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Physiological and Histological Studies on Imidacloprid Toxicity in Male Rabbits

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DECALARATION

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Dedication

To my parents
my mother
my father
my brothers Sami, Sameer, Nabil, Hassan, Osama and Mohmmed
my sisters Somia, Samia, Nabila and Sohear
my friends
my Islamic University and to
all I love

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Physiological and Histological Studies on Imidacloprid Toxicity in Male Rabbits

Abstract

The present study is aimed to investigate the physiological and histological alterations associated with imidacloprid administration in male rabbits. The oral LD_{50} of imidacloprid in the male rabbits was calculated from logarithmic scale and found to be 172 mg/kg body weight. A dose of 1/10 LD_{50} (17.2 mg/kg body weight) imidacloprid was then used to test its toxicity. The experimental group were given orally the dose of 1/10 LD_{50} imidacloprid daily for eight weeks. Control animals were given distilled water. Upon imidacloprid administration, alanine aminotransferase generally showed significant increase throughout the experimental periods with maximum increase in the 2nd and 5th weeks compared to controls (70.8±4.1 v 52.0±3.0 and 65.3±3.9 v 49.8±3.0; % difference=36.2 and 31.1; P=0.017and 0.020, respectively). Aspartate aminotransferase was also increased with significant changes at the 3rd, 4th and 5th weeks (49.2±3.0 v 35.7±2.1; 43.2±2.7 v 34.8±1.5 and 43.0±2.1 v 36.1±1.7; % difference=37.8, 24.1 and 19.1; P=0.012, 0.031 and 0.047). Alkaline phosphatase increased in the first four weeks showing maximum increase during the 2nd week compared to controls (129.5±6.3 v 103.3±3.9, % difference= 25.4, P=0.012). In the last four weeks the enzyme activity decreased with maximum decrease at the 6th week (83.3±.5 v 107.8±5.7, % difference= 22.7, P=0.018). Similar effect was observed for cholinesterase with maximum increase at the 4th week (6167±235 v 4624±168, % difference= 33.4, P= 0.002) and maximum decrease at the 8th week (3831±233 v 4876±155, % difference= 21.4, P= 0.011). Urea was elevated with maximum increase at the 6th week compared to controls (48.2±1.9 v 36.4±3.0, % difference= 32.4, P= 0.011). Such increase was at the 7th week for creatinine (1.20±0.06 v 0.89±0.08, % difference= 34.8, P= 0.025). Protein profile showed statistically significant decrease in total protein levels all over the experimental periods showing maximum decrease at the 3rd week compared to controls (4.5±0.29 v 6.2±0.33, % difference= 27.4, P= 0.009). Similar result was observed for albumin and globulin with maximum changes at the 3rd week

(3.2±0.17 v 4.1±0.18 and 1.40±0.07 v 2.03±0.11; % difference= 22.0 and 31.0; P= 0.010 and 0.003, respectively). Testosterone was significantly lower in imidacloprid-treated rabbits compared to controls particularly at the 3rd and 7th weeks (2.10±0.12 v 3.20±0.18 and 2.40±0.15 v 3.50±0.17; % difference= 34.3 and difference= 31.4; P= 0.002 and 0.003, respectively). On the other hand, the Histopthological alterations were manifested in liver, kidney and testis of the imidacloprid-treated rabbits. Hepatocellular damage as degenerative changes, destruction of architecture of hepatocytes, karyolysis the nuclei and congestion of sinusoids were observed on the treated rabbits. While the effect of imidacloprid was more prominent on kidney tissue. Mild hypertrophy in some individual glomeruli. Kidney section showing dilatation of the inter tubular blood vessels impacted by haemolysed blood, rupture of the renal tubules and necrobiosis in the epithelial cells lining the tubules were observed in the experimental animals. Light microscopic analyses revealed the Histopthological changes on testis tissue. Testis sections from rabbits treated with imidacloprid showed necrotic changes of spermatogonia, primary and secondary spermatocytes. On the other hand disorganization of spermatogenic layers, necrotic Sertoli cells and pyknotic lesions in spermatogonia showed in the treated rabbits. Furthermore, disappearance of interstitial cells of Leydig showed in the treated animals.

Key words: Imidacloprid, toxicity, physiology, histology, male rabbits

دراسة فسيولوجية ونسيجية على سمية الإميداكلوبريد في ذكور الأرانب

المستخلص

تهدف الدراسة الحالية لمعرفة التغيرات الفسيولوجية والنسيجية المصاحبة لإعطاء جرعات من مادة الاميداكلوبرايد لذكور الأرانب. ولقد بينت الدراسة أن قيمة الجرعة النصف مميتة للاميداكلوبرايد في ذكور الأرانب بواسطة الفم هي 172 ملجرام/كجم من وزن الجسم. بناءً على هذه النتيجة تم دراسة سمية الاميداكلوبرايد عند جرعة قيمتها 17.2ملجرام/كجم من وزن الجسم وتساوى 1/10 من الجرعة النصف مميتة. المجموعة التجريبية أعطيت عن طريق الفم جرعة يومية مقدارها 17.2 ملجرام/كجم من وزن الجسم من مادة الاميداكلوبرايد لمدة ثمانية أسابيع. والمجموعة الضابطة تم اعطائها ماء مقطر بواسطة الفم. وبعد اعطاء الاميداكلوبرايد عن طريق الفم لوحظ ارتفاع مستوى الإنزيمات الناقلة للأمين وبدلالة احصائية خلال فترة التجربة وكانت أعلى زيادة في الاسبوع الثاني والخامس مقارنة مع المجموعة الضابطة (70.8±4.1 مقابل 52.0±3.0 و 65.3±3.9 مقابل49.8±3.0؛ 36.2% و 31.1%; P=0.017و , 0.020على التوالي). كما وجد ارتفاع في مستوى إنزيمات الاسبرتيت وبدلالة احصائية في الأسابيع الثالث ،الرابع والخامس (49.2±3.0 مقابل 35.7±2.1؛ 43.2±2.7 مقابل 34.8±1.5 و 43.0±2.1 مقابل 36.1±1.7؛ 37.8% و 24.1%و 19.1%؛ P=0.012 و 0.031 و 0.047 ,على التوالي). انزيم الفوسفاتيز الفاعدي أظهر زيادة في الأربع أسابيع الأولى وكانت أعلى زيادة في الاسبوع الثاني مقارنة مع المجموعة الضابطة (129.5±6.3 مقابل 103.3±3.9؛ 25.4 %؛ P=0.012%). انزيم الفوسفاتيز أظهر نقصان في الأربع أسابيع الأخيرة سجل أعلى نقصان في الاسبوع السادس مقارنة مع المجموعة الضابطة (83.3±.5 مقابل 107.8±5.7؛ 22.7%؛ P=0.018). بطريقة مماثلة لوحظ زيادة في الإنزيم المحلل للأسيتايل كولين في الأربع أسابيع الأولى وسجلت أعلى زيادة في الأسبوع الرابع (6167±235مقابل 4624±168؛ 33.4%؛ P=0.002). كما لوحظ انخفاض في الإنزيم المحلل للأسيتايل كولين في الأربع أسابيع الأخيرة وكان أقل انخفاض في الأسبوع الثامن (3831±233 مقابل 4876±155؛ 21.4%؛ P= 0.011). كما أظهرت النتائج ارتفاع مستوى البولينا وسجل أعلى ارتفاع في الأسبوع السادس مقارنة مع المجموعة الضابطة (48.2±1.9 مقابل 36.4±3.0؛ 32.4%؛ P=0.011). كما لوحظ زيادة في مستوى الكرياتينين وكانت أعلى زيادة في الأسبوع السابع (1.20±0.06 مقابل 0.89±0.08؛ 34.8%؛ P= 0.025). وكذلك فان الدراسة أظهرت نقص ذو دلالة احصائية في مستوى البروتين الكلي خلال فترة التجربة مسجلة أعلى انخفاض في الأسبوع الثالث (4.5±0.29 مقابل 6.2±0.33؛ 27.4%؛ P= 0.009). وبالمثل فقد لوحظ انخفاض في مستوى الألبيومين والجلوبيولين وسجل أعلى نقصان في الأسبوع الثالث مقارنة مع المجموعة الضابطة (3.2±0.17 مقابل 4.1±0.18 و1.40±0.07 مقابل 2.03±0.11؛ 22.0% و 31.0؛ P= 0.010 و 0.003). كما وكذلك فان نتائج التجربة وجدت أن هناك انخفاض في هرمون التستوستيرون وقد سجل اعلى نقصان في الأسبوع الثالث (2.10±0.1 مقابل 3.20±0.18؛ 34.3 %؛ P= 0.002). من ناحية اخرى أظهر الفحص النسيجي للكبد والكلية والخصية أن هناك تغيرات نسيجية مرضية ناتجة عن إعطاء مادة الاميداكلوبرايد تمثلت في حدوث تدمير لخلايا الكبد، تغير التركيب البنائي للخلايا، وتحلل في أنوية العديد من خلايا الكبد واحتقان في المنحنى الجيبي للأرانب المعالجة بالمقارنة مع المجموعة الضابطة. بينما لوحظ تأثير الاميداكلوبريد واضحا على أنسجة الكلية. فقد وجد أن هناك تضخم في الكبة، تمدد في الأوعية الدموية مصحوبا بنزيف دموي، تمزق الأنابيب الكلوية وفقد لأنوية الخلايا المكونة للأنابيب الكلوية للمجموعة التجريبية بالمقارنة مع المجموعة

الضابطة. التحليل بواسطة الميكروسكوب الضوئى أثبت أن هناك تغيرات نسيجية مرضية على أنسجة الخصية، فقد وجد أن هناك تحلل في الخلايا المولدة للنطاف، الخلايا المنوية الأولية و الخلايا المنوية الثانوية. من ناحية اخرى لوحظ تشويش في ترتيب طبقات الخلايا المنوية، تحلل في أنوية خلايا سرتولي، علاوة على ذلك فانه حدث اختفاء لخلايا ليدج في الحيوانات المعالجة.

الكلمات المفتاحية: اميداكلوبريد ، سمية ، فسيولوجية ، نسيجية ، ذكور الأرانب

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CHAPTER I

INTRODUCTION

1.1 Overview

Pesticides are substances or a mixture of substances intended to control a variety of pests such as insects, rodents, fungi, weeds, microorganisms and other unwanted organisms. Pesticides are usually classified into insecticides, fungicides and herbicides. Other categories include rodenticides, termiticides, miticides, disinfectants and insect repellents (Keifer, 1997).

Insecticides are pesticides used against insects. They include ovicides and larvicides used against the eggs and larvae of insects, respectively. The use of insecticides is believed to be one of the major factors behind the increase in agricultural productivity in the 20th century. Nearly all insecticides have the potential to significantly alter ecosystems; many are toxic to humans; and others are concentrated in the food chain (Van Emden et al., 1996).

Insecticides are classified according to the method of application and the way they enter the insect's body: contact insecticides, insecticidal gases, residual insecticides, stomach insecticides and systemic insecticides. Systemic insecticides are absorbed by plant tissues, so that when insects feed on the sap they are poisoned (Daniel and Potter, 1998).

Imidacloprid is a systemic and a new potent insecticide with high insecticidal activity at very low application rates, uses for the control of sucking insects (Kidd and James, 1994). It has many names but the most common is confidor (Meister, 1995 and Tomizawa and Casida, 2005). Both ingestion and contact routes of exposure are effective in controlling insect pests (Cordone and Durkin, 2005). The mechanism of action of imidacloprid has been extensively studied in insects and mammals. Imidacloprid acts as a competitive inhibitor at nicotinic acetylcholine receptors in the nervous system (Liu and Casida, 1993 and Zwart et al., 1994). It effectively blocks the signals that are induced by acetylcholine at the post-synaptic membrane, resulting in

impairment of normal nerve function. Imidacloprid has a higher binding strength to insect nerve receptors than to mammalian receptors (Zwart et al., 1994).

Imidacloprid toxicity is predicted from LD_{50} (a dose that expected to cause death in 50% of animals). This is the first study to determine oral LD_{50} of the imidacloprid in male domestic rabbits. The 1/10 LD_{50} is then used in our toxicity experiments. In addition, few studies addressed the toxic effect of imidacloprid on the functions of several mammalian organs including liver, kidney, brain, and gonads as well as on their histological profile (Eiben, 1991; Eiben & Kaliner, 1991; Thyssen and Machemer, 1999; Svetlana and Koshlukova, 2006; Najafi et al., 2010; Yeh et al, 2010)).

Pesticides are being used in large amounts in the Gaza Strip where the protective measures are poorly followed (Yassin et al., 2002). More than 450 metric tons of pesticides are being used yearly in the Gaza strip. The insecticide represents 70-100 metric tons of these pesticides, 5-7 metric tons of these insecticides are imidacloprid (Ministry of Agriculture, 2009). These highly toxic compounds constitute a real threat on humans. The present work is intended to investigate imidacloprid 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 imidacloprid exposure.

1.2 Objectives

The general objective of the present study is to assess the physiological and histological alterations in rabbit induced by sub lethal dose of imidacloprid (1/10 LD_{50}).

The specific objective are

1. To determine the oral LD_{50} of imidacloprid in male domestic rabbits.
2. To study the effect of imidacloprid on liver function through measurement of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and cholinesterase.

3. To test the effect of imidacloprid on kidney function through determination of urea and creatinine.
4. To assess the effect of imidacloprid on testis function through determination of testosterone.
5. To investigate the effect of imidacloprid on the total protein, albumin and globulin.
6. To examine the histological changes in liver, kidney and testis in response to imidacloprid administration.

1.3 Significance

1. Imidacloprid is being extensively used in agriculture in Gaza Strip with lake of protective measures.
2. Studies on imidacloprid toxicity on mammals including rabbits are very limited in the literature.
3. The results of the present study may be useful to a ware people particularly farmers on the extent of imidacloprid toxicity.

CHAPTER 2

LITERATURE REVIEW

2.1 Definition of pesticide

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

2.2 Classification of insecticides

Insecticides can be classified according to the type of action into organochlorine, organophosphates, carbamates, pyrethroids, neonicotinoids, biological insecticides and antifeedants (Kamrin, 1997).

2.3 Neonicotinoids insecticides

The neonicotinoids, the newest major class of insecticides (Tomizawa and Casida, 2005): neonicotinoids synthetic analogues of the natural insecticide nicotine (with a much lower acute mammalian toxicity and greater field persistence). Neonicotinoids are broad spectrum – systemic insecticides with a rapid action (minutes-hours). They are applied as sprays, seed and soil treatments (Tomizawa and Yamamoto, 1993). Available neonicotinoid insecticides include: Imidacloprid, acetamiprid, clothianidin, dinotefuran, flonicamid, nitenpyram, nithiazine, thiacloprid and thiamethoxam (Tomizawa and Casida, 2003).

2.4 Imidacloprid

2.4.1 Definition

Imidacloprid [1-(6-chloro-3-pyridylmethyl)-nitroimidazolidin-2-ylideneamine] is a new neonicotinoid insecticide (Zwart et al., 1992; Mullins, 1993; Nauen, 1995; Wismer, 2004; Tomlin, 2006; Yeh et al, 2010). Chemical structure of imidacloprid is illustrated in figure 2.1 (Kramer, 2001 and World Health Organization, WHO 2005).

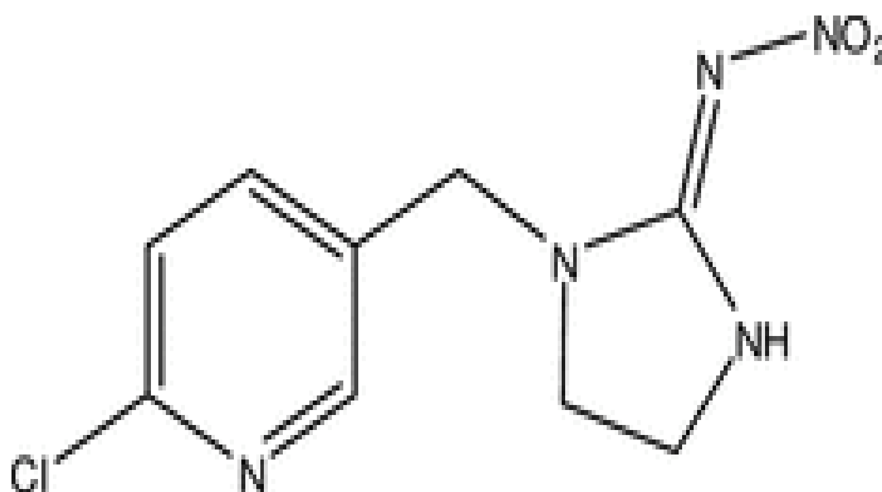


Figure 2.1 Chemical Structures of imidacloprid (Kramer, 2001 and World Health Organization, 2005).

The insecticide imidacloprid is mainly applied as a seed dressing formulation. After root uptake, imidacloprid is translocated acropetally within the xylem and degraded quickly in the plants (Robin and Andreas, 2003). Imidacloprid is an insecticide that was first synthesized in its active form by Bayer HealthCare in Japan in 1986. End-use products have pervaded the market place within the last 5 years (Extension Toxicology Network EXTOXNET, 1995). According to the WHO and United States Environmental Protection Agency this compound is categorized as a “moderately toxic”

requiring a warning or caution labels on marketed products (Avery et al., 1994).

2.4.2 Physical and chemical properties of imidacloprid

Imidacloprid occurs as colorless crystals with a weak characteristic odor. The principal chemical properties of imidacloprid are compiled in Table 2.1 (Kidd and James, 1994 and Tomlin, 2006).

Table 2.1 Physical and chemical properties of imidacloprid (Kidd and James, 1994 and Tomlin, 2006).

Property	Values
Molecular weight	255.7 g/mol
Melting point	136.4-143.8 ℃
Solubility in water	0.61 g/l at 20 ℃
Vapor pressure	3 x 10^{-12} mmHg
Density	1.543 g/cm3

2.4.3 Imidacloprid as acetylcholine receptor

Acetylcholine is an important neurotransmitter in both insects and mammals; it is released at the nerve synapse in response to a membrane depolarization which is the hallmark of nerve transmission. The acetylcholine then binds to a protein receptor in the membrane of the nerve synapse, 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 (Lee and Sine., 2005; Sine and Engel., 2006).

There are two types of acetylcholine receptors (AChR) that bind acetylcholine and transmit its signal.

1. Muscarinic receptors (mAChRs) at which muscarine action mimics the stimulatory action of acetylcholine on smooth muscle and gland. Muscarinic receptors are blocked by atropine. There are five subtypes of muscarinic AChRs based on pharmacological activity M1- M5 (Mohamadi, 2009).

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 (Dani, 2001and Hogg et al., 2003). Nicotinic receptors subdivided in to those found in muscle at neuromuscular junctions and those found in autonomic ganglia and the central nervous system. Each nicotinic cholinergic receptor is made up of five subunits that form central channel. The five subunits came from a menu of 16 subunitsγ, α1-α9, β2-β5, δ, γ and ε, coded by 16 genes (Jones et al., 1999; Karlin, 2002). Some of receptors are homomeric and others are heteromeric (Karlin, 2002). Many of nicotinic acetylcholine receptors are located presynaptically, and some seem to be free in the interstitial fluid. However, others are post synaptic. Some are located on structure other than neurons. The acetylcholine is subsequently destroyed by acetylcholinesterase, and the membrane returns to its normal resting state (Liu and Casida, 1993 and Zwart et al., 1994).

2.4.4 Mechanism of action of imidacloprid

Imidacloprid works by disrupting the nervous system of an insect pest. Imidacloprid kills insects by contact and ingestion (Lagadic et al., 1993). It is used to control sucking insects and is effective against adult or larval stages of various species (Elbert et al., 1990; Lagadic et al., 1993 and Leicht, 1996). Imidacloprid produces neurotoxicity through its action on nicotinic acetylcholine receptor. Imidacloprid is a nAChR agonist (Liu and Casida, 1993 and Zwart et al., 1994). It mimics the action of nicotine in the nervous system, binding or partial binding to specific sub-sites or protein subunits of the nicotinic acetylcholine receptor (nAChR), where nicotine binds which in turn activates nAChR activity producing an unregulated barrage of nerve

impulses, resulting in something akin to a nervous breakdown, and ultimately, death (Tomizawa and Casida 2003, 2004).

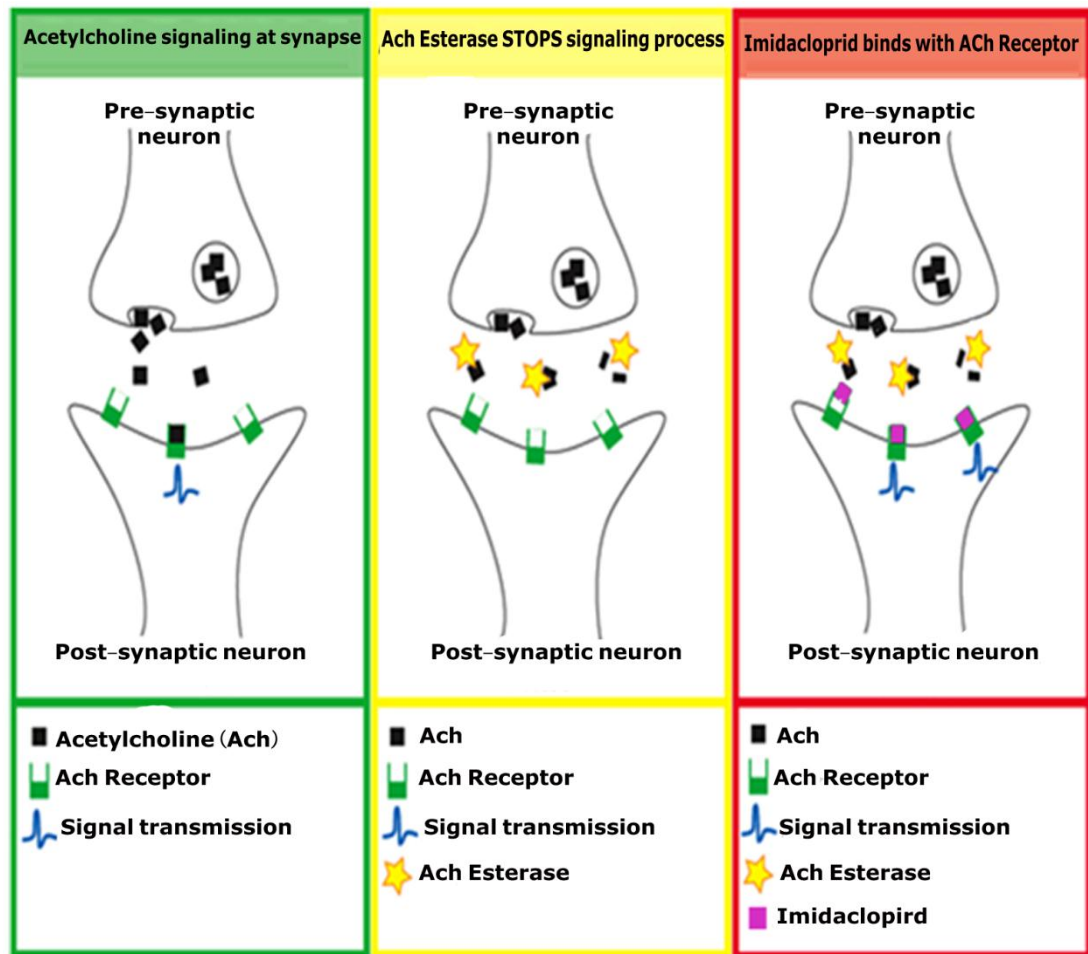


Figure 2.2 Pathophysiology of imidacloprid insecticides poisoning

2.4.5 Symptoms of imidacloprid

The symptoms of imidacloprid poisoning include fatigue, ventricular arrhythmia, hypotension, metabolic acidosis, gastrointestinal irritation, vomiting, mydriasis, hypothermia, leukocytosis, hyperglycemia, muscle weakness including the muscles necessary for breathing, incoordination, tremors, spasms and respiratory difficulties (Wu et al., 2001; Shadnia and Moghaddam, 2008 and Yeh et al., 2010). Other symptoms included decreased motility and lethargy (Doull et al., 1991 and Svetlana and Koshlukova, 2006).

2.4.6 Metabolism of imidacloprid

A proposed metabolic pathway of imidacloprid in rats is shown in Figure 2.3 (Klein, 1987). Two main routes of metabolism responsible for the degradation of imidacloprid were identified. The first is oxidative cleavage, yielding 6-chloronicotinic acid, which is conjugated with glycine to form a hippuric acid-type conjugate. These two metabolites together represented most of the identified metabolites, or about 30% of the recovered radiolabel. Of minor importance in terms of quantity is dechlorination of the pyridinyl moiety, producing the 6-hydroxy nicotinic acid and its methylmercapturic acid derivative. The 6-methylmercapto nicotinic acid conjugated with glycine, and the glycine conjugate constituted 5.6% of the recovered radiolabel. The second important biodegradation step starts with hydroxylation of the imidazolidine ring at the 4 or 5 positions, and about 16% of the recovered radiolabel was identified as the sum of 4- and 5-hydroxy imidacloprid. The loss of water yields the olefinic compound. These biotransformation products and the unchanged parent compound were excreted in urine and faeces, while the guanidine compound was a less important metabolite and was eliminated only in faeces. The nitroso compound is therefore formed in vivo in rats after long-term intake of imidacloprid. In order to confirm this finding, a direct isotope dilution analysis was conducted with the urine of rats and also with the urine of mice that had been fed a diet containing 2000 ppm of imidacloprid for about 1 year. Both analyses clearly demonstrated the presence of the nitroso compound in the urine (Klein, 1990).

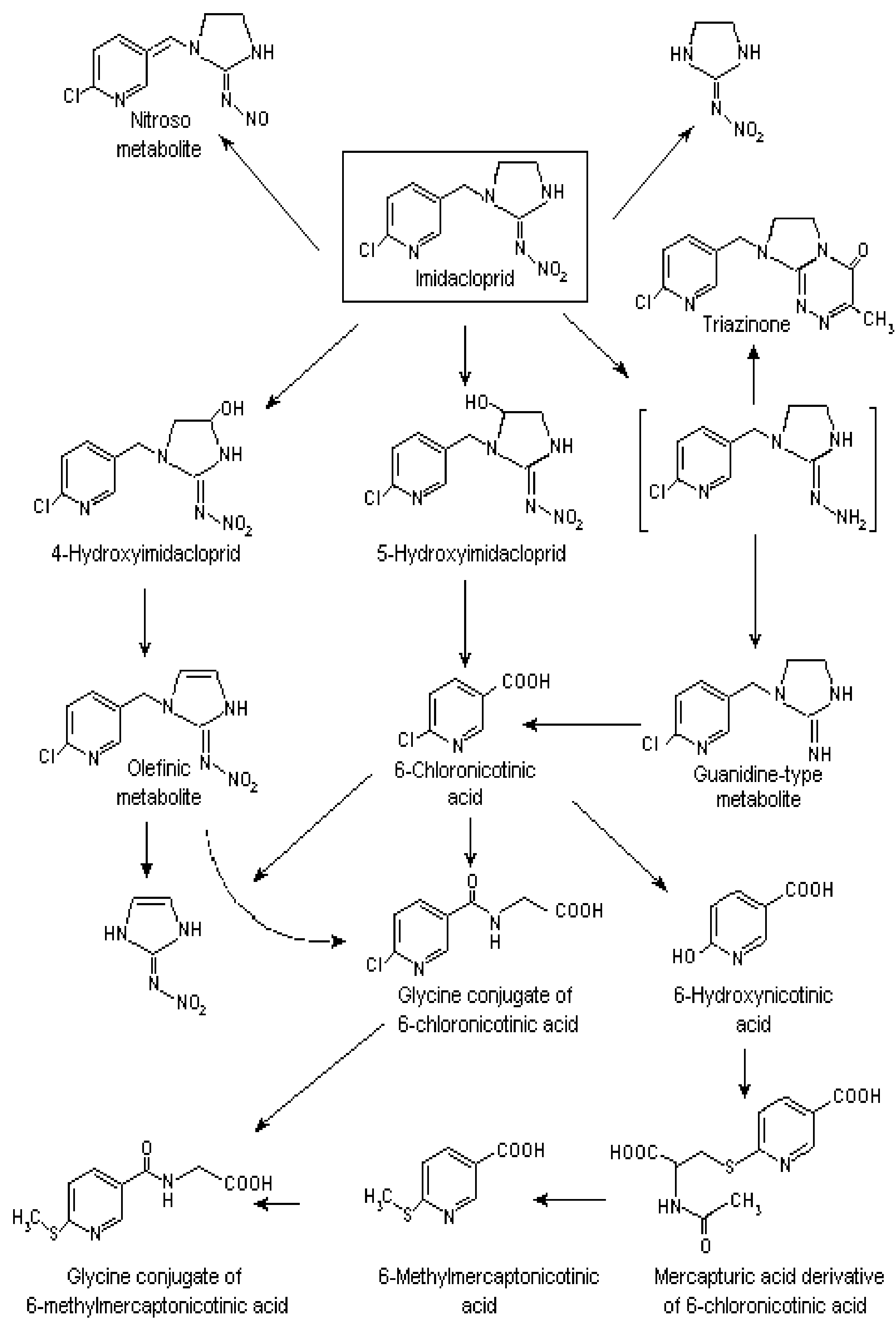


Figure 2.3 Proposed metabolic pathway of imidacloprid (Klein, 1987)

2.4.7 Distribution of imidacloprid

The distribution of the metabolites in liver and kidney at various times after a single oral dose was investigated in rats under the same test conditions as used by Klein (1987). The metabolites were extracted from lyophilized organs with water and methanol, further purified by high-performance liquid chromatography (HPLC) and identified by comparative HPLC with authentic reference compounds in at least two independent chromatographic systems and also by mass and 1H-nuclear magnetic resonance spectroscopic techniques. The metabolites found in the kidney were identical to those identified in urine. Triazinone was not found in the excreta and may have undergone further biodegradation before elimination via the kidney or the bile. The products formed by oxidative mechanisms e.g. 6-chloronicotinic acid increased in the liver during the test period. In kidney, the relative amount of 6-chloronicotinic acid and its glycine conjugate decreased with time, while the amounts of the olefinic metabolite and the mono-hydroxylated derivative 4-hydroxyimidacloprid showed a relative increase. The proportion of the parent compound decreased slowly as it was metabolized (Karl and Klein, 1992).

2.4.8 Fate in humans and animals

Researchers tested imidacloprid absorption using human intestinal cells. Cells rapidly absorbed imidacloprid at a very high rate of efficiency. They concluded that an active transport system was involved (Brunet et al., 2004). Imidacloprid is quickly and almost completely absorbed from the gastrointestinal tract, and eliminated via urine and feces 70-80% and 20-30%, respectively (Kidd and James, 1994).

2.4.9 Uses of imidacloprid

Imidacloprid is used for the control of sucking insects including rice hoppers, aphids, thrips, whiteflies, termites, soil insects, some beetles etc... It is most commonly used on rice, cereal, maize, potatoes, vegetables, sugar beets, fruit, cotton and hops (Kidd and James, 1994). Imidacloprid highly effective for fleas control on cats and dogs (Liu and Casida, 1993; Arther et al., 1997; Dryden et al., 1999 and Larry et al., 2000).

Imidacloprid is being extensively used in the Gaza Strip for the control of sucking insects including termites, citrus leafminer, aphids, thrips and some beetles. It is most commonly used on tomatoes, potatoes, vegetables and citrus (Ministry of Agriculture, 2009).

2.4.10 Imidacloprid toxicity

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 oral LD_{50} value of imidacloprid in rats was estimated to be 450 mg/kg body weight for both sexes (Meister, 1995) and 131 mg/kg in mice (Kidd and James, 1994). Imidacloprid is very low in toxicity via dermal exposure, the dermal LD50 in rats was estimated at greater than 5000 mg/kg (Tomlin, 2006). However no previous study assessed the LD50 in male domestic rabbit, so this is the first study to determine LD_{50} in male domestic rabbits.

2.4.11 Physiological effects of imidacloprid

Groups of 10 male and 10 female Charles-River B6C3F1 mice were given diets containing imidacloprid (purity, 92.8%) at a concentration of 0, 120, 600 or 3000 ppm for up to 107 days (Eiben, 1988). Seven males and seven females at 3000 ppm died; furthermore, several animals displayed a poor general condition was observed. Body-weight gain was reduced and food consumption was increased in males at 600 ppm and males and females at 3000 ppm. At this dose, clinical chemical tests showed significantly decreased urea and cholesterol concentrations in males and lowered alanine aminotransferase activity and glucose concentration in females. Alkaline phosphatase activity was significantly increased in both sexes at 3000 ppm and in females at 120 and 600 ppm. Differences in the weights of the liver, heart, spleen, kidneys, testes and adrenals were observed at 3000 ppm.

Pauluhn (1988) administered imidacloprid (purity, 95.3%) in dust form through the nose only to groups of 10 male and 10 female Wistar rats at analytically determined concentrations of 0, 5.5, 30 and 190 mg/m3 for 6 h/day, 5 days per week for 4 weeks. Body-weight gain was decreased in males at the two higher concentrations. Increased alanine aminotransferase and glutamate dehydrogenase activities were seen in both sexes at the highest concentration, and alanine aminotransferase activity was increased in females at 30 mg/m3 air. The females had increased alkaline phosphatase activity at the two higher concentrations and increased liver weights at 190 mg/m3 of air. The serum alpha1-globulin fraction was reduced in both sexes at the two higher concentrations.

Groups of 10 male and 10 female Wistar rats received diets containing imidacloprid (purity, 95.3%) at a concentration of 0, 150, 600 or 2400 ppm for up to 96 days, equal to 14, 61 and 300 mg/kg bw per day for males and 20, 83 and 420 mg/kg bw per day for females (Eiben and Rinke, 1989). Reduced body-weight gain was observed in males at 600 ppm and in females at 2400 ppm. Elevated alkaline phosphatase and alanine aminotransferase activities and depressed protein, albumin, cholesterol and triglyceride concentrations were found in males and females at 2400 ppm. Depressed protein concentrations were also found in males at 150 and 600 ppm.

Yassin (1998) Investigated and compared the intoxication effects of daily oral administration of 1/10 LD50 of Tamaron, Parathion and confidor for 10 days on serum urea, uric acid and creatinine 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 levels. In general, a 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 content did not show any significant changes in response to the treatment by tamaron, parathion or confidor during all the time intervals studied.

Kaur et al., (2006) studied the repeated oral toxicity of imidacloprid in cow calves. Oral administration of imidacloprid at dose rate of 1 mg/kg/day for 21 consecutive days in cow calves produced very mild toxic symptoms of nasal discharge and occasional regurgitation of ruminal content. Imidacloprid significantly elevated plasma alanine aminotransferase, alkaline phosphatase and had no significant effects on plasma aspartate aminotransferase, acid phosphatase and cholinesterase enzymes. Daily oral administration of imidacloprid failed to induce any significant changes in the levels of total serum protein, blood urea nitrogen, plasma creatinine, blood glucose and plasma cholesterol.

Zaahkook et al., (2009) evaluated toxic effects of imidacloprid insecticide and possible ameliorating role of vitamin C on Japanese quails. The tested quails divided into four groups, the first group served as control, the second group treated with vitamin C only, the third group treated with imidacloprid singly and the fourth group treated with imidacloprid combined with vitamin C for 3 and 6 weeks of treatment and 3 weeks of recovery periods. Highly significant increases were observed in serum glucose level, lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase activities, total lipid and cholesterol in imidacloprid treated group during the experimental period and after imidacloprid combined with vitamin C. These increases were observed after 3 and 6 weeks of treatment. In the same time, significant inhibition in cholinesterase activity in imidacloprid treated group with or without vitamin C was detected. Furthermore , significant decrease were observed in serum total protein , albumin and globulin of groups treated with imidacloprid only, whereas a marked amelioration was detected in these parameters in quails treated with imidacloprid + vitamin C throughout the experimental periods. Creatinine revealed significant increases in imidacloprid treated group during the experimental periods.

The possible protective role of glutathione 0.55 mg/kg body weight against the toxic effect of 1/50 LD50 of imidacloprid insecticide in male Japanese quails was investigated (Helal et al., 2009). Sixty male quails were divided into four groups, the first one serve as control, the second received glutathione only, the third group treated with imidacloprid and the fourth was

administrated both glutathione and imidacloprid conjointly. Birds were treated orally for either three or six week followed a recovery period for 3 weeks. The data obtained revealed a marked increase in serum, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, glucose, total lipid, total cholesterol, and creatinine of quails treated with imidacloprid only, whereas variable level of amelioration were detected in treated groups with glutathione plus imidacloprid, especially in level of glucose, AST activity and creatinine 6 weeks of treatment. On the other hand, a highly significant decrease in total proteins, albumin and globulin were found in the birds treated with imidacloprid alone, but these returned to levels close to normal in the quail treated with glutathione plus imidacloprid. Albumin/globulin ratio and uric acid level were not significantly changed in all groups. In general, there was appreciable improvement after the recovery period.

Najafi et al., (2010) evaluated the effect of daily oral administration of 225 and 112 mg/kg imidacloprid for 60 days on testosterone in the serum of mature male rats. Results showed significant ($p<0.05$) decrease in serum testosterone levels compared to controls. In addition, Lauan (2007) studied the effects of low concentrations of imidacloprid insecticide on the reproductive system of male Nile tilapia. The levels of 1.336 x 10-2 mg/L and 0.1336 mg/L showed significant decrease in plasma testosterone levels of the animal.

A 90 days oral toxicity study of imidacloprid was conducted in female rats with doses of 0, 5, 10, 20 mg/kg/day (Bhardwaj et al., 2010). Decrease in body weight gain was observed at 20 mg/kg/day. The relative body weights of liver, kidney and adrenal was also significantly increased at this dose level. No mortality occurred during treatment period while food intake was reduced at high dose level. In clinical chemistry parameters high dose of imidacloprid has caused significant elevation of aspartate aminotransferase, alanine aminotransferase, glucose and blood urea nitrogen and decreased the activity of AChE in serum and brain. The spontaneous locomotor activity was also reduced at highest dose exposure whereas there were no significant changes in hematological and urine parameters. The brain, liver and kidney of rats exposed with high dose of imidacloprid had showed mild pathological

changes. Based on the morphological, biochemical, hematological and neuropathological studies it is evident that imidacloprid has not produced any significant effects at 5 and 10 mg/kg/day doses but induced toxicological effects at 20 mg/kg/day to female rats. Hence, 10mg/kg/day dose may be considered as no observed effect level for female rats.

2.4.12 Histological effect of imidacloprid

Bloch (1987) and Eiben (1988) showed tubular degeneration of the testis in both dogs and rats in their sub chronic range-finding studies, where imidacloprid was administered at high doses (3000 to 5000 ppm diet).

Groups of 10 male and 10 female Wistar rats received diets containing imidacloprid (purity, 95.3%) at a concentration of 0, 150, 600 or 2400 ppm for up to 96 days, equal to 14, 61 and 300 mg/kg bw per day for males and 20, 83 and 420 mg/kg bw per day for females (Eiben and Rinke, 1989). Histological examination of the liver showed increased incidences of cellular necrosis, round-cell infiltration, swollen cellular nuclei and cytoplasmic lesions in the liver of male at 2400 ppm.

The histological changes in liver and testis of 90 male Japanese quails treated with imidacloprid 1/50 LD50 for 3 and 6 weeks were investigated (Eissa, 2004). Liver showed dilated portal space, large degenerated area, large area of necrosis, dilated sinusoidal spaces, and faintly stained cytoplasmic nuclei. Histological changes occur in testis included thickened tunica albuginea and disappearance of spermatogenetic cells. The seminiferous tubules are devoid of sperms. Some pyknotic nuclei could be observed. Rupture of basement membrane of the seminiferous tubules.

Lauan (2007) studied the effects of low concentrations of carbaryl, chlorpyrifos and imidacloprid on the reproductive system of male Nile tilapia. Five concentrations of each insecticide were used. There were no significant differences on the relative testicular weight of sexually mature male Nile tilapia. The lowest concentrations to induce histological change were 1.336 x 10-6 mg/L for carbaryl, 1.336 x 10-7 mg/L for chlorpyrifos and 336 x 10-4 for imidacloprid. At these levels of treatments, hemorrhagic necrosis was

observed in the peripheral and interlobular interstitium of the seminiferous tubules in the testis of fish exposed to carbaryl and chlorpyrifos. While presence of adipose tissues were observed in the testis of fishes exposed to imidacloprid. Pyknosis and vacuolation occurred in the lobules of treated testes. As the treatment doses disorganization of testes structure also appeared to become severe. Extensive disintegration of interstitium and disorganization of lobules and cysts occurred at 0.1336 mg/L level of treatment of imidacloprid. Hemorrhagic necrosis was observed not only in lobules but at the spermatic duct as well.

Najafi et al., (2010) evaluated the effect of chronic exposure to imidacloprid insecticide on male testicular tissue, sperm morphology and testosterone levels in the serum of mature male rats. Animals were divided into test and control-sham groups. The test group was subdivided into two groups of rats which were administered doses of 225 and 112 mg/kg imidacloprid per group. Each test group received the designated oral dose of imidacloprid once daily, for 60 days while the control-sham group received corn oil (0.2 ml/day) for the same time period. Clinical observations demonstrated decreased movement, occasional trembling, diarrhea and spasms in the test groups. No clinical signs were seen in control-sham rats. Light microscopic analyses revealed increased thickness of tunica albuginea, obvious edema in the sub-capsular and interstitial connective tissue, atrophied seminiferous tubules, arrested spermatogenesis, negative tubular differentiation and repopulation indexes, decreased Leydig cells/mm2 of interstitial tissue, hypertrophy and cytoplasmic granulation of the Leydig cells, vasodilation and thrombosis, elevated death, as well as immature and decreased immotile sperm velocity.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental animals

Healthy adult male domestic rabbits weighing 1000±200 gm were used in the present study. Animals were left for one week before experimentation to adapt to laboratory conditions. They 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 imidacloprid LD_{50}

A total number of 80 rabbits were used for determination of LD_{50} of imidacloprid. Animals were divided into ten groups (8 rabbits/group). The first nine groups (I - IX) were administered different single doses of imidacloprid ranging from 100 to 300 mg/kg body weight as follows:

LD_{50} determination groups	**Dose (mg/kg body weight)**
Group I	100
Group II	125
Group III	150
Group IV	175
Group V	200
Group VI	225
Group VII	250
Group VIII	275
Group IX	300
Group X control group	-

The tenth group was served as control group. Imidacloprid was given orally using a special stomach tube with a smooth tip to protect the interior lining of the oral and buccal cavity from injury. The rabbit was held between its two ears so that the oesophageal opening was clearly and unobstructively opened. The gastric tube was filled with the required dose of imidaclopbrid then smoothly inserted until it's adequately enters the upper part of oesophagus where its contents were emptied. 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 Imidacloprid toxicity experiments

A dose of 1/10 of LD_{50} imidacloprid was given orally to assess imidacloprid toxicity in male domestic rabbit. Animals were divided into two groups: control and experimental groups. Control group comprised 64 rabbits (eight rabbits were housed in each cage) and experimental group included 48 rabbits (six rabbits were housed in each cage). Experimental groups were orally administrated imidacloprid for overall experimental duration of eight weeks. Control animals were given distilled water. Administration of imidacloprid was also done by the special stomach tube. Imidacloprid was purchased from the Palestinian Ministry of Agriculture**.**

3.4 Physiological studies

3.4.1 Blood sampling and processing

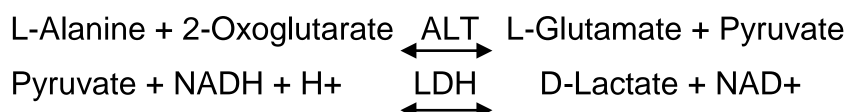
Animals from both experimental and control groups were decapitated weekly. Blood was then collected in centrifuge tubes. The collected blood was allowed to clot and then centrifuged at 3000 r.p.m. for 15 minute. Clear serum samples were separated in glass tubes, labeled and stored in a deep freezer until biochemical analysis. However determination of Alanine Aminotransferase, Aspartate aminotransferase, Alkaline Phosphatase, cholinesterase, urea, and creatinine were carried out on fresh serum.

3.4.2 Determination of enzymes activity

3.4.2.1 Determination of alanine aminotransferase

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

Principle



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

Pyruvate + NADH + H+ $\xrightleftharpoons{LDH}$ 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	85mmol/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 1minute. and start stop watch. Read absorbance again1, 2, and 3 min thereafter at 340 nm.

Calculation

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

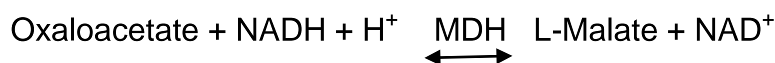
ΔA /min X factor (1745) = ALT activity [U/l]

3.4.2.2 Determination of aspartate aminotransferase

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

Principle

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


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

Reagents

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

Monoreagent preparation

Four parts of R1 were mixed with1 part of R2

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

Procedure

	Sample
Monoreagent	1000 µl
Sample	100 µl

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

Calculation

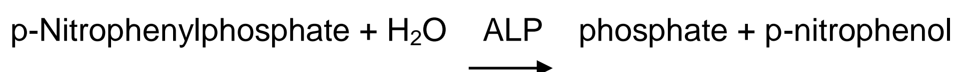
From absorbance reading calculates ΔA /min was calculated and multiply by the corresponding factor:

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

3.4.2.3 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, 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 mol/l
Magnisium acetate	2 mmol /l
Zinc sulphate	0.5 mmol/l
HEDTA	2.5mmol/l

Reagent 2	
p-Nitrophenylphosphate	80 mmol/l

Monoreagent preparation

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

Procedure

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

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

Calculation

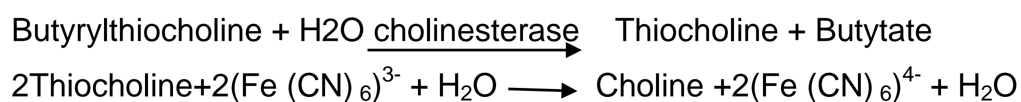
From absorbance reading calculates ΔA /min and multiplies by the corresponding factor:
ΔA /min X factor (2757) = ALP activity [U/l]

3.4.2.4 Determination of cholinesterase

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

Principle

Cholinesterase hydrolyses butyrylthiocholine under release of butyric acid and thiocholine. Thiocholine reduces yellow potassium hexacyanoferrate (III) to colorless potassium hexacyanoferrate (II). The decrease of absorbance is measured at 405 nm.



Reagents

Components	Concentration
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.

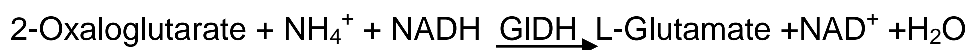
3.4.3 Determination of non- protein nitrogen constituents

3.4.3.1 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 + $2H_2O$ $\xrightarrow{\text{Urease}}$ $2NH_4^+$ + 2HCO3-



2-Oxaloglutarate + NH_4^+ + 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)

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

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

3.4.3.2 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 the.

Principle

Creatinine form 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

Reagents

Component	Concentration
Reagent 1 **Sodium hydroxide**	 0.16 mol/l
Reagent 2	

Picric acid	4.0 mmol/l
Standard	2 mg /dl (177 µmol /l)

Monoreagent preparation

Four parts of R1 were mixed with 1 part of R2

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

Procedure

	Blank	Std./Cal.	Sample
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

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

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

3.4.4 Protein profile

3.4.4.1 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 form a violet blue color complex in alkaline solution. The absorbance of color is directly proportional to concentration.

Reagents

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

Monoreagent preparation

Four parts of R1 were mixed with1 part of R2

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

Procedure

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

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

Calculation

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

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

3.4.4.2 Determination of albumin

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

Principle

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

Reagents

Components	**Concentrations**
Reagent Citrate buffer pH 4.2 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:

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

3.4.4.3 Determination of globulin

Globulin was calculated according the following formula:

Globulin = Total protein - Albumin

3.4.5 Hormone

3.4.5.1 Determination of serum testosterone

Determination of Serum testosterone activity was measured by competitive immunoenzymatic colorimetric method, according to the method of (Tiez, 1986) using ELISA kite for testosterone.

Principle

The testosterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with an antibody directed towards a unique antigenic site on the testosterone molecule. Endogenous testosterone of a patient sample competes with a testosterone horseradish peoxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is reverse proportional to the concentration of testosterone in the sample. After addition of the substrate

solution, the intensity of colour developed is reverse proportional to the concentration of testosterone in the patient sample.

Reagents

1. Microtiter wells, 12x8 (break apart) strips, 96 wells coated with mouse monoclonal anti-testosterone antibody
2. Standard (Standard 0-6), 7 vials, 1 ml, ready to use concentrations: 0 - 0.2 - 0.5 – 1 – 2 – 6 - 16 ng/ml Conversion: 1 ng/ml = 3.467 nmol/l
3. Enzyme conjugate, 1 vial, 25 ml, ready to use testosterone conjugated to horseradish peroxidase
4. Substrate solution, 1 vial, 25 ml,
5. Stop solution, 1 vial, 14 ml, ready to use contains 0.5M H_2SO_4 avoid contact with the stop solution. It may cause skin irritations and burns.
6. Wash solution, 1 vial, 30 ml (40X concentrated)

   Note: Additional standard 0 for sample dilution is available on request.

Procedure

All samples and reagents were allowed to reach at room temperature (~25°C). Reagents mixed by gentle inversion before use. Standards, controls and samples assayed in duplicate.

1. Microtitration strip was marked to be used.
2. Twenty-five µL of the standards, controls and samples were added into each appropriate well.
3. Two hundred µL of conjugate reagent were added into each well using a precision pipette.
4. The wells were mixed for 10 seconds.
5. The wells were incubated for 60 minute at room temperature (~25°C).
6. Each well was aspirated and washed 3 times by added 400 µL of working wash solution.
7. Two hundred µL of substrate solution were added into each well using a precision pipette and gently mixed for 10 seconds.
8. The wells were incubated in the dark for 15 minute at room temperature (~25°C).

9. One hundred μL of stop solution were added into each well using a precision pipette and mixed for 10-20 seconds.
10. The absorbances of the solution in each well were read at 450 nm.

Calculation of results

The absorbance for each standard, control, or samples were obtained, and then the standerd curve prepared by plotted the absorbance readings for each of the standards along the Y-axis versus standerd concentrations in ng/mL along the X-axis, the mean absorbance values for each sample was determined the corresponding concentration of testosterone in ng/mL from the stander curve (Figure 3.1).

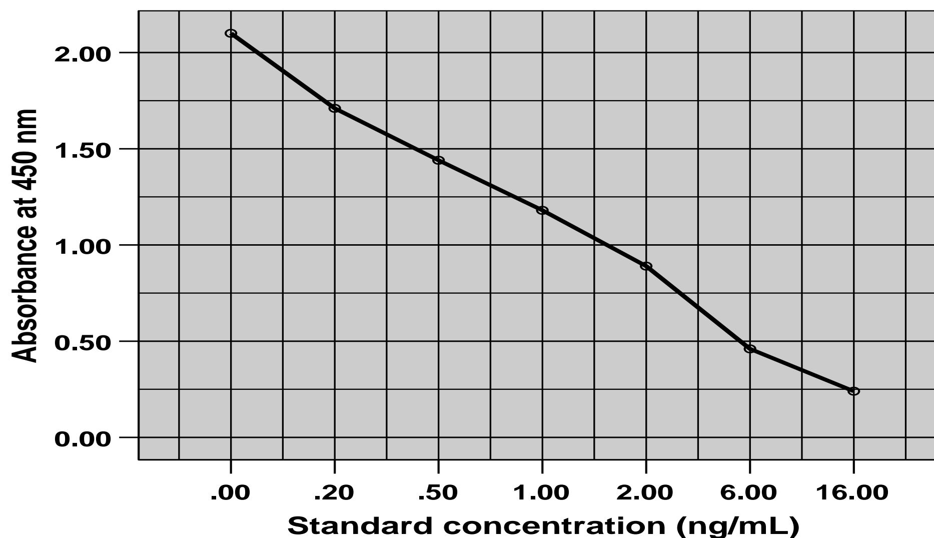


Figure 3.1 Standard curve for testosterone hormone

3.5 Histological Studies

For histological examination, the following steps were carried out weekly from the beginning until the end of experimental duration of eight weeks.

3.5.1 Collection of samples

Following decapitation, liver, kidney and testis were dissected freed from the surrounding connective tissues and organs, then excised. They were immediately immersed in saline solution (0.9% NaCl) for blood removal.

3.5.2 Fixation

The following fixative reagent used for the routine heamatoxylin and eosin stain was 10% buffered formalin (Lillie, 1954).

3.5.3 Processing

Following fixation, dehydration of fixed tissues was done through ascending grades of ethyl alcohol (70%, 80%, 95%, and absolute alcohol). Tissues were then cleared with xylene. This was followed by impregnation with paraffin wax. Having been completely impregnated, the tissues were embedded in paraffin wax, sectioned by a rotatory microtome at a thickness of 2μm, mounted and affixed to slides.

3.5.4 Staining methods

Sections were stained as a routine in Harris's alum heamatoxylin and eosin (Harris, 1900; Allen, 1992)

3.6 Statistical Analysis

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

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

Percentage difference was calculated according to the following formula:

$$\% \text{ difference} = \frac{\text{Mean of treated} - \text{Mean of control}}{\text{Mean of control}} \times 100$$

Graphs

Logarithmic scale of oral LD_{50} of imidacloprid was plotted using Microsoft Excel program 2003.

CHAPTER 4

RESULTS

4.1 Clinical symptoms and morphological notes

Rabbits treated with imidacloprid showed some clinical symptoms including diarrhea, disorientation and drowsiness. In addition administration of imidacloprid causes central nervous system activation mimics the nicotine actions, including tremors and cramps and sometimes death. None of these symptoms were observed in control animals. Concerning morphological changes, imidacloprid-treated rabbits showed hair loss especially in the seventh and eighth weeks of the experiment (Figure 4.1) whereas control animals did not display such change. In addition, the livers of dissected imidacloprid-treated rabbits showed scars of depression (Figure 4.2) whereas those of the control animals showed normal appearance.

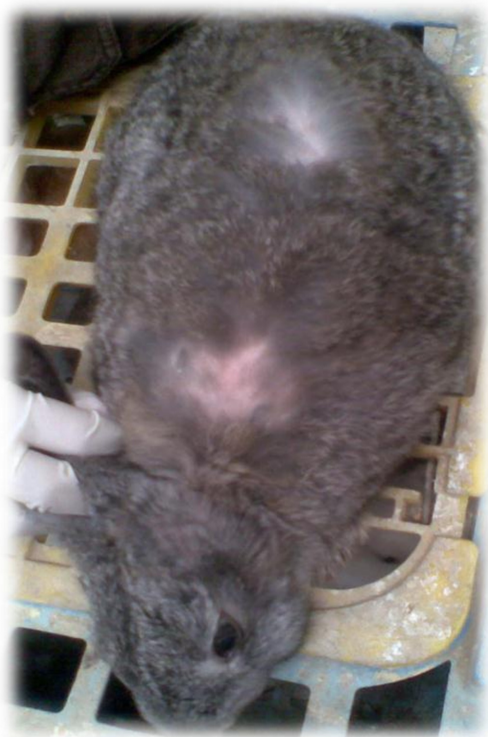


Figure 4.1

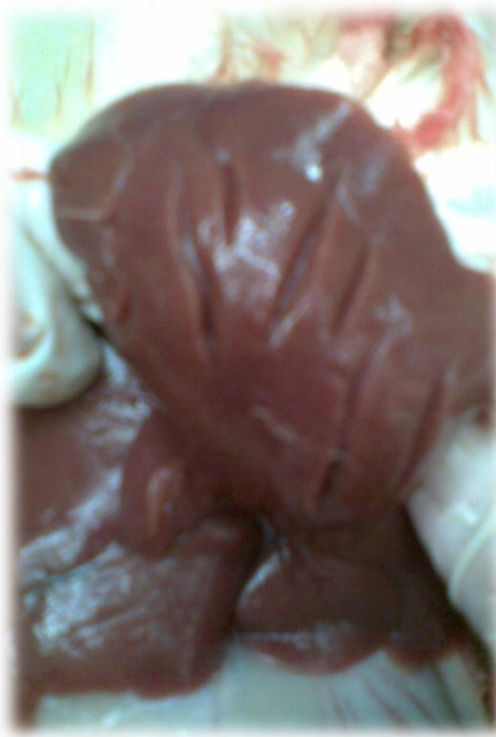


Figure 4.2

4.2 Mortality rate

As indicated in the present study there was ten dead experimental rabbits during the eight weeks experimental duration from the total number of 60 rabbits used in the experiment. Four rabbits died at the first week, two rabbits at the second week, three rabbits at the third week and one rabbit was died at the sixth week. However 48 imidacloprid treated rabbits were used throughout the experimental period of 8 weeks. There was no dead animal in the control group all over the experimental periods studied.

4.3 Oral LD_{50} of imidacloprid

The experimental trials for oral LD_{50} determination of imidacloprid after 48hr of administration in male domestic rabbits revealed that the mortality commenced at 125 mg/kg body weight, recording mortality percentage of 25.0% (Table 4.1). Increasing imidacloprid dose to 150, 175, 200, 225, 250 and 275 resulted in mortality percentages of 50.0, 62.5, 62.5, 62.5, 75.0 and 75.0%, respectively. The mortality rate was a function of dose increase. The maximum concentration of imidacloprid which kill all animals in the group was found to be 300 mg/kg body weight. The calculated oral LD_{50} of imidacloprid in male domestic rabbits from the linear regression was found to be 172 mg/kg body weight (Figure 4.3)

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

Group	Imidacloprid dose(mg/kg body weight)	Number of Animals died/total	%mortality
Group I	100	0/8	0
Group II	125	2/8	25
Group III	150	4/8	50
Group IV	175	5/8	62.5
Group V	200	5/8	62.5
Group VI	225	5/8	62.5
Group VII	250	6/8	75
Group VIII	275	6/8	75
Group IX	300	8/8	100
Group X	Control	0/8	0

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

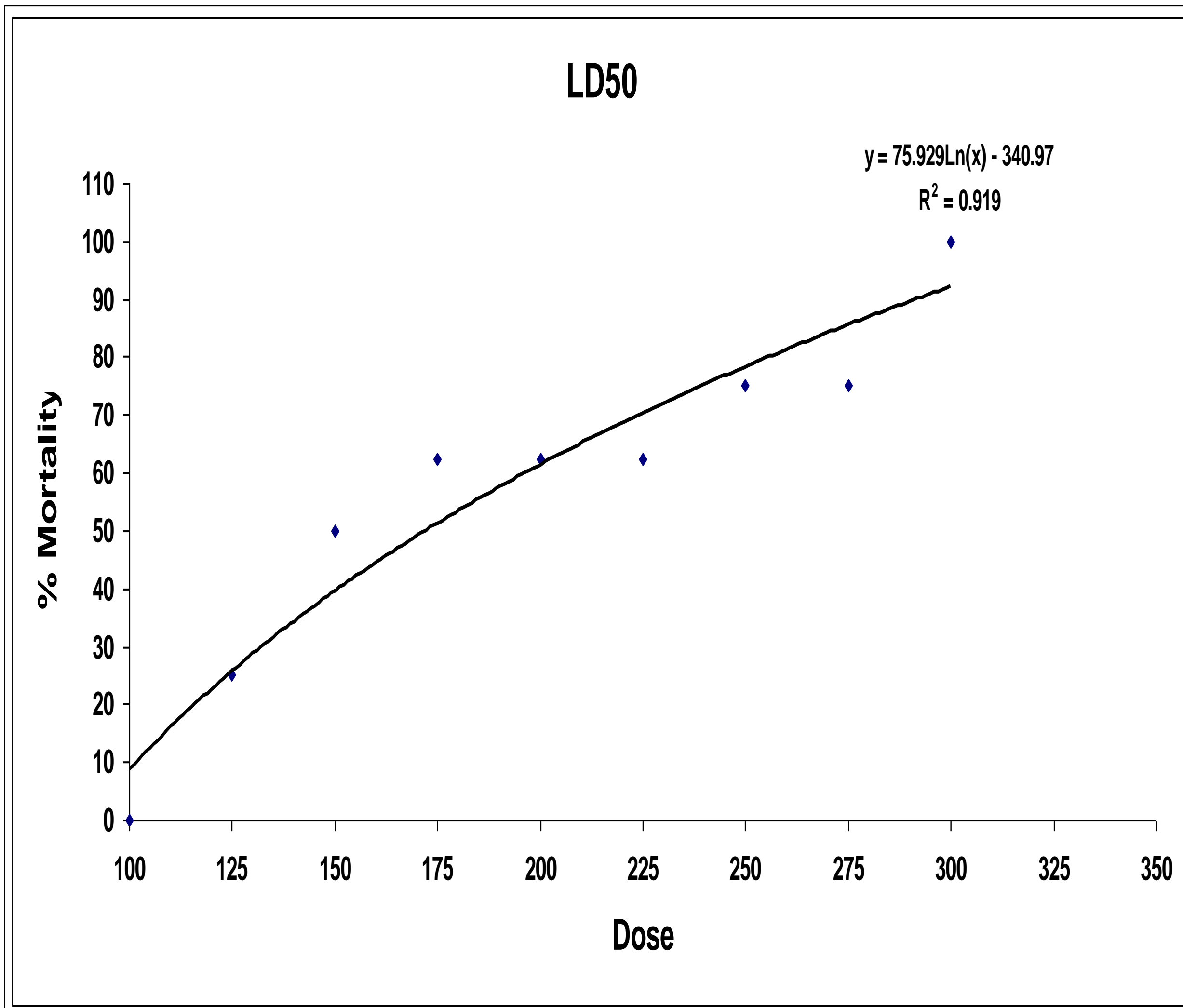


Figure 4.3 Logarithmic scale of oral LD_{50} of imidacloprid in male domestic rabbits (LD_{50}=172 mg/kg body weight)

4.4 Physiological and biochemical parameters

4.4.1 Liver enzymes

4.4.1.1 Alanine aminotransferase

Table 4.2 showed serum alanine aminotransferase (ALT) activity in control and imidacloprid-treated male domestic rabbits for 8 weeks. The normal enzyme activity was 50.3±3.7, 52.0±3.0, 47.6±1.7, 48.4±2.8, 49.8±3.0, 49.3±2.6, 51.7±2.9 and 51.2±1.9 U/L during the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th weeks of the experiment, respectively. Upon imidacloprid administration, ALT activity increased throughout the experimental periods with values of 54.5±3.8, 70.8±4.1, 57.5±2.9, 61.8±3.6, 65.3±3.9, 58.8±3.0, 63.4±3.5 and 58.7±2.7 compared to control levels. The maximum percentage increases of 36.2 and 31.1 in the 2nd and 5th weeks of the experiment, respectively (P=0.017and 0.020, respectively).

Table 4.2 Effect of imidacloprid (1/10 LD_{50}, 17.2 mg/kg body weight) on alanine aminotransferase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Imidacloprid (n=6)	% difference	t-value	P value
1	50.3±3.7	54.5±3.8	8.3	0.722	0.497
2	52.0±3.0	70.8±4.1	36.2	3.495	0.017
3	47.6±1.7	57.5±2.9	20.8	2.654	0.045
4	48.4±2.8	61.8±3.6	27.7	2. 733	0.041
5	49.8±3.0	65.3±3.9	31.1	3.145	0.020
6	49.3±2.6	58.8±3.0	19.3	2.429	0.049
7	51.7±2.9	63.4±3.5	22.6	2.607	0.040
8	51.2±1.9	58.7±2.7	14.6	2.322	0.068

The number of animals (n) was 8 in control group and 6 in imidacloprid-treated animals

All values were expressed as mean±SEM

The level of significance was at P<0.05.

4.4.1.2 Aspartate aminotransferase

The mean values of serum aspartate aminotransferase (AST) activity in control and imidacloprid-treated male domestic rabbits along the experimental period of 8 weeks are presented in Table 4.3. Normal aspartate aminotransferase activities were 33.6±2.1, 33.9±1.8, 35.7±2.1, 34.8±1.5, 36.1±1.7, 36.3±1.8, 35.4±1.3 and 35.8±2.0 U/L at the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th weeks of the experiment, respectively. Oral administration of imidacloprid provoked pronounced increase in the enzyme activity exhibiting values of 37.9±2.2, 37.4±2.1, 49.2±3.0, 43.2±2.7, 43.0±2.1, 40.5±2.3, 39.0±3.5 and 38.9±3.3 U/L. The significant change was only observed at the third, fourth and fifth weeks of the experiment with percentage increases of 37.8, 24.1 and 19.1%, respectively (P=0.012, 0.031 and 0.047).

Table 4.3 Effect of imidacloprid (1/10 LD_{50}, 17.2 mg/kg body weight) on aspartate aminotransferase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Imidacloprid (n=6)	% difference	t-value	P value
1	33.6±2.1	37.9±2.2	12.8	1.437	0.194
2	33.9±1.8	37.4±2.1	10.3	1.238	0.256
3	35.7±2.1	49.2±3.0	37.8	3.830	0.012
4	34.8±1.5	43.2±2.7	24.1	2. 985	0.031
5	36.1±1.7	43.0±2.1	19.1	2.496	0.047
6	36.3±1.8	40.5±2.3	11.8	1.432	0.202
7	35.4±1.3	39.0±3.5	10.2	0.970	0.369
8	35.8±2.0	38.9±3.3	8.7	0.770	0.470

The number of animals (n) was 8 in control group and 6 in imidacloprid-treated animals

All values were expressed as mean±SEM

The level of significance was at P<0.05.

4.4.1.3 Alkaline phosphatase

Table 4.4 illustrates serum alkaline phosphatase (ALP) activity in control and imidacloprid-given male domestic rabbits along the experimental period of 8 weeks. Alkaline phosphatase activity registered for control animals were 101.8±5.4, 103.3±3.9, 105.2±4.3, 104.6±5.2, 105.0±5.5, 107.8±5.7, 109.3±5.1 and 108.0±5.2 U/L at 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th weeks of the experiment, respectively. Oral administration of imidacloprid caused increase in ALP activity in the first four weeks with percentage 5.4, 25.4, 12.2 and 20.6% (P=0.470, 0.012, 0.194, 0.032, respectively). At the 5th, 6th, 7th and 8th weeks of the experiment serum ALP concentrations were decreased with percentage of 11.1, 22.7, 15.4 and 18.1%, respectively. The significant change was observed at 6th and 8th weeks (P=0.018, 0.041, respectively).

Table 4.4. Effect of imidacloprid (1/10 LD_{50}, 17.2 mg/kg body weight) on alkaline phosphatase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Imidacloprid (n=6)	% difference	t-value	P value
1	101.8±5.4	107.3±4.7	5.4	0.770	0.470
2	103.3±3.9	129.5±6.3	25.4	3.530	0.012
3	105.2±4.3	118.0±7.0	12.2	1.560	0.194
4	104.6±5.2	126.1±4.9	20.6	2. 946	0.032
5	105.0±5.5	93.3±6.1	-11.1	1.423	0.205
6	107.8±5.7	83.3±5.5	-22.7	3.246	0.018
7	109.3±5.1	92.5±5.0	-15.4	2.373	0.055
8	108.0±5.2	88.4±4.4	-18.1	2.737	0.041

The number of animals (n) was 8 in control group and 6 in imidacloprid-treated animals

All values were expressed as mean±SEM

The level of significance was at P<0.05.

4.4.1.4 Cholinesterase

The normal values of serum cholinesterase (ChE) in male domestic rabbits throughout the experimental period of 8 weeks are illustrated in Table 4.5. These values were 4532±114, 4491±148, 4605±185, 4624±168, 4843±183, 4794±170, 4916±162 and 4876±155 U/L during the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th weeks of the experiment, respectively. Daily oral administration of imidacloprid progressively increased serum ChE levels to 4737±142, 5170±195, 5686±201 and 6167±235 U/L at the first four weeks. This increase was significant at 2nd, 3rd, 4th weeks (P=0.037, 0.008 and 0.002 respectively). However serum ChE levels decreased gradually to 4049±218, 4255±211, 4335±228 and 3831±233 U/L. This decrease was significant at fifth and eighth weeks (P=0.032 and 0.011, respectively).

Table 4.5 Effect of imidacloprid (1/10 LD_{50}, 17.2 mg/kg body weight) on cholinesterase activity (U/L) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Imidacloprid (n=6)	% differences	t-value	P value
1	4532±114	4737±142	4.5	1.129	0.302
2	4491±148	5170±195	15.1	2.828	0.037
3	4605±185	5686±201	23.5	3.954	0.008
4	4624±168	6167±235	33.4	5. 313	0.002
5	4843±183	4049±218	-16.4	2.781	0.032
6	4794±170	4255±211	-11.2	1.987	0.094
7	4916±162	4335±228	-11.8	2.077	0.083
8	4876±155	3831±233	-21.4	3.897	0.011

The number of animals (n) was 8 in control group and 6 in imidacloprid-treated animals

All values were expressed as mean±SEM

The level of significance was at P<0.05.

4.4.2 Non-protein nitrogen constituents

4.4.2.1 Serum urea

The mean values of serum urea concentration in control and imidacloprid-treated male domestic rabbits are presented in Table 4.6. Urea concentration in control animals exhibited values of 37.7±2.9, 38.3±3.2, 37.0±2.5, 37.3±2.4, 35.3±1.9, 36.4±3.0, 36.2±2.4 and 35.9±2.1 mg/dl at 1st, 2nd, 3rd, 4th, 5th, 7th and 8th weeks of the experiment, respectively. Oral administration of imidacloprid increased serum urea concentration to 41.8±2.7, 43.4±2.0, 38.7±3.2, 41.0±2.9, 43.5±2.5, 48.2±1.9, 45.1±2.8 and 43.0±2.0 mg/dl with percentage increases of 10.9, 13.3, 4.6, 9.9, 23.2, 32.4, 24.6 and 19.8% during the weekly intervals examined compared to controls. Such increase showed significant change during the 5th, 6th and 7th weeks with P value of P=0.041, 0.011 and 0.048, respectively.

Table 4.6 Effect of imidacloprid (1/10 LD50, 17.2 mg/kg body weight) on urea concentration (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Imidacloprid (n=6)	% difference	t-value	P value
1	37.7±2.9	41.8±2.7	10.9	0.977	0.366
2	38.3±3.2	43.4±2.0	13.3	1.447	0.198
3	37.0±2.5	38.7±3.2	4.6	0.411	0.702
4	37.3±2.4	41.0±2.9	9.9	0.976	0.384
5	35.3±1.9	43.5±2.5	23.2	2.590	0.041
6	36.4±3.0	48.2±1.9	32.4	3.642	0.011
7	36.2±2.4	45.1±2.8	24.6	2.484	0.048
8	35.9±2.1	43.0±2.0	19.8	2.423	0.060

The number of animals (n) was 8 in control group and 6 in imidacloprid-treated animals

All values were expressed as mean±SEM

The level of significance was at P<0.05.

4.4.2.2 Serum creatinine

The average values of serum creatinine concentrations in control group of male domestic rabbits as well as in animals treated with imidacloprid along the experimental period of 8 weeks are illustrated in Table 4.7. Normal values recorded for creatinine concentrations were 0.67±0.03, 0.70±0.04, 0.81±0.03, 0.85±0.06, 0.78±0.05, 0.77±0.04, 0.89±0.08 and 0.87±0.04 mg/dl during the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th weeks of the experiment, respectively. Upon imidacloprid administration, serum creatinine concentrations fluctuates across the experimental period reaching its maximum increase during the 7th week of the experiment with percentage of 34.8% (P=0.025).

Table 4.7 Effect of imidacloprid (1/10 LD_{50}, 17.2 mg/kg body weight) on creatinine concentration (mg/dl) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Imidacloprid (n=6)	% difference	t-value	P value
1	0.67±0.03	0.69±0.02	3.0	0.318	0.760
2	0.70±0.04	0.74±0.03	5.7	0.755	0.479
3	0.81±0.03	0.90±0.04	11.1	1.706	0.139
4	0.85±0.06	1.12±0.09	31.8	2. 537	0.044
5	0.78±0.05	0.72±0.02	-7.7	1.203	0.274
6	0.77±0.04	0.65±0.03	-15.6	2.030	0.098
7	0.89±0.08	1.20±0.06	34.8	2.971	0.025
8	0.87±0.04	1.05±0.07	20.7	2.633	0.046

The number of animals (n) was 8 in control group and 6 in imidacloprid-treated animals

All values were expressed as mean±SEM

The level of significance was at P<0.05.

4.4.3 Protein profile

4.4.3.1 Serum total protein

The normal values of serum total protein levels in male domestic rabbits throughout the experimental period of 8 weeks are illustrated in Table 4.8. These values were 5.5±0.22, 5.7±0.20, 6.2±0.33, 5.8±0.24, 5.6±0.26, 6.0±0.31, 6.1±0.30 and 6.0±0.29 g/dL during the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th weeks of the experiment, respectively. Daily administration of Imidacloprid decreased markedly total protein levels to 5.1±0.32, 4.9±0.24, 4.5±0.29, 5.2±0.21, 4.7±0.21, 4.5±0.20, 4.8±0.32 and 5.2±0.25 g/dL showing percentage decreases of 7.3, 14.0, 27.4, 10.3, 16.1, 25.0, 21.3 and 13.3% at the weekly intervals of the experiment compared to controls. This decrease was significant during 2nd, 3rd, 5th, 6th and 7th of the experiment (P=0.045, 0.009, 0.041, 0.010, 0.022).

Table 4.8 Effect of imidacloprid (1/10 LD_{50}, 17.2 mg/kg body weight) on total protein (g/dL) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Imidacloprid (n=6)	% difference	t-value	P value
1	5.5±0.22	5.1±0.32	-7.3	0.974	0.368
2	5.7±0.20	4.9±0.24	-14.0	2.295	0.045
3	6.2±0.33	4.5±0.29	-27.4	3.298	0.009
4	5.8±0.24	5.2±0.21	-10.3	1. 705	0.122
5	5.6±0.26	4.7±0.21	-16.1	2.384	0.041
6	6.0±0.31	4.5±0.20	-25.0	3.278	0.010
7	6.1±0.30	4.8±0.32	-21.3	3.055	0.022
8	6.0±0.29	5.2±0.25	-13.3	1.913	0.114

The number of animals (n) was 8 in control group and 6 in imidacloprid-treated animals

All values were expressed as mean±SEM

The level of significance was at P<0.05.

4.4.3.2 Serum albumin

The mean values of serum albumin concentration in control and imidacloprid-treated male domestic rabbits are presented in Table 4.9. Albumin concentration in control animals exhibited values of 3.7±0.07, 3.8±0.18, 4.1±0.18, 3.8±0.21, 3.6±0.10, 3.9±0.14, 4.1±0.19 and 3.9±0.18 g/dL at 1st, 2nd, 3rd, 4th, 5th, `6th, 7th and 8th weeks of the experiment, respectively. Oral administration of imidacloprid lowered serum albumin concentration to 3.4±0.15, 3.1±0.16, 3.2±0.17, 3.5±0.19, 3.2±0.14, 3.1±0.18, 3.3±0.18 and 3.4±0.21 g/dL with percentage decreases of 8.1, 18.4, 22.0, 7.9, 11.1, 20.5, 19.5 and 12.8% during the weekly intervals examined compared to controls. Such decrease varied throughout the experimental periods showing significant change during the 2nd, 3rd, 6th and 7th weeks of the experiment (P=0.039, 0.010, 0.021 and 0.020, respectively).

Table 4.9 Effect of imidacloprid (1/10 LD_{50}, 17.2 mg/kg body weight) on albumin (g/dL) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Imidacloprid (n=6)	% difference	t-value	P value
1	3.7±0.07	3.4±0.15	-8.1	1.614	0.150
2	3.8±0.18	3.1±0.16	-18.4	2.533	0.039
3	4.1±0.18	3.2±0.17	-22.0	3.740	0.010
4	3.8±0.21	3.5±0.19	-7.9	1. 052	0.333
5	3.6±0.10	3.2±0.14	-11.1	1.832	0.117
6	3.9±0.14	3.1±0.18	-20.5	3.097	0.021
7	4.1±0.19	3.3±0.18	-19.5	3.142	0.020
8	3.9±0.18	3.4±0.21	-12.8	1.910	0.114

The number of animals (n) was 8 in control group and 6 in imidacloprid-treated animals

All values were expressed as mean±SEM

The level of significance was at P<0.05.

4.4.3.3 Serum globulin

The normal values of serum globulin levels in male domestic rabbits throughout the experimental period of 8 weeks are illustrated in Table 4.10. These values were 1.85±0.06, 1.95±0.08, 2.03±0.11, 2.00±0.09, 1.90±0.09, 2.10±0.12, 1.98±0.10 and 2.10±0.08 g/dL during the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th weeks of the experiment, respectively. Daily oral administration of imidacloprid decreased markedly serum globulin levels to 1.74±0.10, 1.70±0.07, 1.40±0.07, 1.73±0.06, 1.50±0.07, 1.48±0.09, 1.50±0.07 and 1.80±0.06 g/dL showing percentage decrease of 5.9, 12.8, 31.0, 13.5, 21.1, 29.5, 24.2 and 14.3% at the weekly intervals of the experiment compared to controls. This decrease was significant all over the experimental periods expect for 1st and 2nd weeks.

Table 4.10 Effect of imidacloprid (1/10 LD_{50}, 17.2 mg/kg body weight) on globulin (g/dL) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Imidacloprid (n=6)	% difference	t-value	P value
1	1.85±0.06	1.74±0.10	-5.9	0.882	0.407
2	1.95±0.08	1.70±0.07	-12.8	2.357	0.051
3	2.03±0.11	1.40±0.07	-31.0	4.753	0.003
4	2.00±0.09	1.73±0.06	-13.5	2. 524	0.045
5	1.90±0.09	1.50±0.07	-21.1	3.464	0.013
6	2.10±0.12	1.48±0.09	-29.5	4.302	0.005
7	1.98±0.10	1.50±0.07	-24.2	3.750	0.010
8	2.10±0.08	1.80±0.06	-14.3	2.737	0.041

The number of animals (n) was 8 in control group and 6 in imidacloprid-treated animals

All values were expressed as mean±SEM

The level of significance was at $P<0.05$.

4.4.4 Hormone

4.4.4.1 Serum testosterone

Table 4.11 demonstrates serum testosterone levels in control and imidacloprid treated male domestic rabbits along the experimental period of 8 weeks. The mean value of testosterone in controls were 3.3±0.13, 3.1±0.17, 3.2±0.18, 3.3±0.15, 3.4±0.18, 3.1±0.15, 3.5±0.17 and 3.4±0.15 ng/ml during the 1st, 2nd, 3rd, 4th, 5th, 6th, 7thand 8th weeks, respectively. Oral administration of imidacloprid daily for 8 weeks decreased testosterone levels all over the experimental periods, recording values of 2.9±0.15, 2.6±0.11, 2.1±0.12, 2.6±0.18, 2.9±0.16, 2.5±0.17, 2.4±0.15 and 2.8±0.12 with percentage decreases of 12.1, 16.1, 34.3, 21.2, 14.7, 19.4, 31.4 and 17.6% compared to control levels. This decrease was significant except for the 1st week (P=0.119)

Table 4.11 Effect of imidacloprid (1/10 LD50, 17.2 mg/kg body weight) on testosterone (ng/ml) in male domestic rabbits.

Experimental period (Week)	Control (n=8)	Imidacloprid (n=6)	% difference	t-value	P value
1	3.3±0.13	2.9±0.15	-12.1	1.878	0.119
2	3.1±0.17	2.6±0.11	-16.1	2.469	0.043
3	3.2±0.18	2.1±0.12	-34.3	5.068	0.002
4	3.3±0.15	2.6±0.18	-21.2	3. 043	0.023
5	3.4±0.18	2.9±0.16	-14.7	2.456	0.049
6	3.1±0.15	2.5±0.17	-19.4	2.691	0.036
7	3.5±0.17	2.4±0.15	-31.4	4.839	0.003
8	3.4±0.15	2.8±0.12	-17.6	2.818	0.037

The number of animals (n) was 8 in control group and 6 in imidacloprid-treated animals

All values were expressed as mean±SEM

The level of significance was at P<0.05.

4.5 Histological investigation

Liver of control groups

Livers of animals from the control group showed an almost uniform gross appearance throughout experimentation. Increase in liver size was detected with the increase of age. Thin connective tissue septa enter the liver dividing it into lobes and lobules. The hepatic lobule is a polygonal prism with a central vein from which radiate branching anastomosing cord or strands of hepatocytes (Figure 4.4). The central veins, from which the hepatic strands are radiating, are larger than the sinusoids. Blood sinusoids are found in between the hepatic cords. Moreover fine bile canaliculi are encountered between the hepatic cells. The liver parenchyma consists of anastomosing sheets of hepatocytes, intralobular and interlobular vessels arranged irregularly throughout hepatic lobules. The hepatic cells are cuboidal or polygonal in shape, with rounded or oval vesicular nucleus and a prominent nucleolus. Some liver cells are binucleated. The cytoplasm is homogeneous, and finely granulated. The hepatic sinusoids are limited by a discontinuous layer of flattened endothelial cells, intervened by certain large phagocytic kupffer cells with a large oval nucleus. There are triangular portal areas in between each group of hepatic lobules. The portal vein is the largest and widest one in this triad. The lumen might appear either empty or containing a few blood cells. The hepatic artery branch has a relatively narrow lumen. The hepatic bile ductule has a distinct wall of low cuboidal or rounded outline epithelial cells surrounded by a thin sheath of connective tissue. Besides, the portal triads contain a few mononuclear cells, mostly lymphocytes dispersed in its scanty connective tissue.

Liver of treated groups

Sections from the liver were prepared from both control and treated rabbits and were examined under the light microscope. The liver sections from treated rabbits showed several changes when compared with those from the control rabbits (Figure 4.4). These changes include congestion in the central vein throughout the first, second, third, fourth and fifth weeks and congestion

in sinusoids during the third week of the experiment. Rabbits treated with imidacloprid displayed degeneration in the hepatocytes clearly in the fifth, sixth, seventh and eighth weeks during the experimental periods. Diffuse kupffer cells between the degenerative hepatocytes showed at forth, sixth and eighth weeks in the imidacloprid-treated rabbits. On the other hand the inflammatory cells infiltration and fibroblastic cells proliferation observed in sixth week. Whereas, some figures showed damage to the trabecular structure of the lobules and the cytoplasm of hepatocytes is filled with vacuoles, Karyolysis and complete pyknosis of many cells are noticed in the seventh and eighth weeks. Furthermore large degenerated area comprised with severe destruction in architecture of hepatocytes showed in the eighth week of the experiment.

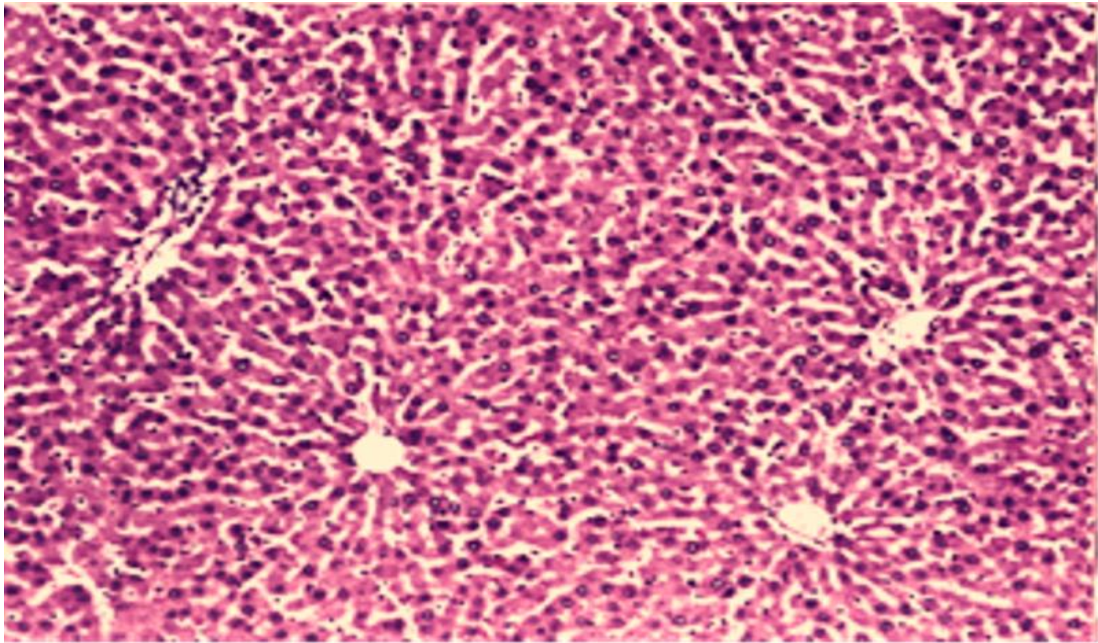


Figure (4.4) Cross section of liver from control rabbit showing normal histological structure of the central vein and surrounding hepatocytes (H&E X160).

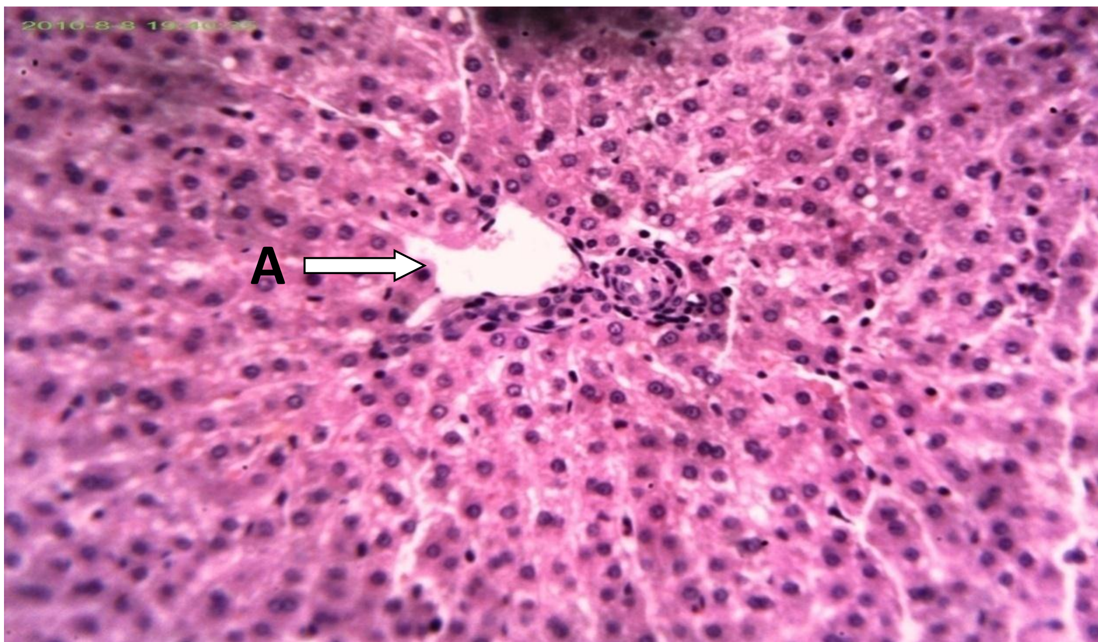


Figure (4.5) Cross section of liver one week post experimentation showing congestion in the central vein (**A**) (H&EX400).

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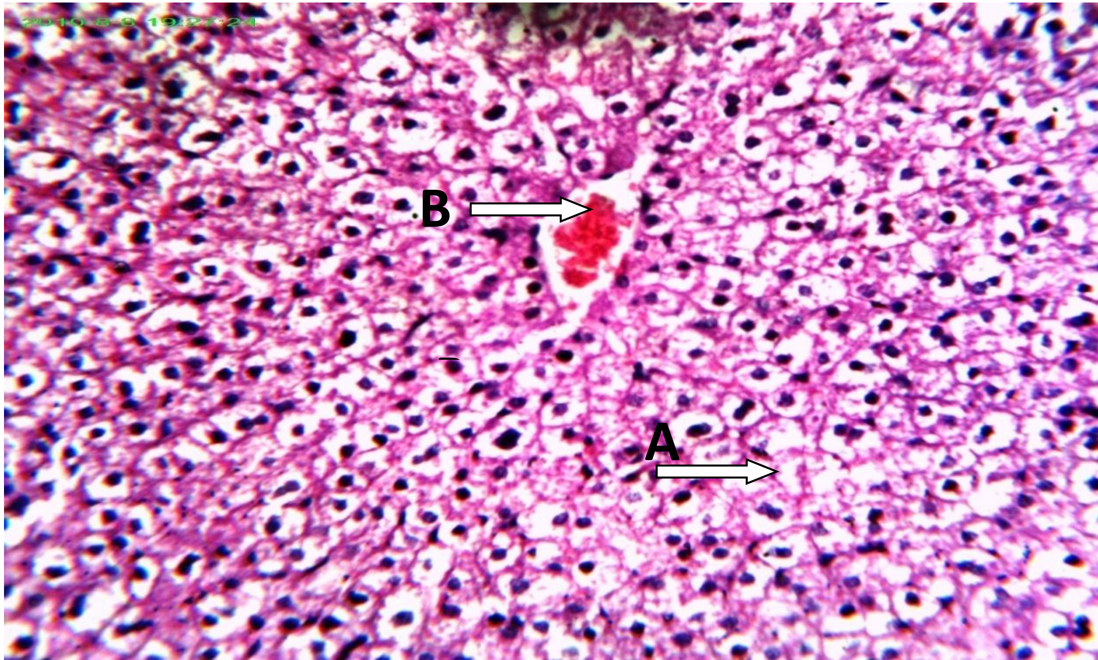

Figure (4.6) Cross section of liver two weeks post experimentation showing degenerative change in the hepatocytes (**A**). Congestion and bleeding in the central vein (**B**) (H&E X400).

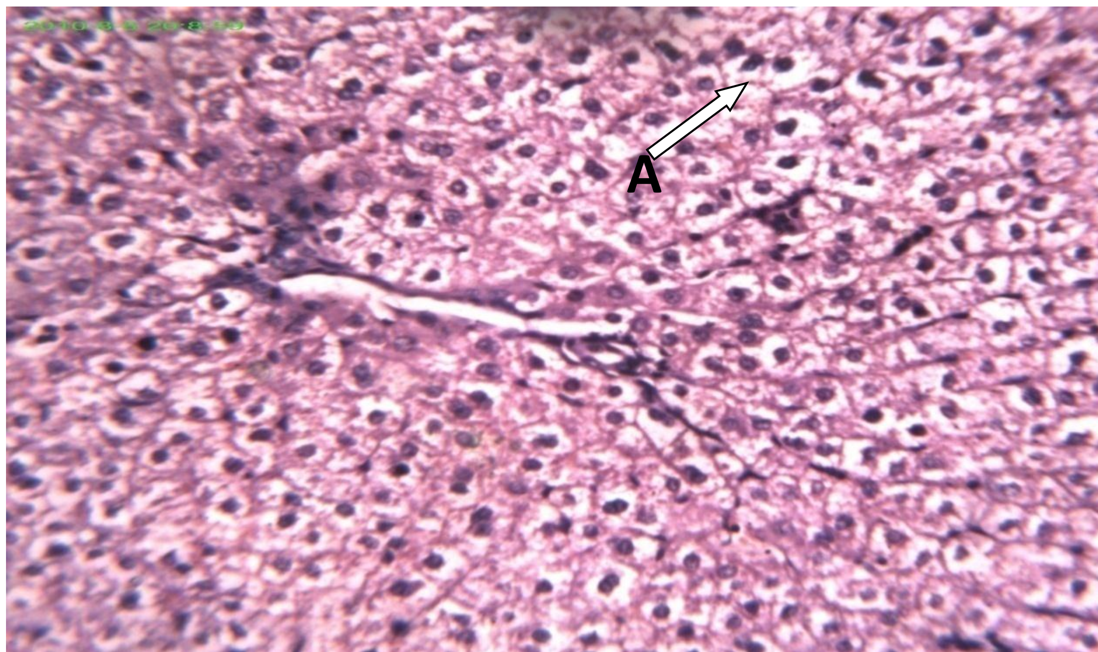

Figure (4.7) Cross section of liver three weeks post experimentation showing congestion in sinusoids, degeneration in the hepatocytes (**A**) (H&E X400).

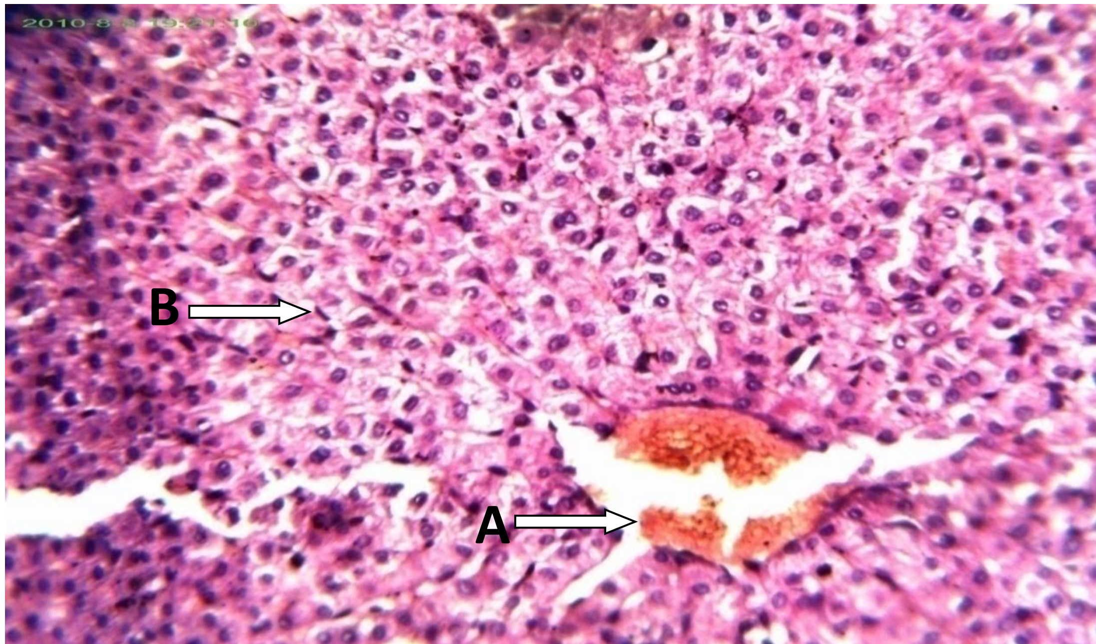

Figure (4.8) Cross section of liver four weeks post experimentation showing congestion and bleeding in the central vein (**A**). Diffuse kupffer cells between the hepatocytes (**B**) (H&E X400).

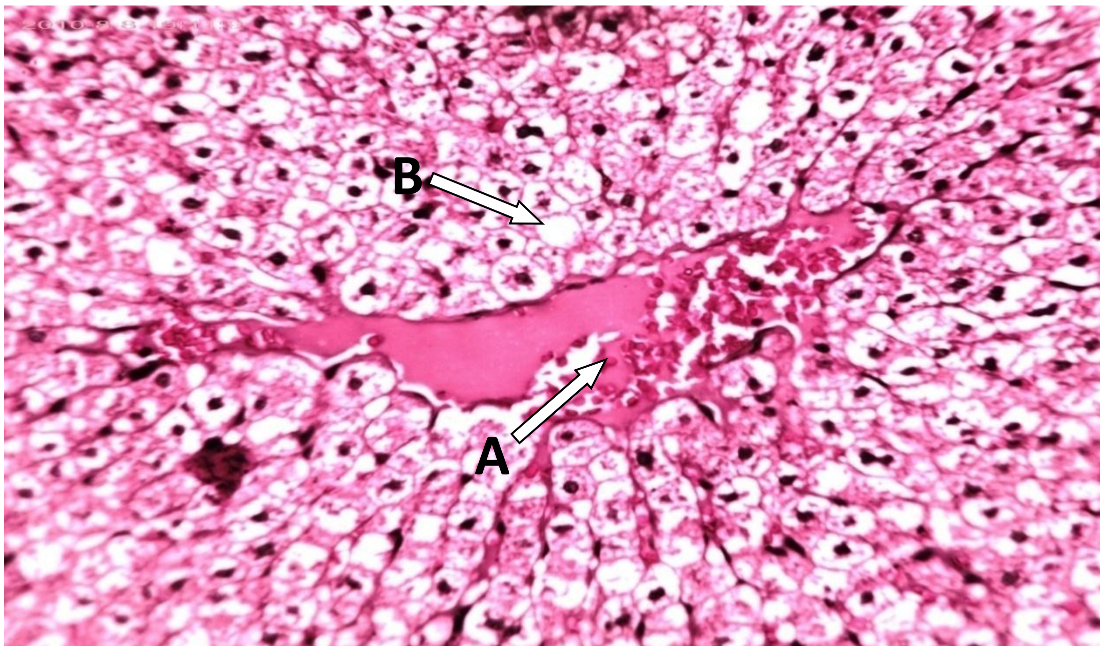

Figure (4.9) Cross section of liver five weeks post experimentation showing congestion and bleeding in the central vein (**A**). Vacuolar degeneration in the hepatocytes surrounding the central vein (**B**) (H&E X400).

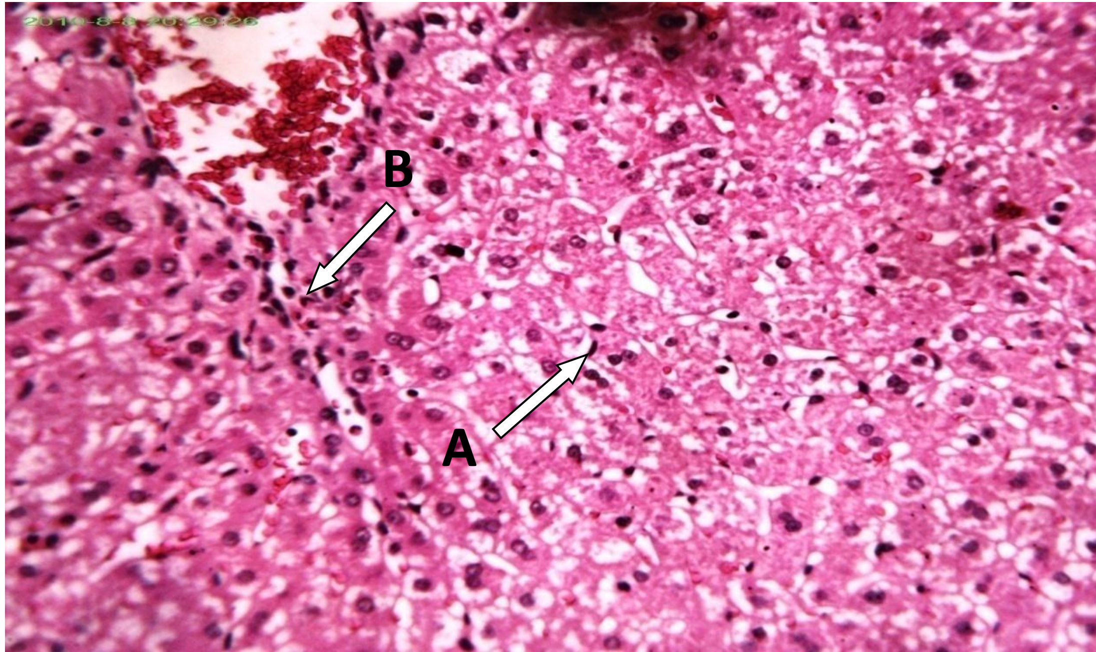

Figure (4.10) Cross section of six weeks post experimentation showing diffuse kupffer cells proliferation in between the degenerated hepatocytes (**A**). The portal area showed congestion in the portal vein, inflammatory cells infiltration (**B**) (H&E X400).

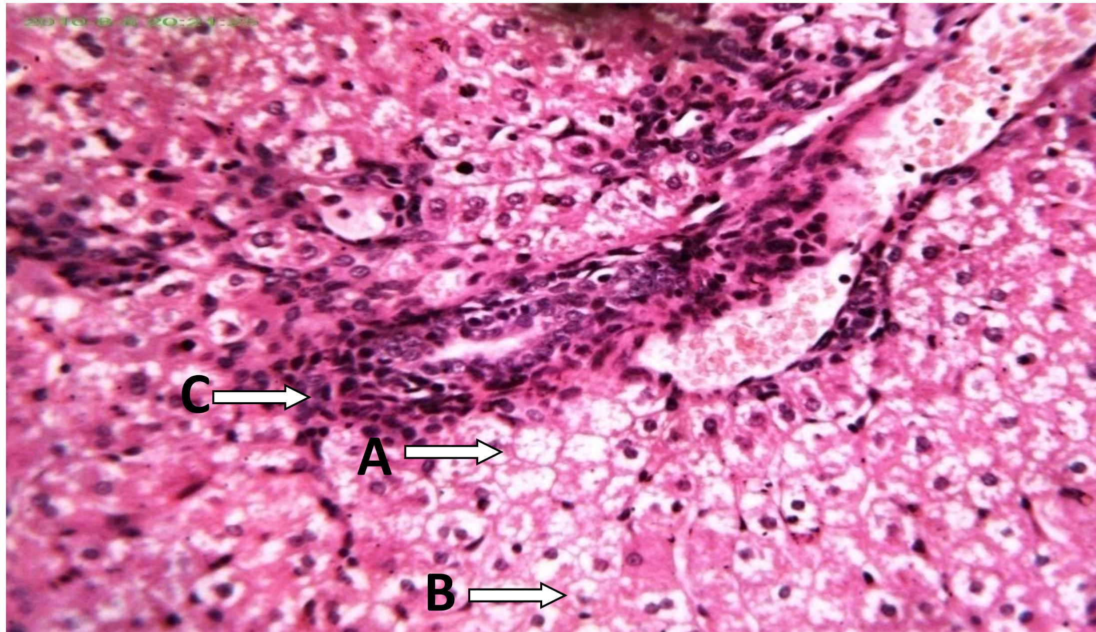

Figure (4.11) Cross section of liver seven weeks post experimentation showing damage to the trabecular structure of the tubules. Vacuolar degeneration in the hepatocytes (**A**). Pyknosis of liver cells are noticed (**B**). Congestion in the portal vein and inflammatory cells infiltration were detected in the portal area (**C**) (H&E X400). .

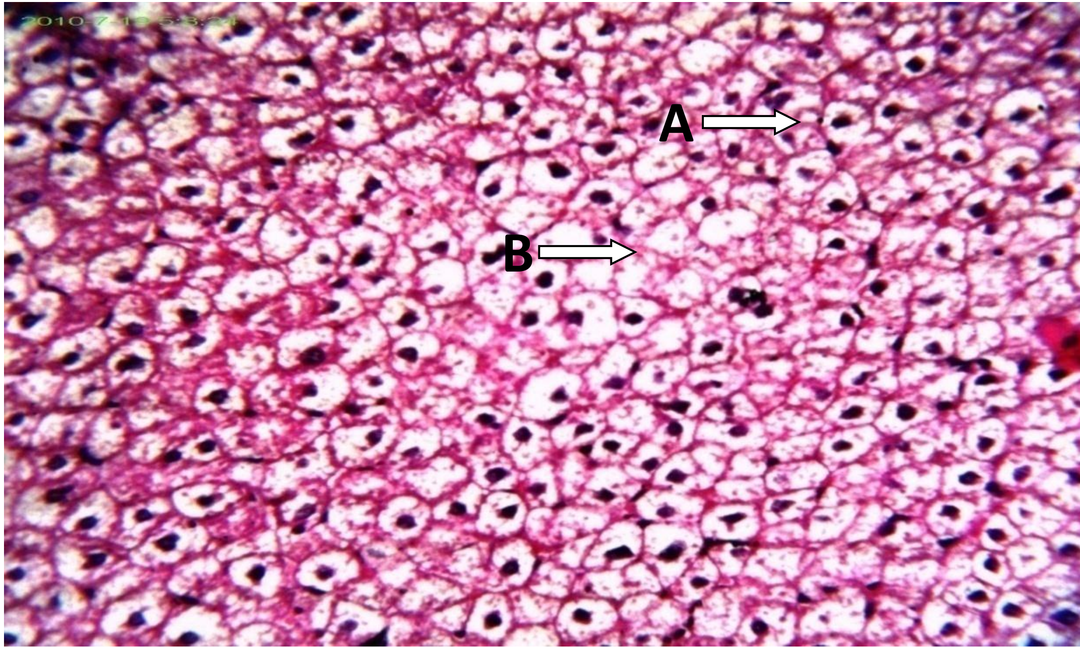

Figure (4.12) Cross section of liver eight weeks post experimentation showing degenerative changes of hepatocytes (**A**). Large area of necrosis (**B**) (H&E X400).

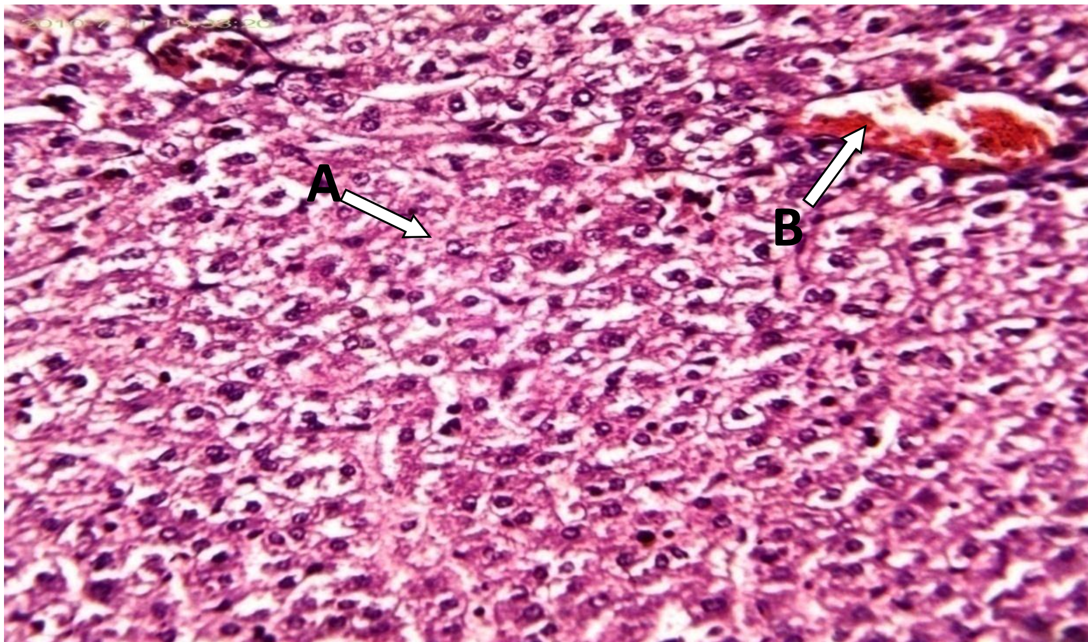

Figure (4.13) Cross section of liver eight weeks post experimentation showing Karyolysis in the nuclei and distortion of the hepatocytes (**A**). Bleeding in the central vein (**B**) (H&E X400).

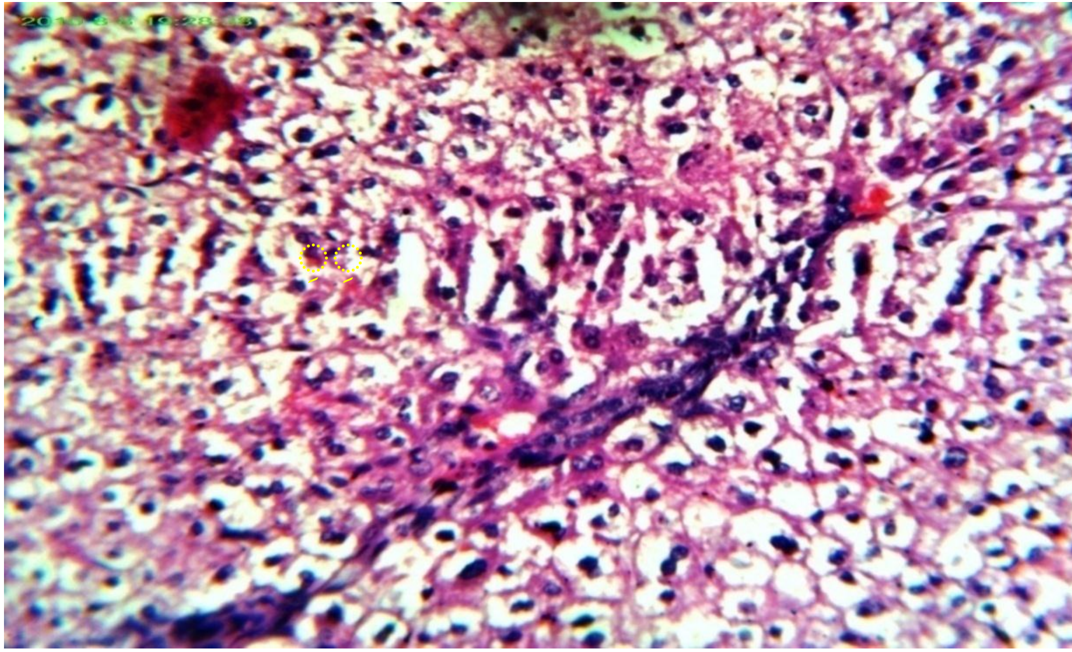

Figure (4.14) Cross section of liver eight weeks post experimentation showing large degenerated area comprised with severe destruction in the architecture of hepatocytes. (H&E X400).

Kidney of control groups

The kidney of control rabbit is a bean- shaped compound – tubular gland consisting of large numbers of uriniferous tubules that constitute the major part of the renal tissue. It is covered by a firm connective tissue-capsule composed of collagenous fibrous and few elastic fibers. The substance of the kidney is differentiated into two regions; and outer cortex and an inner medulla. The latter is formed of conical pyramids, the apices of which are directed loward the renal pelvis. Each medullary pyramid with the corresponding part of the cortex represents a renal lobe. The renal lobes consist of uriniferous tubules and stromal tissue. The latter is formed of a network of connective tissue. The uriniferous tubules are composed of two principal portions; an active part or the nephron, which is the structural and functional unit of kidney, and an excretory part of collecting tubule. The nephron is formed of the renal or Malpighian corpuscle, proximal convoluted tubule, descending and ascending limbs of Henle's loop and distal convoluted tubule. The cortex, in between the medullary rays, consists of Malpighian corpuscles and both proximal and distal convoluted tubules while the medulla consists mainly of the descending and ascending limbs o Henle's loops. However, the collecting tubules are located in both cortical and medullary regions. The Malpighian corpuscle consists of a tuft of blood capillaries, the glomerulus and Bowman's capsule. The latter is a double walled cup formed of two layers of simple squamous epithelium, an outer parietal layer and an inner visceral one separated by a capsular space. The parietal layer is continuous with the wall of the proximal convoluted tubule. It is composed of simple squamous epithelium resting on a thin basement membrane and surrounded by a thin layer of connective tissue. The glomerulus consists of number of capillary loops of an afferent and efferent arteriole separated by reticular connective tissue fibers.

Kidney of treated groups

The kidney sections from imidacloprid-treated rabbits showed many changes when compared with those from the control rabbits (Figure 4.15). Cross section of rabbit's kidney one week post experimentation displayed mild

histological change of the kidney tubules structure. Mild hypertrophy in some individual glomeruli appeared in the second week. Degenerative change in the renal tubule was showed in the third and fourth weeks of the experiment. Dilatation of the inter tubular blood vessels and vascular lumen impacted by haemolysed blood were appeared during the fifth and seventh weeks of the experiment. Rupture of tubules and focal mononuclear leukocyte inflammatory cells infiltrate between tubules observed at the third and sixth weeks in the treated animals. Furthermore, degeneration in the epithelial cells lining the tubules and necrotic of the renal tubule were noticed at the seventh and eighth weeks of the imidacloprid-treated rabbits.

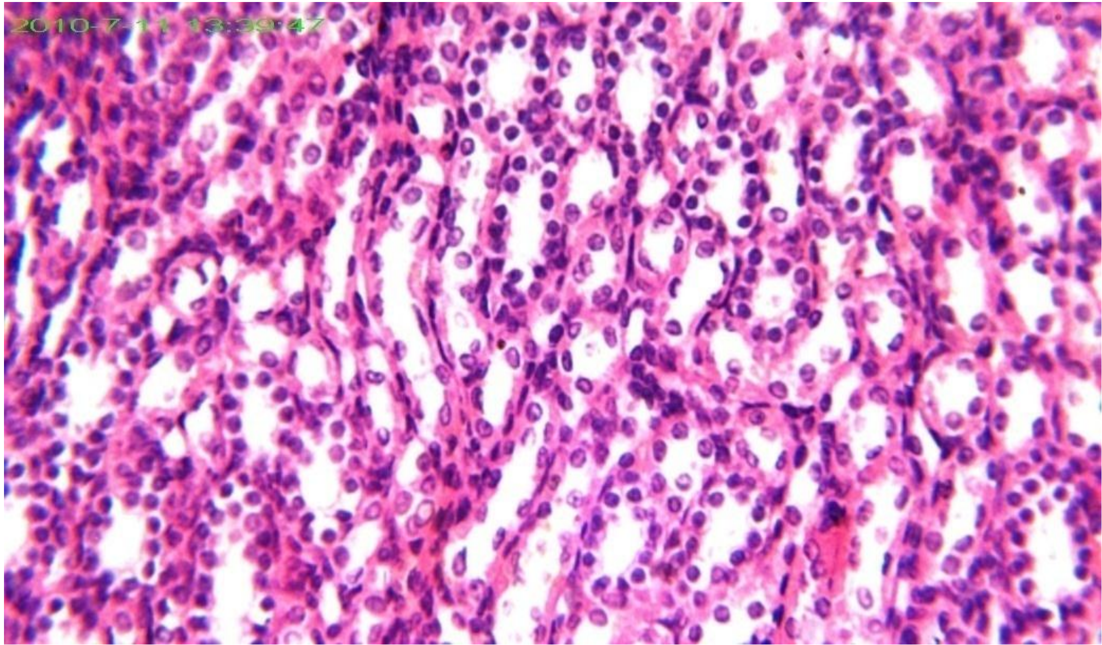

Figure (4.15) Cross section of kidney from control rabbit showing normal histological structure of the renal tubules (H&E X160).

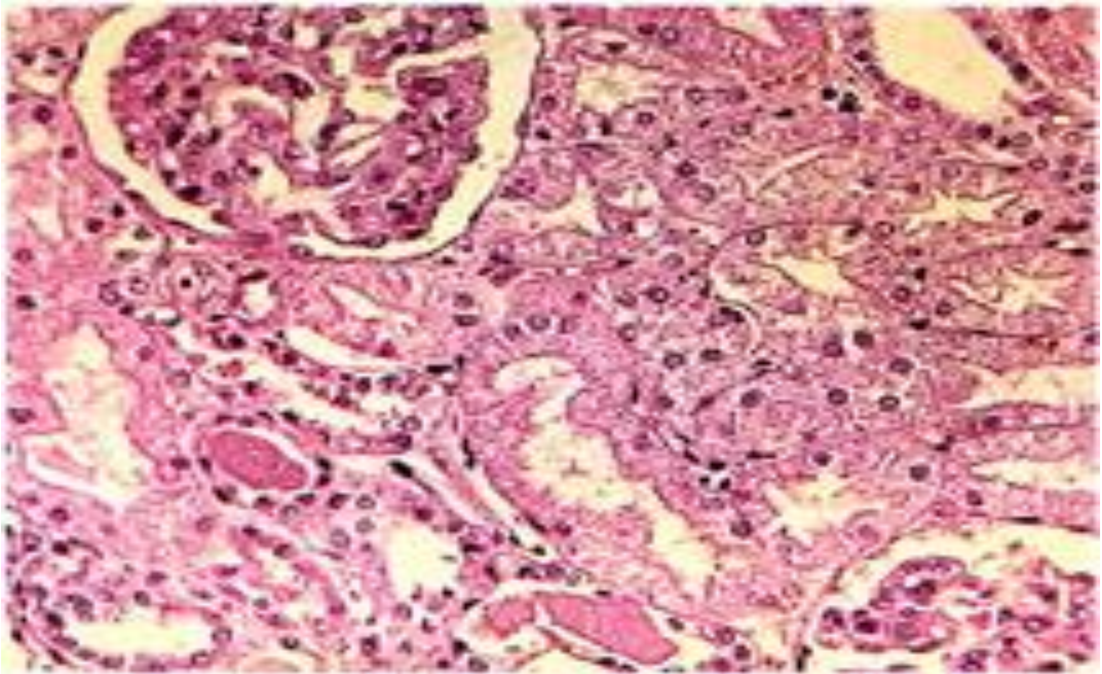

Figure (4.16) Cross section of kidney from control rabbit showing normal histological structure in the cortex (H&E X160).

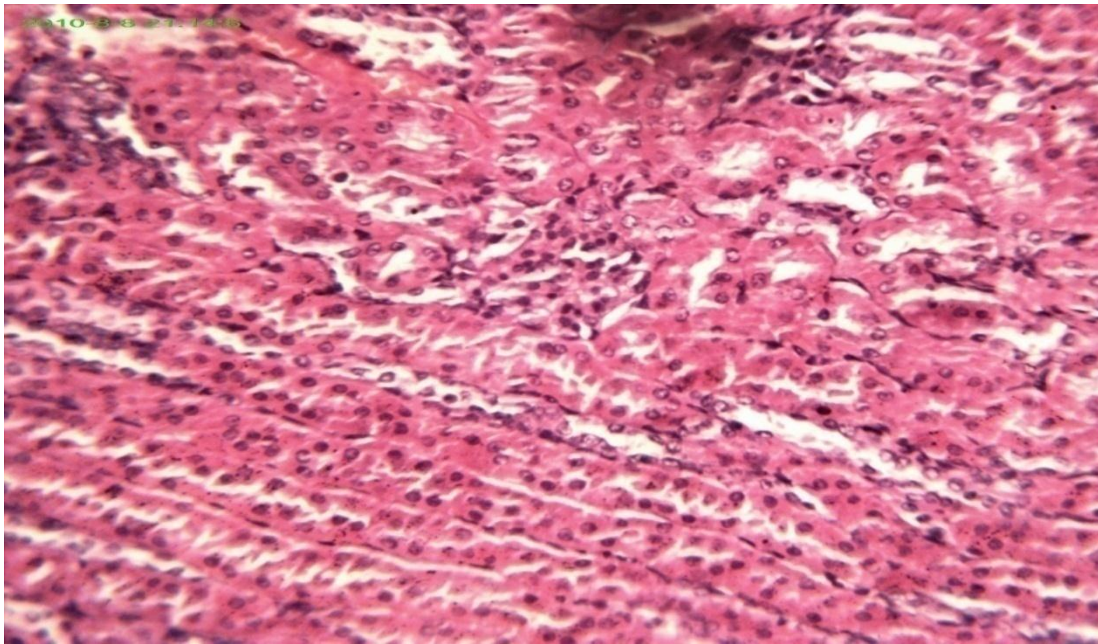

Figure (4.17) Cross section of rabbit kidney one week post experimentation showing mild histological change of the kidney tubules structure. (H&E X160)

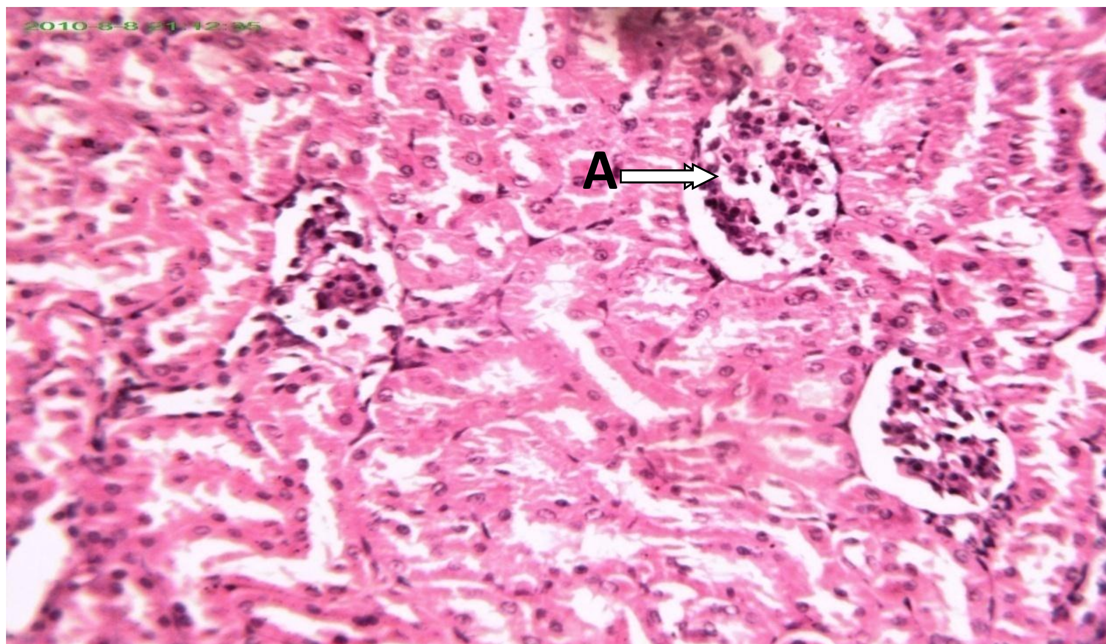

Figure (4.18) Cross section of kidney two weeks post experimentation showing mild hypertrophy in some individual glomeruli (**A**) (H&E X160).

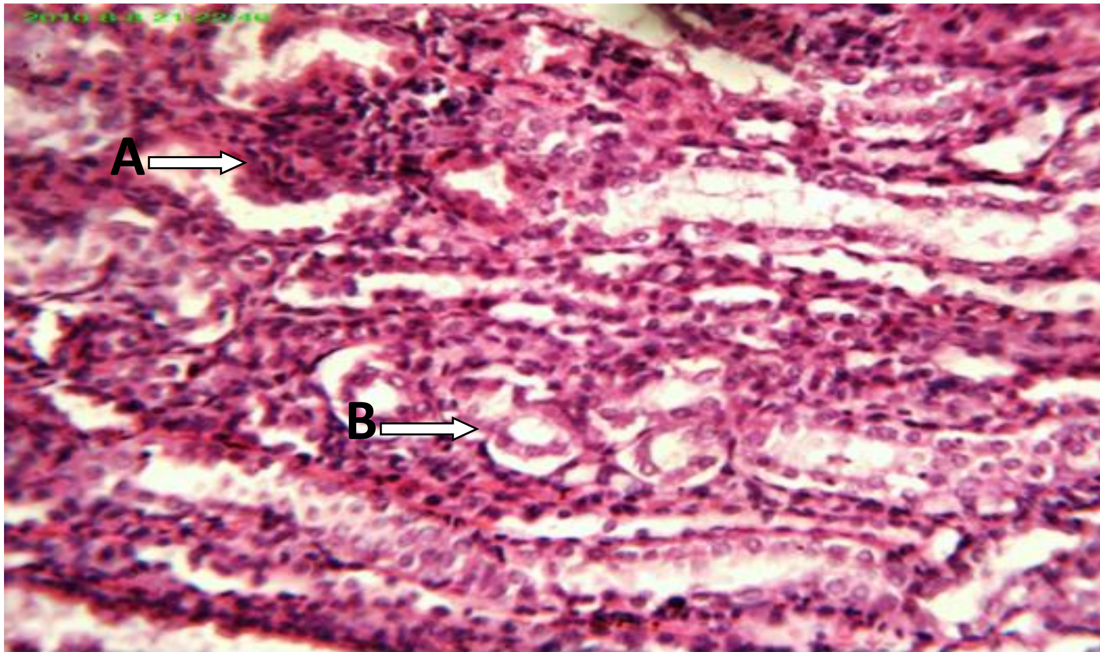

Figure (4.19) Cross section of kidney three weeks post experimentation showing inflammatory cells infiltration (A). Degeneration in the cells lining the tubules (B) (H&E X 160).

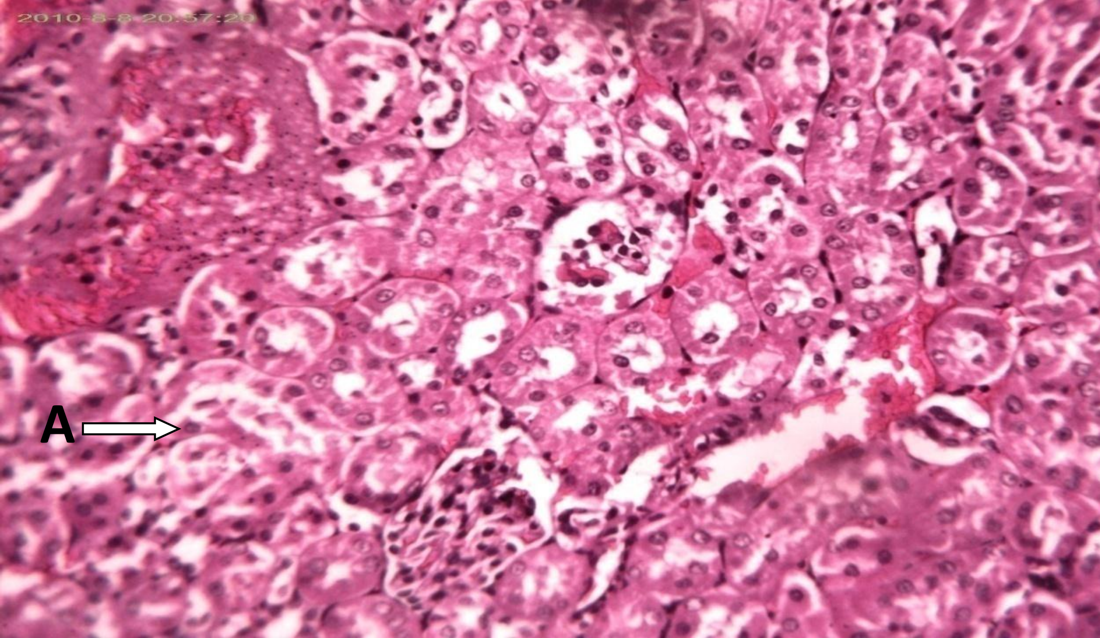

Figure (4.20) Cross section of kidney four weeks post experimentation showing degenerative change in the renal tubule (**A**) (H&E X160).

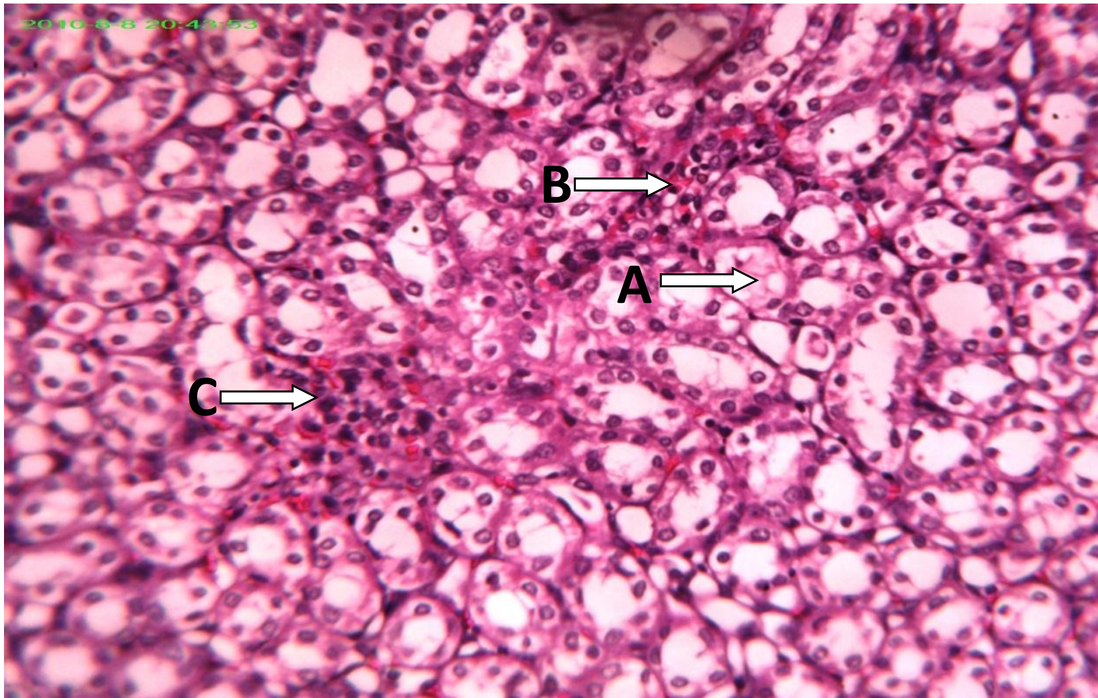

Figure (4.21) Cross section of kidney five weeks post experimentation showing swelling in the lining endothelium (**A**). Focal hemorrhage between the renal tubules (**B**). Inflammatory cells infiltration between the renal tubules (**C**) (H&E X 160).

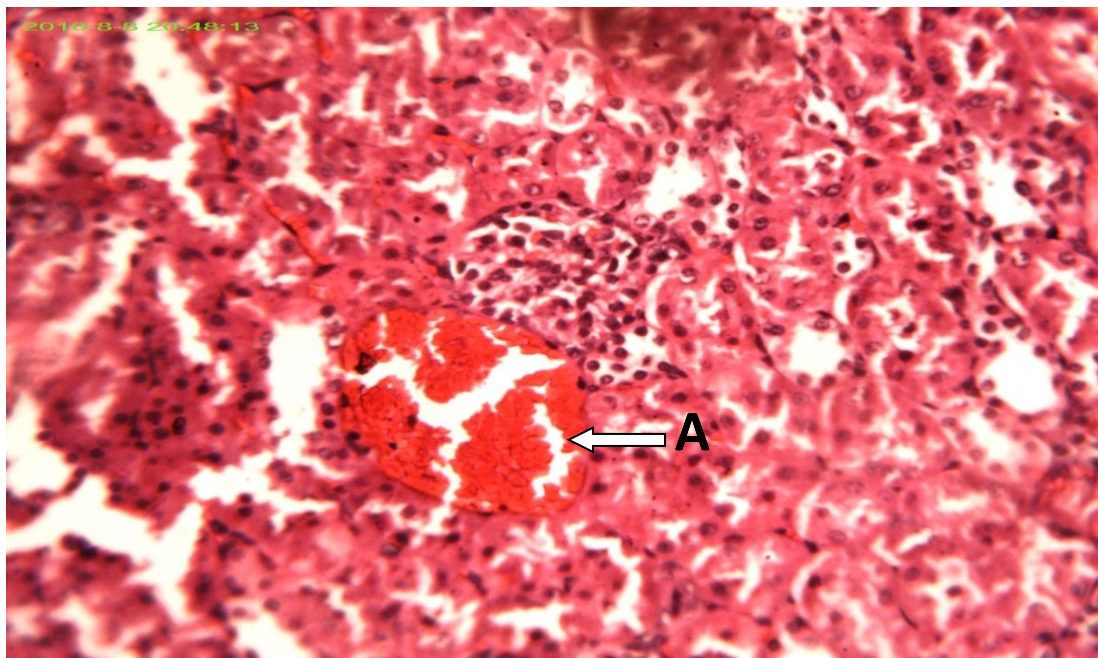

Figure (4.22) Cross section of kidney five weeks post experimentation showing dilatation of the inter tubular blood vessels and vascular lumen impacted by haemolysed blood (**A**) (H&E X400).

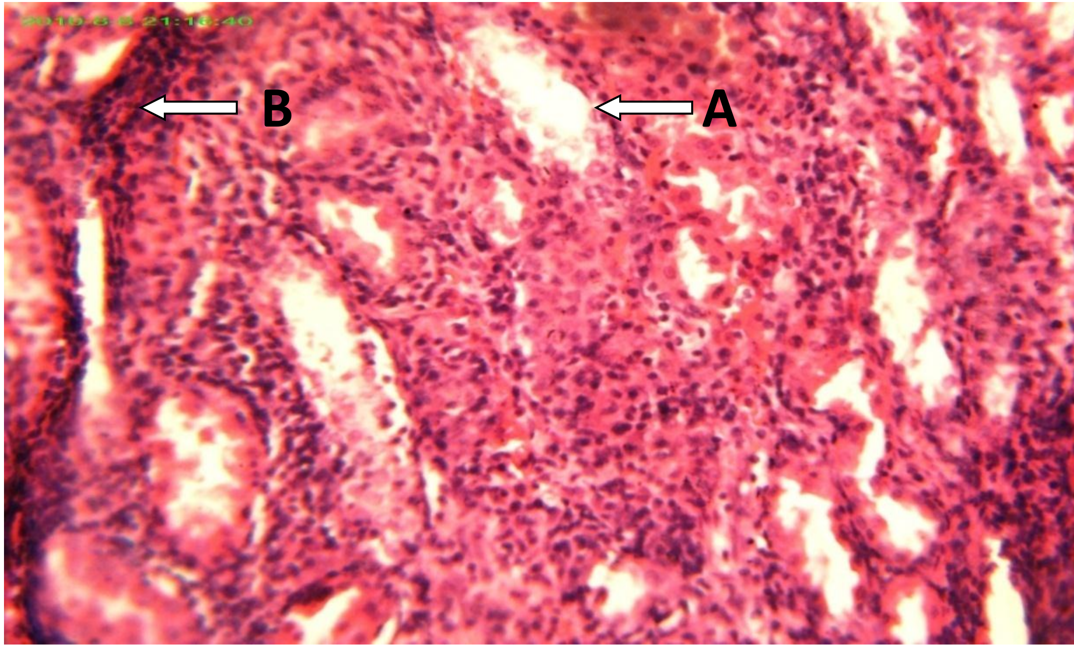

Figure (4.23) Kidney section six weeks after experimentation showing rupture of tubules (**A**). Distinct infiltration of mononuclear cells can be seen in places where tubular epithelium has undergone degenerative changes (**B**) (H&E X400).

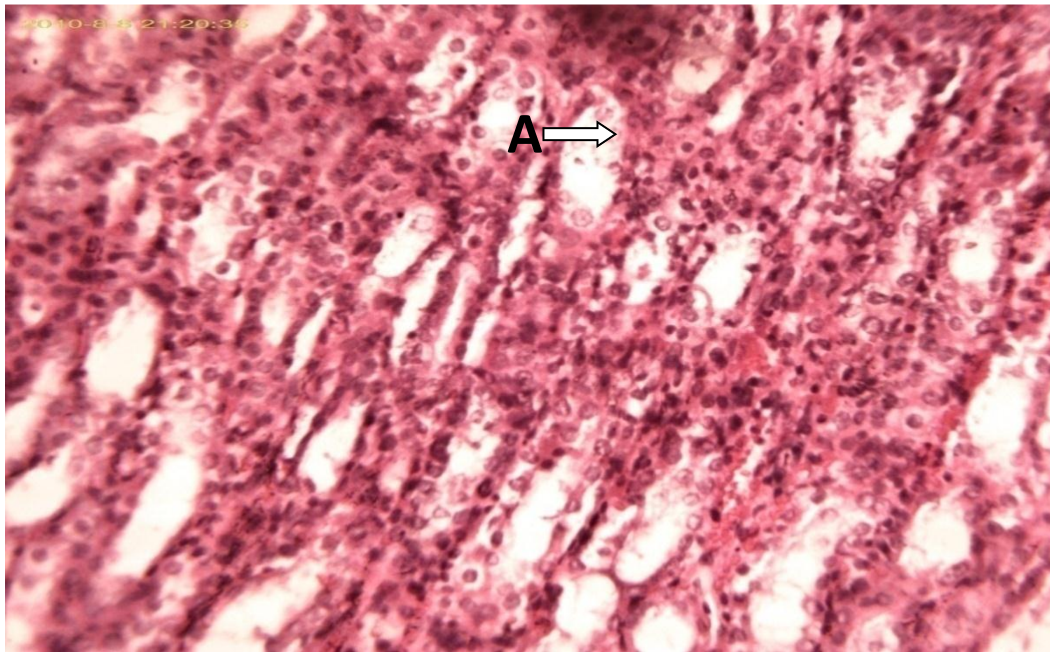

Figure (4.24) Cross section of kidney seven weeks post experimentation showing degeneration and necrosis in the renal tubules (**A**) (H&E X400).

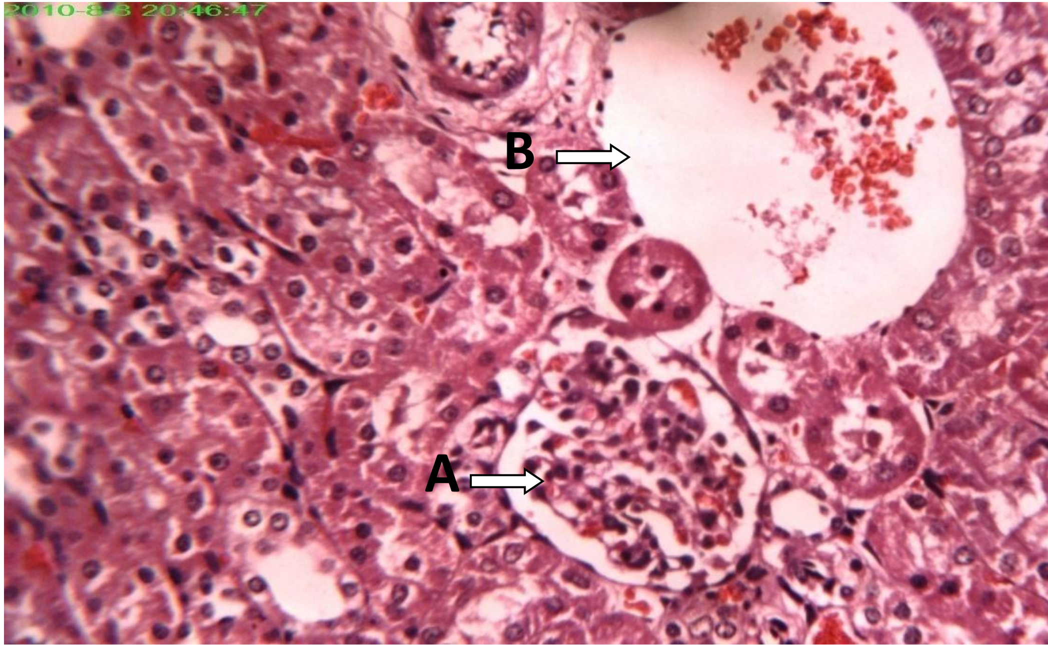

Figure (4.25) Cross section of kidney seven weeks post experimentation showing bleeding in the glomeruli at the cortical portion (**A**). Dilation of blood vessels and vascular lumen impacted by haemolysed blood (**B**) (H&E X400).

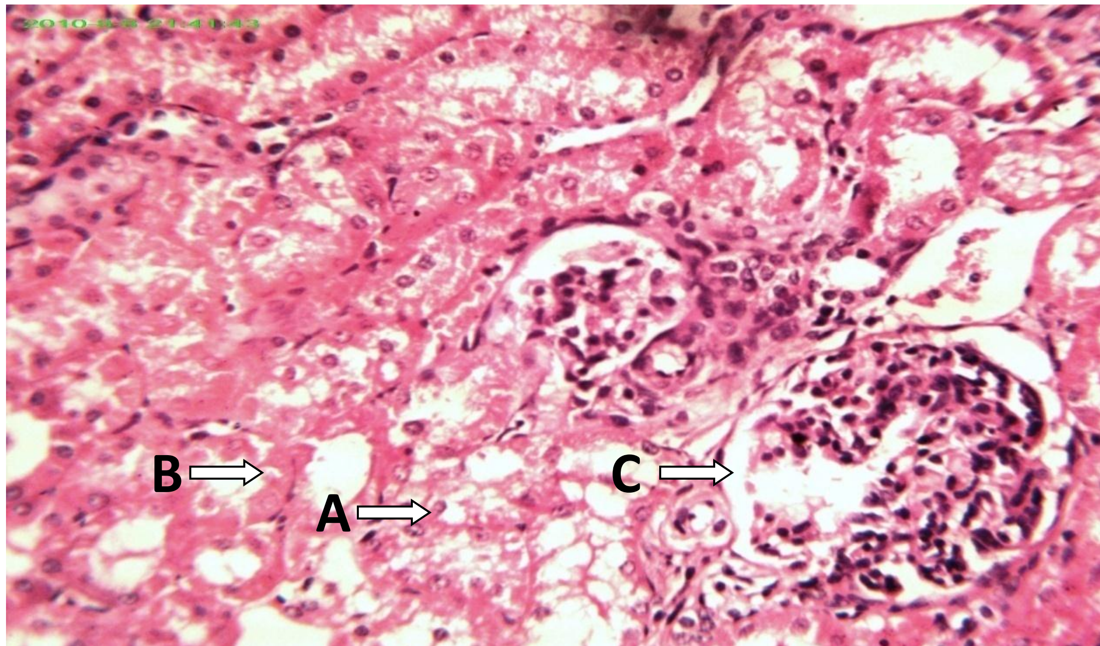

Figure (4.26) Cross section of kidney eight weeks post experimentation showing degeneration in the renal tubules. (**A**). Necrotic of the renal tubules (B). Hypertrophy of the glomeruli (**C**) (H&E X400).

Testis of control group

The internal male genitalia consist of testes with adjoining epididymis, the vas deferens and the accessory sex glands, namely the seminal vesicles, the prostate and bulbourethral glands. The testes surrounded by a thick capsule, the tunica albuginea, from which a conical mass of connective tissue. The tunica albuginea is covered by serosa. From the mediastinum, delicate fibrous septa radiate towards the tunica albuginea and divided the parenchyma of testis into about 300 lobuli testis, each lobule contain 1-4 convoluted seminiferous tubules. Seminiferous tubules enclosed by basal lamina, the insides of the tubules are lined with seminiferous epithelium, which consists of two general types of cells: spermatogenic cells and Sertoli cells. Spermatogonia are the first cells of spermatogenesis, they are contact with the basal lamina of the tubule, spermatogonia differentiate to primary spermatocytes. Primary spermatocytes appear larger than spermatogonia, these cells differentiate to secondary spermatocytes which are smaller than primary spermatocytes. Their division results in the formation of spermatids. Sertoli cells are less numerous than spermatogenic cells and are evenly distributed between them. Their shape is highly irregular. The interstitial tissue between the seminiferous tubules contains blood, lymphatic vessels and various types of cells, with testosterone-producing Leydig cells and macrophages being the major constituent.

Testis of treated groups

Testis sections prepared from rabbits treated with imidacloprid showed degenerative lesions in the spermatoginal cells during the first, second, third and fourth weeks of the experiment. Cross section of seminiferous tubules after five weeks of study displayed necrotic changes of spermatogonia, primary and secondary spermatocytes. On the other hand thickening of basement membrane and the seminiferous tubules are filled with eosinophilic inflammatory cells showed in the sixth week of the experiment. After seven weeks of the study, disorganization of spermatogenic layers, necrotic Sertoli cells and pyknotic lesions in spermatogonia were noticed in the imidacloprid-treated rabbits. Seminiferous tubules after eight weeks of the experiment

showed distortion of the tubules, disappearance of spermatogenetic cells and disappearance of interstitial cells of Leydig. Vasodilation and thrombosis in the vein and the seminiferous tubules are devoid of sperms were also showed in the eighth week of the experiment.

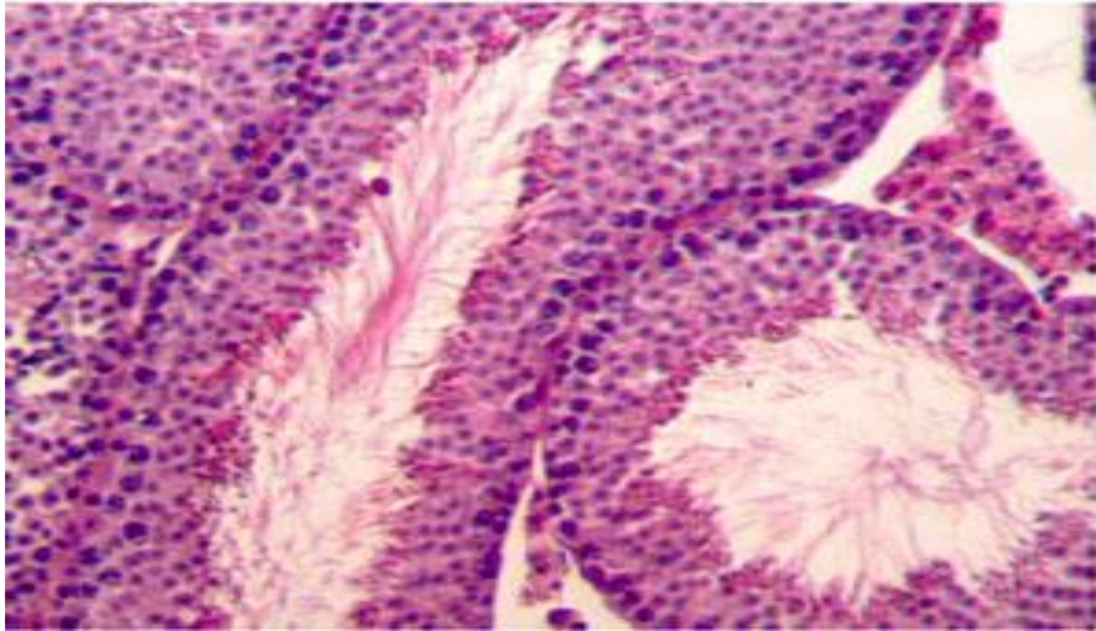

Figure (4.27) Cross section of control seminiferous tubules rabbit showing normal histological structure of the tissue (H&E X400).

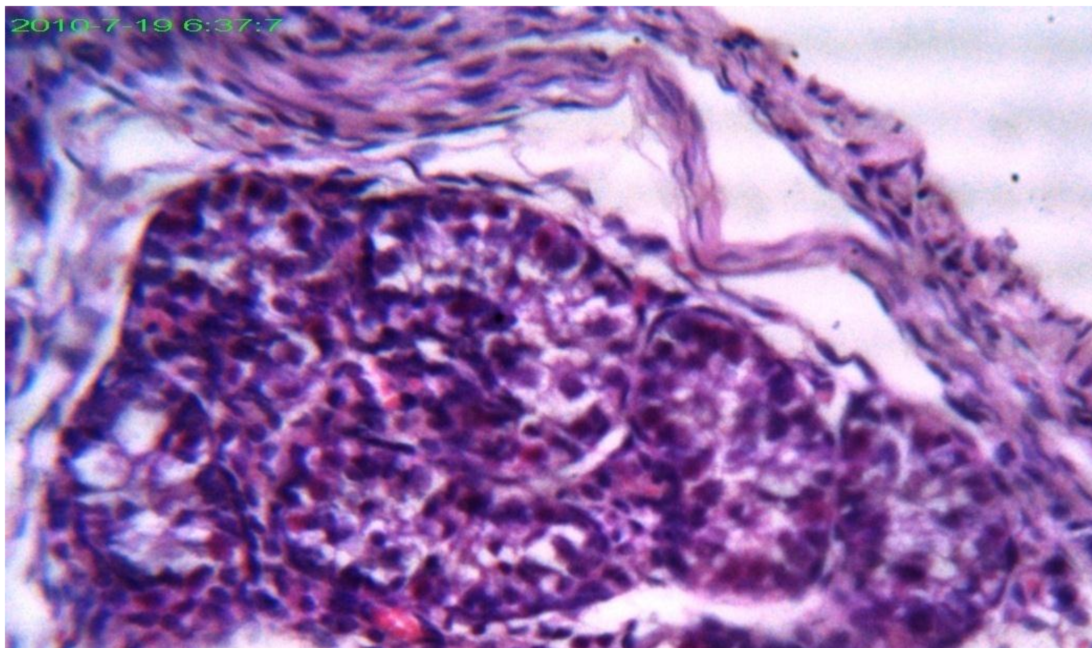

Figure (4.28) Cross section of seminiferous tubules one week post experimentation showing degenerative lesions in the spermatoginal cells. (H&E X400)

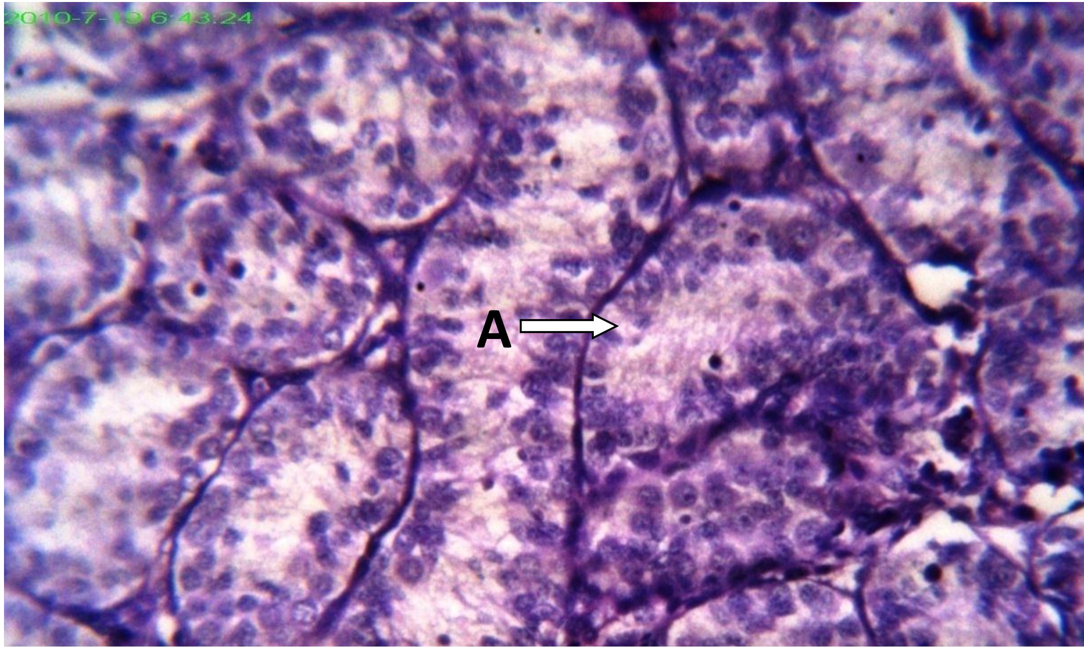

Figure (4.29) Cross section of seminiferous tubules after two weeks of study showing degenerative lesions in the spermatoginal cells (**A**). (H&E X400)

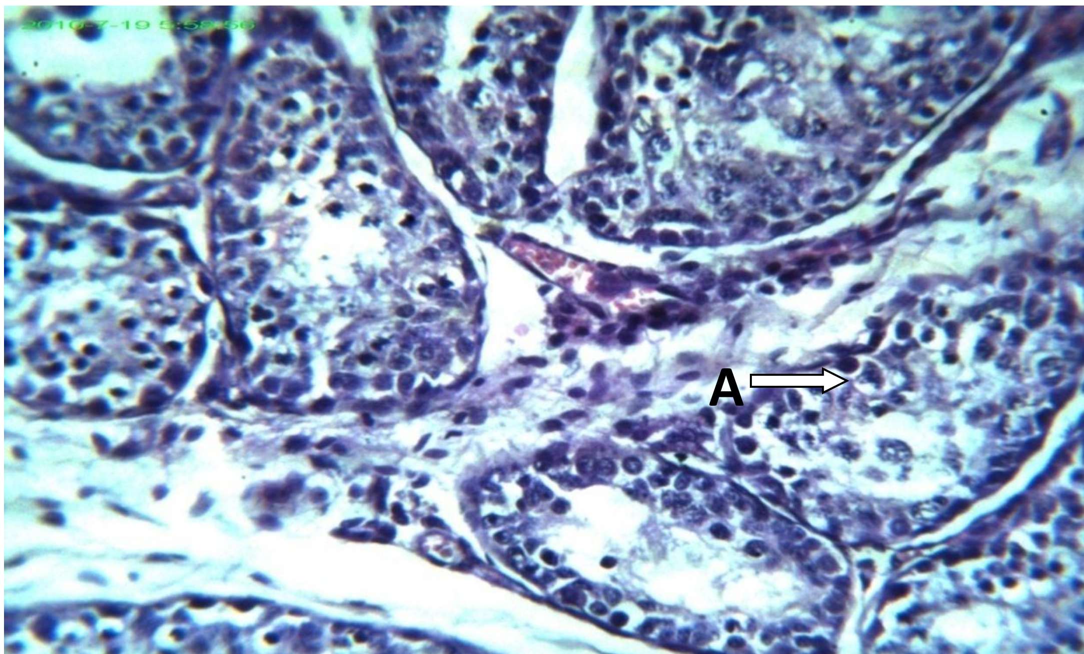

Figure (4.30) Cross section of seminiferous tubules after three weeks of study showing degenerative lesions in the spermatoginal cells (**A**). (H&E X400)

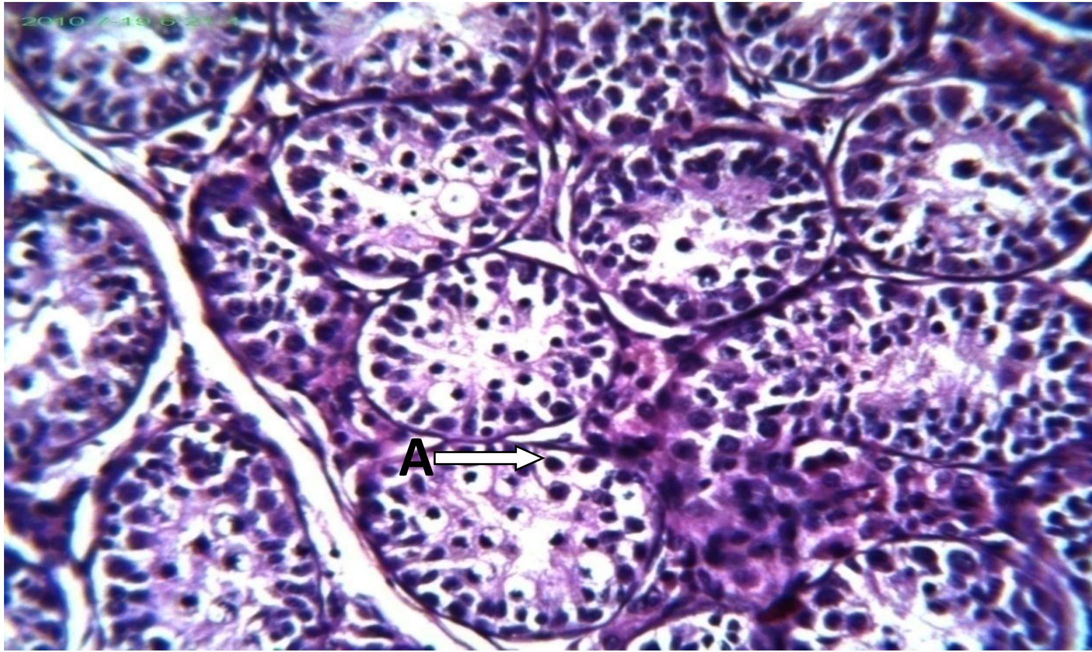

Figure (4.31) Cross section of control seminiferous tubules after four weeks of study showing degenerative lesions in the spermatoginal cells (**A**) (H&E X400).

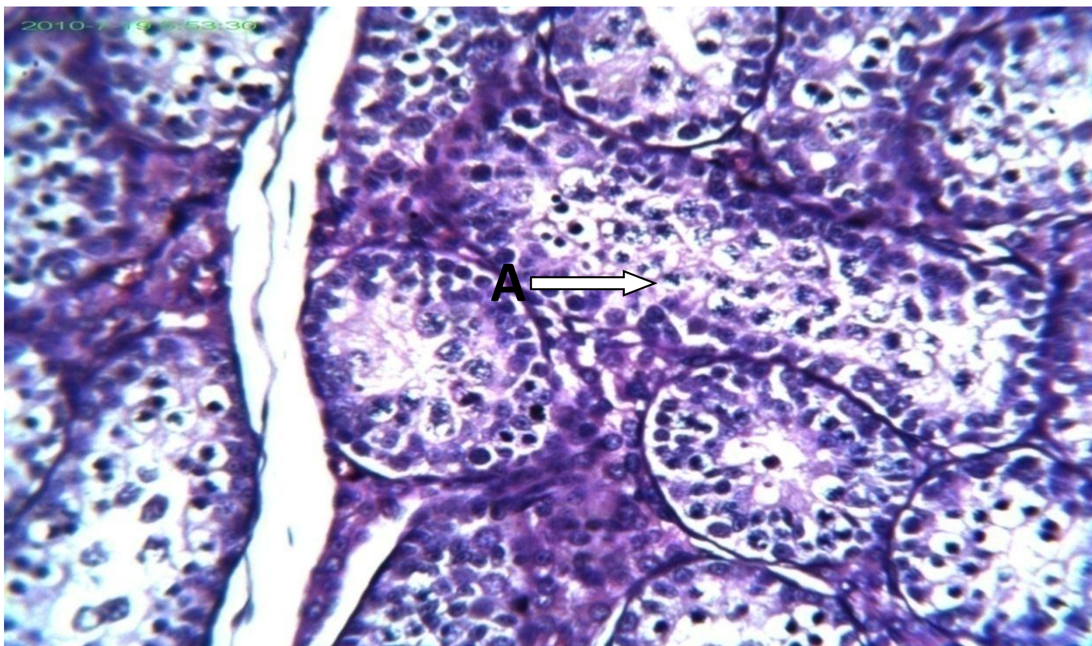

Figure (4.32) Cross section of seminiferous tubules after five weeks of the study showing degeneration changes of spermatogonia, primary and secondary spermatocytes (H&E X400).

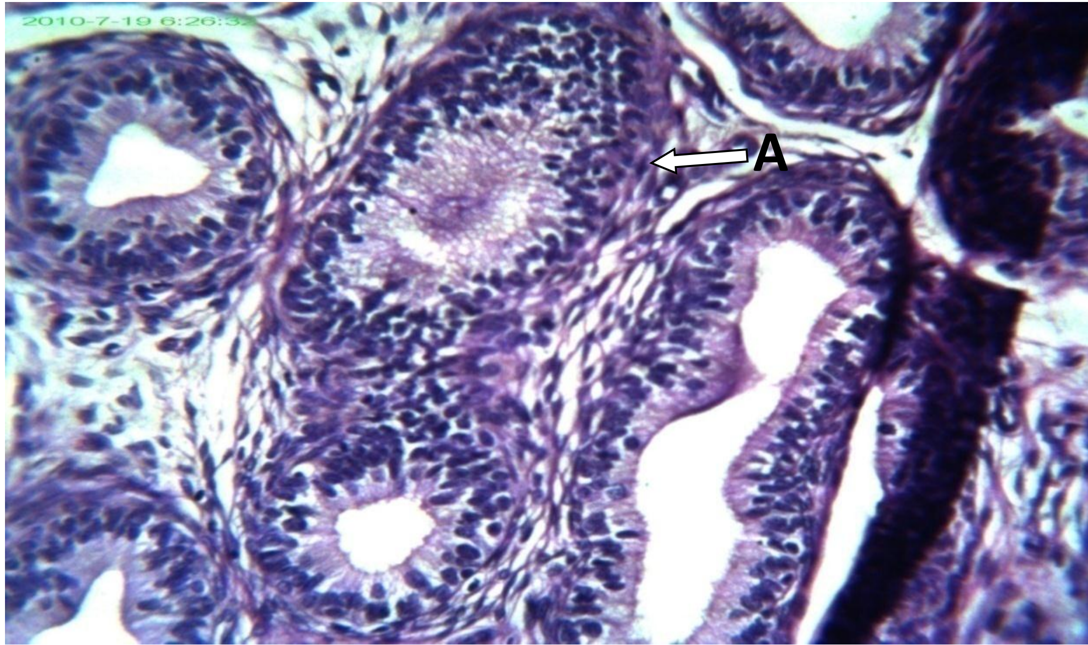

Figure (4.33) Cross section of seminiferous tubules after six weeks of study showing thickening of basement membrane (**A**) (H&E X400).

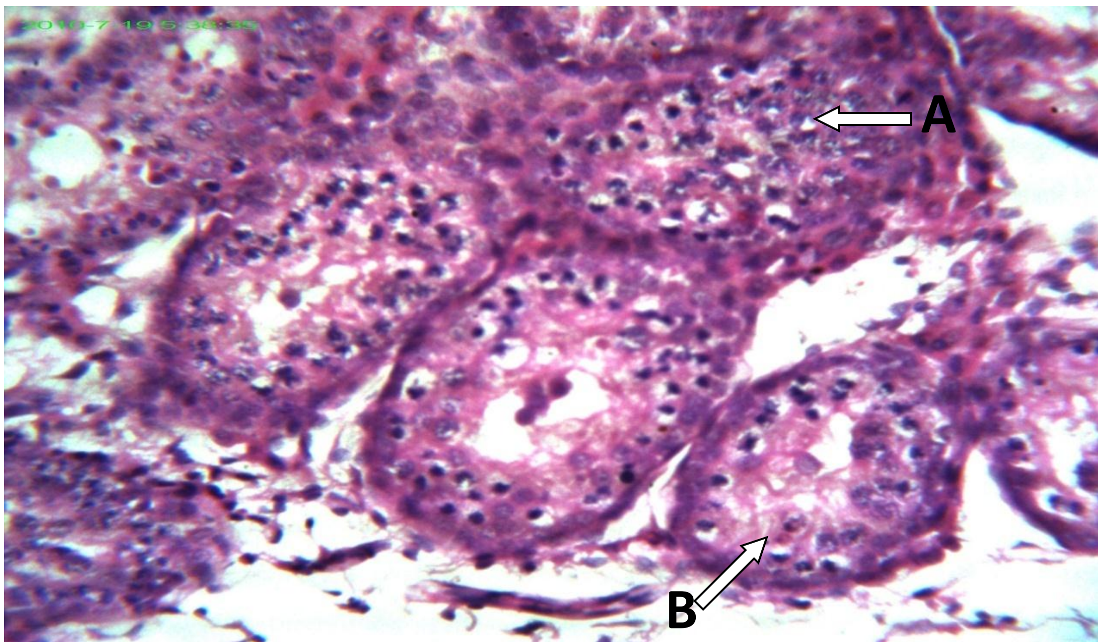

Figure (4.34) Cross section of seminiferous tubules after seven weeks of study showing disorganization of spermatogenic layers (**A**). Pyknotic lesions in spermatogonia (**B**). Necrotic Sertoli cells (H&E X400).

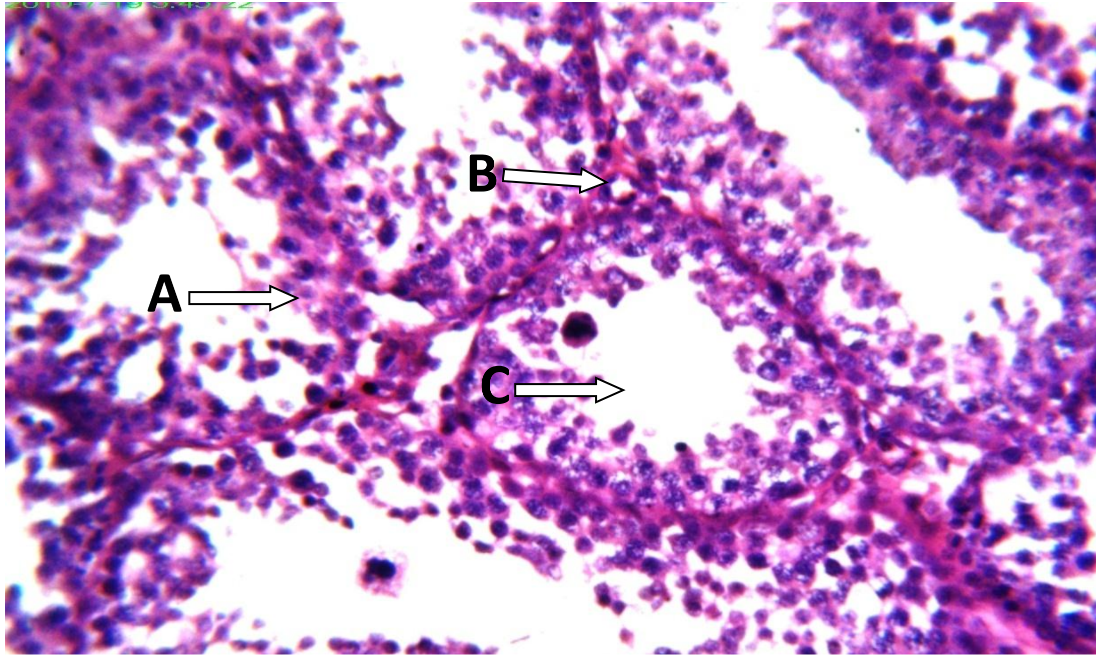

Figure (4.35) Cross section of seminiferous tubules after eight weeks showing distortion and degeneration of the tubules (**A**). Disappearance of interstitial cells of Leydig (B). The seminiferous tubules are devoid of sperms (C) (H&E X400).

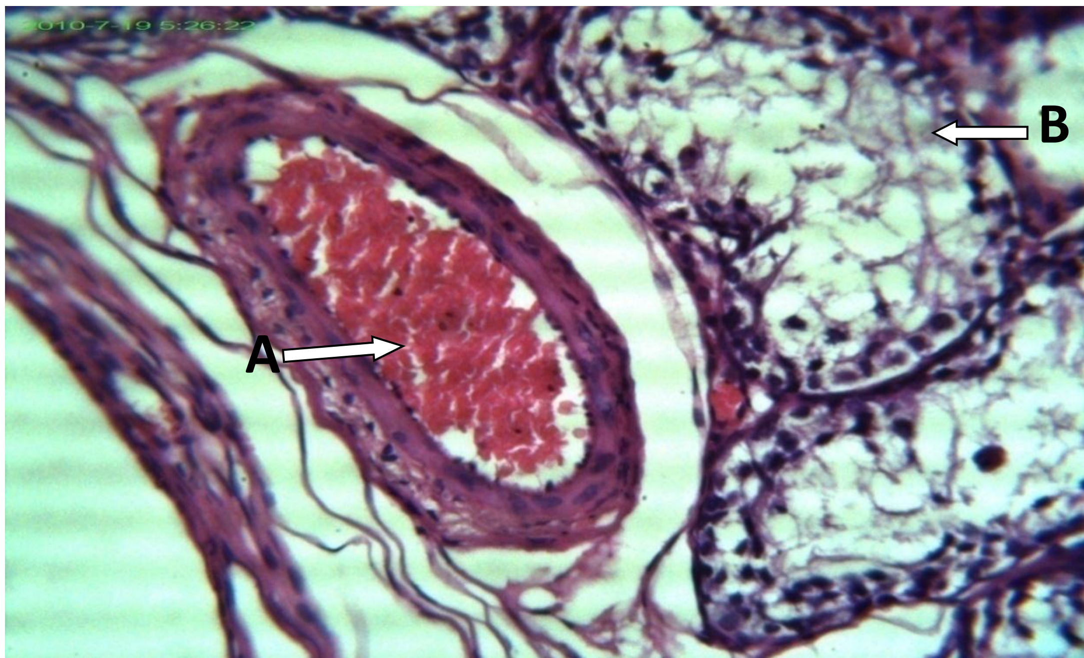

Figure (4.36) Cross section of seminiferous tubules after eight weeks of study showing vasodilation and thrombosis in the vein (A). Disappearance of spermatocyte and Sertoli cells (**B**) (H&E X400).

CHAPTER 5

DISCUSSION

Imidacloprid is a synthetic analog of nicotine (an alkaloid compound found in the leaves of many plants in addition to tobacco, Ware, 2004), which belongs to the class of neonicotinoid insecticides. Imidacloprid has multiple agonist and antagonist effects on neuronal nicotinic acetylcholine receptor channels (Nagata, 1998 and Zhang et al., 2008) and used for the control of sucking insects (Kidd and James, 1994). Imidacloprid has a moderate order of toxicity with respect to ingestion, but appears to be less toxic when absorbed by the skin or inhaled (Mizzel and Sconyers, 1992).

Different insecticides are in wide use worldwide, of which 5% of the world's populations (mainly agro-workers) are directly exposed to these insecticides (David, 2004). In Gaza Strip pesticides including imidacloprid are being excessively used in the agricultural sector (Yassin et al., 2002). Several cases of chronic toxicity or death have been reported and proven among farm workers exposed to different types of pesticides in the Gaza Strip. This may be a result of the use or misuse of these highly toxic compounds, where precautions regarding wearing protective gear during handling and application are poorly followed (Safi, 1998 and Yassin et al., 2002).

Although imidacloprid represents the fourth one among insecticides used in Gaza Strip (Ministry of Agriculture, 2009). Data available on its toxic effects are very restricted. To our knowledge oral LD_{50} was determined in rats and mice (Meister, 1995; Kidd and James, 1994). However, till now no published data are available on oral LD_{50} of imidacloprid in rabbits. Additional toxicity data are needed for complete risk assessment of imidacloprid. Therefore, the present study was aimed to assess oral LD_{50} of imidacloprid in male domestic rabbits and to investigate the effect of imidacloprid on liver, kidney and testis in term of morphological, physiological and histological profiles. The findings can then be extrapolated to human beings to assess the potential hazards in the human populations due to imidacloprid exposure.

5.1 Morphological studies

The present study demonstrates that treatment of rabbits with 1/10 LD_{50} imidacloprid induced some mortalities rate throughout the eight weeks of the experiment study. Such mortality may be attributed to diarrhea noted in the experimental animals. This was in agreement with that found by Najafi et al., (2010) who observed diarrhea in mature male rats in response to chronic exposure of imidacloprid. Gastrointestinal irritation was reported as clinical symptom of imidacloprid toxicity (Yeh et al., 2010). In addition, imidacloprid-treated rabbits showed hair loss especially in the last two weeks (seventh and eighth weeks). This may be coinciding with the significant decrease in protein content observed in the present study. The livers of dissected imidacloprid-treated rabbits showed scars of depressions also in the last two weeks which may be due to the distortion in the liver cells.

5.2 Physiological investigation

5.2.1 Liver enzymes

Data presented in this study showed that the mean levels of serum ALT and AST in the imidacloprid-treated rabbits were significantly higher than those in the controls. Such elevation of liver enzymes as a result of imidacloprid administration was documented by other authors (Pauluhn, 1988; Eiben and Rinke, 1989; Kaur et al., 2006; Zaahkook et al., 2009; Helal et al., 2009; Bhardwaj et al., 2010). Liver is the center of biotransformation and detoxification of foreign compounds and is the most vulnerable to the chemical assaults (Kulkarni and Hodgson, 1980). Serum ALT and AST are considered to be among the most sensitive markers employed in the diagnosis of hepatotoxicity (Kutlu et al., 2005). Pesticide exposure causes liver damage and leakage of cytosolic enzymes from hepatocytes and other body organs into blood (Dewan et al., 2004). 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).

Serum alkaline phosphatase and cholinesterase were increased during the first four weeks and then decreased in the last four weeks. Zaahkook et

al., (2009) and kaur (2006) reported that ALP increased at the first three weeks in animals treated with different doses of imidacloprid. The increase in serum cholinesterase observed in the first four weeks may be attributed to the idea that imidacloprid binds to acetylcholine receptors (Zhang et al., 2008) leading to increase acetylcholine levels. This activates cholinesterase enzyme to break down the excess acetylcholine i.e. increase cholinesterase activity recorded in the first four weeks. With time progression the efficiency of the enzyme decreased in the last four weeks. Such decrease in cholinesterase activity was in agreement with that found by Zaahkook et al., (2009) and Bhardwaj et al., (2010).

5.2.2 Kidney function

The influence of imidacloprid on kidney function of imidacloprid-treated rabbit was assessed through the measurement of urea and creatinine. Urea levels were increased significantly during the last four weeks of the experiment in imidacloprid-treated rabbits compared to the controls. For creatinine this increase was observed during the 4th, 7th and 8th weeks of the experiment in response to imidacloprid administration. Such findings are in agreement with that reported in other studies (Yassin, 1998 and Zaahkook et al., 2009). A creatinine level raised out of proportion to the urea may indicate a pre-renal problem (Delanghe et al., 1989). Urea is formed by the liver as an end product of protein breakdown and it is one marker of the kidney function (Debra Manzella, 2008). Increase in serum urea observed in the present study may be due to 1) impairment in its synthesis as a result of impaired hepatic function, 2) disturbance in protein metabolism and 3) decrease in its filtration rate in the kidney. Creatinine is break-down product of creatine phosphate in muscles, and is usually produced at a fairly constant rate by the body. Creatinine is chiefly filtered out of the blood by the kidneys (Delanghe et al., 1989). Creatinine has been found to be a fairly reliable indicator of kidney function. As the kidneys become impaired for any reason, for example in case of imidacloprid 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 (Ledwith et al., 1997).

5.2.3 Protein profile

As indicated in the present results significant decreases in the levels of total protein, albumin and globulin concentrations were found in rabbits treated with imidacloprid compared to the controls. Similar findings were reported in other studies as a result of oral administration of different doses of imidacloprid (Pauluhn, 1988; Eiben and Rinke, 1989 and Zaahkook et al., 2009). This decrease in serum total protein may be due to lowered synthesis of albumin in liver in response to imidacloprid intake. It was reported that albumin levels are decreased in liver disease (Nyblom et al., 2004). A decrease in globulin is expected as globulin (mostly γ-globulins) may be consumed in the production of antibodies in response to imidacloprid administration.

5.2.4 Testosterone

The current data showed that testosterone hormone level was decreased in imidacloprid-treated rabbits compared to control animals. This result is in accordance with that found by Lauan (2007) and Bhardwaj et al., (2010) in the experimental animals treated with different doses of imidacloprid. The decrease in testosterone level observed in the present data may be explained on the basis of impairment in Leydig cell function. It is well known that testosterone is secreted by Leydig cells (Payne and Shaughnessy, 1996). This was supported by the results of histological changes that there was disappearance of interstitial cells of Leydig in rabbits treated with imidacloprid compared to controls.

5.3 Histological investigation

The previously alterations in the physiological parameter due to imidacloprid administration were correspondingly reflected in the histological findings obtained in the current study from liver, Kidney and testis tissues examination. Liver, Kidney, and gonads have been reported as affected organs for imidacloprid toxicity (Mizzel and Sconyers, 1992). Liver and kidney are the main target of the imidacloprid administration and its metabolites (Klein, 1987). The liver sections from imidacloprid-treated rabbits showed many changes when compared with those from the control rabbits. These changes include congestion in the central vein during the first four weeks of the experiment. This result was corresponded with that observed by (Shakoori et al., 1992). Congestion in the central vein may be attributed to the harmful effect of imidacloprid on heart. It is well known that the mammalian heart is affected by imidacloprid administration (Huang et al., 2006). Vacuolar degeneration in hepatocytes was found in the animals treated with imidacloprid. Such alteration was documented by (Brzoska et al., 2003) in their studies using cadmium and ethanol alcohol to study their effects on liver and kidney tissues of rats. Therefore the cytoplasmic vacuoles could be considered as a sign of metabolic alteration under the influence of imidacloprid administration (Zhang and Wang, 1984) or due to increase permeability of cell membrane leading to an increase of intracellular water. As water sufficiently accumulates within the cell, it produces cytoplasmic vaculation (Shimizu et al, 1996). On the other hand the liver of the imidacloprid-treated rabbits showed dense lymphocytic infiltration especially around the central vein, the increase in these cells may be due to irritability, inflammation and hypersensitivity to imidacloprid administration. Cellular necrosis and sometime pycnosis were reported in the current study. Similar findings are in agreement with that reported by (Eiben and Rinke, 1989 and Eissa,) in the experimental animals treated with different doses of imidacloprid. Necrotic changes resulted from the progressively degradative action of enzymes on the lethally injured cells and denaturation of proteins. The damage from toxic compounds often harms the mitochondria or membrane ion pumps and causes the energy levels in the cells to fall down.

Thus, ATP levels fall, which leads to drop the level of antioxidant (glutathione) in the cell, and a vicious cycle of damage starts (Mitton and Trevithick, 1994). So that the necrotic changes in liver cells may be attributed to the toxic effect of imidacloprid on mitochondria. However nuclear pycnosis and necrosis leading to disintegration of hepatocytes (Persis and Kalaiarasi, 2001). This was supported by the results of histological changes in the liver including the degeneration of hepatocytes showing different sizes of nuclei, architectural alterations, cord disarray. In addition, sinusoidal blood congestion and hemorrhage were all evidence of liver damage. These were evoked by many investigators using various chemicals and toxicants with different animals including fishes (Khogali and Gumaa, 1989; Shakoori et al., 1992; Abdu Rabou, 1996; El-Khatib et al., 2003.).

In the present study the kidney of Imidacloprid-treated rabbits showed several histopathological changes. The kidney tissue showed hypertrophy of the glomerular tufts, degeneration and vacuolation of the cytoplasm of the cells lining the convoluted tubules. Such findings are in agreement with that reported in other study (Zaleska Freljan et al., 1983) on the treatment of mice with bromfenvinphos insecticides. Moreover, the nuclei of some deteriorated cells showed clear signs of degenerated and necrotic tubules due to imidacloprid administration. This result is in agreement with that noticed by (Karim, 1998) who reported the teratogenic effects that have been observed in the kidney of rat fetuses maternally treated with flufenoxuron during organogenesis. Histopathological features found in this study may be attributed to the toxic effect of imidacloprid and its metabolites especially 4-hydroxyimidacloprid and olefinic metabolite on kidney tissue. It is known that olefinic metabolite and 4-hydroxyimidacloprid concentrated in kidney (Karl and Klein, 1992). Dilatation of the inter tubular blood vessels and vascular lumen impacted by haemolysed blood between the renal tubules were also observed on the imidacloprid-treated rabbits. Such alterations were reported by (Abdel Rahman and Zaki, 1992) who noticed deformation of the structure of the glomeruli of the cortical region in mice treated with malathion or sevin insecticides. Vascular dilation found in the interstitial tissue of the imidacloprid-treated rabbits could be a consequence of the action of

vasodilator substances like serotonin and histamine present in most toxins (Videau et al., 2005). On the light of the previous changes one can say that imidacloprid could be toxic at least on male rabbits.

The 8 weeks of imidacloprid administration led to a variety of Histopthological changes in testis tissue. Among the Histopthological symptoms observed were degenerative and pyknotic lesions in the spermatoginal cells. Such findings are in agreement with that reported by (Najafi et al., 2010). Insecticides and pesticides act as reproductive toxicants in male rats and histologically induce severe focal necrosis of the germinal cells associated with tubular atrophy (Swart et al., 1991 and Narayana et al., 2006). This was supported by necrotic and degenerative changes of spermatogonia, primary and secondary spermatocytes in imidacloprid-treated rabbits. Any degeneration event by imidacloprid in the germinal epithelium was able to lead interruption in the mitotic activity of type A spermatogonia cells, which in turn could arrest spermatogenesis and spermyogenesis processes (Najafi et al., 2010). Spermatogenic arrest indicates primary alteration in the Sertoli cells (Videau et al., 2005) whereas necrotic Sertoli cells observed on the animal treated with imidacloprid. Similar results were obtained for endosulfan, malathion, and methomyl (insecticide compounds) when rats were chronically exposed to these compounds (Mahgoub and Azza, 2000 and Saiyed et al., 2003). Any functional damage in sertoli cells could lead to germinal cells degeneration and dissociation. This was supported by the previous findings. Disorganization of spermatogenic cells and the seminiferous tubules are devoid of sperms were also showed in this study. Such findings are in agreement with that reported by (Eissa, 2004 and Lauan, 2007). However Leydig cells of the interstitial connective tissue disappearance in the rabbits treated with imidacloprid. This result is in accordance with that found by (Najafi et al., 2010). According to this results on male reproductive tract of domestic rabbits can be considered the testis as a target for imidacloprid in the treated animals which expose to this compound chronically and it can cause histological damage on testicular tissue and infertility problems in high doses.

CHAPTER 6

CONCLUSIONS

1. The most common clinical symptoms in imidacloprid-treated rabbits were diarrhea, disorientation, drowsiness, tremors, cramps and sometimes death.
2. The mortality increase during experimental period in imidacloprid- treated rabbits compared with controls.
3. Liver enzymes ALT and AST were significantly higher in the treated rabbits all over the experimental periods whereas cholinesterase and ALP levels were significantly increase at the first four weeks and then decreased at the next four weeks compared to the controls.
4. Urea and creatinine levels were increased in response to imidacloprid administration compared to the controls.
5. There were significant decreases in total protein, albumin and globulin upon imidacloprid intake compared to the controls.
6. Serum testosterone levels were significantly lower in imidacloprid-treated rabbits than controls.
7. Liver, kidney and testis of male domestic rabbit can be consider as a target for imidacloprid if animals exposed to this compound chronically and it can cause morphological and histological damage in these tissues.
8. Histopthological changes in liver tissue were manifested in hepatocellular damage as degenerative and destruction of architecture of hepatocytes, dilatation in the blood vessels and vascular congestion of sinusoids. In addition, karyolysis of nuclei was observed.
9. The kidney of imidacloprid-treated showed abnormal feature. Dilatation of the inter tubular blood vessels and vascular lumen impacted by haemolysed blood, rupture of tubules and focal mononuclear leukocyte inflammatory cells infiltrate between tubules, necrobiosis in the epithelial cells lining the tubules.

10. Rabbits treated with imidacloprid showed histological damage on some testicular tissue, necrotic changes of spermatogonia, primary and secondary spermatocytes, disorganization of spermatogenic layers, disappearance of interstitial cells of Leydig and necrotic Sertoli cells.

CHAPTER 7

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