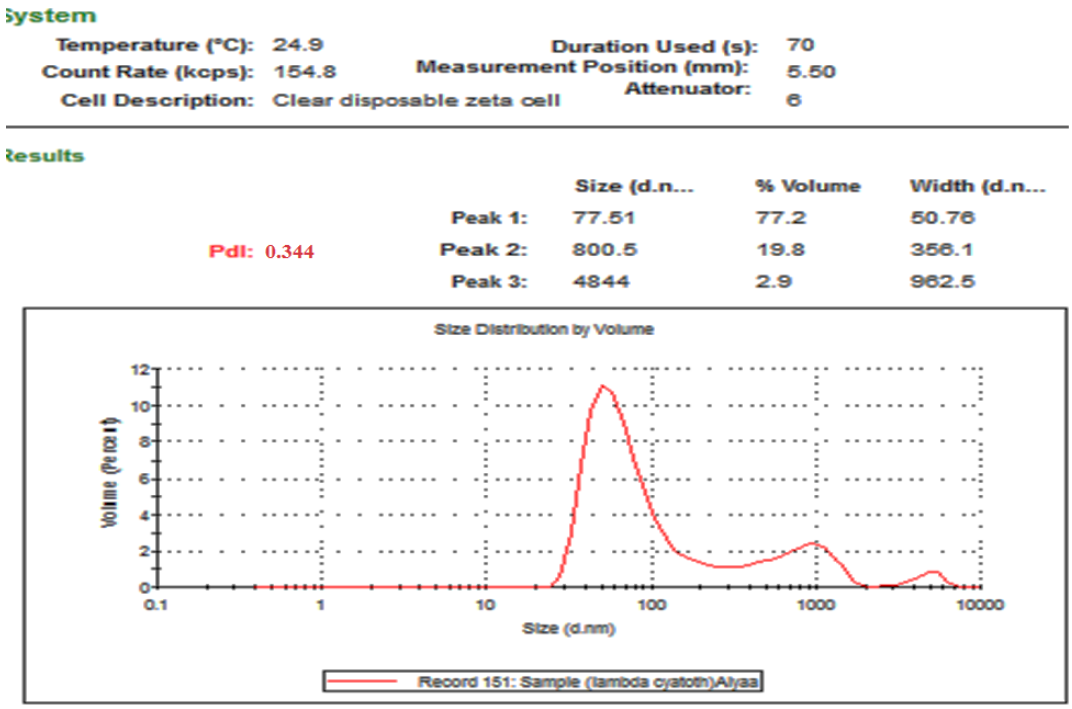
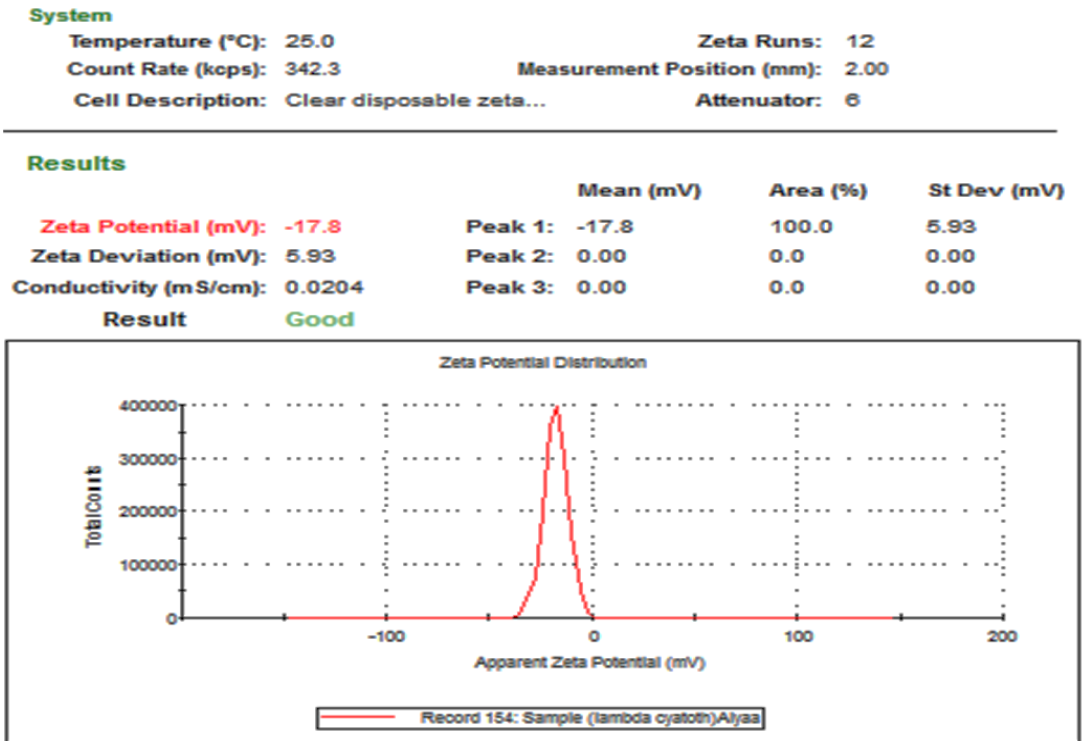


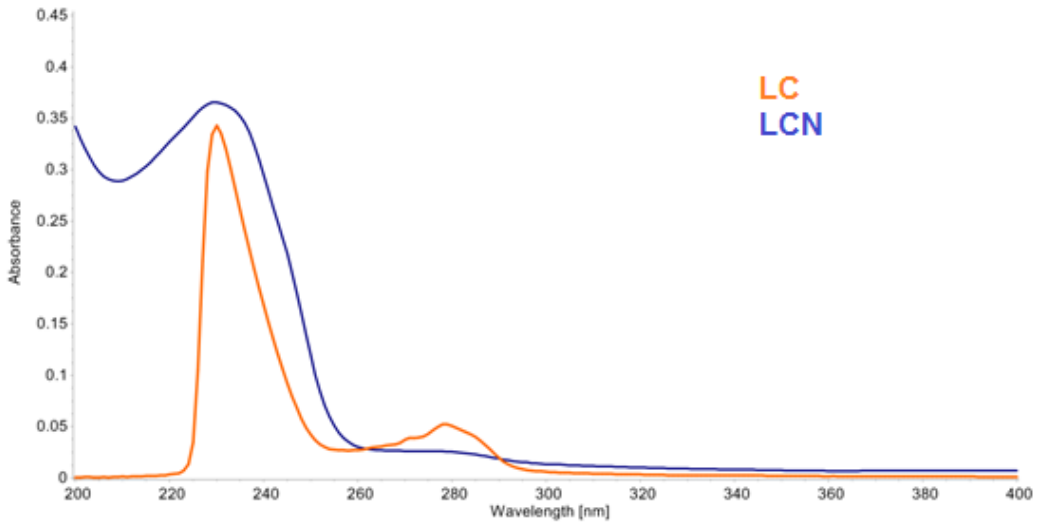
Figure 3: Zeta potential of LCN showed a negative surface charge value.



3- UV-Visible Spectral Analysis

The statement of the composition of conventional lambda and its nanoemulsion formulation of strong absorption band centered at 216 and 245 nm, respectively (Figure 4).

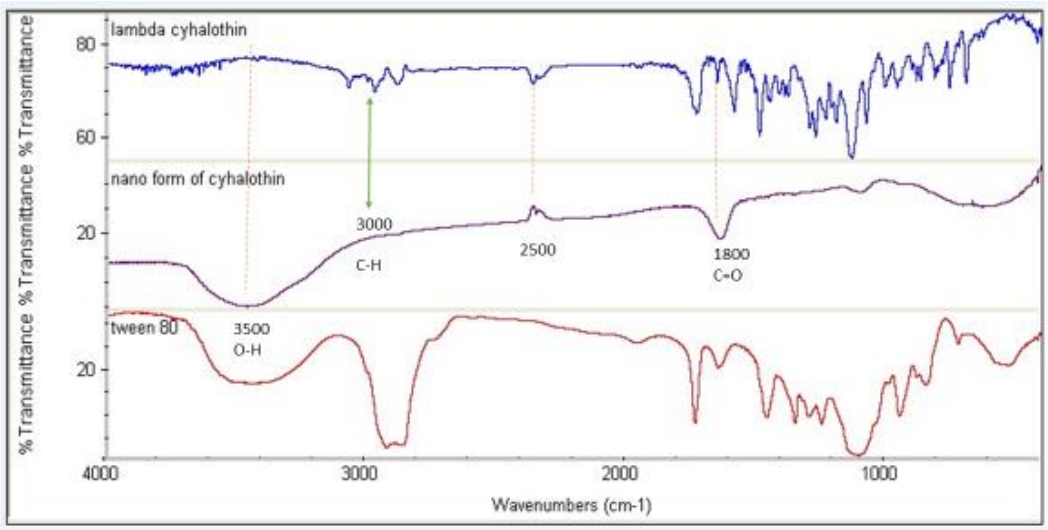
Figure 4: UV-visible spectral analysis of LC and LCN



4- FTIR Spectral Analysis

In the spectrum of the free LC, two peaks appeared at 1800 cm-1 and 2500 cm-1 due to the stretching vibration of the benzene skeleton and represented the stretching vibration of the C=O in ester groups, which were regarded as the characteristic peaks of LC. In the spectra of LCN, the peak at 3500 cm-1 was attributed to the stretching vibration of the O–H group associated with hydrogen bonds. Meanwhile, the peak at 3000 cm-1 represented the deformation vibration of the C–H alkyl group and substituted in benzene structure indicating that the LC has changed and the new form is obtained in LCN (Figure 5).

Figure 5: FTIR spectral analysis of LC and LCN



B .The Larvicidal Activity Bioassay

The larvicidal activity of conventional lambda and its nanoemulsion

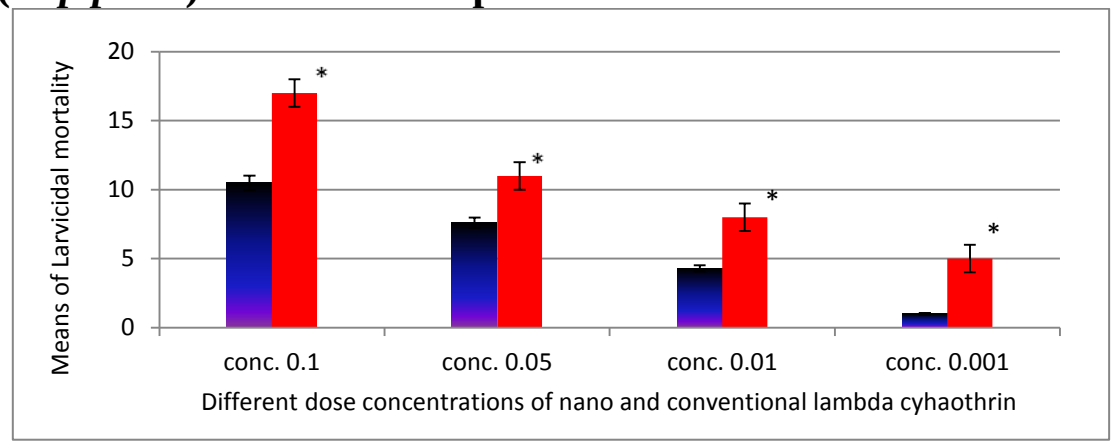
The larvicidal activity of conventional lambda and its nanoemulsion formulation was investigated against the susceptible mosquito larvae (*Culex pipiens*) during 24, 48, and 72 hours of exposure (Table 1 and Figures 6, 7, 8). The statistical analysis of the data of LC and LCN indicated high significant differences between the mortality means. There were also highly significant differences between the means of mortality rates caused by conventional lambda and lambda nanoparticles; hence, the exposure periods exhibited high significant differences between 24, 48, and 72 hours (Table 1 and Figures 6, 7, 8).

Table 1: Larvicidal activity (number of dead larvae) after 24, 48 and 72 hours of exposure to nano and conventional lambda-cyhalothrin.

Exposure		Nano ʎ-Cyhalothrin				Conventional ʎ-Cyhalothrin			
24h	Conc.(µg)	0.1	0.05	0.01	0.001	0.1	0.05	0.01	0.001
	First trial	19	12	10	6	10	7	5	1
	Second trial	15	10	6	4	11	8	4	1
	Third trial	17	11	8	5	10	8	4	1
	Mean	17b*	11b*	8b*	5b*	10.5	7.6	4.3	1
48h	First trial	20	19	15	10	12	10	9	4
	Second trial	16	15	12	8	15	13	7	2
	Third trial	17	11	14	9	13	11	8	3
	Mean	17.5b*	15b*	13.6b*	9b*	13.3	11.3	8	3
72h	First trial	20	20	17	12	19	19	11	8
	Second trial	18	18	17	10	19	18	16	6
	Third trial	19	19	16	11	18	19	15	7
	Mean	19	19	16.6b*	11b*	18	18	14	7

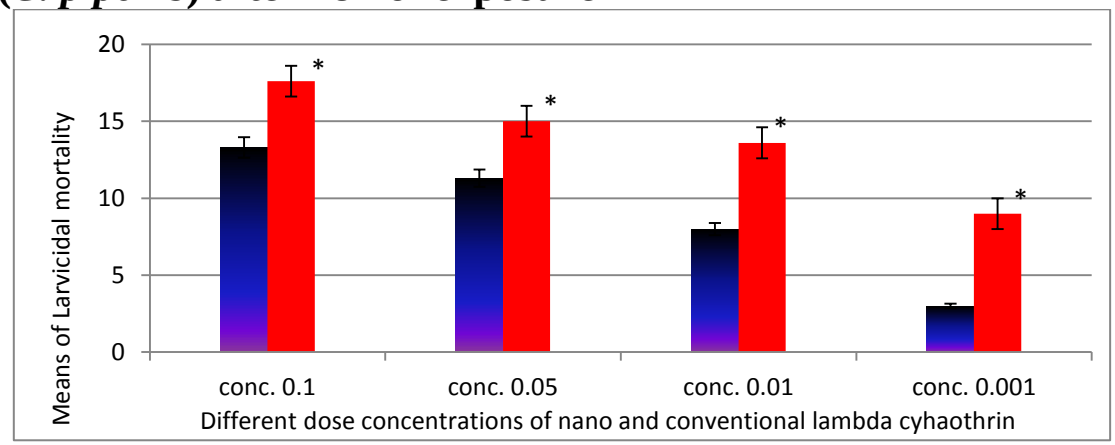
Values are expressed as means ± SE (n=20). *_indicate significant at p≤0.05 , b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group

Figure 6: Larvicidal effect LC annd LCN on mosquito larvae (*C. pipeins*) after 24h of exposure



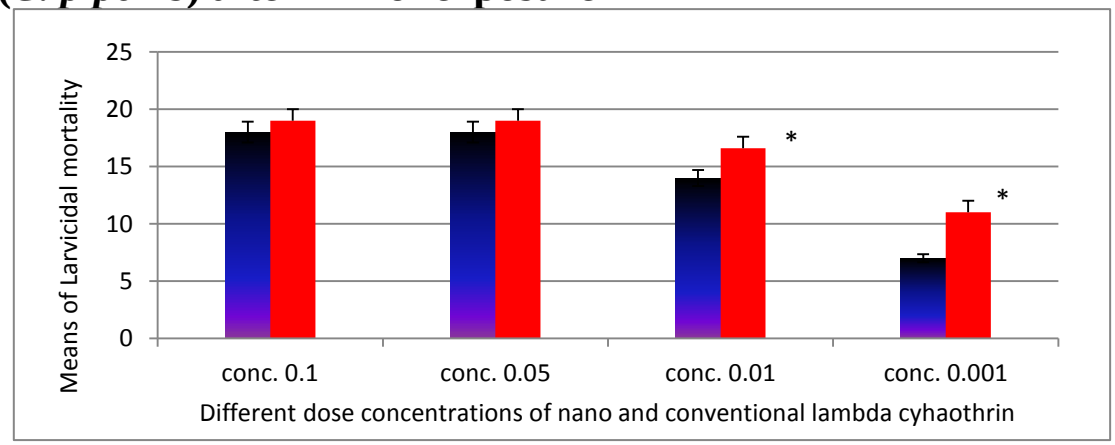
Values are expressed as means ± SE (n=20). *_indicate significant at p≤0.05

Figure 7: Larvicidal effect LC annd LCN on mosquito larvae (*C. pipeins*) after 48h of exposure



Values are expressed as means ± SE (n=20). *_indicate significant at p≤0.05

Figure 8: Larvicidal effect LC annd LCN on mosquito larvae (*C. pipeins*) after 72h of exposure



Values are expressed as means ± SE (n=20). *_indicate significant at p≤0.05

C. Toxicological evaluation of conventional lambda and its nanoemulsion based on three months orally exposure in male rats

Results of two control groups are similar, and presented in tables and figures merged in one final control group.

1-Gravimetric analysis of body and organs:

a- Body weight of male rats (g)

In both groups **conventional ʎ cyhalothrin** treated rats and group of **nano ʎ cyhalothrin** treated rats, the value of final body weight of male rats showed non-significant change during the whole period of experiment in comparison with control group.

The comparison between the results obtained of two groups**,** group of **conventional ʎ cyhalothrin** treated rats and group of **nano ʎ cyhalothrin** treated rats, the value of final body weight of male rats showed non-significant change during whole period of experiment (Table2, Figure 9).

b- Organs / body weight ratio

1- Brain absolute weight (g)

In both groups **conventional ʎ cyhalothrin** treated rats and group of **nano ʎ cyhalothrin** treated rats, the value of brain absolute weight of male rats showed non-significant change during the whole period of experiment in comparison with control group.

The comparison between the results obtained of two groups**,** group of **conventional ʎ cyhalothrin** treated rats and group of **nano ʎ cyhalothrin** treated rats, the value of brain absolute weight of male rats showed non-significant change during the whole period of experiment (Table2).

.

Brain relative weight (g/100 g b.wt.)

The group of **conventional ʎ cyhalothrin** treated rats showed a significant decrease in the value of relative brain weight at **4$^{\underline{th}}$** week of the experiment in comparison with control group.

While in the whole period of experiment, in both groups **conventional ʎ cyhalothrin** treated rats and group of **nano ʎ cyhalothrin** treated rats, the value of brain relative weight showed non-significant change during the whole period of experiment in comparison with control group.

The comparison between the results obtained of two groups, group of **conventional ʎ cyhalothrin** treated rats and group of **nano ʎ cyhalothrin** treated rats, the value of brain relative weight showed non-significant change during the whole period of experiment (Table2, Figure10).

2- Liver absolute weight (g)

The group of **conventional ʎ cyhalothrin** treated rats showed a significant increase in the value of liver absolute weight at **4th** and **10th** week of the experiment in comparison with control group.

But, showed a significant decrease in the value of liver absolute weight at **10th** week of the experiment in comparison with the group of **nano ʎ cyhalothrin** treated rats.

In the group of **nano ʎ cyhalothrin** treated rats, the value of liver absolute weight showed a significant increase during the **10th** week of the experiment in comparison with control group, and in comparison with group of **conventional ʎ cyhalothrin** treated rats (Table2).

Liver relative weight (g/100 g b.wt.)

The group of **conventional ʎ cyhalothrin** treated rats showed a significant increase in the value of liver relative weight at**4th**, **6th** and **12th** week of the experiment in comparison with control group.

In a group of **nano ʎ cyhalothrin** treated rats, the value of liver relative weight showed a significant increase in the value of liver relative weight at**4th**, **10th** and **12th** week of the experiment in comparison with control group.

The comparison between the results obtained of two groups, group of **conventional ʎ cyhalothrin** treated rats and group of **nano ʎ cyhalothrin** treated rats, the value of liver relative weight showed non-significant change during the whole period of experiment (Table2, figure11).

3- Kidney Absolute weight (g)

The group of **conventional ʎ cyhalothrin** treated rats showed a significant increase in the value of kidney absolute weight at **4^{th}** and the **10^{th}** week of the experiment in comparison with control group. Also, showed highly significant increase in comparison with group of **nano ʎ cyhalothrin** treated rats.

In a group of **nano ʎ cyhalothrin** treated rats, the value of kidney absolute weight at **4^{th}** and the **10^{th}** week of the experiment showed a highly significant increase in comparison with group of **conventional ʎ cyhalothrin** treated rat (Table2).

Kidney relative weight (g/100 g b.wt.)

The group of **conventional ʎ cyhalothrin** treated rats showed a significant increase during the**4^{th}**, **6^{th}** , **10^{th}** and **12^{th}** week of the experiment in comparison with control group

In the group of **nano ʎ cyhalothrin** treated rats, the value of kidney relative weight showed a significant increase during the **10^{th}** week of the experiment in comparison with control group.

The comparison between the results obtained of two groups, group of **conventional ʎ cyhalothrin** treated rats and group of **nano ʎ cyhalothrin** treated rats, the value of kidney relative weight showed non-significant change during the whole period of experiment (Table2, Figure12).

4- Testis Absolute weight (g)

The group of **conventional ʎ cyhalothrin** treated rats showed non-significant change in the value of testis absolute weight in comparison with control group and with group of **nano ʎ cyhalothrin** treated rats, during the whole period of experiment.

In a group of **nano ʎ cyhalothrin** treated rats, the value of testis absolute weight showed non-significant change in comparison with control group and with group of **conventional ʎ cyhalothrin** treated rats (Table2).

Testis relative weight (g/100 g b.wt.)

The group of **conventional ʎ cyhalothrin** treated rats showed non-significant change in the value of testis relative weight during the whole period of experiment in comparison with control group and with group of **nano ʎ cyhalothrin** treated rats.

In a group of **nano ʎ cyhalothrin** treated rats, the value of testis relative weight showed non-significant change during the whole period of experiment in comparison with control group and with group of **conventional ʎ cyhalothrin** treated rats (Table2, Figure13).

…

.

.

.

.

.

.

.

.

.

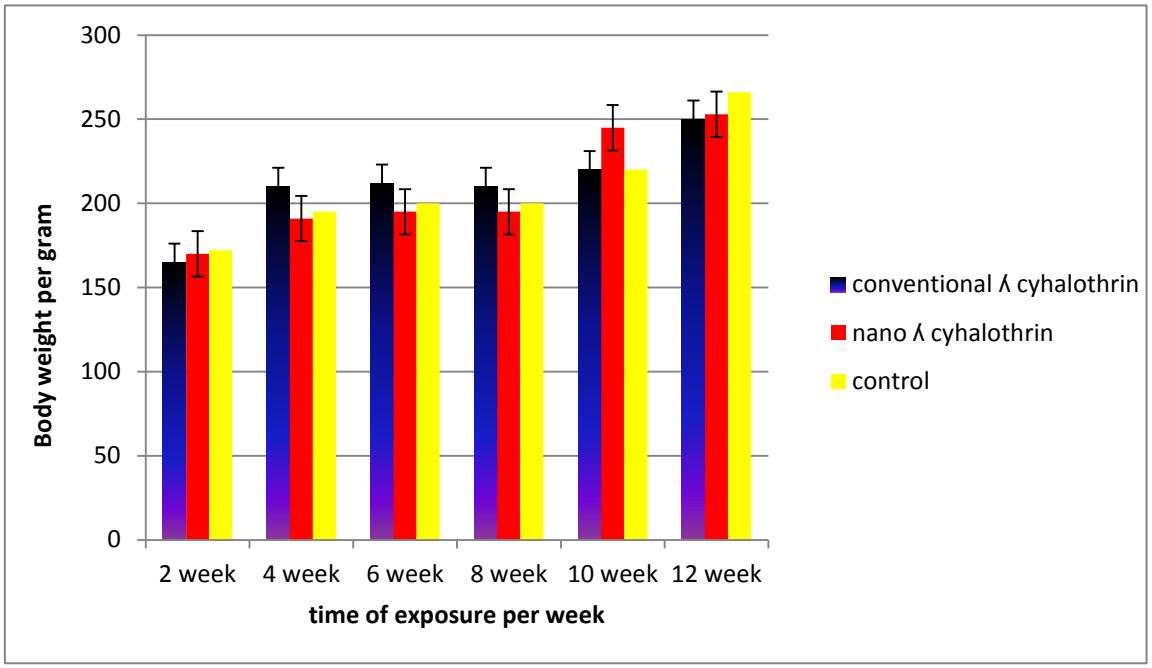
.

Table 2: The effect of three months orally exposure of conventional and nano ʎ cyhalothrin on absolute (g) and relative% body and organ weight in male rats.

Group	Time post-exposure	Body Weight (g)	Organs weight							
			Brain Weight		Liver Weight		Kidney Weight		Testis Weight	
			Absolute(g)	Relative (%)	Absolute(g)	Relative (%)	Absolute(g)	Relative (%)	Absolute(g)	Relative (%)
Conventional ʎ cyhalothrin	2nd week	165 ±2.6	1.5±0.04	0.91±0.02	4.6±0.26	2.77±0.14	1.12±0.09	0.62±0.11	3.82±0. 27	2.27±0.15
	4th week	210±5.7	1.6±0.04	0.76±0.02 a**	7.5±0.77 a*	3.56±0.32 a*	1.65±0.17 a*c**	0.78±0.08 a*	4.23±0.56	2.02±0.29
	6th week	212.5±13.7	1.51±0.13	0.71±0.06	5.25±0.5	2.450±0.07 a*	1.21±0.13	0.56±0.03 a*	3.53±0.47	1.64±0.19
	8th week	210±12.2	1.41±0.06	0.67±0.05	5.75±0.29	2.74±0.04	1.26±0.02	0.60±0.03	3.27±0.36	1.54±0.12
	10th week	220±10	1.56±0.1	0.71±0.08	6.43±0.17 c*	2.92±0.06	1.32±0.03 a**c**	0.60±0.04 a*	3.44±0.14	1.56±0.01
	12th week	250±0.00	1.51±0.07	0.60±0.03	7.40±0.28	2.95±0.11 a*	2.04±0.15	0.81±0.06 a*	3.81±0.06	1.52±0.18
Nano ʎ cyhalothrin	2nd week	170 ±12.9	1.52±0.04	0.90±0.04	4.8±0.45	2.87±0.10	1.01±0.06	0.59±0.03	3.51±0.42	2.03±0.10
	4th week	191.7±11.3	1.6±0.04	1.6±0.04	5.8±0.15	3.07±0.21 a*	1.07±0.07 b**	0.56±0.07	3.55±0.06	1.86±0.10
	6th week	195±2.04	1.50±0.10	0.76±0.05	4.57±0.25	2.34±0.26	1.15±0.09	0.58±0.05	3.05±.040	1.56±0.20
	8th week	195±2.8	1.44±0.45	0.73±0.03	5.18±0.82	2.65±0.19	1.32±0.06	0.67±0.03	3.32±0.42	1.63±0.22
	10th week	245±5	1.64±0.1	0.66±0.05	8.61±0.31 a*b*	3.52±0.20 a*	1.59±0.06 a**b**	0.65±0.04 a*	3.84±0.44	1.57±0.21
	12th week	253±3.3	1.65±0.1	0.64±0.03	7.47±0.18	2.94±0.03 a*	1.72±0.08	0.67±0.02	3.67±0.02	1.45±0.04
Control	2nd week	172±1.52	1.4±0.03	0.86±0.02	5.2±0.04	3.05±0.01	1.08±0.04	0.68±0.03	3.5±0.05	2.03±0.04
	4th week	195±2.8	1.6±0.03	0.81±0.006	5.1±0.10	2.62±0.47	1.12±0.16	0.57±0.07	2.38±0.14	1.12±0.07
	6th week	200±10	1.55±0.35	0.76±0.13	4.10±0.10	2.05±0.05	0.87±0.03	0.43±0.01	2.31±0.19	1.15±0.15
	8th week	200±5.7	1.49±0.07	0.74±0.05	6.02±0.12	3.01±0.07	1.22±0.04	0.61±0.02	2.43±0.09	1.21±0.07
	10th week	220±15.7	1.58±0.02	0.72±0.04	6.10±0.6	2.80±0.38	1.06±0.03	0.48±0.04	2.84±0.16	1.30±0.10
	12th week	266±16	1.50±0.08	0.56±0.01	7.52±1.6	2.62±0.45	1.67±0.36	0.61±0.09	3.72±0.70	1.37±0.16

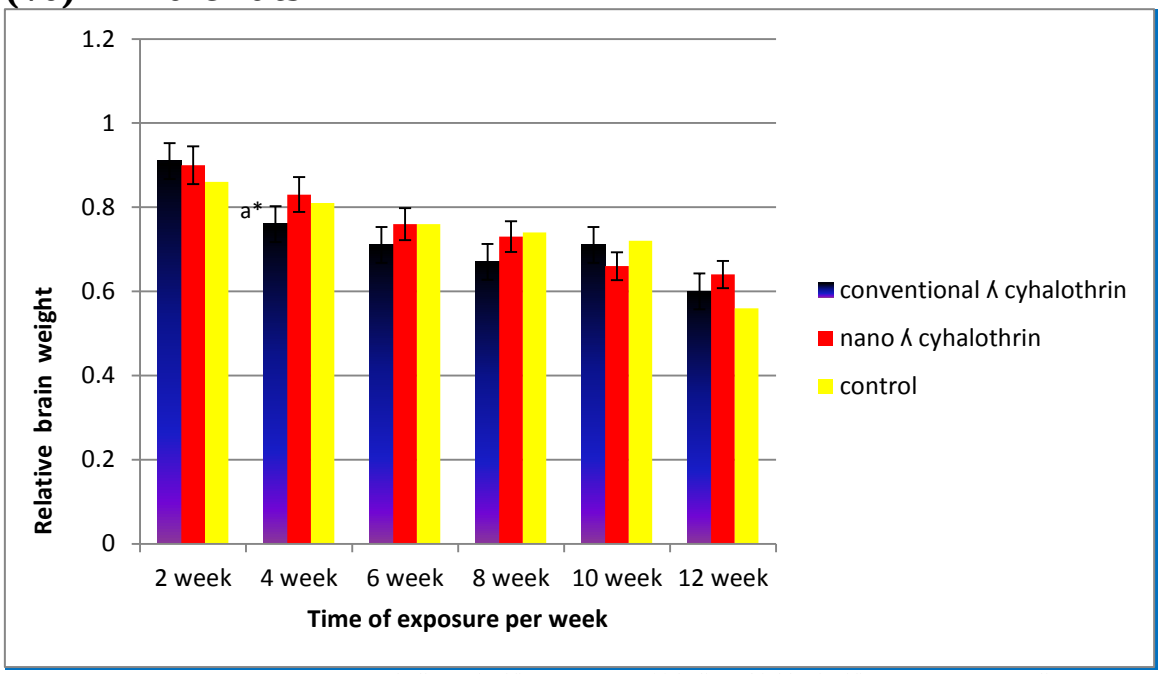
Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 9: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on final body weight (g) in male rats.



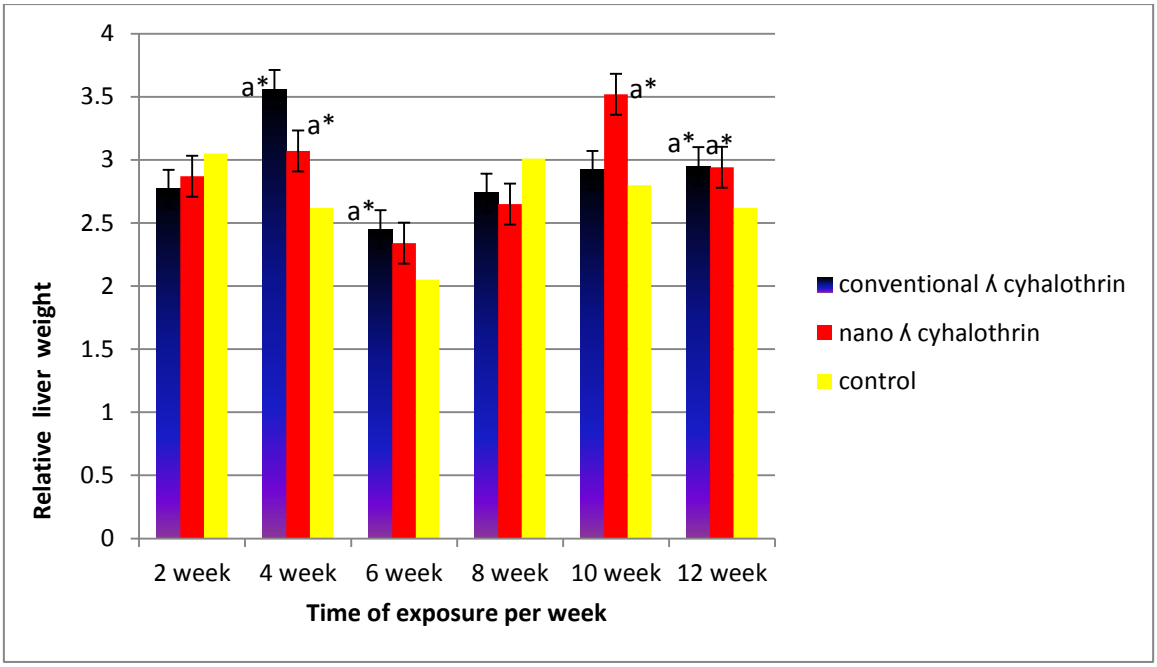
Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 10: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on brain relative weight (%) in male rats



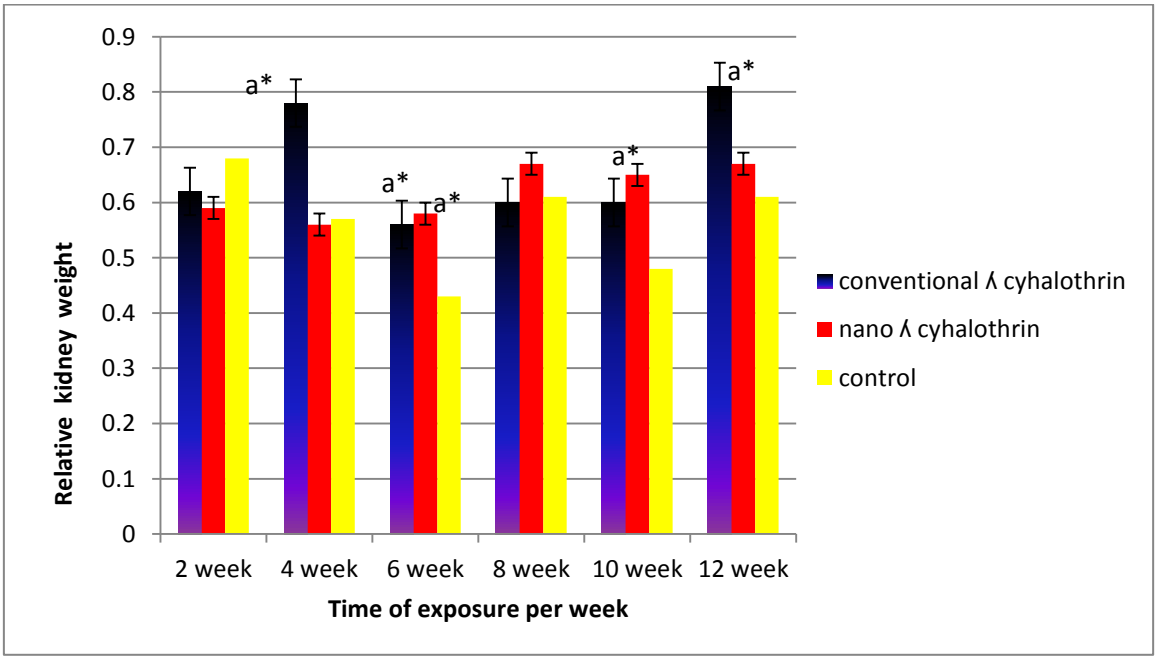
Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 11: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on liver relative weight (%) in male rats



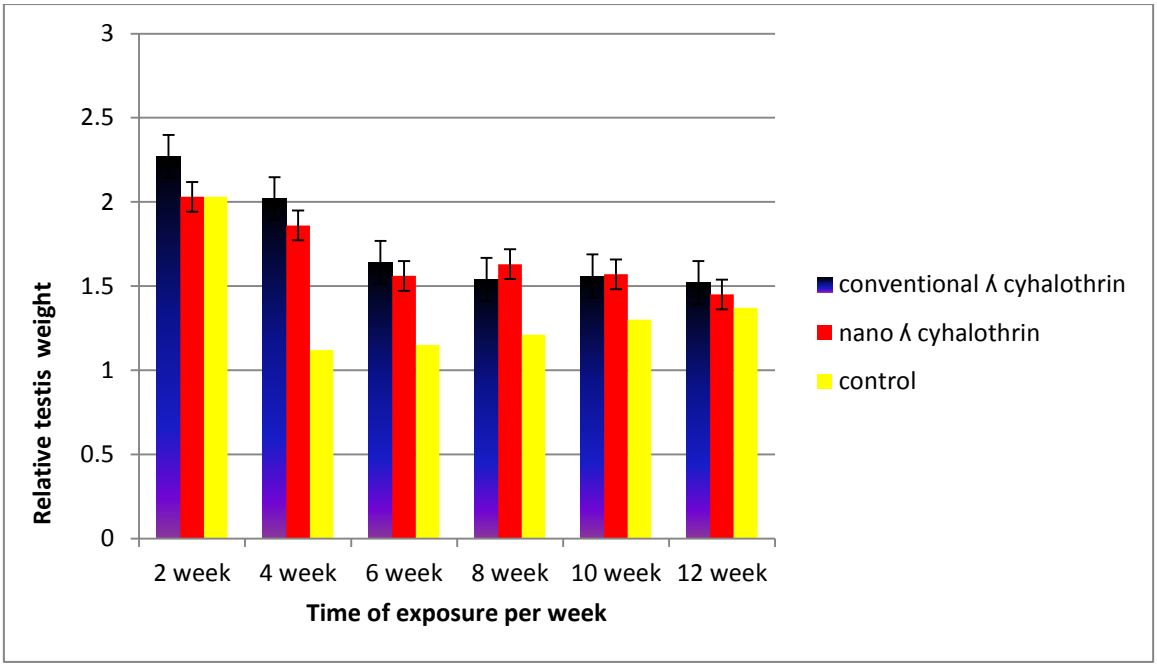
Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 12: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on kidney relative weight (%) in male rats



Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 13: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on testis relative weight (%) in male rat



Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

2- Haematological parameters

In both groups **conventional ʎ cyhalothrin** treated rats and group of **nano ʎ cyhalothrin** treated rats, the value of RBC's counts, Hb content, Hct and MPV values of male rats showed non-significant change during the whole period of experiment in comparison with control group.

The comparison between the results obtained of two groups**,** group of **conventional ʎ cyhalothrin** treated rats and group of nano ʎ cyhalothrin treated rats, the value of RBC's counts, Hb content, Hct and MPV of male rats showed non-significant change during the whole period of experiment (Table3, Figures 14,15,16)**.**

The value of MCH in group of **conventional ʎ cyhalothrin** treated rats showed a significant decrease at **6th** and **8th** week of the experiment in comparison with control group.

In a group of **nano ʎ cyhalothrin** treated rats, the value of MCH showed a significant decrease at $\mathbf{6^{th}}$ and $\mathbf{8^{th}}$week of the experiment in comparison with control group.

The comparison between the results obtained of two groups, group of **conventional ʎ cyhalothrin**

treated rats and group of **nano ʎ cyhalothrin** treated rats, the value of MCH showed non-significant change during the whole period of experiment (Table3, Figure 17).

The value of MCHC in group of **conventional ʎ cyhalothrin** treated rats showed a significant decrease at $\mathbf{8^{th}}$ week of the experiment in comparison with control group.

In a group of **nano ʎ cyhalothrin** treated rats, the value of MCHC showed non-significant change during the whole period of the experiment in comparison with control group.

The comparison between the results obtained of two groups, group of **conventional ʎ cyhalothrin** treated rats and group of **nano ʎ cyhalothrin** treated rats, the value of MCHC showed non-significant change during the whole period of experiment (Table3, Figure 18).

The value of MCV in group of **conventional ʎ cyhalothrin** treated rats showed a highly significant decrease at $\mathbf{2^{nd}}$ and $\mathbf{4^{th}}$ week of the experiment, while showed a significant decrease at $\mathbf{6^{th}}$and $\mathbf{8^{th}}$week of the experiment in comparison with control group.

In a group of **nano ʎ cyhalothrin** treated rats, the value of MCV showed a highly significant decrease at $\mathbf{2^{nd}}$ and $\mathbf{4^{th}}$ week of the experiment, while showed a significant decrease at $\mathbf{6^{th}}$, $\mathbf{8^{th}}$and $\mathbf{10^{th}}$week of the experiment in comparison with control group.

The comparison between the results obtained of two groups, group of **conventional ʎ cyhalothrin** treated rats and group of **nano ʎ cyhalothrin** treated rats, the value of MCV showed non-significant change during the whole period of experiment (Table3, Figure 19).

The value of RDW in group of **conventional ʎ cyhalothrin** treated rats showed a highly significant decrease at **2^{nd}** , **6^{th}**and **8^{th}** week of the experiment, while showed a significant decrease at **4^{th}**, **10^{th}**and **12^{th}**week of the experiment in comparison with control group.

In a group of **nano ʎ cyhalothrin** treated rats, the value of RDW showed a highly significant decrease at **2^{nd}**, and **4^{th}** week of the experiment, while showed a significant decrease at **6^{th}**, **8^{th}**, **10^{th}**and **12^{th}**week of the experiment in comparison with control group.

The comparison between the results obtained of two groups, group of **conventional ʎ cyhalothrin** treated rats and group of **nano ʎ cyhalothrin** treated rats, the value of RDW showed non-significant change during the whole period of experiment (Table3, Figure 20).

The value of Platelets count in group of **conventional ʎ cyhalothrin** treated rats showed a significant increase at **4^{th}** , **6^{th}**and **10^{th}** week of the experiment, in comparison with control group.

In a group of **nano ʎ cyhalothrin** treated rats, the value of Platelets count showed a significant increase at **6^{th}** week of the experiment, in comparison with control group.

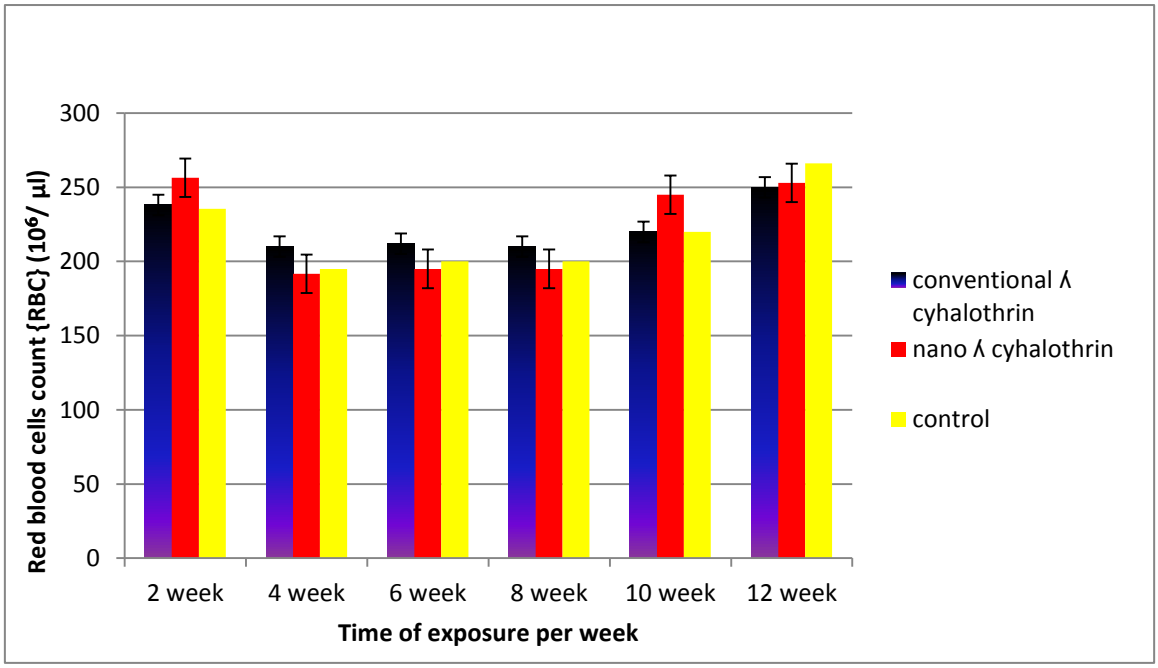
The comparison between the results obtained of two groups, group of **conventional ʎ cyhalothrin** treated rats and group of **nano ʎ cyhalothrin** treated rats, the value of Platelets count showed non-significant change during the whole period of experiment (Table3, Figure 21).

Table 3: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on haematological parameters in male rats.

Group	Time	RBC (10^6 / µl)	HB (g/dl)	HCT (%)	MCH (Pg)	MCHC (g / dL)	MCV (fL)	MPV (fL)	RDW (%)	Platelets (10^3/ µl)
Conventional ʎ cyhalothrin	2nd week	238.1 ±0.28	13.85±1.8	39.7±4.7	18.4±0.15 a*	35.1±0.25	52.4±1 a**	6.20±0.6	33.5±1.05 a**	613.5±10.5
	4th week	210±5.7	13.8±0.6	38.9±1.4	19.5±0.05 a*	35.4±0.25	55±0.1 a**	6.05±0.5 5	36.5±0.0 a*	755.5±17 a*
	6th week	212.5±13.7	12.6±1	36.5±0.7	19.2±0.0 a**	35.2.±0.0	54.4±0.0 a*	5.95±0.05	38.1±0.8 a**	793±49 a*
	8th week	210±12.2	12.35±0.35	37.1±0.7	18.9±0.35 a*	33.4±0.35 a*	56.8±0.5 a*	6.8±0. 6	36.3±2.4 a**	673.5±32
	10th week	220±10	11.8±1.7	35.5±4.7	21.3±1.3	33.1±0.35	64.5±4.7	5.8±0.40	40.2±1.3 a*	692.5±5.5 a*
	12th week	250±0.00	12.43±0.73	37. 7±2.3	20.50±0.9	33±0.34	62.1±3	5.96±0.08	41.6±2.6 a*	634.3±28
Nano ʎ cyhalothrin	2nd week	265.4±0.07	11.35±0.35	33.5±0. 5	18.4±0. 0 a*	35.6±0.3	51.7±0.4 a**	6.85±0.05	31.3±1.35 a**	667.5±61.5
	4th week	191.7±11.3	12.65±0.25	35.8±1	18.6±0.6 a*	35.3±0.4	52.9±1.2 a**	5.9±0.2	35.8±0.35 a**	625.5.5±101
	6th week	195±2.04	15±0.5	44.3±2.5	20.3±0.75 a*	34.1±0.7	59.8±3.3 a*	5.65±0.25	41.5±3.1 a*	709.5±50 *
	8th week	195±2.8	11.1±0.7	32.45±2	19.2±0.05 a*	34.2±0.10	56.2±0.1 a*	6.1±0.4	38.2±1.2 a*	665.5±31
	10th week	245±5	12.25±0.55	37.3±1.7	19.2±0.4	32.9±0.20	58.4±1 a*	6.1±0.20	38.5±0.8 a*	659.5±5.5
	12th week	253±3.3	13.36±0.43	38.4±1.4	20.03±0.26	33±0.20	60.5±0.5	6.16±0.41	40.7±1.1 a*	664±50
Control	2nd week	235.4 ±0.1	12.5±1	35±2	20.5±1	33.5±1	65±1	6.15±0.05	58±2	620±10
	4th week	195±2.8	13.22±1	36.5±1	20.4±2	33.4±2	64.5±1	6.4±2	55±4	635±5
	6th week	200±10	10.95±2.8	38.3±3.5	23.2±0.15	32.9±0.15	65.5±4.1	6.55±0.35	58.15±1.8	690±5
	8th week	200±5.7	12.1±1	35.4±1	22±1	34.1±0.0	64.6±2	6.8±1	47.9±2.1	660.5±0.5
	10th week	220±15.7	12.45±0.55	37.3±1.4	20.9±0.0	33.3±0.15	62.6±0.3	6.45±0.15	44.8±0.9	614±47
	12th week	266±16	11.56±0.4	34.3±1	21.4±0.59	33.6±0.18	63.7±2.1	5.76±0.18	45.1±2.1	635±62

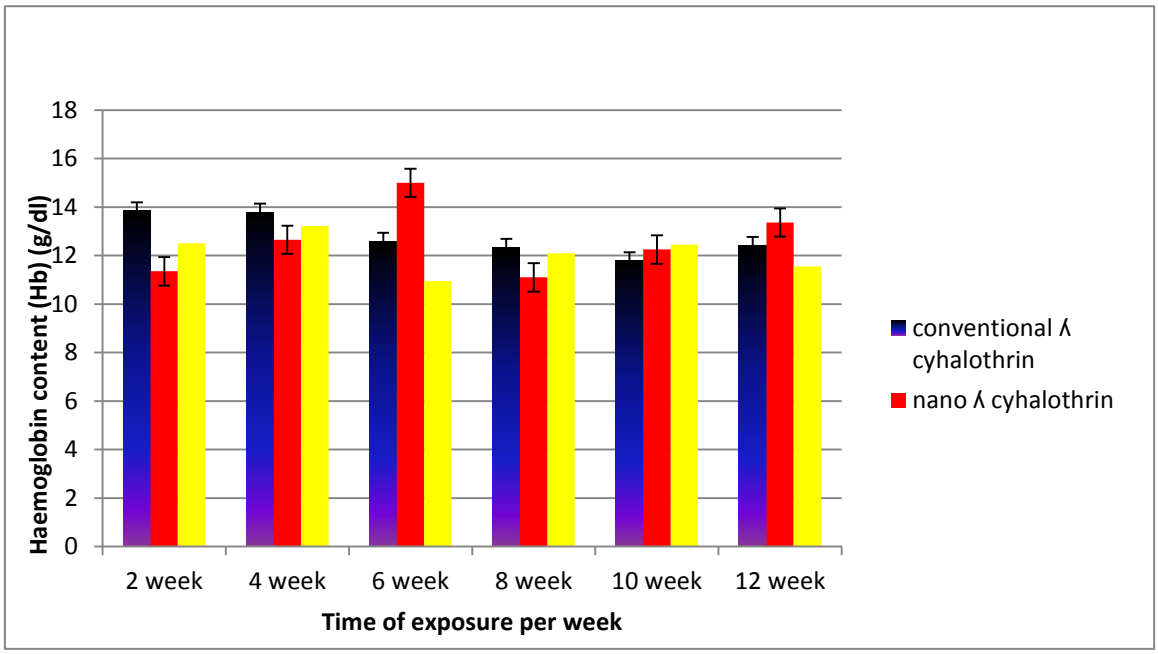
Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 14: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on red blood cells count (RBC) (10^6/ µl) in male rat



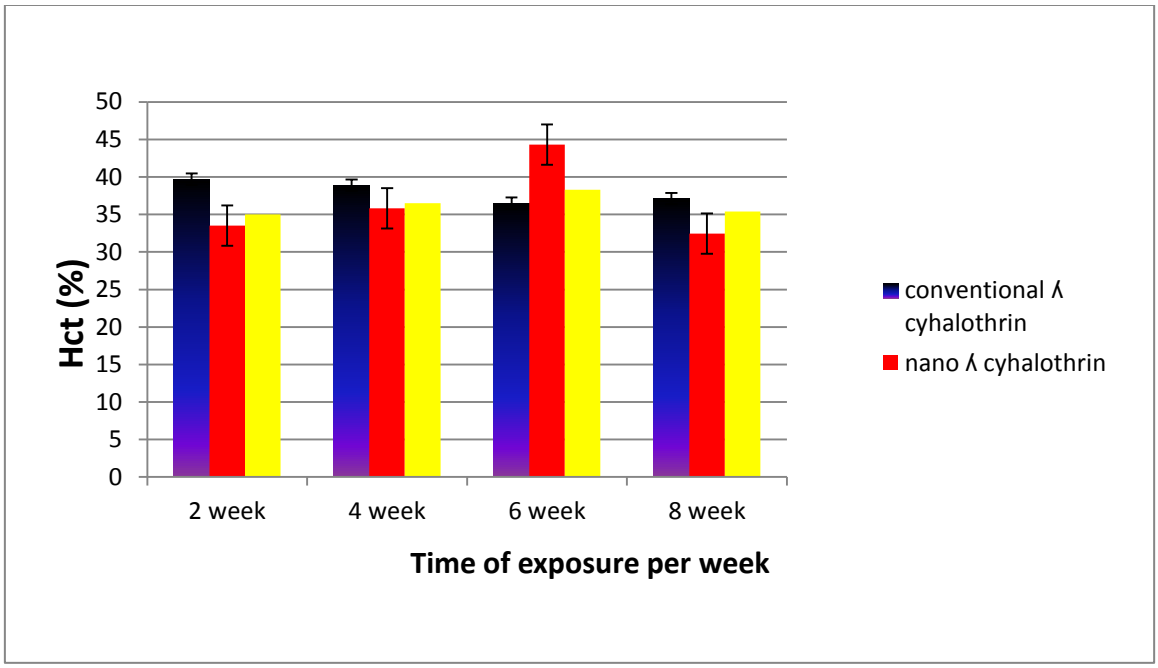
Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 15: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on haemoglobin content (Hb) (g/dl) in male rat



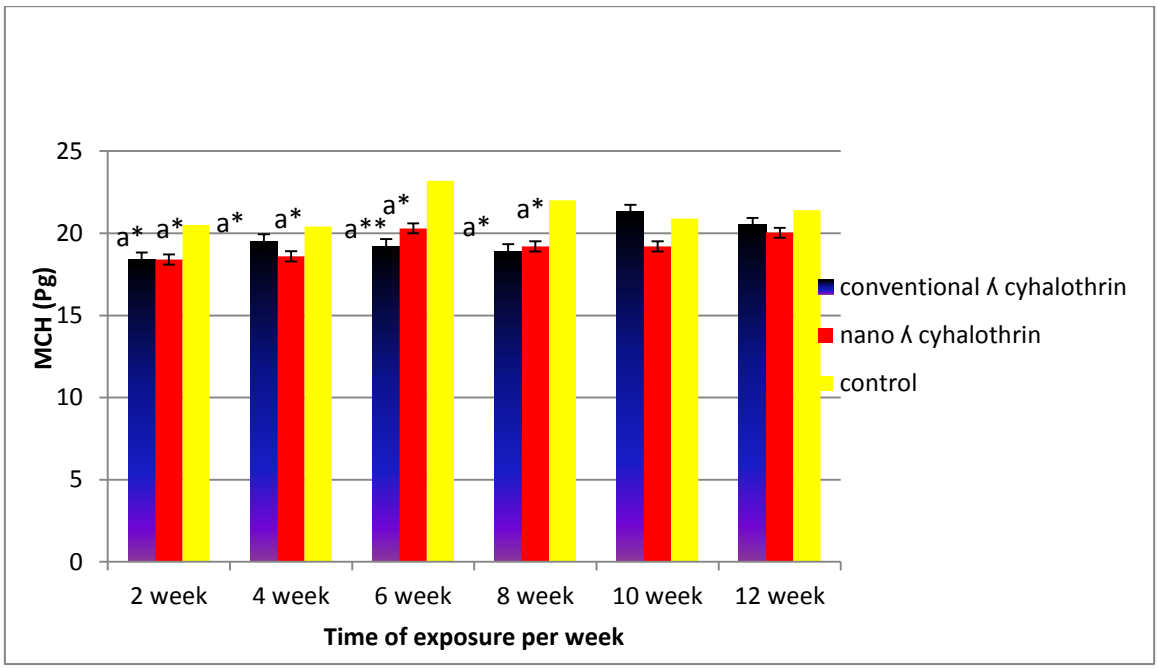
Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 16: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on Hct (%) in male rat



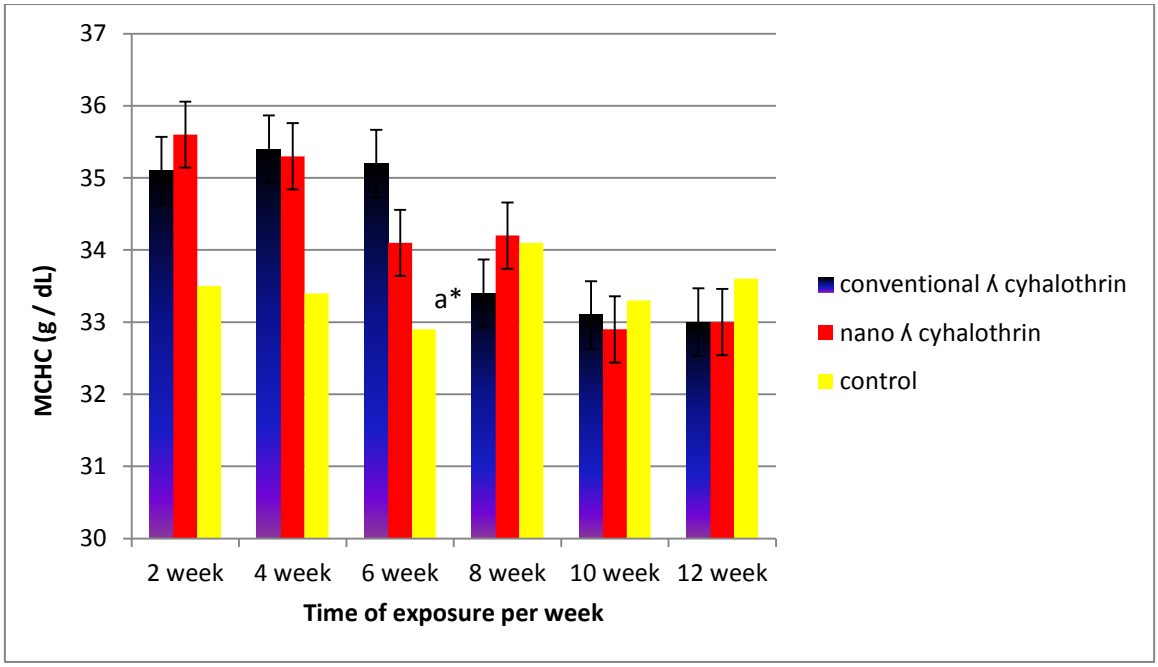
Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 17: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on MCH (Pg) in male rat



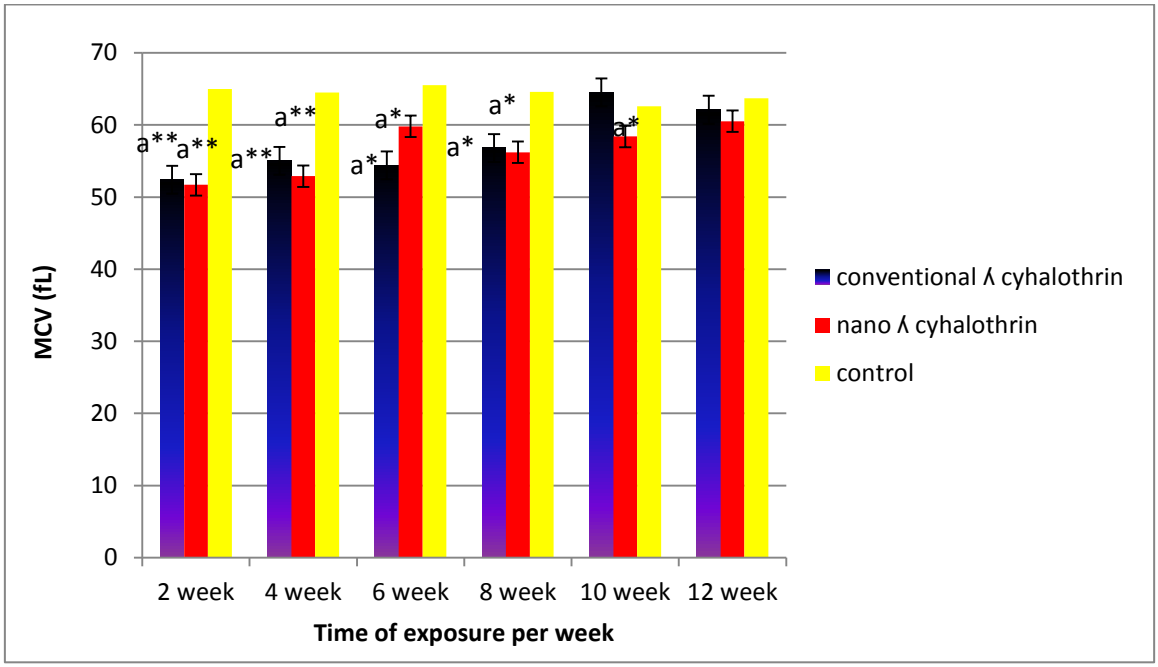
Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 18: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on MCHC (g / dL) in male rat



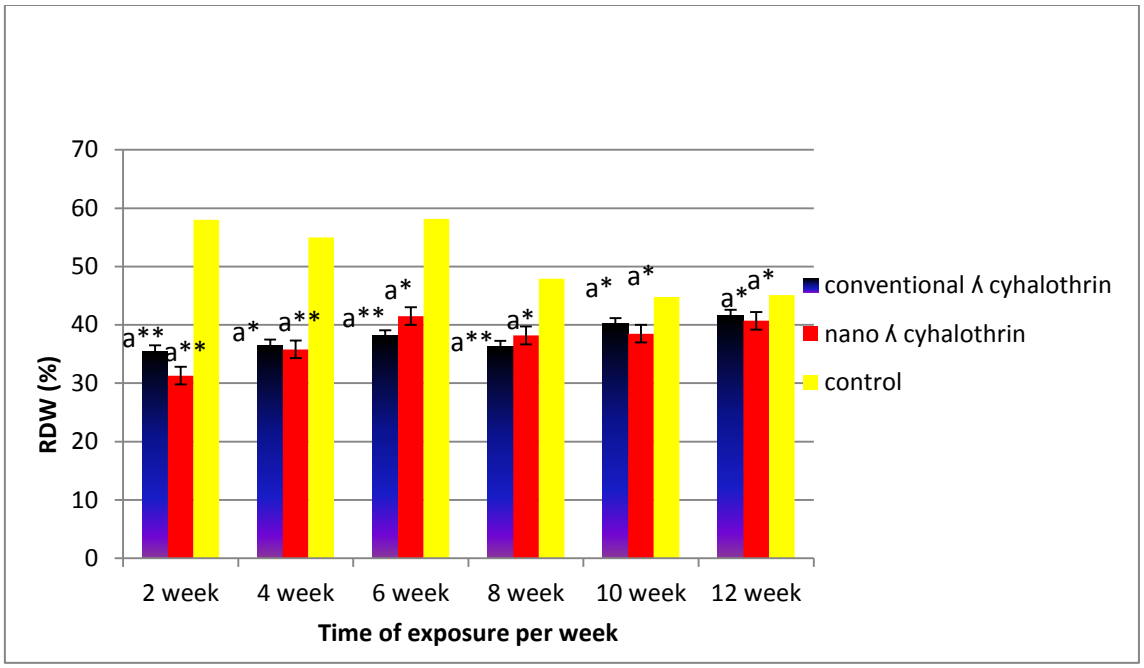
Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 19: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on MCV (fL) in male rat



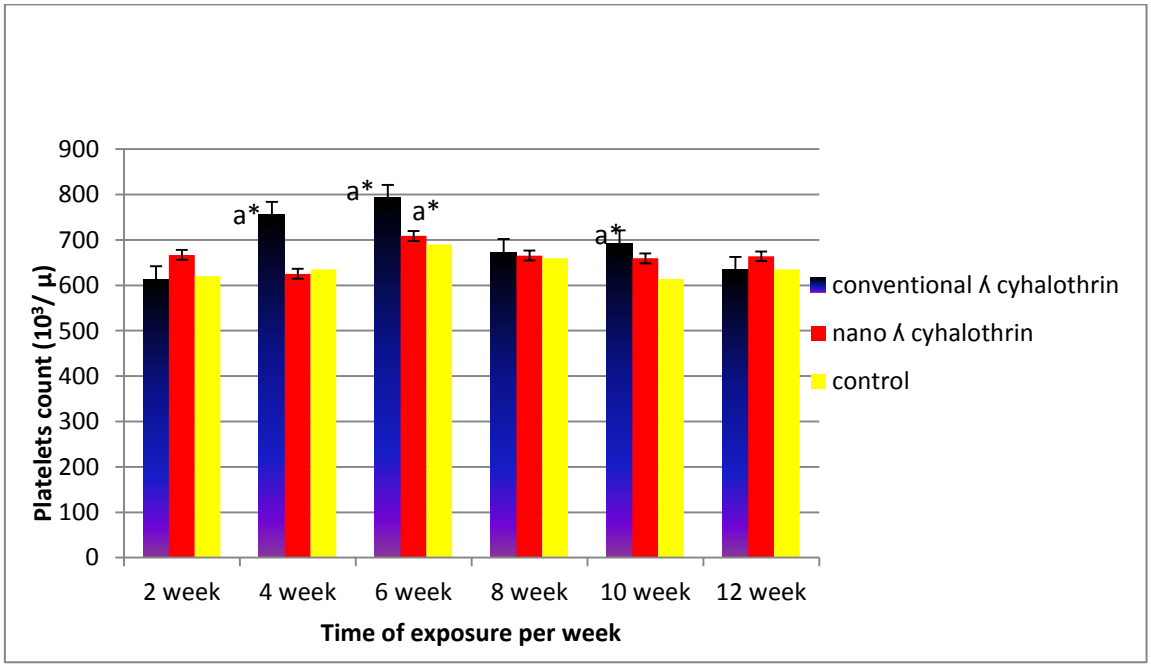
Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 20: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on RDW (%) in male rat



Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 21: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on Platelets count (10^3/ µl) in male rat.



Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Table 4: The effect of three months orally exposure of conventional and nano ʎ cyhalothrin on WBCs/ differential leucocytic count / leukocyte formula /Immunological parameters **in male rats.**

Group	Time	WBC (10^3 / µl)	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)
Conventional ʎ cyhalothrin	2nd week	4.6 ±0.05	3.35±0.15	0.95±0.05	0.27±0.07
	4th week	9.9±1.9a*c*	6.95±1.3a* c*	2.55±0.55a*	0.40±0.00a*
	6th week	6.5±0.9a*	4.85±0.95a*	1.15±0.05	0.50±0.0a*
	8thweek	6.15±0.6a*	4.20±0.2a*	1.6±0.5	0.25±0.05
	10thweek	9.05±0.45a*	6.85±0.35a* c*	1.7±0.20	0.50±0.10a*
	12thweek	9.96±1.12a*	4.73±0.89 a*	1.73±0.17	0.50±0.1a*
Nano ʎ cyhalothrin	2nd week	5.5 ±0.1a*	3.7±0.3	0.8±0.1	0.32±0.02
	4th week	7±3.2a*b*	4.1±1.9a* b*	3.3±1.3a*	0.60±0.10a*
	6th week	8.2±1.6a*	5.25±1.3a*	2.45±0.25a*	0.45±0.05a*
	8thweek	7.5±0.25a*	4.55±0.45a*	2.45±0.85a*	0.55±0.15a*
	10thweek	7.5±2.5a*	4.95±1.4a *b*	2±0.80	0.55±0.25a*
	12thweek	9.26±1.18a*	6.46±0.77a*	2.36±0.44a*	0.43±0.08a*
Control	2nd week	4.8±0.1	3.4±0.05	1.75±0.05	0.3±0.01
	4th week	4.85±0.15	3.45±0.05	1.8±0.1	035±0.05
	6th week	5.5±0.3	3.15±0.35	1.75±0.05	0.20±0.10
	8thweek	4.9±0.1	2.95±0.55	1.85±0.05	0.27±0.02
	10thweek	4.15±1.4	2.6.±1.4	1.9±0.00	0.25±0.05
	12thweek	4.86±0.08	2.93±0.5	1.86±0.96	0.33±0.14

Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group

3- Immunological parameters (differential leucocytes count / leukocyte formula)

The value of WBCs count in group of **conventional ʎ cyhalothrin** treated rats showed a significant increase at $\mathbf{4^{th}}$, $\mathbf{6^{th}}$, $\mathbf{8^{th}}$, $\mathbf{10^{th}}$ and $\mathbf{12^{th}}$ week of the in comparison with control group, Also showed a significant increase at $\mathbf{4^{th}}$ week of the experiment in comparison with **nano ʎ cyhalothrin treated rats.**

In a group of **nano ʎ cyhalothrin** treated rats, the value of WBCs count showed a significant increase at whole period of experiment in comparison with control group, while showed a significant decrease at $\mathbf{4^{th}}$ week of the experiment in comparison with group of **conventional ʎ cyhalothrin** treated rats (Table4, Figure 22).

The value of lymphocytes count in group of **conventional ʎ cyhalothrin** treated rats showed a significant increase at $\mathbf{4^{th}}$, $\mathbf{6^{th}}$, $\mathbf{8^{th}}$, $\mathbf{10^{th}}$and $\mathbf{12^{th}}$ week of the experiment in comparison with control group, Also showed a significant increase at $\mathbf{4^{th}}$, **and** $\mathbf{10^{th}}$ week of the experiment in comparison with **nano ʎ cyhalothrin** treated rats.

In a group of **nano ʎ cyhalothrin** treated rats, the value of lymphocytes count showed a significant increase at $\mathbf{4^{th}}$, $\mathbf{6^{th}}$, $\mathbf{8^{th}}$, $\mathbf{10^{th}}$and $\mathbf{12^{th}}$ week of the experiment in comparison with control group, while showed a significant decrease at $\mathbf{4^{th}}$ and $\mathbf{10^{th}}$ week of the experiment in comparison with group of **conventional ʎ cyhalothrin** treated rats (Table4, Figure 23).

The value of the peripheral neutrophils percentage in group of **conventional ʎ cyhalothrin** treated rats showed a significant increase at $\mathbf{4^{th}}$ week of the experiment in comparison with control group.

In a group of **nano ʎ cyhalothrin** treated rats, the value of the peripheral neutrophils percentage showed a significant increase at $\mathbf{4^{th}}$, $\mathbf{6^{th}}$, $\mathbf{8^{th}}$,and $\mathbf{12^{th}}$ week of the experiment in comparison with control group.

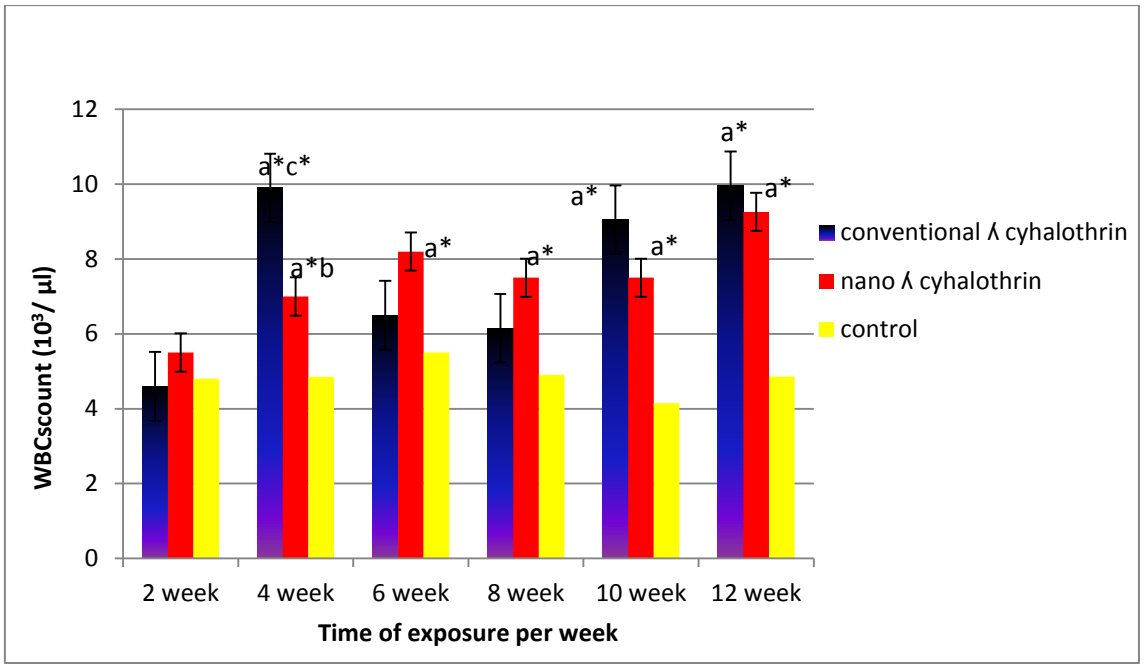
The comparison between the results obtained of two groups, group of **conventional ʎ cyhalothrin** treated rats and group of **nano ʎ cyhalothrin** treated rats, showed non-significant change during the whole period of experiment (Table4, Figure 24).

The value of the peripheral monocytes percentage in group of **conventional ʎ cyhalothrin** treated rats showed a significant increase at $\mathbf{4^{th}}$, $\mathbf{6^{th}}$, $\mathbf{10^{th}}$ and $\mathbf{12^{th}}$ week of the experiment in comparison with control group.

In a group of **nano ʎ cyhalothrin** treated rats, the value of the peripheral monocytes percentage showed a significant increase at$\mathbf{4^{th}}$, $\mathbf{6^{th}}$, $\mathbf{8^{th}}$, $\mathbf{10^{th}}$ and $\mathbf{12^{th}}$ week of the experiment in comparison with control group.

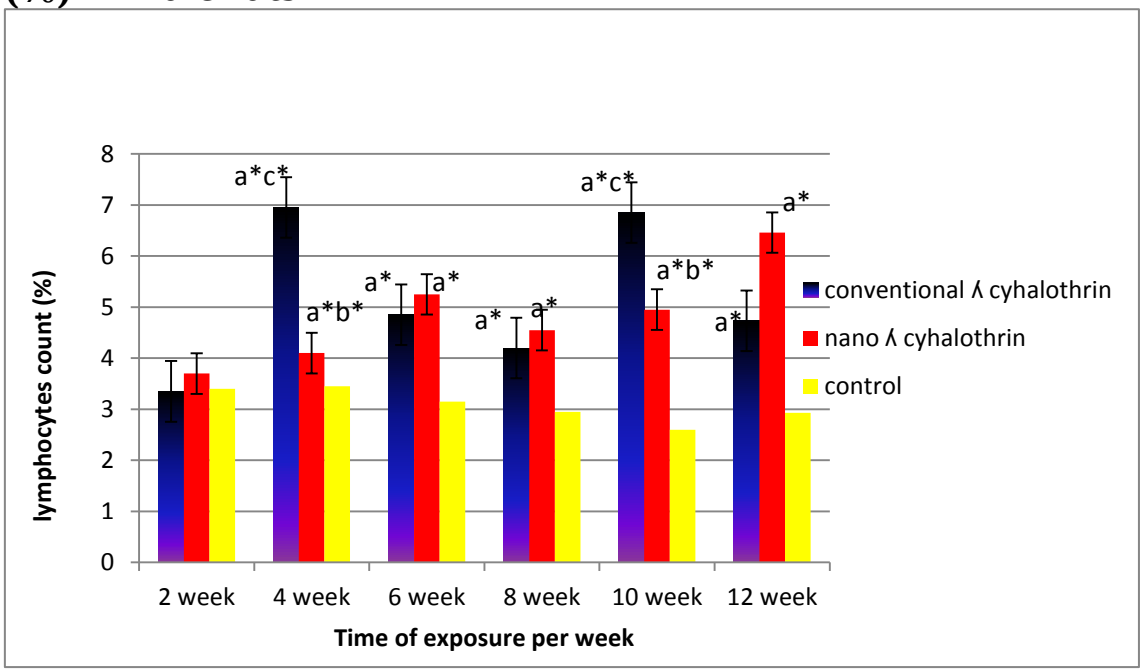
The comparison between the results obtained of two groups, group of **conventional ʎ cyhalothrin** treated rats and group of **nano ʎ cyhalothrin** treated rats, showed non-significant change during the whole period of experiment (Table4, Figure 25).

Figure 22: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on WBCs count (10^3/ µl) in male rat



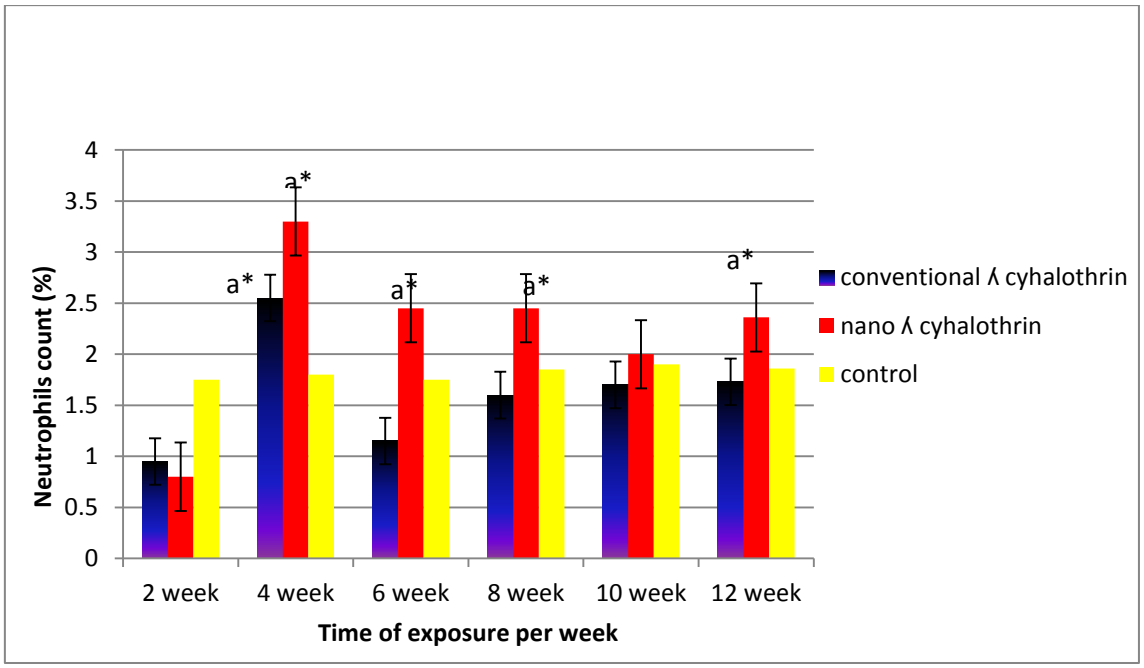
Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 23: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on lymphocytes count (%) in male rats



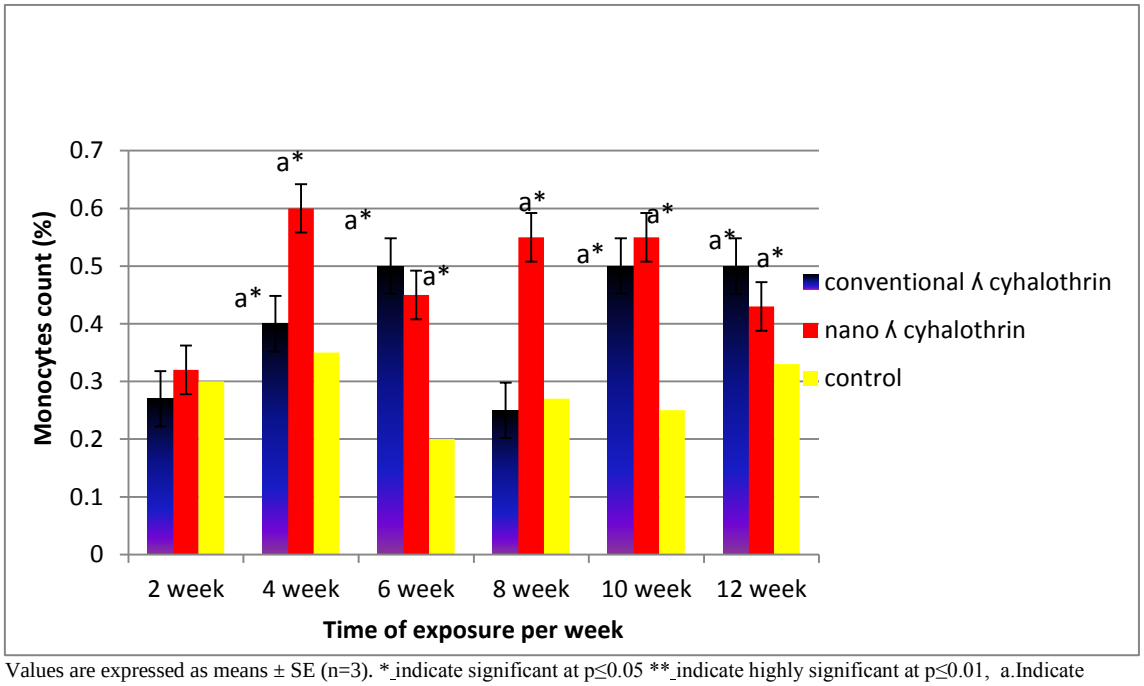
Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 24: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on neutrophils count (%) in male rats



Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 25: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on monocytes count (%) in male rats



Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

2- Oxidative Stress Indicators

Superoxide dismutase activity (SOD)

The group of **conventional ʎ cyhalothrin** treated rats showed a significant decrease in the value of (SOD) concentration during the whole period of experiment in comparison with control group, This decrease became highly significant at **10^{th}** week of the experiment. Also, showed a significant decrease at **8^{th}** week of the experiment in comparison with group of **nano ʎ cyhalothrin** treated rats.

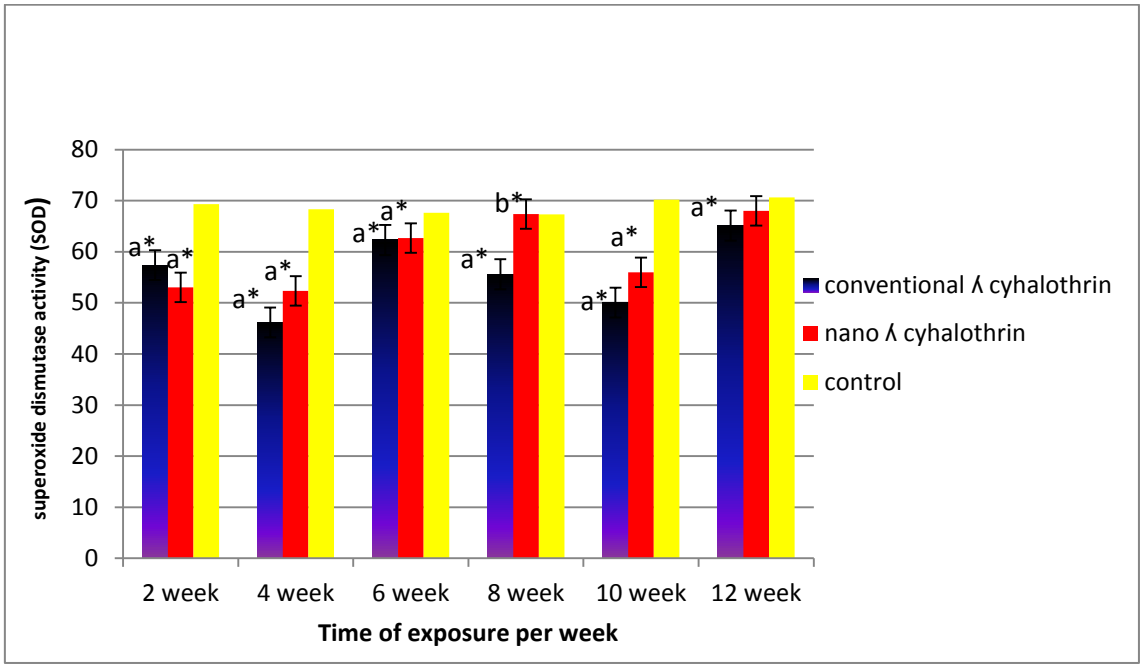
In the group of **nano ʎ cyhalothrin** treated rats, the value of (SOD) concentration showed a significant decrease at **2^{nd}** , **4^{th}**, **6^{th}** and **10^{th}** week of the experiment in comparison with control group. While showed a significant increase at **8^{th}** week of the experiment in comparison with group of **conventional ʎ cyhalothrin** treated rats (Table5, Figure 26).

Table 5: The effect of three months orally exposure of conventional and nano ʎ cyhalothrin on superoxide dismutase activity (SOD) in male rats.

Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group .

Group / Time post-exposure	Conventional ʎ-cyhalothrin	Nano ʎ -cyhalothrin	Control
2^{nd} weeks	57.39±1.64 a*	53.02±7.24a*	69.33±0.66
4^{th} weeks	46.16±8.51a*	52.37±7.05a*	68.33±0.88
6^{th} weeks	62.32±5.75a*	62.66±1.61a*	67.66±0.33
8^{th} weeks	55.60±5.23a*c*	67.93±2.41b*	67.66±0.33
10^{th}weeks	50.06±5.26a**	56±3.91a*	70.23±0.12
12^{th}weeks	65.13±2.52a*	68.03±4.73	70.65±0.20

Figure 26: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on superoxide dismutase activity (SOD) in male rats



Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group .

3- Lipid peroxidation

Malondialdehyde (MDA) level

The group of **conventional ʎ cyhalothrin** treated rats showed a significant increase in the value of (MDA) concentration at **2^{nd}** , **4^{th}**, **6^{th}** and **12^{th}** week of the experiment in comparison with control group. Also, showed a significant increase at **10^{th}** week of the experiment in comparison with group of **nano ʎ cyhalothrin** treated rats.

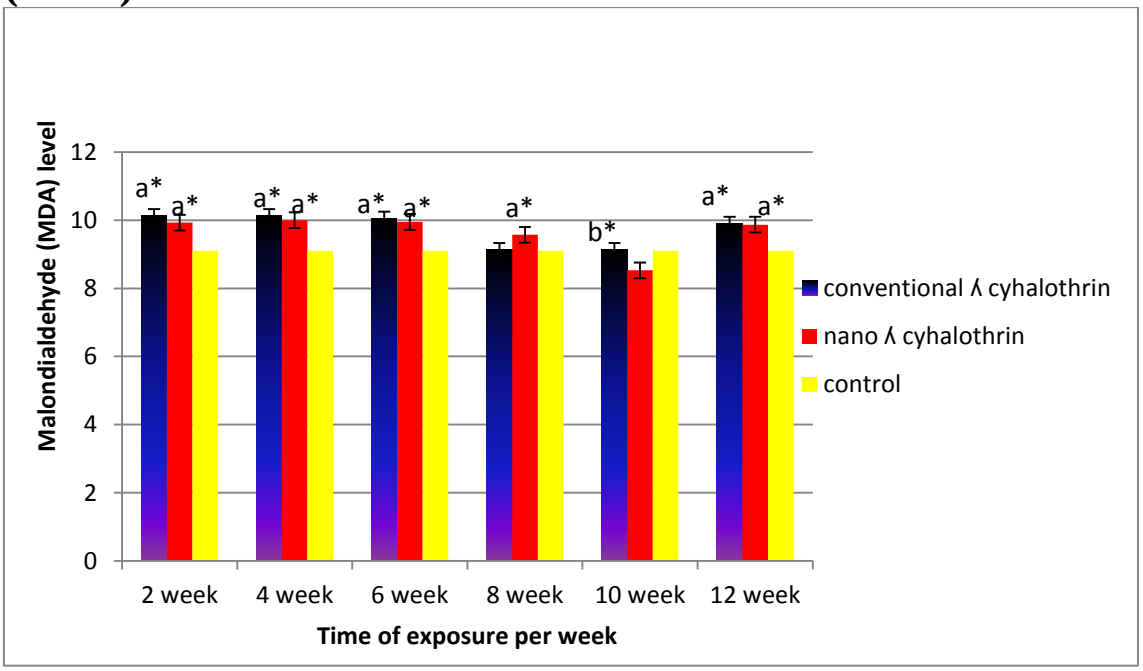
In the group of **nano ʎ cyhalothrin** treated rats, the value of (MDA) concentration showed a significant increase at **2^{nd}** , **4^{th}**, **6^{th}**, **8^{th}** and **12^{th}** week of the experiment in comparison with control group. While showed a significant decrease at **10^{th}** week of the experiment in comparison with group of **conventional ʎ cyhalothrin** treated rats (Table 6, Figure 27).

Table 6: The effect of three months orally exposure of conventional and nano ʎ cyhalothrin on Malondialdehyde (MDA) level in male rats.

Group / Time post-exposure	Conventional ʎ-cyhalothrin	Nano ʎ-cyhalothrin	Control
2nd weeks	10.13±0.033a*	9.93±0.088a*	9.10±0.058
4th weeks	10.13±0.085a*	10±0.058a*	9.10±0.058
6thweeks	10.05±0.155a*	9.95±0.144a*	9.10±0.058
8thweeks	9.13±0.133	9.57±0.120a*	9.10±0.058
10thweeks	9.13±0.133c*	8.53±0.033b*	9.10±0.058
12thweeks	9.90±0.058a*	9.87±0.033a*	9.10±0.058

Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b. Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group .

Figure 27: The effect of three months orally exposure to conventional and nano ʎ cyhalothrin on malondialdehyde (MDA) level in male rats



Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group .

4- Effect on Inflammatory mediators (Immune-toxic effect)

Tumor necrosis factor-α (TNF α) level

The group of **conventional ʎ cyhalothrin** treated rats showed a highly significant increase in the value of (TNF α) concentration at $\mathbf{2^{nd}}$, $\mathbf{4^{th}}$, $\mathbf{6^{th}}$, $\mathbf{8^{th}}$ and $\mathbf{12^{th}}$ week of the experiment in comparison with control group. Also, showed a highly significant increase at $\mathbf{2^{th}}$ week of the experiment in comparison with group of **nano ʎ cyhalothrin** treated rats, But showed a highly significant decrease at $\mathbf{4^{th}}$ and $\mathbf{6^{th}}$ week of the experiment in comparison with group of **nano ʎ cyhalothrin** treated rats.

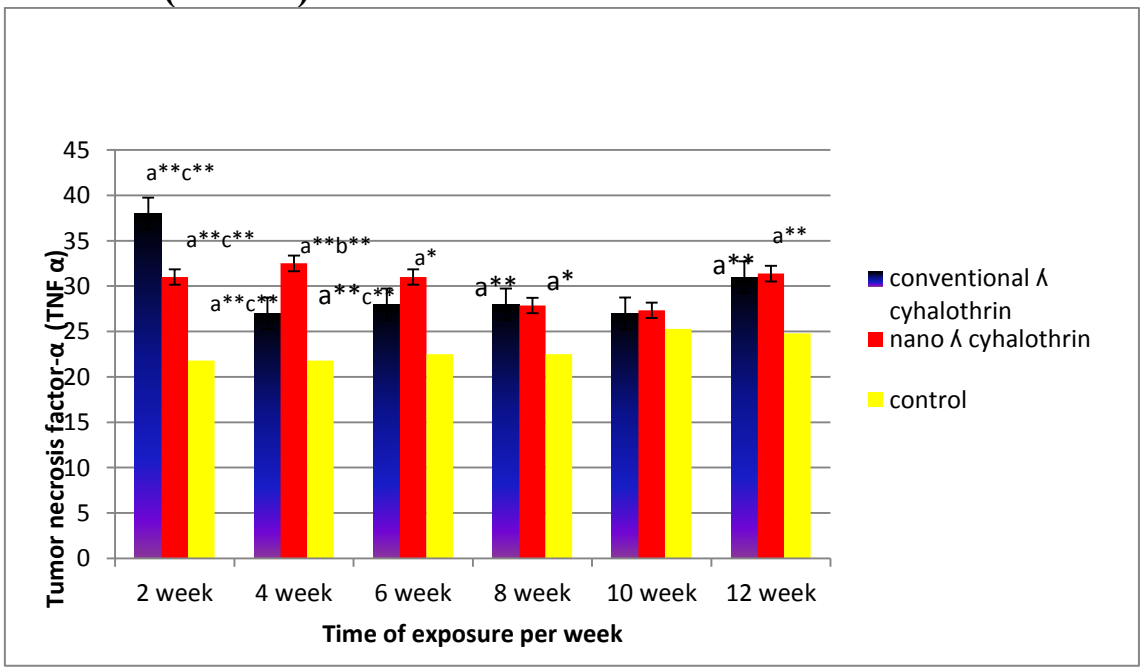
In a group of **nano ʎ cyhalothrin** treated rats, the value of (TNF α) concentration showed a significant increase at $\mathbf{2^{nd}}$, $\mathbf{4^{th}}$, $\mathbf{6^{th}}$, $\mathbf{8^{th}}$ and $\mathbf{12^{th}}$ week of the experiment in comparison with control group. This increase became highly significant at $\mathbf{2^{nd}}$, $\mathbf{4^{th}}$, $\mathbf{6^{th}}$and $\mathbf{12^{th}}$ week of the experiment. Also showed a highly significant increase at $\mathbf{4^{th}}$ and $\mathbf{6^{th}}$ week of the experiment in comparison with group of **conventional ʎ cyhalothrin** treated rats. But, showed a highly significant decrease at $\mathbf{4^{th}}$ week of the experiment in comparison with group of **nano ʎ cyhalothrin** treated rats (Table 7, Figure 28).

Table 7: The effect of three months orally exposure of conventional and nano ʎ cyhalothrin on tumor necrosis factor-α (TNF α) level in male rats.

Group / Time post-exposure	Conventional ʎ-cyhalothrin	Nano ʎ -cyhalothrin	Control
2nd weeks	38±0.36a**c**	31±0.71a**b**	21.8±0.41
4th weeks	27±0.81a**c**	32.5±0.28a**b**	21.8±0.41
6th weeks	28±0.40a**c*	31±0.40a**b*	22.5±1.05
8th weeks	28.75±0.75a**	27.87±1.55a*	22.5±1.05
10th weeks	27.5±0.95	27.32±0.52	25.3±0.54
12th weeks	31±0.40**a	31.37±0.55**a	24.8±0.57

Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

Figure 28: The effect of three months orally exposure of conventional and nano ʎ cyhalothrin on tumor necrosis factor-α (TNF α) level in male rats.



Values are expressed as means ± SE (n=3). *_indicate significant at p≤0.05 **_indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group.

7- Evaluation of DNA damage with comet assay

A- Genotoxicity in blood

The group of conventional ʎ cyhalothrin treated rats showed a non-significant change in the value of comet assay parameters which indicate DNA damage in blood as tail DNA%, tail length (µm), tail moment % and olive tail moment% at whole time of the experiment in comparison with control group.

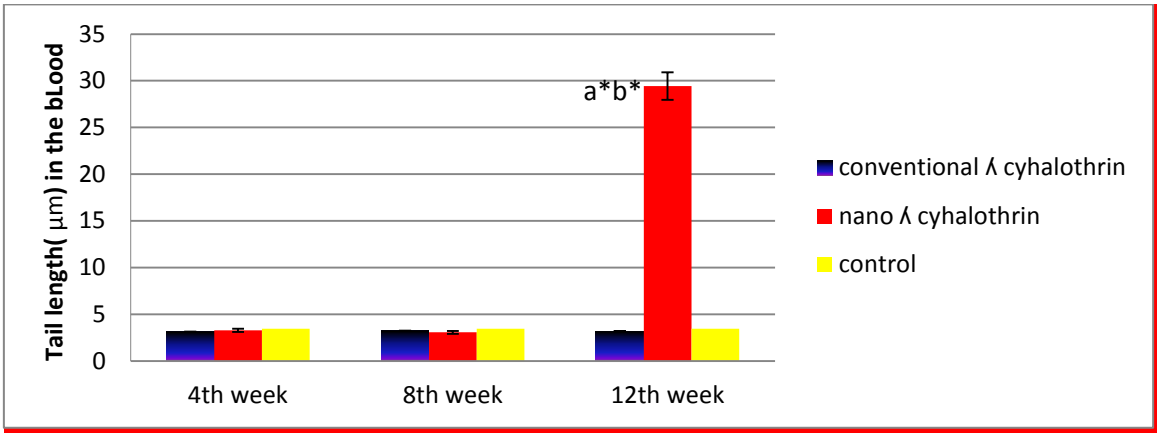
The group of nano ʎ cyhalothrin treated rats showed a significant increase in the value of comet assay parameters which indicate DNA damage in blood as tail DNA%, tail length (µm), tail moment % and olive tail moment% at **12th** week of the experiment in comparison with control group and conventional ʎ cyhalothrin treated rats (Table8, Figures 29, 30).

Table 8: Comet assay parameters in blood cells of rats exposed to conventional and nano ʎ cyhalothrin

	Parameter	Control	Nano ʎ cyhalothrin	Conventional ʎ cyhalothrin
4th week	Tail DNA%	0.063 ± 0.05	0.049 ± 0.05	0.002±0.001
	Tail Length(µm)	3.44 ± 0.30	3.29±0.33	3.13±0.11
	Tail Moment	0.0033 ± 0.0001	0.002 ±0.0017	0.0001±0.0003
	Olive Tail Moment	0.055 ± 0.0001	0.001 ±0.054	0.001±0.0013
8th week	Tail DNA%	0.064±0.03	0.001±0.026	0.11± 0.440.
	Tail Length(µm)	3.44 ± 0.20	0.0001± 3.07	0.35± 3.23
	Tail Moment	0.0025±0.0001	0.0002±0.001	0.0022 ± 0.001
	Olive Tail Moment	0.061±0.001	0.004 ± 0.025	0.045 ± 0.030
12th week	Tail DNA%	0.063±0.05	3.80±1.23a**b*	0.003 ± 0.001c*
	Tail Length(µm)	3.45±0.30	29.43±7.3a**b*	3.18±0.52c*
	Tail Moment	0.0033±0.001	3.10±1.3a**b*	0.0002 ± 0.0001c*
	Olive Tail Moment	0.053±0.001	6.323±2.2a**b*	0.0048 ± 0.001c*

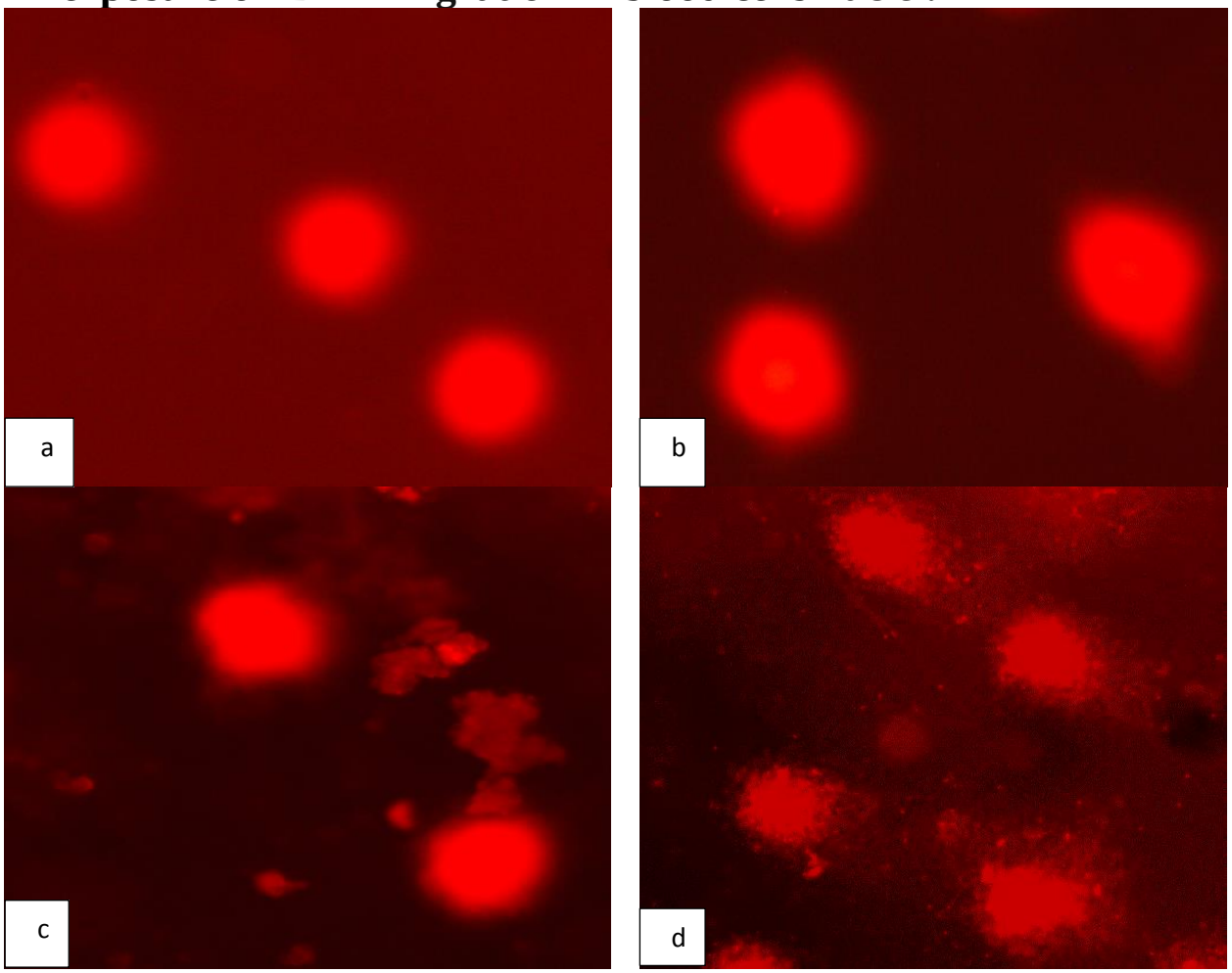
Values are expressed as means ± SE (n=3). * indicate significant at p≤0.05 ** indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group

Figure 29: The effect of conventional and nano ʎ cyhalothrin exposure on DNA migration (Tail length, µm) in blood cells of male rats.



Values are expressed as means ± SE (n=3). * indicate significant at p≤0.05 ** indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group

Figure 30: The effect of conventional and nano ʎ cyhalothrin exposure on DNA migration in blood cells nuclei.



(a) Blood cell nuclei of control rats showing no DNA damage. (b,c) Blood nuclei of rats exposed to conventional lambda cyhalothrin for 12 weeks showing no DNA damage (d) Blood nuclei of rats exposed to nano lambda cyhalothrin for 12 weeks showing DNA damage.

B- Genotoxicity in liver

The group of conventional ʎ cyhalothrin treated rats showed a non-significant change in the value of comet assay parameters which indicate DNA damage in liver as tail DNA%, tail length (µm), tail moment % and olive tail moment% at whole time of the experiment in comparison with control group.

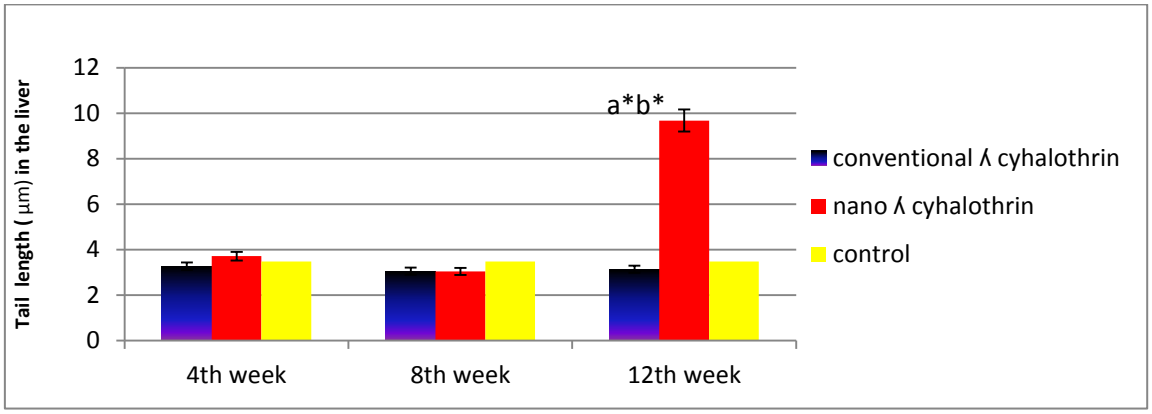
The group of nano ʎ cyhalothrin treated rats showed a significant increase in the value of comet assay parameters which indicate DNA damage in liver as, tail DNA%, tail length (µm), tail moment % and olive tail moment% at **12th** week of the experiment in comparison with control group and in comparison with group of conventional ʎ cyhalothrin treated rats (Table 9, Figures 31, 32).

Table 9: Comet assay parameters in liver of rats exposed to conventional and nano ʎ cyhalothrin

	Parameter	Control	Nano ʎ cyhalothrin	Conventional ʎ cyhalothrin
4th week	Tail DNA%	0.067±0.05	0.0103±0.005	0.0317±0.05
	Tail Length(µm)	3.48 ± 0.30	3.71 ± 0.23	3.27±0.23
	Tail Moment	0.0032 ± 0.001	0.001 ± 0.0001	0.0022±0.001
	Olive Tail Moment	0.621± 0.001	0.015 ± 0.07	0.041±0.007
8th week	Tail DNA%	0.20±0.08	0.016 ± 0.006	0.001 ± 0.0006
	Tail Length(µm)	3.48 ± 0.33	3.04 ± 0.07	3.05 ± 0.001
	Tail Moment	0.149 ± 0.040	0.001 ± 0.0001	0.0002 ± 0.0001
	Olive Tail Moment	0.494 ± 0.093	0.015 ± 0.002	0.0011 ± 0.0007
12th week	Tail DNA%	0.067±0.05	0.56 ± 0.03a*b*	0.0013 ± 0.0005c*
	Tail Length(µm)	3.48 ± 0.30	9.68 ± 2.51a*b*	3.14 ± 0.45c*
	Tail Moment	0.0032 ± 0.001	0.362 ± 0.11a*b*	0.0001± 0.0001c*
	Olive Tail Moment	0.621± 0.001	0.834 ± 0.25a*b*	0.0023 ± 0.0010c*

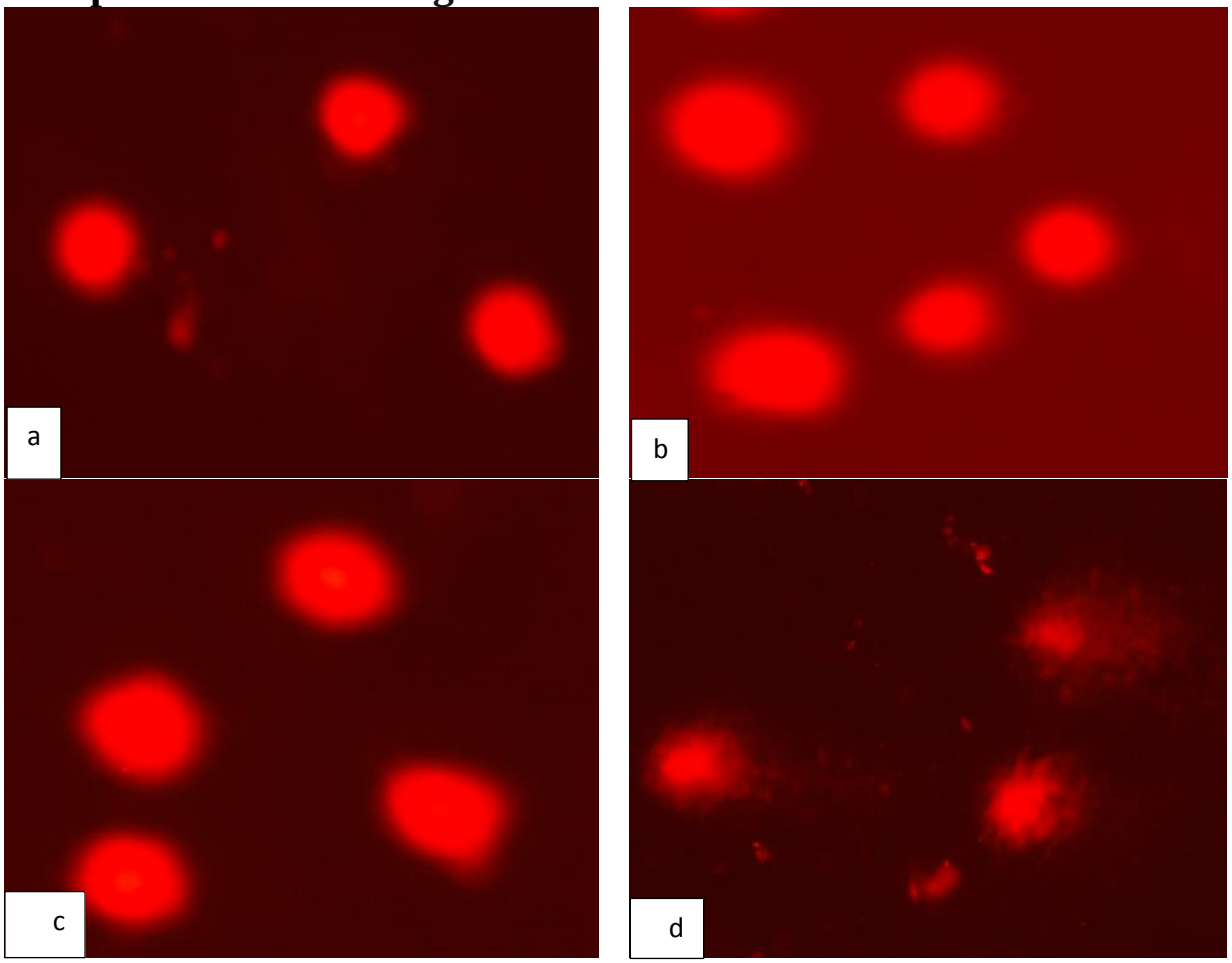
Values are expressed as means ± SE (n=3). * indicate significant at p≤0.05 ** indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group

Figure 31: The effect of conventional and nano ʎ cyhalothrin exposure on DNA migration (Tail length, µm) in liver nuclei of male rats.



Values are expressed as means ± SE (n=3). * indicate significant at p≤0.05 ** indicate highly significant at p≤0.01, a.Indicate significant at p≤0.05 in comparison with the control group, b.Indicate significant at p≤0.05 in comparison with Conventional ʎcyhalothrin - treated group, c.Indicate significant at p≤0.05 in comparison with Nano- ʎcyhalothrin - treated group

Figure 32: The effect of conventional and nano ʎ cyhalothrin exposure on DNA migration in liver nuclei.



(a)Liver nuclei of control rats showing no DNA damage. (b,c) Liver nuclei of rats exposed to conventional lambda cyhalothrin for 12 weeks showing no DNA damage (d) Liver nuclei of rats exposed to nano lambda cyhalothrin for 12 weeks showing DNA damage

7-Histopathological examination

In the group of **conventional ʎ cyhalothrin** treated rats **(Photos.1, b1,b2,b3,b4)**; liver of rats after exposure showed dilatation and congestion of hepatic sinusoids and hydropic degeneration **(Photo.1, b1)**; inter lobular fibrosis were observed **(Photo.1, b2)**; showed marked vacuolar degenerated hepatocytes **(Photo.1, b3)**, inflammatory cell infiltration with mono nuclear cells, marked hydropic degenerated hepatocytes ,inflammatory cell infiltration in portal triad region and vascular degeneration**(Photo.1, b4).**

Liver of **nano ʎ cyhalothrin** treated rats **(Photos.1, c1,c2,c3, c4)**; showed inflammatory cell infiltration with mono nuclear cells **(Photo.1, c1)**, showed focal area of necrosis, infiltrated with mono nuclear cells in portal area **(Photo.1, c2)**, showed marked hydropic degenerated hepatocytes with vacuolation, inflammatory cell infiltration in portal triad region, dilated and congested blood vessel **(Photo.1, c3)**, Kupffer cell activation, focal area of necrosis with mono nuclear cell infiltration **(Photo.1, c4)**

Kidney of **conventional ʎ cyhalothrin** treated rats **(Photos.2, b1,b2,b3,b4)** showed intertubular hemorrhage, perivascular fibrosis **(Photo.2, b1);** showed degenerated renal tubular epithelium in renal medulla **(Photo.2, b2**); showed renal tubular cast **(Photo.2, b3);** showing sever degeneration and necrosis of renal tubular epithelium **(Photo.2, b4).**

Kidney of **nano ʎ cyhalothrin** treated rats **(Photos.2, c1,c2,c3,c4)**; showed swollen and hyper cellularity glomerular tuft **(Photo.2, c1)**; showing mild degenerated renal tubular epithelium in renal medulla, and mild inter tubular hemorrhage **(Photo.2, c2)**; showed swollen and congested glomerular tuft **(Photo.2, c3)**; showed renal medulla with vacuolar degeneration and necrosis in renal tubular epithelium **(Photo.2, c4).**

Brain of **conventional ʎ cyhalothrin** treated rats **(Photos.3, b1,b2,b3,b4)** showed degenerated pyknotic Purkinje cells in cerebellum **(Photo.3, b1)**; showed few degenerated pyramidal cells **(Photo.3, b2)**; showed pyknotic neurons and congestion of blood vessels **(Photo.3, b3)**; showed degenerative and pyknotic pyramidal cells **(Photo.3, b4).**

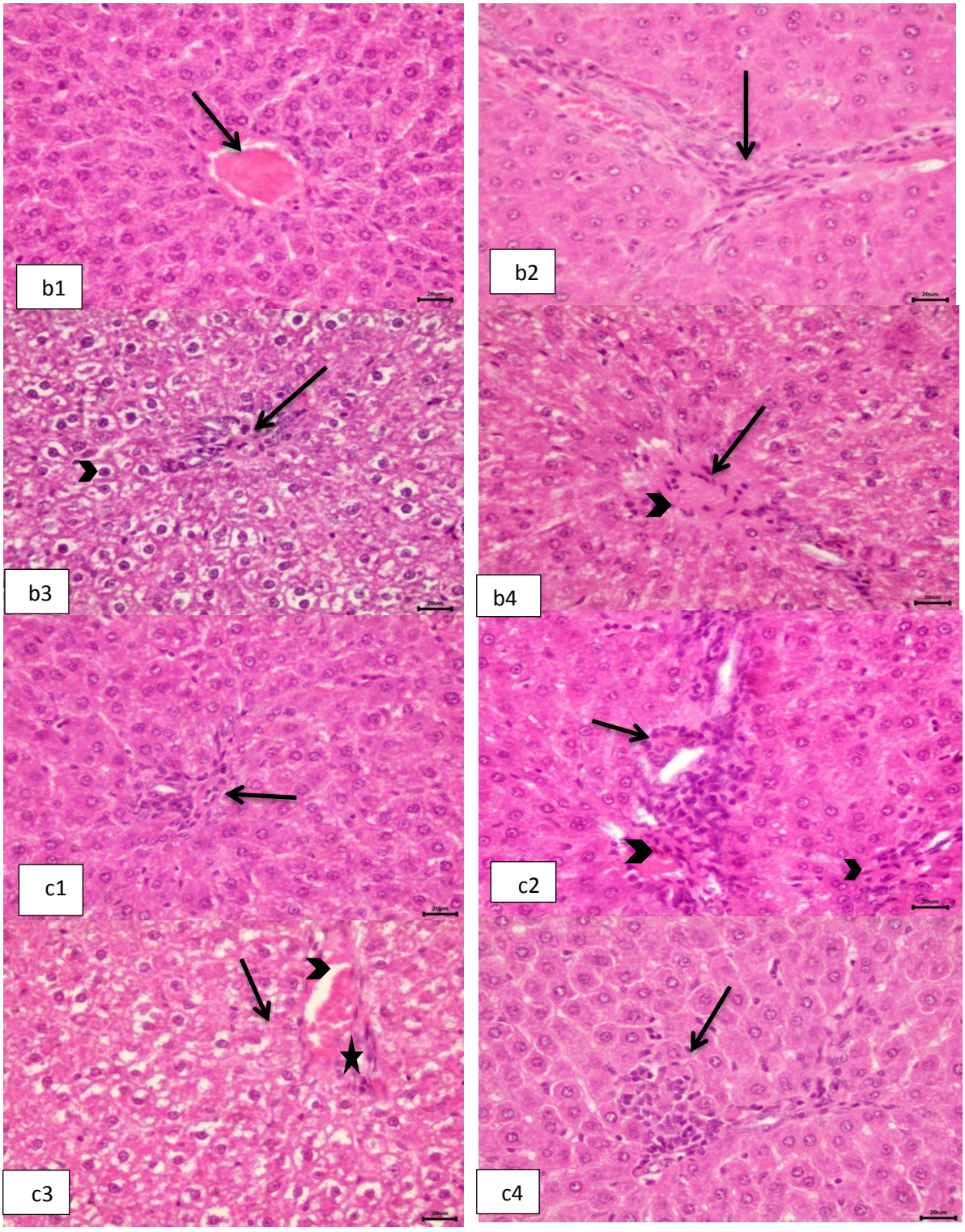
Brain of **nano ʎ cyhalothrin** treated rats **(Photos.3, c1,c2,c3,c4)** showed degeneration in Purkinje cells in cerebellum **(Photo.3, c1)**; showed few pyknotic neurons **(Photo.3, c2)** showed sever perivascular hemorrhage in the cerebral cortex **(Photo.3, c3)** showed degenerated and pyknotic pyramidal cells **(Photo.3, c4)**.

Testis of **conventional ʎ cyhalothrin** treated rats **(Photos.4, b1,b2,b3,b4)** showed degeneration, and disorganization of normal successive stages of spermatogenic cells **(Photo.4, b1**); showed sever interstitial edema **(Photo.4, b2**) showing sever vaculation and disorganization of normal successive stages of spermatogenic cells,, spermatogonia, spermatocytes **(Photo.4, b3**) showing sever degeneration and absence of normal successive stages of spermatogenic cells, spermatogonia, spermatocytes **(Photo.4, b4**).

Testis of **nano ʎ cyhalothrin** treated rats **(Photos.4, c1,c2,c3,c4)** showed disorganization of spermatogonia, spermatocytes, sever vaculation and nuclear pyknosis of germ cells **(Photo.4, c1);** showed hyperplasia of Leydig cell **(Photo.4,c2)**; showed degeneration and disorganization of normal successive stages of spermatogenesis, and Leydig cells degeneration **(Photo.4, c3)**; showed pyknosis and disorganization of spermatogonia, spermatocytes **(Photo.4, c4)**.

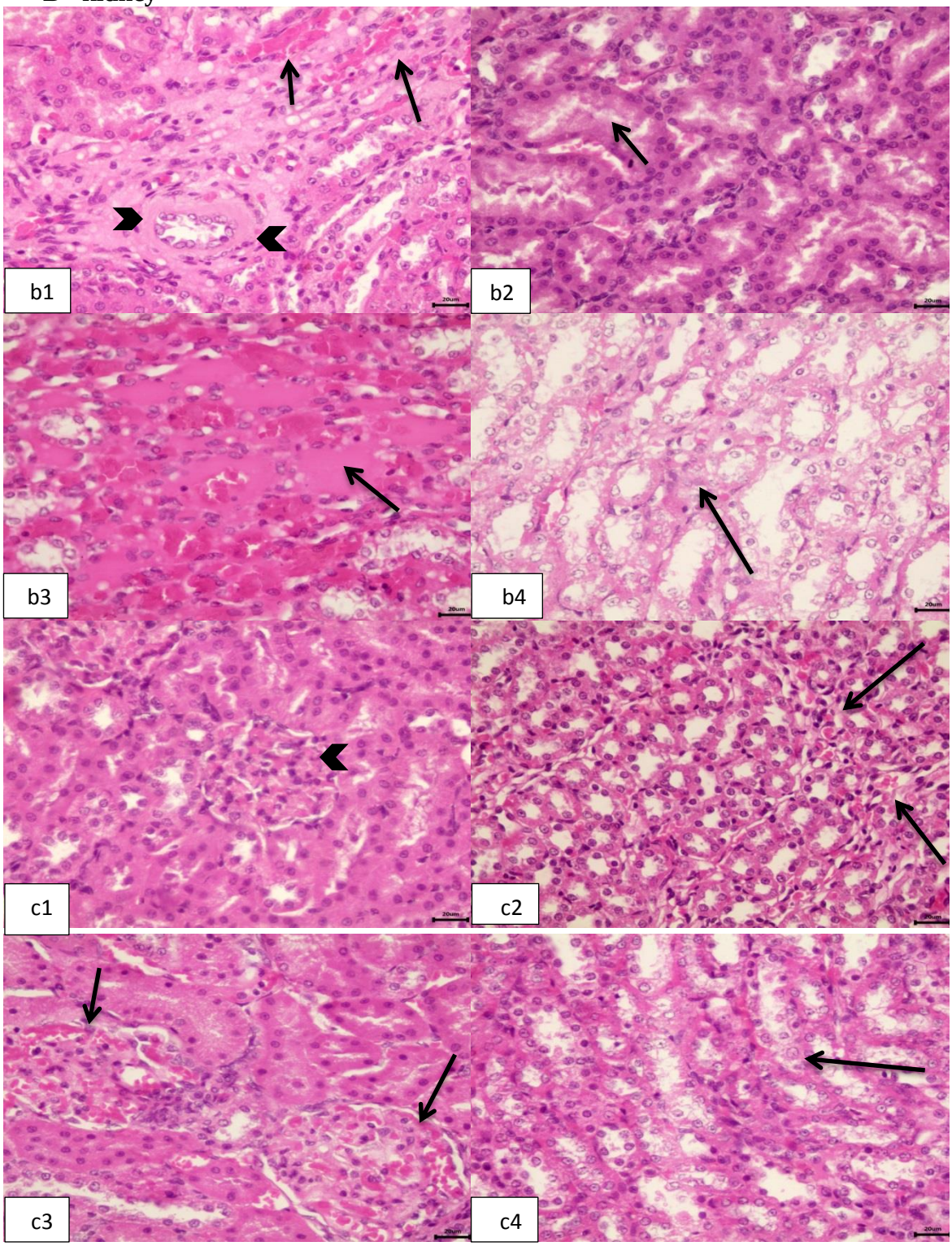
Histopathological examination

A- Liver



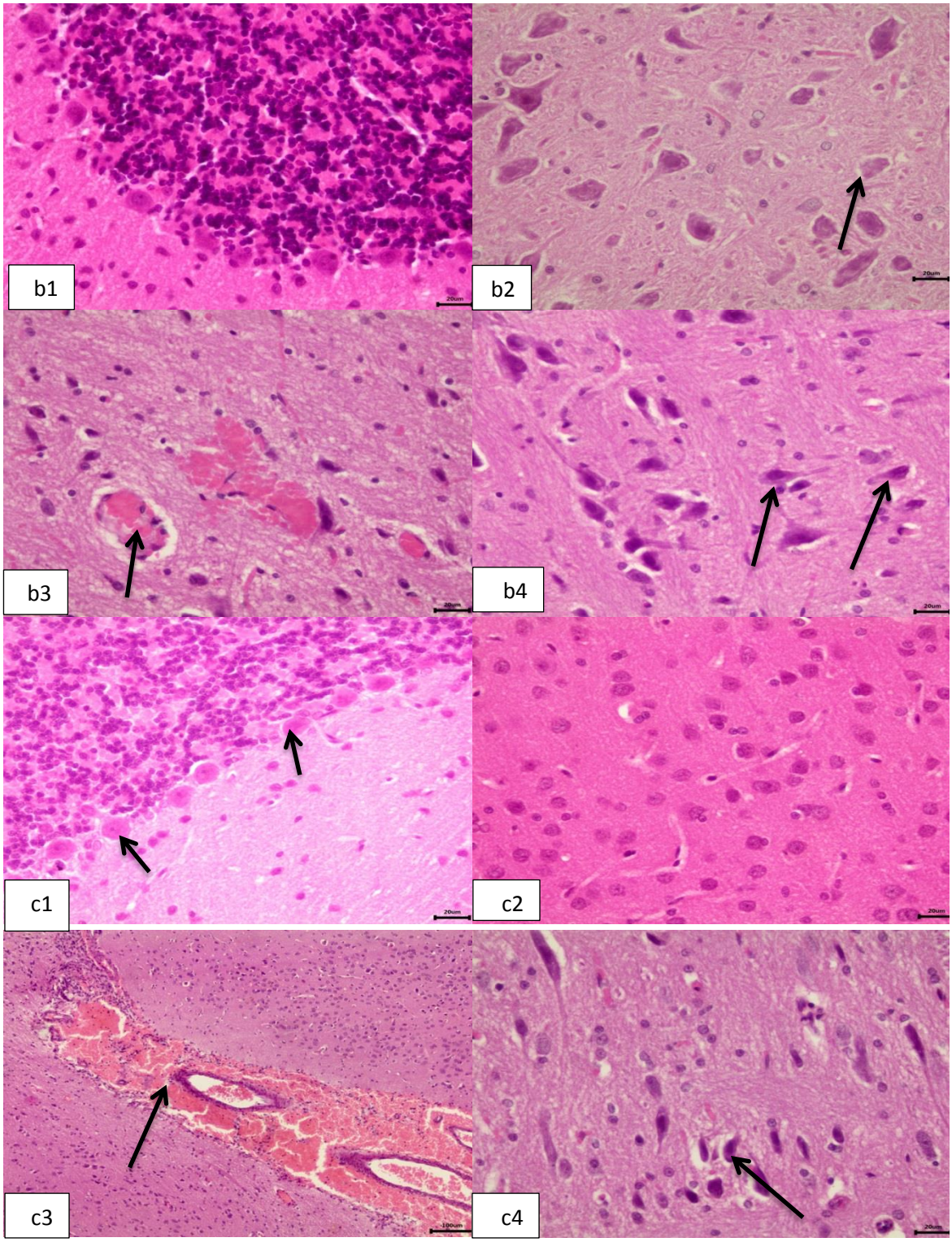
Photomicrograph 1: showing liver of **conventional ʎ cyhalothrin treated rats (b1,b2,b3,b4)** (b1)showing dilatation and congestion of hepatic sinusoids (arrow) and hydropic degeneration; (b2) inter lobular fibrosis were observed (arrow); (b3) showing marked vacuolar degenerated hepatocytes (arrow head) ,inflammatory cell infiltration with mono nuclear cells(arrow) (b4)inflammatory cell infiltration in portal triad region (arrow) and vascular degeneration (arrow head); **liver of nano ʎ cyhalothrin treated rats (c1,c2,c3,c4)** ;(c1) showing inflammatory cell infiltration with mono nuclear cells(arrow) in portal area ,(c2) showing focal area of necrosis (arrow head); infiltrated with mono nuclear cells (arrow) (c3) showing marked hydropic degenerated hepatocytes with vacuolation (arrow) ,inflammatory cell infiltration in portal triad region (star), dilated and congested blood vessel (arrow head) (c4) Kupffer cell activation (arrow), focal area of necrosis with mono nuclear cell infiltration (H&E stain, bar =20µ).

B- kidney



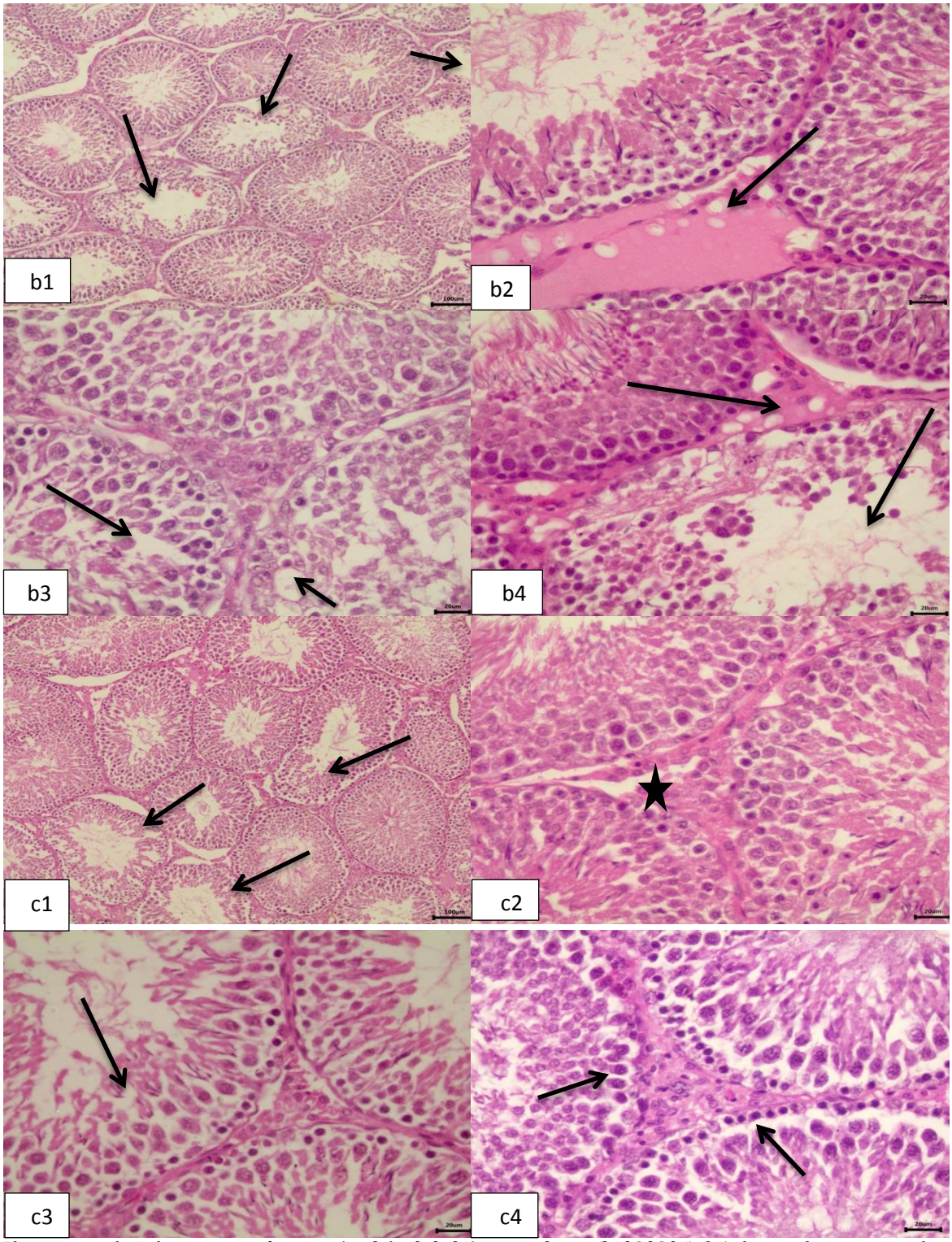
Photomicrograph 2: showing kidney of **conventional ʎ cyhalothrin treated rats (b1,b2,b3,b4)** (b1) showing *intratubular* hemorrhage (arrow), perivascular fibrosis (arrow head); (b2) showing degenerated renal tubular epithelium in renal medulla (arrow); (b3) showing renal tubular cast (arrow); (b4) showing sever degeneration and necrosis of renal tubular epithelium (arrow); **kidney of nano ʎ cyhalothrin treated rats (c1,c2,c3,c4)** ;(c1)showing swollen and hyper cellularity glomerular tuft (arrow head); (c2) showing mild degenerated renal tubular epithelium in renal medulla, and mild inter tubular hemorrhage (arrow) (c3) showing swollen and congested glomerular tuft (arrow); (c4) showing renal medulla with vacuolar degeneration and necrosis in renal tubular epithelium (arrow) (H&E stain, bar =20µ).

C- Brain



Photomicrograph 3: showing brain of **conventional ʎ cyhalothrin treated rats (b1,b2,b3,b4)** (b1) showing degenerated and Pyknotic Purkinje cell in cerebellum; (b2) showing few degenerated pyramidal cells (arrow) (b3) showing Pyknotic neurons and congestion of blood vessels (arrow); (b4) showing degenerative and pyknotic pyramidal cells(arrow); **brain of nano ʎ cyhalothrin treated rats (c1,c2,c3,c4)** ;(c1) showing degeneration in Purkinje cell in cerebellum (arrow);(c2) showing few pyknotic neurons (c3) showing sever perivascular hemorrhage in the cerebral cortex(arrow); (c4) showing degenerated and pyknotic pyramidal cells (arrow) (H&E stain, bar =20µ).

D- Testis



Photomicrograph 4: showing testis of **conventional ʎ cyhalothrin treated rats (b1,b2,b3,b4)** (b1) showing degeneration, and disorganization of normal successive stages of spermatogenic cells, spermatogonia, spermatocytes; (b2) showing sever interstitial edema (arrow); (b3) showing sever vaculation and disorganization of normal successive stages of spermatogenic cells, spermatogonia, spermatocytes ; (b4) showing sever degeneration and absence of normal successive stages of spermatogenic cells, spermatogonia, spermatocytes; **testis of nano ʎ cyhalothrin treated rats (c1,c2,c3,c4)** ;(c1) showing disorganization of spermatogonia, spermatocytes (arrow), sever vaculation and nuclear pyknosis of germ cells; (c2) showing hyperplasia of Leydig cell (star), (c3) showing degeneration and disorganization of normal successive stages of spermatogenic cells, spermatogonia, spermatocytes (arrow) and Leydig cells degeneration; (c4) showing pyknosis (arrow) and disorganization of spermatogonia, spermatocytes (H&E stain, bar =20µ).

Discussion

In recent years, a series of nanopesticides, including inorganic nanomaterials and nanoformulations of traditional active ingredients (i.e., nanoemulsions, nanospheres, and nanocapsules), have been widely investigated and proven to possess superior site-targeted delivery efficiency than their commercial counterparts **(Khandelwal et al., 2016; Sun et al., 2019; Kalia and Kaur, 2019)**. As the first of ten emerging technologies in chemistry, many publications have mainly focused on the preparation of nanopesticides or their application efficacy in the field **(Fernando, 2019)**; however, their environmental and human health hazards, fate, behavior and biological reactivity with non-targeted organisms are controversial **(Huang et al., 2022).** Regulatory authorities require toxicity testing on animals as part of the safety assessment for new drugs, chemicals, biologics, food additives, and medical devices. For new compounds or other chemical entities, organ weight changes are accepted as a sensitive indicator of chemically-induced effects on organs, and are commonly assessed (**Lazic et al., 2020).**

In the present study, nano form of lambda – cyhalothrin are formulated by emulsification solvent evaporation technique similar to **Knieke et al. (2014); Pan et al. (2015); Bhakay et al. (2016) and Wang et al .(2019a).** Nanoemulsions were recognized as formulations constructed from nano-scaled oil or water droplets (exactly beyond the range of 20–200 nm) that spread in an opposite phase as the surfactants lining at the oil/water interface **(Du et al., 2016)**. In our work, the hydrophilic surfactant Tween 80 in 3% concentration was applied and dispersed in deionized water. The pesticide b-Cypermethrin has been contributed in the system water/poly(oxyethylene) nonionic surfactant/methyl decanoate **(Wang et al., 2007)** and this emulsion can be a typical form as a water-insoluble

pesticide delivery system. Furthermore, another b-cypermethrin nanoemulsion preparation has been conducted by utilizing methyl laurate as oil phase, alkyl polyglycoside (APG) and polyoxyethylene 3-lauryl ether (C12E3) as mixed surfactants **(Du et al., 2016; Zhao et al., 2017).** The parameters of the nanosuspension formulation, composition and preparation were precisely researched using the particle size and PDI as determining indices. Our findings of particle size by DLS- zetasizer revealed that an average dynamic nano-size reached to 77. 51 nm diameter less than 100nm. Also, the TEM image recorded that the LCN morphology is nearly spherical and has an average size of 70.3 nm in diameter. These findings were in constant with similar techniques conducted by **Pan et al. (2015),** who reported that among twelve surfactants, four surfactants (SDS, MRES, Tween 80, and PEGNPE) decreased the mean particle size of the nano-preparation less than 200 nm. Zeta potential of Lambda-Cyhalothrin nano suspension declared a negative surface charge value (− 17. 8 mV) which was sufficiently high to eradicate LCN aggregation. This value indicates a stable and well dispersed suspension of LCN that there is no tendency to form aggregates in a short period of time. The high PDI value represented the poor disparity in water and PDI values less than 0.3 introduced a narrow size distribution **(Ahuja, et al., 2015).** The pesticide particles well dispersed via electrostatic repulsion **(Zhang et al., 2015)**. Nonionic surfactants as Tween 80, could inhibit aggregation of particles by adsorbing onto the nanoparticles through the hydrophobic section, which decrease the van Edward attraction between particles (**Wang et al., 2019a).** It declares that interactions between hydrophobic areas of LCN and Tween 80 were substantial for nanoparticles (**Young, et al., 1996; Pan et al., 2015; Cui et al., 2015).**

The strong absorption bands centered at 216 and 245nm are recorded for composition of conventional lambda and its nano-emulsion formulation on

UV-visible spectral analysis. This clearly suggested the formation of LCN nanoparticles embedded in the investigated matrix. For the broadening observation, and according to literature the broad and variable peaks are attributed to formation of new particles **(Abouelkassem et al., 2016).** The recorded two different wave lengths which suggested the presence of new compound has new chemical properties reflected as another peak at different spectrum wave length.

The results of FTIR analysis showed that the addition of new functional groups as O-H and also substitution to another groups as C-H. These structural changes revealed that LCN possess new bonds formed by the conversion of the conventional lambda – cyhalothrin to nano- lambda – cyhalothrin formulation. These results resampled to the previous work conducted by **Qin et al. (2019).**

The data interpretation of larvicidal effect of LC and LCN declared that high significant differences between the obtained mortality means of exposed larvae. There were also high significant differences between the means of mortality rates at variable exposure periods. Hence, the periods of exposure exhibited high significant differences between 24, 48 and 72 hrs, respectively. These results are in agreement with the view of **Patil et al. (2012); Bhan et al. (2014) and Abouelkassem et al. (2016).** In similar to **Desheesh et al. (2019),** they declared that LC encapsulated nanoparticles loaded with poly ethylene glycol are releasing slowly and persistent very efficient against mosquito larvae up to 72 hr than the conventional form. These findings suggested that LCN had an efficient larvicidal effect better than the conventional compound. The reduction of the particle size enlarges the zone area of the particles surface, which give the power to the active ingredients of pesticides to penetrate the biological tissues. Furthermore, nanoparticles added penetration power and enhance the absorption and

accumulation of the pesticide in the tissues **(Ahmed et al., 2019, Wang et al., 2019b).**

It is concluded that there are many factors affected the nano formulation compound composition such as the used solvent, surfactant type, time and temperature of stirring or shearing machine. All these factors induce new properties for the new nano- compound. Each study applied specific factors consequently, unique parameters obtained for its nano product. In this study, we used melt and solvent evaporation method and successfully have provided a new compound of nano-lambada cyalothrin pesticide with novel and unique nano-properties. The produced LCN has a new hydrophilic attendance and better efficacy for pest control resulted in a promising nano-pesticide formulations to improve agricultural practice.

This study aimed to investigate and compared the toxic effect of nano cyhalothrin formulation and conventional compound on non-target vertebrate animal as laboratory animal *Sprauge Dawely* rats. Thus, this study examined some biological impacts related to the possible toxic effects on non-target organisms. In the present study, the results of **body weight** revealed that in both groups conventional and group of nano ʎ cyhalothrin treated rats, showed non-significant change during the whole period of experiment in comparison with control group. Also, the comparison between the results obtained of two groups, group of conventional and group of nano ʎ cyhalothrin treated rats, showed non-significant change during the whole period of experiment**.** In the same line, **Titus et al., (2019)** stated that the mean body weight of *Sprague-Dawley* rats exposed to Cyalothrin at a dose of 30 mg/kg b.wt./day for thirty-five (35) days, showed a non-significant ($P>0.05$) increase in body weight throughout the experimental period. **Boumezrag et al. (2021**) declared that body weight of rabbits orally administrated with 10 and 20 mg/kg b.w/48h of Lambda-cyhalothrin for 25 days. No significant difference of mean body

weight was observed in both LCT- treated groups compared to the control group. **Orlu and Obulor, (2021)** declared that body weight of male mice received the 10mg/kg/bw/day of Lambda-Cyhalothrin for thirty-five (35) days, showed no significant difference (P=0.05) between the bodyweight in control group compared with the treatment groups.

In contrast**, Kumar and Yadav, (2021)** reported that after administration of lambda cyhalothrin during determination of LD50 against the doses 20, 40, 80, 160 and 320 mg/kg b. wt. respectively**,** the body weight of animals was significantly decreased in lambda cyhalothrin treated group**.** The non-significant differences observed in the body weight in this study could be attributed to the effects of insecticide exposure on body weight are contradictory, probably depending on various factors, such as dose and route of administration, species, sex, and treatment duration **(Haratym-Maj, 2003).** The non-significant differences observed in the body weight of the experimental animals shows that lambda cyhalothrin is not a systemic toxin. Therefore, it can silently destroy the vital organs of the body without conspicuous changes in the body weight **(Orlu and Obulor, 2021).**

In toxicological experiments, comparison of **organ weights** between treated and untreated groups of animals have conventionally been used to evaluate the toxic effect of the test article and indeed an important quantitative endpoint in many toxicity studies **(Nirogi et al., 2014).** In the present study, the results of **brain** absolute and relative weight revealed that in both groups conventional and nano ʎ cyhalothrin treated rats, the absolute weight of brain of male rats showed non-significant change during the whole period of experiment in comparison with control group. But the group of conventional ʎ cyhalothrin treated rats showed a significant decrease in the value of relative brain weight at **4$^{\underline{th}}$** week of the experiment in comparison with control group.

In the same line, **Grewal et al. (2010)** reported that cypermethrin at repeated oral doses of 5 and 20 mg/kg/day showed that the relative weight of brain significantly decreased to the extent of 22.95% and 15.09% in male and female rats, respectively. In contrast**, Boumezrag et al. (2021**) declared that body weight of rabbits orally administrated with 10 and 20 mg/kg b.w/48h of Lambda-cyhalothrin for 25 days, the mean brain weight was significantly increased (P<0.05) in the group received LCT at 20 mg/kg b.w compared to the control group. The significant decrease observed in the brain relative weight in this study could be attributed to vacuolization in the cerebral hemisphere as insecticide produced neuronal degeneration and necrosis **(Grewal et al., 2010)**.

In the present study, the results of liver absolute and relative weight revealed that a significant increase in the group of conventional ʎ cyhalothrin treated rats in comparison with control group. In the group of nano ʎ cyhalothrin treated rats, the value of liver absolute and relative weight showed a significant increase in comparison with control group and in comparison with group of conventional ʎ cyhalothrin treated rats. In agreement with **Adam et al. (2020),** who declared that the effects of lambda cyhalothrin on hepato-toxicity indicators at administered doses of 4.16 and 8.32 mg/bw of lambda cyhalothrin respectively for 28 days, there was a dose-dependent significant ($p < 0.05$) increase in liver weight. These results are also in accordance to those reported by **Yousef et al. (2003)**.

In contrast**, Titus et al. (2019)** showed a non-significant (P>0.05) increase in liver weight in rats exposed to cyalothrin at a dose of 30 mg/kg/bw/day for thirty-five days. **Boumezrag et al. (2021**) declared that liver weight of rabbits orally administrated with 10 and 20 mg/kg b.w/48h of Lambda-cyhalothrin for 25 days, showed non-significant change compared to the control group. **Orlu and Obulor, (2021)** declared that liver weight of male

mice received the 10 mg/kg/bw/day of Lambda-Cyhalothrin for thirty-five (35) days, showed no significant difference (P=0.05) between control group compared with the treatment groups. Increased weight of liver recorded in this study might be because of elevated circulation due to raised requirements for the detoxifying compounds that are toxic **(Vemo et al., 2017)**.

In the present study, the results of kidney absolute weight revealed that a significant increase at **4th** and the **10th** week of the experiment in the group of conventional ʎ cyhalothrin treated rats in comparison with control group. Also, showed highly significant increase in comparison with group of nano ʎ cyhalothrin treated rats. The results of kidney relative weight of the group of nano ʎ cyhalothrin treated rats revealed that a significant increase during the **10th** week of the experiment in comparison with control group. In agreement with **Abdel-Mobdy and Abdel-Rahim. (2015)** reported an increase in kidney weight**. Adam et al., (2020)** declared that the effects of lambda cyhalothrin on nephro-toxicity indicators at the doses of 4.16 and 8.32 mg/b.wt. of lambda cyhalothrin respectively for 28 days. Also**, Boumezrag et al. (2021**) declared that kidney weight of rabbits orally administrated with 10 mg/kg b.w/48h of Lambda-cyhalothrin for 25 days, the mean kidney weight was significantly increased (P<0.05) in the group received LCT at 10 mg/kg b.w compared to the control group.

In contrast**, Chakroborty et al. (2019)** suggested that Lambda-cyhalothrin was administered orally at two dose levels (10.83 and 15.17 mg/kg body weight) for consecutive 14 days, Renal toxicity was measured by a significant decrease in renal index. **Titus et al. (2019)** stated that *Sprague-Dawley* rats exposed to Cyalothrin at a dose of 30 mg/kg/b.wt./day for 35 days, showed a non-significant (P>0.05) increase in kidney weight throughout the experimental period**. Orlu and Obulor, (2021)** declared

that kidney weight of male mice received the 10 mg/kg/bw/day of Lambda-Cyhalothrin for thirty-five (35) days, showed no significant difference (P=0.05) between the kidney weight in control group compared with the treatment groups. **Nieradko-Iwanicka and Rutkowski, (2022)** stated that there were not statistically significant differences in kidney mass after receiving 2 mg/kg LCH orally for 8 successive days. The kidneys contributed to the maintenance of the body's homeostasis thanks to the excretion of these unnecessary metabolic products. Concentrating urine in the tubular fluid also increases the concentration of xenobiotics in it. Renal transport and the accumulation and biotransformation of pyrethroid metabolites contribute to the susceptibility of the kidneys to damage by this group of xenobiotics **(Nieradko-Iwanicka and Rutkowski, 2022)**.

In the present study, the results of testis absolute and relative weight in both groups of conventional and group of nano ʎ cyhalothrin treated rats, showed a non-significant change along the time of the experiment in comparison with control group and in comparison with each other. In agreement with **Al-sarar et al. (2014)** who reported that oral LCT administration to adult male mice at 3 doses (0.2, 0.4, and 0.8 mg/kg/day) for 6 weeks resulted in insignificant differences compared with controls. **Li et al., (2018)** reported that twenty-eight-day old male Sprague Dawley rats orally received LCT (0, 0.25, 0.5 or 1 mg/kg body weight/day) for 30 days, testis and epididymal weights showed no significant difference between LCT group and the control. **Titus et al. (2019)** stated that the gonadosomatic indicies of *Sprague-Dawley* rats exposed to Cyalothrin at a dose of 30 mg/kg/bw/day for thirty-five (35) days, showed a non-significant (P>0.05) difference between the control and treatment groups. In contrast**, Hussein et al. (2012)** declared that cyhalothrin was given orally to male rats daily for 30 successive days at two doses (0.1 and 1.0

mg kg^{-1} b.wt.), resulted in a significant decrease in the weight of testes. **Oshoke et al. (2016)** reported that male Wistar rats received by gavage 25, 50, 75 and 100 mg/kg LTC body weight, respectively, over a period of five weeks. A significant decrease in the absolute weight of testes and seminal vesicles were observed. Our results could be contributed to cyhalothrin may not have obvious effect on the gonadosomatic indices but silently destroy target cells of the body over time. Histopathological analysis of the testicles revealed reproductive toxicity **(Titus et al., 2019)**.

This study revealed that the value of RBC's counts, Hb content, Hct and MPV values in both groups conventional and group of nano ʎ cyhalothrin treated rats, showed non-significant change during the whole period of experiment in comparison with control group and with each other. In the same line, **Adam et al. (2020)** declared that the effects of lambda cyhalothrin on hematological characteristics indicators in pregnant does with administered doses of 4.16 and 8.32 mg/b.wt. of lambda cyhalothrin for 28 days. **Boumezrag et al. (2021**) declared that no significant change observed in RBC's counts between the non-treated and treated groups. Similarly, hemoglobin concentration, hematocrit, MCV, MCHC and MCH were not significantly affected after rabbits orally administrated with 10 mg/kg b.wt./48h of ʎ cyhalothrin for 25 days. The inconsistent and the observed differences could be attributed to various factors influencing hematological parameters such as feeding pattern, food utilization, fluids and salts balance, blood sampling and experimental variables **(WHO, 2011)**.

This study revealed that, the value of RDW in group of conventional ʎ cyhalothrin treated rats showed a highly significant decrease at $\mathbf{2^{nd}}$, $\mathbf{6^{th}}$and $\mathbf{8^{th}}$week of the experiment, while showed a significant decrease at $\mathbf{4^{th}}$, $\mathbf{10^{th}}$and $\mathbf{12^{th}}$week of the experiment in comparison with control

group. In a group of nano ʎ cyhalothrin treated rats, showed a highly significant decrease at $\mathbf{2^{nd}}$, and $\mathbf{4^{th}}$ week of the experiment, while showed a significant decrease at $\mathbf{6^{th}}$, $\mathbf{8^{th}}$, $\mathbf{10^{th}}$and $\mathbf{12^{th}}$week of the experiment in comparison with control group. In contrast to **Yahia et al. (2019),** they declared that the effect of exposure to lambda cyhalothrin in a dose of 26 mg/kg body weight for 24 and 48 hours on the hematological parameters of male Sprague Dawley rats showed significant increases (P< 0.01) in hematocrit (HCT %) and mean corpuscular volume (MCV), while other parameters including RDW showed no significant changes.

This study revealed that the value of platelets count in the group of conventional ʎ cyhalothrin treated rats showed a significant increase at $\mathbf{4^{th}}$, $\mathbf{6^{th}}$and $\mathbf{10^{th}}$ week of the experiment, in comparison with control group. In a group of nano ʎ cyhalothrin treated rats, the value of platelets count showed a significant increase at $\mathbf{6^{th}}$ week of the experiment, in comparison with control group. In the same line, **Riaz, and Yousafzai, (2017)** reported that hematological alterations in the rabbits following oral intoxication with cypermethrin at the dose of 75 mg/Kg body weight for seven days and caused an increase 5.76% in platelets count. In contrast, **Ali et al. (2014**) stated that adult female albino rats were treated with Lambda cyhalothrin at 20 and 40 mg/kg.bw, orally, for 14 days. Also caused a significant (P<0.05) reduction in platelets count, pack cell volume (PCV) and lymphocyte count.

This study revealed that the value of total WBCs count in the group of conventional ʎ cyhalothrin treated rats showed a significant increase at $\mathbf{4^{th}}$,$\mathbf{6^{th}}$, $\mathbf{8^{th}}$, $\mathbf{10^{th}}$and $\mathbf{12^{th}}$ week in comparison with control group. Also, showed a significant increase at $\mathbf{4^{th}}$ week of the experiment in comparison with nano ʎ cyhalothrin treated rats. In a group of nano ʎ cyhalothrin treated rats, showed a significant increase at whole period of experiment in

comparison with control group. In the same line, increased WBC count was observed in mice treated with Lambda cyhalothrin **(Fetoui, 2008; Mosbah et al., 2010). Ghosh et al. (2016)** stated that rats were exposed to different doses of lambda cyhalothrin over a period of 14 consecutive days, the dose levels of 7.58, 8.42, 10.83, 15.17, 18.96, and 25.28 mg/kg body wt. for male rats. There were a significant (p<0.001) increase in WBC count in lambda cyhalothrin treated male rats from the dose level of 18.96, and 25.28 mg/kg body wt.

In contrast, **Basir et al. (2011)** investigated the effects of lambda-cyhalothrin in rabbits treated with LCT at 1.0, 4.0 and 8.0 mg/kg b.wt. intraperitoneal, WBC counts and lymphocytes decreased whereas neutrophils, monocytes and eosinophils increased significantly (p <0.05) in treated groups when compared with control group. And also, **Boumezrag et al. (2021**) declared that there is no significant change observed in WBC's counts between the non-treated and treated groups after rabbits orally administrated with 10 mg/kg b.w/48h of Lambda-cyhalothrin for 25 days.

This study revealed that the value of lymphocytes count in the group of conventional ʎ cyhalothrin treated rats showed a significant increase at $\mathbf{4^{th}}$, $\mathbf{6^{th}}$, $\mathbf{8^{th}}$, $\mathbf{10^{th}}$and $\mathbf{12^{th}}$ week of the experiment in comparison with control group, Also showed a significant increase at $\mathbf{4^{th}}$, **and** $\mathbf{10^{th}}$ week of the experiment in comparison with nano ʎ cyhalothrin treated rats.

In a group of nano ʎ cyhalothrin treated rats, showed a significant increase at $\mathbf{4^{th}}$, $\mathbf{6^{th}}$, $\mathbf{8^{th}}$, $\mathbf{10^{th}}$and $\mathbf{12^{th}}$ week of the experiment in comparison with control group. In the same line, **Ramadhas et al. (2014**) reported that adult male Wistar received lambda cyhalothrin at a dose of 8 mg/kg for 21 days orally; resulted in an increase in the lymphocytes percentage. In contrast, **Ibrahim, (2016)** declared that after exposure to different doses (5, 1, 0.2mg/kg/day) of LCT orally, in rat model for 21 days, the high dose

(5mg/kg) of LCT resulted in a significant decrease in the relative lymphocytes when compared to the control animals.

This study revealed that the value of the peripheral neutrophils percentage in the group of conventional ʎ cyhalothrin treated rats showed a significant increase at $\mathbf{4^{th}}$ week of the experiment in comparison with control group. In a group of nano ʎ cyhalothrin treated rats, showed a significant increase at $\mathbf{4^{th}}$, $\mathbf{6^{th}}$, $\mathbf{8^{th}}$,and $\mathbf{12^{th}}$ week of the experiment in comparison with control group. This study revealed that, the value of the peripheral monocytes percentage in group of conventional ʎ cyhalothrin treated rats showed a significant increase at$\mathbf{4^{th}}$, $\mathbf{6^{th}}$, $\mathbf{10^{th}}$ and $\mathbf{12^{th}}$ week of the experiment in comparison with control group. In a group of nano ʎ cyhalothrin treated rats, showed a significant increase at$\mathbf{4^{th}}$,$\mathbf{6^{th}}$, $\mathbf{8^{th}}$, $\mathbf{10^{th}}$,and $\mathbf{12^{th}}$ week of the experiment in comparison with control.

In agreement with **Basir et al. (2011),** who investigated effects of lambda-cyhalothrin in rabbits treated with LCT 1.0, 4.0 and 8.0 mg/kg bw, the neutrophils, monocytes and eosinophils increased, significantly ($p < 0.05$) in treated groups when compared with control. **Ibrahim, (2016)** declared that after exposure to different doses (5, 1, 0.2 mg/kg/day) of LCT orally in rat model for 21 days., the high dose (5mg/kg) of LCT resulted in an increase in the relative granulocytes count when compared to the control animals. **Adam et al. (2020)** declared that the effects of lambda cyhalothrin on hematological characteristics indicators in pregnant does that administered doses of 4.16 and 8.32 mg/b.w. of lambda cyhalothrin for 28 days. It was a significant increase in granulocytes and monocytes values in comparison with control groups.

An increase in the number of leukocytes in the blood of animals irrespective of the pyrethroid applied for intoxication, may result from the mobilization of the immunological system and /or a shift in the leukocytic pool from the spleen to peripheral blood **(Luty et al., 2000; Haratym-**

Maj, 2003). The increase in WBC may be indicative of activation of defense and immune system of the body **(Yousef et al., 2003)**. This might result an increase in release of WBC from bone marrow storage pool into the blood. The rise in WBC count suggests the increased defense mechanism against probable attack of toxic molecules **(Fetoui, 2008; Mosbah et al., 2010).**

This study revealed that the value of superoxide dismutase activity (SOD) in the group of conventional ʎ cyhalothrin treated rats showed a significant decrease during the whole period of experiment in comparison with control group. This decrease became highly significant at 10^{th} week of the experiment. Also, showed a significant decrease at 8^{th} week of the experiment in comparison with group of nano ʎ cyhalothrin treated rats. In the group of nano ʎ cyhalothrin treated rats, the value of SOD concentration showed a significant decrease at 2^{nd} , 4^{th}, 6^{th} and 10^{th} week of the experiment in comparison with control group. In agreement with Fetoui et al. (2008) reported that rats daily received orally 668 ppm LCT 1/10 of LD50 (612 mg/ kg b.wt) and the experimental period was 21 days, resulted in the activities of SOD, GPx, GR and GST were decreased by (34, 4, 5, and 3%, $p < 0.05$), respectively. Aouey et al. (2017) reported that adult rats were orally exposed to 6.2 and 31.1 mg/kg bw of LCT for 7, 30, 45, and 60 days Results revealed that LTC exposure significantly decrease in antioxidant enzyme (CAT, SOD, and GPx) activities in the liver of rats after various treatments. Pawar et al. (2017) reported that Swiss albino mice were received 0.5, 1, 2 mg/kg body weight LCT orally, for 28 days, the activities of superoxide dismutase, catalase and glutathione-S-transferase were depleted significantly in both kidney and brain. Al-Amoudi et al. (2018) reported that adult rats after oral LCT treatment (1/100 LD50, 3 days/week for 4 weeks), the activities of

the antioxidant enzymes SOD and catalase (CAT) decreased significantly in the sera of rats from the group treated with LCT when compared to the control. Chakroborty et al. (2019) declared that Lambda-cyhalothrin was orally administered to rats at two dose levels (10.83 and 15.17 mg/kg body weight). In LCT treated group, the activities of antioxidants enzymes as CAT in the LCT treated low and high dose groups were significantly (p<0.001) decreased compared to the control group. Activities of glutathione peroxidase (GPx) in kidney of LCT treated low and high dose animals were significantly (p<0.05) and (p<0.001) decreased than the control group rats.

Incontrast, Adam et al. (2020) declared that the effects of ʎ cyhalothrin on pregnant does that administered doses of 4.16 and 8.32 mg/b.wt. for 28 days, showed that catalase (CAT), superoxide dismutase (SOD) and total peroxidase(POD) activities registered a dose-dependent significant ($p < 0.05$) increase in ovaries of lambda cyhalothrin-exposed does.

This study revealed that the value of MDA in the group of conventional ʎ cyhalothrin treated rats showed a significant increase at 2^{nd} , 4^{th}, 6^{th} and 12^{th} week of the experiment in comparison with control group. This increase became highly significant at 2^{nd}, 4^{th},and 12^{th} week of the experiment. Also, showed a highly significant increase at 10^{th} week of the experiment in comparison with group of nano ʎ cyhalothrin treated rats .In the group of nano ʎ cyhalothrin treated rats, the value of MDA concentration showed a significant increase at 2^{nd}, 4^{th}, 6^{th} , 8^{th} and 12^{th} week of the experiment in comparison with control group. This increase became highly significant at 2^{nd} , 4^{th},and 12^{th} week of the experiment. In agreement with Fetoui et al. (2008) , who reported that rats daily received orally 668 ppm LCT 1/10 of

LD50 (612 mg/kg.bw) and the experimental period was 21 days, revealed that MDA contents were significantly increased in brain (+31%, $p < 0.01$) and in erythrocytes (+20%, $p < 0.05$) of LCT group compared to those of control group. Madkour, (2012) reported that oral administration of LCT in rats at a dose of 0.6 mg/kg/day for 4 weeks resulted in LPO level in liver was significantly increased in LCT treated animals when compared to normal. Fetoui et al. (2015) declared that that exposure rat to LCT 1/10LD50 (6.23 mg/kg) for a period of 7, 14 and 21 days induced significantly increase in MDA, protein carbonyl (PCO) and NO contents in blood erythrocytes at different times of LCT exposure when compared to control group. Aouey et al. (2017) reported that adult rats were orally exposed to 6.2 and 31.1 mg/kg b.wt. of LCT for 7, 30, 45, and 60 days. Results revealed that LCT exposure significantly increased MDA. Pawar et al. (2017) stated that Swiss albino mice were received 0.5, 1, 2 mg/kg body weight LCT orally for 28 days. It was observed that there was significant (dose-dependent) increase in MDA formed in the kidney and brain after treatment with medium and high doses of LCT in mice. Al-Amoudi et al. (2018) reported that adult rats after oral LCT treatment (1/100 LD50, 3 days/week for 4 weeks), showed that LCT administration significantly increased the level of MDA in the sera of rats from the group treated with LCT when compared to the control. Chakroborty et al. (2019) declared that Lambda-cyhalothrin was administered orally to rats at two dose levels (10.83 and 15.17 mg/kg body weight). In LCT treated group, MDA content increased significantly ($p<0.001$) compared to the control group in a dose-dependent manner.

Our study results revealed the elevated level of lipid peroxidation indicator MDA and the decline of SOD activity which is in accordance with results of **Khalil et al. (2020)** reported elevated level of (MDA) and the decline of antioxidant capacity (TAC), thus triggering the peroxidation of unsaturated fatty acids in the cell membrane of RBCs and WBCs, thus inducing cytotoxicity. This is contributed to LCH is a type II pyrethroid, which has a α-cyano moiety, therefore the release of cyanohydrins, which are unstable under physiological conditions and further decompose to cyanides and aldehydes, which in turn could act as a source of free radicals **(El-Demerdash, 2007)**. The lowered TAC may indicate a deficiency in the defense against ROS, resulting in H2O2 accumulation, which in turn can inhibit superoxide dismutase (SOD) activity, resulting in superoxide radical accumulation. Thus, the excess radicals could readily oxidize the membrane lipids, which culminated in the expressive MDA increase observed in the LCH-exposed group. Lipid peroxidation arises from the reaction of free radicals with lipids and this is considered to be an important feature of the cellular injury brought by free radical attack **(Hoek and Pastorino, 2002).** The results in our study could be contributed to pesticides induce oxidative stress which leading to the generation of free radicals, changes in antioxidants levels and lipid peroxidation **(Ender and Onder, 2006)**. In accordance with the data obtained from this study, pyrethroids have been found to induce oxidative damage in various tissues owing to the formation of ROS and impairments in free oxygen radicals scavenging enzyme systems **(Khalil et al., 2020)**. Antioxidant enzymes cause a primary defense that prevents oxidative damage of biological macromolecules. According to the obtained results the activities of serum SOD treated rats were significantly decreased. These results suggested that LCT has the capability to induce free radicals and oxidative damage as evidenced by alterations in various antioxidant enzymes **(Salama et al.,**

2005). Reduction of antioxidant enzymes levels may be due to the direct effect on the enzymes against LCT-induced ROS generation **(Chakroborty et al., 2019)**.

At the cellular level, ROS are produced via several mechanisms. In this concern, the mitochondria are regarded as the main source of free radical formation in the tissue systems of living animals **(Cadenas and Davies, 2000)**. Several lines of evidence have demonstrated that pyrethroids are able to induce mitochondrial dysfunction, through affecting its essential transport system and/or its component of the respiratory chain. To deal with this, λ cyhalothrin has been reported to be a mitochondrial complex I inhibitor, which would aggravate rises in cellular ROS generation **(Gassner et al., 1997).**

This study revealed that **the mRNA expression levels of the inflammatory cytokines TNF-α** average level in the group of conventional ʎ cyhalothrin treated rats showed a highly significant increase and up-regulation at $\mathbf{2^{nd}}$, $\mathbf{4^{th}}$, $\mathbf{6^{th}}$, $\mathbf{8^{th}}$ and $\mathbf{12^{th}}$ week of the experiment in comparison with control group. Also, showed a highly significant increase in the expression at $\mathbf{2^{nd}}$ week of the experiment in comparison with group of nano ʎ cyhalothrin treated rats, but showed a highly significant decrease at $\mathbf{4^{th}}$ and $\mathbf{6^{th}}$ week of the experiment in comparison with group of nano ʎ cyhalothrin treated rats. In a group of nano ʎ cyhalothrin treated rats, the value of TNF α expression showed a significant up-regulation at $\mathbf{2^{nd}}$, $\mathbf{4^{th}}$, $\mathbf{6^{th}}$, $\mathbf{8^{th}}$ and $\mathbf{12^{th}}$ week of the experiment in comparison with control group. Also showed a highly significant increase at $\mathbf{4^{th}}$ and $\mathbf{6^{th}}$ week of the experiment in comparison with group of conventional ʎ cyhalothrin treated rats. In agreement with **Moustafa and Hussein, (2016**) who documented that male albino rats that were administered commercially and pure doses of LCT as 6.12 mg/kg b.wt. and 0.64 mg/kg b. wt., respectively.

The results indicated that exposure to LCT is capable of inducing an up-regulation in the mRNA expression levels of the inflammatory cytokines TNF-α when compared with the control groups. **Aouey et al. (2017)** reported that adult rats were orally exposed to 6.2 and 31.1 mg/kg bw of LTC for 7, 30, 45, and 60 days, resulted in the levels of tumor necrosis factor-α (TNF-α) and interleukin (IL-6 and IL-1β) gene expressions were significantly increased in the liver of exposed rats compared to controls. In contrast, **Nieradko-Iwanicka and Konopelko, (2020)** reported that albino Swiss mice 16 females and 16 males received LCH orally in oil at a dose of 2 mg/kg b.wt. for 7 days. There was no statistically significant difference in TNFα levels in the kidneys and livers between control and LCH groups.

Tumor necrosis factor alpha is a potent cytokine produced by various cell types including monocytes, in response to inflammation, infection, injury, and other environmental challenges. It plays a unique and pivotal role in regulating apoptotic signaling pathways, and in the control of cell proliferation and inflammation **(Baud and Karin, 2001)**. Moreover, it induces the release of cytokines (interferon-γ, interferon-β, IL-1, IL-6, granulocyte-colony stimulating factor, monocyte colony-stimulating factor, platelet-derived growth factor, platelet-activating factor), prostaglandins, and leukotrienes. So, there is a TNFα increase, and later, there is an Il1ß peak **(Piłat and Mika, 2014)**. Tumor necrosis factor a can induce cell apoptosis through the activation of a caspase cascade **(Chang and Yang, 2000).** The recorded impairment suggested the immunosuppressive effect of these pesticides. The expression of cytokines (TNF-α) as a vital part of the immune system is modulated by infection or inflammation, so assessing their expression levels may help explain the mechanisms of specific contaminants'toxicity **(Van Der Meide and Schellekens, 1996)**. **Zhang et al. (2010)** declared that exposure to synthetic pyrethroids and their

metabolites also led to changes in the secretion levels of tumor necrosis factor a (TNF a) and interleukins (ILs), and again the metabolites showed stronger effects than the parent compounds.

Moustafa and Hussein, (2016) found an increase in inflammatory cytokine (TNF-α) production upon exposure to LCT which supported by our histopathological findings, showed portal areas with round cells infiltration and interstitial aggregation of round cells, this is in accordance with our results. Environmental toxins have been found to be a disruptive cause of the expression patterns of those genes linked to the immune response. In fact, cytokines are considered to be important markers of the inflammation response that are inflicted by environmental chemicals in the mice **(Elsabahy and Wooley, 2013)**.

These findings suggested that ROS produced by LCT and its metabolites activated genes involved in inflammation through the stimulation of a transcription factor, NF-κB, which is situated in the crossover of inflammatory and oxidative stress **(Ruiz et al., 2013; Wang et al., 2016),** LCT and its metabolites may mediate the inflammation process. The positive correlation between LCT metabolites (CFMP and 3-PBA) and inflammatory gene expressions (TNF-α, IL-6, and IL-1β) observed in the study by **Aouey et al. (2017)** which supported these findings. Based on these observations, it is suggested the increased activity of cytokines following LCT exposure as evidence of the oxidative process and the presence of inflammation. These results were correlated with histopathology results, which revealed portal infiltration with inflammatory cells, perivenular sinisoidal dilatation, parenchyma inflammation, and congestion of central veins were seen in LC-treated groups.

The evaluating data of genotoxicity and **DNA damage in both blood and liver** samples revealed that a non-significant change in the value of comet

assay parameters including tail DNA%, tail length (µm), tail moment % and olive tail moment% in the **conventional ʎ cyhalothrin** treated rats along the whole time of the experiment in comparison with the control group. On the other hand, the group of **nano ʎ cyhalothrin** treated rats showed a significant increase in the value of comet assay parameters which indicate DNA damage in both blood and liver samples including significant increase in tail DNA%, tail length (µm), tail moment % and olive tail moment% at **12^{th}** week of the experiment in comparison with control group and the group of **conventional ʎ cyhalothrin** treated rats. The comet assay is a sensitive process utilized in the measurement and identification of DNA condemned at the cellular level. It has been documented that toxicity causes significant DNA damage, leading to abnormal changes in cellular mechanisms (**Sreekumaran et al., 2005).** The percentage of DNA in the tail (tail intensity) shown to be proportionate to the incidence of DNA strand-breaks; the results can be expressed by categorizing cell damage according to the amount of DNA in the tail **(Betti et al., 1993; Tice et al., 2002)**.

Previous studies demonstrated the existence of genotoxic potential of pyrethroid ʎ cyhalothrin as Çelik et al. (2003) indicated that LCT induces structural chromosome aberrations and the occurrence of micronuclei and suggested the in vivo susceptibility of mammals to the genetic toxicity and cytotoxic potential of LCT. The frequency of micronucleated erythrocytes was significantly increased while the nucleolar parameters were repressed by lambda-cyhalothrin treatment on *Garra rufa* (Çavas and Ergene-Gözükara, 2003). The cells were exposed to LCT for 2 h; the dose–response relationship of LCT showed genotoxic effects, where the increase in comet tail length relates to the extent of DNA single strand breaks (Naravaneni and Jamil, 2005). LCT genotoxicity is dose-dependent. This is in accordance with

previous studies where DNA fragmentation was induced by LCT in rat lymphocytes (Sharma et al., 2010) and by cypermethrin in rat brain (Hussien et al., 2013). Fetoui et al. (2015) declared that that exposure rat to LTC at a dose of 6.23 mg/kg for a period of 7, 14 and 21 days induced a noticeable genotoxic effect in rat peripheral blood evidenced by a significant increase in the frequency of micronucleated only at day 21. Dikilitas et al. (2015) reported that the pesticide lambda-cyhalothrin was found genotoxic at all doses. The results showed that lambda-cyhalothrin significantly increased DNA damages in a dose-dependent manner. Aslantürk and Aşkin Çelik, (2016) reported that cytotoxic and genotoxic effects of two pesticide mixtures (lambda-cyhalothrin (LCT) and dimethoate (DIMET)) on human peripheral blood lymphocytes. The LCT induced the DNA damage in lymphocytes, which increased with increasing pesticide concentration. Yahia et al. (2019) reported that of rats after 48 h of the exposure to LCT for 48 h induced severe DNA damage in bone marrow indicated by significant increases (P<0.01) in tail moment, olive tail moment, tail length and % of migrated DNA in tail. Although a number of studies revealed no genotoxic effect tested different pyrethroids (Suralles et al., 1995; Villarini et al., 1998; Hadnagy et al., 1999).

Gao et al. (2006) found that mice administered with these formulations (common formulation, nanoformulation, and nanofunctional formulation) chlorfenapyr through IP at different doses of 4.9, 9.8, or 19.6 mg kg1 exhibited genotoxicity, and the potency of genotoxicity of nanoformulation and nanofunctional formulation was less than that of common formulation. The data also showed that at the same dosage, the effects on DNA damage of peripheral blood lymphocytes of the mouse and chromosome damage in the bone marrow cells of the mouse following chlorfenapyr

nanopreparation was less than the common formulation. However, there was no marked difference in the apoptotic ratio and live cell percentage of liver cells between these two pesticide formulations **(Wan-Jun et al., 2010).**

Genotoxicity of nanomaterials (NMs) could be induced either through direct binding of NMs to chromosomes or DNA or by the generation of ROS inducing DNA lesions. Based on molecular dynamics simulations it has been shown that fullerenes can bind to nucleotides at the free ends and minor grooves of double stranded A-form DNA, thereby permanently disrupting hydrogen bonds and causing deformation **(Zhao et al., 2005).**
More recently, **Li et al. (2013)** demonstrated that NPs with high binding affinity to DNA have a greater potential to interfere with DNA replication. For a great number of NMs the induction of ROS has been shown to be a mode of action through which primary genotoxicity is induced. These ROS can originate from the NM surface (free radicals, transition metals, etc) from interaction of NMs with cellular components such as the mitochondria or through inhibition of the antioxidant defense mechanisms **(Magdolenova et al., 2014**). A possible mechanism of genotoxicity, due to nanomaterials have a greater affinity toward the lipid molecules present in the biological environment. It interaction with lipid bilayers reduced the lipid transition temperature and the membrane fluidity of all types of lipid vesicles. Secondly, these nanomaterials were found capable of covalently binding with the nucleic acid by electrochemical interaction. These results in a reversible electron transfer at the interfacial level generating holes on the nucleic acid bases thereby affecting the length of the strand, terminal phosphates and different types of DNA strands **(Sow and Samadder, 2021).**

The result of histopathological examination revealed that **liver** of conventional ʎ cyhalothrin treated rats showed marked vacuolar degenerated hepatocytes , inflammatory cell infiltration with mono nuclular cells, marked hydropic degenerated hepatocytes, inflammatory cell infiltration in portal triad region and vascular degeneration. Liver of nano ʎ cyhalothrin treated rats showed marked hydropic degenerated hepatocytes with vaculation, inflammatory cell infiltration in portal triad region, dilated and congested blood vessel, hyperplasia in bile duct, and Kupffer cells activation.

Brain of conventional ʎ cyhalothrin treated rats showed pre vascular hemorrhage in the cerebral cortex, some pyknotic Purkinje cell and granular cell layer, congestion of blood vessels, degenerative neurocytes. Brain of nano ʎ cyhalothrin treated rats showed degeneration in Purkinje cell and few pyknotic neuclii, sever pre vascular hemorrhage in the cerebral cortex, degenerated pyramidal cells and pyknotic neurocytes**.**

The liver and brain findings in the present study are in accordance to **El-Bendary et al. (2010),** who revealed that the treated group liver showed congestion of hepatic blood vessels, vascular degeneration with necrosis of hepatic cells. The cells within the group oriented toward the base membrane. Pathological finding in brain showed congestion of meningeal blood vessels, lymphocytic aggregation, degenerative changes of the nerve fibers and fragmentation and necrotic changes of some neurons. Generally histopathological examination revealed vascular congestion, hydrophic degeneration and leukocyte infiltration in the affected organs at the initial stages. In similar regard, **Emmanuel and Kingsley, (2014)** recorded that the brain histo-morphological changes in the cerebrum and cerebellum of adult albino rats under the stress of pyrethroid pesticides as the brain

tissues revealed mild to marked distortion of the cyto-architectural patterns with multifoci of necrosis, severe gliosis involving predominantly astrocytes and olingodendrocytes both in the cerebral and cerebellar tissues. In a parallel consent, **Alrawe and ALzubaidy, (2022)** reported that the administration of dosages of lambda-cyhalothrin 0 control group, 57.12, 114.25, 171.36 mg/kg orally represented 25, 50, 75% of LD50 for 28 days, revealed that the liver and brain showed histopathological changes such as congestion, focal infiltration of mononuclear cells, hemorrhage, coagulative necrosis, and vasogenic edema. In the brain, the lesion was represented by shrunken in purkinji, demyelination of axon and hyper atrophy of astrocyte, the lesion was more severe in both organs when exposed to a high concentration and for longer periods. This results could be contributed to liver is a crucial organ in detoxification processes. However, the morphological frame cannot be interpreted as a specific response to pyrothroides intoxication; instead, it can be attributed to xenobiotics in general. One of the most frequent hepatic alterations observed after exposure to xenobiotics is an increase of vascularization. This physiologic response leads to raises in blood flux and increases of catabolite excretion from pyrothorides metabolism, which occurs mainly through bile **(Bradbury and Coats, 1989; Kolo et al., 2010)**. Local lesions seem the most plausible hypothesis and might be due to an exacerbated ROS production, owing to LCH action on cell mitochondria. The influence of synthetic pyrethroids in the electron transport chain, already discussed, could increase ROS generation, and consequently enhance local cellular lesions. There was a clear cytoplasmic vacuolization in hepatocytes linked to LCH intoxication. Disorganization of typical hepatocytes cords suggests that the organelle distribution was altered, affecting the organ functions as just proposed **(Marinho et al., 2014)**.

In kidney of conventional ʎ cyhalothrin treated rats showed hyper-cellularity in the glomeruli, necrosis of some renal tubular epithelium and mild intertubular mononuclear cell infiltration, intertubular hemorrhage, pre vascular fibrosis, renal tubular cast, sever degeneration and necrosis of renal epithelium in renal medulla. Kidney of nano ʎ cyhalothrin treated rats showed mild intertubular mononuclear cell infiltration, swollen, congested and hyper cellularity glomerular tuft, degenerated renal tubular epithelium in renal medulla, and mild intertubular hemorrhage, renal medulla vacuolar degeneration and necrosis in renal epithelium. In accordance of our findings**, Pawar et al., (2017)** reported that characteristic histopathological lesions noted in the kidney, especially degenerative changes in the convoluted tubules and hyper cellularity of glomerulus. Degenerative changes in convoluted tubules with occlusion of lumen and reduced bowman's space in high-dose group. Tubular degeneration with cystic dilatation of tubules with presence of homogenous pinkish mass was evident in kidneys of mice treated with medium toxic dose which strongly corroborate biochemical perturbations observed after LCT exposure. Parallel to our findings, **Basir et al. (2011)** have also reported similar histopathological changes in the kidney and brain of rabbits following LCT exposure. Further, histological alterations observed in this study are in accordance with other researchers **(Fetoui et al., 2010; Sankar et al., 2012).**

Testis of conventional ʎ cyhalothrin treated rats showed degeneration of lydge cell, disorganization of spermatogonia, spermatocytes and nuclear pyknosis of germ cells, degeneration of lydge cell, degeneration of spermatogenic cells with massive vacuolation, nuclear pyknosis of germ cells, marked interstitial edema Testis of nano ʎ cyhalothrin treated rats showed degeneration of lydge cell, disorganization of spermatogonia,

spermatocytes, mild vaculation, degeneration and disorganization of normal successive stages of spermatogenesis, spermatogonia, spermatocytes. Testis pathological findings are in agreement with **Hussein et al. (2012),** they declared that LCH exposure showed a significant decrease in the numbers of germinal, Lydig and spertmatocyte (primary and secondary) cells when compared to the controls**.** The structures of the seminiferous tubules in cyhalothrin-treatment groups were pathologically damaged. The number of germinal cells was greatly decreased with a disturbance in their diameter. Reactive oxygen species (ROS) caused by insecticide treatment may be involved in the toxicity of various pesticides **(Walsh et al., 2000)**. Increased ROS may decrease the effective concentration of antioxidant, increasing the harmful effects of ROS to reproductive tissue **(Agarwal and Prabakaran, 2005)**. **Sutcu et al. (2007)** have shown that insecticide treatment caused an increase in lipid peroxidation (LPO) in rat erythrocytes. Because spermatozoa have large quantities of polyunsaturated fatty acids (PUFA) in their plasma membranes and their cytoplasm contains low concentrations of scavenging antioxidants **(Agarwal and Prabakaran, 2005)**, a causal relationship is suspected. Thus, it is hypothesized that oxidative damage induced by Cyhalothrin insecticide may be one of these mechanisms**.**

The outcome of this study confirmed that the administration of the conventional and nano form of the insecticide lambda cyhalothrin have non-significant change in rat body weight while they caused an increase in liver and kidney weight. Where, they played an essential role in the metabolism and detoxification and excretion of toxic metabolites. There was normal hemogram but significant leukocytosis was recorded. According to this study result, a lipid peroxidation product MDA showed a significant increase and the antioxidant enzymes SOD revealed a significant decrease, which represent the *oxidative stress state in all*

exposed rats. The *oxidative stress* reflected with the up- regulation of the tumor necrosis factor which is a potent cytokine produced in response to inflammation, infection, injury, and other environmental challenges and plays a unique and pivotal role in regulating apoptotic signaling pathways, and in the control of cell proliferation and inflammation. This is in consequence with pathological finding and lesions detected in liver, kidney, brain and testis. All alterations in parameters including organ weight index, hematological, biochemical and histopathological build up the oxidative DNA damage that was recorded in this study by comet assay.

This work represents the most advanced study to investigate and compare the toxic effect of conventional and nano cyhalothrin (LCN) formulation on non-target vertebrate animal as laboratory animal; *Sprauge Dawely* rat. These findings suggested that there was no significant difference between the of conventional and nano form of the insecticide lambda cyhalothrin in almost parameters, including organ weight index, hematological, biochemical and histopathological examination. Furthermore, there are genotoxicity and DNA damage induced by nano form of lambda-cyhalothrin after 12 weeks of exposure in both investigated organs blood and liver. This effect may have contributed to the smaller size and stronger permeability of nano- formulation would pose unpredictable consequences for non-targeted organisms (**Totsuka et al., 2009).**

Some studies on the nano pesticides formulations on non-vertebrates noted the same criteria. For instance, **Matusse, (2019)** compared the toxicity of a conventional (LC) formulation (CF) and of a more recent lambda-cyhalothrin-loaded nanocapsulates (nano-ECF) on soil biota. In the soil invertebrate trial, collembolans reproduction was greatly inhibited by nano-ECF, and there was no inhibition on reproduction with the CF. The nano-ECF was also about 2X more toxic than the CF to earth worm's

reproduction. As for the higher plants, germination of dicotyledonous species was inhibited only by the nano-ECF. The germination of monocotyledonous was not sensitive to both formulations. The plants did not suffer any inhibition in their growth when exposed to both formulations as well. As for soil enzymatic activity (arylsulfatase, and dehydrogenase) and nitrogen mineralization; only arylsulfatase was affected by the nano-ECF. In opposition to what was expected, the nano-ECF of lambda-cyhalothrin was more toxic than the CF and frequently the difference in toxicity is remarkable. **Huang et al. (2022)** prepared a series of lambda-cyhalothrin (LC)-MCs with nano and micron-scale capsule sizes (average diameters of 209.4 nm, MC-N; 2.41 μm, MC-S; 4.87 μm, MC-M; and 12.41 μm, MC-L). The assessment results showed that the release and sedimentation behavior of LC-MCs in water and toxicity to zebrafish at three life stages were all particle size-dependent. As the diameter distribution of approximately 100 nm extended to the micron scale (~27 μm), the capsules released more slowly and sunk more quickly in water. In addition, micron-sized LC-MC exposure resulted in significantly less fish mortality and malformations of larvae and embryos compared with nanosized LC-MC exposure. The highest accumulation of MC-N in the gill and the severest toxicity to larvae suggested that the smaller size and stronger permeability of nanocapsules would pose unpredictable consequences for nontargeted organisms.

Cu and Cu(OH)2 based nanopesticides had shown some negative effects in spinach *Spinacia oleracea* plants such as alterations in metabolic processes, reduction in antioxidant molecules and total phenolic compounds. Such situations warn us to ensure safety while exposing the nanoparticle based system toward each and every component of a healthy ecosystem **(Zhao et al., 2017)**. **Vignardi et al. (2020)** observed that the highest Cu body

burdens in amphipods exposed to the nanomaterials but found no biologically significant differences among the nano- and conventional forms in terms of sublethal and lethal toxicity using conventional toxicity assays**. Moreover, in a very recent study,** it demonstrates that the overall toxicity of nano-agrochemicals on non-target aquatic species is significantly lower as compared to conventional counterparts. However, further dividing formulations into three categories (organic, bulk and ionic) shows that some nanoformulations can be more toxic when compared to bulk materials but less toxic as compared to ionic formulations while organic nanopesticides do not show a general trend in overall toxicity **(Zhang and Goss, 2022)**

Therefore, this study reveals the limitations of current studies and provides recommendations for future toxicity studies to ensure the effective and sustainable application of nano-agrochemicals, which will be beneficial to both the agrochemical industry and regulatory agencies alike. Therefore, further studies of the mechanisms of genotoxicity and application routes are needed. Moreover, exposure levels of these genotoxic particles in the working environment should be determined.

Summary

The need to protect plants against environmental challenges, abiotic and biotic, leads to the application of nanomaterials and pesticides in the environment. Recently, nanopesticides have been developed to replace classic pesticides. Their wide application in the agricultural practice leads to deposition of nanomaterials (and potential residuals) in the natural environment. The use of nanopesticides is a great challenge for lack of information about their potential ecotoxicity, animal, human and other non-target organisms toxicity.

This study was carried out to, prepare, formulate and characterize the novel nano-emulsion compound of lambda-cyhalothrin, to estimate and compare the larvicidal activity of the novel nano-emulsion lambda-cyhalothrin with, the conventional compound of lambda-cyhalothrin, and to investigate the oxidative stress and genotoxicity of nano lambda-cyhalothrin in albino rats.

Preparation and characterization of the novel compound of lambda-cyhalothrin, **Nano-emulsion Formulation of Lambda – Cyhalothrin** was obtained. The lambda-cyhalothrin-loaded nano-delivery system was developed, using Tween 80 as a hydrophobic surfactant through a solvent evaporation method. The novel compound of lambda-cyhalothrin has unique chemical, physical, and biological properties. The recorded properties of lambda-cyhalothrin nano-emulsion showed spherical particles having an average size of 70.3 nm using transmission electron microscopy. Dynamic light scattering Zetasizer reached 77.51 nm and Zeta potential has a negative surface charge value of -17.8 mV. Lambda-cyhalothrin and lambda-cyhalothrin nano-emulsion showed a strong absorption band at different wavelengths. Fourier transform infrared spectroscopy analysis revealed the addition of new bonds in the lambda-cyhalothrin nano-

emulsion compound. The larvicidal activity of the novel nano-emulsion of lambda-cyhalothrin was estimated and compared with larvicidal activity of the conventional compound of lambda-cyhalothrin and indicated a significant difference between the mortality means in the larvae of *Culex pipiens*. The produced lambda-cyhalothrin nano-emulsion has a new hydrophilic attendance and better efficacy for pest control resulting in promising nano-pesticide formulations to improve agricultural practice.

Building on the superiority and higher efficacy of the produced lambda-cyhalothrin nano-emulsion on target organisms, emerging promise for addressing the problems in agriculture and food production. A great potential of the nanopesticides to partially or totally substitute the conventional agrochemicals, establishes a new era of nano pesticide.

The investigation of the oxidative stress and genotoxicity of nano lambda-cyhalothrin in albino rats by, a total number of 72 healthy adult *Sprague–Dawley* male rats aged 8–10 weeks with an average body weight of 150–200g were divided randomly into three groups,

Conventional-treated rats: 18 rats were given 1/20 of LD50 (79 mg/kg b.wt.) of LC **(Kidd and James, 1991)**, dissolved in 1 ml corn oil as a vehicle, orally, twice weekly**.**

Lambda-cyhalothrin nano- treated rats: 18 rats were given nano λ cyhalothrin which equal **1/80** of LD50 **(Kidd and James, 1991)** orally, twice weekly**.**

Control group [1,2]: 36 rats kept as control and divided into two sub-group have18 rat each. First and second sup-groups administered 1 ml corn oil and 1 ml distilled water, respectively, orally, twice weekly.

The samples were collected according to specific schedule, every 15 days. Three randomly selected rats from each group were euthanized under diethyl ether anesthesia. Blood samples with and without anticoagulant were collected from the descending aorta, and serum samples were harvested and kept at - 20 °C until analysis. Serum samples were prepared for biochemical assay. The liver specimens were collected, and divided into two parts; the first part was used for comet assay, and the second part was preserved in 10% neutral buffered formalin for histopathological examination. Other organs like brain, testis and kidneys samples for histopathological investigation.

Blood collected with EDTA was used for haematological analysis. Haematological variables measured were, total red blood cells count (RBCs), haemoglobin concentration (HGB), red blood cells distribution width (RDW), haematocrit (HCT), main corpuscular volume (MCV) mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets count (PLT), total white blood cells count (WBCs), lymphocytes count and per cent, neutrophils count and per cent, monocytes count and per cent.

Serum samples were obtained from clotted blood collected without anticoagulant after centrifugation. The serum samples were used for determination Superoxide Dismutase (SOD) activity and serum malondialdehyde (MDA) level.

The expression of the inflammatory cytokines TNF-α average level concentration in the serum was determined by enzyme-linked immunosorbent assay (ELISA). DNA damage in the brain and liver was detected using comet assay. Sections from the brain, liver, kidneys and testes were used, immediately after sacrifice for histopathological examination.

Body weight, organ absolute and relative weight, biochemical parameters, comet assay as well as histopathological changes were the criteria used to evaluate the oxidative stress and genotoxicity of nano lambda-cyhalothrin in albino rats.

In the present study, the results of **body weight** revealed that in both groups conventional and group of nano ʎ cyhalothrin treated rats, showed non-significant change during the whole period of experiment in comparison with control group. Also, the comparison between the results obtained of two groups, group of conventional and group of nano ʎ cyhalothrin treated rats, showed non-significant change during the whole period of experiment**.** This could be attributed to the effects of insecticide exposure on body weight are contradictory, probably depending on various factors, such as dose and route of administration, species, sex, and treatment duration**.** The non-significant differences observed in the body weight of the experimental animals shows that lambda cyhalothrin is not a systemic toxin. Therefore, it can silently destroy the vital organs of the body without conspicuous changes in the bodyweight. The results of **brain** absolute and relative weight revealed that in both groups conventional and nano ʎ cyhalothrin treated rats, the absolute weight of brain of male rats showed non-significant change during the whole period of experiment in comparison with control group. But the group of conventional ʎ cyhalothrin treated rats showed a significant decrease in the value of relative brain weight at **4th** week of the experiment in comparison with control group. The significant decrease observed in the brain relative weight in this study could be attributed to vacuolization in the cerebral hemisphere as insecticide produced neuronal degeneration and necrosis.

The results of **liver** absolute and relative weight revealed that a significant increase in the group of conventional ʎ cyhalothrin treated rats in

comparison with control group. In the group of nano ʎ cyhalothrin treated rats, the value of liver absolute weight showed a significant increase in comparison with control group and in comparison with group of conventional ʎ cyhalothrin treated rats. Increased weight of liver recorded in this study might be because of elevated circulation due to raised requirements for the detoxifying compounds that are toxic.

The results of **kidney** absolute weight revealed a significant increase in the group of conventional ʎ cyhalothrin treated rats in comparison with control group. Also, showed highly significant increase in comparison with group of nano ʎ cyhalothrin treated rats. The results of kidney relative weight of the group of nano ʎ cyhalothrin treated rats revealed a significant increase in comparison with control group. The kidneys contributed to the maintenance of the body's homeostasis thanks to the excretion of these unnecessary metabolic products. Concentrating urine in the tubular fluid also increases the concentration of xenobiotics in it. Renal transport and the accumulation and biotransformation of pyrethroid metabolites contribute to the susceptibility of the kidneys to damage by this group of xenobiotics. The results of **testis** absolute and relative weight in both groups of conventional and nano ʎ cyhalothrin treated rats, showed a non-significant change along the time of the experiment in comparison with control group and in comparison with each other. Our results could be contributed to cyhalothrin may not have obvious effect on the gonadosomatic indices but silently destroy target cells of the body over time. histopathological analysis of the testicles revealed reproductive toxicity.

This study revealed that the value of RBC's counts, Hb content, Hct and MPV values in both groups conventional ʎ cyhalothrin treated rats and group of nano ʎ cyhalothrin treated rats, showed non-significant change during the whole period of experiment in comparison with control group

and with each other. The inconsistent and the observed differences could be attributed to various factors influencing hematological parameters such as feeding pattern, food utilization, fluids and salts balance, blood sampling and experimental variables.

This study revealed that, the value of **RDW** in group of conventional ʎ cyhalothrin treated rats showed a significant decrease in comparison with control group. In a group of nano ʎ cyhalothrin treated rats, showed a significant decrease in comparison with control group. This study revealed that the value of **platelets** count in the group of conventional ʎ cyhalothrin treated rats showed a significant increase in comparison with control group. In a group of nano ʎ cyhalothrin treated rats, showed a significant increase in comparison with control group. This study revealed that the value of **total WBCs** count in the group of conventional ʎ cyhalothrin treated rats showed a significant increase in comparison with control group, and in comparison with nano ʎ cyhalothrin treated rats. In a group of nano ʎ cyhalothrin treated rats, showed a significant increase at whole period of experiment in comparison with control group. This study revealed that the value of **lymphocytes** count in the group of conventional ʎ cyhalothrin treated rats showed a significant increase in comparison with control group, and in comparison with nano ʎ cyhalothrin treated rats. In a group of nano ʎ cyhalothrin treated rats, showed a significant increase in comparison with control group. This study revealed that the value of the peripheral **neutrophils** percentage in the group of conventional ʎ cyhalothrin treated rats showed a significant increase in comparison with control group. In a group of nano ʎ cyhalothrin treated rats, showed a significant increase in comparison with control group. This study revealed that, the value of the peripheral **monocytes** percentage in group of conventional ʎ cyhalothrin treated rats showed a significant increase in comparison with control group.

In a group of nano ʎ cyhalothrin treated rats, showed a significant increase in comparison with control group**.**

An increase in the number of leukocytes in the blood of animals irrespective of the pyrethroid applied for intoxication, may result from the mobilization of the immunological system and /or a shift in the leukocytic pool from the spleen to peripheral blood**.** The increase in WBC may be indicative of activation of defense and immune system of the body**.** This might results in increase in the release of WBCs from bone marrow storage pool into the blood. The rise in WBCs count suggests the increase in the defense mechanism against probable attack of toxic molecules.

This study revealed that, the value of **total superoxide dismutase activity** (SOD), in the group of conventional ʎ cyhalothrin treated rats showed a significant decrease during the whole period of experiment in comparison with control group. Also, showed a significant decrease at **8$^{\underline{th}}$** week of the experiment in comparison with group of nano ʎ cyhalothrin treated rats. In the group of nano ʎ cyhalothrin treated rats, showed a significant decrease in comparison with control. This study revealed that **the value of MDA** in the group of conventional ʎ cyhalothrin treated rats showed a significant increase in comparison with control group. In the group of nano ʎ cyhalothrin treated rats, showed a significant increase in comparison with control group. Our study results revealed the elevated level of lipid peroxidation indicator MDA and the decline of SOD activity which could be attributed to, the decline of antioxidant capacity (TAC), thus triggering the peroxidation of unsaturated fatty acids in the cell membrane of RBCs and WBCs, thus inducing cytotoxicity. This is contributed to LCH is a type II pyrethroid, which has a α-cyano moiety, therefore the release of cyanohydrins, which are unstable under physiological conditions and further decompose to cyanides and aldehydes, which in turn could act as a source of free radicals. The lowered TAC may indicate a deficiency in the

defense against ROS, resulting in H_2O_2 accumulation, which in turn can inhibit superoxide dismutase (SOD) activity, resulting in superoxide radical accumulation. Thus, the excess radicals could readily oxidize the membrane lipids, which culminated in the expressive MDA increase, observed in the LCH-exposed group. Lipid peroxidation arises from the reaction of free radicals with lipids and this is considered to be an important feature of the cellular injury brought by free radical attack**.** The results in our study could be contributed to pesticides induce oxidative stress which leads to the generation of free radicals, changes in antioxidants levels and lipid peroxidation. In accordance with the data obtained from this study, pyrethroids have been found to induce oxidative damage in various tissues owing to the formation of ROS and impairments in free oxygen radicals scavenging enzyme systems. Antioxidant enzymes cause a primary defense that prevents oxidative damage of biological macromolecules. According to the obtained results the activities of serum SOD treated rats were significantly decreased. These results suggested that LCT has the capability to induce free radicals and oxidative damage as evidenced by alterations in various antioxidant enzymes.

This study revealed that **the mRNA expression levels of the inflammatory cytokines TNF-*α*** average level in the group of conventional ʎ cyhalothrin treated rats showed a highly significant increase and up-regulation in comparison with control group. In a group of nano ʎ cyhalothrin treated rats, the value of TNF α expression showed a significant up-regulation in comparison with control group. Also showed a highly significant increase in comparison with group of conventional ʎ cyhalothrin treated rats. The recorded impairment suggested the immunosuppressive effect of these pesticides. The expression of cytokines (TNF-α) as a vital part of the immune system is modulated by infection or inflammation, so assessing their expression levels may help explain the mechanisms of

specific contaminants'toxicity. These findings suggested that ROS produced by LCT and its metabolites activated genes involved in inflammation through the stimulation of a transcription factor, NF-κB, which is situated in the crossover of inflammatory and oxidative stress. LCT and its metabolites may mediate the inflammation process. The positive correlation between LCT metabolites (CFMP and 3-PBA) and inflammatory gene expressions (TNF-α, IL-6, and IL-1β) were observed. Based on these observations, it is suggested that the increased activity of cytokines following LCT exposure is an evidence of the oxidative process and the presence of inflammation. These results were correlated with histopathology results, which revealed portal infiltration with inflammatory cells, perivenular sinisoidal dilatation, parenchyma inflammation, and congestion of central veins were seen in LC-treated groups.

The evaluating data of genotoxicity and **DNA damage in both blood and liver** samples revealed a non-significant change in the value of comet assay parameters including tail DNA%, tail length (μm), tail moment % and olive tail moment% in the **conventional ʎ cyhalothrin** treated rats along the whole time of the experiment in comparison with the control group. On the other hand, the group of **nano ʎ cyhalothrin** treated rats showed a significant increase in the value of comet assay parameters which indicate DNA damage in both blood and liver samples including significant increase in tail DNA%, tail length (μm), tail moment % and olive tail moment% at **12^{th}** week of the experiment in comparison with control group and with the group of **conventional ʎ cyhalothrin** treated rats. The results of **conventional ʎ cyhalothrin** could be attributed to, LCT genotoxicity is dose-dependent. Genotoxicity of nanomaterials (NMs) could be induced either through direct binding of NMs to chromosomes or DNA or by the generation of ROS inducing DNA lesions. Based on molecular dynamics simulations it has been shown that NMs can bind to

nucleotides at the free ends and minor grooves of double stranded A-form DNA, thereby permanently disrupting hydrogen bonds and causing deformation. More recently other mechanism, NPs with high binding affinity to DNA have a greater potential to interfere with DNA replication. For a great number of NMs the induction of ROS has been shown to be a mode of action through which primary genotoxicity is induced. A possible mechanism of genotoxicity, due to nanomaterials have a greater affinity toward the lipid molecules present in the biological environment. Their interaction with lipid bilayers reduce the lipid transition temperature and the membrane fluidity of all types of lipid vesicles. Secondly, these nanomaterials were found capable of covalently binding with the nucleic acid by electrochemical interaction. This results in a reversible electron transfer at the interfacial level, generating holes on the nucleic acid bases thereby affecting the length of the strand, terminal phosphates and different types of DNA strands.

The result of histopathological examination revealed that, liver of conventional and nano ʎ cyhalothrin treated rats showed marked hydropic degenerated hepatocytes, inflammatory cell infiltration with mono nuclular cells in portal triad, inter lobular fibrosis, and Kupffer cells activation. Kidney of conventional and nano ʎ cyhalothrin treated rats showed hypercellularity in the glomeruli, necrosis of some renal tubular epithelium and intertubular mononuclear cell infiltration, intratubular hemorrhage, perivascular fibrosis, renal tubular cast, sever degeneration and necrosis of renal tubular epithelium. Brain of conventional and nano ʎ cyhalothrin treated rats showed perivascular hemorrhage in the cerebral cortex, pyknotic and degenerated Purkinje cells in cerebellum, congestion of blood vessels, degenerative neurocytes, degenerated and pyknotic pyramidal cells**.** Testis of conventional and nano ʎ cyhalothrin treated rats showed

hyperplasia of Leydig cells, disorganization of spermatogonia, spermatocytes and nuclear pyknosis of germ cells, degeneration of Leydig cells and marked interstitial edema.

It could be concluded that, this study represents an advanced work to investigate the toxic effect of a nano pesticide formulation on non-target vertebrate animal, laboratory animal (rat). This study provides the environmental impact of some novel nano-pesticides and points to their possible toxic effects on non-target organisms. The results of the present study represent a guide of the eco-toxicological studies for the application of some novel nano-pesticides in the environment.

The finding of this study suggested that there was no significant difference between the oxidative effect of conventional and nano form of the insecticide lambda cyhalothrin in all parameters, including organ weight index, hematological, biochemical and histopathological but the result of comet assay which indicate DNA damage revealed that the nano form cause genotoxic effect and DNA damage after 12 week of exposure.

Finally, there are scarce studies that have been done on the toxicity of nano pesticides. Therefore, the present work was undertaken to understand the relative toxicological differences between these novel nano pesticides and the conventional one. So, what combined toxicity of nanopesticides can produce and what effects of these combined toxicity have on organisms are still poorly studied. Thus we have to raise concerns about this issue, because its potential impact on the environment and humans is far-reaching.

Conclusion

The following points could be concluded from this study:

A. The preparation of the novel nano-emulsion formulation of Lambda – Cyhalothrin was developed. It has unique chemical, physical, and biological properties.

B. There are many factors affected the nano formulation compound composition such as the used solvent, surfactant type, time and temperature of stirring or shearing machine. All these factors induce new properties for the new nano- compound. Each study applied specific factors consequently, unique parameters obtained for its nano product. In this study, we used melt and solvent evaporation method and successfully have provided a new compound of nano-lambada cyalothrin pesticide with novel and unique nano-properties.

C. The characterization of novel nano- formulation confirmed specific properties of LCN included that the spherical particles have average size of 70.3 nm with TEM. DLS- Zetasizer reached to 77.51 nm and Zeta potential has a negative surface charge value (− 17.8 mV). LC and LCN showed strong absorption band at different wave lengths. FTIR analysis revealed addition of new bonds in LCN compound.

D. Larvicidal activity of the novel LCN was estimated and compared with larvicidal activity of the conventional one. It indicated a significant difference between the mortality means in larvae of *C. pipiens*. The produced LCN has a new hydrophilic attendance and better efficacy for pest control resulted in a promising nano-pesticide formulations to improve agricultural practice.

E. The outcome of this investigation confirmed that the administration of the conventional and nano form of the insecticide lambdacyhalothrin have not

significant change in rat body weight while they caused a significant increase in liver and kidney weight.

F. The normal hemogram was obtained but significant decrease RDW diameter of RBCs was recorded,

G. Increasing the value of total Platelets, WBCs, lymphocytes, peripheral neutrophils and monocytes counts.

H. The lipid peroxidation product MDA showed a significant increase and the antioxidant enzymes SOD revealed a significant decrease, which represent the oxidative stress state in all exposed rat groups.

I. This study revealed that **the mRNA expression levels of the inflammatory cytokines of tumor necrosis factor (TNF-α)** showed a significant increase and up-regulation of genes expression during the whole time of the experiment.

J. Genotoxicity and DNA damage in **blood** and **liver** were detected by comet assay parameters which indicated that nano- treated rats showed a significant increase in tail DNA%, tail length (µm), tail moment % and olive tail moment% after12 weeks of exposure in comparison with control group and conventional - treated rats.

K. Alteration in histopathological examination of liver, kidney, brain and testis including marked vacuolar degenerated hepatocytes, hypercellularity in the glomeruli with necrosis of some renal tubular epithelium, and intertubular mononuclear cell infiltration. There is vascular hemorrhage in the cerebral cortex and some pyknotic Purkinje cells. Disruption of germinal epithelium with vacuolization of Leydig cells and decreased spermatogenic cells.

L. The findings of this study suggested that there was no significant difference between the toxic effect of conventional and nano form of the insecticide lambda cyhalothrin in almost parameters during the different times of the experiment, including organ weight index, hematological, biochemical

including oxidative stress and tumer necrosis factor and histopathological examination.

M. Furthermore, there are genotoxicity and DNA damage induced by nano-form of lambda-cyhalothrin LCN after 12 weeks of exposure in both investigated organs blood and liver. This effect may have contributed to the smaller size and stronger permeability of nanoformulation would pose unpredictable consequences for non-targeted organisms.

Recommendations

It is necessary to look deeply at the inappropriate use of pesticides particularly new formulated nano pesticides before they enter the food chain and can affect soil biota, animal and human health as well as the whole eco system. From the previous and mentioned findings of this study, applications and registrations of such chemicals should be limited to a designed program as follow:

1. The potential toxicological effects, and impacts of nanopesticides for environmental and food safety should receive more attention and research. The focus should be on nanoparticulate systems and their interaction with plants and the environment, highlighting reactivity, retention time, levels of bioaccumulation, biodegradation time, waste toxicity, and maxima reduction of leaching and drifting.
2. Concerning the interactions of nanopesticides with biological components of non-target organisms as cell membrane, proteins, saccharides, enzymes and DNA or physiological processes as (absorption, distribution, metabolism, bioaccumulation, and clearance), among other factors, is no less important.
3. Finally, this study reveals the limitations of current studies and provides recommendations for future toxicity studies to ensure the

effective and sustainable application of nano-agrochemicals, which will be beneficial to both the agrochemical industry and regulatory agencies alike. Therefore, further studies of the mechanisms of genotoxicity and application routes are needed. Moreover, exposure levels of these genotoxic particles in the working environment should be determined.

References

Abbassy, M. A., Marzouk, M. A., Mansour, S. A., Shaldam, H. A., and Mossa, A. H. (2014): Impact of oxidative stress and lipid peroxidation induced by lambda cyhalothrin on p450 in male rats: the ameliorating effect of zinc. *J. Environ. Anal. Toxicol*, *4*(4), 1-5.

Abdallah, F. B., Fetoui, H., Zribi, N., Fakhfakh, F., and Keskes, L. (2012): Protective role of caffeic acid on lambda cyhalothrin-induced changes in sperm characteristics and testicular oxidative damage in rats. *Toxicology and Industrial Health*, *28*(7), 639-647.

Abdel-Mobdy, Y. E., and Abdel-Rahim, E. A. (2015): Toxicological influences of lambda cyhalothrin and evaluation of the toxicity ameliorative effect of pomegranate in albino rats. *Global veterinaria*, *14*(6), 913-921.

Abe, J., Nagahori, H., Tarui, H., Tomigahara, Y., Isobe, N, (2017): Metabolism of metofluthrin in rats: I. Identification of metabolites. *Xenobiotica* 5, 1-13.

Abouelkassem, S., El-Borady, O. M., and Mohamed, M. B. (2016): Remarkable enhancement of cyhalothrin upon loading into silver nanoparticles as larvicidal. *International Journal of Contemporary Applied Sciences*, *3*(1), 252–263.

Adam, M.T., Momo, C.M., Narcisse, V.B., Herve, T., Nadège, D.M., Bauland, D.N., Justin, K., and Ferdinand, N. (2020): Haematological Characteristics and Oxidative Stress Indicators in Pregnant Rabbit does (Oryctolagus cuniculus) Exposed to Lambda Cyhalothrin. *EC Veterinary Science*, *5*, 65-75.

Adisa IO, Pullagurala VLR, and Peralta-Videa JR (2019): Recent advances in nano-enabled fertilizers and pesticides: a critical review of mechanisms of action. *Environ Sci Nano* 6:2002–2030.

Agarwal A, and Prabakaran SA (2005): Mechanism, measurement and prevention of oxidative stress in male reproductive physiology. *Indian J. Experim. Biol*. 43: 963-974.

Agathokleous, E., Feng, Z., Iavicoli, I., and Calabrese, E. J. (2020): Nano-pesticides: a great challenge for biodiversity? The need for a broader perspective. *Nano Today, 30*, 100808.

Ahmed, K., Mikhail, W. Z., Sobhy, H. M., Radwan, E. M. M., El Din, T. S., and Youssef, A. (2019): Effect of Lambda-Cyahalothrin as nanopesticide on cotton leafworm, *Spodoptera littoralis* (Boisd.). *Egyptian Journal of Chemistry*, *62*(7), 1263–1275.

Ahuja, B. K., Jena, S. K., Paidi, S. K., Bagri, S., and Suresh, S. (2015): Formulation, optimization and in vitro–in vivo evaluation of febuxostat nanosuspension. *International Journal of Pharmaceutics*, *478*(2), 540–552.

Al-Amoudi, W. M. (2018): Toxic effects of Lambda-cyhalothrin, on the rat thyroid: Involvement of oxidative stress and ameliorative effect of ginger extract. *Toxicology reports*, *5*, 728-736.

Ali A, JA Khan, T Khaliq, I Javed, F Muhammad, B Aslam and MZ Khan, (2014): Hematobiochemical disruptions by lambda-cyhalothrin in rats. *Pak Vet J*, 34(1): 54-57.

Al-Mubaddel, F. S., Haider, S., Al-Masry, W. A., Al-Zeghayer, Y., Imran, M., Haider, A., and Ullah, Z. (2017): Engineered nanostructures: A review of their synthesis, characterization and toxic hazard considerations. *Arabian Journal of Chemistry*, *10*, S376-S388.

Alrawe, S. A., and ALzubaidy, M. H. (2022): Acute and sub-acute toxicity effects of lambda-cyhalothrin in chicks. *Iraqi Journal of Veterinary Sciences*, *36*(1), 191-200.

Al-Sarar, A. S., Abobakr, Y., Bayoumi, A. E., Hussein, H. I., and Al-Ghothemi, M. (2014): Reproductive toxicity and histopathological changes induced by lambda-cyhalothrin in male mice. *Environmental toxicology*, *29*(7), 750-762.

Anadón, A., Martínez, M., Martínez, M.A., Díaz, M.J., and Martínez-Larraňaga, M.R., (2006): Toxicokinetics of lambda-cyhalothrin in rats. *Toxicol. Lett*. 165, 47–56.

Anghinoni, G.; Anghinoni, F.B.G.; Tormena, C.A.; Braccini, A.L.; Mendes, I.D.C.; Zancanaro, L.; and Lal, R. (2021): Conservation agriculture strengthen sustainability of Brazilian grain production and food security. *Land Use Policy 108*, 105591.

Aouey, B., Derbali, M., Chtourou, Y., Bouchard, M., Khabir, A., and Fetoui, H. (2017): Pyrethroid insecticide lambda-cyhalothrin and its metabolites induce liver injury through the activation of oxidative stress and proinflammatory gene expression in rats following acute and subchronic exposure. *Environmental Science and Pollution Research*, *24*(6), 5841-5856.

Aslantürk, Ö. S., and Aşkin Çelik, T. (2016): Protective effect of lycopene against cytotoxic and genotoxic effects induced by mixture of two pesticides (dimethoate and lambda-cyhalothrin) on human peripheral blood lymphocytes. *Caryologia*, *69*(1), 20-28.

Aznar-Alemany, Ò. and Eljarrat, E. (2020): Introduction to pyrethroid insecticides: chemical structures, properties, mode of action and use. *Pyrethroid Insecticides*, 1-16.

Bancroft, J. D., and Gamble, M. (Eds.). (2008): *Theory and practice of histological techniques*. Elsevier health sciences.

Basir, A., Khan, A., Mustafa, R., Zargham Khan, M., Rizvi, F., Mahmood, F., and Yousaf, A. (2011): Toxicopathological effects of lambda-cyhalothrin in female rabbits (Oryctolagus cuniculus). *Human & Experimental Toxicology*, *30*(7), 591-602.

Baud V, and KarinM. (2001): Signal transduction by tumor necrosisfactor and its relatives. *Trends Cell Biol* 11:372–377.

Baun A, Sayre P, Steinhäuser KG, and Rose J (2017): Regulatory relevant and reliable methods and data for determining the environmental fate of manufactured nanomaterials. *NanoImpact* 8:1–10.

Betti, C., Barale, R., and Pool-Zobel, B.L., (1993): Comparative studies on cytotoxic and genotoxic effects of two organic mercury compounds in lymphocytes and gastric mucosa cells of Sprague–Dawley rats. *Environ. Mol. Mutagen*. 22, 172–180.

Bhakay, A., Vizzotti, E., Li, M., Davé, R., and Bilgili, E. (2016): Incorporation of fenofibrate nanoparticles prepared by melt emulsification into polymeric films. *Journal of Pharmaceutical Innovation*, *11*(1), 53–63.

Bhan,S., Mohan, L., and Srivastava, C. N. (2014): Relative larvicidal potentiality of nano-encapsulated temephos and Imidacloprid against Culex quinquefasciantus. *Journal of Asia Pacific Entomology*, *17*(4), 787–791.

Bhoopendra, K., and Nitesh, K. (2014): Immunotoxicity of lambda-Cyhalothrin in Wistar albino rats. *International Journal of Toxicological and Pharmacological Research*, *6*, 47-56.

Bilal M, Xu C, Cao L, Zhao P, Cao C, Li F, and Huang Q (2020): Indoxacarb-loaded fluorescent mesoporous silica nanoparticles for effective control of *Plutella xylostella* L. with decreased detoxification enzymes activities. *Pest Manag Sci* 76:3749–3758.

Boumerzrag, A., Hemida, H., Boumerzrag, F., Smail, F., and Cisse, S. (2021): Pathological and biological effects of treatments with lambda-cyhalothrin in rabbits. *Iraqi Journal of Veterinary Sciences*, *35*(3), 443-450.

Boverhof, D. R., Bramante, C. M., Butala, J. H., Clancy, S. F., Lafranconi, M., West, J., and Gordon, S. C. (2015): Comparative assessment of nanomaterial definitions and safety evaluation considerations. *Regulatory Toxicology and Pharmacology*, *73*(1), 137-150.

Bradbury S, and Coats J (1989): Toxicokinetics and toxicodynamics of pyrethroid insecticides in fish. *Environ Toxicol Chem* 8: 373–380.

Brinke M, Heininger P, and Traunspurger W (2011): A semi-fluid gellan gum medium improves nematode toxicity testing. *Ecotoxicol Environ Saf* 74:1824–1831.

Cadenas, E., and Davies, K.J., (2000): Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic. Biol. Med*. 23, 222–230.

Camara, M. C., Campos, E. V. R., Monteiro, R. A., do Espirito Santo Pereira, A., de Freitas Proença, P. L., and Fraceto, L. F. (2019): Development of stimuli-responsive nano-based pesticides: emerging opportunities for agriculture. *Journal of nanobiotechnology*, *17*(1), 1-19.

Cavas, T., and Ergene-Gozukara, S., (2003) : Evaluation of the genotoxic potential of lambda-cyhalothrin using nuclear and nucleolar biomarkers on fish cells, *Mutat. Res.* 534 (1/2) 93–99

Çelik, A., Mazmanci, B., Çamlica, Y., Aşkin, A., and Çömelekoğlu, Ü. (2003): Cytogenetic effects of lambda-cyhalothrin on Wistar rat bone marrow. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *539*(1-2), 91-97.

Chakroborty B, Pradhan A, Maiti P, and Maiti Choudhury S. (2019): Alleviating impact of taurine on renal lipid peroxidation and oxidative stress in lambda-cyhalothrin exposed rat. *Toxicol Forensic Med Open J*.; 4(1): 1-7. doi: 10.17140/TFMOJ-4-126.

Chang HY, and Yang X. (2000): Proteases for cell suicide: Functions and regulation of caspases. *Microbiol Mol Biol Rev* 64:821–846.

Chatterjee, A., Bhattacharya, R., Chatterjee, S., and Saha, N. C. (2021): λ cyhalothrin induced toxicity and potential attenuation of hematological, biochemical, enzymological and stress biomarkers in Cyprinus carpio L. at environmentally relevant concentrations: A multiple biomarker approach. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *250*, 109164.

Chatterjee, A.K., Chakraborty, R. and Basu, T. (2014): Mechanism of antibacterial activity of copper nanoparticles. *Nanotechnology 25*, 135101.

Chen, L., Chang, J., Wang, Y., Guo, A., Liu, Y., Wang, Q., Zhu, Y., Zhang, Y. and Xie, Z. (2021): Disclosing the future food security risk of China based on crop production and water scarcity under diverse socioeconomic and climate scenarios. *Sci. Total Environ.*, *790*, 148110

Chhipa H (2016): Nanofertilizers and nanopesticides for agriculture. Environ *Chem Lett* 15:15–22.

Chhipa H, and Joshi P (2016) :Nanofertilisers, nanopesticides and nanosensors in agriculture. *Nanosci Food Agric* 1:247–282.

Choi W, Kim S, and Baek YW (2017): Exposure to environmental chemicals among Korean adults-updates from the second Korean National Environmental Health Survey (2012–2014). *Int J Hyg Environ Health* 220(2):29–35.

Côa, F., Bortolozzo, L., Petry, R., Da Silva, G., Martins, C., de Medeiros, A., and Martinez, D. (2020): Environmental toxicity of nanopesticides against non-target organisms: the state of the art. *Nanopesticides*, 227-279.

Corcellas, C., Eljarrat, E., and Barcelo, D., (2015): First report of pyrethroid bioaccumulation in wild river fish: a case study in Iberian river basins (Spain).*Environ.Int*.75, 110-116.

Corcellas, C., Feo, M.L., Torres, J.P., Malm, O., Ocampo-Duque, W., Eljarrat, E.,and Barcelo, D., (2012): Pyrethroids in human breast milk: occurrence and nursing daily intake estimation. *Environ. Int*. 47, 17-22.

Cui, B., Feng, L., Pan, Z., Yu, M., Zeng, Z., Sun, C., Zhao, X., Wang, Y., and Cui, H. (2015): Evaluation of stability and biological activity of solid nanodispersion of lambda-cyhalothrin. *PLoS One*, *10*(8), e0135953.

Deng R, Lin D, and Zhu L (2017): Nanoparticle interactions with co-existing contaminants: joint toxicity, bioaccumulation and risk. *Nanotoxicology* 11:591–612.

Desheesh, M., El-Masry, D., Fargand, M., and Youssef, H. (2019): Larvicidal activity of nano-encapsulated lambda–cyhalothrin against susceptible mosquito larvae (*Culex pipiens*) in comparison with conventional form. *Alexandria Science Exchange Journal*, *40*, 568–573.

Dikilitas, M., Kocyigit, A., Bilinc, H., Taskin, A., and Karakas, S. (2015): Evaluation of genotoxicity of environmentally friendly pesticides on higher cells using the single cell gel electrophoresis (SCGE) assay. *Fresen. Environ. Bull*, *24*(1), 39-47.

Du, Z., Wang, C., Tai, X., Wang, G., and Liu, X. (2016): Optimization and characterization of biocompatible oil-in-water nanoemulsion for pesticide delivery. *ACS Sustainable Chemistry and Engineering*, *4*(3), 983–991.

El-Bendary, H. M., Negm, S. E., Saleh, A. A., Kady, M. M., and Hosam Eldeen, F. A. (2010): Histopathological changes associated with exposure of male mice to pyrethroid pesticide (Lambda cyhalothrin). *Journal of Plant Protection and Pathology*, *1*(9), 697-710.

Elbushra, A.A., and Ahmed, A.E. (2020): Food Security in Sudan: A Historical Analysis of Food Availability. *Iraqi J. Agric. Sci.*, *51*, 422–431.

El-Demerdash, F. M. (2007): Lambda-cyhalothrin-induced changes in oxidative stress biomarkers in rabbit erythrocytes and alleviation effect of some antioxidants. *Toxicol. in Vitro*, 21: 392–397.

Elsabahy M, and Wooley KL (2013): Cytokines as biomarkers of nanoparticle immunotoxicity. *Chem Soc Rev* 42:5552–5576.

Elsharkawy, E. E. (2020): Nanotechnology applications of pesticide formulations. *Journal of Nanomedicine*, *3*(1), 1029.

Emmanuel, I. O. and Kingsley, A. I. (2014): A histomorphologic analysis of pyrethroid pesticide on the cerebrum and cerebellum of adult albino rats. *J. Expt. Clin. Anat*. 13(2), 54-59.

Ender Y., and Onder C. (2006): Effects of dichlorvos on lipid peroxidation in mice on subacute and subchronic periods. Pest. *Biochem. Physiol.*, 86: 106-109.

Etheridge, M., Campbell, S., Erdman, A., Haynes, C., Wolf, S., and McCullough, J. (2013): The big picture on nanomedicine: the state of investigational and approved nanomedicine products. *Nanomedicine: nanotechnology, biology andmedicine*, *9*(1), 1-14.

Ezenwosu, S., Nnamonu, E., Odo, G., Ikele, B., and Ani, O. (2021): Evaluation of lambda-cyhalothrin oxidative stress and gonad histoarchitecture toxicity potency in Clarias gariepinus. *The Journal of Basic and Applied Zoology*, *82*(1), 1-11.

Fernando, G.B., (2019): Ten chemical innovations that will change our world: IUPAC identifies emerging technologies in chemistry with potential to make our planet more sustainable. Chem. *Int. N. Mag.* IUPAC 41, 12–17.

Fetoui, H., Feki, A., Salah, G. B., Kamoun, H., Fakhfakh, F., and Gdoura, R. (2015): Exposure to lambda-cyhalothrin, a synthetic pyrethroid, increases reactive oxygen species production and induces genotoxicity in rat peripheral blood. *Toxicology and industrial health*, *31*(5), 433-441.

Fetoui, H., Garoui, E. M., Makni-Ayadi, F., and Zeghal, N. (2008): Oxidative stress induced by lambda-cyhalothrin (LTC) in rat erythrocytes and brain: attenuation by vitamin C. *Environmental Toxicology and Pharmacology*, *26*(2), 225-231.

Fetoui, H., Makni, M., Garoui, E. M., and Zeghal, N. (2010): Toxic effects of lambda-cyhalothrin, a synthetic pyrethroid pesticide, on the rat kidney: Involvement of oxidative stress and protective role of ascorbic acid. *Experimental and Toxicologic Pathology*, *62*(6), 593-599.

Fetoui, H., Garoui, E. M. and Zeghal, E. (2009): Lambda-cyhalothrin-induced biochemical and histopathological changes in the liver of rats: ameliorative effect of ascorbic acid. *Exp. Toxicol. Pathol.*, 61: 189–196.

Frank, D., Miller, G., Harvey, D., Brander, S., Geist, J., Connon, R., and Lein, P., (2018): Bifenthrin causes transcriptomic alterations in mTOR and ryanodine receptor-dependent signaling and delayed hyperactivity in developing zebrafish (Danio rerio). *Aquat. Toxicol.* 200, 50-61.

Gao, S., Y. Lu, and P. Zhou. (2006): Study on genotoxicity of three chlorfenapyr formulation. *Journal of Environmental and Occupational Medicine* 23: 11–14

Gassner, B., Wûthrich, A., Scholtysik, G., and Solioz, M., (1997): The pyrethroids permethrin and cyhalothrin are potent inhibitors of the mitochondrial complex I. *J. Pharmacol. Exp. Ther*. 281, 855–860.

Ghosh, R., Das, T., Paramanik, A., and Maiti Choudhury, S. (2016): Lambda cyhalothrin elicited dose response toxicity on haematological, hepatic, gonadal and lipid metabolic biomarkers in rat and possible modulatory role of taurine. *Toxicol Forensic Med Open J*, *1*(2), 42-51.

Gilbertson, L., Pourzahedi, L., Laughton, S., Gao, X., Zimmerman, J. B., Theis, T., and Lowry, G. (2020): Guiding the design space for nanotechnology to advance sustainable crop production. *Nature nanotechnology*, *15*(9), 801-810.

Grewal, K., Sandhu, G., Kaur, R., Brar, R., and Sandhu, H. (2010): Toxic impacts of cypermethrin on behavior and histology of certain tissues of albino rats. *Toxicology international*, *17*(2), 94.

Grotto, D., Maria, L.S., Valentini, J., Paniz, C., Schmitt, G., Garcia, S.C., Pomblum, V.J., Rocha, J.B.T. and Farina, M., (2009): Importance of the lipid peroxidation biomarkers and methodological aspects for malondialdehyde quantification. *Quimica Nova*, *32*, pp.169-174.

Hadnagy W, Seemayer NH, Ku¨hn KH, Leng G and Idel H (1999): Induction of mitotic cell division disturbances and mitotic arrest by pyrethroids in V79 cell cultures. *Toxicology Letters* 107(1–3): 81–87.

Handy RD, Cornelis G, and Fernandes T (2012): Ecotoxicity test methods for engineered nanomaterials: practical experiences and recommendations from the bench. Environ *Toxicol Chem* 31:15–31.

Haratym-Maj, A. (2003): Hematological alternations after pyrethroids poisoning in mice. *Annals of agricultural and environmental medicine*, *9*(2), 199-206.

He X, Deng H, and Hwang H (2019): The current application of nanotechnology in food and agriculture. *J Food Drug Anal* 27:1–21.

He X, Fu P, Aker WG, and Hwang HM (2018): Toxicity of engineered nanomaterials mediated by nano–bio–eco interactions. *J Environ Sci Health* C 36:21–42.

Hoek J.B., and Pastorino J.G. (2002): Ethanol oxidative stress and cytokine induced liver damage. *Alcohol*.; 27: 63-68.

Hofmann, T., Lowry, G. V., Ghoshal, S., Tufenkji, N., Brambilla, D., Dutcher, J. R., and Wilkinson, K. J. (2020): Technology readiness and overcoming barriers to sustainably implement nanotechnology-enabled plant agriculture. *Nature Food*, *1*(7), 416-425.

Huang X, Wang A, Luo J, Gao Y, Guan L, Zhang P, Liu F, Mu W, and Li B (2022): Lambda-cyhalothrin-loaded nanocapsules pose an unacceptable acute toxicological risk to zebrafish (*Danio rerio*) at the adult and larval stages but present an acceptable risk to embryos. J Hazard Mater 422:126385.

Hussein, H. K., Elnaggar, M. H., and Al-Zahrani, N. K. (2012): Antioxidant role of folic acid against reproductive toxicity of cyhalothrin in male mice. *Glo. Adv. Res. J. Environ. Sci. Toxicol*, *1*(4), 066-071.

Hussien H.M., Abdou H.M., and Yousef M.I. (2013): Cypermethrin induced damage in genomic DNA and histopathological changes in brain and haematotoxicity in rats: The protective effect of sesame oil. *Brain research bulletin*, *92*,76 83.

Ibrahim, H. M. (2016): Evaluation of the immunotoxic effects of sub-chronic doses of lambda-cyhalothrin in murine model. *MOJ. Immunol*, *3*(6), 00108.

Jambo, Y.; Alemu, A., and Tasew, W. (2021): Impact of small-scale irrigation on household food security: Evidence from Ethiopia. *Agric. Food Secur.*, *10*, 21.

Jassim, Z. E., and Rajab, N. A. (2018): Review on preparation, characterization, and pharmaceutical application of nanosuspension as an approach of solubility and dissolution enhancement. *J Pharm Res*, *12*, 771-4.

Kah M (2015): Nanopesticides and nanofertilizers: emerging contaminants or opportunities for risk mitigation? *Front Chem* 3:64.

Kah M, and Hofmann T (2014): Nanopesticide research: current trends and future priorities. *Environ Int* 63:224–235.

Kah M, Kookana RS, Gogos A, and Bucheli TD (2018): A critical evaluation of nanopesticides and nanofertilizers against their conventional analogues. *Nat Nanotechnol* 13:677–684.

Kah, M., Johnston, L. J., Kookana, R. S., Bruce, W., Haase, A., Ritz, V., and Gubala, V. (2021): Comprehensive framework for human health risk assessment of nanopesticides. *Nature Nanotechnology*, *16*(9), 955-964.

Kah, M., Tufenkji, N. and White, J. C. **(2019):** Nano-enabled strategies to enhance crop nutrition and protection. *Nat. Nanotechnol.* 14, 532–540.

Kalia, A., and Kaur, H., (2019): Nano-biofertilizers: harnessing dual benefits of nano-nutrient and bio-fertilizers for enhanced nutrient use efficiency and sustainable productivity. *Nanosci. Sustain. Agric*. 51–73.

Kaneko, H., (2010): Pyrethroid chemistry and metabolism. In: Krieger, R. (Ed.), Hayyes' Handbook of Pesticide Toxicology, third ed. Elsevier, Amsterdam, The Netherlands (Chapter 76).

Kaneko, H., (2011): Pyrethroids: mammalian metabolism and toxicity. *J. Agric. Food Chem.* 59, 2786-2791.

Khalil, S. R., Abd Elhakim, Y., Abd El-fattah, A. H., Farag, M. R., Abd El-Hameed, N. E., and Abd Elhakeem, E. M. (2020): Dual immunological and oxidative responses in Oreochromis niloticus fish exposed to lambda cyhalothrin and concurrently fed with Thyme powder (Thymus vulgaris L.): Stress and immune encoding gene expression. *Fish & shellfish immunology*, *100*, 208-218.

Khan A, Ahmad L, and Khan MZ. (2012): Hemato-biochemical changes induced by pyrethroid insecticides in avian, fish and mammalian species. *Int J Agric Biol*.; 14, 834-842.

Khan A, Faridi HAM, and Ali M. (2009): Effects of cyper-methrin on some clinico-hemato biochemical and pathological parameters in male dwarf goats. *Exp Toxicol Pathol*.; 61, 151-160.

Khandelwal, N., Barbole, R.S., Banerjee, S.S., Chate, G.P., Biradar, A.V., Khandare, J.J., and Giri, A.P., (2016): Budding trends in integrated pest management using advanced micro- and nano-materials: challenges and perspectives. *J. Environ. Manag*. 184, 157–169.

Khanna, S.K. (2020): Food Availability, Food Security, and Maternal Mental Health. *Ecol. Food Nutr.*, *59*, 1–2

Khashan, H. T. (2016): Determination of a pyrethroid insecticide deltamethrin residues in cattle meat in Baghdad Province/Al-Rusafa. *Int. J. Adv. Res. Biol. Sci*, *3*(11), 193-199.

Khemiri, R., Côté, J., Fetoui, H., and Bouchard, M. (2018): Kinetic time courses of lambda-cyhalothrin metabolites after dermal application of Matador EC 120 in volunteers. *Toxicol. Lett.*, *296*, 132–138.

Kidd H, James DR (Eds.) (1991): The Agrochemicals Handbook, 3rd Ed. Royal Society of Chemistry Information Services, Cambridge, UK., 27-33.

Kidane, L., and Kejela, A. (2021): Food security and environment conservation through sustainable use of wild and semi-wild edible plants: a case study in Berek Natural Forest, Oromia Special Zone, Ethiopia. *Agriculture & Food Security*, *10*(1), 1-16.

Klaine, S.; Koelmans, A..; Horne, N.; Carley, S.; Handy, R..; Kapustka, L.; Nowack, B.; and von der Kammer, F. (2012): Paradigms to assess the environmental impact of manufactured nanomaterials. *Environ. Toxicol. Chem.*, *31*, 3-14.

Kleiven M, and Oughton D (2015): Standard operating procedure: toxicity test with the nematode caenorhabiditis elegans for the NANoREG core nanomaterials, NMBU, ÅS, Norway. file:///F:/Pictures/New%20folder%20(2)/Downloads/SOP+Toxicity+test+with+the+nematode+Caenorhabiditis+elegans+NANoREG_Dec2015.pdf

Knieke, C., Rawtani, A., and Davé, R. N. (2014): Concentrated fenofibrate nanoparticle suspensions from melt emulsification for enhanced drug dissolution. *Chemical Engineering and Technology*, *37*(1), 157–167.

Kolo RJ, Lamai SL, and Ojutiku RO (2010): Subacute toxicity of Karate to Sarotherodon galileus (Linneu, 1758). *J Water Chem Technol* 32:107–112.

Kuder, R.S.; and Gundala, H.P. (2018): Developmental toxicity of deltamethrin and 3-phenoxybenzoic acid in embryo-larval stages of zebrafish (Danio rerio). *Toxicol. Mech. Methods*, *28*, 415–422.

Kumar, V., and Yadav, E. (2021): Assessment of Body weight, brain Weight and Neuro-somatic Index in Albino rat under toxic stress of Lambda Cyhalothrin and its modulation by Green tea. *J. Exp. Zool. India*, *24*(1), 775-780.

Krishna KB, and Prabhakar C. (2011): A review on nanosuspensions in drug delivery. *Int J Pharma and Bio Sci*.; 2: 549-558.

Lazic, S. E., Semenova, E., and Williams, D. P. (2020): Determining organ weight toxicity with Bayesian causal models: Improving on the analysis of relative organ weights. *Scientific reports*, *10*(1), 1-12.

Li AJ, Chen Z, Lin TC, Buck Louis GM, and Kannan K (2020): Association of urinary metabolites of organophosphate and pyrethroid insecticides, and phenoxy herbicides with endometriosis. *Environ Int* 136:105456.

Li L, Xu Z, Kah M, Lin D, and Filser J (2019): Nanopesticides: a comprehensive assessment of environmental risk is needed before widespread agricultural application. *Environ Sci Technol* 53:7923–7924.

Li, H., Fang, Y., Ni, C., Chen, X., Mo, J., Lv, Y., Chen, Y., Chen, X., Lian, Q., and Ge, R. S. (2018): Lambda-cyhalothrin delays pubertal Leydig cell development in rats. *Environmental pollution*, *242*, 709-717.

Li, K., Zhao, X., K. Hammer, B., Du, S., and Chen, Y. (2013): Nanoparticles inhibit DNA replication by binding to DNA: modeling and experimental validation. *ACS nano*, *7*(11), 9664-9674.

Lowry GV, Avellan A and Gilbertson LM, (2019): Opportunities and challenges for nanotechnology in the agri-tech revolution. *Nat Nanotechnol* 14: 517–522

Luty S, Latuszynska J, Obuchowska–Przebirowska D, Tokarska M and Haratym – Maj A (2000): Subacute toxicity of orally applied alpha – cypermethrin in swiss mice. *Annals of Agricultural and Environmental Medicine* **7** 33-41

Lv, Y., Wang, W., Hong, S., Lei, Z., Fang, F., Guo, Q., Hu, S., Tian, M., Liu, B., Zhang, D., Sun, Y., Ma, L., Shen, B., Zhou, D., and Zhu, C., (2016): Comparative transcriptome analyses of deltamethrin-susceptible and -resistant Culex pipiens pallens by RNA-seq. *Molecular Genetics and Genomics*: MGG 291, 309–321.

Macêdo, M. E., Consoli, R. A., Grandi, T. S., Anjos, A. Md, Oliveira, A. Bd, Mendes, N. M., Queiróz, R. O., and Zani, C. L. (1997): Screening of Asteraceae (Compositae) plant extracts for lavicidal activity against Aedes fluviatilis (Diptera: Culicidae). *Memórias Do Instituto Oswaldo Cruz*, *92*(4), 565–570.

Madkour, N. K. (2012): Protective effect of curcumin on oxidative stress and DNA fragmentation against lambda cyhalothrin-induced liver damage in rats. *Journal of Applied Pharmaceutical Science*, *2*(12), 076-081.

Magdolenova, Z., Collins, A., Kumar, A., Dhawan, A., Stone, V., and Dusinska, M. (2014): Mechanisms of genotoxicity. A review of in vitro and in vivo studies with engineered nanoparticles. *Nanotoxicology*, *8*(3), 233-278.

Mancini F, Woodcock BA, and Isaac NJB (2019): Agrochemicals in the wild: identifying links between pesticide use and declines of non-target organisms. *Curr Opin Environ Sci Health* 11:53.

Marinho JFU, Correia JE, Marcato ACC, Pedro-Escher J, and Fontanetti CS (2014): Sugarcane vinasse in water bodies: impact assessed by liver histopathology in tilapia. *Ecotoxicol Environ Saf* 110:239–245.

Markiewicz M, Kumirska J, and Lynch I (2018): Changing environments and biomolecule coronas: consequences and challenges for the design of environmentally acceptable engineerednanoparticles. *GreenChem* 20:41334168.

Martinez MA, Ares I, and Rodriguez JL (2018): Pyrethroid insecticide lambda-cyhalothrin induces hepatic cytochrome P450 enzymes, oxidative stress and apoptosis in rats. *Sci Total Environ* 631–632:1371–1382.

Matusse, C. A. (2019): Comparative toxicity studies on soil biota of conventional lambda-cyhalothrin formulation and lambda-cyhalothrin loaded nanocapsulates. https://repositorio aberto.up.pt/bitstream/10216/123886/2/365632.pdf

Miernicki, M., Hofmann, T., Eisenberger, I., Kammer, Fvonder and Praetorius, A. (2019): Legal and practical challenges in classifying nanomaterials according to regulatory definitions. *Nat. Nanotechnol.* 14, 208–216. The current limitations of the European Union definitions for 'nanomaterial' are outlined along with recommendations for a more coherent approach to classifying nanomaterials for regulatory purposes.

Mitter, N., Worrall, E. A., Robinson, K. E., Li, P., Jain, R. G., Taochy, C., and Xu, Z. P. (2017): Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses. *Nature plants*, *3*(2), 1-10.

Mosbah, R., Boulakoud, M. S., and Yousef, I. M., (2010): Effects of Lambda cyhalothrin on haematological parameters and testicular functions in male rat. *Endocrine Abstracts* **22** P371.

Moustafa, G. G., and Hussein, M. (2016): Lambda cyhalothrin toxicity induces alterations in lipogenic genes and inflammatory factors in rat liver. *Japanese Journal of Veterinary Research*, *64*(1), 25-38.

Muggelberg, L.L., Huff Hartz, K.E., Nutile, S.A., Harwood, A.D., Heim, J.R., Derby, A.P., Weston, D.P., and Lydy, M.J., (2017): Do pyrethroid-resistant Hyalella azteca have greater bioaccumulation potential compared to non-resistant populations? Implications for bioaccumulation in fish. *Environ. Pollut*. 220, 375-382.

Mukherjee, I., Singh, R., and Govil, J.N. (2010): Risk assessment of a synthetic pyrethroid, bifenthrin on pulses. Bull. Environ. *Contam. Toxicol*. 84, 294-300.

Nabuuma, D.; Ekesa, B.; Faber, M.; and Mbhenyane, X. (2021): Community perspectives on food security and dietary diversity among rural smallholder farmers: A qualitative study in central Uganda. *J. Agric. Food Res*, *5*, 100183.

Naravaneni, R., and Jamil, K. (2005): Evaluation of cytogenetic effects of lambda -cyhalothrin on human lymphocytes. *Journal of Biochemical and Molecular Toxicology*, *19*(5), 304-310.

Nieradko-Iwanicka, B., and Konopelko, M. (2020): Effect of Lambdacyhalothrin on Locomotor Activity, Memory, Selected Biochemical Parameters, Tumor Necrosis Factor α, and Interleukin 1ß in a Mouse Model. *International Journal of Environmental Research and Public Health*, *17*(24), 9240.

Nieradko-Iwanicka, B., and Rutkowski, M. (2022): Effect of subacute poisoning with lambda cyhalothrin on vascular endothelial growth factor 2 receptor in mice kidneys. *BMC nephrology*, *23*(1), 1-5.

Nirogi, R., Goyal, V. K., Jana, S., Pandey, S. K., and Gothi, A. (2014): What suits best for organ weight analysis: review of relationship between organ weight and body/brain weight for rodent toxicity studies. *Int J Pharm Sci Res*, *5*(4), 1525-1532.

Nnamonu, E. I., Mgbenka, B. O., and Mbegbu, E. C. (2019): Fertility enhancing potency of omega-3 fatty acids in male rats. *Brazilian Archives of Biology and Technology*, *62*, 1-8.

Okado-Matsumoto, A., and Fridovich, I. (2001): Subcellular distribution of superoxide dismutases (SOD) in rat liver: Cu, Zn-SOD in mitochondria. *Journal of Biological Chemistry*, *276*(42), 38388-38393.

Orlu, E. E., and Obulor, A. O. (2021): Protective Role of Different Local Spices on Lambda Cyhalothrin Induced Nephrotoxicity in Male Mice. *AJOB, 13(4): 61-70.*

Oshoke, F. I., Fadina, O. O., and Fayinminnu, O. O. (2016): Sub-chronic toxicity study of synthetic Pyrethroids(lambda-cyhalothrin) on reproductive organs of male wistar rats. *International Journal of Advance Agricultural Research*, *4*, 38-45.

Özaslan, M.S.; Demir, Y.; Aksoy, M.; Küfrevio˘glu, Ö.I.; and Beydemir, ¸S. (2018): Inhibition effects of pesticides on glutathione-S-transferase enzyme activity of Van Lake fish liver. *J. Biochem. Mol. Toxicol., 32,* e22196.

Özkara A, Akyil D, and Konuk M (2016): Pesticides, environmental pollution, and health. In: Environmental health risk-hazardous factors to living species. *IntechOpen, London*.chapter1.

Pakravan-Charvadeh, M.R.; Mohammadi-Nasrabadi, F.; Gholamrezai, S.; Vatanparast, H.; Flora, C. and Nabavi-Pelesaraei, A. (2021): The short-term effects of COVID-19 outbreak on dietary diversity and food security status of Iranian households (A case study in Tehran province). *J. Clean. Prod.*, *281*, 124537.

Pan, Z., Cui, B., Zeng, Z., Feng, L., Liu, G., Cui, H., and Pan, H. (2015): Lambdacyhalothrin nanosuspension prepared by the melt emulsification-high pressure homogenization method. *Journal of Nanomaterials*, 1–8.

Pandey, S.; Giri, K.; Kumar, R.; Mishra, G.;and Raja Rishi, R. (2018): Nanopesticides: Opportunities in Crop Protection and Associated Environmental Risks. *Proc. Natl. Acad. Sci. India Sect. B Biol. Sci.*, *88*, 1287–1308.

Patil, C. D., Patil, S. V., Borase, H. P., Salunke, B. K., and Salunkhe, R. B. (2012): Larvicidal activity of silver nanoparticles synthesized using Plumeria rubra plant latex against Aedes aegypti and Anopheles stephensi. *Parasitology Research*, *110*(5), 1815–1822.

Pawar, N. N., Badgujar, P. C., Sharma, L. P., Telang, A. G., and Singh, K. P. (2017): Oxidative impairment and histopathological alterations in kidney and brain of mice following subacute lambda-cyhalothrin exposure. *Toxicology and Industrial Health*, *33*(3), 277-286.

Petersen EJ, Diamond SA, and Kennedy AJ (2015): Adapting OECD aquatic toxicity tests for use with manufactured nanomaterials: key issues and consensus recommendations. *Environ Sci Technol* 49:9532–9547.

Piłat, D.; and Mika, J. (2014): The role of interleukin-1 family of cytokines in nociceptive transmission. *BÓL*, *15*, 39–47

Qin, H., Zhou, X., Gu, D., Li, L., and Kan, C. (2019): Preparation and characterization of a novel waterborne lambda-cyhalothrin/alkyd nanoemulsion. *Journal of Agricultural and Food Chemistry*, *67*(38), 10587–10594.

Rabinow, B.E. (2004): Nanosuspensions in drug delivery. *Nat. Rev. Drug Discov*.; 3: 785-796.

Ramadhas, M., Palanisamy, K., Sudhagar, M., and Mani, V. M. (2014): Ameliorating effect of Phoenix dactylifera on lambda cyhalothrin induced biochemical, hematological and hepatopathological alterations in male wistar rats. *Biomedicine & Aging Pathology*, *4*(3), 273-279.

Ratelle M, Côté J, and Bouchard M (2016): Time courses and variability of pyrethroid biomarkers of exposure in a group of agricultural workers in Quebec, Canada. Int Arch Occup Environ Health 89(5):767–783.

Riaz, M., and Yousafzai, A. M. (2017): Toxic effects of insecticides malathion and cypermethrin on hematological parameters in blood of male rabbits (Oryctolagus cuniculus). *Journal of Entomology and Zoology Studies*, *5*(3), 154-158.

Rodrigues SM, Demokritou P, Dokoozlian N, Hendren CO, Karn B, Mauter MS, Sadik OA, Safarpour M, Unrine JM, Viers J, Welle P,White JC, Wiesner MR and Lowry GV. (2017): Nanotechnology for sustainable food production: promising opportunities and scientific challenges. *Environmental Science: Nano*. 4:767–781.

Ross, M. K., and Carr, R. L. (2019): Pyrethroid Insecticides: An Update. *Encyclopedia of Environmental Health*, 429-435.

Ruiz S, Pablo E, Pergola MD, Richard A, Zager MD, Nosratola D, and Vaziri MD (2013): Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease. *Kidney Int* 83:1029–1041.

Salama AK, Osman KA, Saber NA, and Soliman SA. (2005): Oxidative stress induced by different pesticides in the land snails, Helix aspersa. *Pak J Biol Sci*., 8: 92-96.

Sankar P, Telang AG, and Manimaran A. (2012): Protective effect of curcumin on cypermethrin -induced oxidative stress in Wistar rats. *Experimen Toxicol Pathol.*,64:487- 493. DOI: 10.1016/j.etp.2010.11.003.

Saratale RG, Karuppusamy I, Saratale GD, Pugazhendhi A, Kumar G, Park Y, Ghodake GS, Bhargava RN, Banu JR, and Shin HS (2018): A comprehensive review on green nanomaterials using biological systems: recent perception and their future applications. *ColloidsSurf* B 170:20–35.

Sarlak N, Taherifar A, and Salehi F (2014): Synthesis of nanopesticides by encapsulating pesticide nanoparticles using functionalized carbon nanotubes and application of new nanocomposite for plant disease treatment. *J Agric Food Chem* 62:4833–4838.

Sasaki, Y. F., Tsuda, S., Izumiyama, F., and Nishidate, E. (1997): Detection of chemically induced DNA lesions in multiple mouse organs (liver, lung, spleen, kidney, and bone marrow) using the alkaline single cell gel electrophoresis (Comet) assay. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *388*(1), 33-44.

Savary S, Willocquet L, Pethybridge SJ, Esker P, McRoberts N and Nelson A, (2019): The global burden of pathogens and pests on major food crops. *Nat Ecol Evol* 3:430–439

Shan Y, Cao L, Muhammad B, Xu B, Zhao P, Cao C, and Huang Q (2020): Iron-based porous metal-organic frameworks with crop nutritional function as carriers for controlled fungicide release. *J Colloid Interface Sci* 566:383–393.

Sharma D., Saxena P., and Singh V., (2010): Sharma R. Assessment of DNA degradation in lymphocytes of albino rat (*Rattus norvegicus*) under lambda cyhalothrin stress. World Appl. Sci. J.; 11(1): 24-28.

Singh, H., Sharma, A., Bhardwaj, S. K., Arya, S. K., Bhardwaj, N., and Khatri, M. (2021): Recent advances in the applications of nano-agrochemicals for sustainable agricultural development. *Environmental Science: Processes & Impacts*, *23*(2), 213-239.

Soderlund, D.M., (2012): Molecular mechanisms of pyrethroid insecticide neurotoxicity: recent advances. *Arch. Toxicol*. 86, 165-181.

Song, Y., Li, L., Li, C., Lu, Z., Ouyang, F., Liu, L., and Men, X. (2021): Comparative ecotoxicity of insecticides with different modes of action to Osmia excavata (Hymenoptera: Megachilidae). *Ecotoxicology and Environmental Safety*, *212*, 112015.

Sow, P., and Samadder, A. (2021): Nanotechnological Approach in Combating Pesticide Induced Fish Toxicity: Pros and Cons Controversy. In *Proceedings of the Zoological Society* (pp. 1-15). Springer India.

Sreekumaran, R., Paulmurugan, R., and Wilsanand, V., (2005): Genotoxic effects of commonly used pesticides of south India in human lymphocytes. *Pollut. Res*. 24, 7–12.

Stamou, M., Streifel, K.M., Goines, P.E., and Lein, P.J., (2013): Neuronal connectivity as a convergent target of gene x environment interactions that confer risk for Autism Spectrum Disorders. *Neurotoxicol. Teratol*. 36, 3-16.

Su, Y.; Ashworth, V.; Kim, C.; Adeleye, A.S.; Rolshausen, P.; Roper, C.; White, J.; and Jassby, D. (2019): Delivery, uptake, fate, and transport of engineered nanoparticles in plants: A critical review and data analysis. *Environ. Sci. Nano, 6*, 2311–2331

Sun, J., Wang, F., Sui, Y., She, Z., Zhai, W., Wang, C., and Deng, Y. (2012): Effect of particle size on solubility, dissolution rate, and oral bioavailability: evaluation using coenzyme Q10 as naked nanocrystals. *International journal of nanomedicine*, *7*, 5733.

Sun, Y., Liang, J., Tang, L., Li, H., Zhu, Y., Jiang, D.N., Song, B., Chen, M., Zeng, G.M., (2019): Nano-pesticides: a great challenge for biodiversity? Nano Today 28, 100757.

Suralles J, Xamena N, Creus A, Catalan J, Norppa H and Marcos R (1995): Induction of micronuclei by five pyrethroid insecticides in whole-blood and isolated human lymphocyte cultures. *Mutation Research* 341: 169–184

Sutcu R, Altuntas I, Buyukvanli B, Akturk O, Ozturk O, and Koylu H (2007): The effects of diazinon on lipid peroxidation and antioxidant enzymes in rat erythrocytes: role of vitamins E and C. *Toxicol. Indust. Health*. 23: 13-17.

Sutradhar, K.B., Khatun, S., and Luna, I.P. (2013): Increasing possibilities of nanosuspension. *Journal of nanotechnology.:* 346581

Tice, R.R., Hook, G.G., Donner, M., McRee, D.I., and Guy, A.W., (2002): Genotoxicity of radiofrequency signals I. Investigation of DNA damage and micronuclei induction in cultured human blood cells. *Bioelectromagnetics* 23, 113–126.

Titus LK, Orlu EE, and Obulor AO (2019): Evaluation of the Therapeutic Role of *Citrullus lanatus* and *Annona muricata* Fruit Extracts on Cyhalothrin-induced Toxicity. *Journal of Advances in Biology & Biotechnology*; 22(4):1-10.

Totsuka, Y., Higuchi, T., Imai, T., Nishikawa, A., Nohmi, T., Kato, T., and Wakabayashi, K. (2009): Genotoxicity of nano/microparticles in in vitro micronuclei, in vivo comet and mutation assay systems. *Particle and Fibre Toxicology*, *6*(1), 1-11.

Tyne W, Lofts S, and Spurgeon DJ (2013): A new medium for Caenorhabditis elegans toxicology and nanotoxicology studies designed to better reflect natural soil solution conditions. *Environ Toxicol Chem* 32:1711–1717.

Ullah R, Zuberi A, Naeem M, and Ullah S (2015): Toxicity to hematology and morphology of liver, brain and gills during acute exposure of Mahseer (*Tor putitora*) to cypermethrin. Int J Agric Biol 17:199–204.

US EPA (2007) Drew, W. T., Harper, K., Parmar, A., and Dow, M. (2007): Lambda-cyhalothrin. human health risk assessment for the proposed food/feed uses of the insecticide on cucurbit begetables (Group 9), tuberous and corm vegetables (Subgroup 1C), grass Forage, fodder, and hay (Group 17), barley, buckwheat, oat, rye, wild Rice, and pistachios. Petition Numbers 5F6994, 3E6593, and 6E7077. *EPA Office of Prevention, Pesticides, and Toxic Substances Risk Assessment P*.

US EPA *Control of Nanoscale Materials under the Toxic Substances Control Act* (2017): Federal Register; Rules and Regulations, Vol. 82, No. 8 https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/fact-sheet-nanoscale-materials

Valcke M, Karthikeyan S, Walker M, Gagne M, Copes R, and St-Amand A (2020): Regional variations in human chemical exposures in Canada: A case study using biomonitoring data from the Canadian Health Measures Survey for the provinces of Quebec and Ontario. *Int J Hyg Environ Health* 225:113451.

Van Der Meide, P.H.; and Schellekens, H. (1996): Cytokines and the immune response. *Biotherapy*, *8*, 243–249.

Vemo, B. N., Kenfack, A., Ngoula, F., Nantia, E. A., Kodjio, N., Guiekep, A. J. N., and Teguia, A. (2017): Effects of ethanol extract of Bersama engleriana leaves on oxidative stress and reproductive parameters in male Guinea pig (Cavia porcellus) exposed to cypermethrin. *International Journal of Biological and Chemical Sciences*, *11*(5), 2243-2253.

Vignardi, C. P., Muller, E. B., Tran, K., Couture, J. L., Means, J. C., Murray, J. L., and Lenihan, H. S. (2020): Conventional and nano-copper pesticides are equally toxic to the estuarine amphipod Leptocheirus plumulosus. *Aquatic Toxicology*, *224*, 105481.

Villarini M, Moratti M, Paquini R, Scassellati-Sforzolini G, Fatigoni C, and Marcarelli M, (1998): In vitro genotoxic effects of the insecticide deltamethrin in human peripheral blood leukocytes: DNA damage (‘comet’ assay) in relation to the induction of sister-chromatid exchanges and micronuclei. *Toxicology* 130(1998): 129–139

Walsh LP, McCormick C, Martin C, and Stocco DM (2000): Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. *Environ. Health Perspec*. 108: 769-776.

Wang X, Zheng K, Cheng W, Li J, Liang X, Shen J, Dou D, Yin M, and Yan S (2021): Field application of star polymer-delivered chitosan to amplify plant defense against potato late blight. *Chem Eng J* 417:129327.

Wang, C., Cui, B., Guo, L., Wang, A., Zhao, X., Wang, Y., Sun, C., Zeng, Z., Zhi, H., Chen, H., Liu, G., and Cui, H. (2019a): Fabrication and evaluation of lambdacyhalothrin nanosuspension by one-step melt emulsification technique. *Nanomaterials*, 9(2), 145.

Wang, C., Cui, B., Zhao, X., Wang, Y., Zeng, Z., Sun, C., Yang, D., Liu, G., and Cui, H. (2019b): Optimization and characterization of lambda-cyhalothrin solid nanodispersion by self-dispersing method. *Pest Management Science*, *75*(2), 380–389.

Wang, D., Saleh, N. B., Byro, A., Zepp, R., Sahle-Demessie, E., Luxton, T. P., and Su, C. (2022): Nano-enabled pesticides for sustainable agriculture and global food security. *Nature Nanotechnology*, *17*(4), 347-360.

Wang, L., Li, X., Zhang, G., Dong, J., and Eastoe, J. (2007): Oil-in-water nanoemulsions for pesticide formulations. *Journal of Colloid and Interface Science*, *314*(1), 230–235.

Wang, S., Zhang, C., Yang, G., and Yang, Y. (2014): Biological properties of 6-gingerol: a brief review. *Natural product communications*, *9*(7), 1934578X1400900736.

Wang, X.; Martínez, M.A.; Dai, M.; Chen, D.; Ares, I.; Romero, A.; Castellano, V.; Martínez, M.; Rodríguez, J.L.; and Martínez-Larrañaga, M.R., (2016): Permethrin-induced oxidative stress and toxicity and metabolism. A review. *Environ. Res.*, *14*, 86–104.

Wan-Jun, S., Wei-Wei, S., Sai-Yan, G., Yi-Tong, L., Yong-Song, C., and Pei, Z. (2010): Effects of nanopesticide chlorfenapyr on mice. *Toxicological and Environ Chemistry*, *92*(10), 1901-1907.

WHO. (2005): Guidelines for laboratory and field testing of mosquito larvicides. *World Health Organization*. https://apps.w h o . i n t / i r i s / h a n d l e / 1 0 6 6 5 / 6 9 1 0 1 W H O / C D S / W H O P E S /GCDPP/2005.1339 p.

WHO. (2011): Global insecticide uses for vector-borne disease control: A 10- year assessment [2000-2009]. 5th Ed. New York: World Health Organization.

Wielgomas, B.; Nahorski, W.; and Czarnowski, W. (2013): Urinary concentrations of pyrethroid metabolites in the convenience sample of an urban population of Northern Poland. *Int. J. Hyg. Environ. Health*, *216*, 295–300.

Wielgomas, B.; and Piskunowicz, M. (2013): Biomonitoring of pyrethroid exposure among rural and urban populations in northern Poland. *Chemosphere 93*, 2547–2553.

Xu C, Shan Y, Bilal M, Xu B, Cao L, and Huang Q (2020): Copper ions chelated mesoporous silica nanoparticles via dopamine chemistry for controlled pesticide release regulated by coordination bonding. *Chem Eng J* 395:125093.

Xu, Z., Tang, T., Lin, Q., Yu, J., Zhang, C., Zhao, X., and Li, L. (2022): Environmental risks and the potential benefits of nanopesticides: a review. *Environmental Chemistry Letters*, 1-12.

Yahia, D., Ali, M. F., and Abd El-Maguid, D. S. (2019): Estimation of bone marrow DNA damage induced by Lambda cyhalothrin and dimethoate insecticides using alkaline comet assay. *Journal of Advanced Veterinary Research*, *9*(1), 23-28.

Yang X, Flynn R, von der Kammer F, and Hofmann T. (2010): Quantifying the influence of humic acid adsorption on colloidal microsphere deposition onto iron-oxide-coated sand. *Environmental Pollution*; 158: 3498-3506.

Young, R. D. F., Thacker, J. R. M., and Curtis, D. J. (1996): The effects of three adjuvants on the retention of insecticide formulations by cabbage
leaves. *Journal of Environmental Science and Health, Part B, 31*(2), 165–1778.

Yousef, M. I., El-Demerdash, F. M., Kamel, K. I., and Al-Salhen, K. S. (2003): Changes in some hematological and biochemical indices of rabbits induced by isoflavones and cypermethrin. *Toxicology*, *189*(3), 223-234.

Zhang X., Xia Q, and Gu N. (2006): Preparation of all-trans retinoic acid nanosuspensions using a modified precipitation method. *Drug Dev Ind Pharm*.; 32: 857-863

Zhang, B. H., Yin, P. J., Wang, C., Zhang, W. J., Gao, M. M., Xie, D. D., and Yu, W. Y. (2015): Effects of formulation adjuvants for difenoconazole SC on wetting behavior and retention on plant leaves. *Agrochemicals*, *54*(10), 736–739.

Zhang, Y., and Goss, G. G. (2022): Nanotechnology in agriculture: Comparison of the toxicity between conventional and nano-based agrochemicals on non-target aquatic species. *Journal of Hazardous Materials*, 129559.

Zhang, Y., Zhao, M., Jin, M., Xu, C., Wang, C., and Liu, W. (2010): Immunotoxicity of pyrethroid metabolites in an in vitro model. *Environmental toxicology and chemistry*, *29*(11), 2505-2510.

Zhao L, Huang Y, and Keller A (2018): Comparative metabolic response between cucumber (*Cucumis sativus*) and corn (*Zea mays*) to a Cu(OH)2 nanopesticide. *J Agric Food Chem* 66:6628–6636.

Zhao, L., Huang, Y., Adeleye, A., and Keller, A. (2017): Metabolomics reveals Cu (OH) 2 nanopesticide-activated anti-oxidative pathways and decreased beneficial antioxidants in spinach leaves. *Environmental science & technology*, *51*(17), 10184-10194.

Zhao, M., Zhou, H., Hao, L., Chen, H., and Zhou, X. (2022): A high-efficient nano pesticide-fertilizer combination fabricated by amino acid-modified cellulose based carriers. *Pest Management Science*, *78*(2), 506-520.

Zhao, X., Striolo, A., and Cummings, P. (2005):C60 binds to and deforms nucleotides. *Biophysical journal*, *89*(6), 3856-3862.

Zhao, X., Zhu, Y., Zhang, C., Lei, J., Ma, Y., and Du, F. (2017): Positive charge pesticide nanoemulsions prepared by the phase inversion composition method with ionic liquids. *RSC Adv*, *7*(77), 48586–48596.

Zick, S. M., Djuric, Z., Ruffin, M. T., Litzinger, A. J., Normolle, D. P., Alrawi, S., and Brenner, D. E. (2008): Pharmacokinetics of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol and conjugate metabolites in healthy human subjects. *Cancer Epidemiology Biomarkers & Prevention*, *17*(8), 1930-1936.

الملخص العربي

شهد العالم في السنوات القليلة الماضية نمواً ملحوظاً في المستحدثات العلمية والتكنولوجية التي طرأت على الانسان وأصبحت في وقت وجيز جزءاً من حياتنا اليومية ، وأحد أهم هذه التطورات هو علوم وتكنولوجيا النانو.

يتم تعريف تقنية النانو على أنها تقنية متقدمة قائمة على دراسة وفهم علم النانو والعلوم الأساسية مع توفير القدرة التكنولوجية لتخليق المواد النانوية والتحكم في هيكلها الداخلي عن طريق إعادة هيكلة وترتيب الذرات والجزيئات لضمان الحصول على منتجات مميزة وفريدة من نوعها، يتم استعمال النانومتر كوحدة لقياس أطوال الأشياء الصغيرة جدًا التي لا ترى إلا تحت المجهر الإلكتروني. تستعمل هذه الوحدة للتعبير عن أبعاد الأقطار والمقاييس وجزيئات المواد المعقدة والجسيمات المجهرية مثل البكتيريا والفيروسات، النانومتر هو جزء من ألف مليون (مليار) جزء لكل متر، وبعبارة أخرى المتر الواحد يحتوي على مليار جزء من النانو متر. ربما لم تحصل أي تقنية سابقة على قدر كبير من الاهتمام والتوقع بمستقبل واعد مثل تقنية النانو، التي هي بحق تكنولوجيا القرن الحادي والعشرين، كانت المفتاح السحري للتقدم والنمو الاقتصادي . وأصبحت تقنية النانو في طليعة الحقول والمجالات الموجودة في الكيمياء والفيزياء والهندسة وعلم الأحياء والزراعه والطب و سلامه الغذاء، وأعطت أملاً كبيراً وواعداً بناء" على الأسس والمبادئ العلمية التي تتضمن تغييرات واسعة ومتقدمة في تركيب وخصائص العديد من المركبات الكيميائيه والادويه والمبيدات والاسمده الزراعيه.

وحيث ان المبيدات بصورتها التقليديه واحدة من العوامل الرئيسية التي تشارك في تلوث البيئة في عالم اليوم. هذه المواد الكيميائية هي في الاصل مصممة لتكون سامة لمكافحة الآفات وناقلات الأمراض. على الرغم من أن المبيدات قد افادت إلى حد كبير حياة الانسان من خلال تعزيز المنتجات الزراعية و السيطره علي الأمراض المعدية للنباتات والحاصلات الزراعيه وكذلك مكافحه الطفيليات الخارجيه في حيوانات المزرعه والحيوانات المنزليه الاليفه؛ ولكن في المقابل فان استخدامها علي نطاق واسع أساء الي صحه الانسان ومن جانب آخر فان التعرض المهني أو

البيئيي والاتصال طويل الأمد للمبيدات يمكن أن يضر بالحياة البشرية ويمكن أن ينتج عنه اختلال في وظائف الأجهزة الحيويه المختلفة في الجسم، بما في ذلك الجهاز العصبي والمناعي، التناسلي، الكلى، القلب والأوعية الدموية، وأمراض الجهاز التنفسي والغدد الصماء. وفي هذا الصدد، هناك أدلة متزايدة على ارتباط التعرض للمبيدات مع الإصابة بالأمراض المزمنة بما في ذلك السرطان، باركنسون، الزهايمر والتصلب المتعدد، ونتيجه لظهور جيل جديد من المبيدات النانويه المصنعه باستخدام تكنولوجيا النانو وتم ثبوت تفوقها في مكافحه الافات علي المبيدات التقليديه بالعديد من الابحاث داخل مصر وخارجها وجاري تداولها بالسوق الزراعي العالمي و من المحتمل تداولها في السوق الزراعي المصري قريبا، اصبحت الحاجه ملحه لدراسه مدي سميه هذه المبيدات النانويه وتاثيرها علي الانسان والحيوان والبيئه.

يعتبر لامبدا سي هالوثرين احد اهم المبيدات الحشريه المستخدمه علي نطاق واسع في مكافحه والوقايه من عدد كبير من الاصابات الحشريه للمحاصيل الزراعيه مثل الذبابة البيضاء، دودة ثمار العنب، ذبابة الفاكهة، نطاطات الاوراق،فراشة درانات البطاطس، ديدان القطن، نذباب البصل و سوسة النخيل وكذلك مكافحه الطفيليات الخارجيه في حيوانات المزرعه والحيوانات المنزليه الاليفه مما يجعل له تاثير مباشر علي سلامه الغذاء للانسان وصحه الحيوان والانسان، كما يعتبر احد اهم المبيدات المستخدمة فى مصر على نطاق واسع في كل من برامج مكافحة الآفات الزراعية و الصحة العامة.

ولهذا اجريت هذه الدراسه لاستعمال التقنية النانوية في الحصول على مركب نانوي من المبيد التقليدي لمبدا سي هالوثرين مختبريا ودراسة ومقارنه فعاليتهما لمقاومه الافات والاهم لتوضيح تاثير الاجهاد التاكسدي والسميه الجينيه للمبيد الناوني لمبدا سي هالوثرين ومقارنته بالمبيد الاصلي.

وتعتبرهذه الدراسه الاولي من نوعها في تقييم الاجهاد التاكسدي والسميه الجينيه لمبيد نانوي علي الفئران ولقد تم اخيار الفئران البيضاء كموديل (اوحيوان تجارب) لاجراء هذه الدراسه وذلك لانه النموذج الحيواني الاصغر والاقل تكلفه كما ان لدى كل من الإنسان والفأر 30 ألف جين وراثي

يشتركان في كثير منها بينما تتماثل لديهما 90% من الجينات المرتبطة بالأمراض؛ مما يجعل دراسه السميه الجينيه علي الفئران لها دلالات كبيره علي تاثيرها علي الانسان .

تخليق المبيد النانوي الجديد

تم تحضير المبيد النانوي لامبدا سي هالوثرين في صورة مستحلب نانوي عن طريق تقنيه التبخيرللمذيبات في وجود التوين 80 كماده خافضه للتوتر السطحي. يتمتع المبيد النانوي المستحلب المحضرمعمليا الجديد بخواص مختلفه عن المبيد التقليدي لامبدا سي هالوثرين منها سهوله الذوبان في الماء وفاعليه افضل في مكافحه الافات مما يبشر بمستقبل واعد لانتاج وانتشار المبيدات النانويه علي نطاق اوسع. تم تشخيص خصائص المبيد النانوي لامبدا سي هالوثرين المحضر باستعمال تقنيات مطيافيـــة الاشعة تحت الحمراء (FT–IR) وحيود الاشعة السينية (X–Ray diffraction)والمجهر الالكتروني الماسح (TEM) وبلغ قياسه 70.3 نانوميتر.

فاعلية المبيد النانوي الجديد (تجربه السمية ليرقات البعوض)

تم دراسة سمية وكفاءة المبيد النانوي الجديد لامبدا سي هالوثرين كمبيد حشري ومقارنته بفاعلية المبيد التقليدي لامبدا سي هالوثرين؛ حيث تم اختيار يرقات البعوض *Culex pipiens* من السلاله الحقليه وتعريضها لكل من المبيد النانوي الجديد لامبدا سي هالوثرين و المبيد التقليدي لامبدا سي هالوثرين وتم رصد متوسط الوفيات في اليرقات عند 24 ؛48 ؛72 ساعه ثم تحليل النتائج احصائيا وخلصت التجربه الي ان المبيد النانوي الجديد لامبدا سي هالوثرين اثبت فاعلية اقوي في تجربته كمبيد ليرقات البعوض *c.pipiens*

تصميم تجربه السمية

تم اختيار عدد 72 فارا من الذكور من سلاله *Sprague–Dawley* ؛ تتروح اوزانها بين 150–200جراما وعمرها 8–10 أسابيع ثم توزيعها عشوائيا إلى ثلاث مجموعات علي النحو التالي:

مجموعة المبيد التقليدي لامبدا سي هالوثرين: تلقي 18 فأر (4 ملجرام / 100جرام من وزن الجسم) من المبيد التقليدي لامبدا سي هالوثرين ، الذائب في زيت الذرة، مرتين أسبوعيا، عن طريق الفم بواسطة أنبوب المعدة لمدة 12 أسبوعا.

مجموعة المبيد النانوي لامبدا سي هالوثرين: تلقي 18 فأر (0.01 ملجرام / 100جرام من وزن الجسم) من المبيد النانوي لامبدا سي هالوثرين المذاب في الماء المقطر ، مرتين في الأسبوع، عن طريق الفم بواسطه أنبوب المعدة لمدة 12 أسبوعا.

المجموعة الضابطه[1و2]: تم الاحتفاظ ب 36 فأر كمجموعه ضابطه تلقت 18 فأر منها الماء المقطر مرتين اسبوعيا عن طريق الفم لمدة 12 أسبوعا. بالاضافه الي 18 فأر تلقت زيت الذرة مرتين اسبوعيا عن طريق الفم لمدة 12 أسبوعا

تم تسجيل اوزان الفئران أسبوعيا كما تم اختيار 3 فئران عشوائيا من كل مجموعه للذبح كل 15 يوما. تم تجميع عينات من الدم بدون استخدام مانع التخثر لتستخدم في اجراء فحص وعد صوره دم كامله واجراء اختبار الكوميت (التفريد الكهربي للخلايا المنفردة)؛ كما تم تجميع عينات دم دون مضادات التخثر لفصلها بعد الطرد المركزي والحصول على عينات السيرم . تم استخدام عينات السيروم لتحديد مستويات الانزيمات المضاده للاكسده SOD ومؤشر اكسده الدهون MDA ؛ ومؤشر النخر للاورام (TNF-α) .

تم استئصال الكبد والكلي و المخ والخصيتين ثم وزنت و سجلت إلى أقرب مللي غرام واستخدمت أجزاء من الكبد والكلي و المخ والخصيتين مباشرة بعد الذبح للفحص النسيجي. استخدمت أقسام من الكبد بعد ان تم تجهيزها لتكون سائل متجانس من نسيج الكبد يستخدم مع عينات من الدم المجمع بدون مضادات للتخثر لتقدير السميه الجينيه ومدي تضرر الماده الوراثيه DNAعن طريق اجراء اختبار الكوميت (التفريد الكهربي للخلايا المنفردة) او مايطلق عليه اختبار المذنب.

كشفت الدراسه أن تاثير كلا من المبيد النانوي الجديد لامبدا سي هالوثرين و المبيد التقليدي لامبدا سي هالوثرين علي الفئران ادي الي زياده في الوزن النسبي للكبد والكلي خلال فترة التجربة. قد تكون ا هذه الزياده بسبب زياده الدورة الدمويه لمواجهه زياده معدل التخلص من المواد الكيميائيه الناتجة عن تكسير المبيد بواسطه انزيمات الكبد وخروجها مع البول من الكليه، وذلك نتيجه لحاجة الجسم لتلبية متطلبات الطاقة أثناء إزالة السموم.

اوضحت هذه الدراسه ان التعرض لكلا من المبيد النانوي الجديد لامبدا سي هالوثرين و المبيد التقليدي لامبدا سي هالوثرين ادي الي نقص في بعض عناصر صوره الدم مثل قياس توزيع كريات الدم الحمراء RDW؛ بينما كشفت الدراسه عن زياده عددالصفائح الدمويه؛ و كشفت الدراسه ايضا عن زياده عدد كرات الدم البيضاء في العد الكليWBCs وعد كل عنصر منها علي حدي؛

الكريات الأحادية النواة monocytes، العَدِلات neutrophils، واللمفاويات lymphocytes؛ والتي تعد زيادتها دليلا علي رده الفعل المناعيه الناتجه من تعرض الجسم لهجوم السموم.

تعرضت الفئران التي تلقت كلا من المبيد النانوي الجديد لامبدا سي هالوثرين و المبيد التقليدي لامبدا سي هالوثرين لحاله واضحه من الاجهاد التاكسدي والتي ظهرت في صورة نقص في مستويات الانزيمات المضاده للاكسده SOD و زياده في مؤشر اكسده الدهون MDA؛ وزياده في مؤشر النخر للاورام (TNF-α)؛ والذي يتم افرازه من الخلايا المناعيه كرد فعل عند التعرض للاتهاب الشديد والتلوث والسموم والامراض المناعيه والاورام و الذي ينظم رد الفعل المناعي وموت الخلايا. وقد أيد الفحص النسيجي لخلايا الكبد والكلي والمخ والخصيه الي ظهور تلف و انزفه خارج الاورده واحتقان وتورم في الانسجه بالاضافه الي فقدان التركيب المنتظم للخلايا المكونه للكبد بالاضافة للتورم والارتشاح بالانسجه المكونه لخلايا الكلي و تخلل الخلايا المناعيه للانسجه في الكلي كما حدث تلف وتاكل في الخلايا العصبيه و انزفه في انسجه المخ؛ كما اظهر الفحص الميكرسكوبي لانسجه الخصيه وجود تلف واحتقان وتورم في هذه الانسجه بالاضافه الي فقدان التركيب المنتظم للخلايا المكونه للحيوانات المنويه داخل نسيج الخصيه مما ترتب عليه وجود خلل في وظائف كل هذه الاعضاء.

يعد اختبار الكوميت –التفريد الكهربي للخلايا المنفردة– طريقة بسيطة لقياس الكسور في خيوط الحامض النووي DNA في خلايا الكائنات حقيقية الأنوية. و لهذا الاختبار تطبيقات متعددذة فى الكشف عن مختلف المؤثرات الكيميائية و الفيزيائية من حيث قدرتها على إحداث السمية الوراثية، فضلا عن استخدامه فى رصد التلوث البيئي بالمواد ذات السمية الوراثية. و احد اهم اهداف الدراسة الحالية هو تقييم التأثيرات السمية الوراثية للمبيد النانوي الجديد لامبدا سي هالوثرين ومقارنته بالتأثيرات السمية الوراثية للمبيد التقليدي لامبدا سي هالوثرين .

وعلي عكس المتوقع اظهرت هذه الدراسه تاثير المبيد النانوي الجديد لامبدا سي هالوثرين علي احداث الكسور في خيوط الحامض النووي DNA و قدرته على إحداث السمية الوراثية والتي ظهرت بعد الاسبوع الثاني عشر من التعرض للمبيد النانوي لامبدا سي هالوثرين.

خلصت الدراسه الي انه رغم الفاعليه الاقوي للمبيد النانوي الجديد لامبدا سي هالوثرين كمضاد للافات مما يفتح الباب نحو عصر جديد من المبيدات النانويه التي تتميز بتاثير اقوي علي الافات باستخدام كميات اقل من المبيد مما يبلور ويبرز الاهميه الاقتصاديه للمبيد النانوي الجديد لامبدا سي هالوثرين الا ان التعرض للمبيد النانوي الجديد لامبدا سي هالوثرين لا يقل خطوره عن التعرض للمبيد التقليدي لامبدا سي هالوثرين من حيث الاجهاد التاكسدي بل ان تاثير المبيد النانوي الجديد لامبدا سي هالوثرين علي احداث الكسور في خيوط الحامض النووي DNA و قدرته على إحداث السمية الوراثية ظهرت بعد الاسبوع الثاني عشر من التعرض.

ولذلك توصي الدراسه بضروره استمرار البحث والتقصي عن تاثير المبيدات النانويه بمختلف تركيباتها علي الخليه الحيه وبالادق الكائنات الحيه التي لا تمثل الافات المطلوب مكافحتها سواء كانت كائنات مجهريه دقيقه او لا فقاريات او اسماك او حيوانات، و تشير نتائج هذا العمل إلى أنه رغم الفاعليه الاقوي ضد الافات لهذا الجيل الجديد من المبيدات النانويه الا ان تاثيره لا يقل خطورة عن المبيد التقليدي بل اكثر سميه علي الماده الوراثيه مما يدق ناقوس الخطر حول التوسع في استخدام هذا النوع من المبيدات و التريث قبل اصدار التصاريح الازمه لتداولها؛ ومن الجدير بالذكر في هذا الصدد ان منظمه حمايه البيئه الامريكة EPA لم تصدر تقريرها بالسماح بتداول المبيدات النانويه داخل البلاد حتي الان وذلك لنقص الابحاث المنشوره حول تاثير هذه المبيدات النانويه علي البيئه وصحه الحيوان والانسان مما يعد حجر عثره في طريق تقييم المخاطر الناتجه عن استخدامها وانتشاراها واحلالها محل المبيدات التقليديه. ولذلك توصي الدراسه بضروره التقليل من استخدام المبيدات بشكل عام وضروره متابعه النسبه المسموح بها لوجوده علي المحاصيل والفواكه والخضروات ؛ كذلك الاستخدام الامن لها مع الالتزام بفتره الانسحاب الموصي بها من قبل هيئه الصحه العالميه وذللك لضمان تقليل الاضرار الناتجه عنها علي صحه الانسان والحيوان والبيئه.

جامعه اسيوط

كليه الطب البيطري

الاجهاد التأكسدي والسمية الجينية للمبيد النانوي لمدا- سيهالوثرين علي الفئران البيضاء

رسالة مقدمة من

ط.ب علياء أحمد بخيت

ماجيستير العلوم الطبية البيطرية جامعة أسيوط 2017

للحصول علي

درجة الدكتوراه فى العلوم الطبية البيطرية (الطب الشرعي والسموم)

تحت اشراف

الأستاذ الدكتور/ محمود عبدالناصر علي

أستاذ الطب الشرعي والسموم المتفرغ

كليه الطب البيطري

جامعة أسيوط

الأستاذ الدكتورة/ إيمان عزالدوله جابر الشرقاوي

أستاذ الطب الشرعي والسموم

كليه الطب البيطري

جامعة أسيوط

الأستاذ الدكتورة/ ضحي يحيي أحمد أحمد

أستاذ ورئيس قسم الطب الشرعي والسموم

كليه الطب البيطري

جامعة أسيوط

الأستاذ الدكتورة/ منال محمد سيد

رئيس بحوث بمعهد بحوث الصحة الحيوانية أسيوط

2022