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**Faculty of science**  
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**Biological Science/ Zoology**

# **Hepatic and renal toxicity of dimethoate in male domestic rabbit**

**Submitted in Partial Fulfillment for the Degree of Master  
of Science in Biological Sciences-Zoology**

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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 children.*

*To my brothers and sisters for their valuable encouragement.*

*To my university the Islamic university of Gaza which is continuously improving the dedicate research.*

*Prof. Maged M. Yassin for his valuable guidance and advice.*

*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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# *Acknowledgment*

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# *Hepatic and renal toxicity of dimethoate in male domestic rabbit*

## **Abstract**

**Objective:** The present study is aimed to investigate hepatic and renal toxicity of dimethoate in male rabbit.

**Materials and Methods:** The oral LD<sub>50</sub> of dimethoate in the male domestic rabbits was calculated from logarithmic scale and found to be 432 mg/kg body weight. A dose of 1/10 LD<sub>50</sub> (43.2 mg/kg body weight) dimethoate was then used to test its toxicity. The experimental group were given orally the dose of 1/10 LD<sub>50</sub> dimethoate daily for six weeks. Control animals were given distilled water.

**Results:** The overall mortality rate was 11.1% in dimethoate-treated rabbits compared to 2.1% in controls. Clinical signs included disorientation, drowsiness, uncoordinated movements, mild tremor and diarrhea. Final body weight was significantly decreased in dimethoate-intoxicated rabbits. Serum glucose was significantly increased in dimethoate-fed rabbits recording a maximum percentage difference of 26.3% in the 4<sup>th</sup> week of the experiment compared to controls. The activities of serum ALT, AST,  $\gamma$ -GT and ALP were significantly higher in dimethoate-treated rabbits registering maximum percentage differences of 27.8, 35.9, 24.4 and 31.7% during 4<sup>th</sup>, 3<sup>rd</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> weeks of the experiment, respectively. In contrast, serum cholinesterase was markedly decreased showing a maximum percentage difference of 50.1% during the 5<sup>th</sup> week of the experiment. Serum bilirubin was gradually increased exhibiting a maximum percentage difference of 16.7% at the 5<sup>th</sup> week of the experiment. Serum urea and creatinine concentrations were significantly elevated in response to dimethoate administration displaying maximum percentage differences of 31.3 and 26.2% during the 6<sup>th</sup> and 4<sup>th</sup> weeks of the experiment, respectively. Serum total protein, albumin and globulin were significantly decreased upon dimethoate intake recording percentage differences of 28.0, 26.9 and 26.5 at the 4<sup>th</sup>, 6<sup>th</sup> and 3<sup>rd</sup> weeks of experiment, respectively. Serum calcium was significantly increased in

dimethoate-exposed rabbits showing a maximum percentage difference of 18.8% in the 4<sup>th</sup> week whereas serum phosphorus was significantly decreased showing a maximum percentage of 16.1% at 2<sup>nd</sup> week of the experiment.

**Conclusions:** Daily oral administration of 1/10 LD<sub>50</sub> dimethoate caused significance decrease in the body weight, serum Cholinesterase, total protein, albumine, globulin and phosphorus, whereas serum ALT, AST, ALP,  $\gamma$ -GT, urea, creatinine and calcium were significantly increased.

**Key words:** Dimethoate, toxicity, liver, kidney, rabbit.

# دراسة اثر سمية مادة الدايميثويت علي كبد وكلية ذكور الأرانب المنزلية

## ملخص الرسالة

**الهدف:** تهدف الدراسة الحالية لمعرفة مدي سمية مادة الدايميثويت علي كبد وكلية ذكور الأرانب.

**الطرق والادوات:** ولقد بينت الدراسة أن قيمة الجرعة النصف مميتة لدايميثويت في ذكور الأرانب بواسطة الفم هي 432 ملجرام/كجم من وزن الجسم. بناء علي هذه النتيجة تم دراسة سمية الدايميثويت عند جرعة قيمتها 43.2 ملجرام/كجم من وزن الجسم أي ان 1/10 من الجرعة النصف مميتة. المجموعة التجريبية أعطيت عن طريق الفم جرعة يومية مقدارها 43.2 ملجرام/كجم من وزن الجسم من مادة الدايميثويت لمدة ستة أسابيع. والمجموعة الضابطة تم إعطائها ماء مقطر بواسطة الفم.

**النتائج:** كان معدل الوفيات الإجمالية 11.1% في الأرانب المعالجة بالدايميثويت مقارنة مع 2.1% في المجموعات الضابطة. وشملت العلامات السريرية الارتباك، والنعاس، وحركات غير متناسقة، ورعاش خفيف والإسهال. إن النتائج سجلت انخفاض في وزن الجسم النهائي بشكل ملحوظ في الأرانب المعالجة بالدايميثويت. فلو حظ زيادة في مستوي الجلوكوز بدلالة إحصائية في الأرانب المغذية بالدايميثويت وسجلت اعلي نسبة فرق بمعدل 26.3% في الأسبوع الرابع من التجربة مقارنة بالمجموعة الضابطة. كما ان مستوي أنزيمات الكبد  $\gamma$ -GT ، ALP، AST، ALT كانت ذو دلالة إحصائية بنسبة اعلي في الأرانب المعالجة بالدايميثويت مسجلة اعلي نسبة فرق بمعدل 27.8، 35.9، 24.4 و 31.7% في فترة الأسبوع الرابع و الثالث و الثاني و الثالث من التجربة علي التوالي. في المقابل، شوهد انخفاض ملحوظ في مستوي أنزيم كولين استيريز وكانت اعلي نسبة فرق 50.1% خلال الأسبوع الخامس من التجربة. أظهرت الدراسة أيضا ازدياد في مستوي البيليروبين تدريجيا وكانت اعلي نسبة فرق بمعدل 16.7% في الأسبوع الخامس من التجربة. وكان مستوي تركيز كل من اليوريا والكرياتينين مرتفعة بشكل ملحوظ كاستجابة لمادة الدايميثويت المعطاة فظهر اعلي نسبة فرق بمعدل 31.3% و 26.2% خلال الأسبوع السادس والأسبوع الرابع من التجربة علي التوالي. كما انخفض كل من البروتين الكلي والاليومين والغلوبولين بشكل كبير مسجلا اعلي نسبة فرق بمعدل 28.0، 26.9 و 26.5% في الأسبوع الرابع والسادس والثالث من التجربة علي التوالي. كما أوضحت الدراسة زيادة في مستوي الكالسيوم للأرانب المعرضة للدايميثويت فشوهد اعلي نسبة فرق بمعدل 18.8% في الأسبوع الرابع بينما لوحظ انخفاض بدلالة إحصائية في مستوي الفسفور وكانت اعلي نسبة فرق تصل إلي 16.1% في الأسبوع الثاني من التجربة.

**الاستنتاجات:** إن تناول عشر الجرعة من الدايميثويت التي تقتل خمسين في المائة من الأرانب ( $1/10 LD_{50}$ ) عن طريق الفم سببت انخفاض بدلالة إحصائية في وزن الجسم، وأنزيم الكولين استيريز، البروتين الكلي، الألبومين، الجلوبيولين والفسفور، في حين كان مستوي كلا من  $\gamma$ -GT، ALP، AST، ALT ، واليوريا، والكرياتينين والكالسيوم زيادة بدلالة إحصائية.

**الكلمات المفتاحية:** دايميثويت، سم، كبد، كلي، أرنب.

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# CHAPTER 1

## INTRODUCTION

### 1.1 Overview

A pesticide is any substance or mixture of substances that is intended to repel or destroy a pest (United States Environmental Protection Agency, EPA, 2008). Pesticides are a large and diverse group of chemicals (Kamel and Hoppin, 2004) that are used to kill and eradicate rodents, fungi, insects and weeds (Mnif et al., 2011) as well as an important cause of morbidity and mortality in the developing countries (Saxena and Saxena, 2010).

A pesticide may be a chemical substance, biological agent (such as a virus or bacterium), antimicrobial, disinfectant or device used against any pest. Types of pesticides include: Algaecides, avicides, bactericides, fungicides, insecticides, acaricides, molluscicides, nematocides, rodenticides and virucides (Gilden et al., 2010).

Insecticides are pesticides used in particular against insects. One of the most widely used group of insecticides in the world is organophosphate (OP) compounds. Dimethoate (O,O-dimethyl-S-(N-methylcarbamoylmethyl phosphorodithioate) is an organophosphorus insecticide with a contact and systemic action. It is widely used against a broad range of insects and mites on a variety of fruit, vegetable, and field crops and is also used indoor to control houseflies (Meister, 1992). The most common trade names are digon, duragon, rebelate and dimate (Environmental Protection Agency, EPA, 2006).

The toxicological profile of dimethoate demonstrates that dimethoate, like other organophosphates, has anticholinesterase activity in all species tested including mice, rats and dogs (Costa, 2006). Inhibition of brain acetyl cholinesterase (AChE) at synapses by organophosphorus pesticides (OPP) results in accumulation of acetylcholine and over activation of acetylcholine

receptor at neuromuscular junction and in the autonomic and central nervous system. This will manifested in convulsions and even tremors leading in sever cases to death (Lotti, 2001).

Dimethoate is moderately toxic to mammals by ingestion, inhalation and dermal adsorption. Its toxicity is predicted from LD<sub>50</sub> (a dose that expected to cause death in 50% of animals). The reported acute oral LD<sub>50</sub> values for the technical product range from 180 to 330 mg/kg in the rat, while corresponding values in other species are 160 mg/kg in mice, 350-400 mg/kg in guinea pigs and 400-500 mg/kg in rabbits (Gallo and Lawryk, 1991 and Tomlin, 2006). Skin and eye acute percutaneous LD<sub>50</sub> for rats >2000 mg/kg. Inhalation LC<sub>50</sub> (4h) for rats >1.6 mg/l air (Tomlin, 2006).

Several studies addressed the toxic effect of dimethoate on the functions of several mammalian organs including liver and kidney. Dimethoate was reported to alter the level of the marker parameters related to the liver and kidneys in rats and mice (Kossmann et al., 1997 and Gomes et al., 1999). Significant increase in the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP ) and gamma glutamyl transferase ( $\gamma$ -GT) as well as the decrease in the levels of cholinesterase, bilirubin, total protein and albumin in the serum were the major diagnostic symptoms of liver diseases in animals and human (Chatterjea and Shinde, 2005; Attia and Nasr, 2009; Saafi et al., 2011 and Khan et al., 2013). The increase in the uric acid and creatinine in the serum are the major symptoms of glomerular filtration damage (Chatterjea and Shinde, 2005).

Pesticides are being used in large amounts in the Gaza Strip where the protective measures are poorly followed (Yassin et al., 2002). More than 544 metric tons of pesticides are used yearly in the Gaza strip. The insecticide represents 220-225 metric tons of these pesticides, 10-11 metric tons of these insecticides are dimethoate (Personal Communication with Ministry of Agriculture, Palestinian National Authority, 2012). These highly toxic compounds constitute a real threat on humans. The present work is intended

to investigate dimethoate 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 dimethoate exposure.

## **1.2 General objective**

The general objective of the present study is to assess hepatic and renal toxicity of dimethoate in male domestic rabbit.

## **1.3 Specific objectives**

1. To determine the oral LD<sub>50</sub> of dimethoate in male domestic rabbit.
2. To examine the effect of 1/10 LD<sub>50</sub> dimethoate on the general health and weight of male domestic rabbit.
3. To investigate the effect of 1/10 LD<sub>50</sub> dimethoate on serum glucose level.
4. To study the effect of 1/10 LD<sub>50</sub> dimethoate on liver function through measurement of ALT, AST, ALP,  $\gamma$ -GT and cholinesterase as well as bilirubin.
5. To test the effect of 1/10 LD<sub>50</sub> dimethoate on kidney function through determination of urea and creatinine.
6. To investigate the effect of 1/10 LD<sub>50</sub> dimethoate on the total protein, albumin and globulin.
7. To study the effect of 1/10 LD<sub>50</sub> dimethoate on electrolytes, calcium and phosphorus.

## **1.4 Significance**

1. Dimethoate is being widely used in agriculture in Gaza Strip with lack of protective measures.
2. Studies on dimethoate 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 dimethoate toxicity.

# **CHAPTER 2**

## **LITERATURE REVIEW**

### **2.1 Definition of pesticide**

A pesticide is any substance or mixture of substances intended for preventing, destroying or repelling any pest. Pests can be insects, mice and other animals, unwanted plants (weeds), fungi, or microorganisms like bacteria and viruses. Though often misunderstood to refer only to insecticides (Kill insects and other arthropods), the term pesticide also applies to herbicides (kill weeds and other plants that grow where they are not wanted), fungicides (kill fungi including blights, mildews, molds, and rusts), Rodenticides (control mice and other rodents), and various other substances used to control pests. Under United States law, a pesticide is also any substance or mixture of substances intended for use as a plant regulator, defoliant or desiccant (Environmental Protection Agency, EPA, 2006).

### **2.2 Definition and classification of insecticides**

An insecticide is a pesticide used against insects. They include ovicides and larvicides used against the eggs and larvae of insects, respectively. Insecticides are used in agriculture, medicine, industry and the household. The use of insecticides is believed to be one of the major factors behind the increase in agricultural productivity in the 20<sup>th</sup> century (Van Emden and Pealall, 1996). Insecticides can be classified according to the type of action into organochlorine, organophosphates, carbamates, pyrethroids, neonicotinoids, biological insecticides and antifeedants (Brown, 2006).

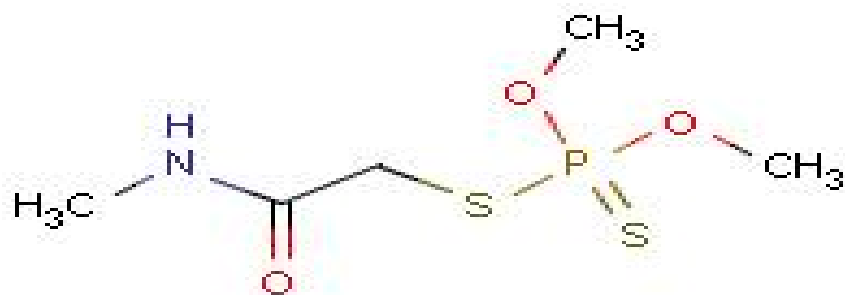
## 2.3 Organophosphorus insecticides

Organophosphorus insecticides are highly toxic compounds containing active phosphorus. They are classified into three groups: phosphorothionate group, in which phosphorus is bound to three oxygens and one sulfur (the double bond). Phosphorothionates include chlorpyrifos, parathion, and tebupirimphos. Compounds in the phosphorodithioate group are like the phosphorothionates but with one of the oxygens replaced by sulfur. Phosphorodithioates include malathion, disulfoton, azinphos-methyl, sulprofos, and dimethoate. The atoms bound to the phosphorus of phosphoroamidothiolates are nitrogen, sulfur, and two oxygens; the double bond is to an oxygen. Examples of phosphoroamidothiolates are acephate and methamidophos (Chambers, 1992).

## 2.4 Dimethoate

### 2.4.1 Definition

Dimethoate is the ISO common name for O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate or 2-dimethoxyphosphinothioylthio-N-methylacetamide (IUPAC). Dimethoate belongs to the class of aliphatic amide organothiophosphate insecticides such as omethoate and mecarbam. It belongs also to the classes of organothiophosphate acaricides. Dimethoate was first described by Hoegberg and Cassaday in 1951 and introduced in market in 1956 (Fischer et al., 1997). It was first registered in the United States in 1962 (EPA, 2006). The chemical structure of dimethoate is illustrated in figure 2.1. (Chemfinder, 2006 and Tomlin, 2006).



**Figure 2.1** Chemical structures of dimethoate (Chemfinder, 2006 and Tomlin, 2006).

World Health Organization (WHO) hazard classification of dimethoate is “Class II, moderately hazardous” (World Health Organization, 2002). EPA has registered dimethoate as a systemic organophosphate insecticide but in 2006 it released Interim Re-registration Eligibility Decision (IRED) document for dimethoate in accordance with FQPA requirements (Bhandare et al., 2011). Dimethoate is designed to be effective by direct contact, ingestion, and inhalation.

#### **2.4.2 Physical and chemical properties of dimethoate**

Dimethoate is a white crystalline solid with a mercaptan odor (EPA, 2006). Dimethoate is a highly mobile, generally non-persistent organophosphate insecticide (USEPA, 2004). Dimethoate has a low environmental persistence (Extension Toxicology Network, 1996b). The principal chemical properties of dimethoate are compiled in Table 2.1 (Kidd and James, 1994 and Tomlin, 2006).

Table 2.1 Physical and chemical properties of dimethoate (Kidd and James, 1994; Young, 2001; European Union Draft Assessment Report, 2005 and Tomlin, 2006).

Property	Values
Molecular weight	229.30 g/mol
Melting point	50.0–51.5 (99.5% purity)°C
Solubility in water	25 g/L at 21°C
Vapor pressure	3 x 10 <sup>-12</sup> mmHg 1.85 x 10 <sup>-6</sup> mmHg at 25°C
Density	1.31 g/cm <sub>3</sub> (purity 99.1% w/w)

Physical and chemical properties of dimethoate.

### 2.4.3 Mechanism of action of dimethoate

Dimethoate like other organophosphates inhibits acetylcholinesterase activity; an enzyme that breaks down the neurotransmitter acetylcholine on synapses and neuromuscular junction.

#### 2.4.3.1 Acetylcholine as a neurotransmitter

Acetylcholine is an important neurotransmitter in both insects and mammals; it is released at the nerve synapse in response to a membrane depolarization which is the hallmark of nerve transmission. The acetylcholine then binds to a protein receptor in the membrane of the nerve synapse (Figure 2.2.A), which then opens/alters an ion channel, which in turn causes changes in the fluxes of ions (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>+</sup>, and Cl<sup>-</sup>) ultimately perpetuating the nerve impulse (Lee and Sine, 2005 and Sine and Engel, 2006). There are two types of acetylcholine receptors (AChR) that bind acetylcholine and transmit its signal:

**1. Muscarinic receptors** (mAChRs) at which muscarine action mimics the stimulatory action of acetylcholine on smooth muscle and gland. Muscarinic

receptors are blocked by atropine. There are five subtypes of muscarinic AChRs based on pharmacological activity M1- M5 (Mohamadi et al., 2009).

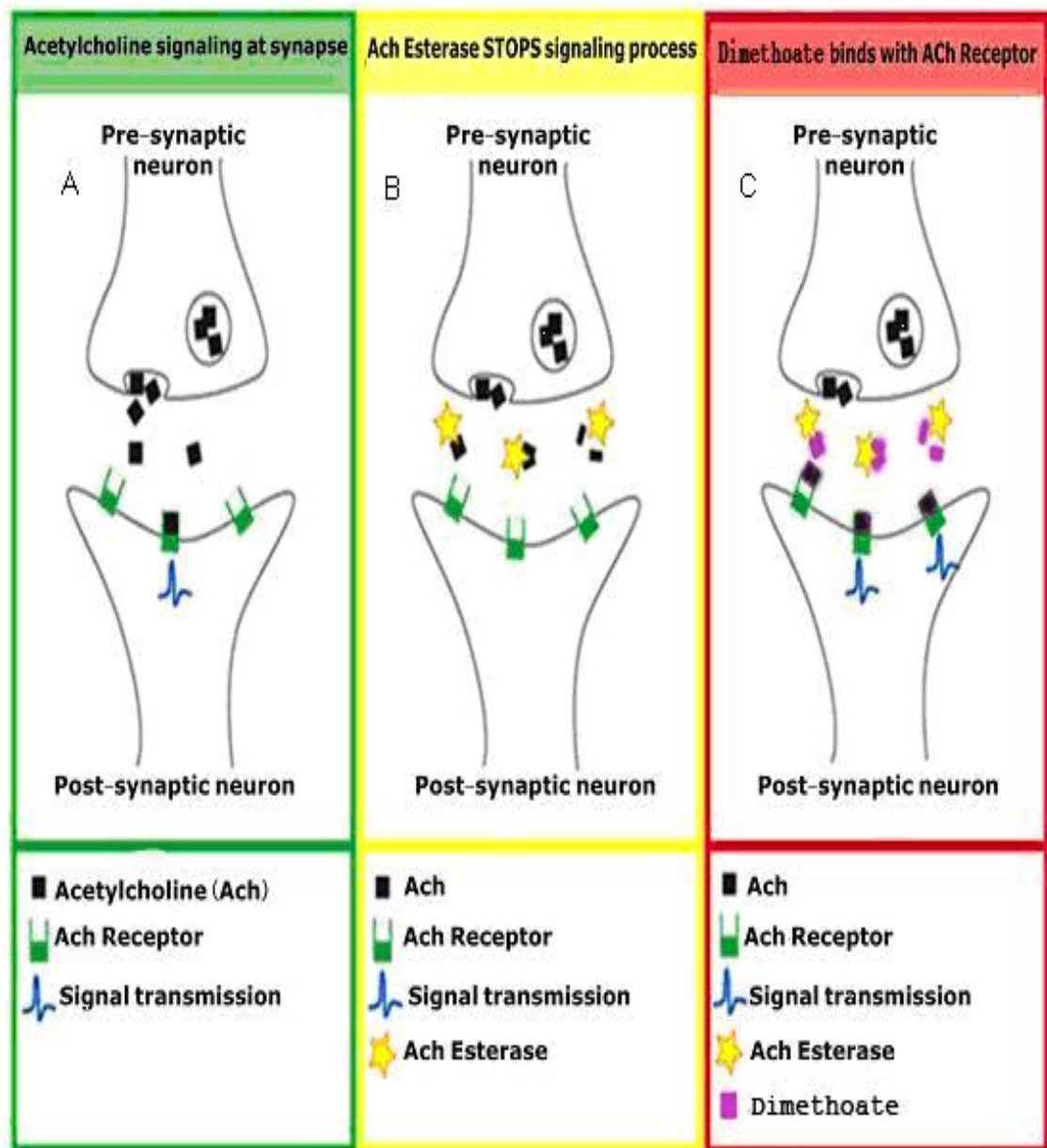
**2. Nicotinic receptors** (nAChRs) which is stimulated by small amount of nicotine whereas a large amount of nicotine blocks the receptor. This effect mimics the action of acetylcholine on nicotinic receptor. The nicotinic acetylcholine receptors are members of a superfamily of ligand-gated ion channels (Dani, 2001 and Hogg et al., 2003). Nicotinic receptors subdivided into those found in muscle at neuromuscular junctions and those found in autonomic ganglia and the central nervous system.

#### **2.4.3.2 Acetylcholinesterase**

As illustrated in Figure 2.2.B, once acetylcholine makes its action, it is subsequently destroyed by acetylcholinesterase enzyme, and the membrane returns to its normal resting state (Liu and Casida, 1993 and Zwart et al., 1994).

#### **2.4.3.3 Acetylcholinesterase as a target for dimethoate**

Dimethoate binds to acetylcholinesterase enzyme in an irreversible manner leading to its inhibition (Figure 2.2.C). Acetylcholinesterase inhibition at synapses results in accumulation of acetylcholine and over activation of acetylcholine receptor at neuromuscular junction and in the autonomic and central nervous system. This will manifest in convulsions and even tremors leading in severe cases to death (Lotti, 2001 and Wiener and Hoffman, 2004).



**Figure 2.2.** Pathophysiology of dimethoate insecticides poisoning.

#### 2.4.4 Toxicity symptoms of dimethoate poisoning

Accumulation of acetylcholine at cholinergic synapses as a result of acetylcholinesterase inhibition producing a range of clinical manifestations, known as the acute cholinergic crisis. The particular clinical features depends on the type of receptors and their location (Eyer, 2003; Eddleston et al., 2006; Karalliedde et al., 2006 and Paudyal, 2008).

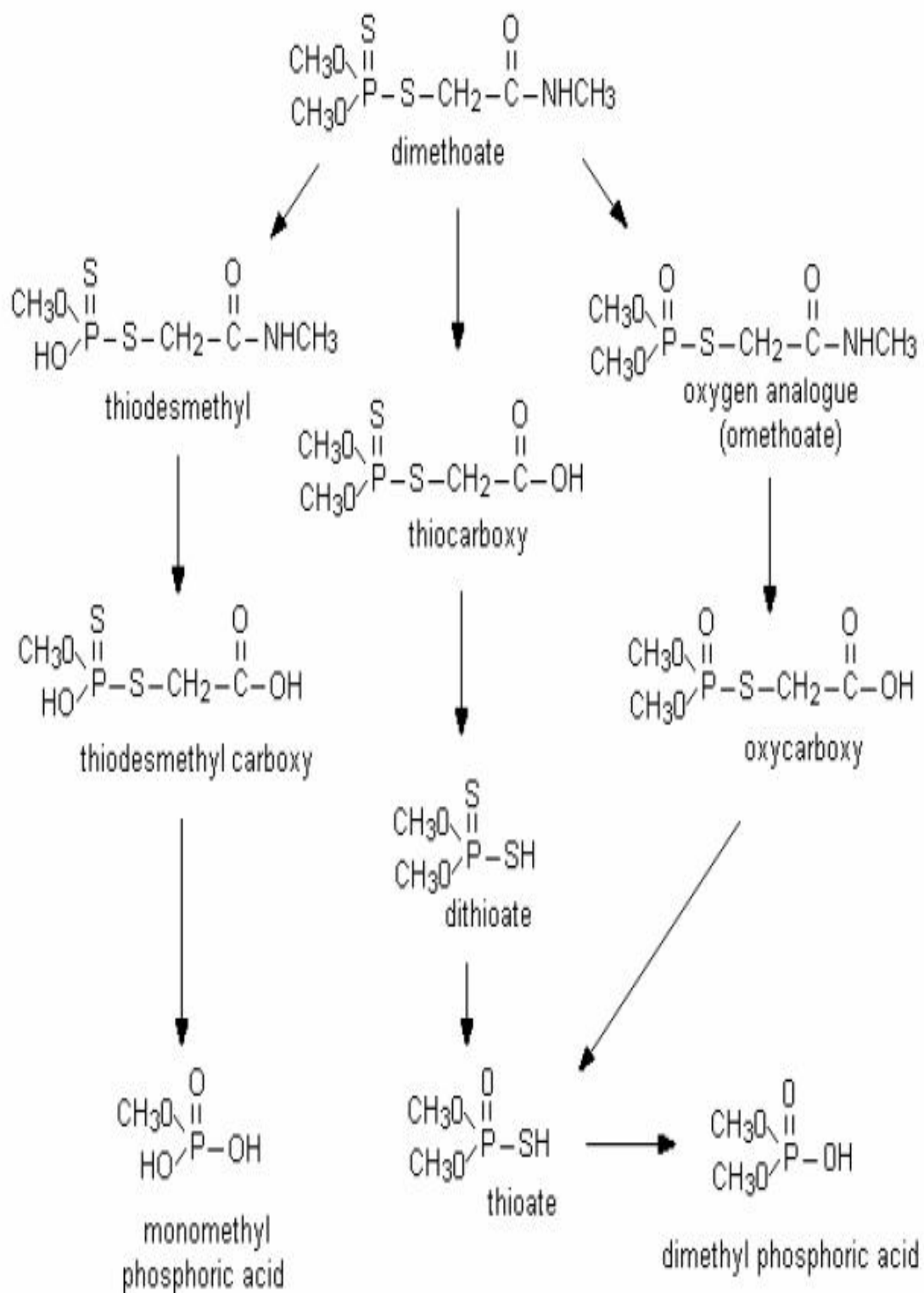
**A. Muscarinic receptors:** diarrhea, urinary frequency, meiosis, bradycardia, bronchorrhoea and bronchoconstriction, emesis, lacrimation, salivation, hypotension and cardiac arrhythmias.

**B. Nicotinic receptors:** fasciculations and muscle weakness, which may progress to paralysis and respiratory failure, mydriasis, tachycardia and hypertension.

**C. Central nervous system:** altered level of consciousness, respiratory failure and seizures.

#### **2.4.5 Metabolism of dimethoate**

Dimethoate is rapidly absorbed from the gastrointestinal tract. The radiolabel dimethoate was found in the liver, bile, kidneys and urine, and not in fat tissues after dosing. The proposed metabolic pathway consisted of hydrolytic (major) and oxidative (minor) pathways (Figure 2.3). The hydrolytic pathway involved cleavage of the C-N bond to yield dimethoate carboxylic acid that was subsequently metabolized to dimethyldithiophosphate, dimethylthiophosphoric acid and dimethyl phosphoric acid. The oxidative pathway involved oxidation of dimethoate to its oxon analogue (Omethoate) that was subsequently metabolized to dimethylthiophosphoric acid and dimethylphosphoric acid. Loss of the methoxy groups of the parent to yield CO<sub>2</sub> was another minor metabolic pathway (USEPA/Office of Pesticide Programs, 1995 and FAO/WHO, 1997).



**Figure 2.3.** Metabolic pathway for dimethoate in rats (Food and Agriculture Organization/ World Health Organization, FAO/WHO, 1997)

#### **2.4.6 Excretion of dimethoate**

About 45% and 5.8% of the radiolabelled dimethoate administered orally were excreted in the urine and the feces, respectively, in rats at 72 h after treatment. The equivalent values in rats after dermal application were 31% and 6.5%, respectively. More than 95% of excreta in the urine after the oral or dermal administration to rats were hydrolytic products (Health Canada Pest Management Regulatory Agency, 2011).

#### **2.4.7 Distribution of dimethoate**

Radiolabeled dimethoate was well absorbed from the gastrointestinal tract following oral administration to rats. Maximum plasma and tissue concentrations were achieved 0.5 hour following dosing and showed a similar distribution in both sexes following a dose of 10 mg/kg body weight. In tissues, radioactivity was primarily detected in the liver and kidney, with the lowest levels found in the brain and fat (Kirkpatrick, 1995).

#### **2.4.8 Uses of dimethoate**

Dimethoate is an organophosphate insecticide more frequently used to kill mites and insects systemically and on contact. It is used against a wide range of insects, including aphids, thrips, planthoppers, and whiteflies on ornamental plants, alfalfa, apples, corn, cotton, tobacco, tomatoes, watermelons, wheat, and other vegetables. It is also used as a residual wall spray in farm buildings for house flies. Dimethoate has been administered to livestock for control of botflies (Hayes and Laws, 1990; Extension Toxicology Network 1996a; Tomlin, 1997 and Srivastav et al., 2010).

#### **2.4.9 Physiological effects of dimethoate**

Reena et al. (1989) investigated chronic effects of a sublethal dose (150 mg/kg body weight) of dimethoate on blood constituents in rats after exposure

of 15 and 30 days. After 30 days of exposure, the levels of blood glucose, cholesterol, urea, total bilirubin and the activities of AST, ALT and amylase markedly increased, but the activities of acid phosphatase and cholinesterase significantly decreased. There was no effect on total plasma protein content. The rats exposed to dimethoate for 30 days showed more prominent changes in all the blood constituents than those exposed for 15 days.

The effect of dimethoate on Wistar rats was studied (Sivaswamy and Balachandran, 1990 and Sivaswamy, 1991). Alkaline phosphatase activity was found to be increased in liver and kidneys, and decreased in the intestines. In addition, Milillo et al. (1993), studied the occasional ingestion of dimethoate by sheep. They found that when 5 sheep were given grass sprayed with dimethoate at 4.5 liters/50 m<sup>2</sup>, there was a fall in serum cholinesterase values for 24h. Furthermore, Hassan et al. (1994) and Attia (1995) reported significant decrease in body weight in response to dimethoate administration in rats.

Abd-Allah (1998) evaluated dimethoate for its mammalian toxicity in albino rats. Dimethoate was administered orally at doses 1/10 and 1/30 LD<sub>50</sub> daily for 30, 60 and 90 days. The body weight gain of the animals was significantly reduced. The activity of serum cholinesterase was significantly inhibited in all the tested periods. Serum AST, ALT and ALP activities were generally increased at all intervals studied. Albumin and total protein concentrations were decreased at 30 and 60 days intervals.

Selmanoglu-Ozmen (2001) reported that ALP and AST were significantly increased whereas cholinesterase was significantly decreased in response to oral administration of dimethoate at a dose of 20.4 mg/kg daily for 3.5 months in Wistar albino male rats. Decreased activity of serum cholinesterase was also detected in Wistar rats in response to 12.5 mg/kg body weight of dimethoate in diet for 4 weeks (Kaspers et al., 2004).

Hypercalcemia and hypophosphatemia were recorded by Mahjoubi-Samet et al. (2005) in rats exposed to 40 mg/kg body weight dimethoate. In addition,

Kamath and Rajini (2007) investigated the effect of repeated sublethal doses of dimethoate on glucose homeostasis in adult rats. They found that daily oral administration of dimethoate (20 and 40 mg/kg body weight) for 30 days induced a significant increase in blood glucose levels which was associated with impaired glucose tolerance.

Sayim (2007) evaluated the subchronic toxicity of orally administered dimethoate in Wistar albino rats, based on biochemical findings in the liver. The animals of the exposed groups were fed with laboratory chow combined with 2, 8 or 20 mg/kg body weight/day dimethoate for 90 consecutive days under controlled laboratory conditions. Results showed that there were decreases in relative liver weights of exposed rats. Although liver total protein levels were significantly increased, liver cholinesterase activities were decreased in all exposed groups.

Mahjoubi-Samet et al. (2008) investigated the effect of dimethoate on kidneys of adult rats and their suckling pups. Female Wistar rats were given daily dimethoate in drinking water 0.2 g/L equivalent to 40 mg/kg bw from day zero until day 10 after delivery. In test group the authors have found higher plasma levels and lower urinary levels of creatinine, and urea than in the controls. In addition, Attia and Nasr (2009) recorded significant inhibition of serum cholinesterase and significant increase in the levels of AST, ALT, ALP and  $\gamma$ -GT in rats received a single dose of dimethoate (75 mg per kg body weight). Urea, creatinine, uric acid and bilirubin levels were increased, whilst serum total protein, albumin and globulin were significantly decreased.

The toxic effects of dimethoate on the biochemical parameters in male rabbits was studied (Salih, 2010). Twenty healthy rabbits (1500-1700 gm) were divided into control group: 10 animals treated with a single daily dose of 5ml corn oil orally for 20 days and dimethoate group: 10 animals treated with a single daily dose of 1/4 of LD<sub>50</sub> of dimethoate (20 mg/kg) in 5ml corn oil orally for 20 days. . Data showed that the treatment with 1/4 of LD<sub>50</sub> of dimethoate resulted in a statistically high significant increase in the levels of serum ALT, AST and ALP as compared to the control. In contrast, total protein and

albumin levels were significantly decreased in the serum of rabbits treated with dimethoate. In addition, serum uric acid and creatinine levels were significantly increased in dimethoate treated rabbits compared to controls.

Saafi et al. (2011) found that daily oral administration of 20 mg/kg body weight dimethoate to males, adult Wistar albino rats caused hepatotoxicity as monitored by the increase in the levels of hepatic markers enzymes (ALT, AST, ALP and  $\gamma$ -GT), as well as in bilirubin. Similarly, AL-Awthan et al. (2012) demonstrated significant increase in the levels of various serum marker enzymes of liver, including AST, ALT and ALP in response to oral administration of 1/50 LD<sub>50</sub> dimethoate to guinea pigs. In addition, Saafi-Ben Salah et al. (2012) showed that feeding of Wistar rats with dimethoate for two months induced a marked renal failure characterized by a significant increase in serum creatinine and urea levels.

# CHAPTER 3

## MATERIALS AND METHODS

### 3.1 Experimental animals

Healthy adult male domestic rabbits weighting 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 x 60 x 60 cm. A commercial balanced diet (Anbar) and water were provided *ad libitum* all over the experimental period.

### 3.2 Determination of dimethoate LD<sub>50</sub>

A total number of 80 rabbits were used for determination of LD<sub>50</sub> of dimethoate. Animals were divided into ten groups (8 rabbits/group). The first nine groups (I - IX) were administered different single doses of dimethoate ranging from 250 to 650 mg/kg body weight as follows:

LD <sub>50</sub> determination groups	Dose (mg/kg body weight)
Group I	250
Group II	300
Group III	350
Group IV	400
Group V	450
Group VI	500
Group VII	550
Group VIII	600
Group IX	650
Group X control group	-

The tenth group was served as control group. Dimethoate 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 rabbit was held between its two ears so that the esophagus opening was clearly and unobstructively opened. The gastric tube was filled with the required dose of dimethoate then smoothly inserted until it's adequately enters the upper part of esophagus where its contents were emptied. The animals were observed for mortality during the 48 hour observation period. The LD<sub>50</sub> was determined by graphical method (Manna et al., 2004).

### **3.3 Dimethoate toxicity experiments**

A dose of 1/10 of LD<sub>50</sub> dimethoate was given orally to assess dimethoate toxicity in male domestic rabbit. Animals were divided into two groups: control and experimental groups. The control group comprised 48 rabbits (eight rabbits were housed in each cage) and the experimental group included 36 rabbits (six rabbits were housed in each cage). The experimental group was orally administrated dimethoate for overall experimental duration of six weeks. Control animals were given distilled water. Administration of dimethoate was also done by the special stomach tube. Dimethoate was purchased from the Palestinian Ministry of Agriculture.

### **3.4 General health**

#### **3.4.1 Mortality rates**

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

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

### 3.4.2 Clinical sings

Clinical symptoms were observed daily by the researcher himself.

## 3.5 Body weight

Animals were individually weighted at weekly intervals in order to detect any changes in their body weights. A sensitive balance (Modal: Ona-15 Teff Istanbul ) was used and weights were recorded to the nearest gram.

## 3.6. Physiological studies

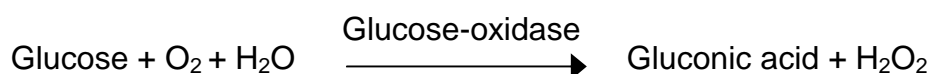
### 3.6.1 Blood sampling and processing

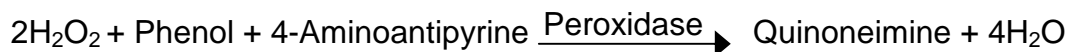
Animals from both experimental and control groups were decapitated weekly. Blood samples was be collected in centrifuge tubes. The collected blood was allowed to clot and then centrifuged at 3000 r.p.m. for 15 minute. Serum samples were separated in glass tubes for biochemical assay.

### 3.6.2 Determination of serum glucose

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

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.





## Reagents

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

## Procedure

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

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

1. Mix well and incubate at 37 °C for 10min. or 20min. at 20-25 °C.
2. Measure the absorbance of sample and std/cal within 60 minutes against reagent blank at wavelength 500 nm.

## Calculation

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

## Reference value (fasting glucose)

(Palestinian Clinical Laboratory Tests Guide, PCLTG, 2005)

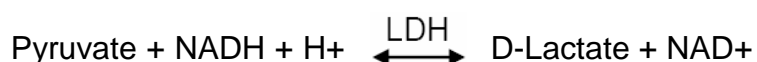
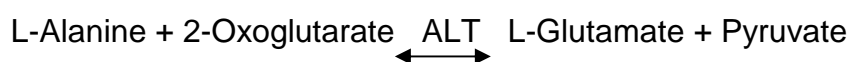
Child	60 – 100 mg/dl
Adult	70 – 110 mg/dl

### 3.6.3 Determination of liver enzymes

#### 3.6.3.1 Determination of serum alanine aminotransferase

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

#### Principle



#### Reagents

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

<b>Sample</b>	
<b>Monoreagent</b>	1000 $\mu$ l
<b>Sample</b>	100 $\mu$ l

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

### Calculation

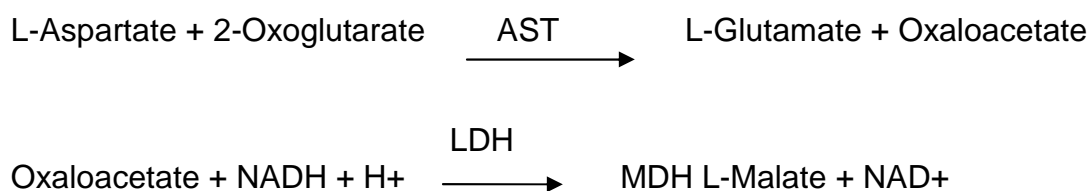
From absorbance reading calculates  $\Delta A$  /min and multiply by the corresponding factor:

$\Delta A$  /min X factor (1745) = ALT activity [U/l]

### 3.6.3.2 Determination of serum 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, 1998a) using DiaSys reagent kits.

### Principle



## Reagents

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

	<b>Sample</b>
<b>Monoreagent</b>	1000 µl
<b>Sample</b>	100 µl

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

## Calculation

From absorbance reading calculates  $\Delta A$  /min was calculated and multiply by the corresponding factor:

$$\Delta A / \text{min} \times \text{factor (1745)} = \text{AST activity [U/l]}$$

### 3.6.3.3 Determination of serum 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



#### Reagents

Components	Concentration
<b>Reagent 1</b>	
2-Amino-2-methyl-1-propanol pH10.4	1.1 mol/l
Magnesium acetate	2 mmol /l
Zinc sulphate	0.5 mmol/l
HEDTA	2.5mmol/l
<b>Reagent 2</b>	
p-Nitrophenylphosphate	80 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	Sample
Monoreagent	1000 µl	1000 µl
Sample	-	20 µl
Dist. Water	20 µl	-

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

## Calculation

From absorbance reading calculates  $\Delta A$  /min and multiplies by the corresponding factor:

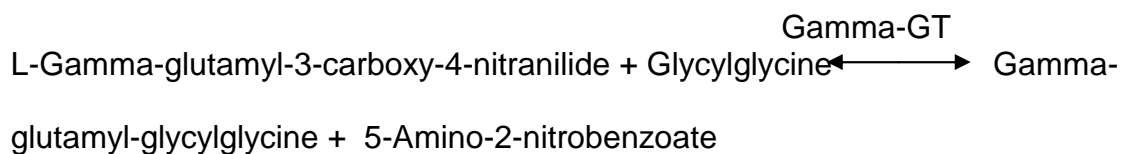
$$\Delta A / \text{min} \times \text{factor (2757)} = \text{ALP activity [U/l]}$$

### 3.6.3.4 Determination of serum gamma glutamyl transferase

Serum gamma glutamyl transferase ( $\gamma$ -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/Persijn (Szasz, 1974 and Persijn and van der Silk, 1974) . 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

Gamma-glutamyl transferase 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  $\gamma$ -GT.



## Reagents

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

## Procedure

### Substrate start

	Blank	Sample
<b>Sample</b>	-	100 µl
<b>Dist. Water</b>	100 µl	-
<b>Reagent 1</b>	1000 µl	1000 µl
<b>Reagent 2</b>	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
<b>Sample/Calibreate</b>		100 µl
<b>Dist. Water</b>	100 µl	
<b>Monoreagent</b>	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  $\Delta A/\text{min}$  and multiply by the corresponding factor from table below:

	According to Szasz	According to IFCC
<b>Substrate start 405 nm</b>	1421	1606
<b>Sample start 405 nm</b>	1158	1309

With calibrator:

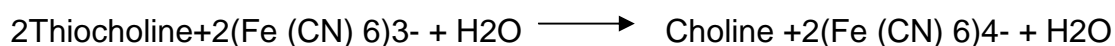
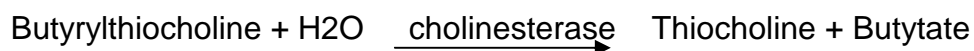
$$\gamma\text{-GT (U/L)} = \frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Calibrator}} \times \text{conc. Calibrator (U/L)}$$

### 3.6.3.5 Determination of serum 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.



## Reagents

Components	Concentration
<b>Reagent 1</b> Pyrophosphate pH 7.6 Potassium hexacyanoferrate(III)	75 mmol/L 2 mmol/L
<b>Reagent 2</b> Butyrylthiocholine	15 mmol/L

## Procedure

	Reagent /blank	Sample
<b>Sample</b>	-	20 µl
<b>Dist. Water</b>	20 µl	-
<b>Reagent 1</b>	1000 µl	1000 µl
Mix, incubate approx.3 min, and then add:		
	Reagent /blank	Sample
<b>Reagent 2</b>	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.

$$\Delta A/\text{min} = [\Delta A/\text{min Sample}] - [\Delta A/\text{min Blank}]$$

## Calculation

Calculate  $\Delta A/\text{min}$  and multiply with 68500 =cholinesterase activity U/L.

### 3.6.4 Determination of serum total 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 are 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 ml	-	-
Working Reagent	1.0 ml	-	1.0 ml	1.0 ml

2- Mix thoroughly and let stand the tubes 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 standard at 540 nm against the reagent blank.

## Calculations

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

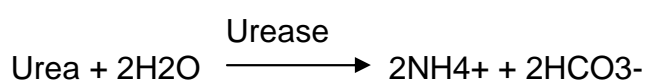
$$\frac{A_{\text{Sample}} - A_{\text{Sample Blank}}}{A_{\text{Standard}}} \times C_{\text{standard}} = C_{\text{Sample}}$$

## 3.6.5 Non-protein nitrogen constituents

### 3.6.5.1 Determination of serum urea

Serum urea was determined by using "Urease-GLDH": enzymatic UV test, according to Thomas method (Thomas, 1998b) using DiaSys reagent kits.

#### Principle



#### Reagents

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

### Calculation

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

### 3.6.5.2 Determination of serum 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 picric acid solution. The difference in absorbance at fixed time during conversion is proportional to the concentration of creatinine in the sample.

Creatinine + picric acid  $\longrightarrow$  Creatinine picric acid complex

## Reagents

Component	Concentration
<b>Reagent 1</b>	
Sodium hydroxide	0.16 mol/l
<b>Reagent 2</b>	
Picric acid	4.0 mol/l
Standard	2 mg/dl (177 µmol /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
<b>Monoreagent</b>	1000 µl	1000 µl	1000 µl
<b>Sample</b>	-	-	50 µl
<b>Std./Cal.</b>	-	50 µl	-
<b>Dist. water</b>	50 µl	-	-

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

## Calculation

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

$$\Delta A = [(A2 - A1) \text{ sample or standard}] - [(A2 - A1) \text{ Blank}]$$

### 3.6.6 Protein profile

#### 3.6.6.1 Determination of serum 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 form a violet blue color complex in alkaline solution. The absorbance of color is directly proportional to concentration.

##### Reagents

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

##### Monoreagent preparation

Four parts of R1 were mixed with 1 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:

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

### 3.6.6.2 Determination of serum 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 from yellow-green to green blue.

## Reagents

Components	Concentrations
<b>Reagent</b>	
Citrate buffer      pH 4.2	30 mmol/L
Bromocresol green	0.26 mmol/L
<b>Standard</b>	5g/dl

## Procedure

	Blank	Sample
<b>Reagent</b>	1000 µl	1000 µl
<b>Sample</b>	-	10 µl
<b>Dist. Water</b>	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:

$$\text{Albumin [g/dL]} = \frac{(\Delta A_{\text{sample}})}{(\Delta A_{\text{standard}})} \times \text{Conc. Std [g/dl]}$$

### 3.6.6.3 Determination of serum globulin

Globulin was calculated according the following formula:

$$\text{Globulin} = \text{Total protein} - \text{Albumin}$$

### 3.6.7 Electrolytes

#### 3.6.7.1 Determination of serum calcium

Serum calcium was determined by Photometric test with cresolphthalein complexone (Thomas, 1998) using DiaSys reagent kit.

##### Principle

Cresolphthalein complexone 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 complexone 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 °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 ml	1 ml	1 ml
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

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

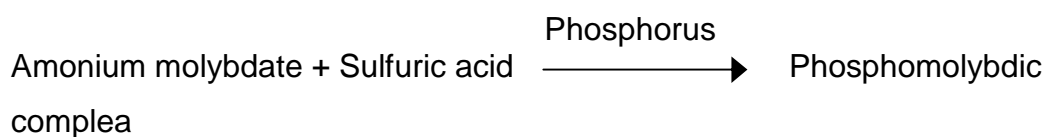
n = standard calcium concentration

### 3.6.7.2 Determination of serum phosphorus

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

### Principle

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



### Reagents

	Components	Concentrations
Reagent	Sulfuric acid	210 mmol/L
	Amonium molybdate	650 umol/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 ml	1 ml	1 ml
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  $P < 0.05$ .

**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<sub>50</sub> of dimethoate were plotted using Microsoft Excel program 2013.

# CHAPTER 4

## RESULTS

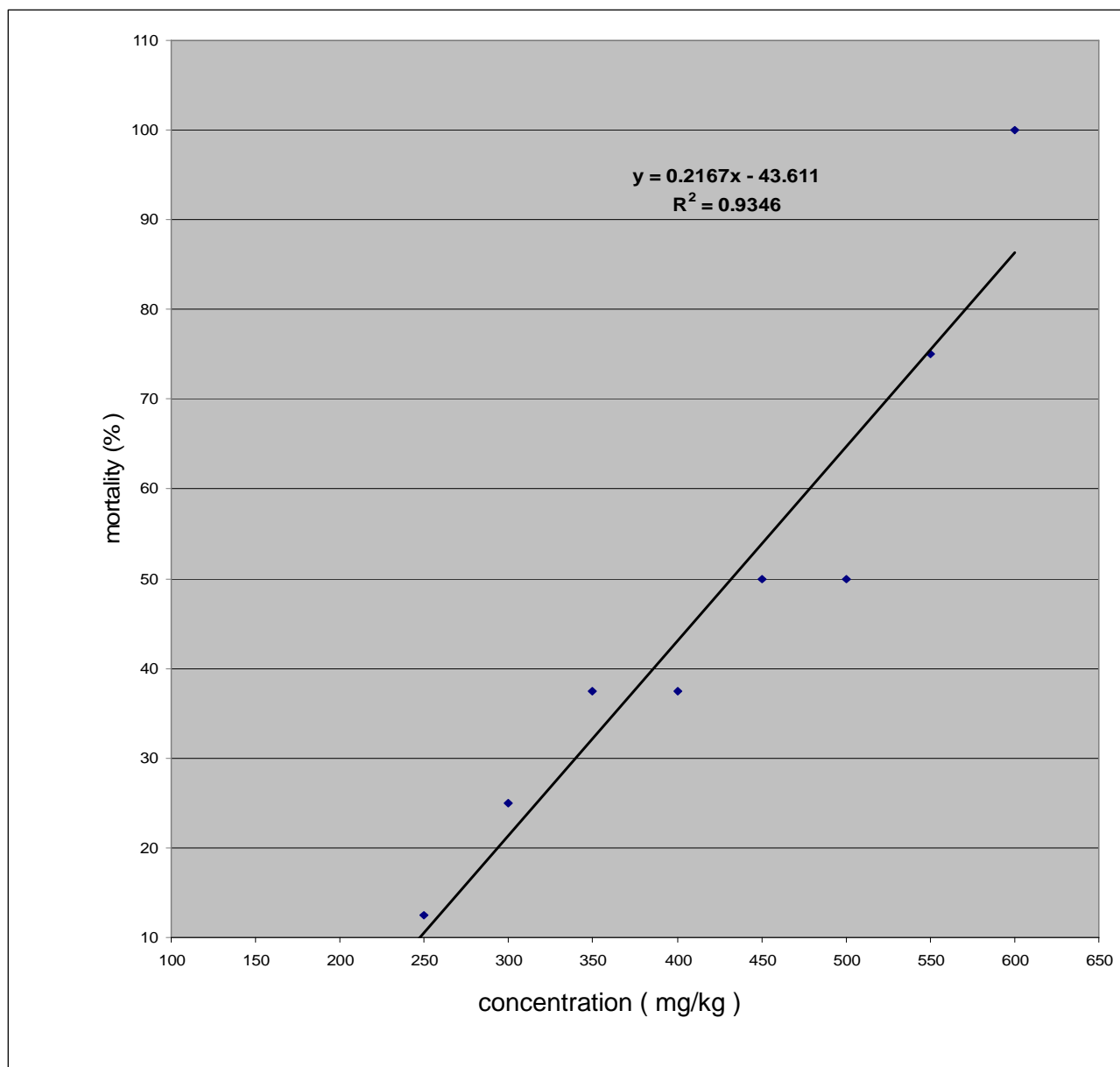
### 4.1 Oral LD<sub>50</sub> of dimethoate

The experimental trials for oral LD<sub>50</sub> determination of dimethoate after 48hr of administration in male domestic rabbits revealed that the mortality commenced at 250 mg/kg body weight, recording mortality percentage of 12.5% (Table 4.1). Increasing dimethoate dose to 300, 350, 400, 450, 500 and 550 mg/kg body weight resulted in mortality percentages of 25.0, 37.5, 37.5, 50.0, 50.0 and 75.0% respectively. The mortality rate was a function of dose increase. The maximum concentration of dimethoate which kill all animals in the group was found to be 600 mg/kg body weight. The calculated oral LD<sub>50</sub> of dimethoate in male domestic rabbits from the linear regression was found to be 432 mg/kg body weight (Figure 4.1).

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

Group	Dimethoate Dose (mg/kg body weight)	Number of Animals died/total	Mortality (%)
Group I	250	1/8	12.5
Group II	300	2/8	25
Group III	350	3/8	37.5
Group IV	400	3/8	37.5
Group V	450	4/8	50
Group VI	500	4/8	50
Group VII	550	6/8	75
Group VIII	600	8/8	100
Group IX	650	8/8	100
Group X	Control	0/8	0

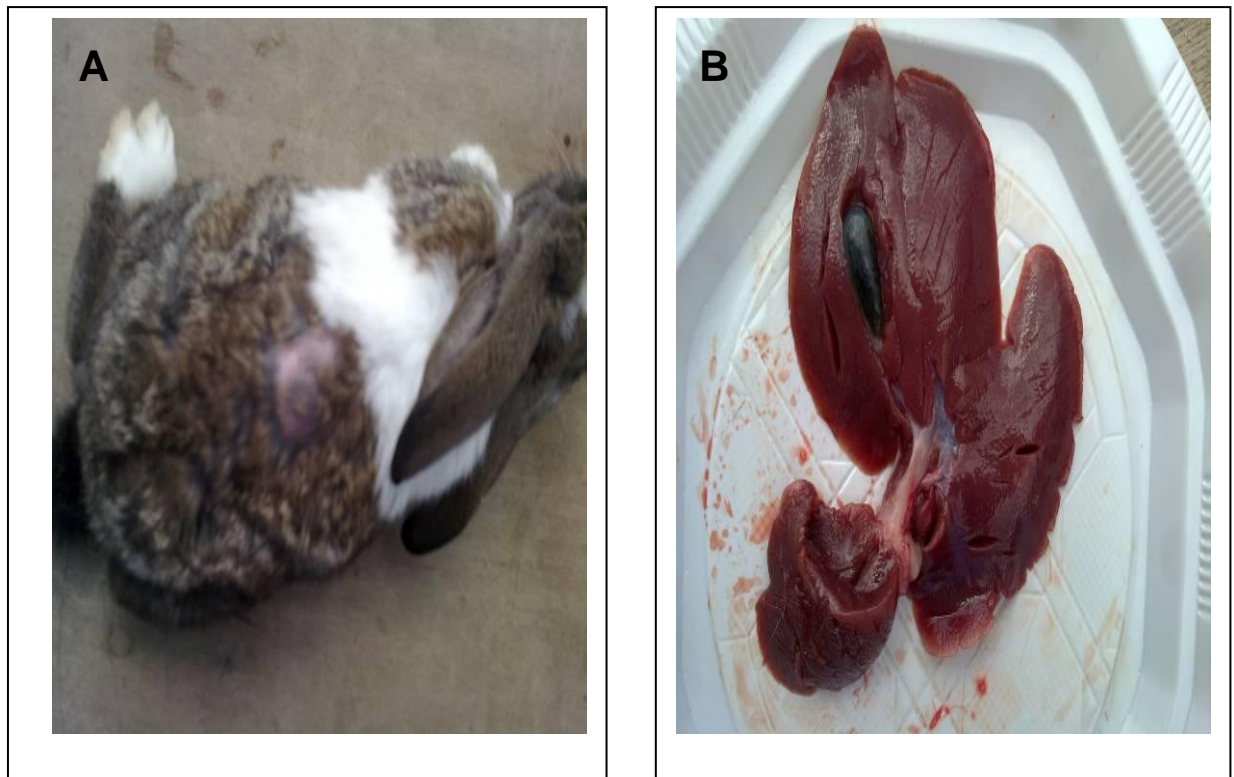
The number of animals administered dimethoate was 8 in each group (I to IX). Control animals were given distilled water and their number was also 8.



**Figure 4.1** Logarithmic scale of oral LD<sub>50</sub> of dimethoate in male domestic rabbits (LD<sub>50</sub>=432 mg/kg body weight).

## 4.2 General health of rabbits

Observation of animals for mortality revealed that only one rabbit died in the control group during the 6<sup>th</sup> week of the experiment recording mortality rate of 1/48 (2.1%). However, the overall mortality rate recorded for an overall rabbits treated with 1/10 LD<sub>50</sub> dimethoate (43.2 mg/kg body weight) was 4/36 (11.1%). One animal died in the 4th week, one animal died in the 5th week and 2 animals died in the 6th week of the experiment. In addition, rabbits in the control group did not show any sign of toxicity. However, dimethoate-fed rabbits showed varying degrees of clinical signs few minutes after dosing. The signs included disorientation, drowsiness, uncoordinated movements, mild tremor and diarrhea. Concerning morphological changes, dimethoate-treated rabbits showed hair loss especially in the fifth and sixth weeks of the experiment (Figure 4.2A) whereas control animals did not display such change. The livers of dissected rabbits also showed scars of depression in response to dimethoate administration (Figure 4.2B) whereas those of the control animals showed normal appearance.



**Figure 4.2:** Morphological effect of 1/10 LD<sub>50</sub> dimethoate (43.2 mg/kg body weight) after 6 weeks on (A) hair and (B) liver of male domestic rabbit.

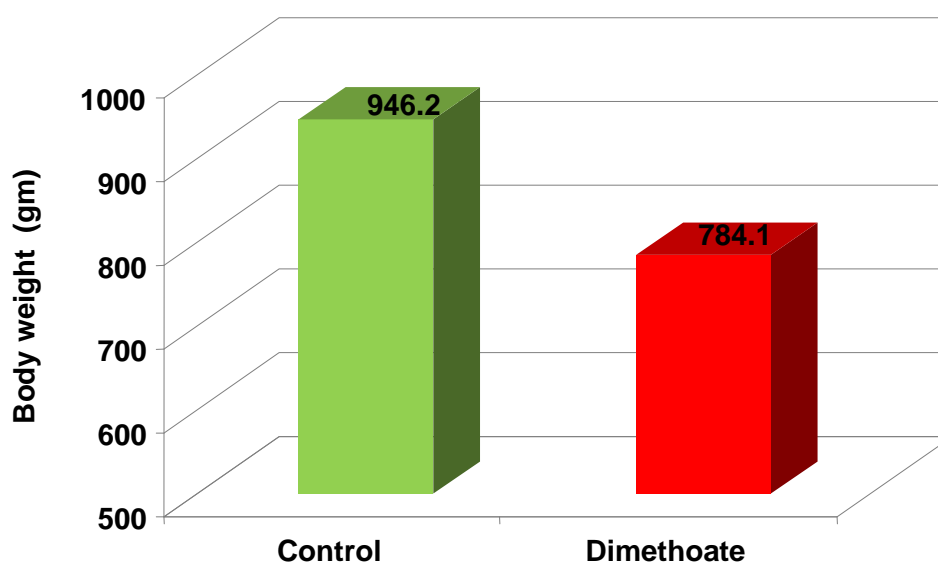
### 4.3 Final body weight

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<sub>50</sub> dimethoate. There was a significant decrease in the body weight of dimethoate-treated rabbits compared to controls (784.1±38.5 Vs 946.2±37.9, %difference=18.7, P=0.013).

Table 4.2 Final body weight of male domestic rabbits after 6 weeks of daily oral administration of 1/10 LD<sub>50</sub> dimethoate.

	Control (n=8)	Dimethoate (n=6)	% difference	t-value	P-value
<b>Body weight (gm)</b>	946.2±37.9	784.1±38.5	-18.7	2.975	0.013

The number of animals (n) was 8 in control group and 6 in dimethoate-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 administration of 1/10 LD<sub>50</sub> dimethoate (43.2 mg/kg body weight)

## 4.4 Biochemical investigation

### 4.4.1 Serum glucose

Table 4.3 demonstrates serum glucose levels in control and dimethoate-treated male domestic rabbits along the experimental period of 6 week intervals. The mean values of glucose level in controls were  $112.8 \pm 3.3$ ,  $114.5 \pm 3.2$ ,  $115.2 \pm 3.8$ ,  $113.7 \pm 4.0$ ,  $116.1 \pm 3.8$  and  $113.4 \pm 2.9$  mg/dl during the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> weeks of the experiment, respectively. Oral administration of 1/10 LD<sub>50</sub> dimethoate daily for 6 weeks caused general increase in glucose level commencing at the second week of the experiment. This increment was significant in the last four weeks of the experiment with a maximum percentage difference of 26.3% in the 4<sup>th</sup> week ( $t=5.423$ ,  $P=0.003$ ).

Table 4.3 Effect of dimethoate (1/10 LD<sub>50</sub>, 43.2 mg/kg body weight) on serum glucose level (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Dimethoate (n=6)	% difference	t-value	P-value
1	$112.8 \pm 3.3$	$107.4 \pm 3.5$	-4.9	1.120	0.306
2	$114.5 \pm 3.2$	$123.8 \pm 4.1$	7.8	1.734	0.144
3	$115.2 \pm 3.8$	$139.7 \pm 5.0$	19.2	3.929	0.011
4	$113.7 \pm 4.0$	$148.1 \pm 4.9$	26.3	5.423	0.003
5	$116.1 \pm 3.8$	$142.1 \pm 4.7$	20.1	4.167	0.014
6	$113.4 \pm 2.9$	$140.9 \pm 5.2$	21.6	4.631	0.010

The number of animals (n) was 8 in control group and 6 in dimethoate-treated animals. All values were expressed as mean $\pm$ 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 alanine aminotransferase (ALT) activity in control and dimethoate-treated male domestic rabbits along the experimental period of 6 weeks are presented in Table 4.4. The normal enzyme activities were  $47.4 \pm 2.3$ ,  $50.3 \pm 2.6$ ,  $48.5 \pm 1.9$ ,  $49.8 \pm 2.2$ ,  $47.1 \pm 1.9$  and  $48.3 \pm 1.7$  U/L during the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> weeks of the experiment, respectively. Upon dimethoate administration, ALT activity increased throughout the experimental periods reaching values of  $53.5 \pm 2.5$ ,  $61.1 \pm 2.8$ ,  $60.8 \pm 2.7$ ,  $65.9 \pm 2.8$ ,  $58.5 \pm 2.5$  and  $61.6 \pm 3.0$  U/L compared to control levels. This increase was generally significant with maximum percentage difference of 27.8 in the 4<sup>th</sup> week of the experiment ( $t=4.259$ ,  $P=0.008$ ).

Table 4.4 Effect of dimethoate (1/10 LD<sub>50</sub>, 43.2 mg/kg body weight) on serum alanine aminotransferase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Dimethoate (n=6)	% difference	t-value	P-value
1	$47.4 \pm 2.3$	$53.5 \pm 2.5$	12.1	1.668	0.134
2	$50.3 \pm 2.6$	$61.1 \pm 2.8$	19.4	2.590	0.041
3	$48.5 \pm 1.9$	$60.8 \pm 2.7$	22.5	3.728	0.010
4	$49.8 \pm 2.2$	$65.9 \pm 2.8$	27.8	4.259	0.008
5	$47.1 \pm 1.9$	$58.5 \pm 2.5$	21.6	3.590	0.012
6	$48.3 \pm 1.7$	$61.6 \pm 3.0$	24.2	4.114	0.009

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

#### 4.4.2.2 Aspartate aminotransferase

Table 4.5 provides mean values of serum aspartate aminotransferase (AST) activity in control and dimethoate-fed rabbits allover the experimental period of 6 weeks. Aspartate aminotransferase activity registered for control animals were  $32.7 \pm 1.4$ ,  $33.2 \pm 1.7$ ,  $35.0 \pm 1.6$ ,  $32.5 \pm 1.7$ ,  $34.1 \pm 1.9$ , and  $33.4 \pm 1.5$  U/L at the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, and 6<sup>th</sup> weeks of the experiment, respectively. Feeding of animals with dimethoate generally provoked significant increase in the enzyme activity throughout the experiment exhibiting values of  $37.9 \pm 1.6$ ,  $40.0 \pm 1.9$ ,  $50.3 \pm 2.4$ ,  $42.4 \pm 2.0$ ,  $43.2 \pm 2.3$ , and  $41.8 \pm 2.5$  U/L ( $P < 0.05$ ). The maximum increase in the enzyme activity was recorded at the 3<sup>rd</sup> week of the experiment showing percentage difference of 35.9%.

Table 4.5 Effect of dimethoate (1/10 LD<sub>50</sub>, 43.2 mg/kg body weight) on serum aspartate aminotransferase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Dimethoate (n=6)	% difference	t-value	P-value
1	$32.7 \pm 1.4$	$37.9 \pm 1.6$	14.7	2.264	0.053
2	$33.2 \pm 1.7$	$40.0 \pm 1.9$	18.6	2.629	0.034
3	$35.0 \pm 1.6$	$50.3 \pm 2.4$	35.9	4.944	0.004
4	$32.5 \pm 1.7$	$42.4 \pm 2.0$	26.4	3.574	0.016
5	$34.1 \pm 1.9$	$43.2 \pm 2.3$	23.5	3.071	0.022
6	$33.4 \pm 1.5$	$41.8 \pm 2.5$	22.3	3.025	0.029

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

#### 4.4.2.3 Alkaline phosphatase

The normal activity of serum alkaline phosphatase (ALP) of male domestic rabbits were  $97.5 \pm 2.7$ ,  $101.6 \pm 3.0$ ,  $95.8 \pm 2.6$ ,  $97.3 \pm 2.9$ ,  $99.2 \pm 3.1$  and  $96.6 \pm 2.8$  U/L at 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, and 6<sup>th</sup> weeks of the experiment, respectively (Table 4.6). Dimethoate intake increased the enzyme activity along the experimental intervals studied, with significant change starting from the second week. The maximum increment in the enzyme activity ( $129.8 \pm 4.5$  U/L) was noted at the 2<sup>nd</sup> week of the experiment with percentage difference of 24.4% in comparison to control level ( $t=4.458$ ,  $P=0.004$ ).

Table 4.6 Effect of dimethoate (1/10 LD<sub>50</sub>, 43.2 mg/kg body weight) on serum alkaline phosphatase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Dimethoate (n=6)	% difference	t-value	P-value
1	$97.5 \pm 2.7$	$105.4 \pm 3.2$	7.8	1.732	0.122
2	$101.6 \pm 3.0$	$129.8 \pm 4.5$	24.4	4.458	0.004
3	$95.8 \pm 2.6$	$112.0 \pm 4.3$	15.6	3.231	0.018
4	$97.3 \pm 2.9$	$118.7 \pm 4.2$	19.8	4.227	0.006
5	$99.2 \pm 3.1$	$123.4 \pm 3.6$	21.7	4.881	0.005
6	$96.6 \pm 2.8$	$111.3 \pm 3.8$	14.1	3.234	0.023

The number of animals (n) was 8 in control group and 6 in dimethoate-treated animals. All values were expressed as mean $\pm$ 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 gamma glutamyl transferase ( $\gamma$ GT) activity in control and dimethoate-intoxicated male domestic rabbits allover the experimental period of 6 weeks. The normal activity of  $\gamma$ GT were  $5.9\pm0.27$ ,  $6.3\pm0.30$ ,  $6.1\pm0.25$ ,  $6.2\pm0.32$ ,  $5.9\pm0.26$  and  $6.2\pm0.29$  U/L at 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> weeks of the experiment, respectively. Daily oral administration of dimethoate increased the enzyme activity throughout the experiment to reach mean values of  $6.7\pm0.32$ ,  $8.2\pm0.37$ ,  $8.4\pm0.41$ ,  $7.7\pm0.35$ ,  $7.1\pm0.29$  and  $8.0\pm0.35$  U/L, respectively. The maximum increase in  $\gamma$ GT activity was registered during the 3<sup>rd</sup> week of the experiment showing percentage difference of 31.7 ( $t=4.757$ ,  $P=0.003$ ).

Table Table 4.7 Effect of dimethoate (1/10 LD<sub>50</sub>, 43.2 mg/kg body weight) on serum gamma glutamyl transferase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Dimethoate (n=6)	% difference	t-value	P-value
1	$5.9\pm0.27$	$6.7\pm0.32$	12.7	1.914	0.092
2	$6.3\pm0.30$	$8.2\pm0.37$	26.2	3.528	0.012
3	$6.1\pm0.25$	$8.4\pm0.41$	31.7	4.757	0.003
4	$6.2\pm0.32$	$7.7\pm0.35$	21.6	3.216	0.018
5	$5.9\pm0.26$	$7.1\pm0.29$	18.5	3.087	0.021
6	$6.2\pm0.29$	$8.0\pm0.35$	25.4	3.984	0.016

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

#### 4.4.2.5 Cholinesterase activity

The mean values of serum cholinesterase (ChE) activity in control and dimethoate-fed rabbits are pointed out in Table 4.8. The normal ChE activities in control animals were  $4615 \pm 138$ ,  $4521 \pm 141$ ,  $4537 \pm 133$ ,  $4762 \pm 139$ ,  $4801 \pm 144$  and  $4627 \pm 136$  U/L during the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> weeks of the experiment, respectively. Oral administration of the organophosphorus pesticide dimethoate provoked progressive significant decrease in the enzyme activity to values of  $3219 \pm 113$ ,  $3020 \pm 105$ ,  $2924 \pm 92$ ,  $3004 \pm 101$ ,  $2877 \pm 86$  and  $2998 \pm 97$  U/L, respectively. The maximum decrease in the mean ChE activity ( $2877 \pm 86$  U/L) was obtained during the 5<sup>th</sup> week of the experiment recording percentage difference of 50.1% ( $t=11.511$ ,  $P=0.000$ ).

Table 4.8 Effect of dimethoate (1/10 LD<sub>50</sub>, 43.2 mg/kg body weight) on serum cholinesterase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Dimethoate (n=6)	% difference	t-value	P-value
1	$4615 \pm 138$	$3219 \pm 113$	-35.6	7.821	0.000
2	$4521 \pm 141$	$3020 \pm 105$	-39.8	8.583	0.000
3	$4537 \pm 133$	$2924 \pm 92$	-43.2	10.027	0.000
4	$4762 \pm 139$	$3004 \pm 101$	-45.3	10.235	0.000
5	$4801 \pm 144$	$2877 \pm 86$	-50.1	11.511	0.000
6	$4627 \pm 136$	$2998 \pm 97$	-42.7	9.758	0.000

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

### 4.4.3 Serum bilirubin

The mean levels of serum bilirubin in control rabbits as well as in rabbits received dimehoate daily for 6 weeks are presented in Table 4.9. The normal levels of bilirubin in control rabbits were  $1.54\pm0.03$ ,  $1.60\pm0.04$ ,  $1.61\pm0.03$ ,  $1.59\pm0.01$ ,  $1.54\pm0.02$  and  $1.62\pm0.05$  mg/dl during the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> weeks of the experiment, respectively. Oral administration of dimethoate caused gradual increase in bilirubin level to reach its maximum percentage difference of 16.7% during the 5<sup>th</sup> week of the experiment ( $t=2.720$ ,  $P=0.042$ ).

Table 4.9 Effect of dimethoate (1/10 LD<sub>50</sub>, 43.2 mg/kg body weight) on serum bilirubin (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Dimethoate (n=6)	% difference	t-value	P-value
1	$1.54\pm0.03$	$1.61\pm0.05$	4.4	1.013	0.340
2	$1.60\pm0.04$	$1.70\pm0.06$	6.1	1.206	0.267
3	$1.61\pm0.03$	$1.76\pm0.08$	9.0	1.766	0.128
4	$1.59\pm0.01$	$1.79\pm0.10$	11.8	2.081	0.083
5	$1.54\pm0.02$	$1.82\pm0.09$	16.7	2.720	0.042
6	$1.62\pm0.05$	$1.85\pm0.07$	13.3	2.565	0.061

The number of animals (n) was 8 in control group and 6 in dimethoate-treated animals. All values were expressed as mean $\pm$ 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 dimethoate-treated male domestic rabbits. Urea concentration in control animals exhibited values of  $37.6 \pm 1.8$ ,  $36.3 \pm 2.0$ ,  $36.0 \pm 2.2$ ,  $35.7 \pm 1.9$ ,  $34.9 \pm 1.7$  and  $35.1 \pm 1.6$  mg/dl during 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> weeks of the experiment, respectively. Daily feeding of dimethoate caused gradual increase in urea concentration all over the experimental intervals examined reaching values of  $41.9 \pm 2.5$ ,  $41.5 \pm 2.7$ ,  $42.0 \pm 2.3$ ,  $43.9 \pm 2.1$ ,  $46.3 \pm 2.6$  and  $48.1 \pm 3.0$  mg/dl, respectively. This increase in urea concentration became significant at the 6<sup>th</sup> week and reached its maximum percentage difference of 31.3% at the end of the experiment ( $t=4.094$ ,  $P=0.009$ ).

Table 4.10 Effect of dimethoate (1/10 LD<sub>50</sub>, 43.2 mg/kg body weight) on serum urea concentration (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Dimethoate (n=6)	% difference	t-value	P-value
1	$37.6 \pm 1.8$	$41.9 \pm 2.5$	10.8	1.262	0.242
2	$36.3 \pm 2.0$	$41.5 \pm 2.7$	13.4	1.469	0.185
3	$36.0 \pm 2.2$	$42.0 \pm 2.3$	15.4	1.913	0.104
4	$35.7 \pm 1.9$	$43.9 \pm 2.1$	20.6	2.744	0.041
5	$34.9 \pm 1.7$	$46.3 \pm 2.6$	28.1	3.323	0.021
6	$35.1 \pm 1.6$	$48.1 \pm 3.0$	31.3	4.094	0.009

The number of animals (n) was 8 in control group and 6 in dimethoate-treated animals. All values were expressed as mean $\pm$ 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 dimethoate along the experimental period of 6 weeks are illustrated in Table 4.11. Normal values recorded for creatinine concentrations were  $0.64\pm0.03$ ,  $0.66\pm0.02$ ,  $0.67\pm0.04$ ,  $0.63\pm0.01$ ,  $0.70\pm0.03$ , and  $0.68\pm0.04$  mg/dl at the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> weeks of the experiment, respectively. Upon dimethoate administration, serum creatinine concentration fluctuates across the experimental period registering its maximum percentage difference of 26.2% during the 4<sup>th</sup> week of the experiment ( $t=3.124$ ,  $P=0.020$ ).

Table 4.11 Effect of dimethoate (1/10 LD<sub>50</sub>, 43.2 mg/kg body weight) on serum creatinine concentration (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Dimethoate (n=6)	% difference	t-value	P-value
1	$0.64\pm0.03$	$0.71\pm0.05$	10.4	1.190	0.268
2	$0.66\pm0.02$	$0.77\pm0.06$	15.4	1.642	0.145
3	$0.67\pm0.04$	$0.77\pm0.04$	13.9	1.568	0.168
4	$0.63\pm0.01$	$0.82\pm0.06$	26.2	3.124	0.020
5	$0.70\pm0.03$	$0.86\pm0.05$	20.5	2.678	0.044
6	$0.68\pm0.04$	$0.78\pm0.04$	13.7	1.580	0.189

The number of animals (n) was 8 in control group and 6 in dimethoate-treated animals. All values were expressed as mean $\pm$ 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  $5.5 \pm 0.22$ ,  $5.6 \pm 0.23$ ,  $6.1 \pm 0.25$ ,  $5.7 \pm 0.27$ ,  $5.6 \pm 0.19$  and  $6.0 \pm 0.23$  gm/dl during the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> weeks of the experiment, respectively. Total protein level showed overt decrease in response to dimethoate administration along the experimental periods tested. This decrease become significant from the 2<sup>nd</sup> week till the end of the experiment, recording its minimal value of  $4.3 \pm 0.23$  at the 4<sup>th</sup> week of the experiment (% difference=28.0,  $t=4.084$ ,  $P=0.006$ ).

Table 4.12 Effect of dimethoate (1/10 LD<sub>50</sub>, 43.2 mg/kg body weight) on serum total protein (gm/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Dimethoate (n=6)	% difference	t-value	P-value
1	$5.5 \pm 0.22$	$5.0 \pm 0.18$	-9.5	1.774	0.114
2	$5.6 \pm 0.23$	$4.8 \pm 0.21$	-15.4	2.545	0.038
3	$6.1 \pm 0.25$	$5.0 \pm 0.20$	-19.8	3.375	0.015
4	$5.7 \pm 0.27$	$4.3 \pm 0.23$	-28.0	4.084	0.006
5	$5.6 \pm 0.19$	$4.5 \pm 0.24$	-21.7	3.668	0.014
6	$6.0 \pm 0.23$	$4.7 \pm 0.19$	-24.3	4.329	0.012

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

#### 4.4.5.2 Serum albumin

The mean values of serum albumin concentration in control and dimethoate-treated male domestic rabbits are shown in Table 4.13. Albumin concentration in control animals exhibited values of  $3.71 \pm 0.14$ ,  $3.64 \pm 0.12$ ,  $3.92 \pm 0.21$ ,  $3.67 \pm 0.18$ ,  $4.03 \pm 0.22$  and  $3.75 \pm 0.17$  gm/dl at 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> weeks of the experiment, respectively. In general, dimethoate intake resulted in gradual significant decrease in albumin concentration recording the minimum concentration of 2.86 gm/dl at the end of the experiment with percentage difference of 26.9% ( $t=4.839$  and  $P=0.008$ ).

Table 4.13 Effect of dimethoate (1/10 LD<sub>50</sub>, 34.2 mg/kg body weight) on serum albumin (gm/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Dimethoate (n=6)	% difference	t-value	P-value
1	$3.71 \pm 0.14$	$3.41 \pm 0.11$	-8.4	1.703	0.127
2	$3.64 \pm 0.12$	$3.16 \pm 0.15$	-14.1	2.398	0.048
3	$3.92 \pm 0.21$	$3.09 \pm 0.09$	-23.7	4.033	0.010
4	$3.67 \pm 0.18$	$3.07 \pm 0.10$	-17.8	2.887	0.028
5	$4.03 \pm 0.22$	$3.21 \pm 0.11$	-22.6	3.382	0.015
6	$3.75 \pm 0.17$	$2.86 \pm 0.07$	-26.9	4.839	0.008

The number of animals (n) was 8 in control group and 6 in dimethoate-treated animals. All values were expressed as mean $\pm$ 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  $1.81 \pm 0.09$ ,  $1.85 \pm 0.08$ ,  $2.10 \pm 0.13$ ,  $1.83 \pm 0.10$ ,  $1.92 \pm 0.11$  and  $2.11 \pm 0.14$  gm/dl during the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup>, and weeks of the experiment, respectively. Oral administration of dimethoate lowered globulin levels to  $1.61 \pm 0.07$ ,  $1.57 \pm 0.07$ ,  $1.61 \pm 0.09$ ,  $1.53 \pm 0.06$ ,  $1.55 \pm 0.08$ , and  $1.64 \pm 0.04$  gm/dl showing percentage differences of 11.7, 16.4, 26.5, 17.9, 21.3 and 25.1% at the weekly intervals of the experiment compared to controls. This decrease was significant all over the experimental periods except for 1<sup>st</sup> week.

Table 4.14 Effect of dimethoate (1/10 LD<sub>50</sub>, 43.2 mg/kg body weight) on serum globulin (gm/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Dimethoate (n=6)	% difference	t-value	P-value
1	$1.81 \pm 0.09$	$1.61 \pm 0.07$	-11.7	1.736	0.121
2	$1.85 \pm 0.08$	$1.57 \pm 0.07$	-16.4	2.568	0.037
3	$2.10 \pm 0.13$	$1.61 \pm 0.09$	-26.5	3.123	0.021
4	$1.83 \pm 0.10$	$1.53 \pm 0.06$	-17.9	2.703	0.035
5	$1.92 \pm 0.11$	$1.55 \pm 0.08$	-21.3	2.935	0.032
6	$2.11 \pm 0.14$	$1.64 \pm 0.04$	-25.1	3.112	0.031

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

## 4.4.6 Electrolytes

### 4.4.6.1 Serum calcium

The mean levels of serum calcium concentration in controls as well as in dimethoate-treated male rabbits are provided in Table 4.15. The normal concentrations of calcium were  $13.9\pm0.5$ ,  $13.7\pm0.3$ ,  $14.0\pm0.4$ ,  $13.5\pm0.3$ ,  $14.1\pm0.5$  and  $13.8\pm0.4$  mg/dl at the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> weeks of the experiment, respectively. Upon dimethoate administration, serum concentration of calcium fluctuates throughout the experiment registering significant increase during the 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> weeks with percentage difference of 14.6, 18.8 and 15.7 respectively ( $t=2.794$ ,  $P=0.048$ ;  $t=2.844$ ,  $P=0.029$  and  $t=2.661$ ,  $P=0.037$ , respectively).

Table 4.15 Effect of dimethoate (1/10 LD<sub>50</sub>, 43.2 mg/kg body weight) on serum calcium (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Dimethoate (n=6)	% difference	t-value	P-value
1	$13.9\pm0.5$	$14.8\pm0.6$	6.3	1.079	0.316
2	$13.7\pm0.3$	$15.6\pm0.8$	12.9	1.937	0.094
3	$14.0\pm0.4$	$16.2\pm0.7$	14.6	2.794	0.048
4	$13.5\pm0.3$	$16.3\pm1.0$	18.8	2.844	0.029
5	$14.1\pm0.5$	$16.5\pm0.8$	15.7	2.661	0.037
6	$13.8\pm0.4$	$15.8\pm0.7$	13.5	2.501	0.054

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

#### 4.4.6.2 Serum phosphorus

Table 4.16 shows the mean of serum phosphorus concentrations in controls as well as in dimethoate-fed male rabbits. The mean concentrations of phosphorus in control animals were  $7.2 \pm 0.4$ ,  $7.4 \pm 0.3$ ,  $7.0 \pm 0.3$ ,  $7.3 \pm 0.2$ ,  $7.4 \pm 0.4$  and  $7.2 \pm 0.3$  mg/dl at the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> weeks of the experiment, respectively. In dimethoate-treated group of animals, serum phosphorus fluctuates along the whole experiment displaying significant decrease during the 2<sup>nd</sup>, 4<sup>th</sup> and 6<sup>th</sup> weeks with percentage difference of 16.1, 14.7 and 15.0 respectively ( $t=2.912$ ,  $P=0.023$ ;  $t=2.887$ ,  $P=0.028$  and  $t=2.857$ ,  $P=0.045$ , respectively).

Table 4.16 Effect of dimethoate (1/10 LD<sub>50</sub>, 43.2 mg/kg body weight) on serum phosphorus (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Dimethoate (n=6)	% difference	t-value	P-value
1	$7.2 \pm 0.4$	$6.7 \pm 0.2$	-7.2	1.348	0.214
2	$7.4 \pm 0.3$	$6.3 \pm 0.2$	-16.1	2.912	0.023
3	$7.0 \pm 0.3$	$6.2 \pm 0.1$	-12.0	2.326	0.059
4	$7.3 \pm 0.2$	$6.3 \pm 0.3$	-14.7	2.887	0.028
5	$7.4 \pm 0.4$	$6.6 \pm 0.1$	-11.4	2.233	0.076
6	$7.2 \pm 0.3$	$6.2 \pm 0.2$	-15.0	2.857	0.045

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

# CHAPTER 5

## Discussion

During the last decade, the extensive use of different pesticides in agriculture and for public health purposes has led to drastic effects in many non-target species including man. One of these pesticides is the organophosphorus insecticide dimethoate which is used in housefly control and against a broad range of agricultural insect and mite pests (Srivastav et al., 2010). Dimethoate poisoning caused an irreversible inhibition of acetylcholinesterase enzyme, resulting in accumulation of acetylcholine and over activation of acetylcholine receptor at neuromuscular junction and in the autonomic and central nervous system. This will manifest in convulsions and even tremors leading in sever cases to death (Wiener and Hoffman, 2004; Costa, 2006 and Karami-Mohajeri et al., 2013).

Although dimethoate is one of the commonest organophosphorus insecticides being used in the agricultural sector in Gaza strip (Yassin et al., 2002 and Personal Communication with Ministry of Agriculture, 2012), limited data are available on this toxic compound. Several cases of organophosphorus poisoning were reported among farm workers and children in Gaza strip which mostly result from use/misuse of this type of pesticides (Yassin et al., 2002 and EL-Shanty, 2009). In this context, Tomlin (2006) satisfied by determining a wide range of oral LD<sub>50</sub> of dimethoate in rabbits. To our knowledge no previous study determine the exact oral LD<sub>50</sub> of dimethoate in domestic rabbits. Therefore, the present work was performed to determine the oral LD<sub>50</sub> of dimethoate and to investigate its toxicological effect on liver and kidney of male domestic rabbits. The findings can then be extrapolated to human beings to assess the potential hazards in the human populations due to dimethoate exposure.

## 5.1 General health of rabbits

The present study demonstrated that oral treatment of rabbits with 1/10 LD<sub>50</sub> dimethoate (43.2 mg/kg body weight) caused an overall mortality rate of 11.1% compared to 2.1% in controls throughout the 6 weeks of the experiment. Such mortality was mostly attributed to diarrhea which may be related to the cholinergic crisis, a consistent sign in organophosphate poisoning. This was in agreement with that found by Saafi et al. (2011) and Noor et al. (2012) who observed diarrhea in mature male rats in response to chronic exposure of dimethoate. In addition, dimethoate-treated rabbits showed hair loss especially in the last two weeks of the experiment. This coincided with the significant decrease in protein content observed in the present study. Heikal et al. (2012) observed hair loss in rats after daily oral administration of 1/10 LD<sub>50</sub> of dimethoate for 4 weeks. The livers of dimethoate-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. Dimethoate is known to induce morphological changes in the liver (AL-Awthan et al., 2012).

## 5.2 Body weight

As indicated in the current data body weight was significantly decreased in dimethoate-supplemented rabbits compared to controls. This finding is in agreement with that obtained by Hassan et al. (1994); Farag et al. (2006); El-Damaty et al. (2012) and Noor et al. (2012). The reduction in body weight in response to dimethoate intake may be a result of the combined action of cholinergic and oxidative stress and/or due to increase degradation of lipids and proteins as a direct effect of organophosphours compound exposure (Saafi et al., 2011; Heikal et al., 2012 and Salama et al., 2013). This explanation is supported by the recorded significant decrease in protein content in dimethoate-treated rabbits compared to controls.

## **5.3 Biochemical investigation**

### **5.3.1 Serum glucose**

Results presented in this study revealed that oral administration of 1/10 LD<sub>50</sub> dimethoate daily for 6 weeks caused general increase in serum glucose levels, which becomes significant in the last four weeks of the experiment. This finding is concurrent with that reported by Hager et al., (2002); Kamath et al. (2008) and Salih (2010). Therefore, glucose homeostasis is affected by dimethoate administration. The mechanism by which this organophosphorus insecticide induces hyperglycemia may involve one or more factors such as: First; reduction in insulin secretion as a result of the destructive action of dimethoate on the beta cells of Langerhans islets in the pancreas (Hager et al., 2002 and Kamath et al., 2008), Second; hepatotoxicity which may lead to damage of hepatic glycogenesis pathway (Sharma et al., 2005 and Saafi et al., 2011). In addition, Kamath and Rajini (2007) observed that dimethoate caused significant increase in blood glucose levels with concomitant inhibition of acetylcholinesterase activity and depletion of reduced glutathione contents in pancreas.

### **5.3.2 Liver enzymes and bilirubin**

Data presented in this study showed that the mean levels of serum ALT, AST,  $\gamma$ -GT and ALP in the dimethoate-treated rabbits were significantly higher than those in the controls. Such elevation of liver enzymes as a result of dimethoate administration was documented by other authors (Chatterjea and Shinde, 2005; Sivapiriyia et al., 2006; Attia and Nasr, 2009; Salih, 2010; Saafi et al., 2011; AL-Awthan et al., 2012 and El-Damaty et al., 2012). Liver is the center of biotransformation and detoxification of foreign compounds and is the most vulnerable to the chemical assaults such as dimethoate poisoning (Kulkarni and Hodgson, 1980; Massoud et al., 2010 and AL-Awthan et al., 2012). Serum ALT, AST and,  $\gamma$ -GT are considered to be among the most sensitive markers employed in the diagnosis of hepatotoxicity (Kutlu et al., 2007 and Saafi et al., 2011). Pesticide exposure causes liver damage and

leakage of cytosolic enzymes from hepatocytes and other body organs into blood (Dewan et al., 2004 and Ncibi et al., 2008). 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,  $\gamma$ -GT and ALP, serum ChE activity was markedly decreased in dimethoate-treated rabbits compared to controls. Such inhibition in ChE in response to organophosphorus dimethoate administrated was obtained by Burford et al. (1990); Bagchi et al. (1995); Hazarika et al. (2003); Timur et al. (2003); Wysocki and Zasadowski (2005); Sivapiriya et al. (2006); Massoud et al. (2011) and Heikal et al. (2012). It is known that organophosphorus pesticides such as dimethoate caused irreversible inhibition of ChE leading to accumulation of acetylcholine and over activation of acetylcholine receptor at neuromuscular junction and in the autonomic and central nervous system. This was manifested in convulsions and even tremors leading in sever cases to death (Ward and Mundy, 1996; Maroni et al., 2000; Lotti, 2001; Eyer, 2003; Kamel and Hoppin, 2004; Tuovinen, 2004; Eddleston et al., 2006; Karalliedde et al., 2006 and Qiang et al., 2010). This result is supported by some mortalities and the clinical signs of anticholinestrase action represented in disorientation, drowsiness, uncoordinated movements, mild tremor and diarrhea observed in animals.

In the present study oral administration of dimethoate caused gradual increase in bilirubin level throughout the experiment. Such increase was reported previously by Attia and Nasr (2009); Ben Amara et al. (2011) and Saafi et al. (2011) in dimethoate-intoxicated rats. The change in serum bilirubin which is accepted as indicator of liver function may provide further evidence on hepatotoxicity induced by the organophosphorus insecticide dimethoate (Saafi et al., 2011 and Khan et al., 2013)

### **5.3.3 kidney function**

The influence of dimethoate on kidney function in dimethoate-treated rabbits was assessed throughout the measurement of urea and creatinine concentration. Urea concentration was generally increased throughout the whole experiment, and this increment becomes significant during the last four weeks of the experiment compared to the control. For creatinine this increase was also observed along the whole experiment with significant increments in 4<sup>th</sup> and 5<sup>th</sup> weeks of the experiment. Such findings are in agreement with that reported in other studies (Radwan et al., 2001; Attia and Nasr, 2009; Salih, 2010; El-Damaty et al. 2012 and Saafi-Ben Salah et al., 2012). A creatinine level raised out of proportion to the urea may indicate a pre-renal problem (Delanghe et al., 1989). 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). 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 (Delanghe et al., 1989). Creatinine has been found to be a fairly reliable indicator of kidney function. As the kidneys become impaired for any reason, for example in case of dimethoate poisoning, the creatinine level in the blood will rise due to poor clearance by the kidneys. A rise in blood creatinine level was observed with damage to functioning nephrons and impaired renal function (Nwanjo et al., 2005 and Garba et al., 2007).

### **5.3.4 Protein profile**

As indicated in the present results significant decreases in the levels of total protein, albumin and globulin concentrations were found in rabbits treated with dimethoate compared to controls. Similar findings were reported in other studies as a result of oral administration of different doses of dimethoate (Attia

and Nasr, 2009 and Salih, 2010). 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 reduce in protein synthesis or increased proteolytic activity or degradation (Yeragi et al., 2003 and Heikal et al., 2012). In addition, the observed decrease in serum proteins could be attributed in part to the damaging effect of dimethoate on liver cells, as confirmed by the increase in activities of serum AST, ALT and  $\gamma$ -GT. It was reported that albumin levels are decreased in liver disease (Nyblom et al., 2004). A decrease in globulin is expected as globulin (mostly  $\gamma$ -globulins) may be consumed in the production of antibodies in response to dimethoate administration (Institoris, et al., 1999).

### **5.3.5 Electrolytes**

The mean serum concentration of calcium showed significant increase in dimethoate-intoxicated rabbits. In contrast, serum phosphorus concentration was significantly decreased in response to dimethoate treatment. Similar results were documented by Logaswamy et al. (2007) and Khan et al. (2013). This indicates that the organophosphorus insecticide dimethoate interferes with calcium and phosphorus homeostasis. Hypercalcemia and hypophosphatemia were recorded by Mahjoubi-Samet et al. (2005) in rats exposed to 40 mg/kg body weight dimethoate. They suggested that exposure to dimethoate altered bone mineral composition especially calcium and phosphorus levels of bone.

# CHAPTER 6

## CONCLUSIONS

1. The calculated oral LD<sub>50</sub> of dimethoate in male domestic rabbits from the linear regression was found to be 432 mg/kg body weight.
2. Daily oral administration of 1/10 LD<sub>50</sub> dimethoate caused an overall mortality rate of 11.1% compared to 2.1% in controls. Clinical sign of dimethoate-intoxicated rabbits were disorientation, drowsiness, uncoordinated movements, mild tremor and diarrhea.
3. The final body weight was significantly decreased in dimethoate-treated rabbits compared to controls.
4. Serum glucose of rabbits was significantly increased in response to dimethoate feeding compared to controls.
5. Liver enzymes ALT, AST, ALP and  $\gamma$ -GT as well as bilirubin were significantly higher in the dimethoate-intoxicated rabbits whereas cholinesterase level was markedly decreased compared to the controls.
6. Serum urea and creatinine concentrations were significantly increased in response to dimethoate administration compared to the controls.
7. There were significant decreases in total protein, albumin and globulin upon dimethoate intake compared to the controls.
8. Hypercalcemia and hypophosphatemia were recorded in dimethoate-intoxicated rabbits.

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