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Mammalian Detrimental Effects of Imidacloprid Residues in Tomato Fruits

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ABSTRACT

Imidacloprid, a neonicotinoid pesticide, was used extensively to control whitefly (*Bemisia tabaci*) on tomato crop worldwide. Current study aimed to determine residue amounts of imidacloprid in tomato fruits after different time intervals of application and to evaluate their detrimental effects on white albino rats. Results revealed that the initial deposit (residue amount after 1 h of last spray application) was 0.316 mg kg^{-1} and decreased to 0.32, 0.23, 0.21, 0.14, 0.12 and 0.11 mg kg^{-1} after 3, 5, 7, 10, 14 and 21 days of last spray, respectively and the half-life time was 10.16 days. Toxicity of repeated sub-lethal doses equal to 0.109, 0.116, 0.210, 0.316 and 42.5 mg kg^{-1} b.wt. day^{-1} for 45 days were tested. Results reported herein revealed significant adverse effects on haematological (PCV (%), RBCs and WBCs), biochemical parameters (total protein (g dL^{-1}) and glucose (mg dL^{-1})) and liver, kidney and cardiac function parameters of male rats at 0.316 and 42.5 mg kg^{-1} b.wt. day^{-1}. Current study highlighted that there was no residual toxicity of imidacloprid after 14 days of last application.

Key words: Residue analysis, toxicity, neonicotinoid pesticides, HPLC analysis, *Solanum lycopersicum*

INTRODUCTION

Imidacloprid is a neonicotinoid insecticide in the chloronicotinylnitroguanidine chemical family (Wismer, 2004; Tomlin, 2006). It is a systemic insecticide with translaminar activity. Imidacloprid is a contact and stomach poison that acts antagonistically through binding to the postsynaptic nicotinic receptors (irreversible blockage of acetylcholine receptors) in the central nervous system. It is classified as type II and III toxicity according to WHO and EPA. Imidacloprid is widely used to control of sucking insects including plant hoppers, aphids, thrips and whiteflies. Also it's effective against soil insects, termites and some species of biting insects, such as rice water weevil and colorado beetle (Wismer, 2004; Tomlin, 2006). In Egypt, imidacloprid is registered under many trade names including best (25% WP), chinook (35% SC), commando (35% SC), confidante (35% SC), confidor (20% SC), gaucho (70% WS), imaxi (35% SC), imidamex (70% WG), imidazed (20% SC), mallet (35% SC), monceren G (37% FS) and nuprid (60% FS) (MALR., 2010) to control aphids, thrips and whitefly on several crops including tomato plants (Omar *et al*., 1994; Schuster *et al*., 1996; El-Khawalka *et al*., 1997; MALR., 2010). Also, it is used extensively as indoors and outdoors foliar spray on tomatoes in Southern Europe (EFSA., 2008).

Imidacloprid is metabolized in plants into an imidazolidine, olfin and nitroso derivatives and they were reported to be more toxic to aphids than imidacloprid itself (Nauen *et al*., 1998).

Imidacloprid moves inside plants mostly through shoots and less via roots, it was reported that more than 85% of the imidacloprid was absorbed by tomato plants was translocated to the shoots after 19 days of application. Moreover, imidacloprid was stored in tomato fruits with no respect to the position of the fruits on the plant (Alsayeda *et al*., 2008). In tomato fruits, imidacloprid, parent compound, was reported in addition to small quantities of the guanidine metabolite while it was detected in tomato leaves with an unidentified polar metabolite (Alsayeda *et al*., 2008). Half-life of imidacloprid ranged between 1.71-2.43 days (Dikshit *et al*., 2003). When imidacloprid was applied on tomato plants at the recommended dose (20 g ai (active ingredient) ha^{-1}), 50-55% disappeared within 3 days of application and the remaining quantity vanished after another 7 days to reach 95-100% dissipation after 10 days (Shallan *et al*., 2004).

However, imidacloprid has selective toxicity to insects and not to mammals due to differences in the structure and the binding affinity at the nicotinic acetylcholine receptor (Chao and Casida, 1997; Tomizawa and Casida, 2005). Mammalian adverse effects of this insecticide were reported including reproductive (Rouchaud *et al*., 1994), teratogenic (Pike *et al*., 1993), mutagenic (Placke and Weber, 1993), carcinogenic (Scholz and Spiteller, 1992) effects. Several side effects were reported after feeding rats with imidacloprid mixed with the diet for three months at doses of 14, 61 and 300 mg kg^{-1} day^{-1} for males and 20, 83 and 420 mg kg^{-1} day^{-1} for females. Effects included reductions in body weight, liver damage and reduced blood clotting function and platelet counts at the doses of 61 mg kg^{-1} day^{-1} in males and 420 mg kg^{-1} day^{-1} in females and the estimated NOAEL was 14 mg kg^{-1} day^{-1} (Eiben and Rinke, 1989). When male rats were fed crushed seeds of broad beans which were treated with a single dose of imidacloprid, there were significant decrease in plasma urea, creatinine, triglycerides, cholinesterase, total protein and albumin (Shallan *et al*., 2004). Jain *et al*. (2006) studied the sub-acute toxicity of imidacloprid on adult male rats following intra-peritoneal administration of 20 and 40 mg kg^{-1} daily for 28 days. Imidacloprid caused an inconsistent effect on total leukocyte count-an increase on the 7th day at the low dose and a decrease up to 28 days at the high dose. Moreover, imidacloprid caused hypoglycaemia at both the dose levels (Jain *et al*., 2006).

Imidacloprid is being considered as safe and a replacement of other highly toxic pesticides to control sucking insects. Therefore, present study aimed to determine its residues amounts in tomato fruits and to study adverse side effects of the residue amounts on haematology, heart, kidney, liver biochemical markers of rats. For 45 days, rats were orally given daily doses equal to the residue amounts of imidacloprid which was detected in tomato fruits after different time intervals of last spray application.

MATERIALS AND METHODS

Insecticides and chemicals: Admire® (20% SC) (imidacloprid (1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine)) was purchased from local pesticide stores and was used in current study. All solvents: Acetonitrile, ethyl acetate, hexane and methylene chloride were HPLC grade and were obtained from local distributors in Egypt.

Field layout and fruit sampling: Imidacloprid treatment had three replicates, each replicate was a 50 m^2 plot. Plots of the tomato plants were sprayed 15 times once every 4-7 days (based on infestation) with the recommended rates of (125 cm^3/100 L of water) of Admire® (20% SC) to control whitefly (MALR., 2010). Spray application started as early as tomato plants were infested with whiteflies after plantation. Control plots were sprayed with water. Agricultural practices were done

following the commercial production program of tomatoes. Five kilograms of tomato fruits were randomly collected from treatment and control plots after 0 and 1 h, 1, 3, 5, 7, 10, 14 and 21 days of last spray application. Fruits were homogenized and a sample of 0.5 kg were placed in polyethylene bags and stored at -20°C until analysis.

Extraction and clean-up: Residues of imidacloprid were analyzed according to Fernandez-Alba *et al*. (1996). Approximately 50 g of tomato fruits were extracted with 200 mL acetonitrile using warring blender for 10 min at the high speed. The mixture was vacuum-filtered through a 12 cm Buchner filter. The filtrate was transferred into a 500 mL separating funnel with 10 mL of phosphate buffer solution pH 7. The separating funnel was shaken vigorously for 1 min and the filtrate was allowed to separate into two phases. The acetonitrile phase was filtered through a layer of sodium sulphate (anhydrous) placed on glass-wool. The acetonitrile extract was evaporated using a rotary evaporator (Unipan vacuum rotary evaporator type 350P, Poland) at 40°C in a water bath. The dried extract was dissolved in a final volume of 5 mL of acetonitrile: water (1:3) and sonicated for 5 min. Extracts were filtered into a 2 mL dark HPLC glass vial using a 1 mL syringe and a 0.2 μm nylon filter (Fisher Scientific, Ottawa, Canada) and was used for HPLC analysis.

Measurement of residues via HPLC: Estimation of imidacloprid residues were done on a Perkin Elmer (series 200) High Pressure Liquid Chromatography (HPLC) equipped with a diode array UV detector at 270 nm (Fernandez-Alba *et al*., 1996). About 20 μL of samples were injected into a Nucleosil 100-5 reverse phase (C18) 5 μm, 250×4 mm column. Mobile phase was acetonitrile: water (25/75, v/v) and the flow rate was 1 mL min^{-1}. Retention time of imidacloprid was 4.8 min. External standard of imidacloprid (technical grade) was used for calibration; standard curve was prepared from 6-8 different concentrations of standard solutions.

Recovery studies: Untreated tomato fruits were spiked with 3 incremental concentrations of the technical grade of imidacloprid (0.75, 1.50 and 3.00 μg) prior to extraction and clean-up. Three replicates of each concentration were passed through the entire process of extraction, clean-up and analysis as described above. The recovery values were calculated according to the following formula and the obtained results were corrected according to the recovery rate:

$$\text{Recovery (\%)} = \frac{\mu g \text{ pesticide residue/g sample found}}{\mu g \text{ pesticide residue/g sample added}} \times 100$$

Calculation of half-life values: Half-life times ($t_{1/2}$) in days were calculated according to the equation of Moye *et al*. (1987):

$$t_{1/2} = \text{In2/K} = 0.693/K$$
$$K = 1/t \times \ln a/m$$

where, K is apparent rate constant, t is time in days, m is residue at x time and a is initial residue.

Method validation: For the estimation of the accuracy and the repeatability of the method, six replicates of spiked tomato samples with a concentration of 0.1 μg L^{-1} of imidacloprid were

processed through the entire analytical procedure. The method accuracy was calculated from the areas obtained in the analysis of the spiked samples as a percentage of those obtained in the analysis of a standard solution with an equivalent concentration. Limits of Detection (LODs) were defined as the concentration of a compound giving a signal-to-noise ratio (S/N) of 3. Limit of Quantification (LOQs) were calculated from S/N ratios (1:10) obtained from the measurement of samples with the lowest concentration level where peaks of studied pesticides were detected (Jabor *et al*., 2007).

Toxicity of residue amounts to rats: Male white albino rats (*Rattus norvegious*) weighing 120±10 g were used. Rats were supplied from a breeding culture located at the Animal Health Research Center (Cairo, Egypt). Rats were housed 4/cage and acclimatized for one week prior treatments with free access to water and diet. Rats were divided into 7 groups each of 4 rats. The rats of each group received daily oral doses of technical grade of imidacloprid for 45 days. Doses were equal to the residue amounts of imidacloprid in tomato fruits after 0, 1 h, 7, 14 and 21 days of last spray application, 0.1 LD_{50} (42.5 mg kg^{-1} b.wt. day^{-1}) and control. The control group was orally administered the same volume of water. Animals were decapitated after 24 h of the last oral treatment. Blood samples were collected into serum separation tubes and EDTA-K_3 tubes. All experimental procedures using the laboratory animals were done according to approved Animal Care and Use Protocol of the High Institute of Public Health, Alexandria University and approved by Damanhour University, Egypt.

Blood hematological parameters: Red Blood Cell counts (RBCs) and White Blood Cell counts (WBCs) were done according to the method of Britton (1963) and Seiverd (1964). The number of RBC's was multiplied by 10^4 to obtain the RBC count for each cm^3. The counts of WBC's were multiplied by 50 to obtain the number of WBC's cm^{-3}. Haematocrit value (PCV) was determined according to the method reported by Bull *et al*. (2001) using a Micro-haematocrit Centrifuge (Bench top High Speed Micro Haematocrit Centrifuge 3 model SH120-I, Microfield Instrument, UK). The PCV value was obtained by reading the packed cell volume on a special graduated haematocrit measurement ruler. The obtained data were expressed as percentages of haematocrit value to the total blood volume.

Measurement of biochemical, liver, kidney and cardiac parameters: Blood glucose, total protein, creatinine and uric acid and enzyme activities of aspartate transaminase (AST) and alanine transaminase (ALT) were determined following the directions of Boehringer Mannheim GMBH Diagnostics Kits. Alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities were determined using Diamond Diagnostics Kits. Determination of γ-glutamyl transferase (GGT) activity was done using Linear Chemicals, SL Kits. Serum creatine kinase (CK) activity was measured using Stanbio Laboratory Kits.

Statistical design and analysis: Results of insecticides residue were analyzed using the General Linear Model (GLM) procedure of statistical analysis system as repeated measures over time (SAS version 9.2). Toxicological results were analyzed using GLM procedure of SAS. Significant means were compared using Tukey-Kramer Honestly Significant Difference (HSD) *post-hoc* multiple comparison test ($p \leq 0.05$) (SAS., 2013).

RESULTS

Recovery of imidacloprid: Fortified tomato samples were used for the calculation of recovery percentages of imidacloprid (Table 1). Recovery percentage of imidacloprid in tomato fruits ranged from 103.2-113%. Similar results was reported for imidacloprid, Fernandez-Alba *et al*. (1996) reported that recovery percentage for imidacloprid were 123, 114 and 102% in pepper, tomato and cucumber fruits, respectively. Also, Watanabe *et al*. (2004) found that the average rates of recovery for imidacloprid were 113.3, 88.0, 82.7 and 87.5% in cucumber, eggplant, lettuce and green pepper, respectively. Recovery percentages were used to correct data in residue studies for both insecticides.

Imidacloprid residue amounts in tomato fruits: Data in Table 2 and Fig. 1 show the amounts of imidacloprid residues in tomato fruits after different time intervals of the last foliage applications. The initial deposit (1 h after last spray application) of imidacloprid was 0.316 mg kg^{-1}. Identical amount was reported after 3 days (0.316 mg kg^{-1}) of last spray then it decreased to 0.233, 0.210, 0.139, 0.116 and 0.109 mg kg^{-1} after 5, 7, 10, 14 and 21 days of last spray

Table 1: Percentages of recovery±SD of imidacloprid insecticide from tomato fruits

Amount (µg)		
Added	Measured ±SD	Recovery (%)±SD
3.00	3.0972±0.0131	103.2±0.436
1.50	1.6950±0.0107	113.0±0.719
0.75	0.7896±0.0205	105.3±2.733

Table 2: Degradation pattern of imidacloprid expressed as the amount of residues and the percentages of loss of residues in tomato fruits at 1 h, 3, 5, 7, 10, 14 and 21 day of last spray application

TAA* (days)	Residues (ppm)	SE	Loss of residues (%)
Initial deposit (after 1 h)	0.316	0.090	0.00
3	0.316	0.081	0.00
5	0.233	0.002	26.26
7	0.210	0.002	33.54
10	0.139	0.067	56.01
14	0.116	0.003	63.29
21	0.109	0.010	65.50
$t_{1/2}$ (d)	10.16		

*TAA: Time after last spray application, n = 3 replicates, percentage of loss of residue = (Initial residues-found residues at different time)/(Initial residues))×100

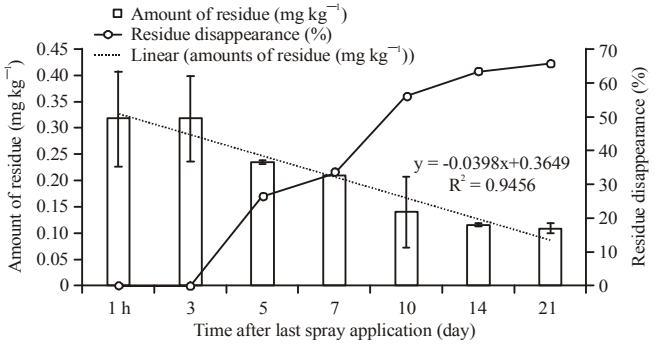

Fig. 1: Disappearance pattern and half-time values of imidacloprid after 1 h, 3, 5, 7, 10, 14 and 21 days of last spray application, $t_{1/2}$ = In2/K = 0.693/K, K = 1/t×ln a/m, where, K is apparent rate constant, t is time in days, m is residue at x time and a is initial residue

application of the insecticide, respectively. Rate of loss of imidacloprid in tomato fruits were 0, 26.26, 33.54, 56.01, 63.29 and 65.50% after 3, 5, 7, 10, 14 and 21 days of the initial deposit, respectively and the half-life time ($t_{1/2}$) of this insecticide was 10.16 days.

Toxicological effects of imidacloprid to male rats: In current study we used imidacloprid (Admire®) to control whiteflies on tomato plants on the basis of 1 foliar spray treatment every 4-7 days for 15 times. After approximately 3 mo of field application of imidacloprid, tomato fruits were sampled and analyzed for imidacloprid residues. Residue amounts that were reported in tomato fruits after 1 h, 7, 14 and 21 days of last spray application and 0.1 LD_{50} were tested against rats. For 45 days, rats were given daily oral doses equal to residue amounts of imidacloprid in tomato fruits and the 0.1 of LD_{50} value. These doses were equal to 0.316, 0.210, 0.116, 0.109 and 42.4 mg kg^{-1} of the technical grade of imidacloprid.

***In vivo* effects of imidacloprid on haematological parameters:** Results in Table 3 show that oral administration of rats to doses equal to residue amounts of imidacloprid after 1 h, 7, 14, or 21 days of last spray did not affect the Packed Cell Volume of blood (PCV %) while it was increased after given rats 42.5 mg kg^{-1} b.wt. day^{-1} (0.1 LD_{50}). All doses of imidacloprid did not affect the RBC counts except doses of 0.316 and 42.5 mg kg^{-1} that decreased it. The WBC numbers were increased after given rats the high doses (0.316 and 42.5 mg kg^{-1} b.wt. day^{-1}) compared to control.

Adverse effects on liver function: Aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) enzyme activities were evaluated in serum of rats treated with doses equal to the residue amounts of imidacloprid in tomato fruits and 0.1 LD_{50} (Table 4). Results showed that the lowest dose of imidacloprid (0.109, 0.116 and 0.21 mg kg^{-1} b.wt. day^{-1}) had no effect on the activity of the tested enzymes. Results revealed that the high doses (0.316 and 42.5 mg kg^{-1} b.wt. day^{-1}) significantly increased ALT and ALP enzyme activities. Only the

Table 3: LS Mean±SE values of haematological parameters; Packed Cell Volume (PCV, %), Red Blood Cell counts (RBCs) and White Blood Cell counts (WBCs) after given rats daily oral doses of imidacloprid for 45 days

TAA[a] (day)	Dose[b]	PCV (%)	RBCs ($\times 10^6$ mm^{-3})	WBCs ($\times 10^2$ mm^{-3})
Control	0.000	38.50±1.25^{bc}	5.09±0.45^{ab}	6.10±0.60^{bc}
21	0.109	36.00±1.25^{c}	6.50±0.45^{a}	5.30±0.60^{c}
14	0.116	35.75±1.25^{c}	5.74±0.45^{a}	5.32±0.60^{c}
7	0.210	36.00±1.25^{c}	5.83±0.45^{a}	5.77±0.60^{b}
1 h	0.316	42.00±1.25^{ab}	4.41±0.45^{c}	7.72±0.60^{a}
0.1 LD_{50}	42.400	44.00±1.25^{a}	4.83±0.45^{bc}	7.70±0.60^{a}

[a]TAA: Time after last spray application, [b]Dose: Daily oral doses in mg active ingredient kg^{-1} body weight (b.wt.) day^{-1}, n = 4 replicates each of 4 animals. Means were compared using Tukey-Kramer honestly significant difference *post-hoc* (HSD) multiple comparison test ($p \leq 0.05$), LS-Means with the same superscript letter are not significantly different

Table 4: Effects of imidacloprid on the activity±SE of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) enzymes in serum of rats administered daily oral sub lethal doses for 45 days

TAA[a] (days)	Dose[b]	AST (U L^{-1})	ALT (U L^{-1})	ALP (U L^{-1})
Control	0.000	21.36±2.04^{b}	22.42±3.64^{c}	49.54±4.61^{c}
21	0.109	22.28±2.04^{b}	16.06±3.64^{c}	49.99±4.61^{c}
14	0.116	20.88±2.04^{b}	20.91±3.64^{c}	54.99±4.61^{c}
7	0.210	21.80±2.04^{b}	18.33±3.64^{c}	56.71±4.61^{bc}
1 h	0.316	22.28±2.04^{b}	35.53±3.64^{b}	63.18±4.61^{b}
0.1 LD_{50}	42.400	24.28±2.04^{a}	42.65±3.64^{ab}	73.98±4.61^{ab}

[a]TAA: Time after last spray application, [b]Dose: Daily oral doses in mg active ingredient kg^{-1} body weight (b.wt.) day^{-1}, n = 4 replicates each of 4 animals. Means were compared using Tukey-Kramer honestly significant difference *post-hoc* (HSD) multiple comparison test ($p \leq 0.05$). LS-Means with the same superscript letter are not significantly different

42.5 mg kg^{-1} b.wt. day^{-1} dose increased the activity of AST while all other tested doses did not show any significant effects on AST activity. Current results showed that imidacloprid induced liver adverse effects.

Adverse effects on content of total protein and glucose: The *in vivo* effects of imidacloprid on total protein (g dL^{-1}) and glucose (mg dL^{-1}) contents were presented in Table 5. Sub-lethal doses of imidacloprid (0.316 and 42.5 mg kg^{-1}) decreased the glucose contents and only the 42.5 mg kg^{-1} dose increased the total protein concentration of blood male rats. Higher doses of imidacloprid (20 mg kg^{-1} day^{-1} for 90 days) increased significantly glucose content of serum of female rats (Abbassy *et al*., 2000). However, Kaur *et al*. (2006) reported that oral administration of imidacloprid did not induce any significant changes in total protein, creatinine and blood sugar levels in treated cow calves.

Adverse effects on kidney function: The effects of imidacloprid on the concentrations of creatinine and uric acid were presented in Table 5. These results revealed that oral doses of imidacloprid did not affect concentration of creatinine or uric acid. Kidney function expressed as creatinine and uric acid levels tended to increase but no significant effect was noticed compared to control.

Adverse effects on cardiac function: The effects of sub-lethal doses of imidacloprid were more pronounced on cardiac function (Table 6). Creatine kinase (CK), γ-glutamyl transferase (GGT) and lactate dehydrogenase (LDH) activities (U L^{-1}) were affected by the imidacloprid insecticide. Imidacloprid doses of 0.109 and 0.166 mg kg^{-1} did not affect CK while residue amounts of 0.210, 0.316 and 42.5 mg kg^{-1} increased its activity compared to control. The GGT enzyme was less affected by imidacloprid doses where only the high dose (42.5 mg kg^{-1}) that reduced its activity

Table 5: Adverse effects of imidacloprid insecticide on the Mean±SE content of total protein, glucose, creatinine and uric acid in the serum of rats administered daily oral doses for 45 days

TAA[a]	Dose[b]	Total protein (g dL^{-1})	Glucose (mg dL^{-1})	Creatinine (g dL^{-1})	Uric acid (mg dL^{-1})
Control	0.000	5.48±0.75[a]	67.74±3.34[a]	0.41±0.06[a]	8.66±0.88[a]
21 days	0.109	5.21±0.75[a]	63.92±3.34[a]	0.42±0.06[a]	8.52±0.88[a]
14 days	0.116	5.04±0.75[a]	59.64±3.34[a]	0.32±0.06[a]	7.67±0.88[a]
7 days	0.210	6.85±0.75[a]	56.76±3.34[ab]	0.56±0.06[a]	9.17±0.88[a]
1 h	0.316	5.21±0.75[a]	42.90±3.34[bc]	0.47±0.06[a]	10.03±0.88[a]
0.1 LD_{50}	42.400	7.42±0.75[a]	35.93±3.34[c]	0.43±0.06[a]	7.06±0.88[a]

[a]TAA: Time after last spray application, [b]Dose: Daily oral doses in mg active ingredient kg^{-1} body weight (b.wt.) day^{-1}, n = 4 replicates each of 4 animals. Means were compared using Tukey-Kramer honestly significant difference *post-hoc* (HSD) multiple comparison test ($p \leq 0.05$). LS-Means with the same superscript letter are not significantly different

Table 6: Adverse effects of imidacloprid insecticide on the activity±SE of enzymes in the serum of rats administered daily oral sub lethal doses for 45 days

TAA[a]	Dose[b]	CK (U L^{-1})	GGT (U L^{-1})	LDH (U L^{-1})
Control	0.000	556.80±51.93[c]	8.11±0.79[ab]	145.12±8.46[a]
21 days	0.109	560.59±51.93[c]	9.21±0.79[a]	99.43±8.46[b]
14 days	0.116	561.38±51.93[c]	6.95±0.79[abc]	98.10±8.46[b]
7 days	0.210	625.59±51.93[b]	5.07±0.79[bc]	86.66±8.46[b]
1 h	0.316	637.97±51.93[b]	5.36±0.79[bc]	94.09±8.46[b]
0.1 LD_{50}	42.400	709.16±51.93[a]	4.92±0.79[c]	74.10±8.46[b]

[a]TAA: Time after last spray application, [b]Dose: Daily oral doses in mg active ingredient kg^{-1} body weight (b.wt.) day^{-1}, n = 4 replicates each of 4 animals. Means were compared using Tukey-Kramer honestly significant difference *post-hoc* (HSD) multiple comparison test ($p \leq 0.05$). LS-Means with the same superscript letter are not significantly different, CK: Creatine kinase GGT: γ-glutamyl transferase and LDH: Lactate dehydrogenase

compared to control. All tested doses significantly decreased the activity of LDH in the serum of treated rats compared to the control. Toor *et al*. (2013) reported no cardiac toxicity of imidacloprid at 1/10th of the LD_{50} oral dose in rats for 30 days.

DISCUSSION

Imidacloprid is reported as a toxic insecticide to plant pollinators (honey bee). Tomato plants has flowers all season around that make it very attractive to honey-bees and other pollinators. So, the application of imidacloprid has severe impacts on non-target organisms (EFSA., 2008; Blacquiere *et al*., 2012). Moreover, movement of imidacloprid from soil to pollen and nectar of squash was reported by Stoner and Eitzer (2012). They reported about 10 ppb when residue was evaluated using the QuEChERS method. Therefore it's important to provide risk assessment studies to monitor adverse effects of this neonicotinoid insecticide in food.

Previous study reported that imidacloprid residues in tomato did not exceed 0.11 mg kg^{-1} after field application in Southern Europe (EFSA., 2008). Our results revealed that the residual amount of imidacloprid after 1 h of last spray application was less than the European Maximum Residual Level (MRL = 0.5 mg kg^{-1}) and the American and Canadian tolerance level (MRL = 1 mg kg^{-1}) (Health Canada, 2013).

Moreover, current study showed significant adverse effects on haematological and biochemical parameters of male rats (PCV (%), RBCs, WBCs, ALT, AST, ALP, total protein, glucose, CK, LDH and GGT) after they were orally giving the neonicotinoid insecticide, imidacloprid (Table 7). All doses of imidacloprid did not affect the RBC counts except doses of 0.316 and 42.5 mg kg^{-1} that decreased it. The WBC numbers were increased after given rats the high doses (0.316 and 42.5 mg kg^{-1} b.wt. day^{-1}) compared to control. Similar results were reported by Ammar *et al*. (2003); they found that 0.1 and 0.25 LD_{50} doses of imidacloprid induced significant increase in total leucocyte count in male albino rats. Oral sub-lethal dose of imidacloprid (0.21 mg kg^{-1}) which was given to rats for 28 days, increased total leukocyte counts (Mohany *et al*., 2012). On the other hand, the sub-acute toxicity of imidacloprid was reported in adult male rats following daily intraperitoneal administration of 20 and 40 mg kg^{-1} daily for 28 days had no effect on PCV (%) and total erythrocyte counts (Jain *et al*., 2006). Also, when female rats administered sub-lethal doses up to 20 mg kg^{-1} b.wt. day^{-1} for 90 days no adverse effects on blood parameters (Bhardwaj *et al*., 2010).

The high doses (0.316 and 42.5 mg kg^{-1} b.wt. day^{-1}) of imidacloprid significantly increased ALT and ALP enzyme activities while only the 42.5 mg kg^{-1} b.wt. day^{-1} dose increased the activity of

Table 7: Summary of statistical analysis of tested haematological and biochemical parameters

Parameters	R^2	CV	F value	P>F
Packed Cell Volume (PCV%)	78.95	4.66	12.86	<0.0001
Red Blood Cells (RBCs)	64.36	16.08	6.19	0.0003
White Blood Cells (WBCs)	70.78	17.00	8.31	<0.0001
Alanine transaminase (ALT)	87.46	23.00	23.90	<0.0001
Aspartate transaminase (AST)	44.38	18.98	2.74	0.0308
Alkaline phosphatase (ALP)	39.06	18.53	2.20	0.0412
Total protein	48.20	23.52	3.19	0.0156
Glucose	85.30	13.71	19.90	<0.0001
Creatine kinase (CK)	34.84	16.71	1.83	0.0268
Lactate dehydrogenase (LDH)	69.28	17.51	7.78	<0.0001
γ-glutamyl transferase (GGT)	65.43	26.74	6.49	0.0002
Creatinine	32.89	26.16	1.68	0.1617
Uric acid	31.90	20.16	1.61	0.1818

AST. Our results along with previous ones suggested that imidacloprid induced liver adverse effects. Imidacloprid induced toxic effects at low doses for example Mohany *et al*. (2012) found that low dose of 0.21 mg kg^{-1} b.wt. day^{-1} for 28 days significantly increased levels of ALT, AST and ALP. Also, Bhardwaj *et al*. (2010) reported increased activity of serum AST and ALT after orally-administering rats with 20 mg kg^{-1} day^{-1} for 90 days. Also, imidacloprid at 1/50th of LD_{50} dose did not cause any histopathological changes in liver but it significantly increase the AST, ALT and ALP in the treated rats for 30 days (Toor *et al*., 2013). One oral dose of imidacloprid equal to 20 mg kg^{-1} b.wt. induced elevation of ALT and AST in blood of female rats (Kapoor *et al*., 2014). Monitoring imidacloprid and its metabolites (6-chloro nicotinic acid and 6-hydroxy nicotinic acid) were analyzed in brain, liver, kidney and ovary organs and blood and urine showed that the maximum concentration of imidacloprid and metabolites in each organ and bodily fluid occurred after 12 h (Kapoor *et al*., 2014). While Vohra *et al*. (2014) reported that when given female rats oral doses of 10 and 20 mg kg^{-1} b.wt. for 60 days did not induce any effects on ALT and AST levels but the high dose (20 mg kg^{-1} b.wt.) showed histopathological effects.

In conclusions, toxicological results reported herein revealed that imidacloprid residue amounts that were measured in tomato fruits after 14 and 21 days of the last spray did not cause any adverse effects on liver, heart and kidney functions of rats given daily oral doses for 45 days. In addition this treatment did not induce any deleterious effect on rats' haematological parameters; Packed Cell Volume (PCV), Red Blood Counts (RBC's) and White Blood Cell counts (WBC's). However, the most severe toxic effect was noticed by orally-administering rats with equal doses to the residue level after 1 h and 7 days of last application of imidacloprid and its 0.1 LD_{50} dose compared to the control.

The severity of imidacloprid damaging action depends entirely on the concentration of the given dose. The damage in liver, heart and kidney functions that we reported herein was supported by previous studies (Abbassy *et al*., 2000; Dere and Polat, 2001). Generally, based on current results the residual toxicity of imidacloprid was found to be not a threat because of residue amounts after 7 days and later did not induce significant adverse effects to tested parameters of rats.

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