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Original Article

Alterations in Hematological Profile of Experimentally Induced Subchronic Thiacloprid Toxicosis in *Gallus domesticus*

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ABSTRACT

Objectives: Thiacloprid, a novel neonicotinoid insecticide is chiefly used as a crop protectant therefore it is likely to cause indirect exposure to poultry through contaminated feed and water because this species is occasionally supplied with feed that is, declared unfit for human consumption. The current study was performed to explore the nonlethal toxic effects of thiacloprid in *Gallus domesticus* on hematological parameters. **Materials and Methods**: Fifty-two birds were randomly divided into nine groups. Groups I to IV of four birds each were kept as healthy control. The Groups V, VI, VII, VIII, IX, and X contained six birds each and were administered thiacloprid at 1 mg/kg/day for 15, 30, 45, 60, 75, and 90 days, respectively. **Results:** Thiacloprid caused variable changes in the hematological parameters. There was a significant decline in the packed cell volume (PCV), hemoglobin (Hb) concentration, and total erythrocyte count (TEC). The PCV declined to the extent of 23.33 ± 0.76% on day 90 from the 0 day value of 29.75 ± 1.26% of experiment. The Hb concentration decreased from 9.93 ± 0.57 g/dl (0 day) to 7.52 ± 0.62 g/dl (90 days). The TEC declined from the 0 day value of 2.41 ± 0.08 × 10^6/mm^3 to 90 days value of 2.08 ± 0.05 × 10^6/mm^3. The total leukocyte count on 0 day was 12.50 ± 0.76 × 10^3/mm^3 and it showed a significant increase from day 45 (17.80 ± 2.67 × 10^3/mm^3) to day 90 (21.33 ± 1.48 × 10^3/mm^3) of thiacloprid treatment. There was a significant rise in value of erythrocyte sedimentation rate to 19.25 ± 1.22 mm/24 h on day 90 of treatment from the 14.42 ± 1.09 mm/24 h on 0 day. The long-term oral administration of thiacloprid produced no significant alterations in the values of erythrocytic indices. **Conclusions:** The repeated oral toxicity on thiacloprid in present investigation suggested that it has an adverse effect on health of birds and is moderately risk insecticide in *G. domesticus*.

Key words: *Gallus domesticus*, insecticide, neonicotinoid, thiacloprid, toxicity

INTRODUCTION

The neonicotinoids, newest major class of insecticides, developed in the past three decades, in particular, have outstanding potency and systemic action for crop production against piercing-sucking pests and they are highly effective for flea control in cats and dogs.[1] The California Department of Foods and Agriculture has identified neonicotinoids as the most likely alternative to the organophosphate insecticides, especially for the control of

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sucking insects. Crop protection and veterinary pest control measures have changed greatly with the recent introduction of neonicotinoids insecticide.[2,3]

Thiacloprid, a novel neonicotinoid insecticide, was developed by Bayer AG and Nihon Bayer Agrochem and first registered in Brazil in 1999. Thiacloprid is N-{3-[(6-chloro-3-pyridinyl) methyl]-1, 3-thiazolan-2-yliden} cyanamide. It is chiefly used to control sucking and biting insects in orchards and vegetable farms. Pests controlled with thiacloprid include aphids, whitefly, beetles, and lepidoptera such as leaf miners. It acts as an acute contact and stomach poison with systemic action and selectively binds and interacts with insect nicotinic acetylcholine receptor site. The selective nature of thiacloprid plays a central role in its safety towards mammalian species.[4]

Thiacloprid was launched in India in 2005 and its effectiveness against insect pests has made this compound very popular. It is chiefly used as a crop protectant, therefore, is likely to cause indirect exposure to poultry through contaminated feed and water because this species is occasionally supplied with feed that is, declared unfit for human consumption.[5] Since there are very few reports on toxicological aspects of this compound in poultry and data generated in one animal species cannot be extrapolated to other species, it is felt necessary to explore the nonlethal toxic effects of thiacloprid in poultry.

MATERIALS AND METHODS

Birds

The present study was conducted on 1½-year-old layer *Gallus domesticus*. The birds were procured and housed in pens at the layer house of the poultry farm, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India. The birds were provided with standard feed and clean water *ad libitum* and were acclimatized to the layer house for 10 days prior to the commencement of the study at ambient temperature of 25°C and 45–55% relative humidity, with 12 h each of dark and light cycles. All procedures were performed in accordance with the guidelines for the care and use of experimental animals of the Committee for Purpose of Supervision of Experiments on Animals.

Chemicals

Technical grade thiacloprid (Alanto 240 SC, Thiacloprid 21.7%), was commercially obtained from the authorized dealer of Bayer CropScience Limited, Sabarkanta, Gujarat, India. All the chemicals used in this study were of high purity. Based on the recommended concentrations of thiacloprid used for crop protection, a suitable nonlethal dose of thiacloprid was selected for the administration by the oral route.

Experimental design

Fifty-two birds were randomly divided into nine groups. The requisite amount of insecticide was suspended in water and administered directly into the proventriculus of the bird by using a catheter with 2 ml glass syringe and/or tuberculin syringe as per given plan.

Groups I to IV of four birds each was kept as a healthy control in which no insecticide was administered, whereas the birds were stressed giving the distilled water by using a catheter with 2 ml glass syringe.

The Groups V, VI, VII, VIII, IX, and X contained six birds each:

- Group V: Thiacloprid at 1 mg/kg/day for 15 days
- Group VI: Thiacloprid at 1 mg/kg/day for 30 days
- Group VII: Thiacloprid at 1 mg/kg/day for 45 days
- Group VIII: Thiacloprid at 1 mg/kg/day for 60 days
- Group IX: Thiacloprid at 1 mg/kg/day for 75 days
- Group X: Thiacloprid at 1 mg/kg/day for 90 days.

The birds were starved overnight and their body weights were recorded before the start of treatment. All the birds were weighed after 15 days and doses of thiacloprid was corrected according to the changes in body weights. To study the hematological parameters, the blood samples were collected directly by cardiac puncture in ethylenediaminetetraacetic acid containing vials on 0, 30, 60, and 90 treatment days from Groups I, II, III, and IV and on 0, 15, 30, 45, 60, 75, and 90 days from Groups V, VI, VII, VIII, IX, and X, respectively.

Methods for estimation of various hematological parameters

Samples were analyzed for hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), and erythrocyte sedimentation rate (ESR). Hb estimation was done by cyanmethemoglobin method and PCV by the microhematocrit method.[6] TEC and TLC were done using Neubauer's hemocytometer and Tualidine blue (0.015%) saline as diluents.[7] Mean corpuscular volume (MCV), mean cell Hb (MCH), and mean corpuscular Hb concentration (MCHC) were calculated.[8]

Statistical calculations

Results were expressed as a mean ± standard error. Statistical significance was determined by one-way analysis of variance. The treatment groups were compared with a control group using Tukey's honest significant difference *post-hoc* test for multiple comparisons with SPSS 16.0 (SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago) for a computer program. The values of $P < 0.05$ were considered statistically significant.

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RESULTS

The effects of daily oral administration of thiacloprid at a dose rate of 1 mg/kg/day for 90 consecutive days on various hematological parameters were investigated in *G. domesticus*. Repeated oral administration of thiacloprid at 1 mg/kg/day produced a significant decline in the PCV and Hb concentration in *G. domesticus* [Figure 1]. The PCV declined to the extent of 23.33 ± 0.76% on day 90 from the 0-day value of 29.75 ± 1.26% of the experiment. The Hb concentration decreased from 9.93 ± 0.57 g/dl (0 day) to 7.52 ± 0.62 g/dl (90 days).

The results of daily oral administration of thiacloprid at a dose rate of 1 mg/kg/day for 90 consecutive days on TEC and TLC are given in Figure 2. Repeated oral exposure of thiacloprid produced a significant decline in the TEC from the 0-day value of 2.41 ± 0.08 × 10^6/mm^3 to the 90-day value of 2.08 ± 0.05 × 10^6/mm^3.

The TLC of *G. domesticus* treated with thiacloprid on 0 day was 12.50 ± 0.76 × 10^3/mm^3 and it showed a significant increase from day 45 (17.80 ± 2.67 × 10^3/mm^3) to day 90 (21.33 ± 1.48 × 10^3/mm^3) of thiacloprid treatment.

Table 1 shows the effect of repeated daily oral administration of thiacloprid (1 mg/kg/day) on the ESR in *G. domesticus*. Daily oral administration of thiacloprid at dose rate of 1 mg/kg/day produced a significant rise in values of ESR to 19.25 ± 1.22 mm/24 h on day 90 of treatment from 14.42 ± 1.09 mm/24 h on 0 day. The long-term oral administration of thiacloprid produced no significant alterations in values of erythrocytic indices as shown in Table 1.

DISCUSSION

The Hb concentration and hematocrit generally provide an accurate reflection of the extent to which the circulating red cell mass is reduced.[9] Brar *et al*.[7] suggested that if the PCV is decreased, the animal is anemic whereas an elevated PCV indicate polycythemia. The decline in Hb suggested that thiacloprid might have interfered with erythropoiesis. The observed results are in agreement to that of Kaur[10] who reported similar changes after the administration of imidacloprid at 0.5 mg/kg/day for 150 days in cow calves. Decline in Hb concentration has also been reported by other workers following the exposure to different insecticides in cockerels,[11] buffalo calves,[12] and rodents.[13] The fall in Hb concentration on subchronic oral exposure to thiacloprid might have occurred because of the interference with heme synthesis as reported with certain toxins and drugs.[14]

The increase in leucocyte count during treatment has been attributed to the occurrence of internal hemorrhage[15] and to the effects on bone marrow and/or the pituitary-adrenal system. Marked leukocytosis has been reported in cow calves[16]

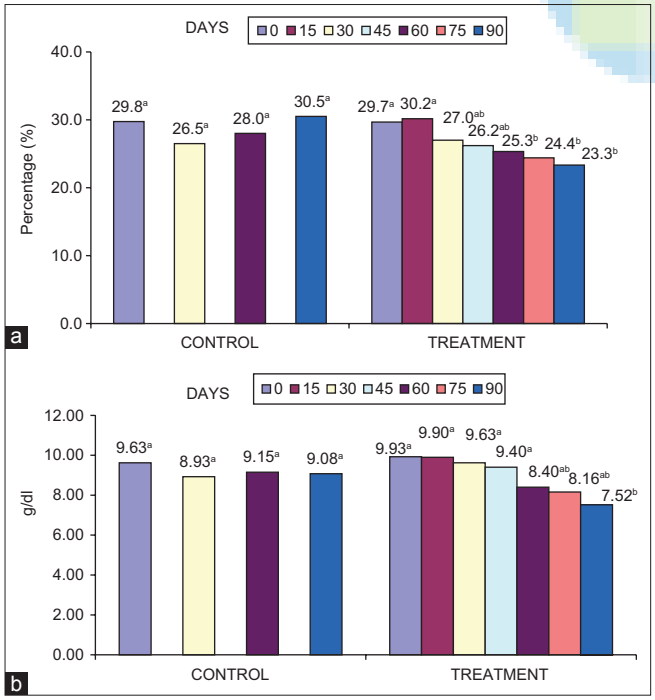


Figure 1: Effect of subchronic oral administration of thiacloprid (1 mg/kg body weight) on (a) packed cell volume (%) and (b) Hemoglobin concentration (g/dl) in *Gallus domesticus*. Mean with at least one common superscript are similar within the group ($P < 0.05$) as compared with control value

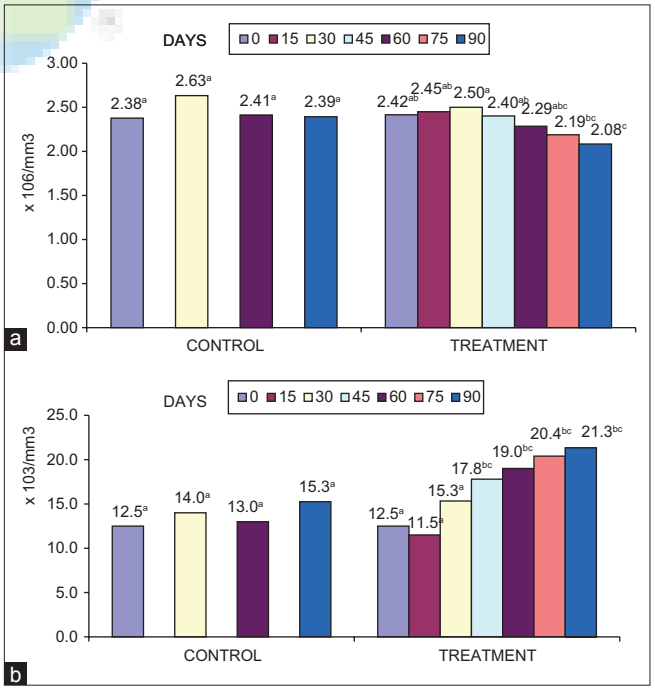


Figure 2: Effect of subchronic oral administration of thiacloprid (1 mg/kg body weight) on (a) total erythrocyte count (×10^3/mm^3) and (b) total leukocyte count (×10^6/mm^3) in *Gallus domesticus*. Mean with at least one common superscript are similar within the group ($P < 0.05$) as compared with control value

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Table 1: Effect of subchronic oral administration of thiacloprid (1 mg/kg/day) on ESR (mm/24 h), mean corpuscular volume (fl), mean corpuscular Hb concentration (g/dl) and mean corpuscular hemoglobin (pg/dl) in *Gallus domesticus*

	Time (days) Treatment						
Groups	0	15	30	45	60	75	90
ESR (mean±SE)							
Control	14.75±0.48^{a} (4)	-	14.88±0.69^{a} (4)	-	14.63±0.55^{a} (4)	-	15.31±0.77^{a} (4)
Thiacloprid	14.42±1.09^{a} (6)	14.79±1.26^{a} (6)	15.88±1.36^{ab} (6)	16.70±0.88^{ab} (5)	17.83±2.57^{ab} (6)	18.10±1.99^{ab} (5)	19.25±1.22^{b} (6)
Mean corpuscular volume (mean±SE)							
Control	126.05±14.15^{a} (4)	-	100.87±11.89^{a} (4)	-	118.48±18.76^{a} (4)	-	129.51±18.47^{a} (4)
Thiacloprid	123.37±6.22^{a} (6)	124.54±6.78^{a} (6)	108.54±12.15^{a} (6)	109.53±10.13^{a} (5)	110.95±6.21^{a} (6)	111.66±4.15^{a} (5)	111.97±6.83^{a} (6)
Mean corpuscular Hb concentration (mean±SE)							
Control	40.56±1.40^{a} (4)	-	34.09±1.60^{a} (4)	-	38.25±1.92^{a} (4)	-	38.28±1.99^{a} (4)
Thiacloprid	41.11±1.96^{a} (6)	44.10±5.25^{a} (6)	38.46±1.44^{a} (6)	39.10±1.26^{a} (5)	36.89±1.48^{a} (6)	37.26±2.25^{a} (5)	36.10±1.51^{a} (6)
Mean corpuscular hemoglobin (mean±SE)							
Control	32.61±2.22^{a} (4)	-	33.74±1.09^{a} (4)	-	33.09±2.77^{a} (4)	-	29.84±1.08^{a} (4)
Thiacloprid	33.56±1.60^{a} (6)	33.02±2.29^{a} (6)	36.13±2.40^{a} (6)	35.94±1.51^{a} (5)	33.97±1.42^{a} (6)	33.30±1.18^{a} (5)	32.45±1.89^{a} (6)

Figures in the bracket indicate a number of birds. Mean with at least one common superscript are similar within the group (P<0.05). SE = Standard error, Hb = Hemoglobin, ESR = Erythrocyte sedimentation rate

and buffalo calves[12] following long-term administration of imidacloprid, phosphamidon, and triazophos, respectively. However, Thaker[16] did not observe any significant alteration in TLC in white leghorn chicks by long-term daily oral administration of endosulfan and malathion.

Insecticides like other toxic chemicals are reported to have an adverse effect on bone marrow causing a decrease in erythrocyte production.[17] Decreased erythrocyte production and hypoplasia of erythropoietic tissues have also been demonstrated in severe uremia (increased blood urea nitrogen) associated with renal damage,[18] as is evident in the present study. Exposure of red blood cells (RBCs) to toxicants results in the production and denaturation of metHb production and in the coalescence of Hb molecules to form Heinz bodies. The attachment of Heinz bodies to the plasma membrane increases membrane rigidity and leads to anemia by means of increased RBC lysis or premature removal from circulation.[19] Erythrocytopenia and hypohemoglobinemia in the present study were indicative of anemic conditions of *G. domesticus*. Similar findings were reported by Kaur[10] after the oral administration of imidacloprid in cow calves for 150 days.

The erythrocytes tend to settle down owing to their tendency for rouleaux formation. Although the rate of fall of RBCs is affected by many factors, increase in plasma proteins, particularly fibrinogen and globulins and decline in erythrocyte count mainly cause an increase in ESR.[7] ESR may also increase in some disease conditions such as acute peritonitis, pleuritis, and hypothyroidism. The findings of the present study are in agreement with those of Kaur.[10] who reported an increase in ESR following the repeated oral administration of imidacloprid in cow calves.[13] Observed no adverse effect on ESR of repeated exposure to triazophos and in buffalo calves.

The RBC indices that are MCV, MCH, and MCHC, are used for the morphological classification of anemias. Erythrocytic volume changes are called microcytic, normocytic, or macrocytic depending on their size. The present findings on erythrocytic indices in thiacloprid treated birds suggested normocytic, nonregenerative anemia which are slightly in contrast to Kaur.[10] who suggested that imidacloprid treated cow calves showed normocytic-hypochromic anemia. Thus, it could be concluded that the repeated oral toxicity on thiacloprid has an adverse effect on the health of birds and is moderately risk insecticide in *G. domesticus*. The insecticide thiacloprid has a wide range of toxicological activities. However, very less work has been done on this insecticide; there is a wide scope for investigation.

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Conflicts of interest

There are no conflicts of interest.

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