إقرار

أنا الموقع أدناه مقدم الرسالة التى تحمل العنوان :

Toxic Effects of Cypermethrin on Liver and Kidney of Male Domestic Rabbits

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Toxic Effects of Cypermethrin on Liver and Kidney of Male Domestic Rabbits

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بناءً على موافقة شئون البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحث/ **علي خالد محمد علوان** لنيل درجة الماجستير في **كلية العلوم قسم العلوم الحياتية – علم الحيوان** وموضوعها:

أثر سمية مبيد Cypermethrin على الكبد والكلية في ذكور الأرانب المحلية

Toxic Effects of Cypermethrin on Liver and Kidney of Male Domestic Rabbits

وبعد المناقشة التي تمت اليوم الاثنين 29 رجب 1436هـ، الموافق 2015/05/18م الساعة الثانية عشرة ظهراً، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

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وبعد المداولة أوصت اللجنة بمنح الباحث درجة الماجستير في **كلية العلوم/ قسم العلوم الحياتية – علم الحيوان.**

واللجنة إذ تمنحه هذه الدرجة فإنها توصيه بتقوى الله ولزوم طاعته وأن يسخر علمه في خدمة دينه ووطنه.

والله ولي التوفيق،،،

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Dedication

I would like first and most to thank almighty God for the blessings and power that made my project a reality, I would like to extend my deepest gratitude to: My parents for their unending love and support, To my wife for her support and patience during the months it has taken me to complete the project, To my brothers and sisters for their valuable encouragement. To blindness Dr. Ali Abdullah Shaheen, who has great credit supporting Lee. To my university the Islamic university of Gaza which is continuously improving the dedicate research. Each and every one of my colleagues , friends and community members who participated in bringing this project to the happy end .

Declaration

I certify that this submission is my own research and that, to the best of my knowledge and belief, it contains material neither previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university of other institute, except where due a acknowledgment has been made in the text.

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Toxic effects of Cypermethrin on liver and kidney of male domestic rabbits

Abstract

Objective: The present study is aimed to investigate the toxic effects of cypermethrin on liver and kidney of male domestic rabbits.

Materials and Methods: The oral LD_{50} of cypermethrin in male domestic rabbit was calculated from logarithmic scale and found to be 665 mg/kg^{-1} body weight. A daily dose of 1/10 LD_{50} cypermethrin (66.5 mg/kg^{-1} body weight) was given orally to 36 animals under experiment for six weeks. Forty eight control animals were given distilled water. Blood samples were collected weekly and analyzed.

Results: The overall mortality rate was 16.7% in cypermethrin-treated rabbits compared to 0.0% in controls. Clinical signs included diarrhea, disorientation, drowsiness and mild tremor. The final body weight was significantly decreased in cypermethrin-intoxicated rabbits. Serum glucose was significantly increased in response to cypermethrin administration recording a maximum percentage difference of 28.1% in the 6^{th} week of the experiment. The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (γ-GT) were significantly higher in cypermethrin-treated rabbits compared to controls, registering maximum percentage differences of 30.1, 38.3, 23.1 and 27.5% during the 4^{th},1^{st}, 2^{nd} and 6^{th} weeks of the experiment, respectively. In contrast, serum cholinesterase (ChE) was markedly decreased recording a maximum percentage difference of 46.5% during the 5^{th} week of the experiment. Serum bilirubin was gradually increased to record a maximum percentage difference of 21.4% in the 5^{th} week. Serum urea and creatinine concentrations were significantly elevated in response to cypermethrin intake displaying maximum percentage differences of 48.8% and 31.3% during the 6^{th} and 5^{th} weeks of the experiment, respectively. Serum total protein, albumin and globulin were significantly decreased upon cypermethrin intoxication exhibiting percentage differences of 31.3, 31.8 and 29.4% at the last

week of the experiment. Serum calcium was significantly decreased in cypermethrin-treated rabbits with a maximum percentage difference of 28.6% at the 6th week whereas phosphorus significantly increased with a maximum percentage difference of 24.3% at the 6th week of the experiment.

Conclusions: Oral daily administration of 1/10 LD_{50} cypermethrin caused significant decrease in body weight, serum cholinesterase, total protein, albumin, globulin and calcium whereas serum glucose, ALT, AST, ALP, γ-GT, bilirubin, urea, creatinine and phosphorous were significantly increased.

Key words: Cypermethrin, toxicity, liver, kidney, male rabbit.

أثر سمية السيبرميثرين علي الكبد و الكلية في ذكور الأرانب المحلية

ملخص الرسالة

الهدف: تهدف هذه الدراسة الحالية لمعرفة مدى سمية السيبرميثرين على كبد وكلي ذكور الأرانب.

الطرق والادوات: ولقد بينت الدراسة بعد التحليل الإحصائي قيمة الجرعة النصف مميتة LD_{50} للسيبرميثرين في ذكور الأرانب التى أعطيت بواسطة الفم هى 665 ملجرام/كجم من وزن الجسم.أعطيت10/1 من الجرعة النصف مميتة من السيبرميثرين (قيمتها 66.5 ملجرام/كجم من وزن الجسم) لعدد 36 أرنب من حيوانات التجربة لمدة ست أسابيع. 48 من حيوانات المجموعة الضابطة تناولت ماء مقطر. عينات الدم تم جمعها أسبوعيا وتم تحليلها.

النتائج: كان معدل الوفيات 16.7٪ في الأرانب المعالجة بالسيبرميثرين بينما المجموعة الضابطة لا يوجد وفيات. ظهرت علامات سريرية كالإسهال و الارتباك و النعاس و الرعاش الخفيف. وقد انخفض وزن الجسم بشكل كبير في ذكور الأرانب المعالجة بالسيبرميثرين. وقد لوحظ زيادة الجلوكوز في الدم بشكل ملحوظ استجابة لمعاملة بالسيبرميثرين حيث سجل أعلي نسبة 28.1 ٪ في الأسبوع السادس من التجربة. كذلك ظهر نشاط ملحوظ لأنزيمات الكبد ناقل الأمين (ALT) و أنزيم الأسبارتيت (AST) وأنزيم الفوسفاتيز(ALP) وناقل جاما جلوتاميت (γ-GT) في المجموعات المعالجة بالمبيد مسجلة أعلي نسب بالترتيب حسب جداولها علي التوالي 30.1 ٪، 38.3 ٪، 23.1 ٪، 27.5 ٪ خلال الأسبوع الرابع و الأول و الثالث و السادس من التجربة علي التوالي. في المقابل شوهد انخفاض مستوى الكولين أستريز (ChE) في البلازما بشكل ملحوظ مسجلا أعلي نسبة 46.5 ٪ خلال الأسبوع الخامس من التجربة. وبينما ازداد البيليروبن في البلازما تدريجيا لتسجيل أقصى نسبة 21.4 ٪ في الأسبوع الخامس من التجربة. وكذلك هناك زيادة ملحوظة في تركيز اليوريا و الكرياتينين في مصل الدم للأرانب المعاملة بالسيبرميثرين حيث سجلت أعلي نسبة 48.8 ٪ و 31.3 ٪ خلال الأسبوع السادس و الخامس من هذه التجربة علي التوالي. وقد انخفض البروتين الكلي و الألبومين و الجلوبيولين في الدم انخفاضا كبيرا مع زيادة سمية السيبرميثرين بالنسب التالية علي الترتيب 31.3 ٪، 31.8 ٪ و 29.4 ٪ في الأسبوع الأخير من التجربة. بينما انخفضت نسبة الكالسيوم في الدم بشكل كبير في الأرانب المعالجة بالسيبرميثرين مسجلة أعلي نسبة 28,6 ٪ في الأسبوع السادس في حين أن الفوسفور زاد بشكل ملحوظ مشكلا أقل نسبة 24.3 ٪ في الأسبوع السادس من التجربة.

الإستنتاجات: إن تناول مبيد السيبرميثرين عن طريق الفم يوميا بجرعة LD_{50}10/1 سببت انخفاض ملحوظ في وزن الجسم و الكولين أستريز (ChE) و البروتين الكلي و الألبومين و الجلوبيولين و الكالسيوم في حين زاد معدل سكر الجلوكوز و ناقل الأمين (ALT) و أنزيم الأسبارتيت (AST) وأنزيم الفوسفاتيز(ALP) وناقل جاما جلوتاميت (γ-GT) والبيليروبين و اليوريا و الكرياتينين و الفوسفور في مصل الدم.

الكلمات المفتاحية: السيبرميثرين ، سمية ،الكبد ،الكلية ،ذكر الأرانب

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Chapter 1

Introduction

1.1 Overview

Synthetic pesticides have become an integral component of various pest eradication programmes for modern farming, various vector borne diseases and household pests. Pyrethroid pesticides represent a major class of very effective multipurpose chemicals, accounting for about 30% of global insecticidal market. Newly designed analogues have been synthesized and launched periodically, boosted up with enhanced potential against pre-existing pests, and those that have become resistant **(Abdou et al., 2009; Assayed et al., 2010; El-Magd et al., 2011 and Adjrah et al., 2013).** Pyrethroid pesticides are broadly divided into type I and II, depending upon absence and presence of an alpha-cyano group, respectively, and their produced behavioural changes **(Spencer et al., 2005; Wolansky et al., 2006 and Saka et al., 2011).**

Cypermethrin is a type II pyrethroid pesticide, used widely in developing and undeveloped nations for almost every aspect of pest control **(Singh and Saxena, 2001; Atessahin et al., 2005; Eraslan et al., 2008 and Bhushan et al., 2013).** The exposure routes of cypermethrin are ingestion, inhalation and dermal absorption **(Noaishi et al., 2013 and Coˆte´ et al., 2014).** Cypermethrin has a maximum chance of accumulating in various food chains and thus imparting related toxicity **(Muthuviveganandavel et al., 2011 and Sangha et al. 2013).**

The toxicity of cypermethrin is predicted form LD_{50} (a dose that expected to cause death in 50% of animals). Oral acute LD_{50} of cypermethrin for rats was estimatied to be 250-4150 and for mice 138 mg kg^{-1} body weight. However, to our best knowledge no previous study assessed the oral LD_{50} in male domestic rabbit. The dermal LD_{50} for rats >4920 and for rabbits >2460

mg kg^{-1} body weight. Inhalation LC_{50} (4 hours) of Cypermethrin for rats 2.5 mg/l (**Tomlin, 2011 and MacBen, 2013).**

Cypermethrin crosses the blood-brain barrier and induces neurotoxicity and motor deficits. The primary symptoms of intoxication with cypermethrin and other synthetic pyrethroids affect mainly the nervous and muscular systems. The most frequent symptoms caused by cypermethrin poisoning include ataxia, hyperreactivity, tremors, paresthesia, exhaustion, hypersalivation, vomiting, diarrhea, urinary incontinence **(Environmental Protection Agency, EPA, 2006 and Grewal et al., 2010).**

Several studies reported the toxic effect of cypermethrin on the functions of several mammalian organs including liver and kidney. cypermethrin was reported to alter the level of the marker parameters related to the liver and kidneys in experimental animals. Significant increase in the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (γ-GT) as well as decrease in the levels of cholinesterase, bilirubin, total protein and albumin were reported in cypermethrin-intoxicated animals **(Gomaa et al., 2011; Adjrah et al., 2013 and Bhushan et al., 2013).** In addition, increase in creatinine and urea concentrations in cypermethrin-treated animals indicated renal damage **(Saxena and Saxena, 2010 and Ahmad et al., 2011).**

Pesticides are being used in large amounts in the Gaza Strip where the protective measures are poorly followed **(Yassin et al., 2002).** More than 435.9 metric tons of pesticides are used yearly in the Gaza strip. The pyrethroids represent 139.2 metric tons of these pesticides, 125.4 metric tons of these insecticides are cypermethrin **(Personal Communication with Ministry of Agriculture, Palestinian National Authority, 2014).** These toxic compounds constitute a real threat on humans. The present work is intended to investigate cypermethrin toxicity in male domestic rabbit. The findings can then be extrapolated to human beings to assess the potential hazards in the human populations due to cypermethrin exposure.

1.2 General objective

The general objective of the present study is to assess toxic effects of cypermethrin on liver and kidney of male domestic rabbit.

1.3 Specific objective

1. To determine the oral LD_{50} of cypermethrin in male domestic rabbit.
2. To examine the effect of 1/10 LD_{50} cypermethrin on general health and body weight of male domestic rabbit.
3. To study the effect of 1/10 LD_{50} cypermethrin on serum glucose.
4. To study the effect of 1/10 LD_{50} cypermethrin on liver function through measurement of ALT, AST, ALP, γ-GT, bilirubin and ChE.
5. To test the effect of 1/10 LD_{50} cypermethrin on kidney function through determination of serum urea and creatinine.
6. To investigate the effect of 1/10 LD_{50} cypermethrin on serum total protein, albumin and globulin.
7. To study the effect of 1/10 LD_{50} cypermethrin on electrolytes, calcium and phosphorus.

1.4 Significance

1. Cypermethrin is being extensively used in agriculture in Gaza Strip with lack of protective measures **(Personal Communication with Ministry of Agriculture, Palestinian National Authority, 2014).**
2. Studies on cypermethrin toxicity on rabbits are limited in the literature.
3. The results of the present study may be useful to aware people particularly farmers on the extent of cypermethrin toxicity.

Chapter 2

Literature Review

2.1 Definition of pesticide

Pesticide is a chemical or biological substance that is intended to prevent or repel or destroy the pests that may damage or disturb the growth or health of living organisms which may be plants or animals. These pests include insects, rodents, fungi, weeds, nematodes, algae, etc. These pesticides are classified on their origin , structure , pests they control or their mode/ site of action **(World Health Organization, WHO, 2009).**

Environmental Protection Agency, EPA, (2012) broadly defined a pesticide as any agent used to kill or control undesired insects, weeds, rodents, fungi, bacteria, or other organisms. Pesticides are classified according to their function: insecticides control insects; rodenticides control rodents; herbicides control weeds; and fungicides control fungi, mold and mildew.

Pesticides can also be considered as either biodegradable pesticides, which will be broken down by microbes and other living organism into harmless compounds, or persistent pesticides, which may take months or years before they are broken down **(EPA, 2013).**

2.2 Types of pesticides

There are two types of pesticides: chemical pesticides and biopesticides **(WHO, 2009):**

2.2.1 Chemical pesticides

Chemical pesticides are further divided into four types based on their origin:

1. Organophosphates: These are the chemical substances which are produced due to the reaction between phosphoric acid and alcohols. They

affect the nervous system by inhibiting the action of the enzyme acetylcholinesterase (AChE). They cause irreversible blockage leading to accumulation of the enzyme which results in overstimulation of muscles. These mainly include insecticides, nerve gases, herbicides, etc.

2. Carbamates: These are esters of carbamic acids. Their mode of action is inhibiting acetylcholinesterase similar to that of the organophosphates but the bond formed for inhibition is less durable and thus reversible. These also include mainly insecticides.

3. Organochlorines: They are derived from chlorinated hydrocarbons. These are endocrine disrupting agents that affect the hormonal system of the body, act as duplicates of the normal hormonal and thus causing adverse health problems. They remain in the environment for a long time by breaking down slowly and accumulating in the fat tissues of animals. A well-known example is dichloro diphenyl trichloroethane (DDT).

4. Pyrethroids: These are potent neuropoisons, endocrine disruptors that can cause paralysis. Pyrethroids are synthetic version of pyrethrin a natural insecticide. They have similar chemical structure and similar mode of action as of pyrethrin which is obtained from chrysanthemum. These are derivatives of ketoalcoholic esters of chrysanthemic and pyrethroic acids and are more stable in sunlight than pyrethrins. These are most popular insecticides as they can easily pass through the exoskeleton of the insect. Examples are cypermethrin, deltamethrin and cyfluthrin.

2.2.2 Biopesticides

They are naturally occurring materials or derived naturally from living organisms or their metabolites, like bacteria, fungi, plants, etc. Biopesticides are classified into three major groups:

1. Microbial pesticides: They have microorganisms acting as pest controllers like bacteria, fungi or viruses. Each of their has specific targets. Widely used are strains of Bacillus Thuringenesis and its subspecies. The mode of action generally is producing a protein that binds to the larval gut receptor which starves the larvae.

2. Biochemical pesticides: They are naturally occurring, nontoxic pest controllers. These include pheromones, natural plant and insect regulators, enzymes, bio repellents or attractants.

3. Plant incorporated protectants: These substances are produced naturally by plants, but the gene necessary for production of pesticide is introduced into the plant through genetic engineering. The substance produced by the plant and the genetic material introduced are together defined as plant incorporated protectants. Biopesticides are used instead of chemical pesticides as their negative effects on the environment health are low compared to chemical pesticides **(WHO, 2009 and EPA, 2013).**

2.3 Routes of entry of pesticides into body

Pesticides can enter the body through inhalation via the respiratory system (volatile or aerosol pesticides), through ingestion (oral) and through dermal exposure by absorption through intact or broken skin **(Christos and Ilias, 2011 and Patil et al., 2012).**

2.4 Cypermethrin

2.4.1 Definition

Cypermethrin is the ISO approved common name for (RS)-α-cyano-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclo-propanecarboxylate, it is a synthetic pyrethroid insecticide containing three chiral centres, giving a racemic mixture of eight isomers comprising four diasterioisomeric pairs. The cypermethrin are alpha-cyano- or type II pyrethroids **(http://www.who.int/whopes/quality/en/Alphacypermethrin eval _april_2006 and Suzan, 2012).**

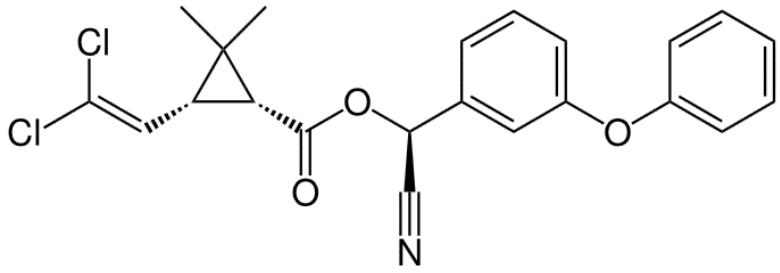

Figure 2.1. Chemical structure of cypermethrin **(Lin et al., 2011)**

2.4.2 Physical and chemical properties of cypermethrin

Cypermethrin occurs as colorless crystals with a mild mercaptan odour .The principal chemical properties of Cypermethrin are compiled in (Table 2.1) **(Tomlin, 2006).**

Table 2.1 Physical and chemical properties of cypermethrin **(Tomlin, 2011).**

Property	Value
Molecular weight	416.3
Molecular formula	$C_{22}H_{19}Cl_2NO_3$
Form	Odorless crystals (pure); yellow-brown viscous semi-solid at ambient temperature.
Melting point	60-80 ^{o}C
Vapor pressure	2.3×10^{-4} mPa (20 ^{o}C)
Specific gravity/density	1.23 (20 ^{o}C)
Solubility: In water In acetone and chloroform In ethanol	 0.004 mg/l (pH7) >450 g/l, 20^{o}C 337 g/l, 20^{o}C

2.4.3 Mechanism of action of cypermethrin

Cypermethrin causes toxicity in many parts of brain, depending upon the doses, time and routes of exposures. Cypermethrin-mediated toxicity appears in experimental animals at all study levels, beginning from the biochemical to anatomical and molecular to phenotypic. Cypermethrin induces neurotoxicity and motor deficits through its action on:

1. Ion channels: Cypermethrin modulates various voltage-gated ion channels including sodium, calcium, chloride and potassium channels. Cypermethrin binds to specific sites of sodium channel, a major site of its action **(Tan et al., 2005).** This binding makes sodium channel open at more hyperpolarized potentials and remain open for longer, allowing more sodium ions to cross and depolarize the neuronal membrane leading to hyper-excitability to the point at which generation of the action potentials is not possible **(Singh et al., 2012).** Unlike sodium channel, cypermethrin alters the kinetics and calcium influx by the inhibition of voltage-gated calcium channel **(Martin et al., 2000),** leading to reduced cellular calcium level and impaired release of neurotransmitter **(Wu et al., 2005).** In addition, cypermethrin suppresses the open state of voltage-gated chloride channel and inhibits GABA dependent uptake of chloride ions (**Ray et al., 1997),** leading to hyper-excitability and neurotoxicity symptoms **(Ullah et al., 2006).** Finally, cypermethrin alters the activity of voltage-gated potassium channel and potassium ion transport across synaptosomes, which regulates the neuronal excitability and ultimately leads to neurotoxicity **(Murakoshi and Trimmer, 1999 and Singh et al., 2012)**.

2. Glutamate receptors, acetylcholine receptors and adenosine triphosphatases: Cypermethrin alters the activity of glutamate and acetylcholine receptors and adenosine triphosphatases. In addition, cypermethrin-induced neurotoxicity is mediated by inhibition of acetylcholinesterase activity **(Saxena and Yadav, 2011 and Sharma et al., 2014).**

3. Gamma-aminobutyric acid (GABA) and dopamine: Several reports have highlighted that cypermethrin antagonizes the inhibitory neurotransmitter GABA, leading to hyper-excitability **(Manna et al., 2005).** In addition, cypermethrin induces oxidative stress leading to dopaminergic neurotoxicity **(Nasuti et al., 2007).**

4. Oxidative stress and DNA damage: The oxidative stress is implicated in the cypermethrin-mediated neurotoxicity. The major contributors of oxidative stress are excessive production of reactive oxygen species and reactive

nitrogen species in the cells or tissues exposed to cypermethrin or reduced level of components of the antioxidant machinery **(Tiwari et al., 2010 and Sharma et al., 2014).** In addition, cypermethrin causes DNA damage and reduces mitotic and nuclear divisions **(Kocaman and Topaktas, 2009 and Hussien et al., 2013).**

2.4.4 Toxicity symptoms of cypermethrin poisoning

Humans: Symptoms of cypermethrin poisoning in humans include facial burning and tingling (Paraesthesia), dizziness, headaches, nausea, anorexia, fatigue and loss of bladder control. With greater exposure, symptoms include muscle twitching, drowsiness, coma and seizures **(Chakravarthi et al., 2007 and Gheshlaghi et al., 2011).**

Laboratory animals: Symptoms of cypermethrin toxicity in laboratory animals include pawing, burrowing, salivation, tremors, writhing and seizures **(Ullah et al., 2006 and Grewal et al., 2010).**

2.4.5 Cypermethrin degradation

Cypermethrin degradation involves an initial hydrolysis of the ester linkage of the cypermethrin molecule to yield 3-phenoxybenzaldehyde as the first hydrolysis product (Figure 2.2) . This metabolite then undergoes the reduction to the phenoxybenzylalcohol and oxidation to form the 3-phenoxybenzoic acid. These metabolites occurred as conjugates with sugars and also in their free form. The parent compound could also be converted into its amide analogue by hydrolysis of the cyano group (but without any further transformation) or to its cis- and trans- hydroxylated derivatives. These components did not appear in their conjugated form.

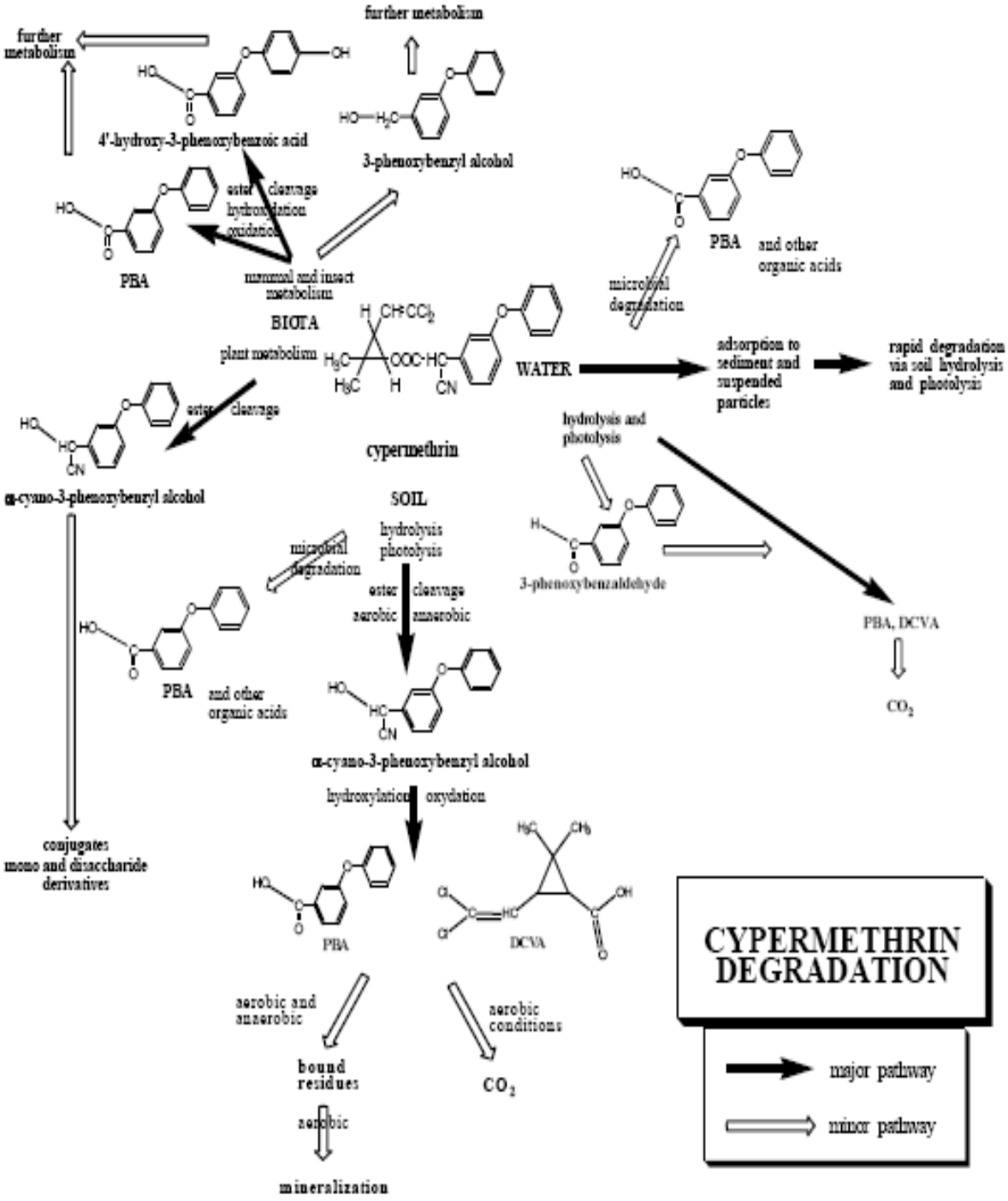

Figure 2.2. Degradation of cypermethrin (Jones, 1992)

2.4.6 Uses of cypermethrin

Cypermethrin is a synthetic pyrethroid insecticide used to kill insects on olive trees, cotton and vegetables, and to kill cockroaches, fleas, and termites in houses and other buildings. Likewise, some veterinary products are based on cypermethrin, which are popularly used for dipping or showering of food animals **(Shah et al., 2007).**

2.4.7 Effects of cypermethrin on liver and kidney

Cox (1996) reported that cypermethrin caused reduction in weight gain and pathological changes in liver of different experimental animals. In this regard, **Kale et al. (1999) and Latuszyńska et al. (2001)** showed that cypermethrin administration provoked inhibition of serum cholinesterase in rats.

In his study entitled "Protective effects of isoflavone on some biochemical parameters affected by cypermethrin in male rabbits", **el-Demerdash et al. (2003)** showed that oral administration of sublethal dose of cypermethrin (24 mg kg^{-1} body weight) every other day for 12 weeks to male New Zealand white rabbits increased the activities of AST, ALT and ALP enzymes in plasma. In addition, **Manna et al. (2004)** reported that oral administration **of** a single LD_{50} dose of cypermethrin increased serum AST, ALT, ALP, and LDH activities, and blood glucose level.

Manna et al. (2006) demonstrated significant increases in the activities of AST, ALT, ALP, LDH and blood glucose level in Wistar rats given consecutive daily oral dose of 14.5 mg kg^{-1} body weight cypermethrin for 60 days. In addition, **Eraslan et al. (2008)** recorded significant increases in the levels of serum glucose and uric acid as well as significant increases in the activity of ALP in Wistar rats treated with a single dose of 125 mg kg^{-1} body weight cypermethrin.

Blood biochemical alteration in albino rats after acute (1day) and sub chronic (7,14 and 21days) treatment of cypermethrin was investigated **(Saxena and Saxena, 2010).** Treatment with cypermethrin caused a significant increase in

serum, free amino acid, total proteins, urea, urea nitrogen, and uric acid, while serum albumin decreased significantly after all the treatments. Serum creatinine declined after acute treatment but increased after 14 and 21 days treatment.

Ahmad et al., (2011) studied the effects of intra-peritoneal administration of cypermethrin on biochemistry and histology of liver and kidneys in male rabbits. Animals in groups B, C and D received low (50 mg kg^{-1} body weight), medium (100 mg kg^{-1} body weight) and high (150 mg kg^{-1} body weight) cypermethrin doses, respectively in mustard oil at weekly interval up to day 71. Group A served as control and received equivalent volume of mustard oil. Increases in AST level in sera of cypermethrin-treated rabbits were accompanied by histological lesions in liver (different stages of degeneration and bile duct hyperplasia). Increased urea and creatinine concentrations and decreased total protein, albumin and globulins in sera of cypermethrin-treated rabbits could be due to renal damage. The renal damage appeared histologically in the form of different lesions (pyknotic nuclei, necrosis, sloughed tubular epithelium, cast deposition & increased urinary space) in cypermethrin-treated rabbits.

The possible protective effect of propolis on hepatotoxic effect caused by cypermethrin in adult male albino rats was evaluated (**Gomaa et al., 2011)**. Rats were classified into 4 groups. Group I (control) subdivided into (a): negative control received 2ml saline orally daily and (b): positive control received orally daily 2 ml corn oil. Group II: received orally daily (cypermethrin 14.5 mg kg^{-1}) dissolved in corn oil. Group III: received orally daily (propolis 200 mg kg^{-1}) dissolved in saline. Group IV: received orally daily (cypermethrin 14.5 mg kg^{-1} dissolved in corn oil + propolis 200 mg kg^{-1} dissolved in saline). After 4 weeks of treatment serum samples were collected for biochemical analysis. Cypermethrin induced a significant increase in the levels of liver enzymes (ALT, AST, ALP), total cholesterol, and malondialdehyde (MDA). While total protein, albumin, triglyceride, very low density lipoprotein-cholesterol (VLDL-c) and antioxidant enzymes were decreased when compared to control rats. Propolis administration with cypermethrin induced a

significant decrease in levels of liver enzymes, total cholesterol and MDA and a significant increase in the levels of antioxidant enzymes, total protein, triglyceride and VLDL-c.

Adjrah et al., (2013) assessed the potential effect of cypermethrin-treated lettuce on the rat liver physiology. Cypermethrin-treated lettuce and three doses of cypermethrin were administered during 28 days to rats. Decrease in rat body weight was noted and animals have soft feces. Plasma concentrations of ALT, AST and total bilirubin increase in rats administered with cypermethrin-treated lettuce. The plasma concentration of total protein is not decreased significantly. In another study, toxicity due to acute (1 day) and subchronic (7, 14, 21 and 28 days) orally administrated doses of cypermethrin in albino rats was evaluated using serum biochemical parameters (AST, ALT, ALP, LDH, total lipids, phospholipids, glycerides, total proteins, cholesterol and bilirubin). The parameters had significantly higher values in treated rats with cypermethrin-associated toxicity **(Bhushan et al., 2013).**

In his study on cypermethrin induced hepatotoxicity in Wistar rats, **Bhatti et al., (2014)** showed that daily oral administration of 250 mg kg^{-1} body weight cypermethrin caused significant elevation in the activities of liver marker enzymes such as serum ALT, AST and LDH. In addition, **Sakr and Albarakai (2014)** showed significant elevation in serum urea and creatinine in albino rat intoxicated with 1/10 LD_{50} cypermethrin for 6 wee

Chapter 3

Materials and methods

3.1 Experimental animals

Healthy adult male domestic rabbits weighing 1000±200 gm were used in the present study. Animals were left for one week before experimentation to adapt to laboratory conditions. Rabbits were kept in metal cages. The dimensions of each cage were 100×60×60 cm. A commercial balanced diet (Anbar) and water were provided *ad libitum* all over the experimental period.

3.2 Determination of cypermethrin LD_{50}

A total number of 80 rabbits was used for determination of LD_{50} of cypermethrin. Animals were divided into ten groups (8 rabbits/group). The first nine groups (I-IX) were administered different single doses of cypermethrin ranging form 400 to 800 mg kg^{-1} body weight as follows:

LD_{50} determination groups	**Dose (mg/kg body weight)**
Group I	400
Group II	450
Group III	500
Group IV	550
Group V	600
Group VI	650
Group VII	700
Group VIII	750
Group IX	800
Group X control group	-

The tenth group were served as control group. cypermethrin was given orally using a special stomach tube with a smooth tip to protect the interior lining of the oral and buccal cavity from injury. The animals were observed for mortality during the 48 hour observation period. the LD_{50} was determined by graphical method **(Manna et al., 2004).**

3.3 Cypermethrin toxicity experiments

A dose of 1/10 LD_{50} of cypermethrin was given orally to assess cypermethrin toxicity in male domestic rabbit. Animals were divided into two groups: control and experimental groups. Control group will comprise 48 rabbits (eight rabbits were housed in each cage) and the experimental group includes 36 rabbits (six rabbits were housed in each cage). Experimental groups were orally administrated cypermethrin daily for overall experimental duration of six weeks. Control animals were given distilled water. Administration of Cypermethrin was also done by special stomach tube. Blood samples were collected weekly and analyzed. Cypermethrin was purchased from the Palestinian Ministry of Agriculture.

3.4 General health of rabbits

Dead animals were recorded in order to calculate the percentage of mortality each week according to the following equation:

$$\% \text{ Mortality} = \frac{\text{Number of dead rabbits}}{\text{Total number of rabbits}} \times 100$$

Clinical symptoms were observed daily by the researcher himself.

3.5 Body weights

Animals were individually weighed at the beginning and the end of the experiment in order to detect any changes in their body weights. A sensitive balance (model: ONA-15, made in Istanbul 1997) was used and weights were recorded to the nearest gram.

33.6. Physiological studies

3.6.1 Blood sampling and processing

Animals from both experimental and control groups were decapitated weekly. Blood was then collected in centrifuge tubes. The collected blood was allowed to clot and then centrifuged at 3000 r.p.m. for 15 minute. Serum samples were separated in glass tubes for biochemical assay.

3.6.2 Determination of serum glucose

Serum glucose was determined by glucose-oxidase procedure **(Trinder, 1969)** using Dialab reagent kits.

Principle

For serum or plasma, couple assay involving both glucose oxidase and peroxidase is frequent employed. In the presence of glucose oxidase, glucose is oxidized to gluconic acid and hydrogen peroxide. Hydrogen peroxide reacts, in the presence of peroxidase, with phenol and 4-aminophenazone to form a quinoneimine dye. The intensity of the pink color formed is proportional to the glucose concentration.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose-oxidase}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2$$

Reagents

Reagent	Components	Concentrations
Reagent 1 Monoreagent	Phosphate Buffer, pH 7.5	250 mmol/l
	Phenol	5 mmol/l
	4-Aminoantipyrine	0.5 mmol/l
	Glucose oxidase	> 10 KU/l
	Peroxidase	> 1 KU/l
Reagent 2	Standard	100 mg/dl

Procedure

1. Pipette into test tubes the following amount as shown in the table below:

Reagent	Blank	Std/Cal	Sample
Standard/Cal	-	10 µl	-
Sample	-	-	10 µl
Reagent 1	1000 µl	1000 µl	1000 µl

2. Mix well and incubate at 37 ^{o}C for 10min. or 20min. at 20-25 ^{o}C.
3. Measure the absorbance of sample and std/cal within 60 minutes against regent blank at wavelength 500 nm.

Calculation

$$\text{Glucose [mg/dl]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Conc. of Std/Cal [mg/dl]}$$

3.6.3 Determination of Liver enzymes

3.6.3.1 Determination of alanine aminotransferase

Serum alanine aminotransferase (ALT) activity was measured by using optimized UV-test according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), according to Guder method **(Guder et al., 2001)** using DiaSys reagent kits.

Principle

L-Alanine + 2-Oxoglutarate $\xleftrightarrow{ALT}$ L-Glutamate + Pyruvate

Pyruvate + NADH + H^+ $\xleftrightarrow{LDH}$ D-Lactate + NAD^+

Reagents

Components	Concentration
Reagent 1	
TRIS pH 7.15	140 mmol/l
L-Alanine	700 mmol/l
LDH (Lactate dehydrogenase)	≥ 2300 U/l
Reagent 2	
2-Oxoglutarate	85 mmol/l
NADH	1 mmol/l

Monoreagent preparation

Four parts of R1 were mixed with1 part of R2
(E.g. 20 ml R1 + 5 ml R2) = Monoreagent

Procedure

Sample	
Monoreagent	1000 µl
Sample	100 µl

Mix, read absorbance after 1 minute and start stop watch. Read absorbance again 1, 2 and 3 min thereafter at 340 nm.

Calculation

From absorbance reading calculates ΔA /min and multiply by the corresponding factor:
ΔA /min X factor (1745) = ALT activity [U/l]

3.6.3.2 Determination of aspartate aminotransferase

Serum aspartate aminotransferase (AST) activity was measured by using optimized UV-test according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), according to Thomas **(Thomas, 1998)** using DiaSys reagent kits.

Principle

L-Aspartate + 2-Oxoglutarate $\xleftrightarrow{AST}$ L-Glutamate + Oxaloacetate

Oxaloacetate + NADH + H^+ $\xleftrightarrow{MDH}$ L-Malate + NAD^+

Reagents

Components	**Concentration**
Reagent 1	
TRIS pH 7.65	80 mmol/l
L-Aspartate	240 mmol/l
MDH (Malate dehydrogenase)	≥ 600 U/l
LDH (Lactate dehydrogenase)	≥ 900 U/l
Reagent 2	
2-Oxoglutarate	12 mmol/l
NADH	0.18 mmol/l

Monoreagent preparation

Four parts of R1 were mixed with1 part of R2

(E.g. 20 ml R1 + 5 ml R2) = Monoreagent

Procedure

	Sample
Monoreagent	1000 µl
Sample	100 µl

Mix, read absorbance was read after 1 min and start stopwatch. Absorbance was read again 1, 2 and 3 min thereafter at 340 nm.

Calculation

From absorbance reading calculates ΔA /min was calculated and multiply by the corresponding factor:

ΔA /min X factor (1745) = AST activity [U/l]

3.6.3.3 Determination of alkaline phosphatase

Serum alkaline phosphatase (ALP) activity was measured by kinetic photometric test, according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), according to the method described by Soldin and his colleagues **(Soldin et al., 2007)** using DiaSys reagent kits.

Principle

p-Nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ phosphate + p-nitroph

Reagents

Components	Concentration
Reagent 1	
2-Amino-2-methyl-1-propanol pH10.4	1.1 mmol/l
Magnisium acetate	2 mmol /l
Zinc sulphate	0.5 mmol/l
HEDTA	2.5mmol/l

Reagent 2 p-Nitrophenylphosphate	 80 mmol/l

Monoreagent preparation

Four parts of R1 were mixed with1 part of R2

(E.g. 20 ml R1 + 5 ml R2) = Monoreagent

Procedure

	Blank	**Sample**
Monoreagent	1000 µl	1000 µl
Sample	-	20 µl
Dist. water	20 µl	**-**

Mix, read absorbance after 1 min and start stopwatch. Read absorbance again 1, 2 and 3 min at 405 nm.

Calculation

From absorbance reading calculates ΔA /min and multiplies by the corresponding factor:

ΔA /min X factor (2757) = ALP activity [U/l]

3.6.3.4 Determination of Serum gamma glutamyl transferase

Serum gamma glutamyl transferase (γ-GT) is an enzyme present in liver and bile duct which is the most sensitive indicator of hepatobiliary diseases. Kinetic photometric test according to Szasz method **(Szasz, 1969)**. The test has also been standardized to the method according to IFCC (international Federation of Clinical Chemistry) **(Schumann et al., 2002)**. Results according

to IFCC are obtained using a special factor or, in case a calibrator (TruCal U) is used, by use of the calibrator value given for the IFCC method.

Principle

γ-GT catalyzes the transfer of glutamic acid to acceptors like glycylglycine in this case. This process releases 5-amino-2-nitrobenzoate which can be measured at 405 nm. The increase in absorbance at this wavelength is directly related to the activity of γ-GT.

L-Gamma-glutamyl-3-carboxy-4-nitranilide + Glycylglycine $\xleftrightarrow{\gamma\text{-GT}}$ Gamma-glutamyl-glycylglycine +5-Amino-2-nitrobenzoate. γ-GT

Reagents

Components	Concentrations
Reagent 1: TRIS Glycylglycine	135 mmol/l 135 mmol/l
Reagent 2: L-Gamma-glutamyl-3-carboxy-4-nitranilide	22 mmol/l

Procedure

Substrate start

	Blank	Sample
Sample	-	100 µl
Dist. Water	100 µl	-
Reagent 1	1000 µl	1000 µl
Reagent 2	250 µl	250 µl

Mix, read absorbance after 2 min and start stop watch. Read absorbance again after 1, 2 and 3 minutes.

Sample start

	Blank	Sample
Sample/Calibreate		100 µl
Dist. Water	100 µl	
Monoreagent	1000 µl	1000 µl

Mix, read absorbance after 2 min and start stop watch. Read absorbance again after 1, 2 and 3 minutes.

Calculation

From absorbance readings calculate ΔA/min and multiply by the corresponding factor from table below:

	According to Szasz	According to IFCC
Substrate start 405 nm	1421	1606
Sample start 405 nm	1158	1309

With calibrator

$$\gamma\text{-GT (U/l)} = \frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Calibrator}} \times \text{conc. Calibrator (U/l)}$$

3.6.3.5 Determination of cholinesterase activity

Serum cholinesterase (ChE) activity was measured by kinetic photometric test, according to the recommendation of German Society of Clinical Chemistry (DGKC), the method described by Ellman and his colleagues **(Ellman et al., 1961)** using DiaSys reagent kits.

Principle

Cholinesterase hydrolyses butyrylthiocholine under release of butyric acid and thiocholine. Thiocholine reduces yellow potassium hexacyanoferrate (III) to

colorless potassium hexacyanoferrate (II). The decrease of absorbance is measured at 405 nm.

$$\text{Butyrylthiocholine} + H_2O \xrightarrow{\text{cholinesterase}} \text{Thiocholine} + \text{Butytate}$$

$$2\text{Thiocholine} + 2(Fe(CN)_6)^{3-} + H_2O \longrightarrow \text{Choline} + 2(Fe(CN)_6)^{4-} + H_2O$$

Reagents

Components	Concentration
Reagent 1 Pyrophosphate pH 7.6 Potassium hexacyanoferrate(III)	 75 mmol/l 2 mmol/l
Reagent 2 Butyrylthiocholine	 15 mmol/l

Procedure

	Reagent /blank	**sample**
Sample	**-**	20 µl
Dist. Water	20 µl	**-**
Reagent 1	1000 µl	1000 µl
Mix, incubate approx.3 min, and then add:		
	Reagent /blank	**Sample**
Reagent 2	250 µl	250 µl

Mix, read absorbance after 2 min and start stop watch. Read absorbance again after 1, 2 and 3 minutes at 405 nm.

ΔA/min = [ΔA/min Sample] – [ΔA/min Blank]

Calculation

Calculate ΔA/min and multiply with 68500 =cholinesterase activity U/l.

3.6.4 Determination of bilirubin

Principle

Both direct and indirect bilirubin couple with diazo in the presence of cetrimide **(Pearlman and lee, 1974).** The terms direct and total refer to the reaction characteristics of serum bilirubin in the absence or presence of solubilizing (accelerating) reagents. The direct and indirect bilirubin is only approximately equivalent to the conjugated and unconjugated fractions.

Reagents

Working reagent: transfer the contents of one reagent BT vial into a reagent AT bottle for total bilirubin determination. Mix thoroughly. Other volumes can be prepared in the proportion: 1 ml reagent BT + 4 ml reagent AT. Stable for 20 days at 2-8 C.

Procedure

1- Pipette into labelled test tube

	Reagent Blank	**Sample Blank**	**Sample**	**Standard**
Distilled water	100 µl	-	-	-
Sample	-	100 µl	100 µl	-
Standard(S)	-	-	-	100 µl
Reagent (AT)	-	1.0 µl	-	-
Working Reagent	1.0 µl	-	1.0 µl	1.0 µl

2- Mix thoroughly and let stand the tubs for 2 min at room temperature.

3- Read the absorbance (A) of the sample blanks at 540 nm against distilled water.

4- Read the absorbance (A) of the sample and of the stranded at 540 nm against the reagent blank.

Calculations

The bilirubin concentration in the sample is calculated using the following formula:

$$\frac{\text{A Sample- A Sample}}{\text{A Standard}} \times \text{C Standard}$$

C Standard = C Sample

3.6.5 Non- protein nitrogen constituents

3.6.5.1 Determination of urea

Serum urea was determined by using "Urease-GLDH": enzymatic UV test, according to Thomas method **(Gutmann and Bergmeyer 1974)** using DiaSys reagent kits.

Principle

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + 2HCO^{3-}$$

$$\text{2-Oxaloglutarate} + NH_4^+ + \text{NADH} \xrightarrow{\text{GlDH}} \text{L-Glutamate} + NAD^+ + H_2O$$

GLDH: Glutamate dehydrogenase.

Reagents

Component	**Concentration**
Reagent 1:	
TRIS pH 7.8	150 mmol/l
2-Oxaloglutarate	9 mmol/l
ADP	0.75 mmol/l
Urease	≥ 7 KU/l
GLDH	≥1 KU/l
Reagent 2: NADH	1.3 mmol/l
Standard	50 mg /dl (8.33 mmol/l)

Monoreagent preparation

Four parts of R1 were mixed with1 part of R2

(E.g. 20 ml R1 + 5 ml R2) = Monoreagent

Procedure

	Blank	**Sample or standard**
Sample or standard	-	10 µl
Monoreagent	1000 µl	1000 µl

Mix and incubate for 60 sec. at 25 C**,** then read absorbance A1. After exactly further 60 sec. read absorbance A2 at 340 nm.

A= (A1-A2) sample or standered

Calculation

$$\text{Urea [mg/dl]} = \frac{\Delta \text{ A sample X conc. Std /Cal [mg/dl]}}{\Delta \text{ A std /cal}}$$

3.6.5.2 Determination of creatinine

Serum creatinine was determined by using kinetic test without deproteinization according to Newman and Price method **(Newman and Price, 1999)** using DiaSys reagent kits.

Principle

Creatinine forms a colored orange-red complex in an alkaline picrate solution. The different in absorbance at fixed time during conversion is proportional to the concentration of creatinine in the sample.

Creatinine + picric acid $\longrightarrow$ Creatinine picrate complex

Reagents

Component	Concentration
Reagent 1 **Sodium hydroxide**	0.16 mmol/l
Reagent 2 Picric acid	4.0 mmol/l
Standard	2 mg/dl (177 mmol /l)

Monoreagent preparation

Four parts of R1 were mixed with 1 part of R2

(E.g.20 ml R1+ 5 ml R2)= Monoreagent

Procedure

	Blank	Std./Cal.	Sample
Monoreagent	1000 µl	1000 µl	1000 µl
Sample	-	-	50 µl
Std./Cal.	-	50 µl	-
Dist. water	50 µl	-	-

Mix and read absorbance A1 after 60 sec against reagent blank at 492 nm, read absorbance A2 after further 120 sec.

Calculation

$$\text{Creatinine concentration [mg/dl]} = \frac{(\Delta A \text{ sample})}{(\Delta A \text{ standard})} \times \text{Conc. Std [mg/dl]}$$

ΔA = [(A2 – A1) sample or standard] – [(A2 –A1) Blank]

3.6.6 Protein profile

3.6.6.1 Determination of total protein

Serum total protein was determined by photometric test according to Thomas method **(Thomas, 1998)** using DiaSys reagent kits.

Principle

Protein together with copper ions forms a violet blue color complex in alkaline solution. The absorbance of color is directly proportional to concentration.

Reagents

Components	**Concentrations**
Reagent 1:	
Sodium hydroxide	80 mmol/l
Potassium sodium tartrate	12.8 mmol/l
Reagent 2:	
Sodium hydroxide	100 mmol/l
Potassium sodium tartrate	16 mmol/l
Potassium iodide	15 mmol/l
Copper sulfate	6 mmol/l
Standard	5 g/dl

Monoreagent preparation

Four parts of R1 were mixed with1 part of R2

(e.g. 20 ml R1 + 5 ml R2) = Monoreagent

Procedure

	Blank	Sample
Monoreagent	1000 µl	1000 µl
Sample	-	20 µl
Dist. water	20 µl	-

Mix, incubate for 5 min at 25°C and read absorbance against the reagent blank within 60 min at 540 nm.

Calculation

The protein concentration in the sample is calculated using the following general formula:

$$\text{Total protein [g/dl]} = \frac{(\Delta \text{ A sample})}{(\Delta \text{ A standard})} \times \text{Conc. Std [g/dl]}$$

3.6.6.2 Determination of albumin

Serum albumin was determined by photometric test according to the method described by Johnson and his colleagues **(Johnson et al., 1999)** using DiaSys reagent kits.

Principle

Serum albumin in the presence of bromecresol green at a slightly acid pH produces a color change of the indicator iron yellow-green to green blue.

Reagents

Components	Concentrations
Reagent	
Citrate buffer pH 4.2	30 mmol/l
Bromocresol green	0.26 mmol/l
Standard	5g/dl

Procedure

	Blank	Sample
Reagent	1000 µl	1000 µl
Sample	-	10 µl
Dist. Water	10 µl	-

Mix, incubate for approx. 10 min. and read the absorbance against reagent blank within 60 min at 540 – 600 nm.

Calculation

Serum albumin concentration in the sample is calculated using the following general formula:

$$\text{Albumin [g/dl]} = \frac{(\Delta \text{ A Sample})}{(\Delta \text{ A Standard})} \text{ X Conc. Std [g/dl]}$$

3.6.6.3 Determination of globulin

Globulin was calculated according the following formula:

Globulin = Total protein - Albumin

3.6.7 Electrolytes

3.6.7.1 Determination of calcium

Serum calcium was determined by photometric test with cresolphthalein complex one **(Thomas, 1998)** using DiaSys reagent kit.

Principle

Cresolphthalein complex one reacts with calcium ions in alkaline medium forming a red-violet color. Interference by magnesium is eliminated by addition of 8-hydroxy-quinoline.

Reagents

Reagent	Components	Concentrations
Reagent 1	Ethanolamine Detergent pH 10.7	600 mmol/l
Reagent 2	2-Cresolphthalein complex one 8-Hydroxyquinoline Hydrochloric acid pH 1.1	0.06 mmol/l 7 mmol/l 20 mmol/l
Reagent 3	Standard:	10 mg/dl

Preparation and stability of working reagent:

Four parts of R1 were mixed with 1 part of R2

Stability: 3 days at 2-8 ^{o}C

Procedure

Wavelength 570 nm, Hg 578 nm (550-590 nm)

Temperature 37°C

Cuvette 1 cm light path

Reading against reagent blank was done

	Blank	**Standard**	**Sample**
Working reagent	1 µl	1 µl	1 µl
Distilled water	20 µl	-	-
Standard	-	20 µl	-
Sample	-	-	20 µl

Mixing and reading the optical density (OD) after a 5 minute incubation was done. The final color is stable for at least 15 minutes.

Calculation

$$\frac{\text{OD Sample}}{\text{OD Standard}} \times n = \text{sample calcium concentration(mg/dl)}$$

n = standard calcium concentration

3.6.7.2 Determination of phosphorus

Serum phosphorus was determined by phosphomolybdate UV end point **(Tietz, 1994)** using Amonium Molybdate Diagnostic kit.

Principle

Determination of inorganic phosphate was made according to the following reaction:

Amonium molybdate + Sulforic acid $\xrightarrow{\text{Phosphorus}}$ Phosphomolybdic complea

Reagents

Reagent	Components	Concentrations
Reagent	Sulfuric acid Amonium molybdate	210 mmol/l 650 mmol/l
Standard	Phosphorus	5 mg/dl

Preparation and stability of working reagent:

The reagent is ready for use

Procedure

Wavelength 340 nm

Temperature 37°C

Cuvette 1 cm light path

Reading against reagent blank was done

	Blank	Standard	Sample
Reagent	1 µl	1 µl	1 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl

Mixing and reading the optical density (OD) after a 5 minute incubation was done. The final color is stable for at least 1 hour.

Calculation

$$\frac{\text{OD Sample}}{\text{OD Standard}} \times n = \text{sample Phosphorus concentration(mg/dl)}$$

n = standard Phosphorus concentration

3.7 Statistical analysis

Data were statistically analyzed using SPSS computer program version 18.0 for windows (Statistical Package for Social Sciences Inc, Chicago, Illinois). Means were compared by independent-sample t-test.

Probability values (P) were obtained from the student's table of "t" and significance was at $P < 0.05$.

The percentage difference was calculated according to the formula:
Percentage difference equals the absolute value of the change in value, divided by the average of the 2 numbers, all multiplied by 100.
Percent difference = (| (V1 - V2) | / ((V1 + V2)/2)) * 100.

Graphs

Body weight graph and logarithmic scale of oral LD_{50} of cypermethrin was plotted using Microsoft Excel program 2015.

Chapter 4

Results

4.1 Oral LD_{50} of cypermethrin

The experimental trials for oral LD_{50} determination of cypermethrin after 48hr of administration in male domestic rabbits revealed that the mortality commenced at 450 mg kg^{-1} body weight, recording mortality percentage of 12.5% (Table 4.1). Increasing cypermethrin dose to 500, 550, 600, 650, 700 and 750 mg kg^{-1} resulted in mortality percentages of 12.5, 25, 25, 37.5, 50.0 and 75%, respectively. The mortality rate was a function of dose increase. The maximum concentration of cypermethrin which kill all animals in the group was found to be 800 mg kg^{-1} body weight. The calculated oral LD_{50} of cypermethrin in male domestic rabbits from the linear regression was found to be 665 mg kg^{-1} body weight (Figure 4.1).

Table 4.1 Mortality percentage of male domestic rabbits after 48hr of oral administration of different doses of cypermethrin.

Group	Cypermethrin Dose (mg kg^{-1} body weight)	Number of Animals died/total	% mortality
Group I	400	0/8	0
Group II	450	1/8	12.5
Group III	500	1/8	12.5
Group IV	550	2/8	25
Group V	600	2/8	25
Group VI	650	3/8	37.5
Group VII	700	4/8	50
Group VIII	750	6/8	75
Group IX	800	8/8	100
Group X	Control	0/8	0

The number of animals administered cypermethrin was 8 in each group (I to IX). Control animals were given distilled water and their number was also 8.

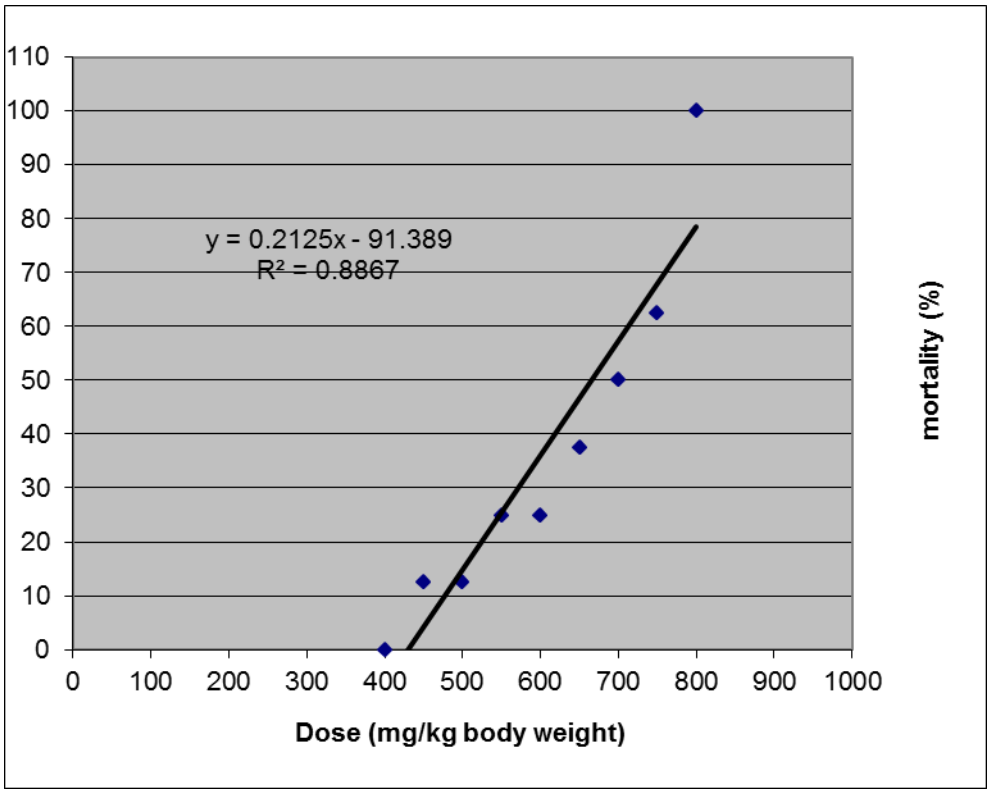

Figure 4.1 Logarithmic scale of oral LD_{50} of cypermethrin in male domestic rabbits (LD_{50}=665 mg kg^{-1} body weight).

4.2 General health of rabbits

To assess cypermethrin toxicity in rabbits, 1/10 LD_{50} cypermethrin (66.5 mg kg^{-1} body weight) was orally administered daily for 6 weeks. The mortality rate recorded for 1/10 LD_{50} cypermethrin-treated rabbits was 0/6 (0%), 1/6 (16.7%), 1/6 (16.7%), 1/6 (16.7%), 2/6 (33.3%) and 1/6 (16.7%) after 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. However, no rabbits were died in the control group during the whole experimental periods studied. In addition, rabbits in the control group did not show any sign of toxicity. However, cypermethrin-treated rabbits showed varying degrees of clinical signs few hours after dosing. The signs included diarrhea, disorientation, drowsiness, weakness, depression and mild tremors. Concerning morphological changes, cypermethrin-treated rabbits showed hair loss especially in the 2nd weeks of the experiment (Figure 4.2 A) whereas control

animals did not display such change. The livers of dissected rabbits also showed scars of depression in response to cypermethrin administration (Figure 4.2 B) whereas those of the control animals showed normal appearance.

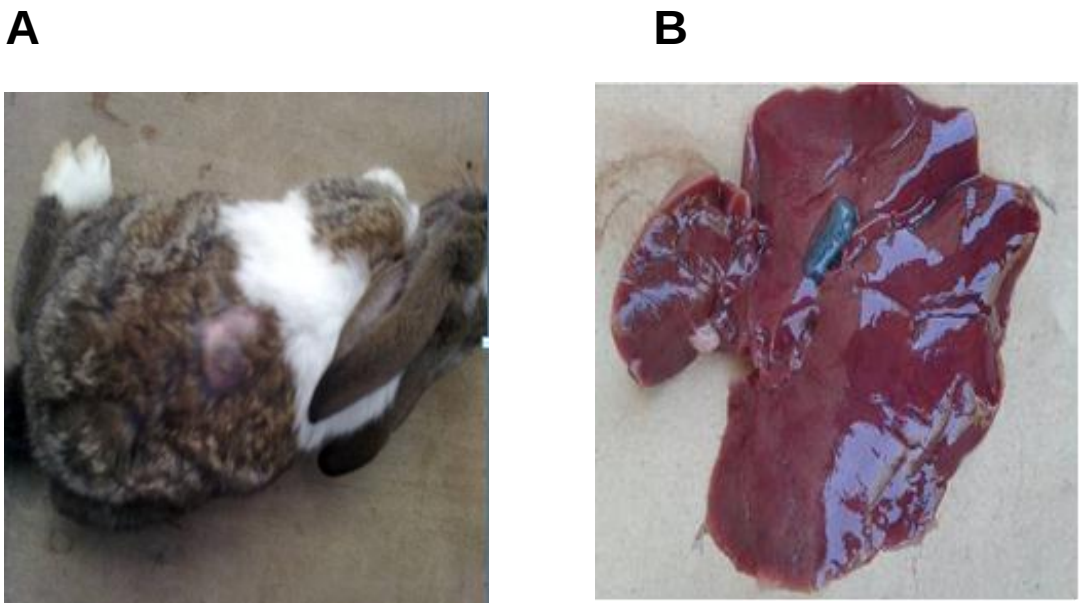

Figure 4.2 Morphological effect of 1/10 LD_{50} of cypermethrin (66.5 mg kg^{-1} body weight) after 6 weeks on hair (A) and liver (B) of male domestic rabbit.

4.3 Final body weight

Table 4.2 and Figure 4.3 illustrate the final body weight of male domestic rabbits after 6 weeks of daily oral administration of 1/10 LD_{50} cypermethrin (66.5 mg kg^{-1} body weight). There was a significant decrease in the body weight of cypermethrin-treated rabbits compared to controls (1357±50.4 *versus* 965±46.3, %difference=33.8, P=0.001).

Table 4.2 Final body weight of male domestic rabbit after 6 weeks of daily oral administration of 1/10 LD_{50} cypermethrin (66.5 mg kg^{-1} body weight).

Parameter	Control (n=8)	Cypermethrin (n=6)	% difference	t-value	P-value
Body weight (gm)	1357±50.4	965±46.3	-33.8	5.373	0.001

The number of animals (n) was 8 in control group and 6 in cypermethrin-treated animals. All values were expressed as mean±SEM. The level of significance was at $P<0.05$.

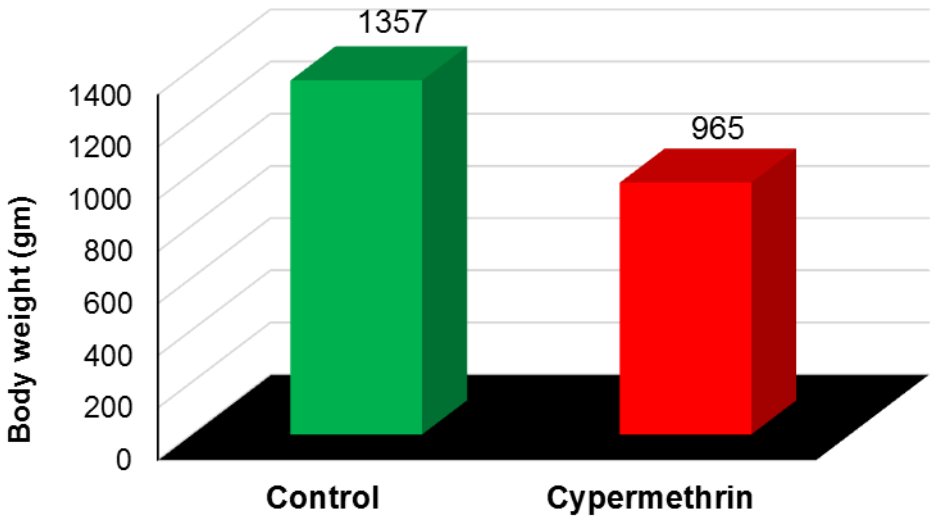

Figure 4.3 Final body weight of male domestic rabbits after 6 weeks of daily oral admininstration of 1/10 LD50 cypermethrin (66.5 mg kg^{-1} body weight)

4.4 Biochemical investigation

4.4.1 Serum glucose

Table 4.3 illustrates the mean values of serum glucose in control and cypermethrin-intoxicated male domestic rabbits all over the experimental period of 6 weeks. The normal glucose levels were 111.8±4.7, 113.0±3.8, 112.5±4.8, 114.4±4.5, 113.7±5.1 and 112.0±5.2 mg/dl at 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Daily oral administration of cypermethrin caused significant increase in glucose level throughout the experiment to reach mean values of 129.1±4.3, 127.8±4.6, 136.8±5.5, 145.1±5.8, 143.1±6.2 and 148.6±6.1 mg/dl, respectively. The maximum increase in glucose level was registered at the end of the experiment showing percentage difference of 28.1% (t=4.589, P=0.001).

Table 4.3 Effect of cypermethrin (1/10 LD_{50}, 66.5 mg kg^{-1} body weight) on serum glucose level (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Cypermethrin (n=6)	% difference	t-value	P-value
1	111.8±4.7	129.1±4.3	14.4	2.718	0.022
2	113.0±3.8	127.8±4.6	12.3	2.466	0.037
3	112.5±4.8	136.8±5.5	19.5	3.264	0.010
4	114.4±4.5	145.1±5.8	23.7	4.140	0.003
5	113.7±5.1	143.1±6.2	22.9	3.618	0.006
6	112.0±5.2	148.6±6.1	28.1	4.589	0.001

The number of animals (n) was 8 in control group and 6 in cypermethrin-treated animals. All values were expressed as mean±SEM. The level of significance was at P<0.05.

4.4.2 Liver enzymes

4.4.2.1 Alanine aminotransferase

The mean values of serum ALT activity in control and cypermethrin -treated male domestic rabbits along the experimental period of 6 weeks are presented in Table 4.4. The normal enzyme activity was 47.2±1.8, 48.5±2.3, 50.0±2.2, 49.3±1.9, 50.4±2.1 and 48.9±2.5 U/L at the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Upon cypermethrin administration, ALT activity was significantly increased throughout the experimental periods reaching mean values of 55.8±2.7, 59.2±2.9, 61.9±3.1, 66.8±2.8, 65.3±3.0 and 62.0±3.3 U/L, respectively. This increase recorded the maximum percentage difference of 30.1% in the 4th week of the experiment (t=5.305, P=0.000).

Table 4.4 Effect of cypermethrin (1/10 LD_{50}, 66.5 mg kg^{-1} body weight) on serum alanine aminotransferase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Cypermethrin (n=6)	% difference	t-value	P-value
1	47.2±1.8	55.8±2.7	16.7	2.630	0.025
2	48.5±2.3	59.2±2.9	19.9	2.957	0.016
3	50.0±2.2	61.9±3.1	21.3	3.164	0.011
4	49.3±1.9	66.8±2.8	30.1	5.305	0.000
5	50.4±2.1	65.3±3.0	25.8	4.178	0.003
6	48.9±2.5	62.0±3.3	23.6	3.354	0.008

The number of animals (n) was 8 in control group and 6 in cypermethrin-treated animals. All values were expressed as mean±SEM. The level of significance was at P<0.05.

4.4.2.2 Aspartate aminotransferase

Table 4.5 provides mean values of serum AST activity in control and cypermethrin -fed male domestic rabbits all over the experimental period of 6 weeks. The activity of AST registered for control animals were 33.8±1.5, 32.5±1.7, 35.1±2.0, 34.0±1.6, 33.9±1.8 and 32.8±1.4 U/L at the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Feeding of animals with cypermethrin provoked significant elevation in the enzyme activity throughout the experiment exhibiting values of 49.8±2.6, 42.4±2.3, 48.9±2.8, 45.3±2.5, 45.7±2.4 and 41.2±2.5 U/L. The maximum elevation in the enzyme activity was recorded in the 1st week of the experiment showing percentage difference of 38.3% (t=5.349, P=0.000).

Table 4.5 Effect of cypermethrin (1/10 LD_{50}, 66.5 mg kg^{-1} body weight) on serum aspartate aminotransferase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Cypermethrin (n=6)	% difference	t-value	P-value
1	33.8±1.5	49.8±2.6	38.3	5.349	0.000
2	32.5±1.7	42.4±2.3	26.4	3.472	0.006
3	35.1±2.0	48.9±2.8	32.9	4.153	0.002
4	34.0±1.6	45.3±2.5	28.5	3.820	0.004
5	33.9±1.8	45.7±2.4	29.6	4.088	0.003
6	32.8±1.4	41.2±2.5	22.7	3.122	0.012

The number of animals (n) was 8 in control group and 6 in cypermethrin-treated animals. All values were expressed as mean±SEM. The level of significance was at P<0.05.

4.4.2.3 Alkaline phosphatase

The normal activity of serum ALP of male domestic rabbits were 97.1±2.9, 102.5±4.0, 97.3±3.8, 98.4±4.1, 100.2±4.5 and 96.8±3.7 U/L at 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively Table 4.6. Cypermethrin intake increased the enzyme activity during the last five weeks of the experiment, showing mean values of 129.3±4.7, 115.8±4.4, 121.1±4.9, 125.0±4.8 and 114.9±4.6 U/L in the 2nd, 3rd, 4th, 5th and 6th weeks, respectively. The significant increment in the enzyme activity started from the 2nd week of the experiment with maximum percentage difference of 23.1% (t=4.092, P=0.003).

Table 4.6 Effect of cypermethrin (1/10 LD_{50}, 66.5 mg kg^{-1} body weight) on serum alkaline phosphatase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Cypermethrin (n=6)	% difference	t-value	P-value
1	97.1±2.9	106.5±3.8	9.2	1.798	0.102
2	102.5±4.0	129.3±4.7	23.1	4.092	0.003
3	97.3±3.8	115.8±4.4	17.4	2.971	0.016
4	98.4±4.1	121.1±4.9	20.7	3.276	0.010
5	100.2±4.5	125.0±4.8	22.0	3.640	0.008
6	96.8±3.7	114.9±4.6	17.1	2.952	0.017

The number of animals (n) was 8 in control group and 6 in cypermethrin -treated animals. All values were expressed as mean±SEM. The level of significance was at P<0.05.

4.4.2.4 Serum gamma glutamyl transferase

Table 4.7 gives the mean values of serum γGT activity in control and cypermethrin-intoxicated male domestic rabbits all over the experimental period of 6 weeks. The normal activity of γGT was 5.95±0.28, 6.34±0.29, 6.05±0.26, 6.20±0.30, 5.90±0.29 and 6.21±0.31 U/L at 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Daily oral administration of cypermethrin increased the enzyme activity throughout the experiment to reach mean values of 7.07±0.31, 8.24±0.38, 7.68±0.40, 7.76±0.39, 7.58±0.35 and 8.19±0.37 U/L, respectively. The maximum increase in γGT activity was registered at the end of the experiment showing percentage difference of 27.5% (t=4.151, P=0.002).

Table 4.7 Effect of cypermethrin (1/10 LD_{50}, 66.5 mg kg^{-1} body weight) on serum gamma glutamyl transferase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Cypermethrin (n=6)	% difference	t-value	P-value
1	5.95±0.28	7.07±0.31	17.2	2.709	0.022
2	6.34±0.29	8.24±0.38	26.1	4.026	0.003
3	6.05±0.26	7.68±0.40	23.7	3.552	0.006
4	6.20±0.30	7.76±0.39	22.3	3.304	0.009
5	5.90±0.29	7.58±0.35	24.9	3.784	0.005
6	6.21±0.31	8.19±0.37	27.5	4.151	0.002

The number of animals (n) was 8 in control group and 6 in cypermethrin-treated animals. All values were expressed as mean±SEM. The level of significance was at P<0.05.

4.4.2.5 Cholinesterase activity

The mean values of serum ChE activity in control and cypermethrin-fed rabbits are pointed out in Table 4.8. The normal ChE activities in control animals were 4618±110, 4527±121, 4569±115, 4770±128, 4644±113 and 4599±120 U/L during the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Oral administration of the cypermethrin pesticide provoked a vi highly significant decrease in the enzyme activity to values of 3145±119, 3106±107, 2979±98, 3053±101, 2891±96 and 2950±102 U/L, respectively. The maximum inhibition in the activity of ChE (2891±96 U/L) was obtained during the 5th week of the experiment recording a percentage difference of 46.5% (t=10.928, P=0.000).

Table 4.8 Effect of cypermethrin (1/10 LD_{50}, 66.5 mg kg^{-1} body weight) on serum cholinesterase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Cypermethrin (n=6)	% difference	t-value	P-value
1	4618±110	3145±119	-37.9	8.932	0.000
2	4527±121	3106±107	-37.2	8.616	0.000
3	4569±115	2979±98	-42.1	10.248	0.000
4	4770±128	3053±101	-43.9	10.270	0.000
5	4644±113	2891±96	-46.5	10.928	0.000
6	4599±120	2950±102	-43.7	10.254	0.000

The number of animals (n) was 8 in control group and 6 in cypermethrin-treated animals. All values were expressed as mean±SEM. The level of significance was at P<0.05.

4.4.3 Serum bilirubin

Table 4.9 shows the mean levels of serum bilirubin in control rabbits as well as in rabbits received cypermethrin daily for 6 weeks. The normal levels of bilirubin in control rabbits were 1.56±0.05, 1.62±0.06, 1.63±0.08, 1.60±0.05, 1.54±0.04 and 1.64±0.06 mg/dl during the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Oral administration of cypermethrin caused gradual increase in bilirubin level to reach its maximum % difference of 21.4 during 5th week of the experiment (t=3.157, P=0.013).

Table 4.9 Effect of cypermethrin (1/10 LD_{50}, 66.5 mg kg^{-1} body weight) on serum bilirubin (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Cypermethrin (n=6)	% difference	t-value	P-value
1	1.56±0.05	1.62±0.07	3.8	0.813	0.435
2	1.62±0.06	1.73±0.09	6.6	1.061	0.316
3	1.63±0.08	1.77±0.10	8.2	1.184	0.267
4	1.60±0.05	1.80±0.12	11.8	1.805	0.105
5	1.54±0.04	1.91±0.14	21.4	3.157	0.013
6	1.64±0.06	1.94±0.11	16.8	2.616	0.028

The number of animals (n) was 8 in control group and 6 in cypermethrin-treated animals. All values were expressed as mean±SEM. The level of significance was at P<0.05.

4.4.4 Non-protein nitrogen constituents

4.4.4.1 Serum urea

Table 4.10 presents the mean values of serum urea concentration in control and cypermethrin-treated male domestic rabbits. Urea concentration in control animals exhibited values of 36.9±2.1, 37.3±1.9, 36.8±1.7, 35.7±2.3, 35.4±1.5 and 34.7±2.0 mg/dl during 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks of the experiment, respectively. Daily feeding of cypermethrin caused progressive significant elevation in urea concentration all over the experimental intervals examined reaching values of 46.8±2.6, 48.2±2.8, 48.8±3.1, 49.0±2.9, 54.9±3.6 and 57.1±3.4 mg/dl, respectively. The increase in urea concentration reached its maximum percentage difference of 48.8% at the last week of the experiment (t=6.031, P=0.000).

Table 4.10 Effect of cypermethrin (1/10 LD_{50}, 66.5 mg kg^{-1} body weight) on serum urea concentration (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Cypermethrin (n=6)	% difference	t-value	P-value
1	36.9±2.1	46.8±2.6	23.7	3.051	0.012
2	37.3±1.9	48.2±2.8	25.5	3.354	0.008
3	36.8±1.7	48.8±3.1	28.0	3.630	0.005
4	35.7±2.3	49.0±2.9	31.4	3.972	0.003
5	35.4±1.5	54.9±3.6	43.2	5.693	0.000
6	34.7±2.0	57.1±3.4	48.8	6.031	0.000

The number of animals (n) was 8 in control group and 6 in cypermethrin-treated animals. All values were expressed as mean±SEM. The level of significance was at P<0.05.

4.4.4.2 Serum creatinine

The mean values of serum creatinine concentrations in control group of male domestic rabbits as well as in animals treated with cypermethrin along the experimental period of 6 weeks are illustrated in Table 4.11. The normal values recorded for creatinine concentrations were 0.66±0.04, 0.64±0.03, 0.67±0.03, 0.64±0.02, 0.70±0.04 and 0.68±0.02 mg/dl at the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks of the experiment, respectively. Upon cypermethrin administration, serum creatinine concentration fluctuates across the experimental period showing significant differences commencing from the 2^{nd} week, exhibiting values of 0.78±0.05, 0.80±0.06, 0.85±0.06, 0.96±0.08 and 0.87±0.07 mg/dl, respectively. The maximum increment of serum creatinine was found during the 5^{th} week of the experiment with percentage difference of 31.3% (t=3.820, P=0.005).

Table 4.11 Effect of cypermethrin (1/10 LD_{50}, 66.5 mg kg^{-1} body weight) on serum creatinine concentration (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Cypermethrin (n=6)	% difference	t-value	P-value
1	0.66±0.04	0.75±0.03	12.8	2.120	0.060
2	0.64±0.03	0.78±0.05	19.7	2.642	0.027
3	0.67±0.03	0.80±0.06	17.6	2.424	0.038
4	0.64±0.02	0.85±0.06	28.2	3.437	0.007
5	0.70±0.04	0.96±0.08	31.3	3.820	0.005
6	0.68±0.02	0.87±0.07	24.5	3.147	0.012

The number of animals (n) was 8 in control group and 6 in cypermethrin-treated animals. All values were expressed as mean±SEM. The level of significance was at P<0.05.

4.4.5 Protein profile

4.4.5.1 Serum total protein

Table 4.12 indicates the normal values of serum total protein levels in male domestic rabbits throughout the experimental period of 6 weeks. These values were 5.58±0.24, 5.67±0.28, 6.04±0.31, 5.69±0.29, 5.68±0.33 and 5.92±0.37 mg/dl during the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Total protein level showed successive decrease in response to cypermethrin administration along the experimental periods tested. This decrease become significant starting from the 3rd week till the end of experiment, recording it's maximum % difference of 31.3 at the last week of the experiment (t=3.724, P=0.004).

Table 4.12 Effect of cypermethrin (1/10 LD_{50}, 66.5 mg kg^{-1} body weight) on serum total protein (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Cypermethrin (n=6)	% difference	t-value	P-value
1	5.58±0.24	5.28±0.22	-5.5	0.892	0.393
2	5.67±0.28	5.16±0.24	-9.4	1.403	0.191
3	6.04±0.31	5.04±0.21	-18.1	2.636	0.025
4	5.69±0.29	4.70±0.22	-19.2	2.705	0.022
5	5.68±0.33	4.35±0.19	-26.5	3.450	0.006
6	5.92±0.37	4.32±0.20	-31.3	3.724	0.004

The number of animals (n) was 8 in control group and 6 in cypermethrin-treated animals. All values were expressed as mean±SEM. The level of significance was at P<0.05.

4.4.5.2 Serum albumin

The mean concentration of serum albumin in control and cypermethrin-treated male domestic rabbits are shown in Table 4.13. Albumin concentration in control animals exhibited mean values of 3.66±0.18 , 3.65±0.19, 3.92±0.23, 4.00±0.22, 3.97±0.25 and 4.01±0.23 mg/dl at 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks of the experiment, respectively. Cypermethrin intake resulted in progressive significant decrease in albumin concentration recording the minimum concentration of 2.91±0.12 mg/dl at the end of the experiment with percentage difference of 31.8% (t=4.026 and P=0.002).

Table 4.13 Effect of cypermethrin (1/10 LD_{50}, 66.5 mg kg^{-1} body weight) on serum albumin (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Cypermethrin (n=6)	% difference	t-value	P-value
1	3.66±0.18	3.37±0.14	-8.3	1.231	0.247
2	3.65±0.19	3.20±0.10	-13.1	2.135	0.059
3	3.92±0.23	3.16±0.08	-21.5	3.065	0.012
4	4.00±0.22	3.12±0.10	-24.7	3.246	0.009
5	3.97±0.25	2.96±0.09	-29.2	3.788	0.004
6	4.01±0.23	2.91±0.12	-31.8	4.026	0.002

The number of animals (n) was 8 in control group and 6 in cypermethrin-treated animals. All values were expressed as mean±SEM. The level of significance was at $P<0.05$.

4.4.5.3 Serum globulin

Table 4.14 demonstrates the normal values of serum globulin levels in male domestic rabbits throughout the experimental period of 6 weeks. These values were 1.96±0.08, 2.04±0.10, 2.13±0.14, 1.99±0.11, 1.93±0.12 and 1.95±0.11 mg/dl during the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Oral administration of cypermethrin lowered globulin levels to 1.84±0.09, 1.82±0.11, 1.74±0.06, 1.59±0.07, 1.56±0.07, and 1.45±0.05 mg/dl, showing percentage differences of 6.3, 11.4, 20.2, 22.3, 21.2 and 29.4% at the weekly intervals of the experiment compared to controls. This decrease was significant all over the experimental periods expect for the 1st and 2nd weeks**.**

Table 4.14 Effect of cypermethrin (1/10 LD_{50}, 66.5 mg kg^{-1} body weight) on serum globulin (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Cypermethrin (n=6)	% difference	t-value	P-value
1	1.96±0.08	1.84±0.09	-6.3	0.972	0.354
2	2.04±0.10	1.82±0.11	-11.4	1.535	0.156
3	2.13±0.14	1.74±0.06	-20.2	2.682	0.023
4	1.99±0.11	1.59±0.07	-22.3	3.038	0.012
5	1.93±0.12	1.56±0.07	-21.2	2.820	0.018
6	1.95±0.11	1.45±0.05	-29.4	3.864	0.003

The number of animals (n) was 8 in control group and 6 in cypermethrin-treated animals. All values were expressed as mean±SEM. The level of significance was at $P<0.05$.

4.4.6 Electrolytes

4.4.6.1 Serum calcium

The mean serum calcium concentrations in controls and in cypermethrin-received male rabbits are provided in Table 4.15. The normal concentrations of calcium were 14.2±0.8, 13.7±0.7, 14.1±0.9, 13.7±0.5, 13.9±1.0 and 14.0±0.9 mg/dl during the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Upon cypermethrin administration, serum concentration of calcium fluctuated throughout the experiment registering, in general, significant decrease during the whole experiment. The maximum decrease of calcium concentration was registered during the last week of the experiment with percentage difference of 28.6 (t=3.433, P=0.006).

Table 4.15 Effect of cypermethrin (1/10 LD_{50}, 66.5 mg kg^{-1} body weight) on serum calcium (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Cypermethrin (n=6)	% difference	t-value	P-value
1	14.2±0.8	11.2±0.5	-23.6	3.113	0.011
2	13.7±0.7	11.4±0.4	-18.3	2.692	0.023
3	14.1±0.9	12.0±0.5	-16.1	2.269	0.047
4	13.7±0.5	12.1±0.8	-12.4	1.928	0.083
5	13.9±1.0	10.6±0.3	-27.0	3.322	0.008
6	14.0±0.9	10.5±0.4	-28.6	3.433	0.006

The number of animals (n) was 8 in control group and 6 in cypermethrin-treated animals. All values were expressed as mean±SEM. The level of significance was at P<0.05.

4.4.6.2 Serum phosphorus

Table 4.16 shows serum phosphorus concentrations in controls as well as in cypermethrin-fed male rabbits. The mean concentrations of phosphorus in control animals were 7.13±0.4, 7.41±0.3, 6.95±0.2, 7.43±0.4, 7.40±0.3 and 7.23±0.2 mg/dl at the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. In cypermethrin-treated group of rabbits, serum phosphorus displayed, in general, gradual increase along the whole experiment. This decrease was significant at the last four weeks showing a maximum percentage difference of 24.3 (t=3.053, P=0.012).

Table 4.16 Effect of cypermethrin (1/10 LD_{50}, 66.5 mg kg^{-1} body weight) on serum phosphorus (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Cypermethrin (n=6)	% difference	t-value	P-value
1	7.13±0.4	8.03±0.5	11.9	1.543	0.154
2	7.41±0.3	8.62±0.5	15.1	2.156	0.057
3	6.95±0.2	8.26±0.6	17.2	2.514	0.031
4	7.43±0.4	8.52±0.4	13.7	2.012	0.072
5	7.40±0.3	9.21±0.6	21.8	2.849	0.017
6	7.23±0.2	9.23±0.7	24.3	3.053	0.012

The number of animals (n) was 8 in control group and 6 in cypermethrin-treated animals. All values were expressed as mean±SEM. The level of significance was at P<0.05.

Chapter 5

Discussion

Pesticides are being extensively used in Gaze strip; a small densely populated area. This constitutes a real threat and impose adverse health effects on many non-target species including humans. One type of these pesticides in use are pyrethroids such as cypermethrin which is effective in controlling insects on vegetable crops, fruit and olives and to kill cockroaches, fleas, and termites in houses **(Palestinian Ministry of Agriculture, 2015).** However, cypermethrin has toxic effects on mammalian nervous system (neuropoison) as well as on the functions of other systems and organs including liver and kidney **(Singh et al., 2012 and Adjrah et al., 2013).** Therefore, investigating cypermethrin toxicity in rabbits can expand our understanding on potential hazards of this insecticide exposure in humans.

5.1 Toxicity of cypermethrin

Although cypermethrin is one of the commonest insecticides being used in the agricultural sector in Gaza Strip **(Yassin et al., 2002 and Palestinian Ministry of Agriculture, 2015)**, limited data are available on this toxic compound. Several cases of pesticides poisoning were reported among farm workers and children in Gaza Strip which mostly resulted from use/misuse of pesticides **(Yassin et al., 2002 and AL-Shanti, 2009)**. In this context, **Tomlin (2011)** satisfied by determining the oral LD_{50} of cypermethrin for rats and mice to be 250-4150 and 138 mg kg^{-1} body weight, respectively. However, to our best knowledge no previous study determine the oral LD_{50} of cypermethrin in domestic rabbits. Therefore, the present work was performed to determine the oral LD_{50} of cypermethrin and to investigate its toxicological effect on liver and kidney of male domestic rabbits. The present results showed that oral LD_{50} of cypermethrin in male domestic rabbit was 665 mg kg^{-1} body weight.

5.2 General health of rabbits

The present study demonstrates that treatment of rabbits with 1/10 LD_{50} cypermethrin induced an overall mortality rate of 16.7% throughout the 6 weeks of the experiment. Such mortality was mostly attributed to diarrhea which may be related to the cholinergic crisis as a result of marked inhibition of ChE enzyme activity detected in the present study in response to cypermethrin intoxication. Such findings are in agreement with that found by **(Wolansky and Harrill 2007; Grewal et al., 2010 and Adjrah et al., 2013).** Who observed that diarrhea was most prominent clinical sign of rats administered with different doses of cypermethrin. In addition, cypermethrin-treated rabbits showed hair loss especially in the last two weeks of the experiment. Similar result was obtained by **Omonona et al., (2015).** This coincided with the significant decrease in protein content observed in the present study. The livers of cypermethrin-treated rabbits showed scars of depressions also in the last two weeks of the experiment which may be due to distortion in the liver cells. cypermethrin in known to induce morphological and histopathological changes in the liver **(Yavasoglu et al., 2006 and Nair et al., 2011 and Mamun., 2014)**.

5.3 Body weight

As indicated in the current data body weight was significantly decreased in cypermethrin-supplemented rabbits compared to controls. This finding is in agreement with that obtained by **Sangha et al., (2013).** The reduction in body weight in response to cypermethrin intake may be a result of the combined action of cholinergic (Reduced food intake and diarrhea) and oxidative stress and/or due to increase or in degradation of lipids and proteins as a direct effect of cypermethrin pesticide exposure **(Adjrah et al., 2013).** This explanation is supported by the recorded significant decrease in protein content in cypermethrin-treated rabbits compared to controls.

5.4 Biochemical investigation

5.4.1 Serum glucose

Results presented in this study revealed that oral administration of 1/10 LD_{50} cypermethrin daily for 6 weeks caused significant increase in serum glucose levels throughout the whole experimental period examined. This finding is in concurrent with that reported by **Paul et al. (2009) and Veerappan et al. (2012).** Therefore, glucose homeostasis is affected by cypermethrin administration. The mechanism by which this Parathyroid insecticide induces hyperglycemia may involve one or more mechanisms: 1) reduction in insulin secretion as a result of the destructive action of cypermethrin on the beta cells of Langerehans islets in the pancreas **(Kalender et al., 2005 and Eraslan et al., 2008)**, 2) impairment in hepatic function due to oxidative changes, which reduce liver ability to glycogenesis **(Abdou et al., 2012 and Bhatti et al., 2014)**, and 3) stimulation of hepatic gluconeogenesis and glycogenolysis **(Veerappan et al., 2012 and Bhanu and Deepak, 2015).**

5.4.2 Liver enzymes and bilirubin

Data presented in this study showed that the mean levels of serum ALT, AST, ALP and γ-GT in the cypermethrin-treated rabbits were significantly higher than those in the controls. Such elevation of liver enzymes as a result of cypermethrin administration was documented by other authors **(Prakash et al., 2009; Bhushan et al., 2013 and Kanbur et al., 2015).** Liver is the center of biotransformation and detoxification of foreign compounds and is the most vulnerable to the chemical assaults such as cypermethrin poisoning **(Mansour and Mossa., 2010 and Heikal et al., 2012).** Serum ALT, AST and γ-GT are considered to be among the most sensitive markers employed in the diagnosis of hepatotoxicity **(Akhtar., 2009; Ambali et al., 2011 and Newairy and Abdou, 2013).** Pesticide exposure causes liver damage and leakage of cytosolic enzymes from hepatocytes and other body organs into blood **(Ncibi et al., 2008; Heikal et al., 2013 and Newairy and Abdou, 2013).** Elevation of liver enzymes may also be due to increased gene expression due to long term requirement of detoxification of pesticides **(Friedman et al., 2003).**

In contrast to elevation of transaminases, γ-GT and ALP, serum ChE activity was markedly decreased in cypermethrin-treated rabbits compared to controls. Such inhibition in ChE in response to cypermethrin administrated was obtained by **(Khan and Tabassum 2003; Wielgomas et al., 2007; Saxena and Yadav 2011 and Sharma et al., 2014).** The inhibition of ChE may reflect the neurotoxic effect induced by cypermethrin which was previously reported by **Singh et al., (2012) and Sharma et al., (2014**)**.** Cypermethrin-induced neurotoxicity is more likely to be a result of cholinergic crises which was manifested in neurobehavioral effects (disorientation, drowsiness, weakness, depression and mild tremors) observed in the present study. In addition, alteration of Ach receptors was proponent to be involucel in cypermethrin-induced neurotoxicity **(Singh et al., 2012).**

Data presented in this study showed that oral administration of cypermethrin to domestic rabbits caused gradual increase in bilirubin level, reaching its significance during the last two weeks of the experiment. Such increase was reported previously by **Bhushan et al., (2013) and Kanbur et al., (2015).** in cypermethrin-intoxicated rats. The change in serum bilirubin which is accepted as indicator of liver function may provide further evidence on hepatotoxicity induced by the cypermethrin insecticide **(Adjrah et al., 2013).** Hyperbilirubinemia may be a result of 1) destruction of red blood cells which release increased amounts of haemoglobin, and subsequent rise in bilirubin content of blood **(Ulaiwi, 2011; Bhushan et al., 2013 and Omonona et al., 2015)** and 2) liver damage and disturbance in bile excretion **(Singh et al., 2005 and Abdou et al., 2012).**

5.4.3 Kidney function

The influence of cypermethrin on kidney function was assessed through the measurement of urea and creatinine. Urea concentration was significantly increased throughout the whole experiment compared to the control. For creatinine this significant increase was also observed along the whole experiment except during the 1st week of the experiment. Such findings are in

agreement with that reported in other studies **(Saxena and Saxena., 2010; Sankar et al., 2012 and Sakr and Albarakai., 2014).** Urea is formed by the liver as an end product of protein breakdown and it is one marker of the kidney function (**Debra Manzella, 2008 and Tawfik and Al-Badr, 2012).** Increase in serum urea observed in the present study may be due to 1) impairment in its synthesis as a result of impaired hepatic function, 2) disturbance in protein metabolism and 3) decrease in its filtration rate in the kidney. The decrease in protein profile observed in the present study may support this explanation. Creatinine is break-down product of creatine phosphate in muscles, and is usually produced at a fairly constant rate by the body. Creatinine is chiefly filtered out of the blood by the kidneys and has been found to be a fairly reliable indicator of kidney function **(Tawfik and Al-Badr, 2012)**. As the kidneys become impaired for any reason, for example in case of cypermethrin poisoning, the creatinine level in the blood will rise due to poor clearance by the kidneys. A rise in blood creatinine level is observed with damage to functioning nephrons and impaired renal function **(Zama et al., 2007 and Ambali et al., 2010)**.

5.4.4 Protein profile

As indicated in the present results, significant decreases in the levels of total protein, albumin and globulin concentrations, commencing from the 3rd week of cypermethrin administration, were found in treated rabbits compared to controls. Similar findings were reported in other studies as a result of oral administration of different doses of cypermethrin **(Lakkawar et al., 2006; Ahmad et al., 2011; Veerappan et al., 2012 and Mhya et al., 2014).** The reduction in serum protein could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver. Also, the protein level suppression may be due to loss of protein either by redaction in protein synthesis or increased proteolytic activity or degradation **(Ncibi et al., 2008 and Shin and Moon, 2010)**. In addition, the observed decrease in serum proteins could be attributed in part to the damaging effect of cypermethrin on liver cells, as confirmed by the increase in activities of serum AST, ALT and γ-GT. It was reported that albumin levels are decreased in liver disease

(Khalifa et al., 2011). A decrease in globulin is expected as globulin (mostly γ-globulins) may be consumed in the production of antibodies in response to cypermethrin administration.

5.4.5 Electrolytes

In general, the mean serum concentration of calcium decreased significantly in cypermethrin-intoxicated rabbits compared to controls. In contrast, serum phosphorus concentration was generally increased in response to cypermethrin treatment. Similar results were documented by **(Atamanalp et al., 2002; Mishra et al., 2010).** This indicates that the insecticide cypermethrin interferes with calcium and phosphorus homeostasis. Hyperphosphatemia and hypocalcemia particularly at the last stages of the experiment were recorded by **Veerappan et al., 2012** in male albino rats exposed to low doses of cypermethrin. This suggests that phosphorous mobilization might have occurred as a result of a shift in the Ca:P ratio in bone which might lead eventually to demineralization of bone matrix.

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

1. The calculated oral LD_{50} of cypermethrin in male domestic rabbits from the linear regression was found to be 665 mg kg^{-1} body weight.
2. Daily oral administration of 1/10 LD_{50} cypermethrin caused an overall mortality rate of 16.7% compared to 0.0% in controls. Clinical signs of cypermethrin-intoxicated rabbits were diarrhea, disorientation, drowsiness and mild tremor.
3. The final body weight was significantly decreased in cypermethrin-treated rabbits compared to controls.
4. Serum glucose was significantly increased in response to cypermethrin feeding compared to controls.
5. Liver enzymes ALT, AST, ALP and γ-GT as well as bilirubin were significantly higher in the cypermethrin-intoxicated rabbits whereas cholinesterase level was markedly decreased compared to the controls.
6. Urea and creatinine concentrations were significantly increased in response to cypermethrin administration compared to the controls.
7. There were significant decreases in total protein, albumin and globulin values upon cypermethrin intake compared to the controls.
8. Hypocalcemia and hyperphosphatemia were recorded in cypermethrin-intoxicated rabbits.

6.2 Recommendations

1. Restriction the use of pesticides in home and farm.
2. The use of more security, such as biological control and the agricultural cycle.
3. Further studies are needed on the health impact other pesticides.

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