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PATHOLOGICAL CHANGES OF CHLORPYRIPHOS INDUCED CHRONIC TOXICITY IN INDIGENOUS CHICKEN

[1,*]Shameem Ara Begum, [1]Tirtha Nath Upadhyaya, [1]Gautam Kumar Baruah, [1]Taibur Rahman, [1]Debesh Chandra Pathak and [1]Sushanta Goswami

*Department of Pathology, College of Veterinary Science, AAU, Khanapara, Guwahati- 781022, Assam, India
Department of Veterinary Pathology, CVSc, Khanapara, Ghy-22, Assam, India

***Corresponding Author**

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Abstract

Chlorpyriphos (CPF; diethyl 3, 5, 6-trichloro-2-pyridyl phosphorothionate) an organophosphate is one of the most widely used insecticides in agriculture worldwide. It has also been used for the control of termites in chicken houses.The objective of the present work was to assess the effect of oral (p.o.) administration of CPF in indigenous chicken. The birds were divided into two major groups -Group I and Group II Group I served as control and Group II was treated with CPF (0.36 mg/kg) orally daily up to 12 weeks. Detailed post mortem examinations of the weekly sacrificed birds were conducted and representative tissues were collected for histopathological examination. Histopathologically, liver tissue of CPF intoxicated chickens showed degeneration, hepatocellular necrosis, congestion, haemorrhages, dilatation of sinusoids and mononuclear cell infiltration. The kidneys showed haemorrhages, cellular swelling, dilatation of Bowman's space and focal coagulative necrosis. Brain showed satellitosis, neuronophagia and degeneration of Purkinje cells. Degenerative changes along with congestion and haemorrhage were observed in intestine, brain and heart. The results indicated that chronic CPF toxicity produces histopathological alterations of different organs in the treated birds.

Keywords: Chickens, Chlorpyriphos, Chronic toxicity, Pathology.

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INTRODUCTION

Insecticides are being used extensively all over the world in the field of agriculture and veterinary practice. However, indiscriminate use of insecticide(s) has led to a widespread concern over the potential adverse effects of these chemicals on animal and human health as these chemicals interfere with the defense mechanisms of the host, which normally ensures its survival against invading pathogens (Kammon 2010 pathobiochem). Organophosphate insecticides are increasingly used as substitutes for organchlorine and carbamate insecticides because of their high efficacy and lower persistence in the environment. Chlorpyriphos (O, O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) is one of about 100 OP insecticides in the market today. It is used to kill insect pests by disrupting their nervous system. Chlorpyriphos (CPF) has an advantage over other products in that it is effective against a wide range of plant-eating insect pest. Chlorpyriphos affects the nervous system of the pest by inhibiting the breakdown of acetylcholine (ACh), a neurotransmitter. When insects are exposed, chlorpyriphos binds to the active site of the cholinesterase (CHE) enzyme, which prevents breakdown of ACh in the synaptic cleft. The resulting accumulation of ACh in the synaptic cleft causes overstimulation of the neuronal cells, which leads to neurotoxicity and eventually death (Karanth and Pope, 2000). Chronic exposure to CPF elicits a number of other toxic affects including hepatic dysfunction, immunological abnormalities, embryotoxicity, genotoxicity, teratogenicity, neurotoxicity, and neurobehaviourial changes (Rahman *et al.,* 2002; Harfod *et al.,* 2005; Verma *et al.,* 2007; Ahmed *et al.,* 2010). As there is paucity of literature on chlorpyriphos induced pathological changes on various organs in indigenous chicken. The objective of this study was to investigate the pathological changes of chronic exposure to chlorpyriphos in indigenous chickens.

MATERIALS AND METHODS

Animal

Three month old unsexed twenty-four indigenous chickens were procured from All India Coordinated Research Project (AICRP) on Poultry, College of Veterinary Science, A.A.U,

Khanapara Guwahati-781022 were wing banded, weighed and reared in the Department of Pathology, College of Veterinary Science, A.A.U, Khanapara Guwahati-781022 with *ad libitum* supply of feed and water treated. The experimental trials were approved by the Institutional Animal Ethics Committee (No.770/ac/CPCSEA/ FVSc/AAU/IAEC/11-12/128), India and conducted under its guidelines at Poultry Research Station, Chennai 600035.

Chemical (Insecticide)

Commercial products of chlorpyriphos (20%) used in this study was procured from Excel Crop Care Private Limited, Mumbai, India.

Treatment groups

In this study, twenty four chickens were randomly segregated into two groups of 12 each and fasted for 6 h prior to dosing. Following the period of fasting, the birds were weighed and the doses were calculated according to the body weight. CPF was diluted in in a tenfold serial dilution with distilled water to obtain a concentration of 0.2 mg /ml (10^{-4}). Fresh preparations were orally administered daily using oral gavage. The first group was given 0.36 mg/kg bw CPF (1.8 ml of 10^{-4} dilution) the daily up to 90 days. Doses were calculated on weekly body weight basis and administered accordingly. The second group was given distilled water via the same route and served as control. The birds were closely watched for the presence of clinical signs, if any, and sacrificed at weekly interval till the end of the experiment.

Gross and histopathological examination

Thorough postmortem examinations of the sacrificed birds were conducted and the presence of gross alteration if any was properly recorded. Representative samples from the liver, kidney, lungs, intestine, heart and proventriculus are collected were collected in 10% neutral formalin. After washing in running water and dehydration in alcohol, tissues were embedded and 5 µm paraffin sections cut and stained with haematoxylin and eosin as per standard method (Luna, 1968).

RESULTS

In chronic toxicity group of chickens immediately after oral dosing the chickens developed increased thirst. Gradually the symptoms disappeared, except for reduced feed intake and gradual reduction in body weight gain. After two months of treatment several of the birds exhibited slightly staggering gait, leg weakness and tremor suffered from diarrhoea. Some of the birds developed curled toes with pale mucous membrane and prominent keel bone. All these symptoms disappeared subsequently towards the end of the experiment. The weekly average body weight of treated and control chickens are summarized in Table 1. It was observed that there was a progressive increase in the average body weight of chickens of both the groups as the birds were in growing stage. But rate of weight gain was less in treated birds in comparison to the control. Statistical analysis revealed significant difference ($P<0.05$) from 7th week onwards to the completion of the experiment.

Liver

Liver changes were found to be progressive. Mild vascular congestion with scattered haemorrhages as well as mild infiltration of mononuclear cells was the alterations observed during the first month of treatment. By the second month the changes were more prominent, and consisted of degeneration of the hepatocytes with a few focal areas of hepatocellular necrosis (Fig. 1.a). Mild to moderate proliferation of the biliary epithelial cells around the portal veins with formation of new bile ducts (Fig. 1.b). Gradually by third month, histopathological changes were found to be more severe. There was dilatation of sinusoids; marked congestion of all the blood vessels, focal to diffuse haemorrhages, and focal infiltration of mononuclear cells. Infiltration of mononuclear cells in the hepatic parenchyma particularly around the blood vessels of the portal region was frequently encountered. Infiltration of mononuclear cells suggested the onset of immunological response by the host. In some areas the hepatic cords were markedly distorted, often with a tendency to form acini. The proliferation of biliary epithelial cells with formation of new bile ducts, mostly in the portal region was striking.

Table 1. Body weight (MEAN ± SE) in control and treated chickens

Time of blood collection (weeks)	Body weight (gms)	
	Control	Chronic
0	824.58±0.15	820.67±0.69
1	875.00±0.68	840.00±0.70
2	880.08±0.01	851.27±0.85
3	900.50±0.49	874.70±0.39
4	908.91±0.33	923.00±0.95
5	921.20±0.50	950.75±0.07
6	930.67±0.11	950.14±0.71
7	943.67±0.56	952.00±0.81*
8	950.91±0.21	958.20±0..22*
9	1000.03±0.32	970.75±0.27 *
10	1120.75±0.55	992.07±0.96 *
11	1133.33±0.90	1008.56±0.50*
12	1146.17±0.69	1010.21±0.76*

*P<0.05

Kidneys

The histopathological changes were found to be mild up to 3rd week of the experimental period, and from 4th week onwards changes became prominent characterized by congestion, focal to diffuse haemorrhage, tubular degeneration, necrosis and cellular swelling (Fig. 1.c). The glomeruli showed congestion and necrosis with dilatation of Bowman's space and vacuolar degeneration. Tubular lesions observed were the direct toxic effect of chlorpyriphos on the cell function.

Lungs

The histopathological changes in lungs appeared to be mild up to 9th week of the treatment period. There were mild vascular congestion and focal haemorrhages. By 10th week of the experimental period, changes revealed marked vascular congestion, and diffuse haemorrhages (Fig. 1.d). There were thickening of interalveolar septa by 10th week. Presence of proteinacious exudates in the lumen of the bronchioles were seen in some birds.

Brain

In chronic toxicity group of chickens, histopathological changes of the brain upto 10th week of experiment included mild vascular congestion, neuronophagia, demyelination and satellitosis.

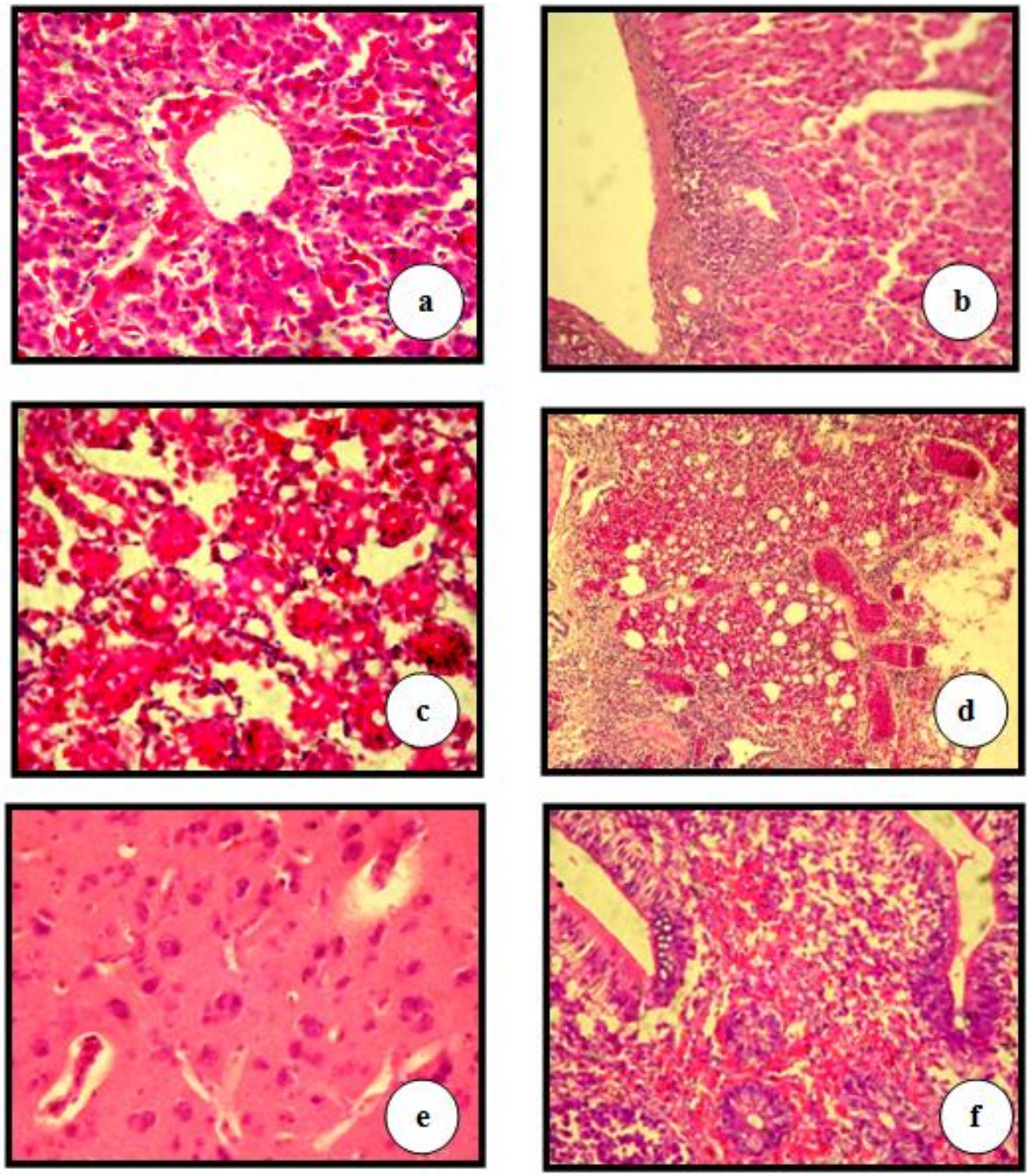

Fig. 1. Histopathological changes observed in indigenous chicken experimentally infected with chlorpyriphos : (a) liver showing haemorrhage, hepatocellular necrosis and dilatation of sinusoids by 8th week. H&E, ×100, (b) liver showing proliferation of biliary epithelium with formation of new bile ducts by 8th week. H&E, ×100, (c) kidneys showing cellular swelling by 8th week. H&E, ×400, (d) lungs showing congestion, haemorrhage and emphysema by 10th week. H&E, ×100, (e) brain showing satellitosis and neuronophagia by 10th week. H&E, ×400, (f) intestine showing haemorrhage, hyperplasia of epithelial cells and dilated intestinal gland filled with mucinous secretions by 10th week. H and E, ×100

Gradually, from 11th week of the experimental period, the changes were more prominent with marked vascular congestion, neuronal degeneration, neuronophagia, satellitosis, gliosis (Fig. 1.e) and demyelination in the cerebrum. In the cerebellum, the Purkinje cells revealed various degrees of degenerative changes. In some birds demyelination was observed. The nuclei of the cells showed hyperchromasia.

Intestine

The microscopic changes of the intestine of chronic toxicity chickens were mainly confined to the caecum.

Up to 8th week of the experimental period, there was presence of mild haemorrhages in the lamina propria along with hyperactive intestinal gland filled with mucinous secretions (Fig. 1.f). Few sections revealed mild infiltration of mononuclear cells in the lamina propria. By 10th week, there was sloughing of the necrotic mucosal epithelium at focal areas.

Proventriculas

In chronic toxicity chickens, proventriculus sections showed hyperplasia of mucosal epithelium, glandular necrosis,

elongation and distension of crypts and infiltration of mononuclear cells in the lamina propria.

Heart

Heart sections showed mild haemorrhage in the myocardium along with separation of muscle fibres.

DISCUSSION

Clinical signs

The clinical signs observed in the present study conform to the findings of several earlier workers in different animals and birds[1]. The subsequent disappearance of leg weakness and unsteady gait, seen in the present study, might be due to development of tolerance to the behavioral effects of repeated exposure to chlorpyriphos. This tolerance has been attributed to an attenuation of cholinergic responsiveness, observed in exposure to a variety of anticholinesterase compounds (Richardson *et al.,* 1993).

Body weight

The decrease in weight gain of the treated birds might be due to development of diarrhoea which impaired absorption of essential nutrients. However, in the present study, there was not any appreciable change in the feed consumption of the birds following chlorpyriphos treatment and it may be anticipated that this effect could possibly be caused by the overall increased degeneration of lipids and proteins as a result of the direct effects of the organophosphate. Reduced body weight gain which might be an indication of direct toxicity or stressogenic activity of these compounds (Goyal *et al.,* 1986).

Liver

Some of the lesions observed in liver in present study are consistent with several workers who reported congestion, vacuolar degeneration and fatty changes, focal to extensive necrosis, hyperplasia of Kupffer cells, dilation of sinusoids, nuclear aberrations, cytoplasmic degranulation and pyknotic nuclei (Choudhary *et al.,* 2003). Hepatotoxic effect of chlorpyriphos in layer chickens also corroborate with the findings of the present study (Kammon, 2010) .

Kidney

The changes recorded in kidney in the present study might be due to effect of metabolites of chlorpyriphos as kidneys are the major route for elimination of chlorpyriphos and sensitive to toxic influences of CPF. Since the kidneys have high oxygen consumption and vulnerable enzyme system along with complicated mechanism of transport for toxins, the highly specialzed ciliated columnar epithelial cells of proximal convoluted tubules are more prone to damage by chlorpyriphos (Tisher and Brenner, 1989). Histopathological lesions observed in the treatment group were indicative of nephrotoxicity of the compound chlorpyriphos and its metabolites. The transient renal injury due to Organophosphates with tubular cell necrosis (Kammon *et al.,* 2010). The presence of necrosis may be related to the depletion of ATP, which finally leads to the death of the cells (Shimizu *et al.,* 1996). Other possible mechanisms for the tubular lesions may involve reactive free radicals or oxidative stress, or both (Kammon *et al.,* 2011).

Lungs

The congestion revealed in lungs in the present study might be a resultant effect of chlorpyriphos toxicity. The changes recorded in lungs had a close resemblance with those observed in broiler chickens (Malik *et al.,* 2002).

Brain

The revealed vacuolization in the neuronal nuclei might be due to impairment of synthetic processes. The observations recorded in brain in the present study corroborates with earlier findings (Kammon *et al.,* 2010).

Intestine

The observed lesions in intestine suggested that chlorpyrifos might have some irritant effects on the epithelial surface of the intestine. The changes reported in intestine had a close resemblance with earlier findings (Krishnamoorthy *et al.,* 2007).

Proventriculus

Krishnamoorthy *et al*. (2007) also described similar lesions in proventriculus. Histopathological examination of tissue sections from different organs in the present study clearly showed that the alterations were influenced by dose and duration of exposure to chlorpyriphos. It was observed that the microscopic lesions initially started in mild form. As the time and cumulative toxin consumption increased, the lesions became more prominent suggesting that chronic toxicity does occur with chlorpyriphos contrary to the literature surveyed. So it needs further elucidation.

Conclusion

Thus it is concluded that, chronic exposure to chlorpyriphos produced histopathological and alterations in chickens. However, the exact mechanism that leads to cellular damage, leading to hepatotoxicity and nephrotoxicity, needs to be elucidated.

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