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ANALYZING THE EFFECTS OF SUB-LETHAL DOSES OF CHLORPYRIFOS AND FOSETYL-ALUMINUM ON HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN JAPANESE QUAIL (*COTURNIX JAPONICA*)

[1]Mudassar Maqbool, [1]Ahmad Ali, [2]Zian Ul Abideen, [2]Tahira Ruby, [1]Tayyaba Khan, [3]Wajeeha Afzal, [1]Sidra Bashir, [4]Sobia Malik, [5]Sheikh Muhammad Azam, [1]Mehroz Ali, [1]Tehseen Abbas, [1]Aziz Ullah, [1]Faiza Waheed, [6]Hafiz Muhammad Sajawal, [1]Hira Gill, [1]Muhammad Zeshan Haider, [1]Sobia Abid

[1]Department of Zoology, The Islamia University of Bahawalpur

[2]Institute of Zoology, Bahauddin Zakariya University Multan

[3]Department of Zoology, University of agriculture Faisalabad

[4]Department of Zoology, The Government Sadiq College Women University, Bahawalpur

[5]Department of Zoology, Division of Science and Technology, University of Education, Lahore,

[6]Department of Zoology, Cholistan University of Veterinary and Animal Sciences Bahawalpur

Abstract

Chlorpyrifos and Fosetyl-Aluminum are widely used pesticides in agriculture for controlling insects and fungi, respectively. Hematological and biochemical parameters serve as optimal biomarkers to determine physiological and chemical alterations. To analyze the possible toxicological effects on the hematology and biochemistry of Japanese Quail (*Coturnix japonica*), Chlorpyrifos and Fosetyl Aluminum were used to understand the potential risks posed by these chemicals to avian species. Adult male birds (n=30), 4-6 weeks old, weighing between 90-100g were randomly divided into 5 groups; (G1) Control (Untreated), treatment groups (G2, G3) were given (0.01 mg/day) as low dose while (G4, G5) were given (0.02 mg/day) as high dose of chlorpyrifos and fosetyl aluminum. On the 10th, 15^{th,} and 20th day of the experiment, quails were slaughtered for blood collection. After 10 days of exposure to chlorpyrifos, notable dose-dependent changes were observed in urea, creatinine, bilirubin, ALT, ALP, WBC, MCV, and granulocyte percentages. By 15 days, additional significant increases were noted in MCH and lymphocyte percentages, while parameters such as RBC count, HCT, MCHC, and platelet count remained unaffected. After 20 days, these trends continued with significant elevations in urea, creatinine, bilirubin, ALT, ALP, WBC, MCV, MCHC, lymphocyte, and granulocyte percentages particularly at higher doses. Similarly, after 10 days of Fosetyl-Aluminum exposure, there were significant increases in urea, creatinine, ALT, ALP, WBC, MCHC, granulocyte percentage, and platelet count, with no significant differences in bilirubin, RBC count, HCT, MCV, MCH, and lymphocyte percentage. By 15 days, additional significant increases were observed in bilirubin, MCV, MCH, MCHC, and lymphocyte percentage, while RBC count, HCT, and platelet count remained unaffected. After 20 days, these trends continued with significant elevations in urea, creatinine, bilirubin, ALT, ALP, WBC, MCV, MCHC, lymphocyte, and granulocyte percentages, with no significant changes in RBC count, hematocrit, and platelet count. These findings provide understanding of the multifaceted effects of treatment and time on liver, kidney, immune, and

hematological functions. Further research is required to understand the mechanisms and clinical significance of these impacts on health of Japanese Quail.

Key Words: Chlorpyrifos, Fosetyl Aluminum, Hepatotoxic effects, Hematology

Introduction

Chlorpyrifos and Fosetyl-Aluminum are commonly used pesticides in agriculture to control insects and fungi, respectively. However, prolonged experiences of these pesticides have been tied to adverse effects on the health of animals and humans, such as developmental delays, neurological disorders, and cancer in humans (Kumar et al., 2023). The Japanese quail is a study organism used in toxicological explorations due to its sensitivity to toxic agents and short generation time. Previous studies have illustrated that when Japanese quail are exposed to Chlorpyrifos and Fosetyl-Aluminum can cause significant changes in hematological and biochemical parameters, indicating the potential for adverse health effects.

Hematology and biochemistry are rapidly becoming undoubtedly important symptomatic and comprehensive instruments for veterinary medicine all over the world. Hematological parameters such as blood cell counts, platelets and hemoglobin etc. serve as optimal biomarkers for assessing oxidative stress and capturing physiological and chemical alterations in diverse species following exposure to different classes of toxic substances, including insecticides and pesticides (Hussain et al., 2011) while biochemical parameters such as urea, creatinine, ALT, and ALP etc. provide an understanding of the fundamental processes of life and plays a significant role in fields like medicine, agriculture, and biotechnology (Alassia, 2023). Deviant levels of these substances can serve as indicators for a wide spectrum of conditions, such as liver or kidney disorders, diabetes, malnutrition, electrolyte imbalances, and numerous other medical ailments. Consequently, serum biochemistry analysis stands as a pivotal tool in clinical medicine, aiding in the diagnosis, monitoring, and treatment of an array of diseases and health conditions (Konecka et al., 2023).

Japanese quail (*Coturnix japonica*) is distinct from the common quail (*Coturnix Coturnix*). Japanese quail has been widely used in toxicological studies due to its sensitivity to toxic agents and short generation time. The Japanese quail is known for its disease resistance and is a suitable bird for commercial meat and egg production, as it matures in about 6 weeks and is usually in full egg production by 50 days of age (Mohammed & Ejiofor, 2015). Quail products are consumed as delicacies on festive occasions, and the domesticated Japanese quail, in contrast to its wild form, has lost all migratory behavior (Tavaniello, 2014).

Organophosphorus pesticides (OPs) are among the most frequently used insecticides in the world, and they are widely accessible commercially for both residential and industrial usage. Uncontrolled usage of organophosphate (OP) pesticides raises the risk of pesticide poisoning globally, particularly in developing countries. Chlorpyrifos (CPF) and Fosetyl- Aluminum (Fos-Al) have been widely employed for pest control in various food crops, including grains, cotton, fruits, nuts, and vegetables, as well as for household pest management. It effectively targets foliar pests like aphids, beetles, and caterpillars, along with soil-dwelling pests such as rootworms and cutworms. CPF poses potential exposure risks through contaminated food and inhalation during processing, with systemic toxicity possible through skin absorption and inhalation of fumes during spraying. Although its residential use has ceased in the US and the EU, residues of CPF have been detected in produce, grains, and vegetables (Tavaniello, 2014).

It works by inhibiting the activity of acetylcholine esterase, an enzyme that regulates the neurotransmitter acetylcholine in the nervous system. This leads to an accumulation of acetylcholine, which can overstimulate the nervous system and cause paralysis or death in insects. However, chlorpyrifos is also toxic to humans and other animals. Exposure to the pesticide can cause a range of health effects, including nausea, dizziness, headaches, muscle weakness, and respiratory paralysis. In high doses, it can cause convulsions, coma, and even death. Concerns about the potential effects of Chlorpyrifos on health and the environment have led to controversy and regulatory actions in recent years. Several countries have banned or restricted its use, and the U.S. Environmental Protection Agency (EPA) has proposed phasing out its use on food crops due to concerns about its impact on children's brain development (Wołejko et al., 2022).

There have been several studies on the effects of fosetyl-aluminum on the hematological and biochemical parameters of various animal models and found that exposure to fosetyl-aluminum caused a significant decrease in red blood cell count, hemoglobin concentration, and hematocrit value in Japanese quail. Fosetyl-aluminum can have adverse effects on the hematological and biochemical parameters of Japanese quail, indicating potential health risks for avian species. The study aims to evaluate the impact of sub-lethal doses of two agrochemicals, Chlorpyrifos and Fosetyl-Aluminum, on the health of Japanese quail by assessing changes in their hematological and biochemical parameters. This research seeks to understand the potential risks posed by these chemicals to avian species, which can serve as indicators of environmental health and can be useful for broader implications for wildlife conservation and ecosystem balance.

2. Materials and Methods

The present research was conducted at Department of Zoology, The Islamia University of Bahawalpur. The study strictly followed the established guidelines and principles set forth by the departmental ethics committee concerning the utilization of animals as research models. Every possible effort was undertaken to ensure the kind treatment and well-being of the experimental subjects throughout the duration of the study.

2.1 Experimental Animal

In the present study, a group of apparently healthy sexually mature adult Japanese quails (n=30) with an approximate weight of 90–100 grams and an age range of 4-6 weeks were obtained from Ahmad-Puri Gate Market, Bahawalpur. These quails were then placed in comfortable metal cages under uniform physical conditions; a photoperiod of approximately 12-14 hours of light, a room temperature of 27±5°C, and a relative humidity of 50–60%. All the birds had access to clean, fresh water and a commercially available standard diet (99 King Feeds) containing 20% protein, provided twice daily with an 8-hour interval.

2.2 Preparation of Stock Solutions & Dose Administration

The stock solutions were prepared from the chemicals Fosetyl-Al and Chlorpyrifos, which were procured from M/S Bayers (Pvt.) Ltd. at Korangi Industrial Area, Karachi, and M/S Pak-China Chemicals Lahore, respectively. In this study, high dose solutions of Chlorpyrifos and Fosetyl-Aluminum were prepared by adding 2 mg of each chemical to 1000 mL of water, resulting in a concentration of 0.002 mg/mL. Low dose solutions were prepared by adding 1 mg of each chemical to 1000 mL of water, yielding a concentration of 0.001 mg/mL. Each bird received 10

mL of these solutions daily. Consequently, birds administered the high dose received 0.02 mg of the chemical per day, while those given the low dose received 0.01 mg per day.

2.3 Experimental Design

Throughout the acclimatization phase, all groups of experimental birds received water and food as per their usual routine, and a daily health check was conducted to monitor for any signs of illness. Following the 07-day acclimatization period, the birds were divided into two main groups: the experimental group (n=24) and the control group (n=6), with the allocation being done randomly. The experimental group was further categorized into subgroups based on the type and concentration of dose to be administered. Each group comprised of 6 birds, making a total of 30 birds across all groups. Group 1 (G1) received fresh water and a standard feed twice a day throughout the experimental period. In Group 2 (G2), a low dose of Fosetyl-Aluminum was administered. Similarly, Group 3 (G3) birds were given a low dose of chlorpyrifos. Group 4 (G4) was treated with a high dose of Fosetyl-Aluminum, and Group 5 (G5) was given a high dose of chlorpyrifos. Each bird in the treated groups received an oral dose of 10 ml daily through a crop tube. The lethal dose (LD50) for the Japanese quail is about 15 mg/kg at 94.5% purity (Suliman et al., 2020).

2.4 Blood Sampling

Birds from the experimental group were randomly chosen for euthanasia and sample collection. This euthanasia was carried out by cutting the jugular vein on the 10^{th}, 15^{th}, and 20^{th} day of the experiment. The blood was collected using EDTA vacutainers for hematological analysis and non-EDTA vials for biochemical analysis. The blood samples were then processed within 24 hours to determine hematological parameters and serum was extracted from the blood to conduct biochemical investigations.

2.5 Analysis of Hematological and Biochemical Parameters

The examination of hematological and biochemical factors involved a comprehensive range of parameters. Hematological measurements involved the assessment of various indicators such as the count of red blood cells (RBC), hematocrit levels (HCT), the average volume of a single red blood cell (MCV), the mean hemoglobin content per red blood cell (MCH), the concentration of hemoglobin per red blood cell (MCHC), Granulocyte (GR%), lymphocyte (Lym%) the count of white blood cells (WBC), and the number of platelets (PLT).

With hematological assessments, biochemical parameters were also scrutinized. These included the analysis of urea, creatinine, bilirubin, alanine aminotransferase, and alkaline phosphatase.

2.6 Statistical Analysis

The data of the current study was portrayed in terms of both the mean and standard error. For each specific group, the data exhibited a normal distribution. Subsequently, each of these parameters underwent statistical analysis through one-way ANOVA using “IBM SPSS Statistics Version 27”.

3. Results

The treatment with chlorpyrifos significantly affected several hematological and biochemical parameters in Japanese Quail, particularly at higher doses (1mg). Notable changes were observed in urea (P=0.009), creatinine (0.005), bilirubin (0.048), ALT (0.048), ALP (0.002), WBC (0.008),

MCV (0.001), and granulocyte percentages (0.018), indicating a dose-dependent response to chlorpyrifos exposure. Parameters like RBC count, HCT, MCH, MCHC, lymphocyte percentage, and platelet count showed no significant differences, suggesting selective impacts of chlorpyrifos

Chlorpyrifos				
Parameter	**Control**	**Low (0.01mg)**	**High (0.02mg)**	**P-Value**
	Mean ± SE	**Mean ± SE**	**Mean ± SE**	
Urea (mg/dL)	9±0.1	10.8±0.2	12±0.4	0.009*
Creatinine (mg/dL)	0.1±0	0.55±0.05	0.65±0.05	0.005**
Bilirubin (mg/dL)	0.995±0.015	1.15±0.05	1.4±0.1	0.048*
ALT (IU/L)	11±0	12.85±0.15	15.15±0.35	0.002**
ALP (IU/L)	86.5±0.5	89±1	103±1	0.002**
WBC (10^6m^3)	45827.5±55.5	51870.5±2894.5	66187±689	0.008*
RBC (10^6/µl)	2.85±0.05	3.1±0.3	3.85±0.15	0.073
HCT %	41.45±1.55	50.2±4.4	51.65±2.05	0.163
MCV (fL)	166.85±0.65	175.95±0.55	180.65±0.55	0.001**
MCH (pg/cell)	35.725±1.325	36.35±1.45	40.05±0.15	0.134
MCHC (g/dL)	28.95±0.55	33.2±0.2	34.2±1.6	0.062
LYM %	51.5±3.5	60.5±0.5	64±2	0.067
GR %	30.5±1.5	37±2	47±2	0.018*
PLT (10^3/µl)	16.85±0.15	21.2±2.2	24.15±0.15	0.06

on quail physiology, (Table 1).

Table 1. Comparison of the studied hematological and biochemical parameters between chlorpyrifos treated and untreated Japanese quail for a period of 10 days

P <0.05 = Least Significant (*) P<0.01 = Significant (**)

After 15 days of exposure, significant differences in several hematological and biochemical parameters were observed between the control and chlorpyrifos-treated groups. Notable changes included increased levels of urea (0.02), creatinine (0.005), bilirubin (0.011), ALT (0.005), ALP (0.008), WBC (0.004), MCV (0.002), MCH (0.01), lymphocyte percentage (0.011), and granulocyte percentage (0.019) in the treated groups, particularly at the high dose. Parameters such as RBC count, HCT, MCHC, and platelet count showed no significant differences, indicating selective impacts of chlorpyrifos on specific physiological aspects of Japanese quail, (Table 2).

Table 2. Comparison of the studied hematological and biochemical parameters between chlorpyrifos treated and untreated Japanese quail for a period of 15 days.

Chlorpyrifos				
Parameter	**Control**	**Low (0.01mg)**	**High (0.02mg)**	**P-Value**
	Mean ± SE	**Mean** ± SE	**Mean** ± SE	
Urea (mg/dL)	8.715±0.615	11.95±0.55	13.2±0.4	0.02*
Creatinine (mg/dL)	0.15±0.05	0.75±0.05	0.75±0.05	0.005**
Bilirubin (mg/dL)	1.05±0.05	1.45±0.05	1.8±0.1	0.011*
ALT (IU/L)	10.8±0.3	13.85±0.65	16.5±0	0.005**
ALP (IU/L)	87.5±0.5	93±2	107±2	0.008**
WBC (10^6m^3)	45815±939	52990±2442	67987.5±444.5	0.004**
RBC (10^6/µl)	3.25±0.45	2.95±0.05	3.6±0.2	0.393
HCT %	43.65±1.55	50.65±4.55	51.6±3	0.312
MCV (fL)	169.6±0.8	176.6±0.8	183±0	0.002**
MCH (pg/cell)	35±0	38.65±0.95	41.9±0.5	0.01*
MCHC (g/dL)	29.3±1.3	34.15±0.05	36.55±1.85	0.063
LYM %	51.5±0.5	65.5±2.5	71±2	0.011*
GR %	30.5±0.5	40±3	50.5±2.5	0.019*
PLT (10^3/µl)	17.65±1.65	22.6±1.9	24.2±1.3	0.129

P <0.05 = Least Significant (*) P<0.01 = Significant (**)

After 20 days of exposure to chlorpyrifos, significant differences in several hematological and biochemical parameters were observed between the control and treated groups. Notable changes included increased levels of urea (0.020), creatinine (0.009), bilirubin (0.006), ALT (0.006), ALP (0.004), WBC (0.004), MCV (0.002), MCHC (0.006), lymphocyte percentage (0.036), and granulocyte percentage (0.032) in the treated groups, especially at the high dose. Parameters such as RBC count, HCT, MCH, and platelet count showed no significant differences, indicating selective impacts of chlorpyrifos on specific physiological aspects of Japanese quail, (Table 3).

Table 3. Comparison of the studied hematological and biochemical parameters between chlorpyrifos treated and untreated Japanese quail for a period of 20 days.

Chlorpyrifos				
Parameter	**Control**	**Low (0.01 mg)**	**High (0.02 mg)**	**P-Value**
	Mean ± SE	**Mean ± SE**	**Mean ± SE**	

Urea (mg/dL)	8.8±0.4	13.1±0.6	15.7±1.2	0.02*
Creatinine (mg/dL)	0.15±0.05	1±0.1	1±0.1	0.009**
Bilirubin (mg/dL)	1.025±0.075	1.7±0.1	2.05±0.05	0.006**
ALT (IU/L)	11.65±0.35	15.1±0.6	17.75±0.35	0.006**
ALP (IU/L)	88±2	97.5±0.5	109±1	0.004**
WBC (10^6m^3)	47780±985	55209.5±2444.5	70148.5±394.5	0.004**
RBC (10^6/µl)	3.05±0.05	3.15±0.05	3.5±0.4	0.461
HCT %	44.3±0.5	51.55±4.25	51.5±3.5	0.32
MCV (fL)	172.1±0.5	176.85±0.65	185.5±1	0.002**
MCH (pg/cell)	36.5±2.5	39.4±0.8	45.2±0.6	0.062
MCHC (g/dL)	27.5±0.5	35±0.7	38.75±1.25	0.006**
LYM %	53±3	66.5±4.5	75±1	0.036*
GR %	29.5±1.5	39±5	53±2	0.032*
PLT (10^3/µl)	18.6±1.4	25.1±1.6	25.6±2	0.102

P <0.05 = Least Significant (*) P<0.01 = Significant (**)

Significant differences were observed in several hematological and biochemical parameters between the control and treated groups of Japanese Quails after 10 days of exposure to Fosetyl-Aluminum. Notable changes included increased levels of urea (0.007), creatinine (0.013), ALT (0.001) ALP (0.008), WBC (0.03), MCHC (0.018), granulocyte percentage (0.042), and platelet count (0.04) in the treated groups, particularly at the high dose. Parameters such as bilirubin, RBC count, HCT, MCV, MCH, and lymphocyte percentage showed no significant differences, indicating selective impacts of f Fosetyl-Aluminum on specific physiological aspects of Japanese quails, (Table 4).

Table 4. Comparison of the studied hematological and biochemical parameters between fosetyl-aluminum treated and untreated Japanese quails for 10 days.

Fosetyl-Aluminum				
Parameter	**Control** **Mean ± SE**	**Low (0.01mg)** **Mean ± SE**	**High (0.02mg)** **Mean ± SE**	**P-Value**
Urea (mg/dL)	9±0.14142	11.3±0.56569	11.9±0.14142	0.007**
Creatinine (mg/dL)	0.1±0	0.4±0	0.5±0	0.013**

Bilirubin (mg/dL)	0.995±0.02121	0.965±0.03536	1.12±0.25456	0.599
ALT (IU/L)	11±0	11.65±0.2121	13.8±0.1414	0.001**
ALP (IU/L)	86.5±0.707	90.5±0.707	92.5±0.707	0.008**
WBC (10^6m^3)	45827.5±78.489	47999±2654.479	54664±1428.356	0.03*
RBC (10^6/µl)	2.85±0.0707	3±0.1414	3.45±0.2121	0.059
HCT %	41.45±2.192	50.7±3.5355	51.75±2.0506	0.054
MCV (fL)	166.85±0.9192	109.75±91.5703	178.4±0.4243	0.474
MCH (pg/cell)	35.725±1.87383	35.15±0.6364	38.4±0.70711	0.139
MCHC (g/dL)	28.95±0.7778	31.95±0.7778	34.8±1.1314	0.018**
LYM %	51.5±4.95	55.5±2.121	61±2.828	0.155
GR %	30.5±2.121	33.5±0.707	40±2.828	0.042*
PLT (10^3/µl)	16.85±0.2121	19.95±0.6364	23.85±2.4749	0.04*

P <0.05 = Least Significant (*) P<0.01 = Significant (**)

After 15 days of exposure to Fosetyl-Aluminum, significant differences were observed including increased levels of urea (0.012), creatinine (0.013), bilirubin (0.041), ALT (0.001), ALP (0.011), WBC (0.012), MCV (0.01), MCH (0.01), MCHC (0.025), lymphocyte percentage (0.006), and granulocyte percentage (0.024) in the treated groups, particularly at the high dose. Parameters such as RBC count, HCT, and platelet count showed no significant differences, (Table 5).

Table 5. Comparison of the studied hematological and biochemical parameters between fosetyl-

Fosetyl-Aluminum				
Parameter	**Control**	**Low (0.01mg)**	**High (0.02mg)**	**P-Value**
	Mean ± SE	**Mean ± SE**	**Mean ± SE**	
Urea (mg/dL)	8.715±0.615	11.3±0.56569	11.9±0.14142	0.012**
Creatinine (mg/dL)	0.15±0.05	0.4±0	0.5±0	0.013**
Bilirubin (mg/dL)	1.05±0.05	0.965±0.03536	1.12±0.25456	0.041*
ALT (IU/L)	10.8±0.3	11.65±0.2121	13.8±0.1414	0.001**
ALP (IU/L)	87.5±0.5	90.5±0.707	92.5±0.707	0.011*
WBC (10^6m^3)	45815±939	47999±2654.479	54664±1428.356	0.012**
RBC (10^6/µl)	3.25±0.45	3±0.1414	3.45±0.2121	0.487
HCT %	43.65±1.55	50.7±3.5355	51.75±2.0506	0.308
MCV (fL)	169.6±0.8	109.75±91.5703	178.4±0.4243	0.01**
MCH (pg/cell)	35±0	35.15±0.6364	38.4±0.70711	0.01**
MCHC (g/dL)	29.3±1.3	31.95±0.7778	34.8±1.1314	0.025*
LYM %	51.5±0.5	55.5±2.121	61±2.828	0.006**
GR %	30.5±0.5	33.5±0.707	40±2.828	0.024*
PLT (10^3/µl)	17.65±1.65	19.95±0.6364	23.85±2.4749	0.497

aluminum treated and untreated Japanese quails for 15 days.
P <0.05 = Least Significant (*) P<0.01 = Significant (**)

After 20 days of exposure to Fosetyl-Aluminum, significant differences were observed in several hematological and biochemical parameters between the control and treated groups. Significant increase was noted in parameters such as urea (0.003), creatinine (0.005), bilirubin (0.005), ALT (0.003), ALP (0.027), WBC (0.036), MCV (0.004), MCHC (0.006), lymphocyte percentage (0.012), and granulocyte percentage (0.013), particularly in the high-dose group. Parameters such as RBC count, hematocrit, and platelet count showed no significant differences, indicating specific physiological impacts of Fosetyl-Aluminum on Japanese quail, (Table 4.6).

Table 6. Comparison of the studied hematological and biochemical parameters between Fosetyl-Aluminum treated and untreated Japanese quails for 20 days.

Fosetyl-Aluminum				
Parameter	**Control**	**Low (0.01mg)**	**High (0.02mg)**	**P-Value**
	Mean ± SE	**Mean ± SE**	**Mean ± SE**	
Urea (mg/dL)	8.8±0.56569	12.3±0.28284	13.05±0.07071	0.003**
Creatinine (mg/dL)	0.15±0.0707	0.55±0.0707	0.85±0.0707	0.005**
Bilirubin (mg/dL)	1.025±0.10607	1.35±0.07071	1.85±0.07071	0.005**
ALT (IU/L)	11.65±0.495	13.35±0.2121	16.5±0.4243	0.003**
ALP (IU/L)	88±2.828	94.5±0.707	99.5±2.121	0.027*
WBC (10^6m^3)	47780±1393	53518±3211.679	58709.5±1492.702	0.036*
RBC (10^6/µl)	3.05±0.0707	3.6±0.2828	3.55±0.495	0.324
HCT %	44.3±0.7071	50.25±5.3033	49.95±0.495	0.245
MCV (fL)	172.1±0.7071	175.6±0.8485	180.8±0.8485	0.004**
MCH (pg/cell)	36.5±3.53553	38.65±0.07071	43.05±0.77782	0.108
MCHC (g/dL)	27.5±0.7071	34.25±1.0607	36.6±1.1314	0.006**
LYM %	53±4.243	61.5±0.707	73.5±2.121	0.012**
GR %	29.5±2.121	35±1.414	45±2.828	0.013**
PLT (10^3/µl)	18.6±1.9799	20.95±5.0205	21.3±2.4042	0.718

P <0.05 = Least Significant (*) P<0.01 = Significant (**)

Discussion

Pesticides are frequently used in agriculture for the consumption of phytosanitary products and pest eradication, ultimately contaminating water resources and soil through spray drift, run-offs and leaching (Barranger et al., 2014). Persistent exposure to such chemical entities not only causes detrimental impacts to non-target organisms but also poses a dangerous situation for humans, mainly due to the residual effect of the pesticides in crops/vegetables (Taylor et al., 2002)
Organophosphate (OP) insecticides are one of the most widely used chemicals in agriculture and public health (Ambali et al., 2010). OPs induce neurotoxicity and tissue damage with observable signs of poisoning. In mammals and birds, the toxic effects of Ops results from the inhibition of cholinesterase enzyme, which leads to the pooling of acetylcholine at the nerve endings and neuromuscular junctions, exhibiting nicotinic, muscarinic, and central nervous system effects resulting in from cholinergic overstimulation (Costa, 2006; Hamm et al., 1998; Richardson, 1995). Prolonged exposure to these insecticides has been shown to cause severe damage to vital organs.

In the present study, we evaluated the toxicity induced by two organophosphorus pesticides, chlorpyrifos and fosetyl-aluminium, and their effects on the hematological and biochemical profile of Japanese quails.

Hemato-biochemical investigations are important for the analysis of the functional status of animals/birds exposed to suspected toxic agents (Omitoyin, 2006). Hematological components such as MCH and MCHC are derived from Hb and RBC, any alteration in Hb and RBC levels would lead to the alteration of MCH and MCHC. MCV indicates the status or size of RBCs (Alwan et al., 2009). The selected pesticides tested in this study led to a significant increase in MCV, MCH, and MCHC concentrations. However, a notable decline was observed in the RBCs count. The increase in MCV and MCH values on treatment with pesticides indicates that the decrease in RBC count may be resulted from destruction of RBCs or their lessened synthesis in the bone marrow (Shakoori et al., 1992). The results of our study are in agreement with those of previous studies on rats (Akhtar et al., 2009), fish (Ghayyur et al., 2019), broiler chicks (Ahmad et al., 2015) and pigeons ((Memon et al., 2024) when exposed to chlorpyrifos. A significant elevation in MCH and MCV levels also indicates macrocytic anemia, which means that red blood cells are larger than typical (Nalina Thimmappa et al., 2019). Macrocytic anemia can be a sign of various health issues, including vitamin deficiencies and liver impairment.

Alterations in WBC counts after exposure to chemical stressors may be associated with a decrease in the nonspecific immunity of organisms. Thus, WBCs are an important blood factor in the adjustment of immunological actions against disease and foreign bodies in various animal species (Ribeiro et al., 2006). The increase in the total WBC counts of Japanese quails after exposure to selected pesticides chlorpyrifos and fosetyl-aluminium in this study can be attributed to the established leucocytosis, which is considered to be of an adaptive value for the tissue under chemical stress. This also helps in the removal of cellular debris of necrosed tissue at a faster rate (Marti et al., 1996). In the presence of foreign substances or under pathological conditions, leucocytosis in the organism may be a consequence of direct stimulation of immunological defense(Marti et al., 1996). The increase in WBC count can also be correlated with an increase in antibody production which helps in survival and recovery of the animal exposed to chemical stressors. Similar trends were observed after chlorpyrifos exposure in house sparrows (Yadav, 2017), (Noreen et al., 2023); (Memon et al., 2024) and rats (Akhtar et al., 2009). Previously, other organophosphorus insecticide-treated cockerels showed an increased number of WBCs to control tissue injuries induced by these insecticides (Hussain et al., 2012). Contrary to the present findings, significantly decreased WBC counts have also been reported in previous studies (Shakoori et al., 1992) on exposure to chlorpyrifos.

The transaminases (AST and ALT) and ALP regulate physiological processes, catalyzing transamination reactions to facilitate xenobiotics and other macromolecules' metabolism. Therefore, alterations in their activities allow direct identification of hepatic damages (Bacchetta et al., 2014; Fırat et al., 2011). In the present study, a significant dose-dependent elevation in liver enzyme levels such as ALP and ALT was recorded in Japanese quails after exposure to both chlorpyrifos and Fosetyl-Aluminium. The increased activities of these enzymes indicate liver damage with enhancement in the permeability of hepatocyte membranes leading to leakage of these enzymes into the bloodstream. It is also said that increased enzyme concentrations are an indication of recent organ damage rather than decreased organ function (Lumeij, 1997). Chlorpyrifos induced elevations of liver enzymes have also been observed in previous studies on birds such as broiler chicks(Ahmad et al., 2015) and indigenous chicken (Begum et al., 2015).

Some studies have also reported the hepatotoxicity induced by chlorpyrifos exposure in rats (Mansour & Mossa, 2010; Saoudi et al., 2021; Tanvir et al., 2016) .

Urea and creatinine are waste products of protein metabolism that are excreted through the kidney. The marked elevation of plasma creatinine and urea levels in the Chlorpyrifos treated organisms indicates functional damage to the kidney and reduced glomerular filtration (Tanvir et al., 2016). Urea levels can also be increased by other factors, such as dehydration, antidiuretic drugs, and diet; however, increased creatinine is more specific to kidney damage (Garba et al., 2007). In this investigation, selected organophosphorus pesticides, chlorpyrifos and fosetyl-aluminium exposure led to an increase in creatinine and blood urea, especially in groups treated with higher concentrations, as compared to control. This could be the result from glomerular dysfunction or increased renal tissue breakdown, or decreased urinary clearance by the kidney. The results of our study are in accordance with those of previous studies which showed chlorpyrifos induced increments in blood urea and creatinine levels in rats (Saoudi et al., 2021; Tanvir et al., 2016) and mice (Ambali et al., 2007).

According to the findings of our study, exposure to both chlorpyrifos and fosetyl-aluminum exhibited toxic impacts on the hemato-biochemical indices of Japanese quails; however, chlorpyrifos had more profound effects comparatively. These results elucidated that organophosphate pesticides induce alterations in different organs of non-target species like birds. These agrochemicals may even be deadly for human beings, who are at the top level of the food chain. It is concluded that regulatory action at the national level and the reinforcement of laws and actions may raise awareness regarding the safe use of such pesticides.

Conclusion

In conclusion, the comprehensive evaluation of sub-lethal doses of Chlorpyrifos and Fosetyl-Aluminum on serum biochemical and hematological parameters in Japanese quails has provided understanding of the potential consequences of pesticide exposure on Japanese quail health. The study illuminated the impact of these chemicals on various physiological markers. Urea, and creatinine levels increased significantly resulting in susceptibility in kidney function and overall protein metabolism. Marginal deviations in bilirubin and ALP suggested subtle perturbations in liver function, consistent with findings from previous studies. RBC count exhibited significant changes, with an increase in high-dose of Chlorpyrifos in groups. WBC count fluctuations and immunosuppressive effects were observed.

Recommendations

The current study was a preliminary study conducted on sub-lethal effects of chlorpyrifos and fosetyl aluminum on the hematological and biochemical parameters of quail. Pesticides not only affect quails but also other organisms such as chickens, rats, rabbits, and even humans. These pesticides can cause not only hematological and biochemical changes but also affects neural functions. Moreover, they serve as a source of oxidative stress and apoptosis. It is necessary to conduct further detailed studies on these pesticides and take necessary steps to reduce their effects.

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