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**Research Article**

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# INDUCED TOXIC EFFECTS OF CHLORPYRIFOS ON HEMATOLOGICAL INDICES IN PIGEONS (*COLUMBA LIVIA DOMESTICA*)

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## ABSTRACT

Agrochemicals are used extensively in agriculture sector across the world; however, they may be eco-toxicological hazards and disrupt the non-target living beings, including birds. Hence, the present study was carried out to evaluate the toxic effects of chlorpyrifos (CPF) insecticide on hematological parameters in pigeons. A total of 120 adult pigeons (*Columba livia domestica*) were used in the study and kept in wooden cages, and all pigeons received the same quantity of (22%) crude protein and water during trials. Pigeons were acclimatized for 15 days and divided into four equal (A, B, C and D) groups, each consisting of 30 birds. Test groups (B-D) were exposed to CPF with oral doses of "1.3 mg, 1.6 mg, and 2.1 mg/kg body weight/day" respectively for twelve successive weeks, whereas (group A) was assigned as a controlled group. At the end of the trials, blood samples from exposed and controlled group birds were obtained from brachial and collected with anticoagulant "K3-EDTA" vacutainer tubes for hematological assessments. Following the 84-day exposure period, notable statistically significant ($p < 0.05$) reduced levels of hemoglobin (Hb) (13.85 g/dl), (12.2 g/dl) and (10.50 g/dl) respectively, in birds of groups B, C and D and significant ($p < 0.05$) reduced level of total erythrocytes count (TEC) and packed cell volume (PCV%) was noted when compared with the control. There was a substantial significant ($p < 0.05$) increase in total leucocyte count (TLC) and total platelet count observed in comparison to the birds of the control group. The results of the present study revealed that alterations in hematological indices exhibited by chlorpyrifos insecticide. This research demonstrated how the indiscriminate use of pesticides damages health and developmental problems in bird ecosystems, and eventually, humans may be in danger for their health problems.

**Keywords:** Toxic effects, Chlorpyrifos, Hematological indices, Pigeons

## INTRODUCTION

Pesticides are frequently used in the agriculture sector to ensure crop growth (Mahmood et al., 2016), and various insecticides are also used to eradicate insects consuming them by using insecticides. Pesticides have been used extensively for a number of years to protect crops from noxious pests. Although using pesticides helps the crops, there's still a danger that it may harm the ecosystem in the surroundings (Alla-Eldin *et al.*, 2016). Insecticide poisoning is a worldwide issue that is particularly challenging in developing countries. In response to the rising need for food, the agricultural sector has been using pesticides extensively and indiscriminately, which is concerning and drawing attention to the possible eco-toxicity of these agrochemicals. Water, soft drinks, dairy, meat products, and food items have all been linked to pesticide residues (Soliman *et al.*, 2015).

The most prevalent morphologically and extensively diversified bird species is the pigeon (Young et al., 2017). The existence of potentially toxic substances in the environment threatens a wide range of living beings, such as birds and aquatic life, endangering their capacity to survive. A significant number of people have expressed concerns about the environment's long-term capacity to preserve its balance and health in connection to the usage of pesticides.

One of the primary sources of environmental pollution is the overuse of pesticides, which have toxic ecological effects on the soundings (Poudel *et al.*, 2020). The CPF is the commonly wide-ranging organophosphorus insecticide, globally used in both residential and agricultural sectors. Because the amino acid sequence of acetylcholinesterase is highly conserved in animals and variations in toxic-kinetic (adsorption, distribution, metabolism, and excretion) effects account for differences in sensitivity among taxa, this insecticide, classified as class II, is toxic to the majority of animal species (Giesy *et al.*, 2014). The CPF insecticide has been introduced into the agricultural sector; it may affect birds, wild animals,

farm animals, pets, and livestock housed in homes (Annabi *et al.*, 2019; Hedau *et al*., 2018). CPF produces excitement by interfering with synaptic transmission and acting as an agonist on nicotinic acetylcholine receptors (Annabi *et al*., 2019). Although the purpose of pesticides is to kill insects, non-targeted organisms may be poisoned if they consume insects and plant debris contaminated with pesticides or if they eat exposed bait containers. In addition, OP, like CPF insecticide exposure, causes damage to the nervous system and musculoskeletal system and problems with the respiratory, circulatory, reproductive, developmental, and immune systems. Organophosphate pesticides harm birds' development, reproduction, and behavior while also decreasing their growth. Additional changes seen in several birds include immunological response deficit, oxidative stress, neurological problems, endocrine disruption, and lesions in diverse organ tissues (Suliman *et al.*, 2020).

In human and animal research, hematological profiles are a crucial measure of an individual's physiological status. Mean corpuscular hematocrit, white blood cell (WBC) and red blood cell (RBC) counts are among the several hematological measures. Red blood cells (RBCs), as well as other characteristics, including hemoglobin (Hb) and platelets, were found to differ across species of birds; breed, sex, and the diet the birds were fed all had an impact on the counts. Hematology evaluation was employed, particularly in birds, to evaluate nutrition and overall health, identify diseases, forecast how long they would last, and determine how well a treatment intervention would work (Orakpoghenor *et al.*, 2021). The purpose of this research work was to look into the harmful effects of chlorpyrifos on pigeon's hematological parameters.

## MATERIALS AND METHODS

**Design of the experiment and the birds:** In our study (120) healthy pigeons (*Columba livia domestica*) were housed for ten days to help them get acclimated. The pigeons received the same amount of clean drinking water, grains, and seeds for their diet. After being acclimated, the pigeons were divided into four groups, A, B, C, and D, each with thirty birds. Before starting the trials, the body weight was measured. Group A pigeons were designated as the control group, while pigeons from groups B, C, and D were designated for CPF exposures.

**The research methodology and pesticide preparations:** Using toxicological techniques, the insecticide concentration (Chlorpyrifos 40EC) was prepared on an LD50 basis. For a continuous 84 days, groups B, C, and D received the oral doses of "1.3 mg, 1.6 mg, and 2.1 mg/kg body weight/day," respectively, by adding one milliliter of maize oil. Pigeons from group A served as the control. In the present research, food consumption, body weight, and toxicity-related clinical signs and mortality were recorded. The pigeons were chosen at random from each group at the hematological assessments.

**Hematological Assessments:** The Department of Zoology, University of Sindh Jamshoro's ethics committee accepted the procedure that was followed for all experimental processes. Blood was taken from the brachial veins of the birds after 84 days of the study. From each of the four groups, pigeons were chosen at random to have blood drawn from their pectoral veins. Using sterile disposable syringes coated with heparin, precisely 2 milliliters of blood were drawn and then transferred to EDTA tubes. Standard hematological procedures were used to determine the hematological indices of the blood samples that were obtained. Hematological procedures to analyze and collect blood samples in order to determine the total erythrocyte counts (TECs), total leucocyte counts (TLCs), hemoglobin (Hb), packed cells volume (PCVs), and platelet values. By the method of (Ghayyur *et al*., 2021).

**Statistical Assessments of Data:** The results were assessed using one-way analysis of variance (ANOVA). Mean ± SD was used to define significant results at the ($P<0.05$) levels through software statistics 8.1; the mean difference between the groups was evaluated using the least significant difference (LSD) test.

## RESULTS AND DISCUSSION

Insecticides and other agrochemicals are widely used in the agricultural sector to protect diverse types of crops that pose a danger to living beings, the environment, and other non-target organisms sharing the same ecosystem. This research was carried out to on how certain insecticides, such as chlorpyrifos, affected pigeon hematology. These agrochemicals produced male fertility disorders, gonad somatic effects, decreased serum testosterone levels, chromosome abnormalities, fetal deformities, and malignancies; it is essential to evaluate the toxicological and safety consequences of insecticides (Hussain *et al.*, 2014). While various anomalies were seen in our study, the pigeons exposed to CPF and the control group's pigeons were found normal and healthy, and no death was noted in them. Following exposure to CPF, drooping wings, diarrhea, a reduction in the frequency of cooing, excessive salivation, and tiredness were all noted. Therefore, the cholinergic toxicity brought on by the pesticide's metabolites may be connected to the behavioral change and significant ($p < 0.05$) decreased feed intake (Table. 1) and symptoms shown in the present research. Earlier findings for Japanese quill exposure to CPF were consistent with those of our results (Hussain *et al.*, 2014). In our investigation, the use of chlorpyrifos (CPF) resulted in toxicity-related death in eight (08) birds from group B, ten (10) birds from group C,

and twelve (12) birds from group D, respectively, in accordance with previously reported results that chlorpyrifos induces behavioral problems in fishes (Ghayyur *et al.,* 2021). Similar behavioral alterations also appeared in previous studies of Japanese quill exposure to chlorpyrifos (Boumezrag *et al.,* 2021; Suliman *et al.,* 2020).

**Table. 1:** Fortnightly and after 84 days' mean values with significant ($p < 0.05$) reduction of feed intake on exposure to CPF in pigeons of groups B, C & $D_1$, and compared to the control

| | **GROUP A (Control)** | **GROUP B** | **GROUP C** | **GROUP D** |
|---|---|---|---|---|
| DAY 14 | 59.57 | 55.5 | 49.28 | 48.21 |
| DAY 28 | 64.78 | 53.64 | 47.5 | 45.85 |
| DAY 42 | 67.53 | 47.5 | 44.07 | 40.42 |
| DAY 56 | 70.17 | 46.12 | 42.21 | 39.31 |
| DAY 70 | 73.31 | 43.53 | 38.33 | 36.83 |
| DAY 84 | 76.81 | 40.24 | 33.26 | 32.13 |
| Mean/Average feed intake per day after 84 days | 68.69 | 47.755* | 42.44** | 40.45** |

Exposures to CPF showed a significant ($P < 0.05$) reduction in body weights of pigeons from treated birds of B, C and D groups as compared to control, whereas a high dose mainly produced more reduction in the body weight (Table. 2)

**Tab. 2:** Mean body weights of CPF-treated pigeons of group B, $C_1$ & D with significant decline ($p < 0.05$) ** as compared to the control group

| **Body Weight (BW)** | **GROUP A (Control)** | **GROUP B** | **GROUP C** | **GROUP D** |
|---|---|---|---|---|
| BW (Grams) | ↑ 66.57 a | ↓ 36.00**b | ↓54.92**c | ↓ 68.55**d |
| (BW) percentage | ↑ 23.98% | ↓ 13.02% | ↓19.97% | ↓24.82% |

Pesticides, especially CPF, are one of the most toxic pesticides to birds, as they elevate the risk of mortality (Patterson *et al.,* 2017). In our study, hemoglobin (Hb) levels showed notable and substantial decreases ($p < 0.05$) in pigeons on exposure to CPF compared to the control group levels. Hemoglobin levels reduced at 19.47%, 29.07%, and 38.95%, respectively, in pigeons from B, C, and D groups (Figure. 1).


**Figure. 1: (Mean) Hemoglobin (Hb) levels pigeon of CPF groups with a significant decrease ($p < 0.05$) ** as compared to the control group.**

It was determined that pesticides containing chlorpyrifos cause significant changes in hemoglobin concentration. Thus, the current study's hematological results demonstrated a considerable decline in packed cell volume (PCV) and total erythrocyte counts (TEC) due to the toxic effects of CPF. In the Japanese quill, comparable outcomes have also been shown (Hussain *et al.,* 2014). The poisonous effects of pesticides on developing erythrocytes and insufficient iron availability may be the cause of the drop in hemoglobin levels (Hussain *et al.,* 2011). The total erythrocyte count was measured in CPF groups of pigeons in the current investigation. However, when comparing the pigeons in the treated groups to the control group, there was a significant ($p < 0.05$) difference in the concentrations of (TEC) levels at 17.35%, 26.17%, and 33.82%, respectively than in the control group (Figure. 2).

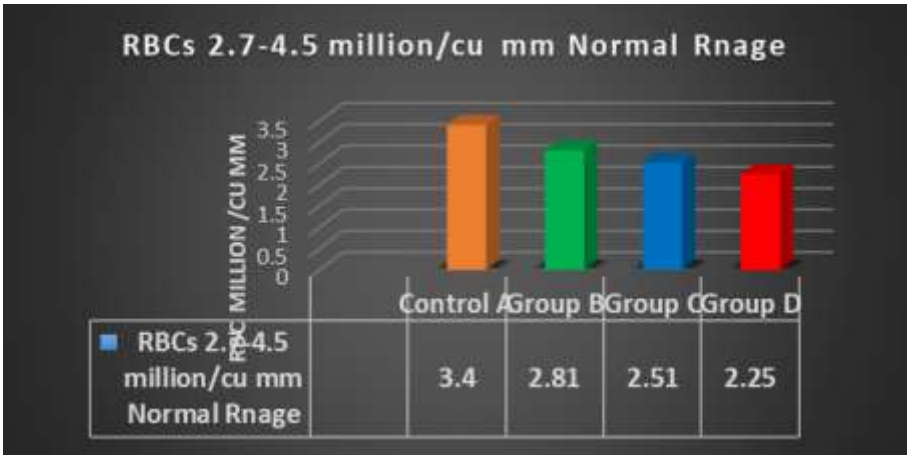


**Figure. 2:** (Mean) total erythrocyte counts (TEC) levels in pigeons of CPF groups with a significant decrease ($p < 0.05$) ** as compared to the control group.

These (TLCs) are the same in the rate of destruction or a decrease in the heme production in the bone marrow (Ahmad *et al.,* 2015). Pesticides may disrupt the erythropoietin tissues and affect the feasibility at cellular levels, which may have contributed to the decrease in TEC and Hb content (Fadina *et al.*, 2017; Zhang *et al.,* 2010). The significant difference ($p <0.05$) in these values of (PCV%) for the pigeons of groups B, C, and D were 20.23%, 29.65%, and 39.45%, respectively; consequently, treated birds showed a noteworthy decrease in (PCV%) as a consequence of the pesticide Chlorpyrifos' hematotoxicity (Figure 3) and it was reported that the proportion of red blood cells (PCV) in the blood depends on both the quantity and quality of RBCs; hence, variations in the quantity and quality of RBCs or hemoglobin will alter the PCV percentage. Previously, FSH treated with acetamiprid and chlorpyrifos showed reduced RBC count, Hb levels, and PCV. As stated in Ghayyur *et al*., 2019; 2021).

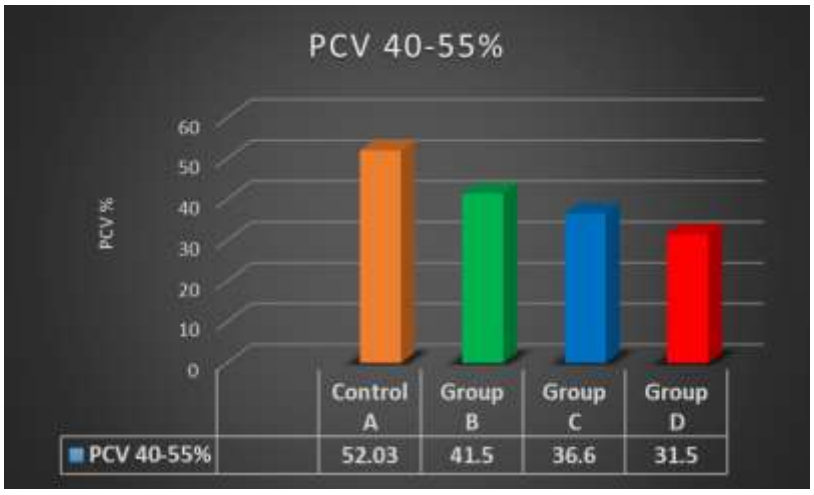


**Figure. 3:** (Mean) packed cell volume (PCV%) levels in pigeons of CPF groups with a significant decrease ($p < 0.05$) ** as compared to the control group.

The mean total leucocyte count (TLC) values for the CPF group's pigeon in our study were also recorded significant ($p < 0.05$) increase likewise palettes conversely, when compared to the levels of the control group (Table.3). Increased production in antibody, which aid in the survival of pigeons exposed to various pesticides, may cause of the increase levels of TLC and platelets (Malik and Maurya, 2014). In the past, cockerels treated with CPF insecticide showed a higher quantity of leukocytes to regulate tissue damage caused by these pesticides (Hussain *et al.*, 2021).

**Table. 3:** (Mean) total leucocyte count (TLC) values and platelets levels in pigeons of CPF groups with significant decrease ($p < 0.05$) ** as compared to the control group**.**

| BIRDS | WBCs (/cu mm) 5000-13000 | Platelets /cu mm (8-10% of RBC) |
|---|---|---|
| Control A1 | 6370a | 28800a |
| Group B1 | 15520**b | 6800**b |
| Group C1 | 16306**c | 6300**c |
| Group D1 | 17820**d | 5300**d |

According to the findings of our study, exposure to CPF exhibited toxic impacts on the hematological indices of pigeons. These results elucidated that CPF insecticide induces alterations in hematological cells. These agrochemicals may even be deadly for human beings, which is at the top level of the food chain. It is concluded that regulatory action at the national level, as well as the reinforcement of laws and actions, may raise awareness regarding the safe use of such insecticides.

## CONCLUSIONS

Keeping in view of our findings, it is concluded that exposure to chlorpyrifos insecticide showed drastic toxic effects on the hematology of pigeons. These results elucidated that CPF insecticide induced alterations in hematological cells and indices. Exposure to CPF exhibited notable statistically significant reduced levels of hemoglobin (Hb), total erythrocytes count (TECs) and packed cell volume (PCV%), respectively. A substantial increase in total leucocyte count (TLC) and total platelet count were also observed. This revealed how the indiscriminate use of pesticides damages health and developmental problems in bird ecosystems, and eventually, human beings may be in serious danger for their health problems. Therefore, the regulatory actions on the country level and reinforcement of the laws and actions may raise awareness regarding the safe use of such insecticides.

## AUTHORS' CONTRIBUTIONS

SA Memon conducted the research, collected all the required data for assessment and evaluation, and wrote the manuscript. N. Memon re-analyzed the data and provided guidance in the conduct of research. N.A Birmani formatted the manuscript and provided guidance in the research work on hematological parameters.

## CONFLICT OF INTEREST

All the authors declare here that they have no conflict of interest.

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