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Effect of Imidacloprid and Tetraconazole on Various Hematological and Biochemical Parameters in Male Albino Rats (*Rattus Norvegious*)

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Abstract

This study was carried out to investigate the toxicity of imidacloprid and tetraconazole in male rats given daily oral doses of these pesticides (for 30 days). Which equal to those residues found in and on cucumber fruits after zero time, (24 hour after application) as well as two doses which equal 0.1, 0.125 of the LD50 value for each pesticide? These doses were (0.943, 0.365, 45, 56.25 mg/kg) for imidacloprid, and (0.174, 0.104, 124.8, 156 mg/kg) for tetraconazole, respectively.Results indicated that imidacloprid and tetraconazole residues on and in cucumber fruits after one hour and 24 hour from the last spray did not cause any significant effects on the activities of AST, ALT, GGT, LDH and ALP enzymes. There is no significant difference on the levels of creatinine, uric acid, total protein, albumin and glucose in the serum of treated rats. There is no effect on Packed Cell Volume (PCV), Red Blood Cell Counts (RBC's), haemoglobin (Hb) and White Blood Cell Counts (WBC's) in the blood of treated rats. Also doses equal to the residues did not cause any harmful effect on concentrations of AChE, T3, T4, TSH and testosterone hormone. The aforementioned results of this part of study indicated that the neonicotinoid insecticide, imidacloprid, and the fungicide tetraconazole, can induce a variety of alterations in the activities of AST, ALT, GGT, LDH and ALP enzymes and the levels of creatinine, uric acid, total protein, albumin and glucose in the serum of treated rats also the concentrations of AChE, T3, T4, TSH and testosterone hormone at higher doses which equal to 0.125 and 0.1of the LD50value for each pesticide. The severity of their action depends entirely on the level of the given doses and the type of the tested pesticides.

Keywords: Imidacloprid; Tetraconazole; Haematological; Biochemical parameters; Hormones; Rats

Introduction

Tetraconazole [(RS)-2-(2, 4-dichlorophenyl)-3-(1H-1, 2, 4-triazole-1-yl) propyl 1, 1, 2, 2-tetrafluoroethyl ether] belongs to the azole group of chemicals and has low acute toxicity. It is broad-spectrum systemic fungicide. It has been registered in Egypt and various countries [1]. This fungicide is steroid demethylation inhibitors acting mainly on the vegetative stages of fungi by blocking the mycelial growth either inside or on the surface of the host plant. Tetraconazole is effective in controlling a broad spectrum of diseases such as powdery mildew and scab on fruit [2]. Imidacloprid [1-(6-chloro-3-pyridylmethyl)-Nnitroimidazolidin-2-ylidene-amine] is an extensively used insecticide for crop protection in the world wide from the last decade due to its low soil persistence and insecticidal activity at low application rate [3,4]. It is fastest growing in sales as insecticide globally because of its low selectivity for insects and apparent safety for humans [5-7]. Its selective toxicity result from its high affinity to insects nicotinic acetylcholine receptors compared to mammals [3,6,8]. Reactive Oxygen Species (ROS) may be involved in the toxicity of various pesticides [9]. Hence, exposure to these insecticides may involve a large segment of the population, which includes agriculture workers and their families, those living in proximity to farms/orchards, and the general population who may be exposed through home application of pesticides or via residues on food Bradman et al. [10-12]. Since the literature concerning the toxicological studies of these pesticides, therefore the present study was carried out to investigate the toxicological effects on hematological and biochemical parameters in adult rats following oral administration by different doses of each pesticide for 30 days, which equal to those residues found in and on cucumber fruits after zero time, (24 hour after application) as well as two doses which equal 0.1, 0.125 of the LD_{50} value for each pesticides. These doses were (0.943, 0.365, 45, 56.25 mg/kg) for imidacloprid, and (0.174, 0.104, 124.8, 156 mg/kg) for tetraconazole, respectively. Imidacloprid is moderately toxic. The oral dose of technical grade imidacloprid that resulted in mortality to half of the test animals (LD_{50}) is 450 mg/kg body weight in rats, and 131 mg/kg in mice. The 24-hour dermal LD_{50} in rats is >5,000 mg/kg. It is considered non-irritating to eyes and skin (rabbits), and non-sensitizing to skin (guinea pigs). Some granular formulations may contain clays as inert ingredients that may act as eye irritants. In acute inhalation toxicity tests with rats, the airborne concentration of imidacloprid that resulted in mortality to half of the test organisms (LC_{50}) is >69 mg/meters cubed air in the form of an aerosol, and >5323 mg/meters cubed air in the form of dust. These values represent the maximum attainable airborne concentrations.

Materials and Methods

Chemicals and bioassay tests

Pesticides used: Imidacloprid (Admire 20% S.C), 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine, Bayer Company (Germany) and *Tetraconazole* (Domark 10% EC), Isagro Company (USA).

Reagents: Kits of ALT/GOT, AST/GPT, ALP, LDH, albumin, total protein, glucose and AChE product by Medical Device Safety Services MDSS GmbH Burckhardtstr 1 Hannover, Germany, were obtained from Vitro Scient, El-Nozha El-Gedida, Heliopolis, Cairo,

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Egypt. Kits of testosterone and Tetraiodothyronine-Thyroxine (T4), Triiodothyronine (T3) and Thyroid Stimulating Hormone (TSH) were obtained from Bio-diagonistic, Dokki, Giza, Egypt.

Different assays were carried out using kits and assay methods were carried out as follow. Counting of red blood corpuscles (RBC's) and White blood cell counts (WBC's) Britton [13] and Seiverd [14], Haemoglobin Eilers [15] Blood glucose measurements Trinde [16], Alanine amino transferase (ALT/GOT) and Aspartate aminotransferase (AST/GPT) Reitman and Frankel [17]. Alkaline phosphatase (ALP) Rec [18], gamma-glutamyl transferase (GGT) Szasz [19] and Whitfield [20] and IFCC [21], Lactate dehydrogenase (LDH) GSCC [22] Albumin Doumas [23] Total protein (TP) Henry [24] Blood glucose measurements Trinder [16] Creatinine Henry [25] uric acid concentrations Barham and Trinder [26] and Fossati et al. [27] Cholinesterase (ChE) Waber [28] Testosterone hormone concentration Ismail et al. [29] and Wisdom [30] and Rajowski et al. [31] and Joshi et al. [32] and Turkes et al. [33] Triiodothyronine (T3) Hormone Burke and Eastman [34] and Utiger [35] and Young et al. [36] and Spector et al. [37] and Cavalieri and Rapoport [38] Thyroxine (T4) Hormone Skelley et al. [39] and Ravel [40] and Robbins [41] and Wistom [30] and Schuurs et al. [42]. Thyroid Stimulating Hormone (TSH) Utiger [43] and Pierce [44] and Burger and Patel [45].

Animals: Male albino rats (*Rattus norvegious*) with an average weight of 100-120 gm were supplied from breeding culture located in the Animal Health Research Center (Cairo).Rats were housed 8/cage and maintained for 25 days' acclimatization prior treatments, and were allowed free access to water and fed on adequate stable diet.

Experiments

Repeated oral dose treatment: Eight groups of male albino rats (4 groups for each pesticide), each consist of 4 rats and received daily oral dose (for 30 days). Doses of imidacloprid equal to the residue found in treated cucumber fruits after 0(1hr), and 24 hr (1 day) from the application of cucumber with the recommended rate of each pesticide, also the treatment with concentrations of 0.125 LD_{50} and 0.1 LD_{50} which equal 0.943, 0.365, 56.25 and 45 mg a.i/kg b.w) respectively. Doses of tetraconazole equal to the residue found in treated cucumber fruits after 0 (1 hr), and 1 day from the application of cucumber with the recommended rate of each pesticide, in addition to the treatment with concentrations of 0.125 LD_{50} and 0.1 LD_{50} which equal 0.174, 0.104, 156 and 124.8 mg a.i/kg b.w) respectively. Two groups of rats given orally the same volumes of water were considerd to be as a control (untreated groups).

Collecting samples: The animals were starved overnight for 12h before blood was collected. Rats were anaesthetized with light ether and venous blood samples were collected by direct heart puncture into sterilized vials. Blood samples allowed setting to clot at 4°C and centrifuged at 1000 g for 30 min. Then 1000 µl aliquots of serum were placed in microfuge tubes and frozen on dry ice. Labeled bags were placed into a -20°C freezer until the time of the assay.

Haematocrit value (Packed cell volume): The haematocrit value determination was done according to Bull et al. [46] and Bull et al. [47] using microhaematocrit centrifuge Model SH120. Wintrobehaematocrit heparinized tubes were packed with blood samples at 2/3 of its volume and stopped at one of its end. Haematocrit tubes contained blood samples were centrifuged at 12000 r.p.m. for seven minutes. Haematocrit value was obtained by reading the packed cell volume on a special graduated haematocrit measurement. The obtained data were expressed as percentages of haematocrit value to the total blood volume.

Statistical analysis

All obtained data were statistically analyzed using Statistical analysis (SAS) software program [48]. Data were analyzed as factorial arrangement of kind of emulsifying and storage period in complete randomized design with three replicates. Comparisons among the means of different treatments were achieved using the least significant difference procedure (LSD) at P=0.05 and 0.01 level as illustrated by Al-Rawi and Khalaf-Allah [49].

Results and Discussions

In vivo effect of imidacloprid and tetraconazole on haematological parameters in treated rats

Results recorded in Tables 1 and 2 showed that the haematological effects in rats given daily oral doses of imidacloprid (0.365, 0.943, 45 and 56.25 mg/kg/day) or tetraconazole (0.104, 0.174, 124.8 and 156 mg/kg/day). Tetraconazole at the low dose (0.104 mg/kg) did not affect the packed cell volume of blood (PCV), while at high doses (0.174, 124.8 and 156 mg/kg a.i./day) caused significant reduction in PCV as compared with controls. In case of imidacloprid, results show that oral administration of the low doses (0.365, 0.943 mg/kg) did not affect the PCV. However, the high doses (45 and 56.25 mg/kg) caused significant reduction of PCV when compared with control. These results were in agreement with those of Jain et al. [50] who found that the sub-acute toxicity of imidacloprid in adult male rats following intraperitoneal administration of 20 and 40 mg/kg daily for 28 days resulted with no effect on packed cell volume on both dose levels. Also, the triazole, fungicide, tetraconazole with the two high doses of 124.8,156 mg a.i./kg b.w/day resulted with no significant increase of RBC's counts, since it gave 6.21 and 6.32 M/mm^3. On the other hand, oral administration

Doses of Imidacloprid (mg a.i/.kg b.w.)	Hematological parameters			
	WBC (x 10^3/mm^3)	RBC (M/mm^3)	Hb (g/dl)	PCV %
0.00 (control)	5.34 ± 0.23	6.23 ± 0.41	14.07 ± 0.56	40.55 ± 0.23
0.365	5.35 ± 0.25	6.34 ± 0.42	14.12 ± 0.46	40.23 ± 1.11
0.943	5.32 ± 0.15	6.45 ± 0.38	14.05 ± 0.81	39.86 ± 0.79
45	6.02 ± 0.41*	6.57 ± 0.31	14.10 ± 0.67	38.72 ± 1.34*
56.25	6.34 ± 0.31*	6.68 ± 0.27*	12.45 ± 0.59*	37.45 ± 1.23*
LSD	0.43	0.16	1.04	1.34

Each value is a mean ± SD; n=8; Statistical difference from the control: *significant at P ≤ 0.05

Table 1: Hematological effects of Imidacloprid in male albino rats given daily oral doses for 30 days.

Doses of tetraconazole (mg a.i/.kg b.w.)	Hematological parameters			
	WBC (x 10^3/mm^3)	RBC (M/mm^3)	Hb (g/dl)	PCV %
0.00 (control)	5.59 ± 0.23	6.13 ± 0.37	14.12 ± 0.30	41.07 ± 0.41
0.104	5.54 ± 0.36	6.09 ± 0.42	14.08 ± 0.27	40.50 ± 1.11
0.174	5.41 ± 0.17	6.15 ± 0.38	14.23 ± 0.59	38.36 ± 0.79*
124.8	7.10 ± 0.09*	6.21 ± 0.26	12.37 ± 0.52*	36.93 ± 1.24*
156	7.48 ± 0.46*	6.32 ± 0.24	11.34 ± 0.61*	35.83 ± 1.08*
LSD	0.51	0.08	1.21	1.57

Each value is a mean ± SD; n=8; Statistical difference from the control: *significant at P ≤ 0.05

Table 2: Hematological effects of tetraconazole in male albino rats given daily oral doses for 30 days.

Doses of imidacloprdmg (a.i/.kg b.w.)	AST (U/L)		ALT(U/L)		GGT (U/L)		ALP (U/L)		LDH (U/L)	
	Mean ± SD	% control	Mean ± SD	% control	Mean ± SD	% control	Mean ± SD	% control	Mean ± SD	% control
0.00 (control)	34.64 ± 0.62	100.00	36.08 ± 0.70	100.00	0.44 ± 0.03	100.00	54.95 ± 6.35	100.00	162.4 ± 3.36	100.00
0.365	34.73 ± 0.51	100.26	35.94 ± 0.45	99.72	0.43 ± 0.03	97.73	54.94 ± 2.90	99.98	163.0 ± 4.67	100.33
0.943	34.90 ± 0.62	100.75	35.98 ± 0.67	99.72	0.46 ± 0.05	104.55	55.30 ± 3.48	100.65	161.7 ± 3.44	99.54
45	35.71 ± 0.50*	103.09	38.78 ± 0.24*	107.48	0.47 ± 0.05	106.82	55.92 ± 9.19	101.77	181.3 ± 1.98*	111.61
56.25	37.22 ± 0.95*	107.45	38.78 ± 0.24*	107.48	0.52 ± 0.07*	118.18	62.74 ± 0.39*	114.18	191.5 ± 4.16*	117.92
LSD	1.13		1.02		0.08		6.13		10.99	

Each value is a mean ± SD; n=8; Statistical difference from the control: *significant at P ≤ 0.05

Table 3: *In vivo* effect of Imidacloprid on some biochemical targets in the serumof male albino rats given repetitive doses for 30 days.

Doses of tetraconazole (mg a.i/.kg b.w.)	AST(U/L)		ALT(U/L)		GGT (U/L)		ALP (U/L)		LDH (U/L)	
	Mean ± SD	% control	Mean ± SD	% control	Mean ± SD	% control	Mean ± SD	% control	Mean ± SD	% control
0.00 (control)	34.78 ± 0.57	100.00	35.47 ± 0.81	100.00	0.46 ± 0.01	100.00	55.92 ± 9.19	100.00	163.69 ± 2.70	100.00
0.104	34.90 ± 0.62	100.35	35.85 ± 0.55	101.07	0.46 ± 0.01	100.00	55.27 ± 4.41	98.84	162.47 ± 3.36	99.25
0.174	34.77 ± 0.65	99.97	35.91 ± 0.45	101.24	0.45 ± 0.02	97.83	54.92 ± 5.19	98.21	162.53 ± 6.49	99.29
124.8	38.11 ± 0.26*	109.57	36.87 ± 0.60*	103.95	0.52 ± 0.03*	113.04	63.92 ± 9.19*	114.30	193.58 ± 4.16*	118.26
156	38.79 ± 0.37*	111.53	37.74 ± 1.86*	106.40	0.57 ± 0.05*	123.91	65.43 ± 3.79*	117.01	203.93 ± 6.49*	124.58
LSD	1.32		1.54		0.05		7.12		10.99	

Each value is a mean ± SD; n=8; Statistical difference from the control: *significant at P ≤ 0.05

Table 4: *In vivo* effect of Tetraconazole on some biochemical targets in the serum of male albino rats given repetitive doses for 30 days.

of imidacloprid at doses of (0.943, 0.365 and 45 mg a.i./kg/day) to rats has no significant changes in RBC's, but the dose at 56.25 mg a.i./kg/day caused significant increase in RBC's counts. These results were in agreement with Shipra Bhardwaj et al. [51] who found that after 90 days of oral administration of imidacloprid in female rats with doses of 0, 5, 10 and 20 mg/kg/day result indicated that no changes were observed in RBC of the treated animals when compared with control. Also theseresult was in agreement with Jain et al. [50] who found that the sub acute toxicity of imidacloprid in adult male rats following intraperitoneal administration with 20 and 40 mg/kg daily doses for 28 days no changes were observedon total erythrocyte counts on both dose levels. In contrast, 1, 2, 4-triazole-Hematological changes, including slightly decreased hemoglobin and/or hematocrit, have also been seen in multiple studies and species in rats at doses of 33 mg/kg/day and above [52]. Also The hematology data did not indicate an apparent treatment-related effect in subchronic or prechronic exposure/Five Crl:CD rats/sex/group were dosed orally by gavages with 0, 70, 200 or 500 mg/kg/day of M 14360 Technical (purity: 92%) for 4 weeks. All of the animals in the 500 mg/kg group and 3 females in the 200 mg/kg group [53]. Oral administration of the two high doses of pesticides, tetraconazole and imidacloprid increase in WBC counts compared with control. On contrary, the two low doses of each compound did not change the WBC counts when compared with control. On the other hand, the two high doses of tetraconazole and the highest dose of imidacloprid caused significant decrease of Haemoglobin (Hb) compared with control values (Tables 1 and 2). The previous results were in agreement with those of Jain et al. [50] who found that imidacloprid, did not cause any significant changes in lymphocyte, neutrophil and eosinophil counts in treated rats. Shipra Bhardwaj et al. [51] found that 90 days of oral administration of imidacloprid in female rats with doses of 0,5,10 and 20 mg/kg/day resulted had no observed changes in WBC of the treated animals as compared withcontrol [54] investigated the effects of tribenuron-methyl on the haematological profile of male albino rats. Tribenuron-methyl was orally administered either at different single or repetitive doses of 5, 25, 50 and 100 mg/kg b. w. resultindicated that, the hematological parameters were insignificantly changed following single oral dosing of either technical or formulated herbicide except the highest dose level (100 mg/kg) of formulated form which case significant decrease in WBC's counts. However, in repetitive doses, such effect was more pronounced. RBC's counts, PCV, MCV, and Hb were significantly decreased. However, WBC's counts insignificantly increased. The haematological parameters were significantly adversely affected following the high dosing level (100 mg/kg b. w.) of either formulated or technical tribenuron-methyl.

In vivo effects of imidacloprid and tetraconazole on the activity of some enzymes in serum of treated rats

The results of serum biochemical parameters of male rats orally administered different doses of imidacloprid for 30 days are shown in Table 3. There were no significant differences in the activity of serum AST, ALT, GGT, ALP and LDH in rats given 0.365 and 0.943 mg/kg/day doses as compared with control. However, a significant increase was noted in the same enzymes in rats given 45 and 56.25 mg/kg/day doses. The previous results were in agreement with those of Shipra Bhardwaj et al. [51] they found that 90 days oral toxicity of imidacloprid in female rats with doses of 5,10 and 20 mg/kg/day caused significant elevation of serum GOT, GPT, glucose and BUN and decreased the activity of AChE in serum and brain. As regards to tetraconazole, there were no significant changes in the activity of the serum enzymes, AST, ALT, GGT, ALP and LDH in the rats given 0.104 and 0.174 mg/kg/day doses as compared with control. However, the high doses, 124.8 and 156 mg/kg/day caused significant increase in the activity of all the tested enzymes as shown in Table 4. These results were in agreement with those of Australian Pesticides and Veterinary Medicines Authority [52]. In this report, male rats received 0, 2, 5, 15 or 40 mg/kg of tetraconazole in the diet for 4 weeks. Did not show any changes in plasma enzymes at the low doses 2, 5, 15 mg/kg and significant increase in the activity of plasma AST and glutamate dehydrogenase at 40 mg/kg. California EPA [53] in the report the serum GOT and GDH activities of the 40 mg/kg group were elevated above those of the control. Also this resulton contrary with another study of Australian

Doses of imidacloprid (mg a.i/.kg b.w.)	Creatinine (mg/dl)		Uric acid (mg/dl)		Total protein (g/dl)		Albumin (g/dl)		Glucose (mg/dl)	
	Mean ± SD	% control	Mean ± SD	% control	Mean ± SD	% control	Mean ± SD	% control	Mean ± SD	% control
0.00 (control)	0.76	100.00	5.70	100.00	6.77	100.00	4.99	100.00	65.54	100.00
0.365	0.77	101.32	5.72	100.35	6.81	100.59	4.96	99.40	64.23	98.00
0.943	0.77	101.32	5.77	101.22	6.80	100.44	4.98	99.80	63.29	96.57
45	0.81	106.58	6.34	111.22	7.70	113.74	4.93	98.79	58.34	89.01
56.25	0.80	105.26	6.71	117.71	8.87	131.02	4.03 ±	80.76	52.34	79.86
LSD	0.04		0.28		0.17		0.88		5.57	

Each value is a mean ± SD; n=8; Statistical difference from the control: *significant at P ≤ 0.05

Table 5: Effect of Imidacloprid on the level of Creatinine, Uric acid, Total protein, Albumin and Glucose in the serum of male albino rats given repetitive doses for 30 days.

Doses of tetraconazole (mg a.i/.kg b.w.)	Creatinine (mg/dl)		Uric acid (mg/dl)		Total protein (g/dl)		Albumin(g/dl)		Glucose (mg/dl)	
	Mean ± SD	% control	Mean ± SD	% control	Mean ± SD	% control	Mean ± SD	% control	Mean ± SD	% control
0.00(control)	0.74	100.00	5.67	100.00	6.65	100.00	4.95	100.00	66.36	100.00
0.104	0.73	98.65	5.81	102.47	6.68	100.45	4.94	99.80	66.01	99.47
0.174	0.71	95.95	5.59	98.59	6.72	101.05	4.95	100.00	64.18	96.71
124.8	0.84	113.51	6.87	121.16	7.83	117.74	3.93	79.39	52.13	78.56
156	0.87	117.57	7.11	125.40	8.54	128.42	3.43	69.29	48.98	73.81
LSD	0.06		0.31		0.15		0.35		5.71	

Each value is a mean ± SD; n=8; Statistical difference from the control: *significant at P ≤ 0.05

Table 6: Effect of Tetraconazole on the level of Creatinine, Uric acid, Total protein, Albumin and Glucose in the serum of male albino rats given repetitive doses for 30 days.

Pesticides and Veterinary Medicines Authority [52]. Rats received 0, 10, 60 or 360 mg/kg of tetraconazole in the diet for 13 weeks. Slightly lower AP, ALT and AST, and slightly higher cholesterol and calcium levels (males) were observed at 360 mg/kg, and changes also occurred at 60 mg/kg.

In vivo effects of imidacloprid and tetraconazole on the levels of total protein, creatinine, uric acid, albumin and glucose in the serum of treated rats

The effects of imidacloprid on the levels of total protein, creatinine, uric acid, albumin and glucose in the serum of treated rats are recorded in Table 5. These results revealed that the low doses of imidacloprid (0.365, 0.943 mg a.i/kg b.w/day) did not cause any significant difference in the concentration of the tested parameters in the serum of treated rats. However, the higher doses of pesticide (45 and 56.25 mg a.i/kg b.w/day) increased significantly the levels of total protein, creatinine and uric acid as compared with control. In contrast, these doses cause significant reduction in the levels of albumin and glucose as compared with control. These results in contrary with those of Barinderjit et al. [55] who reported that oral administration of imidacloprid failed to induce any significant changes in the levels of total protein, creatinine and blood sugar in treated cow calves. Also, Jain et al. [50] found that imidacloprid did not produce any significant changes in both haematological and biochemical indicates after 28 days of administration. In contrary of this study, they found that imidacloprid decrease in the level of creatinine on 28th day. The results of the present study are in contrast with Srivastava and Rampal [56,57] and Jain et al. [58] whom found an increase in blood glucose levels. Also, the present results are in agreement with those of Shallan et al. [59] who found significant decrease in creatinine and cholinesterase (ChE) of male rats plasma after feeding on broad bean dry seeds.The in vivo effects of tetraconazole on the levels of creatinine, uric acid, total protein, albumin and glucose in the serum of rats given different oral doses are recorded in Table 6. There were no significant change in the concentration of creatinine, uric acid, total protein, albumin and glucose in the serum of rats given oral doses 0.104 and 0.174 mg/kg/day doses as compared with control. However, the administration of high doses (124.8 and 156 mg/kg/day) caused significant increase in the levels of total protein, creatinine and uric acid. In contrast, these doses caused significant decrease in the levels of albumin and glucose in the serum of rats as compared with control. The previous results in agreement with those recorded in California EPA [53]. In the clinical chemistry evaluation, the mean serum glucose levels were lower for both sexes in the 70 mg/kg group and for the males in the 200 mg/kg group ($p<0.01$ or 0.001). The total protein and albumin levels of the males in the 70 and 200 mg/kg group were greater than that of the control ($p<0.05$ or 0.01).

In vivo effects of imidacloprid and tetraconazole on the activity of T_3, T_4, Testosterone, and TSH in the serum of treated rats

The effects of, imidacloprid on the concentration activity of T_3, T_4, Testosterone, and TSH in serum of treated rats are given in Table 7. These results revealed that after 30 days, no differences were observed in the concentration of T_3, T_4, Testosterone, and TSH in the serum of rats given the low doses (0.365, 0.943 mg/kg a.i./day) as compared with control. On contrary imidacloprid caused significant reduction in the concentration of T_3, T_4, Testosterone, and TSH in the serum of rats given high doses (45, 56.25 mg/kg a.i. /day). In the same tableimidacloprid produced significant decrease in the concentration of T_4 with percentages 83.50 and 71.57% of control. Also, imidacloprid induced significant decrease in the concentration of Testosterone with percentages 92.37 and 91.31% of controls, and 92.31, 90.77% of control, respectively.Marzouk et al. [60] described the reproductive effects of tribenuron-methyl on male albino rats. Tribenuron-methyl was orally administered in single or repetitive doses. For single dose treatments of technical and formulated forms, testosterone concentration (ng/ml) was insignificantly affected ($P<0.05$). Whereas this effects in repetitive doses were more pronounced,as well as the treatments with 25 and 50 mg/kg formulated and 50 and 100 mg/kg technical, tribenuron-

Doses of imidacloprid (mg a.i/.kg b.w.)	T3 (ng/dl)		T4 (µg/dl)		Testosterone (ng/ml)		TSH (mIU/L)	
	Mean ± SD	% control	Mean ± SD	% control	Mean ± SD	% control	Mean ± SD	% control
0.00 (control)	223.57	100.00	3.94	100.00	4.72	100.00	0.65	100.00
0.365	220.67	98.70	3.86	97.97	4.93	104.45	0.64	98.46
0.943	229.13	102.49	3.90	98.98	4.75	100.64	0.64	98.46
45	211.34	94.53	3.29	83.50	4.36	92.37	0.60	92.31
56.25	197.56 ± 0.89*	88.37	2.82 ± 0.08*	71.57	4.31 ± 0.17*	91.31	0.59 ± 0.02*	90.77
LSD	7.89		0.48		0.33		0.05	

Each value is a mean ± SD; n=8; Statistical difference from the control: * significant at P ≤ 0.05

Table 7: *In vivo* effect of Imidacloprid on some biochemical targets in the serum of male albino rats given repetitive doses for 30 days.

Doses of tetraconazole (mg a.i/.kg b.w.)	T3 (ng/dl)		T4 (µg/dl)		Testosterone (ng/ml)		TSH (mIU/L)	
	Mean ± SD	% control	Mean ± SD	% control	Mean ± SD	% control	Mean ± SD	% control
0.00 (control)	224.12 ± 1.98	100.00	4.11 ± 0.38	100.00	4.75 ± 0.51	100.00	0.68 ± 0.07	100.00
0.104	229.68 ± 1.90	102.48	4.02 ± 0.38	97.81	4.74 ± 0.26	99.79	0.63 ± 0.21	92.65
0.174	227.45 ± 2.89	101.48	3.99 ± 0.26	97.08	4.78 ± 0.13	100.63	0.67 ± 0.11	98.53
124.8	198.67 ± 3.11*	88.64	2.96 ± 0.54*	72.01	4.31 ± 0.46*	90.74	0.53 ± 0.09*	77.94
156	186.54 ± 2.31*	83.23	2.67 ± 0.05*	64.96	4.27 ± 0.21*	89.89	0.50 ± 0.04*	73.53
LSD	8.93		0.52		0.29		0.09	

Each value is a mean ± SD; n=8; Statistical difference from the control: *significant at P ≤ 0.05

Table 8: *In vivo* effect of Tetraconazole on some biochemical targets in the serum of male albino rats given repetitive doses for 30 days

Imidacloprid			Tetraconazole		
Doses of imidacloprid (mg a.i/.kg b.w.)	AChE (U/L)		Doses of tetraconazole (mg a.i/.kg b.w.)	AChE (U/L)	
	Mean ± SD	% control		Mean ± SD	% control
0.00 (control)	1171.22 ± 2.38	100.00	0.00 (control)	1175.18 ± 2.06	100.00
0.365	1170.93 ± 7.62	99.97	0.104	1173.45 ± 8.56	99.85
0.943	1171.39 ± 3.61	100.01	0.174	1169.56 ± 9.56	99.52
45	1102.49 ± 3.71*	94.13	124.8	1125.78 ± 12.93*	95.79
56.25	1035.60 ± 4.91*	88.42	156	1119.57 ± 7.89*	95.27
LSD	27.04		LSD	30.28	

Each value is a mean ± SD; n=8; Statistical difference from the control: *significant at P ≤ 0.05

Table 9: Acetylcholinesterase inhibition of serum of male albino rats given imidacloprid and tetraconazole.

methyl were significant at (P<0.05). Also, treatment with 100 mg/kg formulated tribenuron-methyl caused highly significant increase in serum testosterone concentration (ng/ml) as compared with control group. Results in Table 8 show that tetraconazole had no significant change in the concentration of T_3, T_4, Testosterone and TSH in the serum of rats given oral low doses of (0.104, 0.174 mg/kg a.i./day) from tetraconazole. However, high doses of tetraconazole (124.8, 156 mg/kg/day) caused significant reduction in the concentration of T_3 with percentages of 88.64, 88.23%; T_4 with percentages of 72.01, 64.96%; Testosterone with percentages of 90.74, 89.89% and TSH with percentages of 77.94, 73.53% comparable to control.

In vivo effects of imidacloprid and tetraconazole on the activity of AChE

Oral administration of imidacloprid (45, 56.25 mg/kg/day) and tetraconazole (124.8, 156 mg/kg/day) to rats for 30 days resulted in 5.9-11.6% and 4.2-4.7% inhibition of AChE activity in the serum of treated rats, respectively. The inhibition of AChE was dose dependent but inhibition was not significant at 0.365, 0.943 mg/kg/day from imidacloprid and at 0.104, 0.174 mg/kg/day from tetraconazole doses. However, significant AChE inhibition was observed at the two high doses of each pesticide as shown in Table 9. These finding are in agreement with those obtained by Vodela and Dalvi [61] and Bayoumi et al. [62].

Imidacloprid is quickly and almost completely absorbed from the gastrointestinal tract, and eliminated via urine and feces (70-80% and 20-30%, respectively, of the 96% of the parent compound administered within 48 hours). The most important metabolic steps include the degradation to 6-chloronicotinic acid, a compound that acts on the nervous system as described above. This compound may be conjugated with glycine and eliminated, or reduced to guanidine.

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