Pathology of chlorpyriphos and T-2 toxin on broiler chicken

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ABSTRACT

Forty-eight, newly hatched, unsexed broiler chicks were fed diets containing 45 ppm chlorpyriphos, an organophosphorus compound and 0.5 ppm T-2, a mycotoxin, singly and in combination for 28 days from day of hatch to study pathological effects. Gross, pale, enlarged liver, distended gall bladder and streaks of haemorrhages in the thigh muscles were observed in the chlorpyriphos group, while the chlorpyriphos+T-2 group showed pale and enlarged liver. Histopathological changes observed in the toxin-fed birds during 14th and 28th days of the trial were as follows: liver revealed periportal fibrosis, mononuclear cell infiltration, necrosis of hepatocytes and bile duct hyperplasia in all the toxin-fed birds. Kidney showed tubular epithelial degeneration and necrosis in chlorpyriphos and chlorpyriphos+T-2-fed birds. Hearts of all toxin treated birds showed vacuolar degeneration of myocytes. The chlorpyriphos+T-2-fed birds showed necrosis of oral mucosa with infiltration of heterophils predominantly, along with mononuclear cells. Crop mucosa showed epithelial hyperplasia and keratinisation in all treatment groups. Proventriculus showed hyperplasia of epithelial cells, glandular necrosis and infiltration of mononuclear cells in chlorpyriphos and chlorpyriphos+T-2 groups. The T-2 group showed epithelial necrosis, crypt elongation, diphtheritic membrane formation and mononuclear cell infiltration in lamina propria. Gizzard showed glandular interstitial fibrosis, infiltration of heterophils and mononuclear

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cells in chlorpyriphos, while T-2 groups and chlorpyriphos+T-2 groups showed interstitial glandular fibrosis and hyperplastic reaction. Intestine showed fusion of villi, necrosis, goblet cell hyperplasia and infiltration of mononuclear cells in lamina propria in all toxin-fed birds. Mononuclear cell infiltration, reduced zymogen granules and vacuolar degeneration in chlorpyriphos and chlorpyriphos+T-2 fed birds; mononuclear cell infiltration in T-2 fed birds was observed in pancreas. The chlorpyriphos group alone showed mononuclear cell infiltration in the meninges of brain. The study indicated the pathological effects of these toxins, either alone or in combination, in various organs of broiler chicken at low dose levels.

**Key words:** broiler chicken, chlorpyriphos, T-2 toxin, pathological changes

### Introduction

Poultry feed ingredients are exposed to number of pesticides of residual potential: chlorpyriphos (O, O-diethyl-O-[3,5,6-trichloro-2-pyridyl] phosphorothioate), an organophosphorus compound, which particularly affects the cholinesterase enzyme system. It is a broad spectrum systemic insecticide widely used for the control of pests, mites, flies and lice affecting livestock and poultry (LOOMI et al., 1972) and detected in poultry egg, meat and cow milk and milk products (RAWAT et al., 2003). MALIK et al. (2002) reported that broilers fed 30, 60 and 120 ppm chlorpyriphos from 0 to 6 weeks of age revealed hepatocellular necrosis, desquamation of kidney tubular epithelium, degeneration of myocardium and a few neurons and Purkinje cells in brain. Broilers fed 35, 70 and 140 ppm of chlorpyriphos from 2 to 8 weeks of age showed congestion and haemorrhages of liver, lung, intestine and thigh muscles (YADA V et al., 2003). Limited information is available on chlorpyriphos-induced pathological changes on various organs in broiler chicken.

Mycotoxins comprise a group of structurally diverse fungal secondary metabolites that cause a wide spectrum of pathologic effects in livestock and poultry. The capacity of some mycotoxins to alter the normal immune function when present in feeds at levels below observable overt toxicity is of particular interest (PESTKA and BONDY, 1990). T-2 toxin, a naturally occurring mycotoxin produced by *Fusarium* species, is a 3 hydroxy 4, 15 diacetoxy-8(3-methylbutryloxy), 12, 13 epoxy tricothec-9-ene metabolite. In poultry, T-2 mycotoxicosis reduces growth rate and feed conversion, impairs the immune system and induces pathologic damage to liver and other organs (COULOMBE, 1993). Broilers fed 1ppm of T-2 toxin from 0 to 4 weeks of age revealed histopathological changes in various organs such as liver, kidney, proventriculus, gizzard and intestine (KAMALAVENKATESH, 2003). There is a lack of literature on the combined effects of chlorpyriphos and T-2 toxin in broiler chicken. Available literatures on the individual effects of these toxins indicate that they were relatively at higher dose levels and longer duration of exposure. Hence, the present work was undertaken to study the pathological changes in broilers exposed to these toxins at low dose levels, individually and in combination.
Materials and methods

Forty-eight newly hatched, unsexed commercial broiler chicks (VENCOBB) procured from M/s. Venkateshwarab Hatcheries (P) Ltd, Chennai were wing banded, weighed and housed in battery brooders and supplied with feed and water ad libitum. Birds were randomly divided into four groups of 12 chicks each (i.e. control, chlorpyriphos, T-2, and chlorpyriphos+T-2). Chlorpyriphos pesticide technical grade (96.4%) was procured from De-Nocil Crop Protection Limited, Mumbai and was used in this study. The Fusarium sporotrichioides var sporotrichioides Microbial Type Culture Collection (MTCC) 1894 was subcultured periodically on Sabouraud’s dextrose agar and potato dextrose agar at an interval of 15 days to maintain its viability (BURMEISTER, 1971). The T-2 toxin was produced on wheat substrate. One hundred g. of wheat were placed in 500 mL Erlenmeyer flasks and soaked in 75 mL of water overnight. The flasks were autoclaved (15 psi/15 min), cooled and inoculated with Fusarium sporotrichioides var sporotrichioides. Thirty mL sterile water were then added to each flask and the flasks were incubated at 17 °C for 21 days. After 48 hours of inoculation a whitish mould growth was seen on the surface of wheat, later turning to shades of yellow, and then to a rose colour. Subsequently, the colour changed to carmine-red (JOFFE and PALTI, 1975). After incubation, the mouldy wheat was steamed at 100 °C for 1 hour to kill the spores, followed by drying in a hot-air oven overnight at 60 °C. The dried wheat culture was ground to fine powder and analyzed for its T-2 toxin content by using thin layer chromatography (TAPIA, 1985).

The experimental trials were approved by the Institutional Animal Ethics Committee, India and conducted under its guidelines at the Poultry Research Station, Chennai 600035. Broiler mash containing no toxin binders and tested to be free from aflatoxins, T-2 toxin, ochratoxin-A, cyclopiazonic acid, penicillic acid, citrinin and zearalenone was used. The feed was also tested for pesticide residues and found free from pesticides. The analyses were carried out at our university Central Animal Feed and Food Residue Laboratory, Chennai 600051. Known amounts of chlorpyriphos and T-2 toxin containing wheat culture materials were incorporated in the broiler mash to yield 45 ppm chlorpyriphos and 0.5 ppm T-2, respectively, and fed for 28 days from the day of hatch. The broiler mash fed contained 25% crude protein. Birds were vaccinated against Newcastle disease (ND) F strain vaccine at 5 days of age with one drop (10⁶ EID₅₀/bird) intraocular vaccine from the Institute of Veterinary Preventive Medicine, Ranipet, Tamil Nadu.

Six birds were sacrificed in each group by the cervical dislocation method at the 14th and 28th day of study. After exsanguinations a detailed post-mortem examination was conducted and gross lesions were recorded. Representative pieces of tissue from liver, kidney, heart, oral mucosa, crop, proventriculus, gizzard, intestine, pancreas and brain were collected in 10 per cent formol saline. Paraffin-embedded tissues were sectioned to 5 μ thickness and stained with haematoxylin and eosin for histopathological examination (BANCROFT and STEVENS, 1996).
Results

Gross pathology. Chlorpyriphos-fed birds showed pale and slightly enlarged liver, streaks of haemorrhages in thigh muscles and slightly distended gall bladder. The T-2 and chlorpyriphos+T-2-fed birds showed pale and enlarged liver (Fig. 1).

Fig. 1. Chlorpyriphos+T-2 toxicoses. Two-week-old broiler chicken liver: pale and enlarged

Fig. 2. Chlorpyriphos+T-2 toxicoses. Two-week-old broiler chicken liver: mild periportal fibrosis and bile duct hyperplasia. H&E; ×400.
Liver of chlorpyriphos-fed birds showed periportal fibrosis, bile duct hyperplasia and focal mononuclear cell collection during the 14th and 28th day of trial. In the T-2-fed birds, mild degeneration with focal necrosis, periportal fibrosis, bile duct hyperplasia and periportal lymphocytic infiltration were observed in the liver. Similar lesions were observed in liver of chlorpyriphos+T-2-fed birds during the 14th and 28th day of study (Fig. 2.). Kidney showed tubular epithelial degeneration and necrosis in chlorpyriphos- and chlorpyriphos+T-2-fed birds, while no significant change was

Histopathology. Liver of chlorpyriphos-fed birds showed periportal fibrosis, bile duct hyperplasia and focal mononuclear cell collection during the 14th and 28th day of trial. In the T-2-fed birds, mild degeneration with focal necrosis, periportal fibrosis, bile duct hyperplasia and periportal lymphocytic infiltration were observed in the liver. Similar lesions were observed in liver of chlorpyriphos+T-2-fed birds during the 14th and 28th day of study (Fig. 2.). Kidney showed tubular epithelial degeneration and necrosis in chlorpyriphos- and chlorpyriphos+T-2-fed birds, while no significant change was
observed in T-2-fed birds. All toxin-fed birds showed vacuolar degeneration of cardiac myocytes. Chlorpyriphos+T-2-fed birds alone showed necrosis of oral mucosa, with infiltration of heterophils predominant, along with mononuclear cells. Crop mucosa of all toxin-fed birds showed epithelial hyperplasia and keratinisation during the 14th and 28th day of study. Chlorpyriphos- fed birds showed mild hyperplasia of mucosal epithelium, glandular necrosis, elongation and distension of crypts and infiltration of mononuclear cells in the lamina propria of proventriculus (Fig. 3). The T-2-fed birds
showed epithelial necrosis, diphtheritic membrane formation and crypt elongation on the 14th day and shortening of villi and mononuclear cell infiltration in proventriculus (Fig. 4) on the 28th day of trial. Partial to full thickness necrosis, shortening of villi, epithelial hyperplasia, and mononuclear cell infiltration of proventriculus in chlorpyriphos+T-2 fed birds were observed. Gizzard showed interstitial fibrosis in the glandular area and diffuse infiltration of mononuclear cells (Fig. 5) in chlorpyriphos-fed birds. The T-2-fed birds showed heterophilic infiltration and interstitial fibrosis in the gizzard during the 14th and 28th day of trial. The chlorpyriphos+T-2-fed birds showed epithelial hyperplasia and interstitial glandular fibrosis in the gizzard. Intestine of chlorpyriphos-fed birds showed necrosis, goblet cell hyperplasia, fusion of villi and infiltration of mononuclear cells in the lamina propria, and in T-2-fed birds, catarrhal changes, fusion of villi, crypt hyperplasia and infiltration of mononuclear cells in the lamina propria were observed. The combined toxin-fed group showed fusion of villi and goblet cell hyperplasia of intestine (Fig. 6) on the 14th and 28th day of study, while no comparable literature was available for the combined toxicoses.

Pancreas of chlorpyriphos-fed birds showed mononuclear cell infiltration and reduced zymogen granules. The T-2-fed birds showed mononuclear cell infiltration; combined toxin-fed birds showed reduced zymogen granules, focal collection of mononuclear cells and vacuolar degeneration of pancreatic acinar epithelium during the 14th and 28th day of trial. Similar changes were observed in pancreas in the combined toxin-fed birds during the 14th and 28th day of experimental trial. Chlorpyriphos-fed birds alone showed mononuclear cell infiltration in meninges of brain during the 14th and 28th day of trial.

Discussion

Gross pathology. The gross lesions observed in chlorpyriphos-fed birds were pale and enlarged liver, slightly distended gall bladder and thigh muscle haemorrhages, which was in concurrence with the findings of KAUR et al. (1999) in goats; MEHTA et al. (2003) and YADA V et al. (2003). The T-2- and chlorpyriphos+T-2-fed birds showed pale and enlarged liver, which was in concurrence with the findings of HOERR et al. (1981).

Histopathology. The lesions observed in liver of chlorpyriphos-fed birds were periportal fibrosis, bile duct hyperplasia and focal mononuclear cell collection on the 14th and 28th day of trial. Similar lesions were reported in broilers fed 30, 60 and 120 ppm chlorpyriphos at 6 weeks of age (MALIK et al., 2002) and 35, 70 and 140 ppm chlorpyriphos at 2 to 8 weeks of age (YADA V et al., 2003). Liver of T-2-fed birds showed mild degeneration, with focal necrosis, periportal fibrosis, bile duct hyperplasia and periportal lymphocytic infiltration, which concurred with the findings of HOERR et al. (1981), NIYO et al. (1988) for rabbits and KAMALAVENKATESH (2003). Similar lesions were observed in the combined toxin-fed group, which were not reported earlier. Kidney showed tubular epithelial degeneration
and necrosis in chlorpyriphos- and chlorpyriphos+T-2-fed birds and concurred with earlier reports by BANSAL et al. (1994) in rabbits, and MALIK et al. (2002). Heart muscles showed vacuolar degeneration in all the toxin treated groups, which were not reported earlier for T-2 toxin but which concurred with reports by MALIK et al. (2002) and YADAV et al. (2003) for chlorpyriphos toxicosis. Oral mucosa showed necrosis with infiltration of heterophils predominantly along with mononuclear cells in chlorpyriphos+T-2-fed birds on the 14th and 28th day of the trial, which was in correlation with the findings of WYATT et al. (1972), who reported oral lesions in broilers fed 4 ppm T-2 toxin from 0 to 3 weeks of age. All toxin-fed birds showed epithelial hyperplasia and keratinisation of crop mucosa during the 14th and 28th day of the trial, which was not reported earlier for chlorpyriphos toxicosis but which concurred with reports by KAMALAVENKATESH (2003) for T-2 toxicosis. Chlorpyriphos-fed birds showed slight hyperplasia of mucosal epithelial cells, glandular necrosis, crypt elongation and distension and infiltration of mononuclear cells in lamina propria of proventriculus on the 14th and 28th day of the trial, which were not reported earlier. The T-2-fed group showed epithelial necrosis, diphtheritic membrane formation, crypt elongation on the 14th day, and shortening of villi and mononuclear cell infiltration in the lamina propria of proventriculus on the 28th day of the trial, which correlated with the findings of HOERR et al. (1981) and KAMALAVENKATESH (2003). The chlorpyriphos+T-2-fed birds showed necrosis, mononuclear cell infiltration and epithelial cell hyperplasia, which were not reported earlier. Chlorpyriphos-fed birds showed interstitial fibrosis in the glandular area and focal to diffuse infiltration of mononuclear cells in gizzard, which were not reported earlier. In T-2-fed birds, gizzard showed heterophilic infiltration and glandular interstitial fibrosis, which concurred with reports by KUBENA et al. (1989, 1990) and KAMALAVENKATESH (2003). Combined treatment group showed interstitial fibrosis and hyperplastic reaction, which were not reported earlier. Intestine of chlorpyriphos-fed birds showed necrosis, goblet cell hyperplasia, fusion of villi and infiltration of mononuclear cells in the lamina propria, which concurred with the findings of KAUR et al. (1999) in goats and YADAV et al. (2003). In T-2-fed birds, catarrhal changes, fusion of villi, crypt hyperplasia and infiltration of mononuclear cells in the lamina propria were observed in intestine, which concurred with reports by KAMALAVENKATESH (2003). The combined treatment group showed fusion of villi, goblet cell hyperplasia of intestine on the 14th and 28th day of study, while no comparable literature was available for the combined toxicoses.

Pancreas showed mononuclear cell infiltration and reduced zymogen granules in chlorpyriphos-fed birds, while no comparable literature was available for these changes. The T-2-fed birds showed mononuclear cell infiltration in pancreas and correlated with reports by NARYANASWAMY (1998) and MADHESWARAN (2002) in Japanese quails. The chlorpyriphos+T-2-fed birds showed reduced zymogen granules, focal collection of mononuclear cells and vacuolar degeneration of acinar epithelium, which was not reported.
earlier. Brain of chlorpyriphos-fed birds alone showed mononuclear cell infiltration in meninges, but MALIK et al. (2002) and YADA V et al. (2003) reported perivascular and perineuronal oedema, gliosis and degeneration of a few neurons and Purkinje cells in broilers.

The chlorpyriphos and T-2 toxins individually and in combination induced marked changes in various organs, including digestive tract, and adversely affected the digestion, absorption and assimilation of feed nutrients. The body mass gain of broiler chicken at 4 weeks of age were reduced by 9, 22 and 14 per cent in chlorpyriphos-, T-2- and chlorpyriphos+T-2-fed groups, respectively, when compared to the control in this study. The inherent ability of T-2 toxin in inhibiting protein synthesis through inhibition of peptidyl transferase activity (CORRIER, 1991) might also have attributed to the reduced body mass gain, thus causing economic losses to poultry farmers. The haemagglutination inhibition titres against Newcastle disease virus and stimulation index of splenocytes to concavalin A were decreased significantly in the toxin-fed birds when compared to the control birds in this study (KRISHNAMOORTHY et al., 2005). This indicated the affection of humoral and cellular immunity in toxin-fed birds. These findings indicated a potential threat for predisposition of toxin-fed birds to various infectious diseases. The feed and feedstuffs meant for broilers should be screened for the presence of chlorpyriphos and T-2 toxin before feeding it to them. However, further studies are required to arrive at the minimal individual and combined levels affecting the various organs and interaction of these toxins with common infectious diseases of poultry.

References


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SAŽETAK
Istraživani su patološki učinci organofosforovog spoja klorpirifosa i mikotoksina T-2 na 48 netom izvaljenih tovnih pilića kojima nije određivan spol. Oba spoja davana su u hrani u tijeku 28 dana svaki zasebno i u kombinaciji. Klorpirifos je davan u količini od 45 ppm, a mikotoksin u količini od 0,5 ppm. Patoanatomski ustanovljena je blijeda i povećana jetra, prošireni žučni mjehur i prugasta krvarenja na bedrenom mišiću u skupini koja je dobivala klorpirifos, dok je u skupini koja je dobivala mikotoksin u kombinaciji s mikotoksinom ustanovljena blijeda i povećana jetra. Patohistološke promjene ustanovljene su 14. i 28. dana u skupini pilića koji su dobivali toksin očitovale su se periportalnom fibrozom, infiltracijom mononuklearnih stanica, nekrozom hepatocita i hiperplazijom žučovoda. U piliću koji su dobivali klorpirifos i klorpirifos u kombinaciji s toksinom T-2 ustanovljena je tubularna epitelnal degeneracija i nekroza bubrega. U svih pilića obradivanih toksinom dokazana je vakuolarna degeneracija miocita. Nekroza oralne sluznice s infiltracijom heterofila i mononuklearnim stanicama dokazana je u piliću koji su dobivali klorpirifos i toksin. Sluznica voljke pokazivala je epitelnal hiperplaziju i keratinizaciju u svih obradivanih skupina. Na predželucu je dokazana hiperplazija epitelnalnih stanica, glandularna nekroza i infiltracija mononuklearnih stanica u skupinama koje su dobivale klorpirifos i klorpirifos zajedno s T-2. U skupini kojoj je davan T-2 ustanovljena je epitelnal nekroza, prodljenje kripti, difteroidne naslage i mononuklearna stanična infiltracija u lamini propriji. U želucu je dokazana glandularna intersticijska fibroza, infiltracija heterofila i mononuklearnih stanica u skupinama koje su dobivale zasebno klorpirifos i T-2, dok je u skupini koja je istodobno dobivala klorpirifos i T-2 dokazana izbrišljiva glandularna fibroza i hiperplastične reakcije. Ustanovljeno je spajanje resica, nekroza, djelomična hiperplazija i infiltracija mononuklearnih stanica u lamini propriji crijeva u svih pilića koji su dobivali toksin. Mononuklearna stanična infiltracija, smanjena zimogena zrnca i vakuolarna degeneracija dokazane su u skupini koja je dobivala samo klorpirifos jednako kao i u skupini koja je dobivala istodobno klorpirifos i T-2. Mononuklearna stanična infiltracija dokazana je u gušterači pilića koji su dobivali T-2. Infiltacija mononukularnih stanica dokazana je u moždanim opnama pilića koji su dobivali samo klorpirifos. Istraživanje je pokazalo da se patološki učinci toksina davnih zasebno ili u kombinaciji u vrlo malim dozama očituju na različitim organima tovnih pilića.

Ključne riječi: tovniji pilići, klorpirifos, toksin T-2, patološke promjene