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Individual and combined toxicity effect of indoxacarb and glyphosate on general performance and hematological parameters in Japanese quails

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Abstract
The present study was conducted to assess the general performance, clinical and the hematological observations in sub-acute toxicity of indoxicarb and glyphosate in Japanese quails. Two week old, 252 Japanese quails after one week acclimatization were divided into seven equal groups and group C served as control, whereas group T1 was fed with feed + indoxacarb @ 30 mg/kg of feed, group T2 was fed with feed + indoxacarb @ 60 mg/kg of feed, group T3 was fed with feed + glyphosate @ 250 mg/kg of feed, group T4 was fed with feed +glyphosate @ 500 mg/kg of feed, group T5 was fed with feed + indoxacarb@ 30 mg/kg of feed +glyphosate @500 mg/kg of feed and group T6 was fed with feed +indoxacarb @ 60 mg/kg of feed + glyphosate @ 250 mg/kg of feed for a period of four weeks. The clinical signs observed in combination group were more prominent compared to birds fed with individual indoxacarb or glyphosate at higher doses. Decreases in the body weight in quails was observed in all treatment groups but glyphosate causes significant decrease in body weight compared to indoxacarb. There was marginal reduction in feed intake in quails receiving high dose of indoxacorb and glyphosate alone and combination treated groups. The non-significant alteration in hematological paramenters indicated low toxic minimal adverse toxicity effect of indoxacarb or glyphosate either singly or in combination in Japanese quails for a period of 28 days.

Keywords: glyphosate, hematology, indoxacarb, Japanese quails, toxicity

Introduction

Indoxacarb (S)-methyl 7-chloro-2, 5- dihydro-2-[(methoxycarbonyl) [4 (trifluoromethoxy) henyl] amino]-carbonyl] indeno [1, 2-e] [1, 3, 4] oxadiazine-4a-(3H)-carboxylate (C22H17ClF3 N3O7) is a new insecticide of class oxadiazine having strong activity against lepidopteron pests of fruits, vegetables, corn, tree cotton, peanut, soybean, alfalfa and other crops recommends it`s wide use in agriculture and horticulture [1]. The activated metabolite of indoxacarb has a unique mode of action by blocking of sodium channels of nerve cells, resulting in paralysis and death of the target pest species [2]. The neuronal cholinergic nicotinic receptors are the one of the primary target sites of the insecticide in mammals [3].

Glyphosate (N-[phosphonomethyl]-glycine, H203P-CH2-NH-CH2-C02H) is a unique broad-spectrum post emergence herbicide being used worldwide for the control of terrestrial weeds [4]. It was primarily developed for noncrop agriculture use; however, it has also been used in connection with insecticide tolerant crop varieties and in forestry management practices for site preparation and herbaceous weed control [5]. Generally, glyphosate is slightly toxic to mammals and fish, but it may have an impact on poultry industry through feed residual effect. A higher concentration of glyphosate was detected when glyphosate was sprayed several times during the crop cycle, and when treatments approached the flowering stage [6].

The use of insecticide in combinations for agricultural pest control and public health is increasing day by day due to the challenges posed by insecticide resistance to the existing chemicals. Past research in agriculture sciences showed excellent effects of mixing of glyphosate with numerous insecticides (including indoxacarb) in various agricultural crops; however no information is available on toxicopathology of combined effect of indoxacarb and glyphosate in avian, animals and humans. Therefore, there is a need to study toxicopathology of indoxacarb and glyphosate to evaluate health hazards and toxicity associated with dietary

exposure in birds. This study will given for sight on pesticides and herbicides toxicities in animals and birds in Indian scenario. In view of this it was felt necessary to explore the toxic effect of indoxacarb and glyphosate in quail.

Materials and Methods

Experimental animals

The study was conducted in 252, two week old Japanese quails procured from Venketashwara Hatchery Pvt. Ltd., Pune. Before start of study, the experimental protocol was approved from Institutional Animal Ethics Committee (IAEC).

Experimental design

In the present experiment, total 252 Japanese quails were first acclimatized under identical hygienic and managemental conditions for a period of one week. The birds were divided into seven equal groups, each comprising of 36 quails. All the birds were maintained under identical managemental conditions throughout the experimental period and fed with ad-lib respective dietary treatment for a period of six weeks. Group C served as control, whereas group T1 was fed with feed + indoxacarb @ 30 mg/kg of feed, group T2 was fed with feed + indoxacarb @ 60 mg/kg of feed, group T3 was fed with feed + glyphosate @ 250 mg/kg of feed, group T4 was fed with feed +glyphosate @ 500 mg/kg of feed, group T5 was fed with feed + indoxacarb@ 30 mg/kg of feed +glyphosate @500 mg/kg of feed and group T6 was fed with feed +indoxacarb @ 60 mg/kg of feed + glyphosate @ 250 mg/kg of feed for a period of four weeks i.e. from 4th week to 7th week of age.

Feeding and Watering

Individual feed ingredients were procured from M/s. Shrikrupa Poultry Feeds, MIDC-38, Amravati, Maharashtra and feed was prepared as per requirement of quails [7]. All birds of different group were maintained on ad-lib water and respective treatment diet for at period of 28 days with identical managemental and hygienic conditions.

Indoxacarb and Glyphosate

Indoxacarb was procured from Gharda Chemicals Pvt. Ltd., Khed, Dist. Ratnagiri (Batch No.- IDC670T0430B) while glyphosate was procured from Krishi Rasayan Export Pvt. Ltd., New Delhi (Batch No.- S/KRE/L/T-01).

Clinical observations

Birds of all the groups were kept under close observation for recording clinical symptoms if any and the general performance was recorded on the basis of mean weekly body weights and feed consumption during the experimental period of 28 days.

Haematological observations

At the end of 4th week of experimental period, blood samples from six birds of each group were collected in EDTA (1mg/ml) and were analyzed for hematological parameters viz. hemoglobin (Hb), packed cell volume (PCV), total erythrocyte counts (TEC), total leucocyte counts (TLC) and differential leucocyte counts (DLC). The microhematocrit values of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were derived from the values of Hb, PCV and TEC [8].

Statistical analysis

Data obtained for weekly body weight, weekly feed consumption and different parameters under hematology were statistically analyzed by using Complete Randomized Design [9].

Results and Discussion

Clinical observations

During the experimental studies, control, T1 and T3 group birds did not show any of the clinical signs of pathological importance while birds of group T2, T4, T5 and T6 fed with high doses of indoxacarb and glyphosate or their combination showed clinical symptoms of dullness, depression, anorexia, ruffled feathers, crowding towards the corner of shade and retarded growth after second week of experiment and wasthese symptoms were observed up to 4th week of experiment. The clinical signs observed in combination group were more prominent compared to birds fed with individual high dose indoxacarb or glyphosate. Literature scanned did not reveal information of toxicity of indoxacarb and glyphosate in quails. Somewhat similar clinical signs were reported by previous researchers indicating neurotoxicty (increased activity, restlessness, burrowing of mouth in litter, itching, pilo erection) of indoxacarb [10, 11, 12, 13] in rat and mice. The variation in signs observed might be possibly due to variation in species, dose, duration and route / mode of administration of toxins.

Mean weekly body weight

The mean weekly body weight showed significant differences among the control and different treatment groups from 1st week to the end of 4th week of experiment. However, the pooled mean body weight for 1st to 4th week of experiment fed with indoxacarb and glyphosate at different level either individually or in combination did not reveal significant differences (Table 1). Results indicated significant higher body weight in control group at 1st, 3rd and 4th week of experiment compared to other treatment groups. From this it can be concluded that indoxacarb and glyphosate decreases the body weight in quails but glyphosate causes significant decrease in body weight compared to indoxacarb. Similarly reduction in body weights of rats fed indoxacarb at different dose levels and for different periods has been observed by previous workers [14, 15, 16]. Present findings of decreased body weight in glyphosate fed quails are in agreement earlier study in male rats [17] and suggested that higher concentration of glyphosate in feed result in poor absorption of dietary components from gastrointestinal tract and also uncoupling of oxidative phosphorylation occurs as a result of glyphosate ingestion which results in reduction in weight gain.

Mean weekly feed consumption

The average weekly feed consumption was found to be decreased marginally in dose dependant manner from 2nd week to 4th week of experiment in all toxin treated groups. The remarkable decrease in feed consumption was observed in third and fourth week particularly in high dose indoxacarb and glyphosate alone treated groups and also in their combination groups when compared with control groups (Table 2). The result of the present study indicated that there was marginal reduction in feed intake in quails receiving high dose of indoxacorb and glyphosate alone and combination treated groups which might be due to lower palatability or adverse generalized effect of both the toxicants. Similar to the results of the present study, several workers reported

reduction in feed consumption in rats fed indoxacarb at different doses was recorded by earlier workers [18, 15, 19, 20].

Haematological observations

At the end of 4th week of experiment the mean values of Hb, PCV, TEC, MCV, MCH and MCHC did not differ significantly among control and different treatment group birds (Table 3). However, mean hemoglobin values were found to be numerically lower in group T2, T4 and T1 when compared with control group birds indicating mild anemia. Results thus indicated that indoxacarb and glyphosate toxicity numerically decreases the hemoglobin values. No specific trend of increase or decrease of PCV was recorded either in individual toxicant or in combination group. Numerically lower values of TEC were recorded in T2 and T4 group indicating mild adverse effect of high dose indoxacarb and glyphosate at on erythrocyte count. Numerically increased value of MCV was recorded in group T1, T2, T4 and T5 indicated mild macrocytic anemia. The numerically increase MCH was recorded in group T1 (21.05±1.62) and T5 (18.01±1.00) fed with indoxacarb @ 30 mg/kg of feed either singly or in combination with glyphosate @ 500 mg/kg of feed. The numerically increase value of MCHC was recorded in T1 group while numerically decreased MCHC was recorded in group T2. Data thus indicated that short duration toxicity showed non-significant decrease in values of MCHC. The present finding on 4th week of experiment are in agreement with earlier worker [19, 15] in subchronic toxicity of indoxacarb in mice and rats. Contrary to the present findings significant decrease in TEC in Indoxacarb treated group rats was also recorded by some researchers [20, 21, 13]. The present findings of glyphosate group is in agreement with one of the study in male rats dosed @ 25000 ppm glyphosate [17]. From the result recorded either in individual toxicant or in combination indicated minimal effect of indoxacarb and glyphosate on erythrocyte parameters.

The significantly ($p<0.05$) lower TLC value was recorded in group T4 (11.83±1.31) fed with glyphosate alone @ 500 mg/kg of feed followed by group T5 (12.27±0.73) indicating toxic effect of glyphosate @ 500 mg/kg of feed either individually or in combination with lower dose of indoxacarb @ 30 mg/kg of feed in Japanese quails. Contrary to the present findings no changes in total leukocyte counts in rats due to experimental glyphosate toxicity study was recorded by few workers [17, 22, 23]. The mean values of lymphocytes, heterophil, monocytes and eosinophil counts were found to be differ significantly among control and different treatment groups but were within normal range and did not illustrate any significant correlation between different treatment groups. It was observed that birds fed with glyphosate either individual or in combination with indoxacarb decreases the lymphocyte production for 4 week period of experiment. The heterophil count in treatment groups T3, T4, T5 and T6 was found to be significantly ($p<0.05$) increased. While values in group T1 and T2 differ non significantly but were numerically higher when compared with group C. Systemic stress due to toxicity may leads to endogenous release of corticosteroids which may lead to non inflammatory neutrophilia [8] and might be the reason for significant increase of heterophil count in all treatment groups. The similar hematological findings were also recorded during chlorpyrifos toxicity in broilers and suggested that these mild alteration in body weight, feed consumption and hematological parameters might be due to mild to moderate toxic changes to vital organs particularly liver, spleen and kidney which may directly or indirectly related to haematopoiesis [24].

Table 1: Weekly body weights (g) in different groups

Groups	Weeks of experiment					
	0	1	2	3	4	Pooled mean 1th to 4th weeks
C	112.42±1.78	153.78±1.82^{a}	184.5±2.33^{ab}	209.25±3.46^{a}	223.86±4.12^{a}	176.76±20.00
T1	109±2.55	149.78±2.56ab	182.33±3.14^{b}	201.19±3.54^{ab}	216.81±3.88^{ab}	171.82±19.27
T2	109.11±2.32	150.83±2.56^{ab}	177.89±2.79^{b}	196.97±3.53^{abc}	203.83±7.20^{bc}	167.73±17.29
T3	105±2.61	145.44±2.89^{bc}	178.61±3.14b	196±5.48^{bc}	205.78±4.57^{bc}	165.57±18.33
T4	108.92±2.61	141.61±2.50^{c}	178.5±3.25^{b}	187.64±6.67^{c}	202.67±7.08b^{c}	163.87±17.03
T5	106.19±1.86	148.56±2.19a^{bc}	178.78±2.23^{b}	201.81±3.29^{ab}	209.61±3.7^{ab}	168.99±18.95
T6	106.11±3.04	146.11±3.98^{bc}	193.53±5.30^{a}	193.54±5.30^{bc}	189.72±11.116^{c}	165.80±17.41
Significance	NS	*	**	*	*	NS
CD(0.05)		7.534	9.170	12.844	17.905	

a,b,c Means with different superscripts in a column differ significantly *P < 0.05 ; ** P < 0.01
Mean bearing same superscript in column do not differ significantly. NS- Non significant

Table 2: Weekly feed consumption (g) per bird in different groups

Groups	Weeks of experiment				
	1st	2nd	3rd	4th	Pooled mean 1st to 4th weeks
C	125	154	175	202	164.00± 16.29
T1	121	152	172	185	157.50±13.93
T2	122	143	165	175	151.25±11.82
T3	125	148	170	189	158.00±13.82
T4	125	140	161	182	152.00±12.43
T5	126	145	162	179	153.00±11.36
T6	122	141	158	177	149.50±11.75
Significance					NS

a,b,c Means with different superscripts in a column differ significantly *P < 0.05 ; ** P < 0.01
Mean bearing same superscript in column do not differ significantly. NS- Non significant

Table 3: Hematological observations in different groups at the end of 4th week of experiment

Group	Hematological observations											
	Hb (g/dL)	PCV (%)	TEC (10^{6}cumm)	TLC (10^{3}cumm)	MCV (fL)	MCH (Pg)	MCHC (%)	L (%)	H (%)	M (%)	E (%)	B (%)
C	10.47± 0.20	44.67± 1.76	5.91± 0.39	15.19± 0.77^{ab}	78.00± 8.32	18.19± 1.51	23.58± 0.88	39.67± 0.67^{a}	45.5± 0.76^{c}	9.5± 0.43^{a}	4.83± 0.31^{bc}	0.50± 0.34
T1	9.6± 0.10	48.17± 1.9	4.70± 0.39	12.81± 1.154^{bcd}	104.09± 4.23	21.05± 1.62	20.11± 0.93	41.17± 0.70^{a}	49.17± 0.48^{bc}	6.0± 0.58^{bcd}	3.33± 0.33^{d}	0.50± 0.22
T2	9.15± 0.36	47.33± 2.04	5.30± 0.18	14.71± 0.83^{bc}	89.73± 4.58	17.36± 0.85	19.39± 0.54	39.0± 0.58^{a}	48.5± 1.18^{c}	8.0± 1.53^{ab}	4.0± 0.26^{cd}	0.50± 0.22
T3	10.23± 0.6	46.67± 3.04	5.98± 0.23	13.18± 0.87^{bcd}	77.91± 3.77	17.14± 0.83	22.37± 1.81	32.0± 0.58^{c}	53.33± 0.62^{ab}	7.33± 0.67^{abc}	6.17± 0.48^{b}	1.17± 0.40
T4	9.33± 1.71	44.0± 3.61	5.16± 0.33	11.83± 1.37^{d}	88.89± 12.57	17.88± 3.28	22.24± 4.54	31.0± 0.58^{c}	54.17± 0.60^{a}	5.5± 0.22 ^{cd}	8.17± 0.31^{a}	1.17± 0.31
T5	10.37± 0.35	50.67± 2.62	5.85± 0.38	12.27± 0.73 ^{cd}	87.89± 5.27	18.01± 1.00	20.67± 1.07	34.0± 1.98 ^{bc}	56.17± 2.86^{a}	4.33± 0.62^{d}	5.17± 0.91^{bc}	0.33± 0.21
T6	10.42± 0.34	40.67± 2.86	5.51± 0.25	17.84± 0.82 ^{a}	74.10± 4.71	19.1± 0.95	26.05± 1.49	35.33 ±1.8^{4b}	53.5± 2.22^{ab}	6.0± 0.68^{bcd}	4.33± 0.67^{cd}	0.67± 0.33
Significance	NS	NS	NS	**	NS	NS	NS	**	**	**	**	NS
CD(0.05)				2.727				3.300	4.342	1.850	2.221	

a,b,c Means with different superscripts in a column differ significantly *P < 0.05 ; ** P < 0.01
Mean bearing same superscript in column do not differ significantly. NS- Non significant

Conclusion

This study thus indicated low toxicity of indoxacarb and glyphosate either individually or in combination on clinical and haematological observations in Japanese quails.

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