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Impact of chlorpyrifos on health biomarkers of broiler chicks

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ABSTRACT

The present study aimed to investigate the deleterious effects of chlorpyrifos (CPF) in experimentally exposed broiler birds. The experiment was carried out on one day old (n = 120) broiler chicks. The CPF was reconstituted in corn oil as vehicle (1 ml/kg) to obtain a final concentration of a single dose to the birds 5, 10 and 20 mg/kg body weight (BW) for fourteen days of the experiment through the stomach tube. The control group was given corn oil 1 ml/kg only. Birds exposed to high dose (20 mg/kg BW) showed signs of toxicity (salivation, lacrimation, gasping, convulsions, frequent defecation and tremors). The birds exposed to 10 and 20 mg/kg showed significantly ($P \leq 0.05$) decreased body weight. Significantly ($P \leq 0.05$) decreased hematological parameters i.e. total erythrocyte counts, hemoglobin concentration, hematocrit and total leukocyte were observed in the high dosed group as compared to control and other low dosed fed birds. Serum protein and albumin showed a significant ($P \leq 0.05$) increase in high dosed CPF fed birds. Non significant results were observed in the case of globulin. The acetylcholinestrease (AChE) activity was significantly ($P \leq 0.05$) decreased in blood, serum and plasma in CPF fed birds compared to control birds. In CPF fed birds as compared to control birds we found significantly ($P \leq 0.05$) higher levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Necrotic and degenerative changes were observed on histopathological investigations of spleen, kidneys, bursa of Fabricius, thymus and brain tissues in CPF exposed birds. In conclusion the chlorpyrifos induced toxicopathological effects on health biomarkers of broiler chicks.

1. Introduction

In Pakistan, approximately more than 108, 39, 30, 6 and 5 types of insecticides, weedicides, fungicides, rodenticides and acaricides, respectively are used [1] where in the last two decades the use of insecticides increased and the number of sprays per crops has touched the level of more than ten, which is dangerous for human health [2]. Due to scarcity of data, underreporting and misdiagnosis of insecticide toxicity the number of cases may be higher than the reported one in countries like Pakistan [1,3].

Organphosphorous (OP) pesticides are excessively used in domestic and garden applications to eradicate/control fruit flies, mosquito eradication and as a topical treatment for head lice [4,5]. The main routes of toxicity of organophosphate are through inhalation, ingestion or through skin contamination [6]. Long term exposure to these chemicals induces countless abnormalities and shortens the life span of organisms [7]. Due to moderate environmental persistence, high selectivity toward insects and relatively low toxicity to mammals, organophosphorus is extensively used in agro-livestock production [8–10]. The mechanism of toxic action of organophosphate compounds in mammals and birds is through inhibition of the target enzyme cholinesterase, which leads to accumulation of acetylcholine at nerve terminals and neuromuscular junctions. This accumulation leads to cholinergic overstimulation manifested as muscarinic, nicotinic, and central nervous system effects [11,12]. The most important diagnostic or biomarker endpoint of organophosphate exposure and poisoning is decreased cholinesterase activity in the blood (erythrocytes, plasma or serum) and other tissues like brain [13]. Birds have no cholinesterase activity in erythrocytes; therefore, the extent of their exposure to organophosphate poisoning is based on cholinesterase activity in plasma and nervous tissue [14].

Chlorpyrifos [O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)-phosphorothioate] has a broad spectrum activity within the groups of OP insecticides, therefore it is a major concern with agriculture, and public health. Neurotoxicity is the main manifestation of chlorpyrifos (CPF) due to long term exposure or acute intoxication [15].

Hemato-biochemical investigations are important for the analysis of the functional status of animals/birds to suspected toxicant agents [16]. Chlorpyrifos decreases the body weight gain and is also involved in hepatic injury as revealed by increased activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in rats and layer chicken [17,18]. Many studies have investigated the chronic toxicity of chlorpyrifos in birds and have noted adverse effect on

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fertility, hatchability, embryonic deformities and body weight [19,20]. Some of the hematological studies have revealed that there is no change in the hematological parameters but the values of serum biochemistry were increased significantly in a dose dependent manner [21].

Most of the studies of CPF toxicity have been conducted on rats and data regarding CPF toxicity in broiler birds are not well known. Studies on CPF toxicity in birds investigated the hematological or biochemical aspects only and a single study reported the relationship of CPF and cholinesterase activity in broiler chicks [22]. Few studies demonstrated the protein status in broiler chicks exposed to CPF [23,24]. Therefore, the present study was designed to comprehensively investigate the clinical signs, hemato-biochemical alterations, enzyme variations, cholinesterase activity and toxicopathology induced by CPF in broiler birds. Our findings evidenced that CPF induced significant alterations on health biomarkers of chicken than control birds, with a significant decrease in acetylcholine concentration and internal organ damage.

2. Materials and methods

2.1. Experimental birds, management and treatment protocol

The in vivo experiment was approved by the Institutional Biosafety and Bioethics Committee (IBCC) of the University of Agriculture, Faisalabad, Pakistan and the research was planned in accordance with national legislation regarding the safety and welfare of animals. For this purpose, one-day old (n = 120) broiler chicks were procured from local hatchery and kept in separate cages under similar management and housing conditions i.e. temperature (25–32 °C) and humidity (55–65%). All the birds were provided ration containing 17% crude protein [25] and water *ad libitum* throughout the experiments (42 days). Routine vaccination schedule for broiler birds was followed during the experiment. This experiment was performed during 2012–2013.

Chlorpyrifos (CPF 92%) was purchased from the M/S Ali Akbar Group of Industries, Raiwind Road, Lahore, Pakistan. The CPF was reconstituted in corn oil as vehicle. After four days of acclimatization, all the birds were randomly divided into four equal groups. Birds in the first three groups received chlorpyrifos at doses of 5, 10 and 20 mg/kg body weight (BW) for 14 days of the experiment through crop tubing, respectively. The fourth (control) group was given corn oil 1 ml/kg BW.

2.2. Physical parameters examined

All the birds were monitored daily for clinical signs (salivation, lacrimation, gasping, convulsions, frequent defecation and tremors) and the severity of clinical signs was categorized as mild, moderate and severe. At the end of the experiment a cumulative picture of clinical signs was developed for description. The body weight of all the birds was recorded weekly. Data of feed intake were taken on a daily basis and presented on a weekly basis at the end of the experiment. The birds (n = 5) in each group were sacrificed by cervical dislocation at 0, 14, 28 and 42 days of the experiments for biochemical and histopathological studies. A summary of sampling schedule during the CPF toxicity experiment for various tests is described below:

Parameters studied	Sampling days				
	0	7	14	28	42
Hematology	✓	–	✓	✓	✓
Enzymes analysis (AST and ALT)	✓	–	✓	✓	✓
Protein analysis	✓	–	✓	✓	✓
Acetylcholinesterase	✓	✓	✓	✓	✓
Histopathology	✓	–	✓	✓	✓

2.3. Hemato-biochemical parameters studied

The blood samples were collected from wing vein of birds in EDTA (1 mg/ml) coated vacuum tubes and used for analysis of hematological procedures [26]. Erythrocyte counts were conducted with the help of a hemocytometer using the technique described by Natt and Herrick [27]. Specialized solution (Natt and Herrick) was used for counting both RBC and WBC. It is the proportion of blood cells occupied by the RBCs and its determination was carried out by microhematocrit method [26]. Hemoglobin concentration (Hb) was measured by spectrophotometery. Drabkin's solution [28] was used in the procedure at 540 nm wavelength. The volume of 20 µl blood from each bird was mixed in 5 ml Drabkin's solution. In the reaction mixture the color was developed and its absorbance was measured by spectrophotometer at 540 nm.

Serum was collected from blood samples without addition of anticoagulant and stored at –20 °C. The serum concentration of the enzyme ALT was measured by using the commercially available kit (ALAT-IFFC MOD, Ref # 12032, Germany) and for AST (ASAT-IFFC MOD, Ref # 12021, Germany) was used. For total protein analysis, a commercially available kit (Fluitest® TP, Ref # 9106, Germany) was used. A commercially available kit (Fluitest® ALB BCG, Ref # 9136, Germany) was used for the analysis of total albumin. Globulin reading was obtained by subtracting the total albumin from the total serum proteins.

2.4. Acetylcholinesterase assay

The acetyl cholinesterase activity was measured at 0, 14, 28 and 42 days of the experiment and was also noted on the 7th day as well to check the acute/early phase effect of CPF on acetylcholine inhibition/activity in broilers. The acetylcholinesterase assay was performed within 2 hours following the collection of blood, plasma and serum with BioAssay Systems' QuantiChromTM Acetylcholinesterase Assay (DACE-100, Hayward) based on an improved method [29]. In the assay the thiocholine produced by the action of acetylcholinesterase forms a yellow color with 5,5′-dithiobis (2-nitrobenzoic acid). The intensity of the product color was measured at 412 nm and was proportional to the enzyme activity in the samples (blood, serum and plasma).

2.5. Gross lesions and histopathology

The gross and histopathological lesions were observed following CPF treatment and in control birds (n = 5 each group) at 0, 14, 28 and 42 days. The visceral organs (brain, thymus, spleen, bursa of Fabricious and kidneys) were weighed, examined for the gross lesions and fixed in 10% neutral buffered formalin as described previously [30]. Tissue specimens for histopathology were processed by the routine method of dehydration and paraffin embedding techniques [31]. Sections of 4–5 µm thickness were cut and stained with hematoxylin and eosin [32].

2.6. Statistical analysis

Two Factor Factorial Design was used for statistical analysis. The data were subjected to analysis of variance and different group means were compared by using Tukey's test using R 2.14.1 statistical computer package with the level of significance $P \leq 0.05$. The data were presented as mean ± SD and mean ± SE as indicated.

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