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Hepatic and Renal Toxicity of Methamidophos in Male Domestic Rabbit

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اسم الطالب: محمد سعيد محمد النجار

Student's name

التوقيع:

Signature

التاريخ: ٢٢/٧/٢٠١٥

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Hepatic and Renal Toxicity of Methamidophos in Male Domestic Rabbit

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Prepared by:

Mohammed S. AlNajjar

B.Sc. Zoology

Suprevisor:

Prof. Dr. Maged M. Yassin

Professor of Physiology

Faculty of Medicine

The Islamic University of Gaza

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بسم الله الرحمن الرحيم

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نتيجة الحكم على أطروحة ماجستير

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

(تأثير مبيد Methamidophos على الكبد والكلى في ذكور الأرانب المنزلية)

(Hepatic and Renal Toxicity of Methamidophos in Male Domestic Rabbit)

وبعد المناقشة العلنية التي تمت اليوم الثلاثاء 06 رمضان 1436هـ، الموافق 2015/6/23م الساعة الحادية عشرة صباحاً بمبنى طيبة، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

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د. كمال جادالله النبريص	مناقشاً داخلياً	.....
د. محمود إسماعيل الحبيبي	مناقشاً خارجياً	.....

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

والله ولي التوفيق،،،

مساعد نائب الرئيس للبحث العلمي والدراسات العليا

أ.د. فؤاد علي العاجز

P.O. Box 108, Rimal, Gaza, Palestine fax: +970 (8) 286 0800 فاكس Tel: +970 (8) 286 0700 ص.ب. 108 الرمال، غزة، فلسطين هاتف
public@iugaza.edu.ps www.iugaza.edu.ps

Dedication

To my great parents who have always supporting me

To my brothers and sisters

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

To my beloved sons hashem and qassem,

And To all of them dedicate this work.

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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mohammed	Mohammed Samir Alnajjar	15/6/2015

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Hepatic and Renal Toxicity of Methamidophos in Male Domestic Rabbit

Abstract

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

Materials and Methods: The oral LD_{50} of methamidophos in male domestic rabbit was calculated and found to be 20.5 mg kg^{-1} body weight. The daily dose of 1/10 LD_{50} methamidophos (2.1 mg kg^{-1} body weight) was given orally to 36 animals under experiment for six weeks. Thirty six control animals were given distilled water. Blood samples were collected weekly and analyzed.

Results: The overall mortality rate was 11.1% in methamidophos-treated rabbits compared to 0.0% in controls. Clinical signs included diarrhea, disorientation, drowsiness, weakness, depression and mild tremors. The final body weight was significantly decreased in methamidophos-intoxicated rabbits. Serum glucose was significantly increased in response to methamidophos administration recording a maximum percentage difference of 27.1% in the 4^{th} week of the experiment. The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (γ-GT) were significantly higher in methamidophos-fed rabbits compared to controls, registering maximum percentage differences of 31.2, 44.7, 26.7 and 45.6% during the 6^{th}, 4^{th}, 4^{th} and 3^{rd} weeks of the experiment. In contrast, serum cholinesterase (ChE) was significantly decreased recording a maximum percentage difference of 77.8% at the end of the experiment. Serum bilirubin was gradually increased to record a maximum percentage difference of 19.3% in the 5^{th} week. Serum urea and creatinine concentrations were significantly elevated in response to methamidophos intake displaying maximum percentage differences of 45.8% and 31.7% during the 4^{th} week of the experiment. Serum total protein, albumin and globulin were significantly decreased upon methamidophos intoxication exhibiting percentage differences of 27.0, 25.9 and 24.3% at 5^{th} week of the experiment. Serum calcium was significantly decreased in methamidophos-treated rabbits with a maximum percentage difference of 17.4% at the end of the experiment, whereas phosphorus was significantly increased with a maximum percentage difference of 23.8% at the 4^{th} week of the experiment.

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

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

أثر سمية التمارون علي الكبد و الكلية في ذكور الأرانب المحلية

الملخص

هدف الدراسة: تهدف الدراسة الحالية إلى تمحيص الآثار السمية لمبيد التمارون على الكبد والكلية في ذكور الأرانب المحلية.

المواد و الخطوات: قيمة الجرعة النصف مميتة في مبيد التمارون في ذكور الأرانب المحلية حسبت من المقياس أللوغاريتمي ووجدت 20.5 مليجرام لكل كجم من وزن الجسم. الجرعة اليومية من 10/1 الجرعة النصف مميتة هي 2.1 مليجرام لكل كجم من وزن الجسم كانت تعطى عن طريق الفم إلى 36 حيوان تحت التجربة لستة أسابيع. 36حيوان ضابط تم إعطاؤهم ماء مقطر. عينات الدم كانت تجمع اسبوعيا وتحلل إحصائيا.

النتائج: كان نسبة الوفيات في الأرانب المعالجة بالتمارون 11.1٪ مقارنة بالضابطة 0.0% . ظهرت علامات سريريه كالإسهال و الارتباك و النعاس والضعف والاكتئاب و الرعاش الخفيف. وقد انخفض وزن الجسم بشكل كبير في ذكور الأرانب المعالجة بالتمارون خلال الأسابيع الأخيرة. وقد لوحظ زيادة نسبة الجلوكوز في الدم حيث سجلت أعلي نسبة في الأسبوع الرابع من التجربة 27.1 ٪. كذلك ظهر نشاط ملحوظ لأنزيمات الكبد ناقل الأمين (ALT) و أنزيم الأسبارتيت (AST) وأنزيم اللالكالين فوسفاتيز(ALP) وناقل جاما جلوتاميت (γ-GT) في المجموعات المعالجة بالمبيد مسجلة أعلي نسب بالترتيب حسب جداولها علي التوالي (31.2 ٪)، (44.7 ٪)، (26.7 ٪) و(45. ٪) خلال الأسبوع السادس و الرابع و الرابع و الثالث من التجربة علي التوالي. في المقابل وجد انخفاض مستوى الكولين استيريز (ChE) في البلازما بشكل ملحوظ مسجلا أعلي نسبة عند نهاية التجربة 77.8 ٪ . وبينما ازداد البيليروبين في البلازما تدريجيا ليسجل أقصى نسبة 19.3٪ في الأسبوع الخامس من التجربة. وكان هناك زيادة ملحوظة في تركيز اليوريا و الكرياتينين في مصل الدم للأرانب المعاملة بالتمارون حيث سجلت أعلي نسبة 45.8 ٪ و 31.7 ٪ خلال الأسبوع الرابع لكل منهما وقد انخفض البروتين الكلي و الألبومين و الجلوبيولين في الدم انخفاضا كبيرا مع زيادة سمية التمارون بالنسب التالية علي الترتيب 27.0 ٪، 25.9 ٪ و 24.3 ٪ في الأسبوع الخامس من التجربة. في حين انخفضت نسبة الكالسيوم في الدم بشكل كبير في الأرانب المعالجة بالتمارون مسجلة أعلي نسبة عند نهاية التجربة 17.4٪ في حين أن الفسفور زاد بشكل ملحوظ مشكلا أعلى نسبة في الأسبوع الرابع من التجربة 23.8 ٪.

الخاتمة: إن تناول مبيد التمارون عن طريق الفم بجرعة LD_{50}10/1 قد أدي إلي انخفاض كبير في وزن الجسم و كذلك أنزيمات الكبد و الكلية مثل الكولين استيريز (ChE) و البروتين الكلي و الألبومين و الجلوبيولين و الكالسيوم في حين زاد معدل سكر الجلوكوز و ناقل الأمين (ALT) و أنزيم الأسبارتيت (AST) وأنزيم الفوسفاتيز (ALP) وناقل جاما جلوتاميت (γ-GT) والبيليروبين و اليوريا و الكرياتينين و الفوسفور في مصل الدم.

الكلمات المفتاحية : التمارون – سمية – الكبد – الكلية – ذكر الأرانب

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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 et al., 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. Methamidophos is a highly active, systemic, residual organophosphate insecticide/acaricide/avicide with contact and stomach action **(Watts, 2011).** The chemical name of methamidophos is O,S-Dimethylphosphora-midothiolate with common trade names are tamaron, monitor and nitofol **(Extension Toxicology Network, EXTOXNET, 2009).**

Methamidophos is highly toxic via oral, dermal and inhalation routes of exposure. Acute oral LD_{50} of methamidophos (a dose that resulted in the mortality of half of the test organisms) is estimated to be 21 and 16 mg/kg body weight for male and female rats, respectively, 30-50 mg/kg body weight in guinea pigs and in the range of 10-30 mg/kg body weight in rabbits, cats and dogs. However, no previous study assessed the exact or narrow range of oral LD_{50} in male domestic rabbit. The dermal LD_{50} for rats 130 mg/kg. Inhalation LC_{50} (4 hours) for rats 0.2 mg/l (**Tomlin, 2011 and MacBen, 2013).**

As organophosphate compound, methamidophos 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 **(Lugokenski et al., 2012 and Kumar et al., 2015).**

Several studies reported the toxic effect of methamidophos on mammalian organs including liver and kidney. Methamidophos was reported to alter the physiological and histological aspects related to the liver and kidneys in experimental animals as well as in humans **(Satar et al., 2004; Satar et al., 2005; de Castro and Chiorato, 2007; Khan et al., 2010 and Araoud et al., 2014).** Signs and symptoms of methamidophos poisoning may include weakness, headache, blurred vision and confusion. Nausea, vomiting, abdominal pain, diarrhea, excessive sweating, and salivating may develop. Difficulty in breathing may be experienced. On severe poisoning, there will be muscle spasms, unconsciousness and convulsion. Breathing may stop, followed by death **(Kumar et al., 2010 and Watts, 2011).**

Pesticides are being used in large amounts in the Gaza Strip where the protective measures are poorly followed **(Serag El Din et al., 2014).** More than 544.4 metric tons of pesticides are used annualy in the Gaza Strip. The insecticide represents 232.5 metric tons of these pesticides, 27 metric tons of these insecticides are methamidophos **(Ministry of Agriculture, Palestinian National Authority, 2015).** This highly toxic compound constitute a real threat on humans. The present work is intended to investigate methamidophos 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 methamidophos exposure.

1.2 General objective

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

1.3 Specific objectives

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

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

7. To study the effect of 1/10 LD_{50} methamidophos on serum calcium and phosphorus.

1.4 Significance

1. Methamidophos is being extensively used in agriculture in Gaza Strip with lack of protective measures.
2. Studies on methamidophos toxicity on liver and kidney of 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 methamidophos 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 **(World Health Organization, WHO, 2011).**

A pesticide is broadly defined as any agent used to kill or control undesired insects, weeds, rodents, fungi, bacteria, or other organisms. Pesticides are classified according to their function: insecticides control insects; rodenticides control rodents; herbicides control weeds; and fungicides control fungi, mold and mildew **(Environmental Protection Agency, 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 and 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 and methamidophos **(Gupta, 2006; Tomlin, 2011 and Kumar et al., 2015).**

2.4 Methamidophos

2.4.1 Definition and structure

Methamidophos (O,S-dimethyl phosphoramidothioate) is an organophosphorus insecticide which contains an asymmetric center at the phosphorus atom and one radical attached to the central phosphorus through a connection P O R and the other through a connection P S R (Figure 2.1). Methamidophos is widely used in agriculture, both in developed and developing countries **(Lin et al., 2006 and Emerick et al., 2012).**

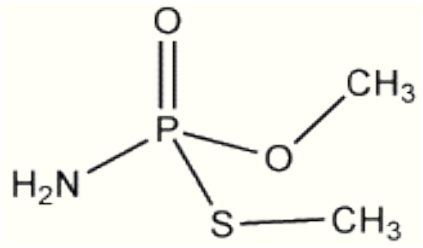

Figure 2.1 Chemical structure of methamidophos **(Emerick et al, 2012).**

2.4.2 physical and chemical properties of methamidophos

Methamidophos is colorless crystals with a mercaptan-like odor. The principal chemical properties of methamidophos are compiled in Table 2.1 **(Tomlin, 2011).**

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

Property	Value
Molecular Weight	141.1 g/mol
Melting Point	44.9 ^{o}C
Solubility in Water	> 200 g/l at 20 ^{o}C
Vapor Pressure	2.3 mPa at 20 ^{o}C
Density	1.27 g/cm^{3}

2.4.3 Mechanism of action of methamidophos

Methamidophos like other organophosphates inhibits acetylcholinesterase activity; an enzyme that breake 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.

Acetylcholine receptors: acetylcholine 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+, K+, Ca+, and Cl-) ultimately perpetuating the nerve impulse **(Sine and Engel, 2006 and Jha et al., 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, 2009 and Ockenga et al., 2013 and 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; Pohanka, 2013 and Holt et al., 2015).**

2.4.3.2 Acetylcholinesterase

As illustrated in Figure 2.2.B, once acetylcholine makes its action, it is subsequently destroyed by the enzyme acetylcholinesterase, and the membrane returns to its normal resting state **(Pohanka, 2011 and Colović et al., 2013).**

2.4.3.3 Acetylcholinesterase as a target for methamidophos

Methamidophos 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 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 **(Lugokenski et al., 2012 and Kumar et al., 2015).**

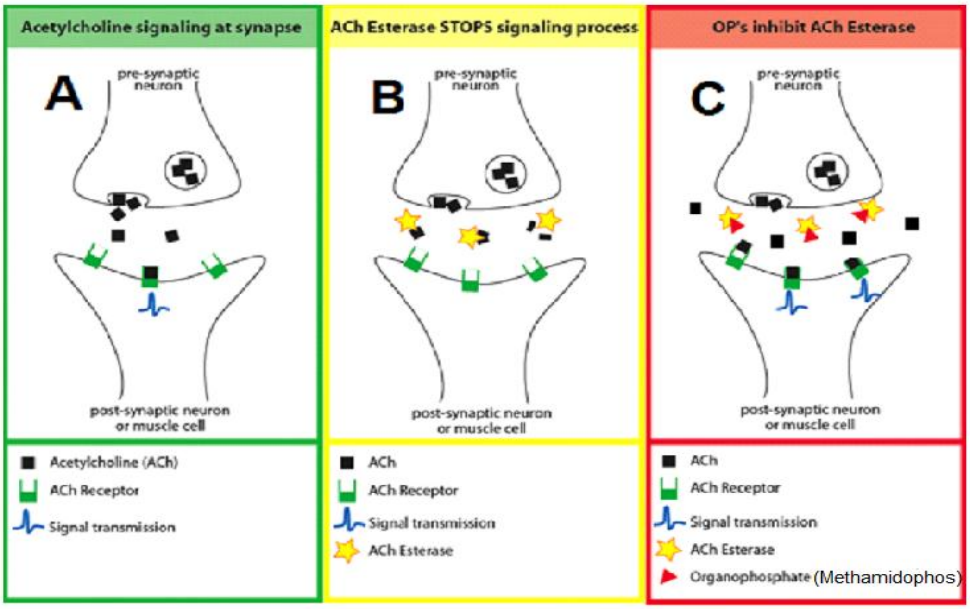

Figure 2.2 Pathophysiology of organophosphorus methamidophos pesticide poisoning **(Organophosphate pesticides and chlid health, 2007)**.

2.4.4 Toxicity symptoms of methamidophos poisoning

Methamidophos is highly toxic via oral, dermal and inhalation routes of exposure**.** Accumulation of acetylcholine at cholinergic synapses as a result of acetylcholinesterase (AChE) inhibition 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 **(Eddleston et al., 2006; Paudyal, 2008 and Christensen et al., 2009).**

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 **(Watts, 2011).**

2.4.5 Metabolism and toxicokinetics of methamidophos

In mammals, orally administered methamidophos is rapidly and almost completely absorbed from the gastrointestinal tract. Then methamidophos is distributed to all tissues, with highest concentrations in the liver and carcass and low levels in adipose tissue. **(Larsen, 2002; The National Advisory Committee for Acute Exposure Guideline Levels, 2009 and Suemizu, 2014).** Methamidophos showed low potential for accumulation in other tissues examined. Urine is the major route of excretion of methamidophos; approximately 70-77% is excreted in urine and approximately 20% in faeces, mostly within 24 hours **(Chang et al., 2009 and Kumar et al., 2015)**. At 24 hour after oral administration of 15 mg/kg body weight radiolabelled methamidophos to rats, only 1.4% of the dose was recovered as unchanged methamidophos. Major metabolites in urine are phosphoric acid (27.5%), O,S-dimethyl thiophosphoric acid (22.5%), O-methyl thiophosphoric acid amide (18%), S-methyl phosphoramidothioic acid (12.1%), S-methyl thiophosphoric acid (11.2%) and 7.3% unknown. Methamidophos is metabolized primarily by hydrolysis, with cleavage of the P-N bond yielding dimethyl phosphoric acid derivatives **(Kumar et al., 2015).**

2.4.6 Uses of methamidophos

Methamidophos is a broad-spectrum systemic organophosphate insecticide, with contact and stomach action. It is used globally on cotton, rice, citrus, maize, grapes, soybeans, tobacco, vegetables, hops, peaches, bananas and pineapple **(Watts, 2011)**. Methamidophos is used to control chewing, mining, and sucking insects such as aphids, leafhoppers, leaf-eating caterpillars, flea beetles, worms, whiteflies, thrips, cabbage looper, Colorado potato beetle, potato tubeworms, armyworms, mites, leafhoppers, and many others **(Gilbert, 2014)**. In Gaza strip methamidophos is being commonly used for the control of insects on wide range of crops including fruits (almonds, peach, apricot, apples and pears), citrus fruit, vegetables (potatoes, sweet potatoes, tomatoes and pepper) and flowers **(Ministry of Agriculture, 2015).**

2.4.7 Methamidophos toxicity

Methamidophos is classified as a class I compound, and must bear the signal word "Danger- Poison" on commercial products. Pesticides in this toxicity class are restricted use pesticides. Tolerances for residues of methamidophos on raw agricultural products range from 0.5 ppm in or on melons to 1.0 ppm in or on broccoli and tomatoes

(EXTOXNET, 2009). A common measure of acute toxicity is the lethal dose (LD_{50}). The oral LD_{50} (Median lethal dose, is a statistically derived single dose of a substance that can be expected to cause death in 50 % of animals when administered by the oral rout) is expressed in terms of weight of test substance per unit weight of test animal mg/kg body weight. The lower the LD_{50} value, the more toxic the substance. Acute oral LD_{50} of methamidophos was estimated to be 21 and 16 mg/kg body weight for male and female rats, respectively; 30-50 mg/kg body weight in guinea pigs and in the range of 10-30 mg/kg body weight in rabbits, cats and dogs. However, no previous study assessed the exact or narrow range of oral LD_{50} in male domestic rabbit. The dermal LD_{50} for rats 130 mg/kg. Inhalation LC_{50} (4 hours) for rats 0.2 mg/l (**Tomlin, 2011 and MacBen, 2013).**

2.4.8 Effect of methamidophos on liver and kidney

Methamidophos has toxic effects on mammalian organs including liver and kidney. Methamidophos was reported to alter the physiological and histological aspects related to the liver and kidneys in experimental animals as well as in humans **(Satar et al., 2005 and Khan et al., 2010).**

Yassin (1998) investigated and compared the intoxication effects of daily oral administration of 1/10 LD_{50} tamaron, parathion and confidor for 10 days on serum urea, uric acid, creatinine and glucose of rabbit. The daily oral administration of any of the three insecticides for 10 days caused a general increase of urea concentration in rabbits blood serum compared to the control level. In general, significant increase of urea content was observed from the third day of inoculation. Insecticides administration also raised up the concentration of uric acid and the highest serum content of uric acid was noticed in the tenth day of insecticides treatment. However, creatinine and glucose levels showed no significant increases in response to the treatments by tamaron, parathion or confidor during all the time intervals studied. In addition, **Wu et al. (2001)** reported reduced levels of erythrocytes and plasma cholinesterase in response to methamidophos exposure.

Exposure to different organophosphorus pesticides including methamidophos was studied **(Yassin, 2003).** Serum cholinesterase activity was significantly lowered whereas the activities of AST, ALT, AP and lactate dehydrogenase in the serum were significantly increased compared to controls. Concentrations of serum urea and uric acid were significantly increased. Serum creatinine was increased in comparison with controls, but the change was not significant. Serum levels of sodium, potassium, chloride and

magnesium did not change significantly compared to control levels. However, there was significant decrease in calcium levels and significant elevation in serum inorganic phosphorus.

Satar et al. (2004 and 2005) found that the mean level of cholinesterase was significantly lower in male Wistar albino rats received 30 mg/kg methamidophos compared to controls. In addition, **de Castro and Chiorato (2007)** studied the maternal exposure of 1-4 mg/kg methamidophos on several developmental measures in Wistar male and nulliparous female Rats. They reported significant decrease in the body weight gain of rats treated with methamidophos compared to controls.

The effect of exposure to organophosphorus including methamidophos was investigated **(Khan et al., 2010).** They revealed a significant decrease in plasma cholinesterase levels. A relatively high level of methamidophos was detected in plasma. Serum AST, ALT, creatinine, □-GT, malondialdehyde, total antioxidant, and C-reactive protein were significantly raised in response to methamidophos exposure compared to controls ($P<0.001$). The authors concluded that exposure to organophosphorus pesticides study caused derangement of hepatic and renal function.

In their study to test the protective or reactivation capability of some oximes on human and rat cholinesterases, **Lugokenski et al. (2012)** reported marked inhibition of rat brain AChE, human erythrocyte AChE and human plasma butyryl cholinesterase (BChE) in response to methamidophos treatment.

Araoud et al. (2014) investigated the role of vitamin E on nephrotoxicity and hepatotoxicity induced by oral administration of methamidophos in Wistar rats for 4 weeks. Methamidophos treatment resulted in a significant decrease in body weight of the experimental animals compared to controls. Moreover, methamidophos-treated rats had significantly lower BChE ($P<0.01$) and paraoxonase 1 activities compared with the control group ($P<0.05$). However, methamidophos-treated rats had significantly higher alkaline phosphatase activity compared with untreated rats ($P<0.05$). Methamidophos-treated rats had also significantly higher urea ($P<0.01$) and uric acid levels ($P<0.05$) compared with the control group. Vitamin E administration ameliorated the adverse effects of methamidophos on rat liver and kidney.

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 methamidophos LD50

A total number of 80 rabbits were used for determination of LD_{50} of methamidophos. Animals were divided into ten groups (8 rabbits/group). The first nine groups (I-IX) were administered different single doses of methamidophos ranging from 5 to 45 mg/kg body weight as follows:

LD50 determination groups	Dose (mg/kg body weight)
Group I	10
Group II	12.5
Group III	15
Group IV	17.5
Group V	20
Group VI	22.5
Group VII	25
Group VIII	27.5
Group IX	30
Group X control group	0

The tenth group was served as control group. Methamidophos 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 LD50 was determined by graphical method **(Manna et al., 2004).**

3.3 Methamidophos toxicity experiments

A dose of 1/10 of LD50 methamidophos was given orally to assess methamidophos toxicity in male domestic rabbit. Animals were divided into two groups: control and

experimental groups. Control group comprised 36 rabbits (six rabbits were housed in each cage) and experimental group also included 36 rabbits (six rabbits were housed in each cage). Experimental groups were orally administrated methamidophos daily for overall experimental duration of six weeks. Control animals were given distilled water. Administration of methamidophos was also done by special stomach tube. Blood samples were collected weekly and analyzed. methamidophos 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.

3.5 Body weight

Animals were individually weighed at the beginning and the end of the experiment in order to detect any changes in their body weights. A sensitive balance (model: ONA-15, made in Istanbul 2013) 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 frequently employed. In the presence of glucose oxidase, glucose is oxidized to gluconic acid and hydrogen peroxide. Hydrogen peroxide reacts, in the presence of peroxidase, with

phenol and 4-aminophenazone to form a quinoneimine dye. The intensity of the pink color formed is proportional to the glucose concentration.

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

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

Reagents

Reagent	Components	Concentrations
Reagent 1 Monoreagent	Phosphate Buffer, pH 7.5 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 ^{oC} for 10min. or 20min. at 20-25 ^{oC}.
3. Measure the absorbance of sample and std/cal within 60 minutes against regent blank at wavelength 500 nm.

Calculation

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

3.6.3 Determination of Liver enzymes

3.6.3.1 alanine aminotransferase

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

Principle

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

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

Reagents

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

Working mixture

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 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^+

Reagents

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

Working mixture

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 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-nitrophenol

Reagents

Components	**Concentration**
Reagent 1	
2-Amino-2-methyl-1-propanol pH10.4	1.1 mmol/l
Magnisium acetate	2 mmol /l
Zinc sulphate	0.5 mmol/l
HEDTA	2.5mmol/l
Reagent 2	
p-Nitrophenylphosphate	80 mmol/l

Working mixture

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 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 $\xrightleftharpoons{\gamma\text{-GT}}$ Gamma-glutamyl-glycylglycine +5-Amino-2-nitrobenzoatę.

Reagents

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

Procedure

Substrate start

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

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

Sample start

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

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

Calculation

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

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

With calibrator

□-GT (U/l) = ΔA/min Sample X conc. Calibrator (U/l)

ΔA/min Calibrator

3.6.3.5 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 + Butyrate

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

Reagents

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

Procedure

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

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

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

Calculation

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

3.6.4 Determination of 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 is only approximately equivalent to the conjugated and unconjugated fractions.

Reagents

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

Procedure

1- Pipette into labelled test tube

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

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

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

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

Calculations

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

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

C Standard = C Sample

3.6.5 Determination of Non- protein nitrogen constituents

3.6.5.1 Urea

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

Principle

Urea + $2H_2O$ $\xrightarrow{\text{Urease}}$ $2NH4^+ + 2HCO3^-$

2-Oxaloglutarate + $NH4^+$ + NADH $\xrightarrow{\text{GLDH}}$ L-Glutamate +NAD^+ +H_2O

GLDH: Glutamate dehydrogenase.

Reagents

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

Working mixture

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 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 difference in absorbance at fixed time during conversion is proportional to the concentration of creatinine in the sample.

Creatinine + picric acid ⟶ Creatinine picrate complex

Reagents

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

Working mixture

Four parts of R1 were mixed with 1 part of R2

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

Procedure

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

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

Calculation

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

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

3.6.6 Determination of Protein profile

3.6.6.1 Total protein

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

Principle

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

Reagents

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

Working mixture

Four parts of R1 were mixed with1 part of R2

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

Procedure

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

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

Calculation

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

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

3.6.6.2 Albumin

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

Principle

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

Reagents

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

Procedure

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

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

Calculation

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

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

3.6.6.3 Determination of globulin

Globulin was calculated according the following formula:

Globulin = Total protein - Albumin

3.6.7 Determination of some Electrolytes

3.6.7.1 Calcium

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

Principle

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

Reagents

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

Preparation and stability of working reagent:

Four parts of R1 were mixed with 1 part of R2

Stability: 3 days at 2-8 oC

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 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 + Sulfuric acid $\xrightarrow{\text{Phosphorus}}$ Phosphomolybdate+ ammonium sulfate

Reagents

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

Preparation and stability of working reagent:

The reagent is ready for use

Procedure

Wavelength 340 nm

Temperature 37°C

Cuvette 1 cm light path

Reading against reagent blank was done

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

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

Calculation

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

n = standard Phosphorus concentration

3.7 Statistical analysis

Data were statistically analyzed using SPSS computer program version 18.0 for windows (Statistical Package for Social Sciences Inc, Chicago, Illinois).

Means were compared by independent-sample t-test.

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

The percentage difference was calculated according to the formula:

Percentage difference equals the absolute value of the change in value, divided by the average of the 2 numbers, all multiplied by 100.

Percent difference = (| (V1 - V2) | / ((V1 + V2)/2)) * 100.

Graphs

logarithmic scale for oral LD50 determination of methamidophos and body weight graph were plotted using Microsoft Excel program 2013.

Chapter 4

Results

4.1 Oral LD_{50} of methamidophos

The experimental trials for oral LD_{50} determination of methamidophos after 48hr of administration in male domestic rabbits revealed that the mortality commenced at 12.5 mg kg^{-1} body weight, recording mortality percentage of 12.5% (Table 4.1). Increasing methamidophos dose to 15, 17.5, 20, 22.5 and 25 resulted in mortality percentages of 12.5, 37.5, 37.5, 50 and 75.0%, respectively. The mortality rate was a function of dose increase. The maximum concentration of methamidophos which kill all animals in the group was found to be 27.5 mg kg^{-1} body weight. The calculated oral LD_{50} of methamidophos in male domestic rabbits from the linear regression was found to be 20.5 mg kg^{-1} body weight (Figure 4.1).

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

Group	methamidophos Dose (mg kg^{-1} body weight)	Number of Animals died/total	% mortality
Group I	10	0/8	0
Group II	12.5	1/8	12.5
Group III	15	1/8	12.5
Group IV	17.5	3/8	37.5
Group V	20	3/8	37.5
Group VI	22.5	4/8	50
Group VII	25	6/8	75
Group VIII	27.5	8/8	100
Group IX	30	8/8	100
Group X	Control	0/8	0

The number of animals administered methamidophos was 6 in each group (I to IX). Control animals were given distilled water and their number was also 6.

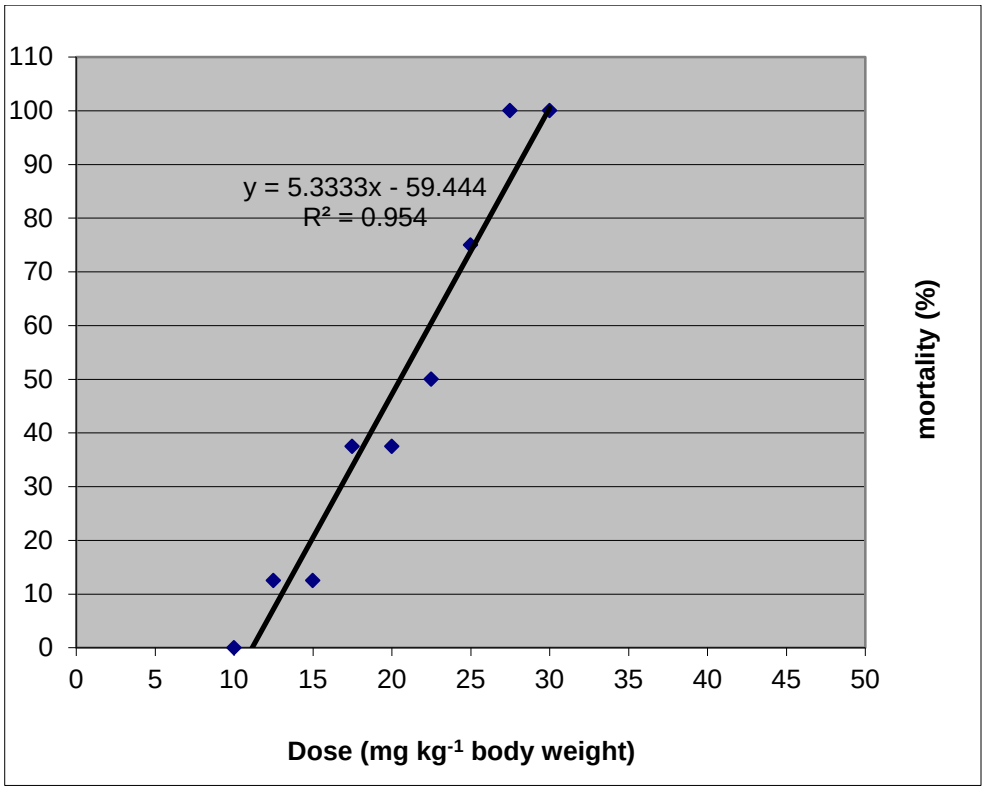

Figure 4.1 Determination of LD_{50} value for methamidophos after 48h of administration from linear correlation between methamidophos concentration versus mortality percentage (%) (LD_{50}=20.5 mg kg^{-1} body weight).

4.2 General health of rabbits

To assess methamidophos toxicity in rabbits, 1/10 LD_{50} methamidophos (2.1 mg kg^{-1}) was orally administered daily for 6 weeks. The mortality rate recorded for 1/10 LD_{50} methamidophos-treated rabbits was 0/6 (0%), 0/6 (0%), 0/6 (0%),1/6 (16.7%), 1/6 (16.7%) and 2/6 (33.3%) 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 of the experiment. In addition, rabbits in the control group did not show any sign of toxicity. However, methamidophos-intoxicated rabbits showed varying degrees of clinical signs few hours after dosing. The signs included diarrhea, disorientation, drowsiness, weakness, depression and mild tremor. Concerning morphological changes, methamidophos-treated rabbits showed dermal abnormalities particularly during the fifth and sixth weeks of the experiment whereas control animals did not display such abnormalities. The livers of methamidophos-treated rabbits also showed scars of depression (Figure 4.2) whereas those of the control animals showed normal appearance.

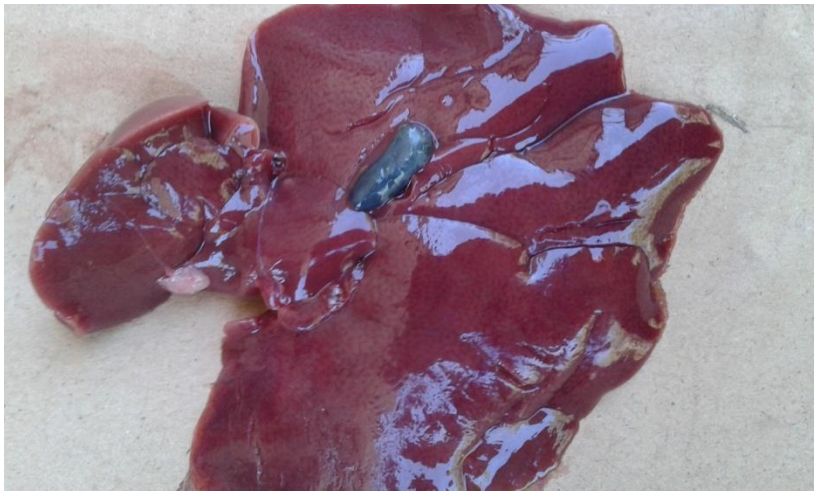

Figure 4.2 Effect of 1/10 LD_{50} methamidophos (2.1 mg kg^{-1} body weight) on liver morphology of male domestic rabbit.

4.3 Final body weight

Table 4.2 and Figure 4.3 provide the final body weight of male domestic rabbits after 6 weeks of daily oral administration of 1/10 LD_{50} methamidophos (2.1 mg kg^{-1} body weight). There was a significant decrease in the body weight of methamidophos-treated rabbits compared to controls (915±32.8 *versus* 1170±45.5, %difference=24.5, P=0.003).

Table 4.2 Final body weight of male domestic rabbit after 6 weeks of daily oral administration of 1/10 LD_{50} methamidophos (2.1 mg kg^{-1} body weight).

Parameter	Control (n=6)	methamidophos(n=6)	% difference	t-value	P-value
Body weight (gm)	1170±45.5	915±32.8	24.5	4.316	0.003

- The number of animals (n) was 6 in control group and 6 in methamidophos-treated group. All values were expressed as mean±SEM. P<0.05: Significant.

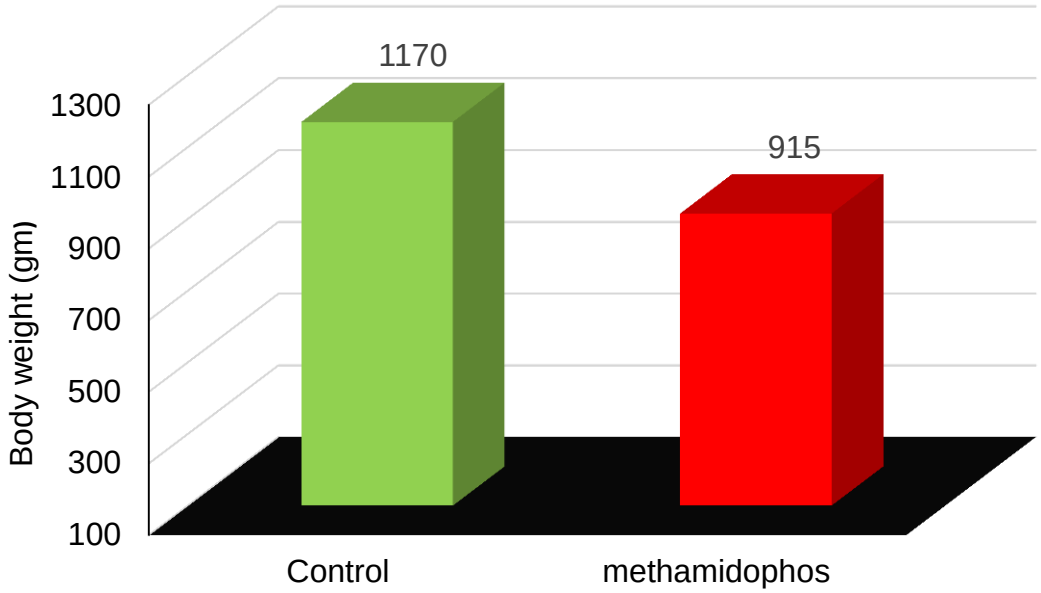

Figure 4.3 Final mean body weight of male domestic rabbits after 6 weeks of dialy oral administration of 1/10 LD50 methamidophos (2.1 mg kg-1 body weight)

4.4 Biochemical investigation

4.4.1 Serum glucose

Table 4.3 illustrates serum glucose levels in control and methamidophos-treated male domestic rabbits along the experimental period of 6 week intervals. The mean values of glucose level in controls were 113.7±4.6, 114.8±5.3, 115.2±4.9, 114.0±5.7, 115.7±5.1 and 117.4±4.8 mg/dl during the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks of the experiment, respectively. Oral administration of 1/10 LD_{50} methamidophos (2.1 mg kg^{-1} body weight) daily for 6 weeks caused general increase in glucose level commencing all over experiment at period examined. This increase was significant in the last five weeks of the experiment with a maximum percentage difference of 27.1% during the 4^{th} week (t=4.024, P=0.003).

Table 4.3 Effect of methamidophos (1/10 $LD_{50,}$ 2.1 mg kg^{-1} body weight) on serum glucose level (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=6)	Methamidophos(n= 6)	% difference	t-value	P-value
1	113.7±4.6	115.2±5.4	1.3	0.219	0.832
2	114.8±5.3	135.7±6.2	16.7	2.548	0.031
3	115.2±4.9	133.4±6.0	14.6	2.357	0.043
4	114.0±5.7	149.7±6.9	27.1	4.024	0.003
5	115.7±5.1	143.1±6.7	21.2	3.262	0.010
6	117.4±4.8	137.0±7.1	15.4	2.367	0.042

The number of animals (n) was 6 in control group and 6 in methamidopho-treated animals. All values were expressed as mean±SEM. P<0.05: Significant, P>0.05:not Significant.

4.4.2 Liver enzymes

4.4.2.1 Alanine aminotransferase

The mean values of serum ALT activity in control and methamidophos-treated male domestic rabbits along the experimental period of 6 weeks are presented in Table 4.4. The normal enzyme activity was 47.5±1.9, 48.0±2.2, 47.8±1.7, 50.2±2.0, 47.4±2.3, and 48.7±2.1 U/l at the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks of the experiment, respectively. Upon methamidophos administration, ALT activity was increased throughout the experimental periods reaching mean values of 51.8±2.6, 55.7±2.4, 56.1±3.0, 61.4±3.2, 57.0±2.9 and 66.7±3.6 U/l,respectively This increase was significant during the last five weeks of the experiment recording the maximum percentage difference of 31.2% in the 6^{th} week of the experiment (t=4.467, P=0.002).

Table 4.4 Effect of methamidophos (1/10 LD_{50}, 2.1 mg kg^{-1} body weight) on serum alanine aminotransferase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=6)	Methamidophos(n= 6)	% difference	t-value	P-value
1	47.5±1.9	51.8±2.6	8.7	1.340	0.213
2	48.0±2.2	55.7±2.4	14.8	2.370	0.042
3	47.8±1.7	56.1±3.0	16.0	2.484	0.035
4	50.2±2.0	61.4±3.2	20.1	3.057	0.014
5	47.4±2.3	57.0±2.9	18.4	2.639	0.027
6	48.7±2.1	66.7±3.6	31.2	4.467	0.002

The number of animals (n) was 6 in control group and 6 in methamidopho-treated animals. All values were expressed as mean±SEM. P<0.05: Significant, P>0.05:not Significant.

4.4.2.2 Aspartate aminotransferase

Table 4.5 provides mean values of serum AST activity in control and methamidophos-fed male domestic rabbits all over the experimental period of 6 weeks. The AST activity registered for control animals were 32.6±1.6, 33.7±1.5, 35.0±2.0, 33.2±1.8, 34.5±1.7 and 33.8±1.4 U/l at the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks of the experiment, respectively. Treatment of animals with methamidophos provoked significant elevation in the enzyme activity throughout the experiment exhibiting values of 38.9±2.1, 49.2±2.8, 46.5±2.6, 52.3±3.0, 45.4±2.8 and 45.2±2.9 U/l. The maximum elevation in the enzyme activity was recorded at the 4^{th} week of the experiment showing percentage difference of 44.7% (t=5.717, P=0.001).

Table 4.5 Effect of methamidophos(1/10 LD_{50}, 2.1 mg kg^{-1} body weight) on serum aspartate aminotransferase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=6)	Methamidophos(n= 6)	% difference	t-value	P-value
1	32.6±1.6	38.9±2.1	17.6	2.556	0.031
2	33.7±1.5	49.2±2.8	37.4	5.084	0.001
3	35.0±2.0	46.5±2.6	28.2	3.614	0.006
4	33.2±1.8	52.3±3.0	44.7	5.717	0.001
5	34.5±1.7	45.4±2.8	27.3	3.511	0.007
6	33.8±1.4	45.2±2.9	28.8	3.753	0.005

- The number of animals (n) was 6 in control group and 6 in methamidopho-treated animals. All values were expressed as mean±SEM. P<0.05: Significant, P>0.05:not Significant.

4.4.2.3 Alkaline phosphatase

The normal activity of serum ALP of male domestic rabbits are illustrated in table 4.6. They were 96.4±4.1, 102.0±4.5, 97.1±4.0, 98.2±3.8, 100.3±4.6 and 96.5±4.7 U/l at 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks of the experiment, respectively. Methamidophos intake increased the enzyme activity overall the experiment, showing mean values of 105.9±5.0, 114.8±4.9, 114.6±5.7, 128.4±6.5, 125.5±5.4 and 117.0±6.1 U/l, respectively. The significant increment in the enzyme activity started from the 3^{rd} week of the experiment with

maximum percentage difference of 26.7% during the 4th week of the experiment (t=4.115, P=0.003).

Table 4.6 Effect of methamidophos (1/10 LD_{50}, 2.1 mg kg^{-1} body weight) on serum alkaline phosphatase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=6)	Methamidophos (n=6)	% difference	t-value	P-value
1	96.4±4.1	105.9±5.0	9.4	1.483	0.172
2	102.0±4.5	114.8±4.9	11.8	1.907	0.089
3	97.1±4.0	114.6±5.7	16.5	2.520	0.033
4	98.2±3.8	128.4±6.5	26.7	4.115	0.003
5	100.3±4.6	125.5±5.4	22.3	3.490	0.007
6	96.5±4.7	117.0±6.1	19.2	2.681	0.025

- The number of animals (n) was 6 in control group and 6 in methamidophos-treated animals. All values were expressed as mean±SEM. P<0.05: Significant, P>0.05:not Significant.

4.4.2.4 Serum gamma glutamyl transferase

Table 4.7 gives the mean values of serum γGT activity in control and methamidophos - intoxicated male domestic rabbits along the experimental period of 6 weeks. The normal activity of γGT was 6.04±0.29, 6.15±0.32, 5.89±0.25, 6.10±0.34, 5.82±0.28 and 6.09±0.31 U/l at 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Daily oral administration of methamidophos increased the enzyme activity throughout the experiment to reach mean values of 7.34±0.38, 8.26±0.45, 9.37±0.56, 8.58±0.51, 9.06±0.54 and 8.01±0.47 U/l, respectively. The maximum increase in γGT activity was registered at the 3rd week of the experiment showing percentage difference of 45.6% (t=6.039, P=0.001).

Table 4.7 Effect of methamidophos (1/10 LD_{50}, 2.1 mg kg^{-1} body weight) on serum gamma glutamyltransferase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=6)	Methamidophos (n=6)	% difference	t-value	P-value
1	6.04±0.29	7.34±0.38	19.4	2.721	0.024
2	6.15±0.32	8.26±0.45	29.3	3.879	0.004
3	5.89±0.25	9.37±0.56	45.6	6.039	0.001
4	6.10±0.34	8.58±0.51	33.8	4.158	0.002
5	5.82±0.28	9.06±0.54	43.5	5.720	0.001
6	6.09±0.31	8.01±0.47	27.2	3.511	0.007

The number of animals (n) was 6 in control group and 6 in methamidophos-treated animals. All values were expressed as mean±SEM. P<0.05: Significant.

4.4.2.5 Serum Cholinesterase activity

The mean values of serum ChE activity in control and methamidophos-intoxicated rabbits are pointed out in Table 4.8. The normal ChE activities in control animals were 4534±168, 4526±159, 4663±171, 4537±152, 4701±163 and 4542±148 U/l during the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks of the experiment, respectively. Oral administration of the organophosphorus pesticide methamidophos provoked a progressive significant decrease in the enzyme activity to values of 2603±117, 2442±106, 2455±102, 2230±98, 2228±94 and 1997±98 U/l, respectively. The maximum inhibition in ChE activity was obtained during the six week of the experiment recording a percentage difference of 77.8% (t=13.887, P=0.001).

Table 4.8 Effect of methamidophos (1/10 LD_{50}, 2.1 mg kg^{-1} body weight) on serum cholinesterase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=6)	Methamidophos (n=6)	% difference	t-value	P-value
1	4534±168	2603±117	-54.1	9.013	0.001
2	4526±159	2442±106	-59.8	10.375	0.001
3	4663±171	2455±102	-62.0	10.495	0.001
4	4537±152	2230±98	-68.2	12.075	0.001
5	4701±163	2228±94	-71.4	12.291	0.001
6	4542±148	1997±89	-77.8	13.887	0.001

The number of animals (n) was 6 in control group and 6 in methamidophos-treated animals. All values were expressed as mean±SEM. P<0.05: Significant.

4.4.3 Serum Total bilirubin

The mean levels of serum bilirubin in control rabbits as well as in rabbits received methamidophos daily for 6 weeks are presented in Table 4.9. The normal levels of bilirubin in control rabbits were 1.62±0.05, 1.71±0.06, 1.63±0.08, 1.60±0.03, 1.69±0.04 and 1.64±0.03 mg/dl during the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks of the experiment, respectively. Oral administration of methamidophos caused gradual increase in bilirubin level to reach its maximum % difference of 19.3 during 5^{th} week of the experiment (t=2.745, P=0.024).

Table 4.9 Effect of methamidophos(1/10 LD_{50}, 2.1 mg kg^{-1} body weight) on serum bilirubin (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=6)	Methamidophos(n= 6)	% difference	t-value	P-value
1	1.62±0.05	1.70±0.06	4.8	0.996	0.348
2	1.71±0.06	1.86±0.09	8.3	1.342	0.217
3	1.63±0.08	1.81±0.10	10.5	1.424	0.192
4	1.60±0.03	1.83±0.12	13.4	1.951	0.087
5	1.69±0.04	2.05±0.13	19.3	2.745	0.024
6	1.64±0.03	1.92±0.10	15.7	2.462	0.039

The number of animals (n) was 6 in control group and 6 in methamidophos-treated animals. All values were expressed as mean±SEM. P<0.05: Significant, P>0.05:not Significant.

4.4.4 Non-protein nitrogen constituents

4.4.4.1 Serum urea

Table 4.10 presents the mean values of serum urea concentrations in control and methamidophos-treated male domestic rabbits. Urea concentrations in control animals exhibited values of 37.1±1.8, 37.5±1.6, 36.9±1.7, 35.7±1.4, 34.2±1.5 and 35.3±2.0 mg/dl during 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks of the experiment, respectively. Daily intake of methamidophos caused significant elevation in urea concentration all over the experimental intervals examined reaching values of 45.0±2.4, 47.6±2.8, 50.6±3.1, 56.9±4.0, 50.7±3.6 and 50.0±2.9 mg/dl, respectively. The maximum increase of urea

concentration was recorded at the 4th week of the experiment with percentage difference of 45.8% (t=5.428, P=0.001).

Table 4.10 Effect of methamidophos(1/10 LD_{50}, 2.1 mg kg^{-1} body weight) on serum urea concentration (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=6)	methamidophos(n=6)	% difference	t-value	P-value
1	37.1±1.8	45.0±2.4	19.2	2.697	0.025
2	37.5±1.6	47.6±2.8	23.7	3.213	0.011
3	36.9±1.7	50.6±3.1	31.3	4.073	0.003
4	35.7±1.4	56.9±4.0	45.8	5.428	0.001
5	34.2±1.5	50.7±3.6	38.9	4.570	0.001
6	35.3±2.0	50.0±2.9	34.5	4.334	0.002

The number of animals (n) was 6 in control group and 6 in methamidophos-treated animals. All values were expressed as mean±SEM. P<0.05: Significant.

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 methamidophos along the experimental period of 6 weeks are illustrated in Table 4.11. The normal values recorded for creatinine concentrations were 0.62±0.02, 0.60±0.03, 0.57±0.01, 0.61±0.02, 0.58±0.01, and 0.61±0.04 mg/dl at the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Upon methamidophos administration, serum creatinine concentrations were increased to mean values of 0.70±0.04, 0.72±0.04, 0.73±0.05, 0.84±0.06, 0.75±0.05 and 0.76±0.03 mg/dl, respectively. Similar to urea, creatinine registered its maximum increase in the 4th week of the experimental with % difference of 31.7% (t=4.191, P=0.003).

Table 4.11 Effect of methamidophos(1/10 LD_{50}, 2.1 mg kg^{-1} body weight) on serum creatinine concentration (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=6)	methamidophos(n=6)	% difference	t-value	P-value
1	0.62±0.02	0.70±0.04	12.1	1.958	0.086
2	0.60±0.03	0.72±0.04	18.2	2.667	0.029
3	0.57±0.01	0.73±0.05	24.6	3.284	0.011
4	0.61±0.02	0.84±0.06	31.7	4.191	0.003
5	0.58±0.01	0.75±0.05	25.6	3.367	0.010
6	0.61±0.04	0.76±0.03	21.9	3.064	0.015

- The number of animals (n) was 6 in control group and 6 in methamidophos-treated animals. All values were expressed as mean±SEM. P<0.05: Significant, P>0.05:not Significant.

4.4.5 Protein profile

4.4.5.1 Serum total proteins

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 5.81±0.28, 5.76±0.29, 6.04±0.31, 5.92±0.26, 6.10±0.33 and 5.83±0.32 mg/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 methamidophos administration along the experimental periods tested. This decrease become significant starting from the 2nd week till the end of experiment, recording it's a maximum % difference of 27.0 at the 5th week of the experiment (t=4.120, P=0.003).

Table 4.12 Effect of methamidophos(1/10 LD_{50}, 2.1 mg kg^{-1} body weight) on serum total protein (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=6)	methamidophos(n= 6)	% difference	t-value	P-value
1	5.81±0.28	5.27±0.21	-9.7	1.541	0.162
2	5.76±0.29	4.90±0.18	-16.1	2.528	0.035
3	6.04±0.31	4.93±0.16	-20.2	3.167	0.013
4	5.92±0.26	4.71±0.19	-22.8	3.756	0.006
5	6.10±0.33	4.65±0.14	-27.0	4.120	0.003
6	5.83±0.32	4.69±0.17	-21.7	3.105	0.015

- The number of animals (n) was 6 in control group and 6 in methamidophos-treated animals. All values were expressed as mean±SEM. P<0.05: Significant, P>0.05:not Significant.

4.4.5.2 Serum albumin

The mean concentration of serum albumin in control and methamidophos-intoxicated male domestic rabbits are shown in Table 4.13. Albumin concentration in control animals exhibited mean values of 3.72±0.18, 3.63±0.16, 3.91±0.23, 3.85±0.21, 3.97±0.25 and 3.75±0.20 mg/dl at 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. In general, methamidophos intake resulted in successive significant decrease in albumin

concentration recording a value of 3.06±0.09 mg/dl at the 5th week of the experiment with percentage difference of 25.9% (t=3.431 and P=0.009).

Table 4.13 Effect of methamidophos(1/10 LD_{50}, 2.1 mg kg^{-1} body weight) on serum albumin (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=6)	methamidophos(n=6)	% difference	t-value	P-value
1	3.72±0.18	3.43±0.14	-8.1	1.293	0.232
2	3.63±0.16	3.15±0.11	-14.2	2.406	0.043
3	3.91±0.23	3.21±0.10	-19.7	2.794	0.023
4	3.85±0.21	3.10±0.13	-21.6	3.040	0.016
5	3.97±0.25	3.06±0.09	-25.9	3.431	0.009
6	3.75±0.20	3.03±0.13	-21.2	2.961	0.017

- The number of animals (n) was 6 in control group and 6 in methamidophos-treated animals. All values were expressed as mean±SEM. P<0.05: Significant, P>0.05:not Significant.

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 1.99±0.08, 2.06±0.10, 2.09±0.13, 1.96±0.11, 2.03±0.14 and 1.98±0.11 mg/dl during the 1st, 2nd, 3rd, 4th, 5th and 6th weeks of the experiment, respectively. Oral administration of methamidophos lowered globulin levels to 1.87±0.09, 1.84±0.11, 1.73±0.06, 1.60±0.07, 1.59±0.05, and 1.61±0.06 mg/dl showing percentage differences of 6.2, 11.3, 18.8, 20.2, 24.3 and 20.6 % at the weekly intervals of the experiment compared to controls. This decrease was significant all over the experimental periods expect for the 1st and 2nd weeks**.**

Table 4.14 Effect of methamidophos(1/10 LD_{50}, 2.1 mg kg^{-1} body weight) on serum globulin (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=6)	methamidophos(n= 6)	% difference	t-value	P-value
1	1.99±0.08	1.87±0.09	-6.2	0.990	0.351
2	2.06±0.10	1.84±0.11	-11.3	1.498	0.172
3	2.09±0.13	1.73±0.06	-18.8	2.681	0.028
4	1.96±0.11	1.60±0.07	-20.2	2.809	0.023
5	2.03±0.14	1.59±0.05	-24.3	3.061	0.016
6	1.98±0.11	1.61±0.06	-20.6	2.887	0.020

- The number of animals (n) was 6 in control group and 6 in methamidophos-treated animals. All values were expressed as mean±SEM. P<0.05: Significant, P>0.05:not Significant.

4.4.6 Electrolytes

4.4.6.1 Serum calcium

The mean serum calcium concentrations in controls and in methamidophos-received male rabbits are provided in Table 4.15. The normal concentrations of calcium were 13.9±0.8, 13.7±0.9, 14.1±0.6, 13.8±0.7, 14.0±1.0 and 13.8±0.7 mg/dl during the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks of the experiment, respectively. Upon methamidophos administration, serum concentration of calcium fluctuates throughout the experiment registering significant decrease during the 4^{th} and 6^{th} weeks with percentage differences of 14.8 and 17.4 (t=2.431, P=0.041 and t=2.631, P=0.030, respectively).

Table 4.15 Effect of methamidophos(1/10 LD_{50}, 2.1 mg kg^{-1} body weight) on serum calcium (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=6)	methamidophos(n= 6)	% difference	t-value	P-value
1	13.9±0.8	12.5±0.6	-10.6	1.402	0.198
2	13.7±0.9	12.0±0.4	-13.2	1.875	0.096
3	14.1±0.6	12.5±0.7	-12.0	1.793	0.112
4	13.8±0.7	11.9±0.4	-14.8	2.431	0.041
5	14.0±1.0	12.5±0.3	-11.3	1.540	0.162
6	13.8±0.7	11.6±0.3	-17.4	2.631	0.030

- The number of animals (n) was 6 in control group and 6 in methamidophos-treated animals. All values were expressed as mean±SEM. P<0.05: Significant, P>0.05:not Significant.

4.4.6.2 Serum phosphorus

Table 4.16 shows serum phosphorus concentrations in controls as well as in methamidophos-fed male rabbits. The mean concentrations of phosphorus in control animals were 7.30±0.4, 7.46±0.3, 6.95±0.2, 7.43±0.2, 7.44±0.4 and 7.18±0.3 mg/dl at the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks of the experiment, respectively. Methamidophos-treated group of rabbits showed general increase in serum phosphorus along the experiment displaying significant increase commencing from the 2^{nd} week of the experiment. The maximum increase in serum phosphorus was detected during the 4^{th} week of the experiment showing percentage difference of 23.8 (t=3.276, P=0.011).

Table 4.16 Effect of methamidophos(1/10 LD_{50}, 2.1 mg kg^{-1} body weight) on serum phosphorus (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=6)	methamidophos(n= 6)	% difference	t-value	P-value
1	7.30±0.4	8.18±0.5	11.4	1.517	0.168
2	7.46±0.3	8.79±0.4	16.3	2.560	0.034
3	6.95±0.2	8.45±0.6	19.5	2.757	0.025
4	7.43±0.2	9.44±0.7	23.8	3.276	0.011
5	7.44±0.4	8.97±0.4	18.6	2.652	0.029
6	7.18±0.3	8.82±0.5	20.5	2.801	0.023

- The number of animals (n) was 6 in control group and 6 in methamidophos-treated animals. All values were expressed as mean±SEM. P<0.05: Significant, P>0.05:not Significant.

CHAPTER 5

Discussion

Pesticides are synthetic chemicals of potential toxicity that frequently used in the Gaza Strip to combat insects, rodents and plant pests and other creatures that can pose problems for agriculture and for public health. One of these pesticides is the organophosphorus insecticide methamidophos which is commonly used in Gaza Strip to combat insects on wide range of crops including fruits (almonds, peach, apricot, apples and pears), citrus fruit, vegetables (potatoes, sweet potatoes, tomatoes and pepper) and flowers **(Ministry of Agriculture, 2015).** However, the use/misuse of this highly toxic compound caused several cases of death among farm workers and children in Gaza strip **(Yassin et al., 2002 and EL-Shanty, 2009)**. Despite that, limited data are available on toxic effect of methamidophos on various mammalian systems and organs in Gaza strip as well as worldwide. Therefore, investigating methamidophos toxicity on liver and kidney of rabbits can expand our understanding on health hazards of this insecticide exposure in humans.

5.1 Toxicity of methamidophos

Acute oral LD_{50} of methamidophos was estimated to be 21 and 16 mg/kg body weight for male and female rats, respectively, 30-50 mg/kg body weight in guinea pigs and in the range of 10-30 mg/kg body weight in rabbits, cats and dogs **(Tomlin, 2011 and MacBen, 2013)**. However, to our best knowledge no previous study assessed the exact or narrow range of oral LD_{50} in male domestic rabbit. In the present study, the logarithmic scale showed that the oral LD_{50} of methamidophos in male domestic rabbits was 20.5 mg kg^{-1} body weight i.e. lies in the range of previously estimated oral LD_{50} of methamidophos in rabbits. This confirms the fact that methamidophos is a highly toxic pesticide and coincides with the idea that the lower the LD_{50} value, the more toxic is the pesticide. **Extension Toxicology Network (2009)** classified methamidophos as a class I compound and reported that its use must be restricted**.**

5.2 General health of rabbits

The present study demonstrates that treatment of rabbits with 1/10 LD_{50} methamidophos induced an overall mortality rate of 11.1% 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 **(Kumer et al., 2010 and Narang et al.,**

2015). In addition, methamidophos-treated rabbits showed dermal abnormalities especially in the last two weeks of the experiment. It is accepted that organophosphorus pesticides suppress the immune system making the skin vulnerable to attack of various pathogens **(Pore et al., 2011 and Díaz-Resendiz et al., 2015).** The livers of methamidophos-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. Methamidophos is known to induce morphological and histopathological changes in the liver **(Foudoulakis et al., 2013 and Araoud et al. 2014).**

5.3 Body weight

As indicated in the current data body weight was significantly decreased in methamidophos-supplemented rabbits compared to controls. This finding is in agreement with that obtained by **de Castro and Chiorato, (2007) and Araoud et al. (2014).** The reduction in body weight in response to methamidophos intake may be a result of the combined action of cholinergic (redused food intake and diarrhea) and oxidative stress and/or due to increase degradation of lipids and proteins as a direct effect of organophosphours pesticide exposure **(Mossa et al., 2011 and Sharma et al., 2015).** This explanation is supported by the recorded significant decrease in protein content in methamidophos-treated rabbits compared to controls.

5.4 Biochemical investigation

5.4.1 Serum glucose

Results presented in this study revealed that oral the daily administration of 1/10 LD_{50} methamidophos for 6 weeks caused general significant increase in serum glucose levels. This finding is in concurrent with that reported by **Colak et al. (2014)**. Therefore, glucose homeostasis is affected by methamidophos administration. The mechanism by which this organophosphorus insecticide induces hyperglycemia may involve one or more mechanisms: 1) reduction in insulin secretion as a result of the destructive action on the beta cells of Langerehans islets in the pancreas **(Gulalp et al., 2007 and Ambali et al., 2011)**, 2) impairment in hepatic function due to oxidative changes, which reduce liver ability to glycogenesis **(Slotkin et al., 2005b and Goel et al., 2006)**, 3) stimulation of hepatic gluconeogenesis and glycogenolysis **(Abdollahi et al., 2004)**, and 4) activation of the hypothalamus-pituitary-adrenal (HPA) axis. The activation of HPA axis by organophosphorus pesticides may cause secretion of glucocorticoids from adrenal cortex

that in turn increases blood glucose by induction of gluconeogenesis pathway **(Rahimi and Abdollahi, 2007)**.

5.4.2 Liver enzymes and bilirubin

Data presented in this study showed that the mean levels of serum ALT, AST, ALP and □-GT in the methamidophos-treated rabbits were significantly higher than those in the controls. Such elevation of liver enzymes as a result of methamidophos administration was documented by other authors **(Dilshad et al., 2008 and Araoud et al., 2014)**. Liver is the center of biotransformation and detoxification of foreign compounds and is the most vulnerable to the chemical assaults such as methamidophos poisoning **(Mansour and Mossa, 2010 and Heikal et al., 2012b)**. Serum ALT, AST and □-GT are considered to be among the most sensitive markers employed in the diagnosis of hepatotoxicity **(Akhtar, 2009; Ambali et al., 2011 and Newairy and Abdou, 2013)**. Pesticide exposure causes liver damage and leakage of cytosolic enzymes from hepatocytes and other body organs into blood **(Ncibi et al., 2008; Heikal et al., 2013 and Newairy and Abdou, 2013)**. Elevation of liver enzymes may also be due to increased gene expression due to long term requirement of detoxification of pesticides **(Friedman et al., 2003).**

In contrast to elevation of transaminases, □-GT and ALP, serum ChE activity was significantly decreased in methamidophos-treated rabbits compared to controls. Such inhibition in ChE in response to organophosphorus methamidophos administrated was previously obtained (**Emerick et al., 2012; Maretto et al., 2012; Foudoulakis et al., 2013 and Araoud et al., 2014).** It is known that organophosphorus pesticides such as methamidophos 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 manifested in cholinergic symptoms including diarrhea, convulsions and even tremors leading in severe cases to death **(Watts, 2011; Foudoulakis et al., 2013 and Kumar et al., 2015).** This result is supported by some mortalities and the clinical signs of anticholinestrase action represented in diarrhea, disorientation, drowsiness, weakness, depression and mild tremors observed in methamidophos-treated rabbits**.**

In the present study oral administration of methamidophos caused general increase in total bilirubin level throughout the experiment. Such increase was reported previously by

Ahmed (2006); Dilshad et al. (2008) and Jayusman et al. (2014) in organophosphorus pesecticide-intoxicated rats. Bilirubin which is a product of haemoglobin degradation is a marker of hepatobiliary injury **(Ozer et al., 2008).** The increase of total bilirubin in plasma may be attributed to the impairment of hepatocellular function in acute or subacute hepatic necrosis and may provide further evidence on hepatotoxicity induced by the organophosphorus insecticide methamidophos **(Satar et al., 2004; Khan et al,. 2010 and Araoud et al., 2014).**

5.4.3 Kidney function

The influence of methamidophos on kidney function was assessed through the measurement of urea and creatinine. Urea concentration was significantly increased throughout the whole experiment compared to the control. For creatinine this significant increase was also observed along the whole experiment except during the 1st week. Such findings are in agreement with the study of **Araoud et al. (2014)** who found that methamidophos-treated groups of Wistar male rats (low 1/50 LD_{50} and high doses 1/10 LD_{50}) showed significantly higher urea and uric acid levels compared with untreated controls. Urea is formed by the liver as an end product of protein breakdown and it is one marker of the kidney function (**Debra Manzella, 2008 and Tawfik and Al-Badr, 2012**). Increase in serum urea observed in the present study may be due to 1) impairment in its synthesis as a result of impaired hepatic function, 2) disturbance in protein metabolism and 3) decrease in its filtration rate in the kidney. The decrease in protein profile observed in the present study may support this explanation. Creatinine is break-down product of creatine phosphate in muscles, and is usually produced at a fairly constant rate by the body. Creatinine is chiefly filtered out of the blood by the kidneys and has been found to be a fairly reliable indicator of kidney function (**Tawfik and Al-Badr, 2012).** As the kidneys become impaired for any reason, for example in case of methamidophos poisoning, the creatinine level in the blood will rise due to poor clearance by the kidneys. A rise in blood creatinine level is observed with damage to functioning nephrons and impaired renal function **(Zama et al., 2007 and Ambali et al., 2010)**.

5.4.4 Protein profile

As indicated in the present results significant decreases in the levels of total protein, albumin and globulin concentrations, commencing from the 2nd week of methamidophos administration, were found in experimental animals compared to the controls. Similar

findings were reported in other studies as a result of organophosphorus intoxication **(Peeples et al., 2005; EL-Shanty, 2009 and Ahmad and Gautam, 2014)**. The reduction in serum protein could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver. Also, the protein level suppression may be due to loss of protein either by reduce in protein synthesis or increased proteolytic activity or degradation **(Ncibi et al., 2008 and Shin and Moon, 2010)**. In addition, the observed decrease in serum proteins could be attributed in part to the damaging effect of methamidophos on liver cells, as confirmed by the increase in activities of serum AST, ALT and □-GT. It was reported that albumin levels are decreased in liver disease **(Khalifa et al., 2011)**. Impairment in liver function that responsible for synthesis of plasma proteins.

5.4.5 Electrolytes

The mean serum concentration of calcium was generally decreased in methamidophos-intoxicated rabbits and this decrease became significant at 4^{th} and 6^{th} weeks of the experiment. In contrast, serum phosphorus concentration was significantly increased in response to methamidophos treatment allover the experimental period except during the first week. Alterations in serum electrolytes were reported by **Yassin (2003); Turabi et al. (2008) and Meijer et al. (2014)** in response to exposure to organophosphorus pesticides including methamidophos. This indicates that the organophosphorus insecticide methamidophos interferes with calcium and phosphorus homeostasis. Hypocalcemia and hyperphosphatemia observed in the present study may be attributed to disturbance of parathyroid glands and calcitonin cells and may alter bone mineral composition especially calcium and phosphorus levels of bone **(Tripathi and Srivastav, 2012)**. In addition, **Yassin (2003)** suggested that high exogenous contribution of phosphorus driven from the organophosphorus methamidophos administration may contribute to hyperphosphatemia recorded in the serum of rabbit.

CHAPTER 6
CONCLUSIONS

1. The calculated oral LD_{50} of methamidophos in male domestic rabbits was found to be 20.5 mg kg^{-1} body weight.
2. Daily oral administration of 1/10 LD_{50} methamidophos (2.1 mg kg^{-1} body weight) caused an overall mortality rate of 11.1% compared to 0.0% in controls.
3. Clinical signs of methamidophos-intoxicated rabbits were diarrhea, disorientation, drowsiness, weakness, depression and mild tremor.
4. The final body weight was significantly decreased in methamidophos-treated rabbits compared to controls.
5. Serum glucose was significantly increased in response to methamidophos administration compared to controls.
6. Liver enzymes ALT, AST, ALP and □-GT as well as bilirubin were significantly higher in the methamidophos-intoxicated rabbits whereas cholinesterase level was significantly decreased compared to the controls.
7. Urea and creatinine concentrations were significantly increased in response to methamidophos administration compared to the controls.
8. There were significant decreases in total protein, albumin and globulin values upon methamidophos intake compared to the controls.
9. Hypocalcemia and hyperphosphatemia were recorded in methamidophos- intoxicated rabbits.

Recommendations

1. Restriction the use of pesticides in home and farm.
2. Use of more secure alternatives of pesticides such as biological control and the agricultural cycle.

3. Further studies are needed on health impact of other pesticides

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