

Hepatotoxicity of Imidacloprid in Male Rabbit: Physiological and Histological Aspects

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Abstract: The present study was aimed to investigate the histopathological and physiological alterations in the liver of male rabbit in response to imidacloprid administration. Rabbits were given orally the dose of 1/10 LD₅₀ imidacloprid daily (mg/100/L) for 8 weeks. Control animals were given distilled water. Upon imidacloprid administration, alanine amino transferase 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 = 0.017 and 0.020, respectively). Aspartate amino transferase 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 phosphates increased in the first 4 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 4 weeks, the enzyme activity decreased with maximum decrease at the 6th week (83.3±5.5 v 107.8±5.7, % difference = 22.7, p = 0.018). Similar changed were 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).

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

INTRODUCTION

Pesticides are substances or a mixture of substances intended to control a variety of pests such as insects, rodents, fungi, weeds, micro organisms 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).

Imidacloprid is used for the control of sucking insects including rice hoppers, aphids, thrips, whiteflies, termites and soil insects. 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; Ritzhaupt *et al.*, 2000).

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 (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; 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; 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 LD₅₀ (a dose that expected to cause death in 50% of animals). This is the first study to determine oral LD₅₀ of the imidacloprid in male domestic rabbits. The 1/10 LD₅₀ is then used in the toxicity experiments. In addition, few studies addressed the toxic effect of imidacloprid on the functions of several

mammalian organs among them were the liver. (Eiben and Rinke, 1989; Eiben and Kaliner, 1991; Najafi *et al.*, 2010; Yeh *et al.*, 2010).

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 amino transferase, 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. 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.

A 90 days oral toxicity study of imidacloprid was conducted in female rats with doses of 0, 5, 10 and 20 mg/kg/day (Bhardwaj *et al.*, 2010). Decrease in body weight gain was observed at 20mg/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.

Pesticides are being heavily used in large amounts in the Gaza strip where the protective measures are poorly followed (Yassin *et al.*, 2002). More than 450 metric ton of pesticides are being used yearly in the Gaza strip. The insecticide represents 70-100 metric ton of these pesticides (5-7) metric ton of these insecticides are imidacloprid. These highly toxic compounds constitute a real threat on humans. The present research 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.

MATERIALS AND METHODS

Healthy adult male domestic rabbits weighing 1000 ± 200 g were used in the present study. Animals were left for 1 week before experimentation to adapt to laboratory conditions. They were kept in metal cages. The dimensions of each cage were $100 \times 60 \times 60$ cm. A commercial balanced diet (Anbar) and water were provided *ad libitum* all over the experimental period.

Experimental design: The study had 2 phases: The 1st was to determine the oral LD₅₀ of imidacloprid and the second was to assess the physiological and histological alterations in rabbit induced by sub lethal dose of imidacloprid (1/10 LD₅₀).

Determination of imidacloprid LD₅₀: A total number of 80 rabbits were used for determination of LD₅₀ of imidacloprid. Animals were divided into 10 groups (8 rabbits/group). The first 9 groups (1-10) were administered different single doses of imidacloprid ranging from 100-300 mg kg⁻¹ body weight as shown in Table 1.

The 10th 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 2 ears so that the esophageal opening was clearly and unobstructively opened. The gastric tube was filled with the required dose of imidacloprid then smoothly inserted until it's adequately enters the upper part of esophagus where its contents were emptied. The animals were observed for mortality during the 48 h observation period. The LD₅₀ was determined by graphical method (Manna *et al.*, 2004).

Table 1: The 1st 9 groups were administered different single doses of imidacloprid

Dose (mg kg ⁻¹ body weight)	LD ₅₀ determination groups
100	1
125	2
150	3
175	4
200	5
225	6
250	7
275	8
300	9
	10 control group

Imidacloprid toxicity experiments: After determination of imidacloprid LD₅₀, a dose of 1/10 of LD₅₀ imidacloprid was given orally to assess imidacloprid toxicity in male domestic rabbit. Animals were divided into 2 groups: Control and experimental groups. Control group comprised 48 rabbits (6 rabbits were housed in each cage) and experimental group included 64 rabbits (8 rabbits were housed in each cage). Experimental groups were orally administrated imidacloprid for overall experimental duration of 8 weeks. Control animals were given distilled water. Administration of imidacloprid was also done by the specialstomach tube.

Morphological studies

Growth appearance: Growth appearance of both control and experimental animals were noticed.

Mortality rate: The percentage of mortality rates was calculated.

Physiological and biochemical analysis

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, i.e., for 15 min. Clear serum samples were separated in glass tubes, labeled and stored in a deep freezer until biochemical analysis. However, determination of almandine aminotransferase, aspartate aminotransferase, alkaline phosphatase, cholinesterase were carried out on fresh serum.

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.

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 using DiaSys reagent kits (Lothar, 1998).

Determination of alkaline phosphates: Serum Alkaline Phosphates (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 using DiaSys reagent kits (Soldin, 2007).

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

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:

$$\text{Difference (\%)} = \frac{\text{Mean of treated} - \text{Mean of control}}{\text{Mean of control}} \times 100$$

Histological studies: For histological examination, liver was dissected freed from the surrounding connective tissues and organs, then excised. It was immediately immersed in saline solution (0.9% NaCl) for blood removal. The following fixative reagent used for the routine heamatoxylin and eosin stain was 10% buffered formalin. 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 rotator microtome at a thickness of 2 μm , mounted and affixed to slide. Sections were stained as a routine in Harris's alum heamatoxylin and eosin (Casselman, 1959; Allen, 1992).

RESULTS

Oral LD₅₀ of imidacloprid: The experimental trials for oral LD₅₀ determination of imidacloprid after 48 h of administration in male domestic rabbits revealed that the mortality commenced at 125 mg kg⁻¹ body weight, recording mortality percentage of 25.0% (Table 2). 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⁻¹ body weight. The calculated oral LD₅₀ of imidacloprid in male domestic rabbits from the linear regression was found to be 172 mg kg⁻¹ body weight. The number of animals administered imidacloprid was (8) in each group (1-10). Control animals were given distilled water and their number was also 8.

Table 2: Mortality percentage of male domestic rabbits after 48 h of oral administration of different doses of imidacloprid

Groups	Imidacloprid dose (mg kg ⁻¹ body weight)	No. of animals died/total	Mortality (%)
1	100	8/0	0.0
2	125	8/2	25.0
3	150	8/4	50.0
4	175	8/5	62.5
5	200	8/5	62.5
6	225	8/5	62.5
7	250	8/6	75.0
8	275	8/6	75.0
9	300	8/8	100.0
10	Control	8/0	0.0

Table 3: Effect of imidacloprid (1/10 LD₅₀, 17.2 mg kg⁻¹ 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	3.7±50.3	3.8±54.5	8.3	0.722	0.497
2	3.0±52.0	4.1±70.8	36.2	3.495	0.017
3	1.7±47.6	2.9±57.5	20.8	2.654	0.045
4	2.8±48.4	3.6±61.8	27.7	2.733	0.041
5	3.0±49.8	3.9±65.3	31.1	3.145	0.020
6	2.6±49.3	3.0±58.8	19.3	2.429	0.049
7	2.9±51.7	3.5±63.4	22.6	2.607	0.040
8	1.9±51.2	2.7±58.7	14.6	2.322	0.068

Table 4: Effect of imidacloprid (1/10 LD₅₀, 17.2 mg kg⁻¹ 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	2.1±33.6	2.2±37.9	12.8	1.437	0.194
2	1.8±33.9	2.1±37.4	10.3	1.238	0.256
3	2.1±35.7	3.0±49.2	37.8	3.830	0.012
4	1.5±34.8	2.7±43.2	24.1	2.985	0.031
5	1.7±36.1	2.1±43.0	19.1	2.496	0.047
6	1.8±36.3	2.3±40.5	11.8	1.432	0.202
7	1.3±35.4	3.5±39.0	10.2	0.970	0.369
8	2.0±35.8	3.3±38.9	8.7	0.770	0.470

The number of animals was 6 per time interval for each control and imidacloprid-treated animals. All values are expressed as mean±SE p<0.05 significant

Morphological results

Growth appearance: Imidacloprid-treated rabbits showed hair loss, especially in the 7th and 8th weeks of the experiment (Fig. 1) whereas control animals did not display such change. In addition, the livers of dissected imidacloprid-treated rabbits showed scars of depression whereas those of the control animals showed normal appearance (Fig. 2).

Mortality rate: There was 10 dead experimental rabbits during the 8 weeks experimental duration from the total number of 60 rabbits used in the experiment. The 4 rabbits died at the 1st week, 2 rabbits at the 2nd week, 3 rabbits at the 3rd week and 1 rabbit was died at the 6th 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.



Fig. 1: Imidacloprid-treated rabbits showed hair loss



Fig. 2: Gross control liver animals

Physiological and biochemical results

Alanine aminotransferase: Table 3 pointed out that administration of imidacloprid caused progressive increase in Alanine aminotransferase (ALT) activity 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 = 0.017 and 0.020, respectively)

Aspartate aminotransferase: As depicted from Table 4, treatment of animals with imidacloprid caused increase in Aspartate aminotransferase (AST). The significant change was only observed at the 3rd, 4th and 5th 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 5: Effect of imidacloprid (1/10 LD₅₀, 17.2 mg kg⁻¹ 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	5.4±101.8	4.7±107.3	5.4	0.770	0.470
2	3.9±103.3	6.3±129.5	25.4	3.530	0.012
3	4.3±105.2	7.0±118.0	12.2	1.560	0.194
4	5.2±104.6	4.9±126.1	20.6	2.946	0.032
5	5.5±105.0	6.1±93.3	-11.1	1.423	0.205
6	5.7±107.8	5.5±83.3	-22.7	3.246	0.018
7	5.1±109.3	5.0±92.5	-15.4	2.373	0.055
8	5.2±108.0	4.4±88.4	-18.1	2.737	0.041

Table 6: Effect of imidacloprid (1/10 LD₅₀, 17.2 mg kg⁻¹ body weight) on cholinesterase activity (U/L) in male domestic rabbits

Experimental period (week)	Control (n = 8)	Imidacloprid (n = 6)	Difference (%)	t-value	p-value
1	114±4532	142±4737	4.5	1.129	0.302
2	148±4491	195±5170	15.1	2.828	0.037
3	185±4605	201±5686	23.5	3.954	0.008
4	168±4624	235±6167	33.4	5.313	0.002
5	183±4843	218±4049	-16.4	2.781	0.032
6	170±4794	211±4255	-11.2	1.987	0.094
7	162±4916	228±4335	-11.8	2.077	0.083
8	155±4876	233±3831	-21.4	3.897	0.011

The number of animals was 6 per time interval for each control and imidacloprid-treated animals. All values are expressed as mean±SE p<0.05 significant

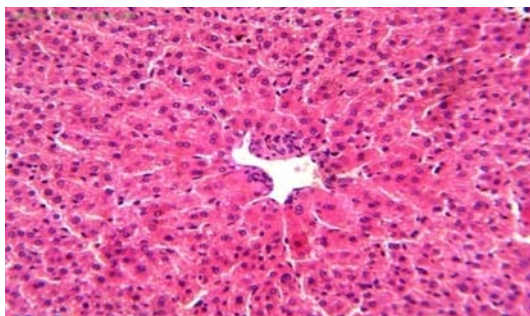


Fig. 3: Cross section of liver from control rabbit showing normal histological structure of the central vein and surrounding hepatocytes (H&E×160)

Alkaline phosphates: The level of Alkaline Phosphatase (ALP) increases in response to administration of imidacloprid. Such changes were significant only at 6th and 8th weeks (p = 0.018, 0.041, respectively (Table 5).

Cholinesterase: Table 6 pointed out that daily oral administration of imidacloprid progressively increased serum ChE levels to 4737±142, 5170±195, 5686±201 and 6167±235 U L⁻¹ at the 1st 4 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⁻¹. This decrease was significant at 5th and 8th weeks (p = 0.032 and 0.011, respectively).

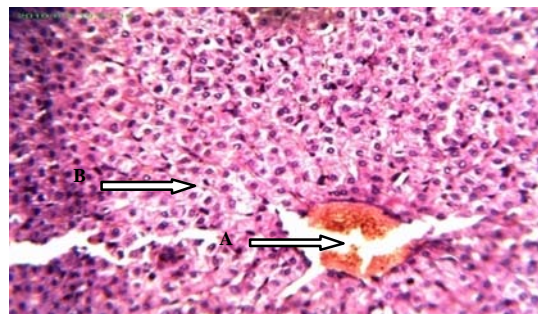


Fig. 4: Cross section of liver 4 weeks post experimentation showing congestion and bleeding in the central vein (A) associated with diffuse kupffer cells proliferation in between the hepatocytes (B) (H&E X 160)

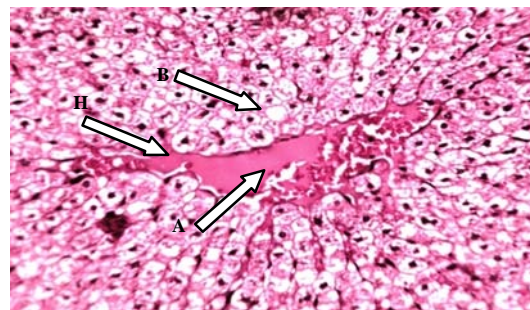


Fig. 5: Cross section of liver 5 weeks post experimentation showing congestion and bleeding in the central vein (A) associated with vacuolar degeneration in the surrounding hepatocytes (B) and hydropic degeneration (H) (H&E X 400)

Histological results: Histological analysis of the control liver sections showed normal parenchyma architecture with cords of hepatocytes, portal tracts and central veins (Fig. 3). Histological analysis of experimental liver sections after the 4th week showed congestion and bleeding in the central vein (A) associated with diffuse kupffer cells proliferation in between the hepatocytes (B) (Fig. 4). Sections of the 5th week of study showed sinusoidal congestion and hepatocytes edema associated with vacuolar degeneration in the surrounding hepatocytes (Fig. 5). Histological analysis of the liver after 6 weeks post experimentation showed diffuse kupffer cells proliferation in between the degenerated hepatocytes, congestion in the portal vein, inflammatory cells infiltration and fibroblastic cells proliferation (Fig. 6). The analysis of the liver sections at the 7th week of study showed severe sinusoidal congestion with severe cytoplasmic vacuolation (severe hepatocytes edema) and damage to the orbicular structure of the lobules.

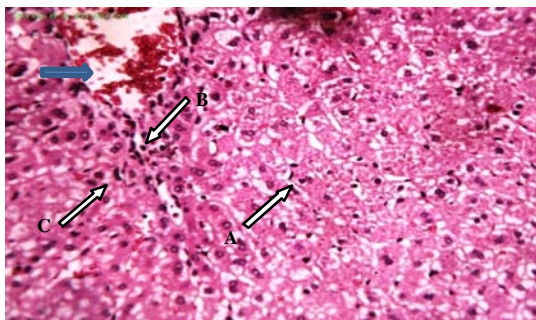


Fig. 6: Cross section of 6 weeks post experimentation showing diffuse kupffer cells proliferation in between the degenerated hepatocytes (A). The portal area showed congestion in the portal vein, inflammatory cells infiltration (B) afibroblastic cells proliferation (C) and and hemolysis of blood vessel (blue arrow) (H&E X 160)

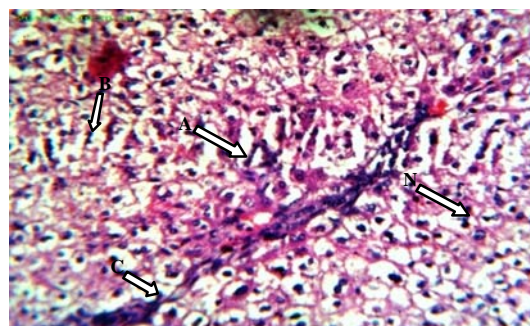


Fig. 9: Cross section of liver 8th weeks post experimentation showing severe destruction of architecture of hepatocytes (A), degenerative changes of hepatocytes (B) and diffuse Kupffer cells proliferation observed between the cells (C) and necrotic cells (N) (H&E×400)

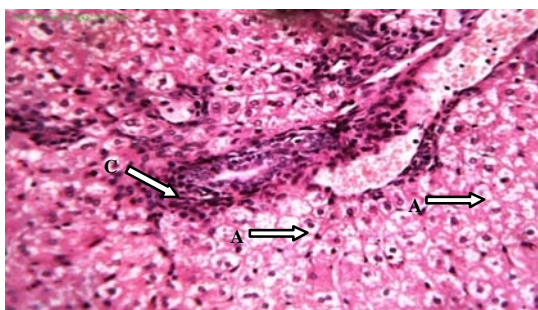


Fig. 7: Cross section of liver 7 weeks post experimentation showing damage to the trabecular structure of the lobules. The cytoplasm of hepatocytes is filled with vacuoles. Karyorrhexis (A) and complete pyknosis (B) of many cells are noticed. Inflammatory cells infiltration and congestion in the portal vein were detected in the portal area (C) (H&E X 400)

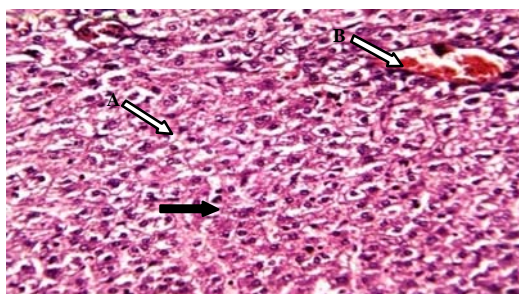


Fig. 8: Cross section of liver 8th weeks post experimentation showing damage to the hepatocytes and the nuclei showed Karyolysis (A), bleeding in the central vein (B) and a necrotic cell (Black arrow) (H&E X 400)

Karyorrhexis, complete pyknosis of some cells, inflammatory cells infiltration and congestion in the portal vein were also noticed (Fig. 7). Microscopic sections of the liver at the 8th week of study showed severe destruction of architecture of hepatocytes, degenerative changes of hepatocytes and diffuse kupffer cells proliferation observed between the cells (Fig. 8 and 9).

DISCUSSION

Imidacloprid is a synthetic analog of nicotine (an alkaloid compound found in the leaves of many plants in addition to tobacco which belongs to the class of neonicotinoid insecticides (Ware and Whitacre, 1992). Imidacloprid has multiple agonist and antagonist effects on neuronal nicotinic acetylcholine receptor channels and used for the control of sucking insects (Nagata, 1998; Zhang *et al.*, 2008; 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 (Mizell 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 (James, 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; Yassin *et al.*, 2002).

Although, imidacloprid represents the fourth one among insecticides used in Gaza strip. Data available on its toxic effects are very restricted. To the knowledge oral LD₅₀ was determined in rats and mice (Meister, 1995; Kidd and James, 1994). However, till now no published data are available on oral LD₅₀ of imidacloprid in rabbits. Additional toxicity data are needed for complete risk assessment of imidacloprid. Therefore, the present study was aimed to assess oral LD₅₀ of imidacloprid in male domestic rabbits and to investigate the effect of imidacloprid on liver organ 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.

The present study, demonstrates that treatment of rabbits with 1/10 LD₅₀ imidacloprid induced some mortalities rate throughout the 8 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 2 weeks (7th and 8th weeks). Hair loss is one of the 1st signs of poi-soning. This may be coinciding with the significant decrease in protein content. In severe infections were clinical signs are apparent; these may include fur loss and an abnormal molting pattern which is due to due to inflammation and tissue destruction. The livers of dissected imidacloprid-treated rabbits showed scars of depressions also in the last 2 weeks which may be due to the distortion in the liver cells (US EPA, 1993).

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 researchers (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 1st 4 weeks and then decreased in the last 4 weeks. Zaahkook *et al.* (2009) and Kaur (2006) reported that ALP increased at the 1st 3 weeks in animals treated with different doses of imidacloprid. The increase in serum cholinesterase observed in the 1st 4 weeks may be attributed to the idea that imidacloprid binds to acetylcholine receptors leading to increase acetylcholine levels (Zhang *et al.*, 2008). This activates cholinesterase enzyme to break down the excess acetylcholine, i.e., increase cholinesterase activity recorded in the 1st 4 weeks. With time progression the efficiency of the enzyme decreased in the last 4 weeks. Such decrease in cholinesterase activity was in agreement with that found by Zaahkook *et al.* (2009) and Bhardwaj *et al.* (2010).

Liver is the main target of the imidacloprid administration and its metabolites (Klein, 1987). 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 tissues examination. Liver has been reported as affected organs for imidacloprid toxicity (Mizell and Sconyers, 1992).

The histopathological changes investigated in this study reflected some significant effects with variable in 10 sites. These changes include congestion in the central vein during the 1st 4 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 cytoplasm 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 cytoplasm vacuolation (Shimizu *et al.*, 1996). Results also showed degenerative changes of hepatic parenchymal cells. This is well corroborated by significant increase in serum activity of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT). These increases in

serum level of these enzymes agree with earlier research of Bhat *et al.* (1998) on minocycline and Aubrecht *et al.* (1997) on hygromycin B.

Other changes reported were mostly hepatocytic vacuolation, intercellular vacuolation and infiltration of lymphocytes around the central veins. To a lesser extent, there were nuclear deaths or pyknosis and hepatocytic ruptures as the liver is the most active mammalian organ in xenobiotic metabolism and contains a larger variety of enzymes for this action. Accordingly, its role in metabolic conversions is its susceptibility to chemical injury (Shakoori, 1990). Besides, 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 hyper sensitivity to imidacloprid administration.

Further finding to histopathology indicated that there were cellular necrosis and sometime pyknosis were reported in the current study. Similar findings are in agreement with that reported by Eiben and Rinke (1989) and Eissa (2004) 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 pyknosis and necrosis leading to disintegration of hepatocytes (Persis and Kalaierasi, 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 and cord disarray.

The architectural alterations of the liver including cord disarray, hypertrophy and disintegration of hepatocytes showing different sizes of nuclei, lymphocytic infiltration in addition to 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 (Persis and Kalaierasi 2001).

On the light of the previous changes one can say that imidacloprid could be toxic at least on male rabbits.

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