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Hepatic and Renal Toxicity of Dichlorvos in Male Domestic Rabbits

سمية مبيد ديكلورفوس على كبد وكلية ذكور الارانب المنزلية

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إقـــــرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

Hepatic and Renal Toxicity of Dichlorvos in Male Domestic Rabbits

سمية مبيد ديكلورفوس على كبد وكلية ذكور الارانب المنزلية

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Abstract

Objective: The present study is aimed to assess hepatic and renal toxicity of dichlorvos in male domestic rabbits

Materials and Methods: The oral LD_{50} of dichlorvos in male domestic rabbit was calculated from linear regression and found to be 11.6 mg/kg body weight. A daily dose of 1/10 LD_{50} of dichlorvos (1.2 mg/kg body weight) were given to the animals under experiment for six weeks. Control animals were given distilled water. Blood samples were collected weekly and analyzed.

Results: The overall mortality rate was 6.3% in dichlorvos-treated rabbits compared to no mortalities in controls. Clinical signs included diarrhea, reduced food intake, weakness, disorientation, drowsiness and mild tremors. The growth rate was significantly decreased in dichlorvos-intoxicated rabbits. Serum glucose was significantly increased in response to dichlorvos administration recording a maximum percentage difference of 26.7% in the 4th week of the experiment. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were significantly higher in dichlorvos-fed rabbits compared to controls, registering maximum percentage differences of 29.4% and 38.6%, respectively during the 5th week of the experiment. Alkaline phosphatase (ALP) and gamma glutamyl transferase (γ-GT) were also significantly increased recording maximum percentages of 27.8% and 38.1 in the 4th and 6th weeks of the experiment, respectively. In contrast, serum cholinesterase (ChE) was progressively decreased recording a maximum percentage difference of 72.6% at the end of the experiment. Serum bilirubin was gradually increased to record a maximum percentage difference of 23.3% in the 5th week. Serum urea and creatinine concentrations were significantly elevated in response to dichlorvos intake displaying maximum percentage differences of 35.4% and 29.6% during the 6th and 5th weeks of the experiment, respectively. Serum total protein, albumin and globulin were significantly decreased upon dichlorvos intoxication exhibiting percentage differences of 31.2, 30.2 and 31.4% at 4th week of the experiment. Serum calcium was significantly increased in dichlorvos-treated rabbits with a maximum percentage difference of 24.3% at the 4th week whereas phosphorus was significantly decreased with a maximum percentage difference of 28.6% at the 6th week of the experiment.

Conclusions: Dichlorvos caused hepatic and renal toxicity in rabbit through alterations of liver and kidney functions.

Key words: Dichlorvos, toxicity, liver, kidney, rabbit.

ملخص الرسالة

سمية مبيد ديكلورفوس على كبد وكلية ذكور الارانب المنزلية

هدف الدراسة: تقييم سمية مبيد ديكلورفوس على كبد وكلية ذكور الارانب المنزلية .

الخطوات: قد بينت الدراسة بعد التحليل الإحصائي قيمة الجرعة المميته لنصف العدد من العينة التجريبية LD_{50} في ذكور الأرانب بواسطة الفم ١١,٦ ملجرام/كجم من وزن الجسم.أعطيت الأرانب جرعة قيمتها ١,٢ملجرام/كجم من وزن الجسم و تساوي ١٠/١ من الجرعة المميته لنصف العدد من العينة التجريبية يوميا لمدة ست أسابيع بينما المجموعة الضابطة تناولت الماء. عينات الدم كانت جمعت أسبوعيا وتم تحليلها.

النتائج: كان معدل الوفيات ٦،٣٪ في الأرانب المعالجة بالديكلورفوس بينما الضابطة لم توجد بها وفيات . ظهرت علامات سريرية كالإسهال و الارتباك و النعاس و الرعاش الخفيف. وقد انخفض وزن الجسم بشكل كبير في ذكور الأرانب المعالجة بالديكلورفوس خلال الأسابيع الأخيرة. الجلوكوز في الدم زاد بشكل ملحوظ استجابة لمعاملة بالديكلورفوس حيث سجل أعلي نسبة مئوية للاختلاف .٢٦,٧ ٪ في الأسبوع الرابع من التجربة. نشاط الانزيم الناقل للالانين امين (ALT) زاد بشكل ملحوض في الارانب التي تناولت الديكلورفوس مقارنة مع المجموعة الضابطة مسجلة اعلى اختلاف بنسبة مئوية ٢٩,٤٪ خلال الاسبوع الخامس من التجربة، الانزيم الناقل للاسبرتيت امين (AST) زاد ليسجل اعلى نسبة مئوية للاختلاف ٣٨,٦٪ في الاسبوع الخامس ، أنزيم الكالاين فوسفاتيز(ALP) ايضا زاد ليسجل اعلى نسبة للاختلاف ٢٧,٨٪ في الاسبوع الرابع وايضا الانزيم الناقل للجاما جلوتاميت (γ-GT) زاد ليسجل اعلى نسبة اختلاف ٣٨,١٪ في الاسبوع السادس .في المقابل انزيم كولين استريز في مصل الدم نقص بشكل ملحوظ مسجل اعلى نسبة للاختلاف ٧٢,٦٪ خلال الاسبوع السادس من التجربة ، البليروبين في مصل الدم زاد تدريجيا ليسجل اعلى نسبة اختلاف ٢٣,٣٪ في الاسبوع الخامس، تركيزات اليوريا والكرياتنين ارتفعت بشكل ملحوظ استجابة لتعاطي الديكلورفوس مسجلة اعلى نسبة مئوية للاختلاف ٣٥،٤٪ و ٢٩،٦٪خلال الاسبوع السادس والخامس من التجربة على التوالي ، البروتين الكلي والالبومين والجلوبيولين في مصل الدم انخفظوا بشكل ملحوظ في نتيجة للديكلورفوس ليسجلوا نسبة اختلاف ٣١،٢ و٣٠،٢ وكذلك ٣١،٤٪ في الاسبوع الرابع والرابع والرابع على التوالي ، الكالسيوم ارتفع بشكل ملحوظ في مصل دم الارانب المعاملة بالديكلورفوس باعلى نسبة اختلاف ٢٤،٣٪ في الاسبوع الرابع بينما الفوسفور انخفض بشكل ملحوظ مع اعلى نسبة اختلاف ٢٨،٦٪ في الاسبوع السادس من التجربة

الخاتمة: إن تناول مبيد ديكلورفوس عن طريق الفم يوميا بجرعة ١٠/١ LD_{50} قد أدي الي انخفاض كبير في وزن الجسم و كذلك أنزيمات الكبد و الكلية مثل الكولين أستريز (ChE) و البروتين الكلي و الألبومين و الجلوبيولين و الفوسفور في حين زاد معدل سكر الجلوكوز والانزيم الناقل للانين امين (ALT) و الانزيم الناقل للاسبارتيت امين (AST) وأنزيم الكالاين فوسفاتيز(ALP) والانزيم الناقل لجاما جلوتاميت (γ-GT) والالبومين و اليوريا و الكرياتينين و الكالسيوم في مصل الدم.

الكلمات المفتاحية : الديكلورفوس– سمية – الكبد – الكلية – الأرنب

Dedication

I would like first and most to thank almighty God for

the blessings and power that made my project a reality

To my great parents spirits who have always live with me

To my brothers and sisters

Special Dedication to my wife who encouraged me to accomplish this thesis

To my university the Islamic university of Gaza which is

continuously improving the dedicate

Each and every one of my colleagues , friends and

community members who participated in bringing this

project to the happy end .

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Bassam Khalil Al malihi

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List of Abbreviations

DDVP	Dichlorovinyl dichloromethyl phosphate
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
ChE	Cholinesterase

Chapter 1

Introduction

Chapter 1

Introduction

1.1 Overview

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 (Gilden, Huffling., Sattler., 2010). These pesticides are classified based on their origin or structure or pests they control the mode/ site of action as insecticides, rodenticides and fungicides. Pesticides are also classified into two major types: chemical and biopesticides (Environmental Protection Agency " EPA", 2013).

Insecticides are chemical compounds used against insects. They include ovicides and larvicides used against the eggs and larvae of insects, respectively. One of the most widely used groups of insecticides in the world is organophosphate compounds. Dichlorvos is an organophosphate insecticide that is used in crop and food storage areas, green houses, barns, in workplaces and in the home. Veterinaries use dichlorvos to control parasites on pets (Agency for Toxic Substances and Disease Registry, ATSDR, 2011). The chemical name of dichlorvos is 2,2-dichlorovinyl dimethyl phosphate (DDVP). Registered trade names include atgard, dichlorman, divipan, herkol, vapona, and nuvan (Musa., Hati., Mustapha., & Magaji., 2010).

Dichlorvos is highly toxic by inhalation, dermal absorption, and ingestion. Because dichlorvos is volatile, inhalation is the most common route of exposure (Lazarini., Lima., Guedes., & Bernardi., 2004). As with all organophosphates, dichlorvos is readily absorbed through the skin. The toxicity of dichlorvos is predicted from LD50 (a dose that expected to cause death in 50% of animals). The oral LD50 for dichlorvos is 61 to 175 mg/kg in mice, 100 to 1090 mg/kg in dogs, 15 mg/kg in chickens, 25 to 80 mg/kg in rats, 157 mg/kg in pigs, and 11 to 12.5 mg/kg in rabbits. The dermal LD_{50} for dichlorvos is 70.4 to 250 mg/kg in rats, 206 mg/kg in mice, and 107 mg/kg in rabbits. The 4-hour LC50 for dichlorvos is greater than 0.2 mg/L in rats (Tomlin, 2011).

As organophosphate compound, dichlorvos inhibits the activity of the enzyme acetylcholinesterase (AChE) which is essential in the normal transmission of nerve impulses. Inactivation of AChE results in the accumulation of acetylcholine at cholinergic receptor sites, causing a cholinergic crisis that can lead to death (Binukumar., & Gill., 2010 ., Barrett ., Jaward., 2012).

Several studies reported the toxic effect of dichlorvos on the functions of several mammalian organs including liver and kidney. Dichlorvos 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) as well as decrease in the levels of cholinesterase were reported in dichlorvos -intoxicated rats (Tembhre., Gour., Ahirwar., & Namdeo., 2012 ., Uthman et al., 2013). Alterations of protein profile as well as urea and creatinine concentrations were also document (Uthman et al., 2013 ; Ojo et al., 2014a).

Pesticides are being used in large amounts in the Gaza Strip where the protective measures are poorly followed (Serag El Din., Yassin., & Al-Shanti., 2014). More than 544.4 metric tons of pesticides are used annually in the Gaza Strip. The insecticide represents 232.5 metric tons of these pesticides; 14.2 metric tons of these insecticides are dichlorvos (Ministry of Agriculture, Palestinian National Authority, 2015). This highly toxic compound constitutes a real threat on humans. The present work is intended to investigate dichlorvos hepato- and renal toxicity in male domestic rabbits. The findings can then be extrapolated to human beings to assess the potential hazards in the human populations due to dichlorvos exposure.

1.2 General objective

The general objective of the present study is to assess hepatic and renal toxicity of dichlorvos in male domestic rabbits**.**

1.3 Specific objective

1. To determine the oral LD_{50} of dichlorvos in male domestic rabbit.

2. To examine the effect of 1/10 LD_{50} dichlorvos on general health and growth rate of male domestic rabbit.

3. To investigate the effect of 1/10 LD_{50} dichlorvos on serum glucose.

4. To study the effect of 1/10 LD_{50} dichlorvos on liver function through measurement of ALT, AST, ALP, γ-GT, ChE and bilirubin.

5. To test the effect of 1/10 LD_{50} dichlorvos on kidney function through determination of serum urea and creatinine.

6. To verify the effect of 1/10 LD_{50} dichlorvos on serum total protein, albumin and globulin.

7. To assess the effect of 1/10 LD_{50} dichlorvos on electrolytes, calcium and phosphorus.

1.4 Significance

1. Dichlorvos is being used in agriculture in Gaza Strip with lake of protective measures (Palestinian Ministry of Agriculture, 2015).

2. Studies on dichlorvos toxicity on rabbits are limited in the literature.

3. The results of the present study may be useful to a ware people particularly, farmers on the extent of dichlorvos toxicity.

Chapter 2

Literature Reviews

Chapter 2

Literature Reviews

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 (World Health Organization, WHO, 2011). Pesticides were identified according to their function into: insecticides control insects; rodenticides control rodents; herbicides control weeds; and fungicides control fungi, mold and mildew (EPA, 2013). 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 Definition and classification of insecticides

An insecticide is a pesticide used against insects in all developmental form. They include ovicides and larvicides used against the eggs and larvae of insects, respectively. Insecticides are used in agriculture, medicine, industry, and general home use. Insecticides can be classified according to the type of action into organochlorine, organophosphates, carbamates, pyrethroids, neonicotinoids, biological insecticides and antifeedants (Brown., 2006 & WHO., 2011).

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, methamidophos and dichlorvos (Tomlin., 2011 ., Ofordile . Okoye., & Raphael., 2014).

2.4 Dichlorvos

2.4.1 Definition and structure

Dichlorvos is an organophosphorus insecticide with chemical structure O.-Odimethyl-O-2, 2-dichloro-vinyl phosphate (DDVP) and a chemical formula of $C_4H_7Cl_2O_4P$ (Figure 2.1, Musa et al., 2010 ., Ofordile et al., 2014).

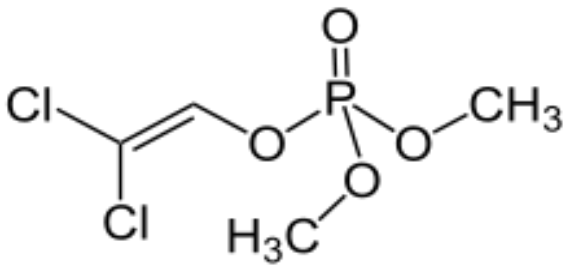

Figure 2.1. Chemical structure of dichlorvos (Ofordile et al., 2014)

2.4.2 Physical and chemical properties of dichlorvos

Dichlorvos is a colorless to amber liquid with an aromatic odor. It is slightly soluble in water and glycerol, and miscible with aromatic and chlorinated hydrocarbon solvents and alcohols. Physical and chemical properties of dichlorvos is summarized in Table 2.1. (Tomlin., 2011).

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

Property	Value
Molecular Weight	220.98
Melting Point	140 ^{o}C at 20 mm Hg
Solubility in Water	16,000 mg/l at
Vapor Pressure	0.012 mm Hg at 20 ^{o}C
Density	1.415 at 25/4 ^{o}C (water = 1)

2.4.3 Mechanism of action of dichlorvos

Dichlorvos like other organophosphates inhibits acetylcholinesterase activity; an enzyme that break 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 or at neuromuscular junction in response to a membrane depolarization which is the hallmark of nerve transmission (Figure 2.2). Acetylcholine then binds to a protein receptor in the membrane of the nerve synapse or neuromuscular junction, which then opens/alters an ion channel, which in turn causes changes in the fluxes of ions (Na+, K+, Ca+, and Cl-) ultimately perpetuating the nerve impulse (Sine & Engel., 2006., Jha., Gupta., Zucker., & Auerbach., 2012). 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 &Ockenga., Kühne., Bocksberger., Banning., & Tikkanen., 2013 ; Xu et al., 2015).
2. Nicotinic receptor (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. Nicotinic receptors subdivided into those found in muscle at neuromuscular junctions and those found in autonomic ganglia and the central nervous system (Gotti et al., 2010., Azazh., 2011., Pohanka & Dobes., 2013 ., Holt et al., 2015).

2.4.3.2 Acetylcholinesterase

As illustrated in Figure 2.2, once acetylcholine makes its action, it is subsequently destroyed by the enzyme acetylcholinesterase, and the membrane returns to its normal resting state (Pohanka, 2011 ; Colović., Krstić., Lazarević-Pašti., Bondžić., & Vasić., 2013).

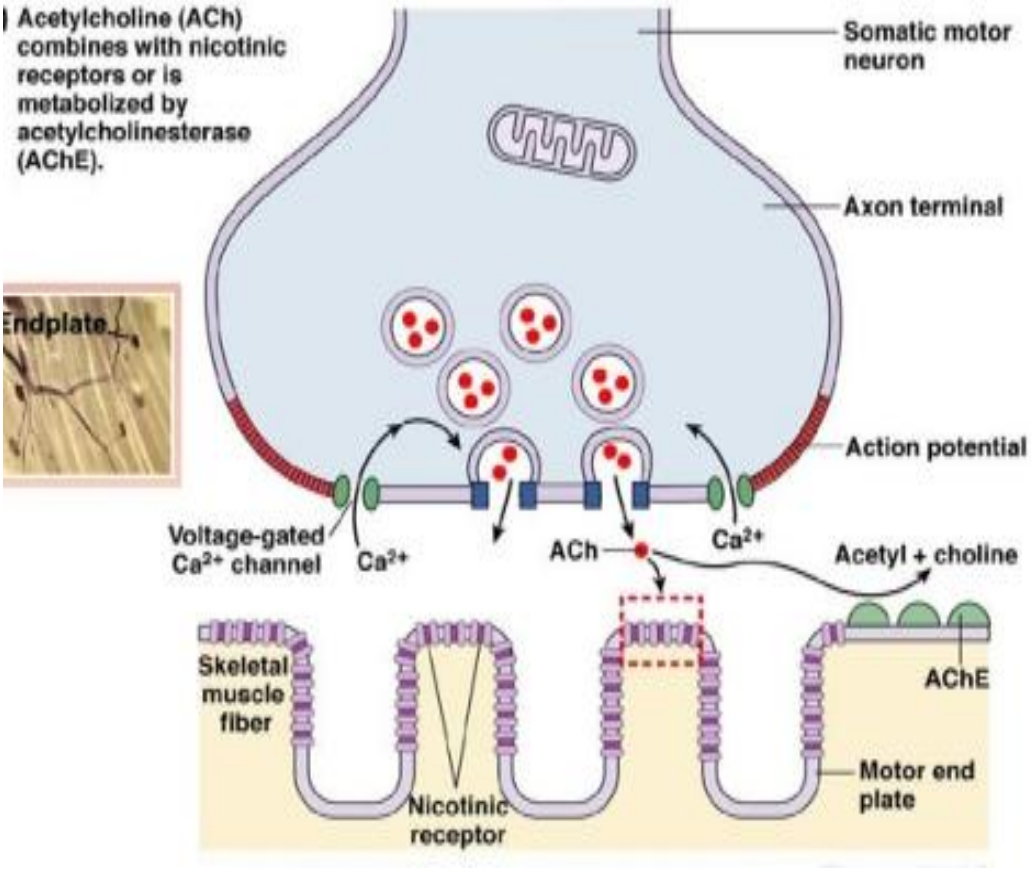

Figure 2.2. Acetylcholine as a neurotransmitter and its inactivation by acetylcholinesterase (Azazh, 2011).

2.4.3.3 Acetylcholinesteraseas a target for dichlorvos

Dichlorvos binds to acetylcholinesterase enzyme in an irreversible manner leading to its inhibition. Acetylcholinesterase inhibition at synapses results in accumulation of acetylcholine and 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 (Binukumar ., & Gill., 2010 ., Tembhre., Gour., Ahirwar., & Namdeo., 2012).

2.4.4 Toxicity symptoms of dichlorvos poisoning

Accumulation of acetylcholine at cholinergic synapses as a result of acetylcholinesterase (AChE) inhibition by dichlorvos producing a range of clinical manifestations, known as the acute cholinergic crisis; headache, restlessness, insomnia, anxiety and other non-specific symptoms. The particular clinical feature

depends on the type of receptors and their location (Kumar et al., 2010., Balali-Mood., & Saber., 2012; Seabury., Sullivan., Stork., & Holland., 2013 .,Narang et al., 2015).

A. Muscarinic receptors: diarrhoea, urinary frequency, intestinal motility, miosis, bronchorrhoea and bronchoconstriction, emesis, lacrimation, salivation, hypotension and secretory gland stimulation, cardiac arrhythmias and bradycardia.

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

C. Central nervous system: altered level of consciousness, respiratory failure and seizures. Severe poisoning results in slurred speech, tremors, ataxia, convulsions, depression of respiratory and circulatory centers and, eventually, coma and death.

2.4.5 Metabolism and excretion of dichlorvos

Dichlorvos is rapidly metabolized by enzymes in the skin, lung, liver, intestine, kidney, heart, skeletal muscles, brain and blood. Dichlorvos breaks down via two enzymatic mechanisms. The first is glutathione-independent, catalyzed by "A"-type esterases, and produces dimethyl phosphate and dichloroacetaldehyde. The second is glutathione-dependent and results in formation of desmethyl dichlorvos and S-methyl glutathione (Figure 2.3). Subsequent degradation of desmethyl dichlorvos to dichloroacetaldehyde and monomethyl phosphate is also catalyzed by "A"-type esterases. S-methyl-glutathione is broken down to methylmercapturic acid and excreted in the urine of animals treated with dichlorvos. The vinyl moiety of the dichlorvos molecule undergoes two routes of biotransformation: conversion to dichloroethanol and subsequent formation of dichloroethanol glucuronide; or dehalogenation and incorporation of the carbon atoms into various metabolic pathways in the body. These pathways result in the production of hippuric acid, urea, carbon dioxide, and other endogenous compounds. Metabolites of dichlorvos are excreted in the urine and feces (ATSDR, 1997 ; Australian Pesticides & Veterinary Medicines Authority, 2008).

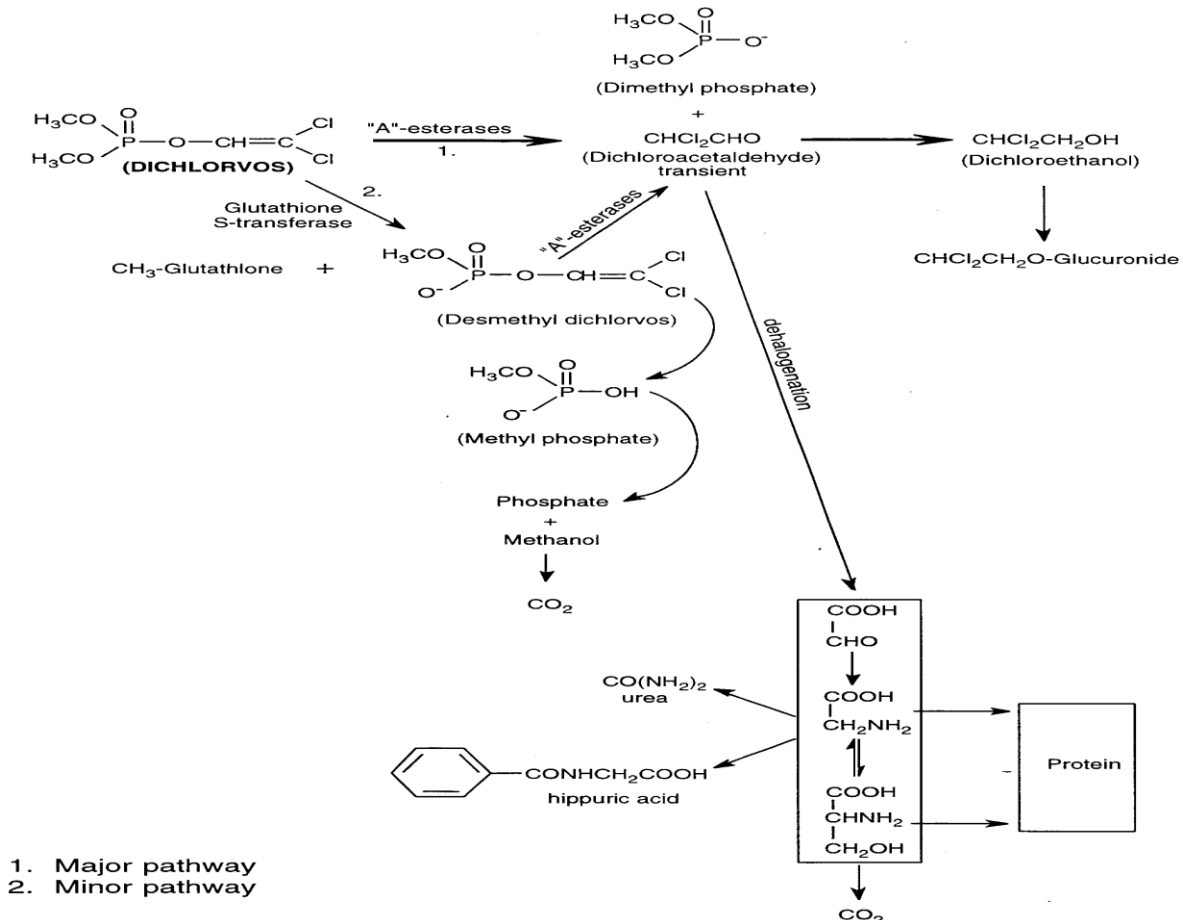

Figure 2.3. Pathways of dichlorvos metabolism (ATSDR, 1997)

2.4.6 Effects of dichlorvos on liver and kidney

Ogutcu et al. (2008) assessed dichlorvos-induced hepatotoxicity in rats and the protective effects of vitamins C and E. Vitamin C (200 mg/kg) + vitamin E (200 mg/kg), dichlorvos (1.6 mg/kg), or a combination of vitamin C (200 mg/kg) + vitamin E (200 mg/kg) + dichlorvos (1.6 mg/kg) was given to rats via oral gavage for 7weeks. When rats of the dichlorvos-treated group and the vitamins + dichlorvos-treated group were compared with the control group, body weights were decreased and liver weights were increased significantly at the end of the 4th and 7th week. Serum total protein, albumin, triglyceride, low density lipoproteincholesterol (VLDL-cholesterol) levels were decreased, and serum ALP, ALT, AST, γGT, lactate dehydrogenase (LDH), and total cholesterol levels were increased significantly at the end of the 4th and 7th week in the dichlorvos- and vitamins + dichlorvos-treated rats. There was a statistically significant difference for all biochemical parameters when the vitamins +

dichlorvos-treated group was compared with the dichlorvos-treated group at the end of the 4th and 7th week.

Celik et al. (2009) studied the effects of sublethal concentrations of dichlorvos on hematological constituent (RBC, WBC, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet counts, hemoglobin and hematocrite levels) and serum damage marker enzymes (AST, ALT, ALP and lactate dehydrogenase) in rats at subacute period under laboratory conditions. Dichlorvos at dosages of 5 and 10 ppm was administered orally to six male rats ad libitum during the tests for 4 weeks consecutively. According to the results, dichlorvos treatments increased significantly the levels of serum marker enzyme activities, whereas they did not change hematologic constituent except for WBC number treated with both dosages of dichlorvos.

In their study entitled "Protective efficacy of 2-PAMCl, atropine and curcumin against dichlorvos induced toxicity in rats", Yadav et al. (2012) demonstrated that serum AST and ALT activities were significantly elevated in dichlorvos-exposed group of rats as compared to control group. Conversely, significant inhibition in serum AchE activity was recorded in dichlorvos-exposed group.

Kemabonta., & Akinhanmi., (2013) investigated the toxicological effects of three pesticides: chlorpyrifos, dichlorvos and alphacypermethrin on body weight, haematology, systemic, biochemical parameters and semen of adult mice. Mice were exposed 8 hrs daily to the fumes of these pesticides and fed pelleted mice feeds for 8 weeks. There was a decrease in weight of mice exposed to the insecticides with chlorpyrifos being the most affected. Mice exposed to alphacypermethrin and the control showed no physiological changes while those exposed to chlorpyrifos and dichlorvos showed skin lesions and inflamed bladder respectively. Histology of tissues of the brain, liver, lung and kidney showed edema, inflammations, congestions, nephritis and necrosis. Haematological parameters showed significant decrease in RBC, haemoglobin and packed cell volume except the WBC, which increased insignificantly. Biochemical parameters showed insignificant increase in AST, ALP, creatinine and urea levels except the Alanine phosphatase (ASP) which

showed a significant increase. Semen analysis revealed decreased sperm motility and an increase in abnormal sperm cells.

Uthman et al. (2013) studied the effects of dichlorvors (1%) on the biochemistry and histology of liver of white albino rats at sub-acute period. Forty clinically healthy adult (Wister strain) albino rats consisting of both sexes, weighing 150-210 gm were randomly divided into treatment and control groups, each consisting of twenty rats. Each group was further divided into four experimental days of first, third, seventh and fourteenth days so that each experimental day has five treatment and five control rats. The pesticide solution at daily oral dose of 7.5 mg/kg was administered. At the end of these experimental periods, blood samples were taken for biochemical tests and liver biopsy for histology. There was significant increase in the levels of serum marker enzyme ALT, AST and ALP as well as in total protein, especially in early treatment days. The histopathology revealed generalized hepatic vascular congestion, mild periportal inflammation, lymphocytic infiltration and focal micro vesicular steatosis.

Ojo et al. (2014a) evaluated the protective effectof *Alstonia boonei* against dichlorvos-induced nephrotoxicity in wistar rats. Dichlorvos was orally administered at a dose of 50 mg/kg body weight for 14 days. Serum concentrations of creatinine and urea were significantly increased in dichlorvos-treated rats compared to control group. However, significant decrease in serum total protein was registered in dichlorvos-treated rats. In addition, Brown et al. (2015) assessed some biochemical indices in albino rats exposed to acute toxic effect of dichlorvos at doses of 3.7, 7.4, 11.1, 14.8 and 18.5 mg/kg, respectively. Statistically significant dose dependent increases in ALT, AST, γ-GT, ALP, urea and creatinine, were observed when compared with control value. There was a significant decrease in cholinesterase levels that was also dose dependent when compared with the control value.

Chapter 3

Materials and Methods

Chapter 3

Materials and Methods

3.1 Experimental animals

Healthy adult male domestic rabbits weighting 1000±200 mg 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 dichlorvos LD_{50}

A total number of 80 rabbits were used for determination of LD_{50} of dichlorvos. Animals were divided into ten groups (8 rabbits/group). The first nine groups (I-IX) were administered different single doses of dichlorvos ranging from 3 to 19 mg/kg body weight as follows:

Table (3.1): Determination of dichlorvos LD_{50}

LD_{50} determination groups	Dose (mg/kg body weight)
Group I	3
Group II	5
Group III	7
Group IV	9
Group V	11
Group VI	13
Group VII	15
Group VIII	17
Group IX	19
Group X control group	0

The tenth group was served as control group. dichlorvos 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 Dichlorvos toxicity experiments

A dose of 1/10 of LD_{50} dichlorvos was given orally to assess dichlorvos toxicity in male domestic rabbit. Animals were divided into two groups: control and experimental groups. Control group comprised 48 rabbits (8 rabbits were housed in each cage) and experimental group included 48 rabbits (8 rabbits were housed in each cage). Experimental groups were orally administrated dichlorvos daily for overall experimental duration of 6 weeks. Control animals were given distilled water. Administration of dichlorvos was also done by special stomach tube. Blood samples were collected weekly and analyzed. Dichlorvos 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 Growth rate

Animals were individually weighed at the beginning and the end of each week 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.

3.6. 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.

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

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

Table (3.2): Reagents used to determination of serum glucose

Reagent	Components	Concentrations
Reagent 1 Monoreagent	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
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.
4. 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 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^+

Table (3.3): Reagents used to determination of alanine aminotransferase

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 $\xrightarrow{AST}$ L-Glutamate + Oxaloacetate

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

Table (3.4): Reagents used to determination of aspartate aminotransferase

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 NADH	 12 mmol/l 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

Table (3.5): Reagents used to determination of alkaline phosphatase

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

Table (3.6): Reagents used to determination of Serum gamma glutamyl transferase

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.

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

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

Table (3.7): Reagents used to determination of cholinesterase activity

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:

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

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 (Thomas, 1998) using DiaSys reagent kits.

Principle

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

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

GLDH: Glutamate dehydrogenase.

Table (3.8): Reagents used to determination of urea

Component	Concentration
Reagent 1: 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
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 ⟶ Creatinine picrate complex

Table (3.9): Reagents used to determination of creatinine

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

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

Table (3.10): Reagents used to determination of total protein

Components	Concentrations
Reagent 1:	
Sodium hydroxide	80 mmol/l
Potassium sodium tartrate	12.8 mmol/l

Reagent 2: Sodium hydroxide Potassium sodium tartrate Potassium iodide Copper sulfate	 100 mmol/l 16 mmol/l 15 mmol/l 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:

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

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.

Table (3.11): Reagents used to determination of albumin

Components	Concentrations
Reagent Citrate buffer pH 4.2 Bromocresol green	 30 mmol/l 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.

Table (3.12): Reagents used to determination of calcium

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 complex

Table (3.13): Reagents used to determination of phosphorus

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 Distilled water	1 µl 10 µl	1 µl -	1 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

Chapter 4

Results

Chapter 4

Results

4.1 Oral LD_{50} of dichlorvos

The experimental trials for oral LD_{50} determination of dichlorvos after 48 hr of administration in male domestic rabbits revealed that the mortality commenced at 5 mg/kg body weight, recording mortality percentage of 12.5% (Table 4.1). Increasing dichlorvos dose to 7, 9, 11, 13, 15 and 17 resulted in mortality percentages of 12.5, 25.0, 50.0, 50.0, 75.0 and 87.5%, respectively. The mortality rate was a function of dose increase. The maximum concentration of dichlorvos which killed all animals in the group was found to be 19 mg/kg body weight. The calculated oral LD_{50} of dichlorvos in male domestic rabbits from the linear regression was found to be 11.6 mg/kg body weight (Figure 4.1).

Table (4.1): Mortality percentage of male domestic rabbits after 48 hr of oral administration of different doses of dichlorvos.

Group	Dichlorvos Dose (mg/kg body weight)	Number of Animals died/total	% mortality
Group I	3	0/8	0
Group II	5	1/8	12.5
Group III	7	1/8	12.5
Group IV	9	2/8	25.0
Group V	11	4/8	50.0
Group VI	13	4/8	50.0
Group VII	15	6/8	75.0
Group VIII	17	7/8	87.5
Group IX	19	8/8	100
Group X	Control	0/8	0.0

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

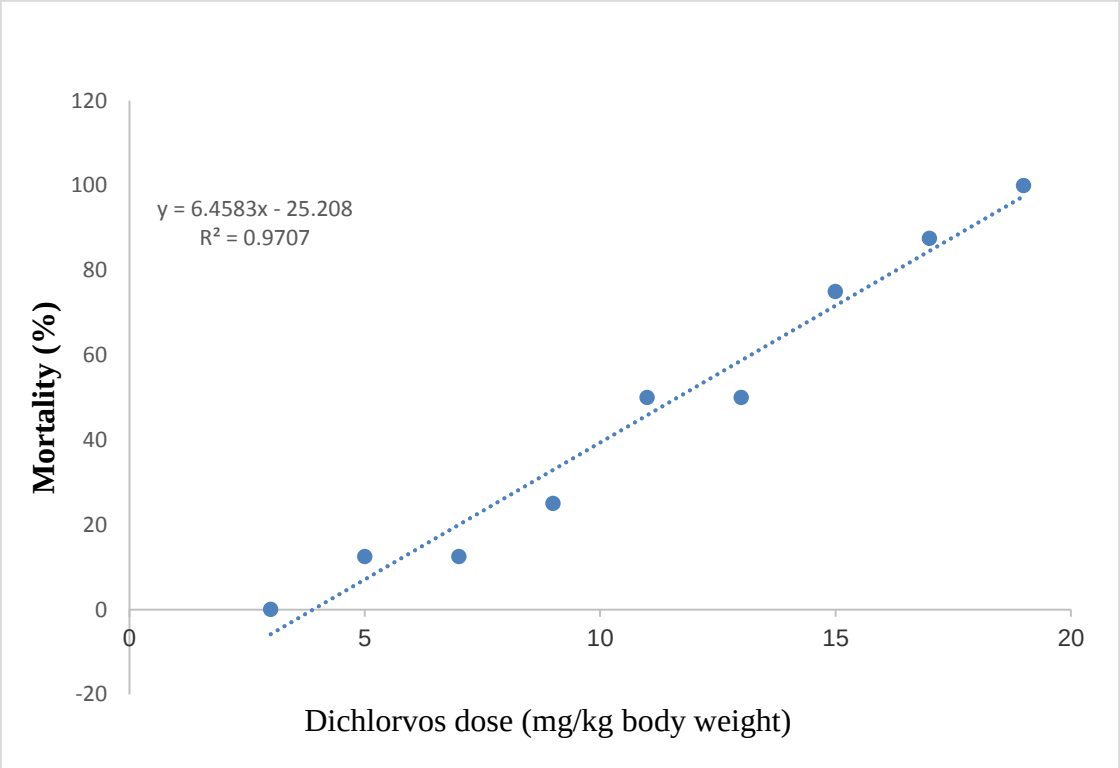

Figure 4.1 Liner Regression of oral LD_{50} of dichlorvos in male domestic rabbits (LD_{50}=11.6 mg/kg body weight).

4.2 General health of rabbits

To assess dichlorvos toxicity in rabbits, 1/10 LD_{50} dichlorvos (1.2 mg/kg) was orally administered daily for 6 weeks. The mortality rate recorded for 1/10 LD_{50} dichlorvos-treated rabbits was 0/8 (0%), 0/8 (0%), 0/8 (0%), 0/8 (0%), 1/8 (12.5%) and 2/8 (25.0%) after 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks of the experiment, respectively. However, none of the rabbits died in the control group. In addition, rabbits in the control group did not show any sign of toxicity. However, dichlorvos-fed rabbits showed varying degrees of clinical signs few minutes after dosing. The signs included diarrhea, reduced food intake, weakness, disorientation, drowsiness and mild tremors Concerning morphological changes, dichlorvos-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 dichlorvos administration (Figure 4.2B) whereas those of the control animals showed normal appearance.

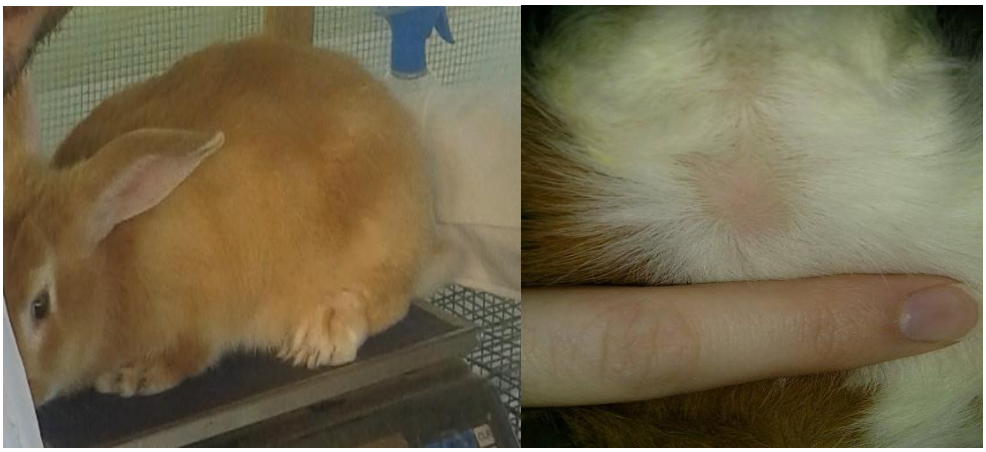

Figure 4.2A: Effect of 1/10 LD_{50} dichlorvos (1.2 mg/kg) on hair of male domestic rabbits. Control (left) and dichlorvos-treated rabbits (right)

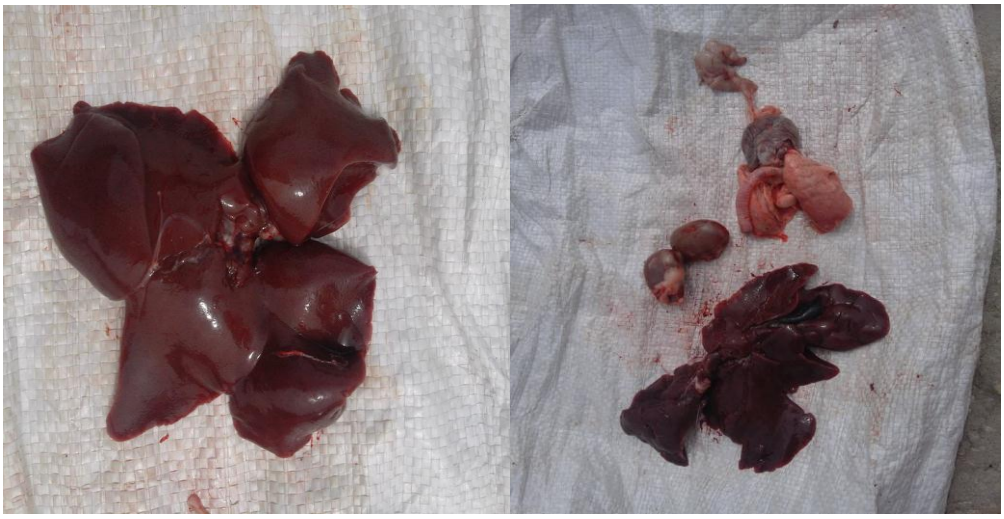

Figure 4.2B: Effect of 1/10 LD_{50} dichlorvos (1.2 mg/kg) on liver appearance of male domestic rabbits. Control (left) and dichlorvos-treated rabbits (right)

4.3 Growth rate

Figure 4.2 illustrates the growth rate of controls and 1/10 LD_{50} (1.2 mg/kg body weight) dichlorvos-treated male domestic rabbits at weekly intervals for 6 weeks. There was a prominent decrease in the growth rate of dichlorvos-fed rabbits with respect to controls.

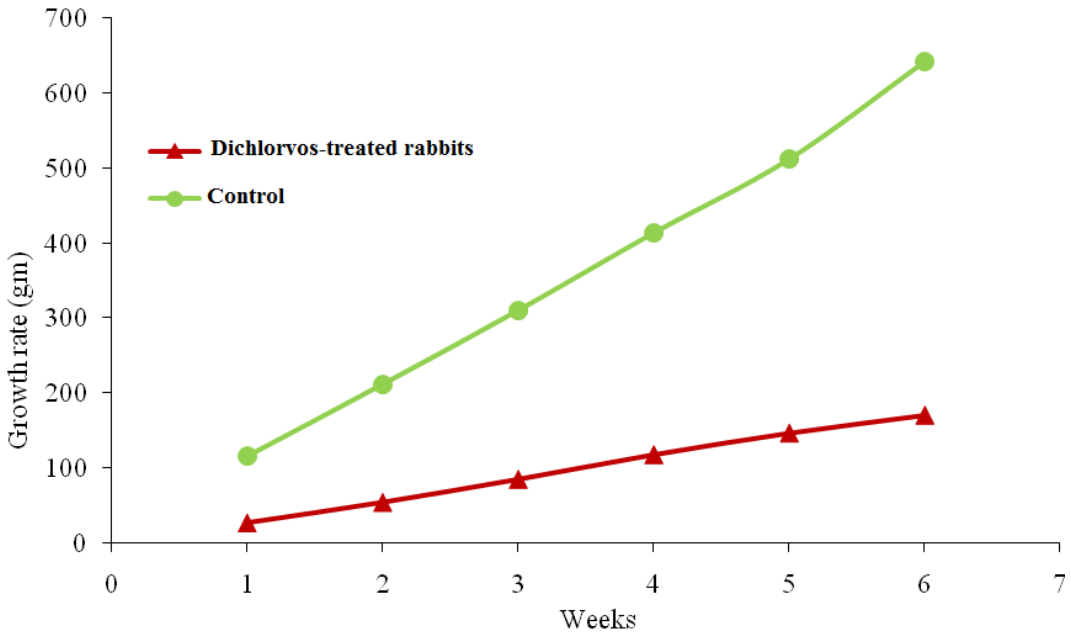

Figure 4.3 Growth rate of control and dichlorvos-treated rabbits during the 6 weeks experimental intervals

4.4 Biochemical investigation

4.4.1 Serum glucose

Table 4.3 provides serum glucose levels in control and dichlorvos-treated male domestic rabbits along the experimental period of 6 week intervals. The mean values of glucose level in controls were 114.0±3.8, 116.4±4.4, 114.8±4.6, 115.3±4.3, 117.1±5.2 and 116.6±5.5 mg/dl during the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Oral administration of 1/10 LD_{50} dichlorvos (1.2 mg/kg body weight) 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 26.7% in the 4th week (t=4.428, P=0.001).

Table (4.2): Effect of dichlorvos (1/10 LD_{50}, 1.2 mg/kg body weight) on serum glucose level (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	dichlorvos (n=8)	% difference	t-value	P-value
1	114.0±3.8	111.2±4.0	-2.5	0.504	0.624
2	116.4±4.4	129.2±5.7	10.4	1.818	0.094
3	114.8±4.6	134.1±6.6	15.5	2.457	0.030

4	115.3±4.3	150.8±7.1	26.7	4.428	0.001
5	117.1±5.2	146.5±7.3	22.3	3.342	0.006
6	116.6±5.5	141.1±6.9	19.0	2.742	0.018

The number of animals (n) was 8 in control group and 8 in dichlorvos-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 dichlorvos-treated male domestic rabbits along the experimental period of 6 weeks are pointed out in Table 4.4. The normal enzyme activity was 48.5±1.8, 50.3±2.6, 49.8±2.4, 47.4±2.0, 49.0±2.7, and 51.1±2.9 U/l at the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Upon dichlorvos administration, ALT activity was increased throughout the experimental periods reaching mean values of 52.3±2.4, 61.0±3.2, 59.7±3.5, 55.5±3.1, 65.9±4.0 and 66.6±3.8 U/l. This increase was significant during the last five weeks of the experiment recording the maximum percentage difference of 29.4% in the 5th week of the experiment (t=3.613, P=0.004).

Table (4.3): Effect of dichlorvos (1/10 LD_{50}, 1.2 mg/kg body weight) on serum alanine aminotransferase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	dichlorvos (n=8)	% difference	t-value	P-value
1	48.5±1.8	52.3±2.4	7.5	1.372	0.195
2	50.3±2.6	61.0±3.2	19.2	2.665	0.021
3	49.8±2.4	59.7±3.5	18.1	2.396	0.034
4	47.4±2.0	55.5±3.1	15.7	2.268	0.043
5	49.0±2.7	65.9±4.0	29.4	3.613	0.004
6	51.1±2.9	66.6±3.8	26.3	3.279	0.007

The number of animals (n) was 8 in control group and 8 in dichlorvos-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 illustrates mean values of serum AST activity in control and chlorpyrifos-fed male domestic rabbits all over the experimental period of 6 weeks. AST activity registered for control animals were 32.8±1.6, 33.2±1.9, 35.0±2.0, 32.4±1.8, 34.1±1.5 and 33.4±1.7 U/l at the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Feeding of animals with dichlorvos provoked elevation in the enzyme activity with significant changes starting from the second week of experiment. The maximum elevation in the enzyme activity was recorded at the 5th week of the experiment showing percentage difference of 38.6% (t=5.158, P=0.000).

Table (4.4): Effect of dichlorvos (1/10 LD_{50}, 1.2 mg/kg body weight) on serum aspartate aminotransferase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	dichlorvos (n=8)	% difference	t-value	P-value
1	32.8±1.6	37.1±1.8	12.3	1.881	0.084
2	33.2±1.9	43.0±2.5	25.7	3.189	0.008
3	35.0±2.0	43.4±2.3	21.4	2.852	0.015
4	32.4±1.8	45.3±2.6	33.2	4.235	0.001
5	34.1±1.5	50.4±3.0	38.6	5.158	0.000
6	33.4±1.7	45.9±2.8	31.5	3.982	0.002

The number of animals (n) was 8 in control group and 8 in dichlorvos-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 mean normal activity of serum ALP of male domestic rabbits were 104.1±4.5, 102.8±4.1, 103.5±4.8, 100.3±3.9, 105.0±4.6 and 104.7±5.0 U/l at 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively (Table 4.6). Dichlorvos intake increased the enzyme activity during the whole experiment, showing mean values of 118.0±5.6, 113.9±4.8, 123.2±5.5 and 132.7±6.7, 130.4±6.8 and 127.6±6.3 U/l, respectively. The significant increment in the enzyme activity started from the 3rd week of the experiment and the maximum percentage difference of 27.8% was found during the 4th week (t=4.356, P=0.001).

Table (4.5): Effect of dichlorvos (1/10 LD_{50}, 1.2 mg/kg body weight) on serum alkaline phosphatase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	dichlorvos (n=8)	% difference	t-value	P-value
1	104.1±4.5	118.0±5.6	12.5	1.953	0.075
2	102.8±4.1	113.9±4.8	10.2	1.802	0.097
3	103.5±4.8	123.2±5.5	17.4	2.692	0.020
4	100.3±3.9	132.7±6.7	27.8	4.356	0.001
5	105.0±4.6	130.4±6.8	21.6	3.204	0.008
6	104.7±5.0	127.6±6.3	19.7	2.890	0.014

The number of animals (n) was 8 in control group and 8 in dichlorvos-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 demonstrates the mean values of serum γGT activity in control and dichlorvos-intoxicated male domestic rabbits all over the experimental period of 6 weeks. The mean normal activities of γGT were 5.68±0.24, 5.79±0.22, 5.71±0.26, 6.12±0.34, 5.90±0.30 and 6.05±0.29 U/l at 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks of the experiment, respectively. In general, daily oral administration of dichlorvos significantly increased the enzyme activity throughout the experiment to reach mean values of 6.72±0.34, 6.48±0.31, 6.60±0.29, 7.94±0.43, 8.33±0.52 and 8.90±0.53 U/l, respectively. The maximum increase in γGT activity was registered at the end of the experiment showing percentage difference of 38.1% (t=5.016, P=0.000)

Table (4.6): Effect of dichlorvos (1/10 LD_{50}, 1.2 mg/kg body weight) on serum gamma glutamyltransferase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	dichlorvos (n=8)	% difference	t-value	P-value
1	5.68±0.24	6.72±0.34	16.8	2.540	0.026
2	5.79±0.22	6.48±0.31	11.2	1.863	0.087
3	5.71±0.26	6.60±0.29	14.5	2.235	0.045
4	6.12±0.34	7.94±0.43	25.9	3.338	0.006
5	5.90±0.30	8.33±0.52	34.2	4.301	0.001
6	6.05±0.29	8.90±0.53	38.1	5.016	0.000

The number of animals (n) was 8 in control group and 8 in dichlorvos-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 dichlorvos-fed rabbits are presented in Table 4.8. The mean normal ChE activities in control animals were 5024±219, 4911±198, 4874±183, 5003±197, 4738±170 and 5036±201 U/l during the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Oral administration of the organophosphorus pesticide dichlorvos provoked a progressive significant decrease in the enzyme activity to values of 4375±181, 3918±162, 3396±158, 2874±136, 2312±109 and 2353±102 U/l, respectively. The maximum decrease in the ChE activity was obtained at the last week of the experiment recording a percentage difference of 72.6% (t=12.014, P=0.000).

Table (4.7): Effect of dichlorvos (1/10 LD_{50}, 1.2 mg/kg body weight) on serum cholinesterase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	dichlorvos (n=8)	% difference	t-value	P-value
1	5024±219	4375±181	-13.8	2.230	0.048
2	4911±198	3918±162	-22.5	3.698	0.005
3	4874±183	3396±158	-35.7	5.953	0.000
4	5003±197	2874±136	-54.1	8.861	0.000
5	4738±170	2312±109	-68.8	11.948	0.000
6	5036±201	2353±102	-72.6	12.014	0.000

The number of animals (n) was 8 in control group and 8 in dichlorvos-treated animals. All values were expressed as mean±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 dichlorvos daily for 6 weeks are shown in Table 4.9. Oral administration of dichlorvos caused gradual increase in bilirubin level. This increase was significant in the last 4 weeks of the experiment. The maximum increase in bilirubin level was recorded during the 5th week of the experiment with percentage difference of 23.3 (t=3.463, P=0.006).

Table (4.8): Effect of dichlorvos (1/10 LD_{50}, 1.2 mg/kg body weight) on serum bilirubin (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	dichlorvos (n=8)	% difference	t-value	P-value
1	0.92±0.03	0.97±0.05	5.3	1.014	0.334
2	0.95±0.02	1.04±0.06	9.0	1.520	0.159
3	0.94±0.04	1.07.±0.04	12.9	2.111	0.061
4	0.88±0.03	1.10±0.06	22.2	3.366	0.007
5	0.91±0.02	1.15±0.07	23.3	3.463	0.006
6	0.96±0.05	1.19±0.06	21.4	3.240	0.009

The number of animals (n) was 8 in control group and 8 in dichlorvos-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 dichlorvos-treated male domestic rabbits. Urea concentration in control animals exhibited values of 37.9±1.6, 40.1±1.8, 38.6±1.5, 39.4±1.4, 40.8±2.0 and 41.2±1.9 mg/dl during 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks of the experiment, respectively. Daily feeding of dichlorvos caused successive increase in urea concentration all over the experimental intervals examined reaching values of 40.8±2.0, 47.6±2.4, 47.1±2.9, 50.3±3.1, 57.2±3.3 and 58.9±3.0 mg/dl, respectively. The increase in urea concentration was significant during the last weeks and reached its maximum percentage difference of 35.4% at the end of the experiment (t=5.051, P=0.000).

Table (4.9): Effect of dichlorvos (1/10 LD_{50}, 1.2 mg/kg body weight) on serum urea (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	dichlorvos (n=8)	% difference	t-value	P-value
1	37.9±1.6	40.8±2.0	7.4	1.153	0.276
2	40.1±1.8	47.6±2.4	17.1	2.496	0.032
3	38.6±1.5	47.1.±2.9	19.8	2.571	0.029
4	39.4±1.4	50.3±3.1	24.3	3.268	0.008
5	40.8±2.0	57.2±3.3	33.5	4.329	0.001
6	41.2±1.9	58.9±3.0	35.4	5.051	0.000

The number of animals (n) was 8 in control group and 8 in dichlorvos-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 dichlorvos along the experimental period of 6 weeks are indicated in Table 4.11. The normal values recorded for creatinine concentrations were 0.68±0.03, 0.74±0.4 0.70±0.02, 0.69±0.03, 0.72±0.04, and 0.70±0.5 mg/dl at the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Upon dichlorvos administration, serum creatinine increased with fluctuation across the experimental period reaching its maximum value of 0.97±0.07 mg/dl during the 5th week of the experiment with percentage difference of 29.6% (t=3.685, P=0.004).

Table (4.10): Effect of dichlorvos (1/10 LD_{50}, 1.2 mg/kg body weight) on serum creatinine (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	dichlorvos (n=8)	% difference	t-value	P-value
1	0.68±0.03	0.72±0.04	5.7	1.027	0.328
2	0.74±0.04	0.89±0.06	18.4	2.490	0.033
3	0.70±0.02	0.81.±0.05	14.6	2.314	0.044
4	0.69±0.03	0.89±0.05	25.3	3.141	0.010
5	0.72±0.04	0.97±0.07	29.6	3.685	0.004
6	0.70±0.05	0.92±0.03	27.2	3.479	0.006

The number of animals (n) was 8 in control group and 8 in dichlorvos-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 displays the mean normal values of serum total protein levels in male domestic rabbits throughout the experimental period of 6 weeks. These values were 6.57±0.30, 6.80±0.28, 6.74±0.33, 6.39±0.36, 7.11±0.37 and 7.04±0.39 g/dl during the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Total protein level

showed an overt decrease in response to dichlorvos administration along the experimental periods tested. This decrease become significant starting from the 3rd week till the end of experiment, recording it's a maximum % difference of 31.2 at the 4th week of the experiment (t=4.308, P=0.002).

Table (4.11): Effect of dichlorvos (1/10 LD_{50}, 1.2 mg/kg body weight) on serum total protein (g/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	dichlorvos (n=8)	% difference	t-value	P-value
1	6.57±0.30	5.82±0.27	-12.1	1.893	0.088
2	6.80±0.28	6.12±0.25	-10.5	1.833	0.096
3	6.74±0.33	5.35.±0.23	-23.0	3.331	0.008
4	6.93±0.36	5.06±0.22	-31.2	4.308	0.002
5	7.11±0.37	5.78±0.26	-20.6	3.004	0.013
6	7.04±0.39	5.43±0.21	-25.8	3.651	0.005

The number of animals (n) was 8 in control group and 8 in dichlorvos-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 dichlorvos-treated male domestic rabbits are exhibited in Table 4.13. Albumin concentration in control animals exhibited mean values of 3.58±0.18, 4.04±0.22, 3.98±0.20, 4.12±0.24, 4.17±0.23 and 4.14±0.22 g/dl at 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Dichlorvos intake resulted in significant decrease in albumin concentration recording the minimum concentration of 3.04±0.11 g/dl at the 4th week of the experiment with percentage difference of 30.2% (t=4.021 and P=0.003).

Table (4.12): Effect of dichlorvos (1/10 LD_{50}, 1.2 mg/kg body weight) on serum albumin (g/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	dichlorvos (n=8)	% difference	t-value	P-value
1	3.85±0.18	3.47±0.12	-10.4	1.823	0.098
2	4.04±0.22	3.59±0.11	-11.8	1.868	0.091

3	3.98±0.20	3.20.±0.15	-21.7	3.178	0.010
4	4.12±0.24	3.04±0.11	-30.2	4.021	0.003
5	4.17±0.23	3.38±0.10	-20.9	3.065	0.012
6	4.14±0.22	3.27±0.10	-23.5	3.418	0.007

The number of animals (n) was 8 in control group and 8 in dichlorvos-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 2.69±0.12, 2.83±0.11, 2.75±0.12, 2.80±0.14, 2.92±0.15 and 2.88±0.17 g/dl during the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Oral administration of dichlorvos lowered globulin levels to 2.40±0.11, 2.56±0.13, 2.20±0.10, 2.04±0.08, 2.39±0.10, and 2.88±0.17 g/dl showing percentage differences of 11.4, 10.0, 22.2, 31.4, 20.0 and 26.3 % 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 (4.13): Effect of dichlorvos (1/10 LD_{50}, 1.2 mg/kg body weight) on serum globulin (g/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	dichlorvos (n=8)	% difference	t-value	P-value
1	2.69±0.12	2.40±0.11	-11.4	1.836	0.096
2	2.83±0.11	2.56±0.13	-10.0	1.710	0.118
3	2.75±0.12	2.20±0.10	-22.2	3.284	0.009
4	2.80±0.14	2.04±0.08	-31.4	4.579	0.001
5	2.92±0.15	2.39±0.10	-20.0	2.928	0.015
6	2.88±0.17	2.21±0.08	-26.3	3.735	0.004

The number of animals (n) was 8 in control group and 8 in dichlorvos-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 dichlorvos-received male rabbits are provided in Table 4.15. The normal concentrations of calcium were 10.2±0.3, 10.5±0.4, 10.0±0.3, 10.1±0.2, 10.3±0.5 and 10.1±0.4 mg/dl during the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Upon dichlorvos administration, serum concentration of calcium fluctuates throughout the experiment registering significant increase, in general, throughout the experimental periods examined. The maximum increase in calcium concentration was recorded during the 4th week of the experiment with percentage difference of 24.3 (t=3.348, P=0.007).

Table (4.14): Effect of dichlorvos (1/10 LD_{50}, 1.2 mg/kg body weight) on serum calcium (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	dichlorvos (n=8)	% difference	t-value	P-value
1	10.2±0.3	11.4±0.5	11.1	1.865	0.092
2	10.5±0.4	12.6±0.7	18.2	2.671	0.023
3	10.0±0.3	11.8±0.6	16.5	2.577	0.028
4	10.1±0.2	12.9±0.8	24.3	3.348	0.007
5	10.3±0.5	12.0±0.4	15.2	2.401	0.037
6	10.1±0.4	11.6±0.5	13.8	2.242	0.049

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

4.4.6.2 Serum phosphorus

As depicted from Table 4.16, serum phosphorus concentrations were generally showed gradual decrease in dichlorvos--treated group with respect to control group. The significant change was registered during the last 4 weeks of the experiment. The maximum decrease in phosphorus concentration was found at the end of the experiment displaying percentage difference of 28.6 (t=3.868, P=0.003).

Table (4.15): Effect of dichlorvos (1/10 LD_{50}, 1.2 mg/kg body weight) on serum phosphorus (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	dichlorvos (n=8)	% difference	t-value	P-value
1	7.08±0.34	6.23±0.27	-12.8	1.976	0.076
2	7.13±0.30	6.43±0.25	-10.3	1.801	0.102
3	7.35±0.39	6.14±0.24	-17.9	2.631	0.025
4	7.27±0.38	5.86±0.22	-21.5	3.135	0.011
5	7.40±0.43	5.81±0.21	-24.1	3.325	0.008
6	7.51±0.45	5.63±0.18	-28.6	3.868	0.003

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

Chapter 5

Discussion

Chapter 5

Discussion

A pesticides are being extensively used in Gaza Strip for the purpose of increasing agricultural yield and improving income. These highly toxic compounds, usually used or/and misused with poorly protective measures, imposed a real threat on human health (Serag El Din et al., 2014). One of these pesticides is the organophosphorus insecticide dichlorvos which is commonly used in Gaza Strip to combat insects on many crops (Ministry of Agriculture, Palestinian National Authority, 2015). Previous studies reported several cases of organophosphorus poisoning among farm workers and children in Gaza strip which mostly result from use/misuse of this type of pesticides (Yassin et al., 2002 ; EL-Shanty, 2009). However, limited data are available on the extent of toxicity of dichlorvos. Therefore, the present work was performed to determine the oral LD_{50} of dichlorvos 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 dichlorvos exposure.

5.1 Toxicity of dichlorvos

The logarithmic scale showed that the oral LD_{50} of dichlorvos in male domestic rabbits was 11.6 mg/kg body weight. This value lies in the range of previously estimated LD_{50} of dichlorvos in rabbits (Tomlin, 2011). The low value of LD_{50} registered for dichlorvos confirmed the fact that this organophosphorus insecticide is highly toxic. This coincides with the idea that the lower the LD_{50} value, the higher the toxicity of the pesticide. Dichlorvos was classified as a highly hazardous pesticide in terms of being neurotoxin, carcinogenic, genotoxin, endocrine disruptor, toxic to reproductive and immune system as well as to the environment (Zabrodskii et al., 2008., Masoud et al., 2011., Barrett ., Jaward., 2012., Dirican & Kalender, 2012 ., Watts., 2014).

5.2 General health of rabbits

The present study demonstrates that treatment of rabbits with 1/10 LD_{50} dichlorvos induced an overall mortality rate of 6.3% throughout the 6 weeks of the experiment.

Such mortality was mostly attributed to diarrhea and reduced food intake which may be related to the cholinergic crisis, a consistent sign in organophosphate poisoning (Kumar et al., 2010 ; Narang et al., 2015). In Addition, Dichlorvos-treated rabbits showed dermal abnormalities and hair loss particularly in the last two weeks of the experiment. Similar observations were reported (Kemabonta ; Akinhanmi, 2013). The hair loss noticed in experimental rabbits is coincided with the significant decrease in protein content observed in the present study. In general, it is accepted that organophosphorus pesticides suppress the immune system making the skin vulnerable to attack of various pathogens (Pore et al., 2011 ., Diaz-Resendiz et al., 2015). The livers of dichlorvos-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. Dichlorvos is known to induce morphological and histopathological changes in the liver (Ogutcu et al., 2008 ., Kurtulus et al., 2012).

5.3 Growth rate

Data presented in the current study revealed a prominent decrease in the growth rate of dichlorvos-supplemented rabbits with respect to controls. This decrease in body weight is in agreement with that obtained by Ogutcu et al. (2008) ; Kemabonta ; Akinhanmi (2013). The reduction in body weight in response to dichlorvos intake may be a result of the combined action of cholinergic (reduced food intake and diarrhea) and oxidative stress and/or due to increase degradation of lipids and proteins as a direct effect of organophosphours compound exposure (Ogutcu et al. (2008); Mossa et al., 2011 ., Sharma et al., 2015). This explanation is supported by the recorded significant decrease in protein content in dichlorvos-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} dichlorvos 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 in concurrent with that reported by Joshi & Rajini (2012)., Lakshmanan et al. (2013). Therefore, glucose homeostasis is affected by dichlorvos administration. The

mechanism by which the organophosphorus insecticide dichlorvos induces hyperglycemia may involve one or more mechanisms: 1) reduction in insulin secretion as a result of the destructive action of dichlorvos on the pancreas (Nozha et al., 2005 ., Chaturvedi et al., 2015), 2) impairment in hepatic function due to oxidative changes, which reduce liver ability to glycogenesis (Bui-Nguyen et al., 2015), 3) stimulation of hepatic gluconeogenesis and glycogenolysis (Pore et al., 2011 ., Lakshmanan et al., 2013), ; 4) activation of the hypothalamus-pituitary-adrenal (HPA) axis. The activation of HPA axis by organophosphorus causes secretion of glucocorticoids from adrenal cortex that in turn increases blood glucose by induction of gluconeogenesis pathway (Rahimi.,& Abdollahi, 2007).

5.4.2 Liver enzymes and bilirubin

As shown in this study, the mean levels of serum ALT, AST, ALP and γ-GT in the dichlorvos-treated rabbits were significantly higher than those in the controls. Such elevation of liver enzymes as a result of dichlorvos administration was documented by other authors (Ogutcu et al., 2008; Celik et al., 2009; Yadav et al., 2012
., Kemabonta ., Akinhanmi., 2013 ., Holy et al., 2015a). Liver is the center of biotransformation and detoxification of foreign compounds and is the most vulnerable to the chemical assaults such as dichlorvos poisoning (Hodgson., 2004 ., Holy et al., 2015b). Serum ALT, AST and γ-GT are considered to be among the most sensitive markers employed in the diagnosis of hepatotoxicity (Ogutcu et al., 2008; Ambali et al., 2011 ., Newairy.,&Abdou, 2013). Pesticide exposure, including dichlorvos, causes liver damage and leakage of cytosolic enzymes from hepatocytes and other body organs into blood stream (Yadav et al., 2012., Newairy., & Abdou, 2013 ., Holy et al., 2015a). Elevation of liver enzymes may also be due to induction of oxidative stress or to increased gene expression due to long term requirement of detoxification of pesticides (Friedman et al., 2003 .,Ojo et al., 2014b).

In contrast to elevation of transaminases, γ-GT and ALP, serum ChE activity was markedly decreased in dichlorvos-treated rabbits compared to controls. Such inhibition in ChE in response to organophosphorus dichlorvos administrated was reported by Mousa, 2009; Binukumar & Gill (2010) ., Dere et al., (2010). In addition, Holy (2015a) showed that the decrease in ChE in dichlorvos-treated albino rats was dose dependent when compared with the control value. It is known that

organophosphorus pesticides such as dichlorvos cause 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 is usually manifested in convulsions and even tremors leading in severe cases to death (Lotti, 2001., Varo et al., 2003., Chedi., & Aliyu, 2010 ., Colović et al., 2013). Therefore, ChE level in serum is useful as a test of liver function and as an indicator of organophosphorus insecticide poisoning. This conclusion is supported by some mortalities and the clinical signs of anticholinestrase action represented by diarrhea, reduced food intake, weakness, disorientation, drowsiness and mild tremors which were observed in dichlorvos-treated rabbits.

In the present study oral administration of dichlorvos caused general increase in bilirubin level throughout the experiment. Such increase was reported previously by Wu ., & Deng (2009)., Ahmad ., & Gautam (2014) ., Vanaja ., & Palanimuthu (2014) and El-Saad et al., (2016) in organophosphorus poisoning, including dichlorvos. The change in serum bilirubin which is accepted as indicator of liver function may provide further evidence on hepatotoxicity induced by the organophosphorus insecticide dichlorvos (Ogutcu et al., 2008., Celik et al., 2009 ., El-Saad et al., 2016).

5.4.3 Kidney function

The effect of dichlorvos on kidney function was assessed through the measurement of urea and creatinine. Urea concentration was generally increased throughout the whole experiment, and this increment was significant during the last five weeks of the experiment compared to the control. Similar result was registered for creatinine. Such findings are in agreement with that reported in other studies (Ojo et al., 2014a ., Holy et al., 2015a). he 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 ., Mansour ., & Mossa (2010). 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 and Debra Manzella, 2008). 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 dichlorvos 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., Ambali et al., 2010 ., Ojo et al., 2014a).

5.4.4 Protein profile

As indicated in the present results significant decrease in the levels of total protein, albumin and globulin concentrations were found in rabbits treated with dichlorvos compared to the controls. Similar findings were reported in other studies as a result of oral administration of different doses of dichlorvos (Ogutcu et al., 2008., Ojo et al., 2014a). This suggests that exposure to an OP insecticide, such as dichlorvos, may influence total protein and albumin metabolism. 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 (Lakshmanan et al., 2013 ., Deka., & Mahanta, 2015). In addition, the observed decrease in serum proteins could be attributed in part to the damaging effect of dichlorvos 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).

5.4.5 Electrolytes

The mean serum concentration of calcium showed significant increase in dichlorvos-intoxicated rabbits. In contrast, serum phosphorus concentration was significantly decreased in response to dichlorvos treatment. Raheja ., & Gill (2002) showed that chronic dichlorvos administration caused significant rise in the intrasynaptosomal calcium levels. In addition, Bui-Nguyen et al. (2015) reported that dichlorvos exposure altered the transcript abundance of the calcium storage and signaling pathway proteins. This indicates that the organophosphorus insecticide dichlorvos

interferes with calcium homeostasis. Hypophosphatemia and hypercalcemia particularly at later intervals of the experiment were recorded by Tripathi .,& Srivastav (2012) in male Wistar rats exposed to the organophosphorus dichlorvos. They suggested that exposure to organophosphorus pesticide may disturb parathyroid glands and calcitonin cells and may alter bone mineral composition especially calcium and phosphorus levels of bone.

Chapter 6
Conclusions

Chapter 6

Conclusions

1. The calculated oral LD_{50} of dichlorvos in male domestic rabbits from the linear regression was found to be 11.6 mg/kg body weight.
2. Daily oral administration of 1/10 LD_{50} dichlorvos caused an overall mortality rate of 6.3% compared to no mortalities in controls. Clinical signs of dichlorvos-intoxicated rabbits were diarrhea, reduced food intake, weakness, disorientation, drowsiness and mild tremors.
3. The growth rate was significantly decreased in dichlorvos-treated rabbits compared to controls.
4. Serum glucose was significantly increased in response to dichlorvos feeding compared to controls.
5. Liver enzymes ALT, AST, ALP and γ-GT as well as bilirubin were significantly higher in the dichlorvos-intoxicated rabbits whereas cholinesterase level was markedly decreased compared to the controls.
6. Urea and creatinine concentrations were significantly increased in response to dichlorvos administration compared to the controls.
7. There were significant decreases in total protein, albumin and globulin values upon dichlorvos intake compared to the controls.
8. Hypercalcemia and hypophosphatemia were recorded in dichlorvos- intoxicated rabbits.

Recommendations

1. Restriction the use of pesticides in home and farm and use of more secure alternatives such as biological control.
2. Further studies are needed using other types of pesticides.

Chapter 7
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