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TOXICITY OF BAYTHROID-TM : HEMATOLOGICAL EFFECTS IN GROWING CHICKS OF *GALLUS DOMESTICUS*

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Abstract: Baythroid (TM), a mixture of methamidophos and cyfluthrin, was administered orally to chicks as two concentrations (strong and weak) for 40 hours and 9 days, respectively. In strong dose (10 mg/kg body wt.) experiment the blood samples were collected from control and treated groups at 5, 10, 20 and 40 hours following administration of a single dose of insecticide. In weak dose experiment (5 mg/kg body wt./day), the blood was collected at 3, 6 and 9 days of insecticide administration and used to study various hematological parameters such as hemoglobin (Hb), total erythrocytic count (TEC), total leukocytic count (TLC) and hematocrit (Het) estimation. The above data were used to evaluate mean corpuscular volume (MCV), mean corpuscular Hb (MCH) and mean corpuscular Hb concentration (MCHC). Baythroid-TM treatment as strong dose produce significant increase in Hb (32%, 41%, 28% and 30%) and MCH (22%, 32%, 25% and 27%) contents at 5, 10, 20 and 40 hours, respectively. Rise in case of Het (29%) and TEC (9%) was also observed, which was significant only at 5 hours treatment. The TLC also showed an increase, which was 39%, 48%, 34% and 20% at 5, 10, 20 and 40 hours treatments, respectively. Weak dose of insecticide produced significant decrease in TEC (16%, 15% and 11%) and Hb content, which was 24%, 16% and 21% at 3, 6 and 9 day treatments, respectively. MCV and TLC showed an increase only at 3 and 9 days insecticide feeding durations, respectively. It is concluded that 10 mg dose level of Baythroid-TM, administered for short duration has somewhat stimulatory effect on hemopoietic tissue in chicks, which may be a protective response but weak dose administered for relatively long duration indicated suppression of blood components.

Key words: Insecticides, pesticides, pyrethroid, organophosphate blood cells, RBC, WBC, hematological indices, hemopoietic, vertebrate, aves.

INTRODUCTION

The present pesticides, in addition to their outstanding role in insect pest control, are also responsible for induction of variety of toxic effects in the environment and its various components (Gomes *et al.*, 1999; Khalaf-Allah, 1999; Stiller *et al.*, 1999). The pesticides currently in use are mostly organophosphates (OP) pyrethroids and their mixture. As a result of prolong unmanaged use, these pesticides are gradually becoming ineffective against insects due to induction of defence systems (Ben-Cheikh *et al.*, 1998; Chandre *et al.*, 1998; Park and Kamble, 1998; Wirth, 1998; Koffi *et al.*, 1999; Lee *et al.*, 1999). Gradually higher concentrations of these chemicals are required to kill the insect pests, the practice, which is environmentally more hazardous

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significant ($P<0.001$) after 5 hours of toxicant feeding (Tables I-II). In long term (weak dose) experiment, significant decline (24, 16 and 21%) in Hb content was observed at 3, 6 and 9 days ($P<0.05$), respectively (Tables III-VI). The Hct in both strong (10 mg/kg body weight) and weak dose (5 mg/kg body weight/day) Baythroid-TM treatments, did not show any severe alteration except 29% rise within 5 hours in case of strong dose (Tables I-II) and 19% decline at 9 days in weak dose treatment (Tables III-VI), while non-significant changes were observed at 3 and 6 days.

Table I: Effect of Baythroid-TM *(10 mg/kg/body weight)* administered for a total period of 40 hours on some hematological parameters of chick, *Gallus domesticus*.

Parameters[b]	Control (n=4)	Insecticide Treatment (Hours)			
		5 (n=5)	10 (n=5)	20 (n=5)	40 (n=5)
TEC	2.46[a]	2.67**	2.60	2.54	2.51
($X10^6/\mu l$)	±0.03	±0.03	±0.03	±0.06	±0.04
Hb content	5.94	7.86***	8.40**	7.63*	7.74**
(g/dl)	±0.17	±0.24	±0.54	±0.55	±0.38
Hct (%)	30.08	38.80*	35.47	32.49	31.64
	±2.67	±1.56	±1.69	±1.42	±1.01
MCV (fl)	120.73	144.38	138.03	127.79	125.78
	±9.22	±5.46	±5.13	±6.10	±3.12
MCH (pg)	24.07	29.46**	31.73**	30.04***	30.56***
	±0.80	±0.95	±1.79	±0.87	±1.50
MCHC (%)	20.70	20.79	22.71	23.48	23.85
	±1.11	±0.74	±0.55	±2.33	±1.50
TLC	20.30	28.20***	29.95***	27.10**	24.25**
($X10^3/\mu l$)	±0.57	±0.32	±0.44	±0.63	±0.82

a: Mean ± SEM; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$ (Student's 't' test).

Abbreviations used: Hb, hemoglobin; Hct, hematocrit; TEC, total erythrocytic count; TLC, total leukocytic count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; fl, femtolitre = 10^{-15} litre; pg, picogram = 10^{-12}g.

Total erythrocytic count did not exhibit any severe change in strong dose experiment, however weak dose of toxicant administered for 9 days proved more toxic then strong dose. A significant decrease of 15.7%, 15.5% and 11% was observed in TEC at 3, 6 and 9 days treatments, respectively (Tables III, VI).

Experimental procedure

Two doses of Baythroid TM were administered to chicks (orally) for two different durations *i.e.*, for 40 hours in short-term experiment and for 9 days in long-term experiment.

In short-term (strong dose) experiment a group of twenty-four chicks were used. Twenty chicks were divided into four groups of five birds each while a group of four animals were used for control experiments. All chicks were weighed and a strong dose of Baythroid-TM 10 mg/kg body wt. was administered orally to 20 mice with the help of blunt glass pipette. After the stipulated periods of 5, 10, 20 and 40 hours, a group of five treated birds were dissected, their blood samples were collected in anticoagulant (EDTA) containing eppendorfs.

For weak dose (long-term) experiment another group of 24 chicks was administered with Baythroid-TM @ 5 mg/kg body wt./day for 9 days. After regular intervals of 3, 6 and 9 days, a group of five chicks was taken out and dissected. The blood samples were collected as above for hematological studies.

Methodology used

The hemoglobin (Hb) content of the blood was estimated according to Vankampen and Zijlstra (1961). The hematocrit (Hct) was analyzed by microhematocrit method of Strumia *et al.* (1954), while the total erythrocytic count (TEC) and total leukocytic count (TLC) was performed according to routine clinical methods as described in Dacie and Lewis (1986). The above hematological values were then used to determine different hematological indices *i.e.*, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) as mentioned in Dacie and Lewis (1986).

RESULTS

Mortality

Administration of Baythroid-TM as weak dose (5 mg/kg body wt./day) produced 20% mortality in chicks at 3 and 9 days treatment groups, separately. The overall mortality of experimental animals in long-term experiment was 13.3%. No mortality was observed in short-term (10 mg/kg body wt./day) experiment.

Hematological effects

Baythroid-TM as strong dose produced significant increase of 32%, 41%, 28% and 30% in Hb content after 5, 10, 20 and 40 hours, respectively. The difference was highly

significant ($P<0.001$) after 5 hours of toxicant feeding (Tables I-II). In long term (weak dose) experiment, significant decline (24, 16 and 21%) in Hb content was observed at 3, 6 and 9 days ($P<0.05$), respectively (Tables III-VI). The Hct in both strong (10 mg/kg body weight) and weak dose (5 mg/kg body weight/day) Baythroid-TM treatments, did not show any severe alteration except 29% rise within 5 hours in case of strong dose (Tables I-II) and 19% decline at 9 days in weak dose treatment (Tables III-VI), while non-significant changes were observed at 3 and 6 days.

Table I: Effect of Baythroid-TM *(10 mg/kg/body weight)* administered for a total period of 40 hours on some hematological parameters of chick, *Gallus domesticus*.

Parameters[b]	Control (n=4)	Insecticide Treatment (Hours)			
		5 (n=5)	10 (n=5)	20 (n=5)	40 (n=5)
TEC	2.46[a]	2.67**	2.60	2.54	2.51
($X10^6/\mu l$)	±0.03	±0.03	±0.03	±0.06	±0.04
Hb content	5.94	7.86***	8.40**	7.63*	7.74**
(g/dl)	±0.17	±0.24	±0.54	±0.55	±0.38
Hct (%)	30.08	38.80*	35.47	32.49	31.64
	±2.67	±1.56	±1.69	±1.42	±1.01
MCV (fl)	120.73	144.38	138.03	127.79	125.78
	±9.22	±5.46	±5.13	±6.10	±3.12
MCH (pg)	24.07	29.46**	31.73**	30.04***	30.56***
	±0.80	±0.95	±1.79	±0.87	±1.50
MCHC (%)	20.70	20.79	22.71	23.48	23.85
	±1.11	±0.74	±0.55	±2.33	±1.50
TLC	20.30	28.20***	29.95***	27.10**	24.25**
($X10^3/\mu l$)	±0.57	±0.32	±0.44	±0.63	±0.82

a: Mean ± SEM; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$ (Student's 't' test).

Abbreviations used: Hb, hemoglobin; Hct, hematocrit; TEC, total erythrocytic count; TLC, total leukocytic count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; fl, femtolitre = 10^{-15} litre; pg, picogram = 10^{-12}g.

Total erythrocytic count did not exhibit any severe change in strong dose experiment, however weak dose of toxicant administered for 9 days proved more toxic then strong dose. A significant decrease of 15.7%, 15.5% and 11% was observed in TEC at 3, 6 and 9 days treatments, respectively (Tables III, VI).

Table II: Percent increase (+) or decrease (-) in different hematological parameters of chick, *Gallus domesticus*, after Baythroid-TM administration *(10 mg/kg body weight)* for a total period of 40 hours.

Parameters[a]	Insecticide Treatment (hours)			
	5	10	20	40
TEC	+8.53**	+5.69	+3.25	+2.03
Hb Content	+32.32***	+41.41**	+28.45*	+30.30**
Hct	+28.65*	+17.91	+8.01	+5.18
MCV	+19.58	+14.32	+6.49	+4.18
MCH	+22.39**	+31.90**	+24.80***	+26.96***
MCHC	+0.43	+9.71	+13.43	+15.21
TLC	+38.91***	+47.53***	+33.49**	+19.45**

*P<0.05; **P<0.01; ***P<0.001 (Student's 't' test).

For abbreviations, see Table I

Table III: Effect of Baythroid-TM *(5 mg/kg/body weight/day)* administered for a total period of 3, 6 and 9 days on some hematological parameters of chick, *Gallus domesticus*.

Parameters[b]	3 days		6 days		9 days	
	Control (n=3)	Treated (n=5)	Control (n=3)	Treated (n=5)	Control (n=3)	Treated (n=5)
TEC	2.09[a]	1.72*	1.98	1.60**	1.97	1.70*
($X10^6/\mu l$)	±0.09	±0.07	±0.08	±0.06	±0.07	±0.05
Hb content	7.92	6.02*	8.26	6.95*	8.38	6.61*
(g/dl)	±0.56	±0.47	±0.33	±0.41	±0.44	±0.31
Hct (%)	40.83	43.20	42.30	37.51	41.13	33.54**
	±1.62	±2.02	±1.88	±1.79	±1.52	±0.87
MCV (fl)	195.36	251.16*	238.04	228.72	213.11	195.00
	±9.66	±10.23	±14.62	±9.11	±14.97	±14.52
MCH (pg)	38.82	35.00	42.58	42.38	43.42	38.43
	±2.71	±1.42	±2.36	±3.27	±3.37	±4.25
MCHC	19.40	13.94*	19.53	18.53	20.37	19.71
(g/dl)	±1.44	±1.20	±2.15	±1.32	±0.81	±1.33
TLC	32.67	38.60	25.50	36.50**	31.83	39.25*
($X10^3/\mu l$)	±1.97	±3.71	±2.02	±1.46	±2.32	±1.97

a: Mean ± SEM (Student's 't' test) *P<0.05; **P<0.01; ***P<0.001.

For abbreviations, see Table I.

The only hematological parameter which showed prominent change in both insecticide treatments was TLC, which was increased with high significance by 39%, 48%, 34% and 20% at 5, 10, 20 and 40 hours treatments in strong dose (Tables I-II). In weak dose insecticide treatment, a significant increase of 43% and 23% was noticed at 6 and 9 day treatments, respectively, while a non-significant rise of 18% was observed at 3 days (Tables III-VI).

Table IV: Percent increase (+) or decrease (-) in different hematological parameters of chick, *Gallus domesticus*, after Baythroid-TM administration *(5 mg/kg body weight)* for the total period of 9 days.

Parameters[a]	Cyfluthrin Treatment (days)		
	3 (n=5)	6 (n=5)	9 (n=5)
TEC	-15.69*	-15.46**	-10.88*
Hb content	-23.89*	-15.85*	-21.12*
Hct	+5.80	-11.32	-18.49**
MCV	+28.58*	-4.89	-8.49
MCH	-9.99	-0.46	-11.49
MCHC	-28.14*	-5.12	-3.24
TLC	+18.15	+43.14**	+23.31*

*P< 0.01; **P< 0.01; ***P< 0.001 (Student's 't' test)
For abbreviations, see Table I.

Hematological indices almost reflected the same pattern of changes as were observed in case of TEC, Hb and Hct. Tables I-VI indicate the effect of Baythroid-TM as strong and weak doses, on hematological indices values of chick. The MCV and MCHC did not show any sign of toxicity throughout the experiment. In contrast MCH exhibited the prominent rise of 22%, 32%, 25% and 27% at 5, 10, 20 and 40 hours after insecticide feeding. The last two values were highly significant (Tables I-VI).

DISCUSSION

Two different doses of Baythroid-TM administered for various durations produced variable effects on hematological parameters. The TEC and Hct did not show any significant shift at 10 mg dose level, administered for short duration except 9% and 29% increase at 5 hours treatment respectively. Haemoglobin content on the other hand, showed 28-41% increase from 5 to 40 hours treatment. The rise in TEC and Hct was also found in rabbits administered with Karate (cyhalothrin) for 15 days @ 10 mg/kg body wt./day (Shakoori *et al.*, 1992). Ali *et al.* (1997) showed increase in Hb and MCHC following malathion toxicity in chick of *Gallus domesticus*. This increase may be a protective response in chicks to counter the toxic effects and for induction of defense system of the body. In 9 day insecticide treatment, however, TEC and Hb decreased

significantly, which were 11-16% in former case and 16-24% in later. Decrease was also found in Hct (19%) and MCHC (28%) at 9th and 3rd day, respectively. The results indicated that Baythroid-TM administration at 10 mg dose level for short duration has somewhat stimulatory effect on hemopoietic system at least for initial few hours, while significant alteration were observed in 5 mg dose level administered for 9 days.

This type of data showed that this dual target site mixture of OP and pyrethroid insecticides, induced its toxicity gradually after few days exposure even at low dose (5 mg/kg body wt./day). Toxicant induced hematologic disorders such as RBC, WBC counts, Hct, MCH, MCV porphyries, porphyrinurias, aplastic anemia and increased risks of leukemia or lymphoma has been reported (Shakoori *et al.*, 1988; Ali and Shakoori, 1981, 1990; Lisiewicz, 1993; Khalaf-Allah, 1999; Ali *et al.*, 1997, 2000). In another study mixture of endosulfan, dimethoate and carbaryl, given to rats orally induced significant alteration in red cell count, white cell counts and Hb content, while no change was observed when these pesticides were administered alone at 100 fold ADI level (Akay *et al.*, 1999), while MCV, MCH, MCHC and erythrocyte sedimentation rate remained unaffected.

The findings in the present experiment differ from the studies of Shakoori *et al.* (1990a,b) with bifenthrin (a pyrethroid) in rabbits, which showed the significant decrease in Hb content, TEC and MCHC in 30 days study with sublethal dose. Similar decrease was also observed in rat and fish with other pesticides like malathion, aldrin, gamma-BHC, endrin and cypermethrin (Ali *et al.*, 1988; Ali and Shakoori, 1990; Shakoori *et al.*, 1988; Reedy and Bashamohideen, 1989; Guilhermino *et al.*, 1998). Ahmad *et al.* (1995) reported decrease in Hb and Hct values following administration of fenpropathrin (Danitol) for 30 days in Chinese grass carp. Similar decrease in Hb, TEC and Hct was also shown in another study with DDT in rats administered @ 10, 20 and 100 mg/kg body wt. for 48 hours, 15 days and 18 months, respectively (Ali and Shakoori, 1994). There was a significant increase in TLC in both treatments, which was more prominent in 10 mg dose level administered for 40 hours duration. Increase in TLC is a typical protective response of vertebrate systems against toxic insult (Ali and Shakoori, 1990, 1994; Shakoori *et al.*, 1990a,b). Ahmad *et al.* (1995) also reported the significant increase in WBC count with sublethal dose of Danitol (a pyrethroid) in fish, which may enhance the detoxification process.

The present study revealed that administration of double target site mixture of OPs and pyrethroid (Baythroid-TM) insecticides to chicks, slight stimulatory effects in case of 10 mg dose level fed for 40 hours, while low dose fed for 9 days showed significant inhibitory effects on hemopoietic system in chicks.

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